LIÈGE université
Psychologie, Logopédie
& Sciences de l'Éducation

# Neuroimaging and Electrophysiology of the Association Between Sleep, the Locus Coeruleus, and Alzheimer's Disease


**Nasrin Mortazavi**

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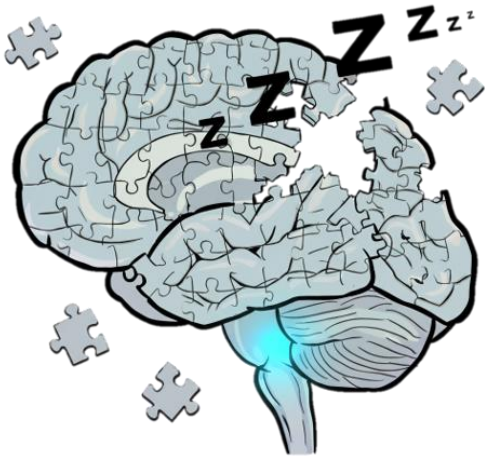
Belgium


**Supervisors:**
Dr. Gilles Vandewalle
Dr. Christine Bastin


A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Psychological Sciences at the Faculty of Psychology and Educational Sciences.

Academic year 2025-2026

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

E. Pugh

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## Abstract

The locus coeruleus (LC) is a brainstem nucleus that is the principal source of norepinephrine in the brain. It plays a central role in many aspects of brain function including the regulation of sleep and wakefulness. Animal studies show that LC neurons exhibit state-dependent firing patterns, being most active during wakefulness, relatively less active during non-rapid eye movement (NREM) sleep, and virtually silent during REM sleep. These state-dependent modifications are required for the transitions between wakefulness, NREM and REM sleep. Moreover, variations of LC activity during sleep are associated with oscillatory brain activity taking the form of sleep spindles and slow waves while experimental LC activation during sleep in animals induces so-called microarousals, highlighting its capacity to modulate sleep continuity and microarchitecture. The LC is also one of the first brain regions to show abnormal accumulation of tau protein, a key feature of Alzheimer's disease (AD) neuropathology — decades before symptom onset. The LC may therefore underlie the emerging link in the literature between sleep and neurodegeneration and its individual characteristics may constitute a potential early marker and modulator of neurodegenerative progression.

The contribution of LC function to sleep variability, particularly across aging and in relation to the vulnerability to AD remains however insufficiently characterized in humans. The overall aim of this thesis is to explore the contribution of LC function and its neural circuits to sleep features in humans and their relevance to aging and the genetic susceptibility to AD.

In a first study, we examined the relationship between LC activity—measured during wakefulness using 7-Tesla functional MRI (7T fMRI) in different cognitive contexts—and electroencephalogram (EEG) features of rapid eye movement sleep (REMS) in 52 younger and older adults. We found that the

activity of the LC during wakefulness is significantly associated with REMS theta oscillations and the EEG sigma power preceding REMS, with task- and age-specific patterns. The results suggest that an optimal activity of the LC during wakefulness may reflect its optimal activity during sleep and support the quality of REM sleep in humans.

A second study extended this work by investigating the cross-talk between the LC and hypothalamus, i.e. their effective connectivity in fMRI, using the same dataset. We first show a mutual influence between the LC and the anterior-superior and posterior parts of the hypothalamus, supporting the idea that the connectivity patterns observed in animal models between these structures are also present in humans. Importantly, in older adults, stronger connectivity from the anterior hypothalamus to the LC was associated with reduced generation of theta oscillations during REM sleep, as well as with other types of oscillations during sleep, indicating that age-related modulation of the LC circuitry may underlie variability in sleep.

In a third study, we focused on sleep microarousals, consisting of brief cortical activations during sleep that are related in part to LC activity, and their association with genetic risk for developing AD in a large dataset comprising 540 healthy younger and late middle-aged individuals. We used polygenic risk scores (PRS) for AD and detailed classification of arousal subtypes based on their associations with sleep stage transitions and muscle activities. Results yield significant interaction between PRS and specific arousal types in older adults. Notably, arousals association with sleep stage transitions and muscle activity were negatively associated with PRS, while arousals associated with sleep stage transition but not with muscle activation were positively associated with PRS. Further analyses showed that these arousal subtypes relate to the longitudinal cognitive decline, further linking arousal dynamics, LC-related sleep physiology, and AD risk.

Together, these studies reinforce the view that LC and its associated circuits play a critical and age-dependent role in modulating sleep physiology in humans. Furthermore, alterations in element of the microstructure of sleep associated with LC activity, such as sleep microarousals, may serve as early electrophysiological markers of the vulnerability to AD. These findings deepen our understanding of the neurophysiological mechanisms connecting sleep, aging, and neurodegeneration, and may contribute to identifying novel potential targets to detect and maybe delay early AD neuropathology progression.

## Résumé

Le locus coeruleus (LC) est un noyau du tronc cérébral qui constitue la principale source de noradrénaline dans le cerveau. Il joue un rôle central dans de nombreux aspects du fonctionnement cérébral, notamment dans la régulation du sommeil et de l'éveil. Les études animales montrent que les neurones du LC présentent des schémas de décharge dépendants de l'état : ils sont les plus actifs durant l'éveil, relativement moins actifs durant le sommeil à ondes lentes (NREM), et pratiquement silencieux durant le sommeil paradoxal (REM). Ces modulations dépendantes de l'état sont nécessaires aux transitions entre l'éveil, le sommeil NREM et le sommeil REM. De plus, les variations de l'activité du LC pendant le sommeil sont associées à une activité oscillatoire cérébrale prenant la forme de fuseaux de sommeil et d'ondes lentes, tandis qu'une activation expérimentale du LC durant le sommeil chez l'animal induit les « micro-éveils », mettant en évidence sa capacité à moduler la continuité et la microarchitecture du sommeil. Le LC est également l'une des premières régions cérébrales à présenter une accumulation anormale de protéine tau, caractéristique clé de la neuropathologie de la maladie d'Alzheimer (MA), et ce plusieurs décennies avant l'apparition des symptômes. Le LC pourrait donc sous-tendre le lien émergent dans la littérature entre sommeil et neurodégénérescence, et ses caractéristiques propres pourraient constituer un marqueur précoce potentiel ainsi qu'un modulateur de la progression neurodégénérative.

La contribution de la fonction du LC à la variabilité du sommeil, en particulier au cours du vieillissement et en lien avec la vulnérabilité à la MA, reste toutefois insuffisamment caractérisée chez l'humain. L'objectif global de cette thèse est d'explorer la contribution de la fonction du LC et de ses circuits neuronaux aux caractéristiques du sommeil chez l'humain, ainsi que leur pertinence pour le vieillissement et la susceptibilité génétique à la MA.

Dans une première étude, nous avons examiné la relation entre l'activité du LC — mesurée durant l'éveil à l'aide de l'IRM fonctionnelle à 7 Tesla (IRMf 7T) dans différents contextes cognitifs — et les caractéristiques électroencéphalographiques (EEG) du sommeil paradoxal (REMS) chez 52 jeunes adultes et adultes âgés. Nous avons constaté que l'activité du LC pendant l'éveil est significativement associée aux oscillations thêta du REMS ainsi qu'à la puissance sigma de l'EEG précédant le REMS, avec des schémas spécifiques à la tâche et à l'âge. Les résultats suggèrent qu'une activité optimale du LC durant l'éveil pourrait refléter son activité optimale durant le sommeil et soutenir la qualité du sommeil paradoxal chez l'humain.

Une deuxième étude a prolongé ce travail en explorant les interactions entre le LC et l'hypothalamus, c'est-à-dire leur connectivité effective en IRMf, en utilisant le même ensemble de données. Nous avons d'abord mis en évidence une influence réciproque entre le LC et les parties antéro-supérieure et postérieure de l'hypothalamus, soutenant l'idée que les schémas de connectivité observés dans les modèles animaux entre ces structures sont également présents chez l'humain. Fait important, chez les adultes âgés, une connectivité plus forte de l'hypothalamus antérieur vers le LC était associée à une réduction de la génération d'oscillations thêta durant le sommeil paradoxal, ainsi qu'à d'autres types d'oscillations pendant le sommeil, indiquant que la modulation liée à l'âge du circuit LC pourrait sous-tendre la variabilité du sommeil.

Dans une troisième étude, nous nous sommes concentrés sur les micro-éveils du sommeil, qui consistent en de brèves activations corticales pendant le sommeil et qui sont en partie liées à l'activité du LC, ainsi que sur leur association avec le risque génétique de développer la MA, dans un vaste ensemble de données comprenant 540 individus jeunes et âgés en bonne santé. Nous avons utilisé des scores de risque polygénique (PRS) pour la MA

et une classification détaillée des sous-types de micro-éveils en fonction de leurs associations avec les transitions de stades du sommeil et les activités musculaires. Les résultats mettent en évidence une interaction significative entre les PRS et certains types spécifiques de micro-éveils chez les adultes âgés. Notamment, les micro-éveils associés aux transitions de stades de sommeil et à l'activité musculaire étaient négativement associés aux PRS, tandis que les micro-éveils associés aux transitions de stades du sommeil mais sans activation musculaire étaient positivement associés aux PRS. Des analyses complémentaires ont montré que ces sous-types de micro-éveils sont liés au déclin cognitif longitudinal, reliant davantage la dynamique des micro-éveils, la physiologie du sommeil liée au LC et le risque de MA.

Dans l'ensemble, ces études renforcent l'idée que le LC et ses circuits associés jouent un rôle crucial et dépendant de l'âge dans la modulation de la physiologie du sommeil chez l'humain. En outre, les altérations d'éléments de la microstructure du sommeil associées à l'activité du LC, telles que les micro-éveils, pourraient servir de marqueurs électrophysiologiques précoces de la vulnérabilité à la MA. Ces résultats approfondissent notre compréhension des mécanismes neurophysiologiques reliant le sommeil, le vieillissement et la neurodégénérescence, et pourraient contribuer à identifier de nouvelles cibles potentielles pour détecter, voire retarder, la progression précoce de la neuropathologie de la MA.

## Main Scientific Contribution

**Publications as first author**

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Mortazavi, N., Zubkov , M., Chylinski, D., Collette, F., Bastin, C., Maquet, P., Vandewalle, G., Talwar, P. (2025). Sleep Arousals are associated with the polygenic risk for developing Alzheimer's Disease and with Cognitive Decline in healthy late middle-aged individuals. Submitted to Sleep.

**Conference presentations as first author**

Mortazavi, N., Talwar, P., Koshmanova, E., Sharifpour, R., Beckers, E., Berger, A., Campbell, I., Paparella, I., Balda Aizpurua, F., Zubkov, M., Lamalle, L., & Vandewalle, G. (25 September 2024). Locus coeruleus activity during wakefulness is associated with sigma power prior to REM sleep [Poster presentation]. The 27th Conference of the European Sleep Research Society (ESRS), Seville, Spain.

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Is Associated with Rapid Eye Movement Sleep Intensity [Poster presentation]. GIGA DAY, Liege, Belgium.

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Mortazavi, N., Koshmanova, E., Sharifpour, R., Berger, A., Paparella, I., Campbell, I., Beckers, E., Talwar, P., Sherif, S., & Vandewalle, G. (2022). Brain correlates of perceptual switch during perception rivalry: an ultrahigh field 7T functional magnetic resonance imaging study. [Poster presentation].The Brain Conference.

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## List of abbreviations

| Abbreviation | Description |
|---|---|
| **1KGP** | 1000 Genomes Project |
| **AAL** | Automated Anatomical Labeling |
| **AASM** | American Academy of Sleep Medicine |
| **Aβ** | Amyloid-β |
| **AD** | Alzheimer's disease |
| **BAI** | Beck Anxiety Inventory |
| **BDI** | Beck Depression Inventory |
| **BF** | Basal Forebrain |
| **BOLD** | Blood-Oxygen-Level-Dependent |
| **BMI** | Body Mass Index |
| **CAT12** | Computational Anatomy Toolbox |
| **CI** | Confidence Interval |
| **CSF** | Cerebrospinal Fluid |
| **DCM** | Dynamic Causal Modelling |
| **DARTEL** | Diffeomorphic Anatomical Registration Through |
| **DSST** | Digit Symbol Substitution Test |
| **ECG** | Electrocardiogram |
| **EOG** | Electrooculogram |
| **EMG** | Electromyogram |
| **EEG** | Electroencephalogram |
| **FCSRT** | Free and Cued Selective Reminding Test |
| **FA** | Flip Angle |
| **FDR** | False Discovery Rate |
| **fMRI** | functional Magnetic Resonance Imaging |
| **GLM** | General Linear Model |
| **GLMM** | Generalized Linear Mixed Model |
| **GWAS** | Genome-wide Association Studies |
| **HRF** | Hemodynamic Response Function |
| **ISF** | Interstitial Fluid |
| **LC** | Locus Coeruleus |

| | |
|---|---|
| **LD** | Linkage Disequilibrium |
| **LH** | Lateral Hypothalamus |
| **LDT** | Laterodorsal Tegmental nucleus |
| **MAF** | Minor Allele Frequency |
| **MNI** | Montreal Neurological Institute |
| **MP2RAGE** | Magnetization-Prepared with 2 Rapid Gradient |
| **MT-TFL** | Magnetization Transfer–weighted Turbo-Flash |
| **MTC** | Magnetization Transfer Contrast |
| **MST** | Mnemonic Similarity Task |
| **MCI** | Mild Cognitive Impairment |
| **MMSE** | Mini-Mental State Examination |
| **MoCA** | Montreal Cognitive Assessment |
| **MRI** | Magnetic Resonance Imaging |
| **NE** | Norepinephrine |
| **NREM** | Non-Rapid Eye Movement |
| **NFTs** | Neurofibrillary Tangles |
| **OSA** | Obstructive Sleep Apnea |
| **PET** | Positron Emission Tomography |
| **PPT** | Pedunculopontine Tegmental nucleus |
| **PG** | Periaqueductal Gray |
| **PSG** | Polysomnography |
| **PSQI** | Pittsburgh Sleep Quality Index |
| **PRS** | Polygenic Risk Score |
| **p-tau** | Phosphorylated tau |
| **PTSD** | Post-Traumatic Stress Disorder |
| **ROI** | Region Of Interest |
| **SNP** | Single Nucleotide Polymorphism |
| **SPM12** | Statistical Parametric Mapping |
| **SUVR** | Standardized Uptake Value Ratio |
| **SCN** | Suprachiasmatic Nucleus |
| **SWA** | Slow-Wave Activity |
| **SWE** | Slow-Wave Energy |
| **SWS** | Slow-Wave Sleep |
| **TIV** | Total Intracranial Volume |

| | |
|---|---|
| **TMN** | Tuberomammillary Nucleus |
| **TMT** | Trail Making Test |
| **TR** | Repetition Time |
| **TE** | Echo Time |
| **TST** | Total Sleep Time |
| **VTA** | Ventral Tegmental Area |
| **VLPO** | Ventrolateral Preoptic Area |
| **WASO** | Wake After Sleep Onset |

# Theoretical introduction

## Chapter 1: Sleep

### General aspects

Sleep is a fundamental aspect of life and is essential for survival of animals and humans. Historically, sleep was considered as a relaxing state of the brain with the only scope of granting rest. Research in the past decades showed however that sleep is an active state of the brain defined by behavioral changes and/or specific states of brain electrical activity with a rather specific etiology and function. Sleep appears to be a universal phenomenon in the animal kingdom. It has been observed in a wide range of distantly related animals, including invertebrates, fruit flies, fish, reptiles, birds and mammals (Yamazaki et al., 2020). Most of our current understanding of sleep is based on animal studies, highlighting the need for further research in humans.

Many roles have been attributed to sleep, such as brain thermoregulation, energy conservation, tissue restoration and immune defense (Dang-Vu et al., 2006). Several recent studies also agree on the importance of sleep in the proper development of our cognitive abilities. Indeed, when we sleep, a window of opportunity is offered to our brain to sort and reinforce recently encoded information without being interrupted by new external information. This process is called consolidation, leading to the formation of lasting memory (Girardeau & Lopes-dos-Santos, 2021). Sleep also provides an opportunity for our body to eliminate brain waste, strengthen and modulate our immune defenses, promote growth, and improve our physical and psychological performance such as learning and concentration when awake (Jessen et al., 2015). The formulation of the glymphatic system hypothesis in 2013, a macroscopic system for eliminating waste products from the brain based on glial cells supporting neurons, gives sleep a main role in the

“cleansing” of cerebrospinal fluid (CSF). Indeed sleep would provide an opportunity for the CFS to get rid of metabolic waste and soluble proteins accumulated during wakefulness that could lead to the onset of neurodegenerative diseases ,such as Alzheimer's disease (Jessen et al., 2015). The reason why this glymphatic activity would be even more effective during our sleep would be related to the decrease in norepinephrine (NE) levels in our brain, which would lead to an expansion of the extracellular space, a decrease in fluid flow resistance, and therefore an increase in the incoming flow of CSF and the outflow of interstitial fluid (ISF). In other words, this modulation of circulation could be influenced by noradrenergic tone (Reddy & van der Werf, 2020).

In the case of sleep disturbances, our cognitive abilities can face harmful effects. Indeed, in the event of insufficient or poor quality of sleep, our ability to be attentive during wakefulness is significantly reduced, the risk of the appearance of episodes of irritability, anxiety and other emotional and behavioral disorders can also be increased (Medic et al., 2017). Chronic sleep insufficiency can further weaken our immune defenses, promote the increase in cardiovascular disease and the risk of obesity leading to forms of late-onset diabetes (Buxton & Marcelli, 2010). Therefore, sleep plays a crucial role in maintaining good health (Medic et al., 2017).

In this section, we will describe electroencephalogram (EEG), the different stages across which one goes over the sleep-wake cycle with their main EEG and physiological characteristics, mechanisms that govern sleep–wake cycles, and the changes of sleep in aging and Alzheimer’s disease (AD).

**Electroencephalogram (EEG)**

The EEG is a tool that is very commonly used to characterize sleep by recording the spontaneous electrical activity of the brain through the use of multiple electrodes placed on the scalp. The EEG only gives us direct access to the electrical activity of the surface of the cerebral cortex, including the frontal, parietal, occipital and external temporal cortex regions. The subcortical regions, such as the amygdala, thalamus, cerebellum, locus coeruleus (LC) and basal ganglia are therefore not directly accessible, though their influence on the activity of the cortex can be detected. The signals recorded through the EEG are largely the product of synchronized postsynaptic currents, generated at the neuron dendrites (Zielinski et al., 2016). Although EEG offers good temporal resolution, in the order of milliseconds, it does not offer very good spatial resolution. This means that the location of the source of the electrical signals remains difficult to isolate (Zielinski et al., 2016). The EEG signal would therefore be a mixture of surface signals and deeper signals, for which sophisticated algorithms are necessary to differentiate them. In clinical practice, the EEG is a tool providing essential information on neurological characteristics that modulate wakefulness and sleep states, but above all EEG is also instrumental in illustrating dysfunctions associated with various sleep disorders, such as narcolepsy, obstructive sleep apnea, REM sleep behavior disorder, insomnia, and periodic leg movement (Jain & Ganesan, 2024).

A standard EEG configuration involves positioning electrodes on the scalp following the international 10-20 system (Carden, 2009), which is based on four anatomical reference points: the nasion, located at the junction of the frontal and nasal bones; the inion, a prominent point on the occipital bone;

and the left and right mastoids. Electrodes are arranged at intervals of either 10% or 20% of the total distance measured from front to back and side to side of the head (distance between two mastoids), with the central electrode placed at the intersection of these two two measured distances. By convention, electrodes on the left side of the head are labeled with odd numbers, whereas those on the right are labeled with even numbers (**Figure 1.1**).

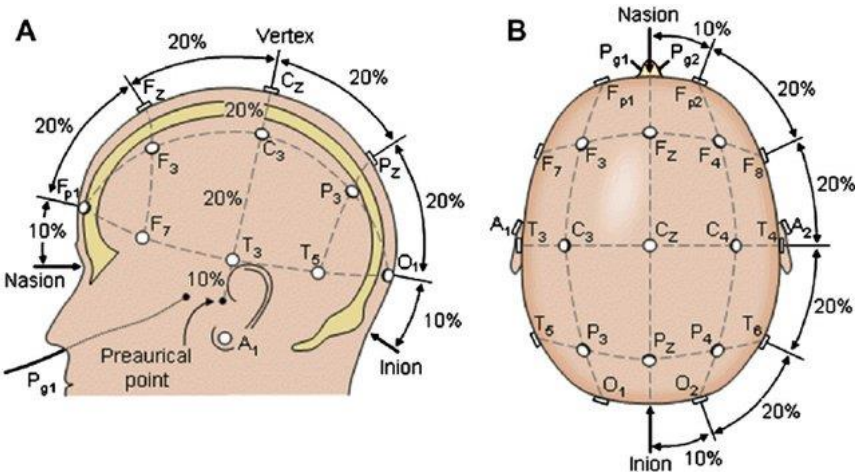


***Figure 1.1.*** *The 10-20 electrode placement system, where electrodes are positioned at intervals equal to 10 or 20% of the total distance from front to back and from left to right across the scalp (Foldvary-Schaefer & Grigg-Damberger, 2012).*

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

- Alpha waves are usually found around the frequency range between 8 and 12 Hz and are mostly present during quiet wakefulness with eyes closed. They are generally best observed in the posterior and occipital regions, with a typical amplitude of about 20 – 60μV. Indeed, alpha rhythms are pronounced when the eyes are closed, and are eliminated when the eyes are open, because visual input and cognitive tasks such as thinking or calculating demand focused attention, which suppresses alpha activity. There is also a decrease in these rhythms during sleep (Constant & Sabourdin, 2012; Sazgar et al., 2019; Zielinski et al., 2016)
- Beta waves are characterized by rapid oscillations of 16 to 25 Hz and an amplitude of about 30 μV. These rhythms are present during states of increased vigilance and critical reasoning. In other words, they represent the characteristic of the awake and alert subject who is actively thinking (Constant & Sabourdin, 2012; Zielinski et al., 2016).
- Delta waves have a frequency range between 0.5 – 4 Hz and a large amplitude (> 75 μV). These are the most important rhythms during the deepest stages of NREM sleep (i.e., stage N3) and are not commonly observed during wakefulness. The intensity of the non-rapid eye movement (NREM) sleep delta rhythm is often used as an indicator of sleep intensity, in other words, it is an indicator of the quality and depth of sleep in a given individual. Delta activity during NREM sleep arises from the synchronous alternation between cortical 'up' states (periods of neuronal firing) and 'down' states (neuronal silence) (Zielinski et al., 2016).

- Gamma waves represent fast oscillations (25-50 Hz), they are generally of low amplitude. They are classically recorded during wakefulness. They are associated with consciousness and are increased in the cortex after sensory stimuli. Gamma rhythms are also present during the ascending phase of slow waves during NREMS, and are enhanced after sleep loss. Gamma rhythms are enhanced with processing and learning (Adamantidis et al., 2019; Constant & Sabourdin, 2012).
- Sigma waves have a frequency of 12 to 16 Hz that appear mostly during the N2 and N3 stages of NREM sleep. Sleep spindles, which are brief but powerful bursts of synchronous neuronal firing, occur in sigma range. It has been shown that the thalamic reticular nucleus (TRN) generates sleep spindles in reciprocal interactions with thalamocortical neurons (Fernandez & Lüthi, 2020). These spindles are thought to be actively involved in the consolidation of declarative memory (long-term memory) through the process of reconsolidation. Indeed, spindles are involved in plasticity and long-term memory. Increased spindle activity is observed during NREM sleep after learning declarative tasks and procedural motor skills (Geva-Sagiv & Nir, 2019; Zielinski et al., 2016). Spindles are typically categorized into two subtypes based on their frequency and topographical distribution: Slow spindles (~12–14 Hz), which are most prominent over frontal regions and fast spindles (~14–16 Hz), which are typically maximal over central and parietal regions (Andrillon et al., 2011; Mölle et al., 2011).

- Theta waves are found approximately around the 4 to 8 Hz frequency range with an amplitude of 50 to 100 μV. Theta rhythms are present in adults in light sleep (N1 and N2) and represent a dominant component during rapid eye movement (REM) sleep. Theta rhythm also tends to increase with rising sleep pressure during wakefulness. These rhythms could also be associated with limbic activity (memory and emotions) (Constant & Sabourdin, 2012). Abnormalities in theta rhythms also occur in diseases associated with sleep disorders, such as AD. In addition, people with REM sleep behavioral disorders exhibit dysregulated, asymmetrical theta activity (Sazgar et al., 2019; Zielinski et al., 2016).

**Wakefulness and stages of sleep and related brain structures**

In 1968, Rechtschaffen and Kales (Rechtschaffen & Kales, 1968) defined three stages of sleep: the waking stage, REM sleep, and NREM sleep. The NREM sleep was subdivided into four stages: S1, S2, S3 and S4. Since the S3 and S4 stages are similar in many ways, the American Academy of Sleep Medicine (AASM) revised the R&K rules in 2007 and used the N1, N2, N3 to represent the different substages of NREM sleep, combining both S3 and S4 into the N3 stage (Adamantidis et al., 2019; Huang et al., 2021; Moser et al., 2009). Since this revision, sleep stages are conventionally scored in 30-second epochs based on standard polysomnographic criteria, allowing each epoch to be classified as wakefulness, NREM stages (N1, N2, N3), or REM sleep. It is considered that the normal adult brain first enters sleep through the NREM stages, followed by REM sleep, thus forming a sleep cycle. The sequence of sleep stages may vary, however, meaning one may directly go to N2 stage and not N1, there may be no periods of N2 between N3 and REM, or one may not

go to N3 before reaching REM or may go directly to REM at sleep onset. The NREM and REM stages of sleep then alternate through 4 to 6 cycles lasting 70 to 110 minutes each. Typically, the first part of our sleep is dominated by NREM sleep and the second part is dominated by REM sleep, taking a greater proportion in sleep cycles as sleep continues (Monderer et al., 2014). This sleep staging was made possible through specific physiological parameters detected on the EEG, electromyography (EMG) and electro-oculography (EOG) performed during polysomnography (PSG). PSG is a complete and reference medical examination of sleep. It is generally performed during a subject's sleep, making it possible to study the different physiological aspects of sleep and to characterize them, but also to diagnose and evaluate sleep-related disorders, such as obstructive sleep apnea disorder or insomnia disorder. The PSG is scored by assigning a sleep stage to each 30-second epoch recorded (Monderer et al., 2014). The stages of sleep throughout a night's sleep can be graphically represented by a sleep hypnogram (**Figure 1.2**).

*Wakefulness*

The state of wakefulness can be staged when a subject is in a state of high alertness with his eyes open or when he is relaxed with his eyes closed. When the eyes are open, the EEG shows fast, low-amplitude mixed-frequency activity, in which beta rhythms are dominant. The EOG tracing consists of movements and blinks of the eyes, and the EMG activity of the chin is increased. Once the eyes are closed, alpha rhythms predominate in the EEG and are most prominent in the occipital brain region (Iber et al., 2007). This alpha activity decreases with concentration or opening of the eyes. While

having eyes closed prior to sleep onset, the Wake (W) stage is reached when 50% of the epoch has alpha activity in the occipital region. It is estimated that a normal adult spends less than 5% of the night at this stage (Stampi et al., 1995).

A variety of brain structures and pathways are involved in the wake stage. Wakefulness is primarily dependent on the ascending arousal system, which was first described by Moruzzi and Magoun (Moruzzi & Magoun, 1949). This system is made up of two major pathways that emerge from the brainstem reticular formation: one innervates the thalamus and the other extends into the posterior hypothalamus and forebrain (Jones, 2005). Wake promotion is attributed to the action of monoaminergic, cholinergic and glutamatergic neurons (Saper & Fuller, 2017). Glutamatergic and cholinergic neurons located in the parabrachial nucleus, pedunculopontine tegmental nucleus (PPT), and supramammillary nucleus send projections to the basal forebrain (BF), which itself comprises both cholinergic and GABAergic neurons that innervate the cerebral cortex (Kroeger et al., 2017; Pedersen et al., 2017). Notably, parvalbumin-expressing GABAergic neurons within the BF have been found to strongly enhance wakefulness and promote faster EEG oscillations (Anaclet et al., 2015). Similarly, activation of cholinergic neurons in the BF has been shown to suppress slow-wave EEG activity (L. Chen et al., 2016). Furthermore, GABAergic neurons in the lateral hypothalamus have recently been implicated in promoting arousal (Venner et al., 2016). Norepinephrine in the locus coeruleus (LC), serotonin in the dorsal raphe nucleus, acetylcholine in the pedunculopontine tegmental nucleus (PPT), histamine in the tuberomammillary nucleus (TMN), and orexin in the lateral hypothalamus also contribute to maintaining wakefulness and regulating the transition between wakefulness and NREM sleep (Saper & Fuller, 2017). For instance,

lateral hypothalamus orexin neurons which have wake-promoting properties indirectly inhibit lateral hypothalamus neurons expressing melanin-concentrating hormone, which have sleep promoting properties (Apergis-Schoute et al., 2015).

*NREM sleep*

NREM sleep is subdivided into stages based on brain wave activity, muscle tone, and eye movements. NREM is characterized by a drop in body temperature, slowed breathing and heart rate, and a decreased reaction to environmental stimuli.

*Stage N1*

The N1 stage of sleep is characterized by light sleep or drowsiness. It is identified by the fact that less than 50% of the epoch is occupied by alpha activity on the EEG. The EEG also shows low-voltage and mixed-frequency activity, mainly in the theta frequency (4 to 8 Hz). The EOG channel often shows slow, rolling eye movements, and EMG activity is decreased. In normal adults, stage N1 should take up 2-5% of sleep, but patients with excessive sleep fragmentation, such as those with sleep apnea, often have a higher percentage of stage N1 sleep (Monderer et al., 2014).

*Stage N2*

Stage N2 of sleep is defined by the presence of K complexes (sharp, large amplitude, biphasic wave of more than 0.5 sec duration) or sleep spindles on the EEG. The background EEG shows low-amplitude mixed-frequency activity. The EOG usually shows no eye movement. However, slow eye movements may persist from the N1 stage. EMG activity is reduced from the N1 stage and

awakening. The N2 stage typically makes up 45-55% of the sleep in adults (Monderer et al., 2014).

*Stage N3*

Stage N3 of sleep represents the deepest stage of sleep. This stage together with N2 is also known as slow-wave sleep (SWS). The N3 stage is defined by the presence of slow waves with the frequency of 0.5 - 4 Hz and a minimum amplitude of 75µV, which are most prominent over frontal derivations (Berry et al., 2012) . These slow waves encompass two distinct types of oscillations commonly differentiated in the literature: slow oscillations (<1 Hz) and delta waves (1–4 Hz) (Adamantidis et al., 2019). These waveforms are governed by distinct regulatory mechanisms (Lee et al., 2004). More recently, a novel classification approach based on the transition frequency (inverse of the time between the hyperpolarised and depolarised states) has been proposed as a way to distinguish between two types of slow waves: "slow switchers" and "fast switchers" (Bouchard et al., 2021).

Slow-wave activity must occupy at least 20% of the epoch to meet the criteria for the N3 stage. EMG is active, but decreased compared to wakefulness and N1 stage, and there is no eye movement. The N3 stage occupies 15 to 25% of the sleep in healthy subjects (Monderer et al., 2014). The nature of sleep oscillations suggests that glymphatic activity is primarily concentrated during the N3 sleep phase. During this phase, slow oscillatory brain waves would promote the increase in CSF volume in the interstitial spaces, leading to an increase in glymphatic clearance of 80 to 90% compared to the waking state. These results highlight the importance of SWS in the glymphatic function of our brain (Reddy & van der Werf, 2020).

A key physiological hallmark of NREM sleep is the coupling between slow waves and sleep spindles. Spindles tend to occur during the up states of slow waves, and this temporal coordination is believed to play a critical role in memory consolidation and synaptic plasticity (Klinzing et al., 2016; Mölle et al., 2011).

Research indicates that various structures, including the LC, hypothalamus, thalamus, cerebral cortex, basal forebrain, cerebellum, caudal brainstem, spinal cord, and peripheral nerves, play a role in regulating and modulating NREM sleep. During NREM stage 2, the onset and cessation of individual spindle sequences are influenced by corticothalamic activity. Though most cortical neurons are wake-active, one population is especially active during NREM sleep. These cells produce neuronal nitric oxide synthase (nNOS) and are a small subset of the broader population of GABAergic cortical interneurons. The nNOS neurons are thought to respond to homeostatic sleep drive and synchronize slow cortical rhythms via long-range, intracortical projections and release of GABA and nitric oxide (Morairty et al., 2013). Additionally, both cortical and thalamic mechanisms contribute to the production of EEG delta waves, which emerge in the deep sleep stage (N3). While the cortex has traditionally been regarded as the primary source of this activity, delta oscillations can also originate from thalamocortical neurons (De Andrés et al., 2011).

High concentrations of adenosine in the preoptic area of hypothalamus also promote the activation of inhibitory GABAergic neurons which strongly innervates arousal-promoting brain structures such as LC (Scammell et al., 2017). This region is also involved in the NREM response to sleep deprivation (Alam et al., 2014). Preoptic area of hypothalamus contains neurons that are

activated during sleep compared to wakefulness (Szymusiak et al., 2007). The activation of neurons in the preoptic area promotes NREM sleep (Zhang et al., 2015).

*REM sleep*

REM sleep is characterized by an active brain with a loss of muscle tone. The EEG shows a low-voltage, mixed-frequency pattern, and prominent theta activity. The EMG of the chin indicates that the tone is markedly decreased and the EOG shows rapid eye movements. There is a cessation of K-complexes, sleep spindles, and high-amplitude waves. During REM sleep, sawtooth waves, which are characterized as sequences of 2–6 Hz sharply contoured waves are observed. Sawtooth waves are most prominent in central brain regions and typically occur just before rapid eye movement bursts. REM sleep accounts for 20-25% of total sleep time in healthy adults (Monderer et al., 2014).

The main region involved in REM control is the brainstem, where both REM-promoting and REM-inhibiting neural structures are located (Scammell et al., 2017). The sublaterodorsal nucleus plays a crucial role in regulating REM sleep. Glutamatergic neurons of this nucleus produce the muscle paralysis of REM sleep by exciting GABAergic/glycinergic neurons in the ventromedial medulla and spinal cord that hyperpolarize motor neurons (Luppi et al., 2012; Scammell et al., 2017). Mice with lesions in this region exhibits shorter REM sleep bouts with impaired muscle atonia, showing that this structure is essential for REM generation and REM-related paralysis as well (Krenzer et al., 2011). Cholinergic neurons of the pedunculopontine and laterodorsal tegmental nuclei also promote REM sleep and may help drive the typical fast EEG activity observed during this sleep stage (Van Dort et al., 2015). During

wake and NREM sleep, REM is repressed through inhibition of sublaterodorsal nucleus neurons by GABAergic neurons of the ventrolateral periaqueductal grey (vlPAG) and adjacent lateral pontine tegmentum, and by monoaminergic neurons of the LC and raphe nuclei. By contrast during REM sleep, these structures are inhibited in order to activate the sublaterodorsal nucleus, which then inhibits, together with the medulla, the ventrolateral periaqueductal grey matter via GABAergic innervations. This mutual inhibition between the sublaterodorsal nucleus and the ventrolateral periaqueductal grey matter is at the basis of NREM-REM switch, a mechanism which is regulated at a higher level by the activation of cholinergic neurons in the pedunculopontine tegmentum and laterodorsal tegmentum (Van Dort et al., 2015). The LC activity also dynamically changes during sleep to shape the alternation between REM and non-REM sleep. The LC activity is reduced during NREM sleep compared to wakefulness and the LC becomes nearly silent during REM sleep (Gompf et al., 2010; Osorio-Forero et al., 2022). In this regard, Hobson and colleagues observed that the transition from NREM to REM sleep was preceded by increased activity in a population of cholinergic neurons within the gigantocellular tegmental field (FTG), occurring simultaneously with the suppression of neuronal firing in the posteroventral LC (Hobson et al., 1975). Based on these findings, they proposed a computational model in which REM sleep is regulated through a reciprocal inhibitory interaction between REM-OFF LC-NE neurons and REM-ON cholinergic neurons in the FTG (McCarley & Hobson, 1975).

In addition, the lateral hypothalamus produces melanin-concentrating hormone (MCH) which is considered to stabilize REM sleep (Adamantidis & de Lecea, 2023). MCH neurons are sleep-active, with maximal firing rates during REM sleep (Hassani et al., 2009). Activation of MCH neurons increases

the transitions from NREM to REM sleep and prolonged the durations of REM sleep episodes, indicating that MCH neuron activation enhances the initiation and maintenance of REM sleep (Weber & Dan, 2016).

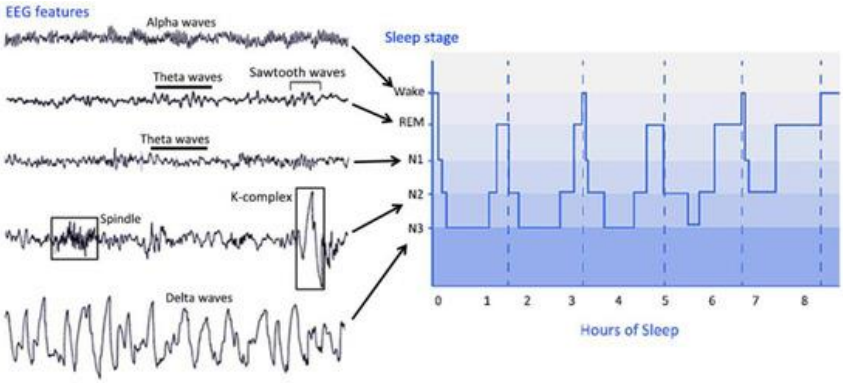


***Figure 1.2. EEG features and hypnogram of sleep stages.*** *This figure illustrates the progression of sleep stages over an 8-hour night, depicted on the right as a hypnogram. Each line on the hypnogram corresponds to a specific sleep stage, ranging from wakefulness to REM and the three stages of NREM sleep (N1, N2, N3), and shows the cyclical nature of sleep architecture throughout the night. On the left, characteristic EEG features of each stage are displayed. REM: Rapid Eye Movement sleep, N1: Non-REM Sleep Stage 1, N2: Non-REM Sleep Stage 2, N3: Non-REM Sleep Stage 3 (Pan et al., 2021).*

### Sleep-wake regulation by homeostatic and circadian systems

One of the fundamental physiological mechanisms that govern sleep–wake cycles includes the brain's homeostatic and circadian systems, which control when sleep and wakefulness occur (Borbély, 1982).

Circadian regulation of sleep follows a roughly 24-hour cycle. Sleep is most restorative when aligned with the circadian "rest" phase—nighttime for

humans. During the day, the circadian system helps sustain alertness despite increasing sleep pressure; hence, this circadian system promotes alertness/wakefulness during the day and sleep at night in humans (Postnova & Sanz-Leon, 2025). The suprachiasmatic nucleus (SCN) in the hypothalamus acts as the master clock, syncing body rhythms to the light–dark cycle (Dibner et al., 2010). Light is the main environmental cue that influences the circadian rhythm (Khalsa et al., 2003), which explains how we adapt to new time zones or feel more active during daylight. The circadian oscillator naturally runs on a cycle slightly longer than 24 hours, but exposure to the daily light–dark cycle resets it to a 24-hour rhythm (Czeisler & Gooley, 2007). Social cues like meal timing and physical activity can also impact circadian rhythms, though they primarily act through peripheral clocks in organs such as the liver, pancreas, and muscles, rather than directly influencing the SCN (Dibner et al., 2010). The SCN communicates to the dorsomedial hypothalamus which acts on the sleep regulatory centers such as the VLPO and the brain areas responsible for hormone release, thermoregulation, and feeding (Saper et al., 2005). Proper alignment between circadian rhythms, sleep, and environmental cues is essential for health; misalignment is linked to sleep disorders and disease (Jorgensen et al., 2020). Damage to the SCN disrupts these 24-hour rhythms, leading to irregular sleep–wake patterns, as seen in SCN-lesioned monkeys (Edgar et al., 1993) and in patients with AD or Huntington's disease (Harper et al., 2008; Morton et al., 2005).

In addition to supporting wakefulness, the circadian system may also actively promote sleep, particularly in the second half of the night. Evidence suggests that MCH neurons, located in the lateral hypothalamus and maximally active during REM sleep, may serve as a key circadian-modulated pathway for sleep promotion, especially REM sleep. A reflection of this circadian influence is the

prevalence of REM sleep in the latter part of the night, when circadian sleep propensity is still high despite declining homeostatic pressure (Konadhode et al., 2013).

The homeostatic sleep drive increases sleep pressure as time awake increases. Once sleep begins, this pressure gradually decreases, with the rate of decline influenced by the amount of prior wakefulness, thus reducing the pressure for sleep. A key indicator of this homeostatic process is the slow wave activity (spectral power in the 0.5–4 Hz range, SWA) in NREM sleep, which rises with prolonged wakefulness and diminishes during NREM sleep (Borbély, 1982). Although the mechanisms behind sleep homeostasis are not yet fully uncovered, glymphatic clearance—the brain's waste removal system—is believed to play a role (Jessen et al., 2015). During wakefulness, byproducts of brain activity build up and can become harmful; sleep helps eliminate them (Xie et al., 2013). Several of these metabolic waste products accumulate during waking hours and are cleared from the brain during sleep, aided by the expansion of the extracellular space and increased cerebrospinal fluid flow. These processes likely contribute to the rising sleep pressure and may link disrupted clearance to neurodegenerative diseases like AD, where β-amyloid plaques and tau tangles interfere with brain cleanup (Tarasoff-Conway et al., 2015).

Sleep homeostasis is also closely tied to synaptic homeostasis, as proposed by the synaptic homeostasis hypothesis. According to this model, wakefulness is associated with widespread synaptic potentiation driven by learning and environmental interaction, while sleep and particularly SWS facilitates a global downscaling of synaptic strength, preserving the most relevant synapses while restoring cellular energy balance. This synaptic

renormalization may be essential for memory consolidation and overall brain plasticity (Tononi & Cirelli, 2014). In addition, sleep pressure is influenced by brain metabolism, with energy depletion during wakefulness contributing to the build-up of homeostatic drive. The accumulation of sleep-promoting substances, or hypnogenic molecules, further supports sleep initiation and maintenance. Among them, adenosine is one of the most well-characterized; it accumulates extracellularly in the basal forebrain during wakefulness and promotes sleep by inhibiting arousal-related neurons (Reichert et al., 2016).

Together, the homeostatic and circadian drives influence relevant brain regions to regulate sleep-wake cycle. During the day, rising circadian wake signals help maintain alertness despite increasing homeostatic sleep pressure. At night, the circadian signal decreases, allowing for sustained sleep even as homeostatic pressure declines (Edgar et al., 1993; Postnova & Sanz-Leon, 2025). Misalignment between the sleep, the circadian rhythm, or the light–dark environment can disrupt sleep quality and duration as often happens in shift workers (Rajaratnam et al., 2013) or individuals who are blind and cannot perceive light (Flynn-Evans et al., 2014).

**Sleep and aging**

Normal aging is accompanied by non-pathological changes in sleep patterns. As people get older, their total sleep time (TST) tends to decrease compared to younger individuals. This decline continues until around the age of 60, after which sleep duration stabilizes through the later decades of life. These changes may result from a mix of biological shifts in sleep regulation, alterations in sleep-related behaviors, and a higher prevalence of sleep disorders (Lavoie et al., 2018).

As people age, the proportion of sleep spent in deep sleep within NREM sleep (i.e., N3) and in REM sleep both decline, while lighter stages of NREM sleep (particularly stage N1 and N2) become more predominant. The time required to fall asleep also tends to increase modestly with age. Sleep efficiency (percentage of time spent asleep while in bed), also continues to show an age-dependent decline beyond age 90 years (Miner & Kryger, 2016). Furthermore, older adults experience more frequent microarousals during the night and an increase in time awake after sleep onset (WASO) (**Figure 1.3**). However, their ability to return to sleep after these arousals does not increase compared to younger adults (Ohayon et al., 2004). Daytime napping also becomes more common with age. In addition, the amplitude of circadian rhythms is reduced. Starting around the age of 20, circadian rhythms begin to shift earlier—older adults often feel sleepy earlier in the evening and wake up earlier in the morning (Roenneberg et al., 2007). In addition, reductions in SWA occurs (Mander et al., 2013), specifically older subjects show lower slow wave density and amplitude than young subjects (Carrier et al., 2011). Besides, in the older individuals, EEG power density is reduced in frequencies below 14.0 Hz in SWS and the delta-theta (0.25 – 7.0 Hz) and low alpha (8.25 – 10.0 Hz) band in REM sleep (Landolt et al., 1996).

Non pathological age-related sleep changes may increase vulnerability to sleep disorders such as insomnia (Miner & Kryger, 2016). Sleep disorders such as sleep-disordered breathing and insomnia are more prevalent among older adults and can significantly impact daily functioning and the ability to live independently. Research indicates that addressing these sleep issues can lead to symptom improvement in older individuals in the context of comorbid medical and mental health conditions (Lavoie et al., 2018).

Another change that occurs during old age is related to melatonin. This "sleep" hormone is secreted in the dark, by the pineal gland triggered by the SCN. The circadian pattern of melatonin production and release is closely linked to the sleep-wake cycle (Nakagawa et al., 1992; Wehr et al., 2001). The onset of nighttime melatonin secretion is initiated 2 h in advance to the individual's habitual bedtime and has been shown to correlate with the onset of evening sleepiness (Zhdanova et al., 1996). Conversely, when retinal cells perceive light, its synthesis is inhibited (Brainard et al., 2001). The timing of the nighttime increase in melatonin secretion aligns with the onset of sleep. Additionally, the sleep-promoting effects of externally administered melatonin suggest that this hormone, produced by the pineal gland, plays a key role in the natural regulation of sleep. With aging, melatonin production gradually decreases (Pandi-Perumal et al., 2005). Reduced nighttime melatonin secretion has been proposed as a contributing factor to the decline in sleep maintenance commonly observed in older adults (Tozawa et al., 2003). Studies have suggested that the decline in melatonin production during aging may be due to pathological processes. Indeed, it has been established that the pineal gland, the main centre for the production of melatonin, tends to calcify during age, which alters its function (Vasey et al., 2021).

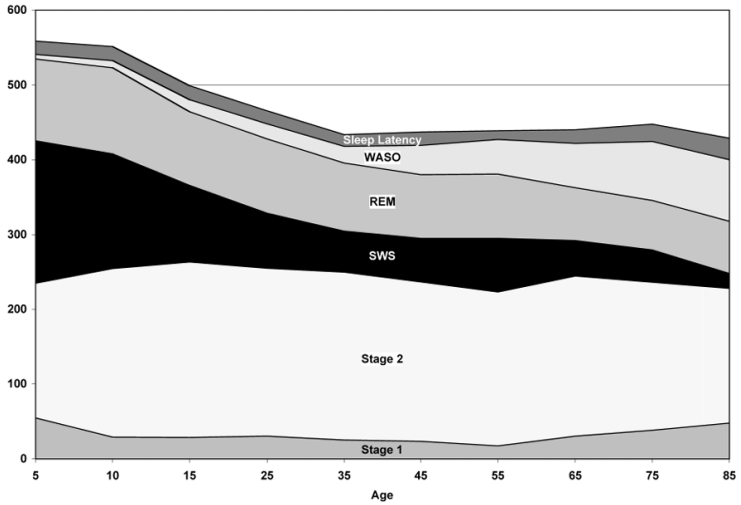


***Figure 1.3.*** *Illustration of the Age-related trends for the general mean time spent in each sleep stage (N1/N2/N3/REM), as well as wake after sleep onset (WASO), sleep latency and total sleep time (in minutes) (Ohayon et al., 2004).*

## Sleep and Alzheimer's disease (AD)

AD is a progressive neurodegenerative disorder. While it is typically identified by the emergence of cognitive symptoms, it is also marked by a prolonged asymptomatic phase during which amyloid-β (Aβ) accumulates as insoluble extracellular plaques, tau proteins aggregate within neurons, and neuronal as well as synaptic loss occurs, ultimately leading to cognitive decline and dementia (Bateman et al., 2012; Jack et al., 2013; Vos et al., 2013). Studies indicated that Aβ and tau protein accumulations as well as neurodegeneration starts before the onset of cognitive symptoms, occurring during the asymptomatic or preclinical stage of AD (Price & Morris, 1999; Sperling et al., 2011). Age is the greatest risk factor for AD with the risk doubling every 5 years after the age of 65 (Jorm & Jolley, 1998).

Neuropathological hallmarks of AD, such as amyloid plaques, neurofibrillary tangles (NFTs), and in some cases Lewy bodies, can disrupt neuronal network function in brain regions involved in sleep-wake regulation, thereby contributing to the sleep disturbances observed in AD. Studies showed decreased total sleep time, sleep efficiency, the time spent in deep sleep within NREM sleep (i.e., N3) and in REM sleep compared to lighter stages of NREM sleep (particularly stage N1 and N2), as well as REM density, and increased number of awakenings, wake time after sleep onset, N1 percentage, sleep latency, and REM latency in AD patients compared with controls. Importantly, reductions in SWS and REM sleep percentage have been significantly correlated with the severity of cognitive impairment in individuals with AD (Zhang et al., 2022). Studies have also reported that individuals with mild cognitive impairment (MCI), a prodromal stage of AD, exhibit similar alterations in PSG measured sleep parameters. These include increased stage N1 sleep, wake after sleep onset (WASO), sleep latency, and REM latency, along with decreased total sleep time, sleep efficiency, and REM sleep (D'Rozario et al., 2020). Disrupted circadian rhythms is frequently observed in patients with AD and is considered a major contributor to their sleep disturbances (McCurry et al., 2004; Vitiello et al., 1992). For example, a 24-hour PSG study in AD patients demonstrated pronounced fragmentation of the sleep-wake cycle, characterized by extended periods of wakefulness during the night and frequent napping throughout the day (Prinz et al., 1982).

It is also reported that patients with AD exhibited reduced sleep spindle activity compared to healthy controls, and this reduction was associated with lower cognitive function on both the Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MoCA) (Liu et al., 2020). Besides, it is shown that sleep spindles were generally reduced in both normal aging and

AD, with AD patients showing an additional, more pronounced reduction in fast spindles (14–16 Hz) compared to age-matched healthy individuals. Furthermore, the mean intensity of fast spindles was found to be positively correlated with immediate recall performance in patients with AD, suggesting a potential link between spindle activity and memory function in this population (Rauchs et al., 2008).

Furthermore, in cognitively normal older adults, greater amyloid accumulation (as measured by positron emission tomography (PET) and CSF Aβ42 concentrations) has been associated with self-reported short sleep duration (Spira et al., 2013), excessive daytime sleepiness (Carvalho et al., 2018), prolonged sleep latency (Branger et al., 2016; Brown et al., 2016), lower sleep quality (Sprecher et al., 2015, 2017), the presence of OSA (Sharma et al., 2018), and reduced sleep efficiency along with more frequent daytime napping (Ju et al., 2013). Moreover, SWA has been shown to decrease in cognitively normal individuals with evidence of amyloid deposition (Mander et al., 2015). In a separate study involving both cognitively normal and mildly cognitively impaired older adults, reductions in SWA were associated with both amyloid and tau pathology, with the effect being more pronounced for tau (Lucey et al., 2019). This pattern aligns with findings in mouse models, where the development of tau pathology is associated with decreased SWA (Holth et al., 2017). Additionally, polysomnographic features such as reduced sleep spindles and diminished coupling between slow oscillations and spindles have emerged as potential markers of early tau pathology (Kam et al., 2019; Winer et al., 2019). Besides, earlier occurrence of spindles on slow-depolarisation slow wave is associated with higher medial prefrontal cortex Aβ burden and is related to greater longitudinal memory decline in healthy old individuals (Chylinski et al., 2022).

Sleep disturbances have also been linked to future risk of both cognitive decline and AD pathology (Shi et al., 2018). For instance, older women who reported sleeping five hours or less per night demonstrated poorer cognitive performance over the following two years compared to those who slept seven hours per night (Tworoger et al., 2006). Research using actigraphy has demonstrated that greater wakefulness after sleep onset in cognitively normal older adults influences the link between amyloid accumulation and memory outcomes, including performance on the selective reminding test (Molano et al., 2017) as well as both immediate and delayed recall (Wilckens et al., 2018). Additionally, sleep disorders like obstructive sleep apnea (OSA), sleep periodic limb movement disorder (PLMD), and insomnia have been linked to an increased risk of future cognitive impairment and AD (Leng et al., 2016; Osorio et al., 2011; Yaffe et al., 2011). For instance, individuals with greater than moderate or severe OSA, have more risk of cognitive impairment and dementia over 5 years compared to those with mild or no OSA (Yaffe et al., 2011). Collectively, these findings support the hypothesis that sleep disturbances may not only result from AD pathology but also actively contribute to it, indicating a possible bidirectional relationship. Sleep disruption may accelerate AD progression by increasing Aβ and tau production and aggregation, as well as by impairing their clearance from the brain. In mouse models, for instance, sleep deprivation resulted in elevated ISF Aβ concentrations, and after 21 days, this was associated with an increase in amyloid deposition in the form of insoluble plaques (Kang et al., 2009). Studies in humans have demonstrated that even a single night of sleep deprivation, as well as targeted disruption of slow SWS, can lead to a 10–30% increase in CSF Aβ levels (Ju et al., 2017; Lucey et al., 2018; Ooms et al., 2014). Sleep deprivation in humans, as well as chemogenetically-induced

wakefulness in mice, has been shown to elevate tau concentrations in mice ISF, human CSF, and human plasma up to 50% (Barthélemy et al., 2020; Benedict et al., 2020; Holth et al., 2019).

Sleep disturbances have been shown to be associated with increased inflammatory activation (Irwin et al., 2016). Research suggests that inflammation, which may promote Aβ accumulation and tau pathology, could serve as a biological risk factor for MCI preceding the AD onset (Y. Chen & Yu, 2023; Lo et al., 2016; Wood, 2018). As a result, inflammation is hypothesized to represent a biologically plausible pathway linking sleep disruption to an increased risk of AD (Ahnaou & Drinkenburg, 2021; Spira et al., 2014). It has also been proposed that treating sleep disturbances could help reduce inflammation, potentially slowing or mitigating the progression of AD (Irwin & Vitiello, 2019).

Another proposed mechanism linking sleep to elevated CSF concentrations of Aβ and tau involves reduced clearance during sleep. This hypothesis suggests that bulk fluid flow referred to as the glymphatic system facilitates the transport of solutes from the ISF to the CSF, thereby clearing neuronal waste products from the brain parenchyma (Iliff et al., 2012). During sleep, glymphatic system fluid flow in mice has been shown to increase, enhancing the clearance of soluble Aβ from the brain (Xie et al., 2013). This enhanced glymphatic activity during sleep has also been implicated in modulating tau pathology, as reduced clearance has been associated with increased tau accumulation in a mouse model of traumatic brain injury (Iliff et al., 2014). In mice, aging has been reported to impair the function of the glymphatic system, potentially reducing the brain's ability to clear waste products like Aβ and tau (Kress et al., 2014). The brain's ability to clear Aβ would be further

diminished following the aggregation of insoluble amyloid plaques, trapping Aβ within brain tissue (Patterson et al., 2015).

There is also substantial evidence supporting the involvement of the orexin system in the development of amyloid deposition and AD. Orexin-A and orexin-B (also known as hypocretin-1 and hypocretin- 2) are wake-promoting neuropeptides (Tsujino & Sakurai, 2009). Orexin deficiency can be seen in individuals with narcolepsy. In humans, individuals with narcolepsy show reduced levels of CSF Aβ, total tau, and phosphorylated tau (p-tau), as well as lower amyloid deposition on PET imaging, when compared to age- and sex-matched controls (Gabelle et al., 2019; Jennum et al., 2017). Furthermore, genetic knockout of the orexin gene in mice resulted in increased sleep duration and a significant reduction in amyloid pathology within the brain (Roh et al., 2014). Additional studies in mice have shown that treatment with almorexant, a dual orexin receptor antagonist, led to reduced concentrations of soluble Aβ, whereas intra-cerebroventricular administration of orexin resulted in increased soluble Aβ levels (Kang et al., 2009). Although the impact of orexin receptor antagonists on soluble CSF Aβ and tau levels or amyloid deposition has not yet been examined in humans, existing findings indicate that inhibiting orexin signaling may influence amyloid pathology in the brain (Lucey, 2020).

Given these findings and the extended asymptomatic phase of preclinical AD, sleep disturbances are hypothesized to serve either as early markers of underlying AD pathology and/or as contributing mechanisms that increase the risk of developing AD, suggesting a potential bi-directional relationship between sleep and the disease, **Figure 1.4**) (Lucey, 2020).

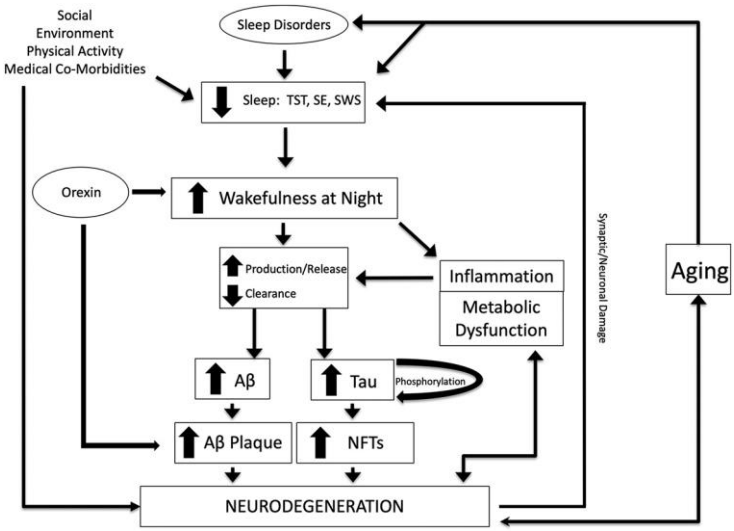


**Figure 1.4. Hypothetical Model of the Relationship Between Sleep and Alzheimer's Disease**

*This model illustrates how various factors—such as aging, sleep disorders, and environmental influences—contribute to sleep disturbances and increased nighttime wakefulness. Reduced sleep duration and quality lead to increased production of amyloid-β (Aβ) and release of tau, along with impaired clearance of these proteins from cerebrospinal fluid (CSF). These changes promote the accumulation of amyloid plaques and tau pathology. Additionally, sleep loss alters tau phosphorylation, which may further exacerbate tau-related damage. Sleep disturbances may also interact with inflammation and metabolic dysfunction, influencing Aβ and tau levels and contributing to neurodegeneration. The resulting neuronal and synaptic damage from Aβ plaques and tau tangles feeds back into the system, further disrupting sleep. Orexin, a neuropeptide that regulates wakefulness, has also been shown to exacerbate amyloid pathology, potentially playing a role in this feedback loop.*

*TST: Total sleep time; SE: sleep efficiency; SWS: slow wave sleep; Aβ: amyloid-β; NFTs: neurofibrillary tangles (Lucey, 2020).*

## Chapter 2: The Locus coeruleus

### Structure and function

The locus coeruleus (LC) was first described in the late 18th century by French anatomist Félix Vicq d'Azyr and later named for its dark, bluish appearance in post-mortem brain samples "locus coeruleus" meaning "blue spot" in Latin. It was not until the middle of the twentieth century (1964) that it was discovered that the blue pigmentation that earned its name "locus coeruleus" was, in fact, due to the presence of neuromelanin granules, a by-product of the metabolism of norepinephrine (NE). The LC is a small, elongated, rod-shaped, bilateral nucleus, which is located in the dorsal edge of the pons, near the floor of the fourth ventricle, and consists of densely packed noradrenergic neurons. Measuring approximately 14.5 mm in length and 2.5 $mm^2$ in diameter (Fernandes et al., 2012), the LC contains between 22,000 and 50,000 pigmented neurons (Beardmore et al., 2021; German et al., 1988). It is the brain's primary source of NE; and the widespread neuronal projections of the LC across the brain enable this small nucleus to influence activity in various cortical regions, thereby supporting wakefulness, attention, sensory processing, memory, arousal, muscle tone, and respiratory function. At the same time, it suppresses sleep-promoting brain areas through the release of NE (Poe et al., 2020; Sara, 2009).

Like other neurotransmitter systems, NE exerts its effects through multiple receptors located in target tissues. Conventionally, three main subtypes of noradrenergic receptors have been identified: $\alpha_1$, $\alpha_2$, and $\beta$. $\alpha_1$ and $\beta$ are excitatory and exist primarily at postsynaptic sites while $\alpha_2$ is inhibitory and exist in both pre- and post-synaptic sites (Berridge & Waterhouse, 2003). Recent molecular and pharmacological research has identified multiple

subtypes of β, $\alpha_1$, and $\alpha_2$ adrenergic receptors, highlighting their greater diversity. Currently, three β-receptor subtypes ($\beta_1$–$\beta_3$), three $\alpha_1$ subtypes ($\alpha_{1a}$, $\alpha_{1b}$ and $\alpha_{1d)}$, and four $\alpha_2$ subtypes ($\alpha_{2A–D}$) have been identified (Berridge & Waterhouse, 2003). Through its various receptors and signaling pathways, NE acts as a powerful neuromodulator. It primarily lowers baseline neuronal activity while enhancing responsiveness to new stimuli. NE also supports synaptic plasticity, including long-term potentiation, in brain regions such as the neocortex, hippocampus, amygdala, and cerebellum (Benarroch, 2018; Hagena et al., 2016).

LC neurons have two distinct modes of activity during wakefulness: phasic and tonic. These two modes differ in the spike discharge pattern and the norepinephrine release characteristics (Aston-Jones & Bloom, 1981). Tonic mode consists of irregular but constant baseline activity (1-6 spikes per second). Phasic mode is characterized by short (< 300 ms) bursts of high frequency activity (10-15 spikes per second) followed by a long period of sustained inhibition of spontaneous activity. While phasic activity may occur spontaneously, it is also associated with relevant stimuli. LC activity changes between these modes throughout behavioral states (Aston-Jones & Cohen, 2005). Studies suggested an inverted-U relationship between LC activity and performance. At very low LC tonic discharge, performance is poor because the animals are non-alert. Performance is best at moderate LC tonic and at high levels of phasic activity (phasic activity) after goal-relevant stimuli. Performance is poor in high tonic mode (no phasic activity). This pattern resembles the classical Yerkes-Dodson association between arousal and performance (Aston-Jones et al., 1999) (**Figure 2.1**).

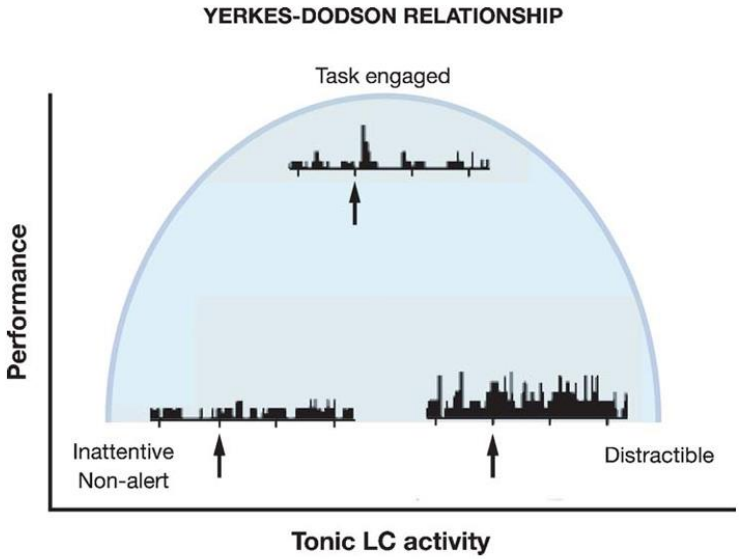


***Figure 2.1. Inverted-U relationship between locus coeruleus (LC) activity and performance on tasks that demand focused attention.*** *When LC tonic discharge is very low, animals tend to be drowsy and inattentive, leading to poor performance. Optimal task performance occurs when LC tonic activity is at a moderate level and is accompanied by prominent phasic LC responses to goal-relevant stimuli; this state is referred to as the phasic LC mode. In contrast, when LC tonic activity is excessively high and phasic responses are absent (tonic LC mode), performance again deteriorates. This pattern closely mirrors the classical Yerkes-Dodson relationship between arousal and performance (Aston-Jones & Cohen, 2005).*

### Imaging LC

Due to its small size and deep location within the brain, visualizing the LC in vivo has been challenging. However, advancements in ultra-high-field magnetic resonance imaging (MRI) have enabled detailed imaging of the brain's smallest structures. These technological developments have facilitated the exploration of the LC's structural and functional properties with

greater precision. As a result, LC-specific MRI sequences, which are sensitive to neuromelanin, have become increasingly effective in capturing high-resolution images of this nucleus, allowing for more accurate studies of its role in health and disease (**Figure 2.2**) (Priovoulos et al., 2018).

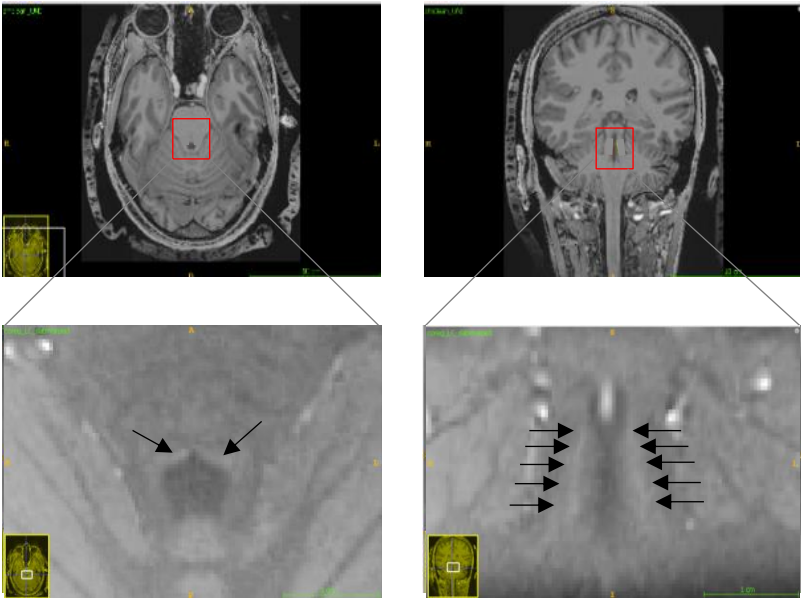

***Figure 2.2. Visualization of human LC using a LC-specific MRI sequence.***

**LC and sleep-wake regulation**

The LC-NE system is a component of the reticular formation, a network of nuclei involved in both ascending and descending pathways. While the descending pathways are associated with sensory and motor regulation (such as nociception and muscle tone) (Van Egroo et al., 2022) and are not the focus here, this section centers on the role of the LC-NE system within the ascending pathways.

Within the neurobiological network governing sleep and wakefulness, LC-NE neurons project to several key regions, including cholinergic and GABAergic neurons in the basal forebrain, GABAergic neurons in the ventrolateral preoptic area (VLPO) of the anterior hypothalamus, orexin-producing neurons in the lateral hypothalamus, serotonergic neurons in the dorsal raphe, and cholinergic neurons in the pedunculopontine tegmental (PPT) nucleus (Samuels & Szabadi, 2008; Saper & Fuller, 2017; Van Egroo et al., 2022). The locus coeruleus itself receives inputs from over 100 distinct brain regions (Schwarz et al., 2015). Specifically within the sleep–wake circuitry, LC-NE neurons are influenced by orexinergic neurons of the lateral hypothalamus, GABAergic neurons of the VLPO and ventrolateral hypothalamus, histaminergic neurons of the tuberomammillary nucleus (TMN), dopaminergic neurons of the ventral tegmental area (VTA), serotonergic neurons of the dorsal raphe, cholinergic neurons of the PPT and laterodorsal tegmental (LDT) nuclei, and dopaminergic neurons of the periaqueductal gray matter (Samuels & Szabadi, 2008; Saper & Fuller, 2017; Van Egroo et al., 2022) (**Figure 2.3**).

In addition, a population of GABAergic neurons has been identified in the dendritic zone surrounding the LC—referred to as the pericerulear or peri-LC region—as well as interspersed among LC-NE neurons. These GABAergic neurons play a critical inhibitory role by modulating the tonic and phasic activity of LC-NE neurons at the local level (Breton-Provencher & Sur, 2019; Van Egroo et al., 2022). The LC's anatomical connections with multiple sleep- and wake-regulating nuclei position it as a key player in initiating and sustaining sleep and wakefulness, along with the behavioral and

electrophysiological characteristics linked to these states (Van Egroo et al., 2022).

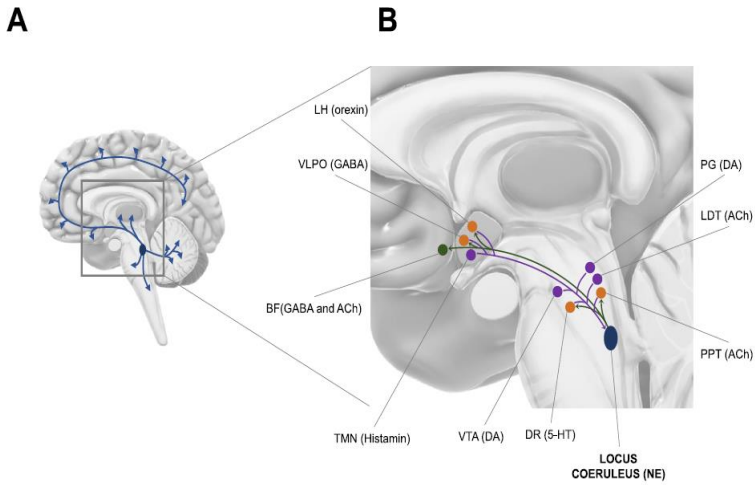


***Figure 2.3. Afferent and Efferent Connections of the Brainstem Locus Coeruleus (LC)***

*(A) The LC projects widely throughout the brain, reaching regions such as the hippocampus, amygdala, thalamus, and neocortex.*

*(B) Diagram of the LC's interactions with various sleep–wake regulatory centers. Purple dots indicate nuclei projecting to the LC; green dots represent nuclei receiving output from the LC; orange dots denote nuclei with reciprocal connections to the LC. Note that the spatial arrangement of nuclei is illustrative and does not correspond to exact anatomical positions.*

*LC efferent pathways target cholinergic and GABAergic neurons in the basal forebrain (BF), GABAergic neurons in the ventrolateral preoptic area (VLPO) of the anterior hypothalamus, orexin-producing neurons in the lateral hypothalamus (LH), serotonergic neurons in the dorsal raphe (DR), and cholinergic neurons in the pedunculopontine tegmental (PPT) nucleus.*

*Afferent inputs to the LC originate from orexinergic neurons in the LH, GABAergic neurons in the VLPO, histaminergic neurons in the tuberomammillary nucleus (TMN), dopaminergic neurons in the ventral tegmental area (VTA), serotonergic neurons in the DR, cholinergic neurons in the PPT and laterodorsal tegmental (LDT) nuclei, and dopaminergic neurons in the periaqueductal gray (PG) (Van Egroo et al., 2022).*

Electrophysiological recordings in rats, cats, and monkeys have consistently shown that the firing rates of LC neurons during NREM and REM sleep are substantially lower than during wakefulness. While LC activity is reduced during NREM sleep, it remains detectable; however, the LC becomes nearly silent during REM sleep **(Figure 2.4)**. LC-NE neurons have been shown to predict transitions from sleep to wakefulness, exhibiting bursts of activity in the seconds leading up to both spontaneous and stimulus-induced awakenings (Aston-Jones & Bloom, 1981). This state-dependent firing pattern has been supported by findings that NE concentrations in regions such as the pons, amygdala, and hippocampus are highest during wakefulness, reduced during quiet wakefulness, and lowest during sleep (Van Egroo et al., 2022). Notably, some studies observed that not all presumed LC neurons decreased their activity during NREM and/or REM sleep. Additionally, LC firing rates are also diminished during quiet, as opposed to active, wakefulness (Osorio-Forero et al., 2022). A study in humans also showed that higher LC activity during wakefulness as assessed by fMRI is linked to poorer self-reported sleep quality and lower power over the EEG theta band during REM sleep in the healthy middle-aged individuals. Besides, greater LC structural integrity, measured by LC contrast, was related to better habitual subjective sleep quality in both young and old individuals (Koshmanova et al., 2023).

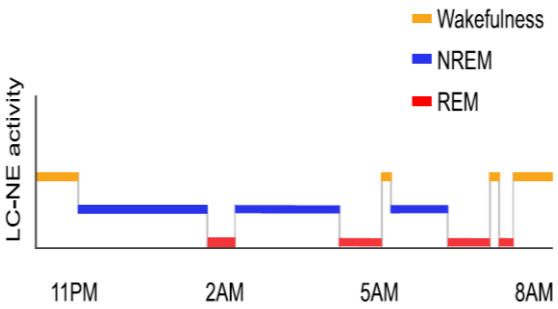


***Figure 2.4- Overnight LC-NE Activity in sleep.*** *LC-NE Activity and Sleep Macrostructure. The tonic firing rate of LC-NE neurons is maximal during wakefulness, decreases during NREM sleep, and is nearly absent during REM sleep (Van Egroo et al., 2022).*

Recent investigations have revealed that during NREM sleep, certain LC neurons exhibit a slow (~50-second) oscillatory rhythm marked by alternating periods of low and high firing, with activity levels that can match or even transiently exceed those observed during quiet wakefulness (Osorio-Forero et al., 2025). During NREM sleep, tonic LC-NE activity exhibited consistent fluctuations in relation to sleep spindles. Specifically, LC-NE neurons showed a marked reduction in firing in the seconds preceding spindle onset, a pronounced increase in activity during the spindle itself, and a subsequent decline in discharge following spindle termination (Aston-Jones & Bloom, 1981). Importantly, LC activity must decrease sufficiently to allow for the transition into REM sleep, and this drop is often preceded by increased spindle activity (Osorio-Forero et al., 2025). During REM sleep and the seconds before spindles that the LC is inactive, are marked by heightened activity in limbic circuits, pronounced synaptic plasticity (Ribeiro et al., 1999,

2002), and rich cognitive content, in the form of vivid dream reports (Poe et al., 2020). While LC activity during post-learning sleep has not been extensively studied, the limited existing data indicate a nuanced pattern of activation and suppression, both potentially critical for sleep- dependent memory consolidation (Born et al., 2006; Poe, 2017; Ribeiro et al., 1999). In particular, LC activity appears to be timed so that NE is released at forebrain synapses during the peaks of slow oscillations in NREM sleep, supporting the strengthening or preservation of memory-related circuits (Sara, 2010). Conversely, LC inactivity just prior to NREM spindles and during REM replay periods may permit synaptic depotentiation, a process necessary to reshape memory networks (Booth & Poe, 2006; Poe et al., 2000, 2010). Disruptions to either of these patterns (i.e., LC activation during NREM slow oscillations or LC silencing before spindles and during REM) may impair memory consolidation processes (Poe et al., 2020).

Notably, conditions like post-traumatic stress disorder (PTSD), insomnia, and opioid withdrawal exhibit sleep disturbances that may be linked to an overactive LC during sleep (Vanderheyden et al., 2014), potentially contributing to emotional dysregulation and hippocampal memory impairments (Wassing et al., 2019). Similarly, early-stage AD is associated with increased LC activity during sleep, which may play a role in the characteristic sleep disturbances observed early in the disease. In later stages, the extensive degeneration of the LC may result in insufficient NE release at forebrain synapses, compromising memory maintenance during slow oscillation reactivation and potentially accelerating memory loss (Poe et al., 2020).

## LC and arousals

*Arousals description & detection*

The definition of an arousal by the American Academy of Sleep Medicine (AASM) describes it as "an abrupt shift of EEG frequency including theta, alpha and/or frequencies greater than 16Hz but not spindles that lasts at least 3 seconds, with at least 10 seconds of stable sleep preceding the change" (Berry et al., 2012). Arousals can occur in three stages of NREM and as well as REM sleep. Arousals can be found on an epoch that is classified as wakefulness between lights-off and lights-on as long as it has at least 10s of stable sleep preceding it and all scoring criteria are met. In this regard, a minimum of 10s of stable sleep has to separate two distinct arousals. Also, arousals in NREM sleep may occur with or without any concomitant increase in the EMG signal amplitude, whereas arousals in REM sleep requires EMG increase lasting at least 1 second. Arousal scoring should take into account data obtained from the frontal, central, and occipital electrode derivations to ensure comprehensive and accurate assessment of arousal events across different brain regions. Arousal scoring can be enhanced by incorporating supplementary data from the recording, such as electrocardiogram (ECG), respiratory events or additional EEG channels. However, these sources of information cannot serve as the sole basis for identifying arousals, and they do not alter the established arousal scoring criteria (**Figure 2.5**). The 10 seconds of stable sleep necessary before scoring an arousal can start in the previous epoch, even if that epoch has been scored as stage wakefulness. An arousal can still be scored even if it occurs just before a transition to wakefulness; in such cases, both the arousal and the transition to wake are scored (Berry et al., 2012).

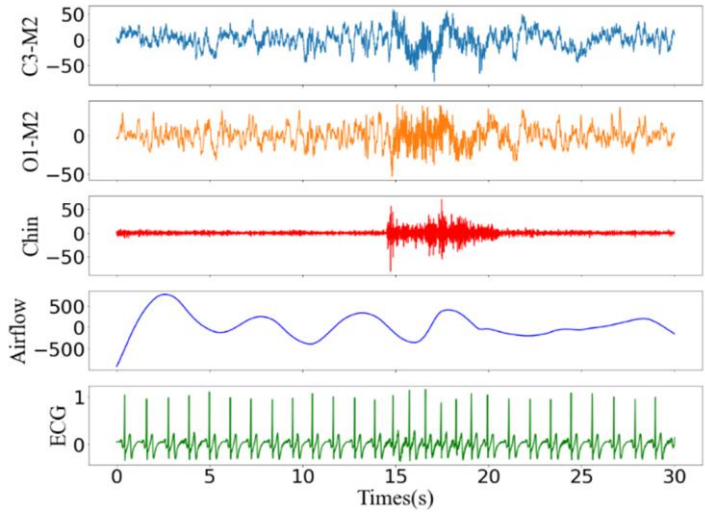


***Figure 2.5. Diagram illustrating EEG channel activity during an arousal event*** *(Qian et al., 2021).*

The 3-second duration criterion for scoring arousals, while somewhat arbitrary and not based on a physiological threshold, arises from methodological concerns in arousal detection. Scoring arousals is partly subjective, as it involves individual interpretation of increases in theta, alpha, or frequencies above 16 Hz. Shorter events tend to reduce scorer agreement. One study examined how different arousal definitions affected interscorer reliability and found significant variability depending on the criteria used. Reliability improved when arousals were accompanied by EMG activity, leg movements, or respiratory events, and decreased when EEG arousals were brief (Loredo et al., 1999). These findings emphasize how the chosen definition and its clarity directly influences arousal detection and affects the consistency of findings across studies.

The literature on arousals is highly complex and varied, largely due to the use of differing definitions of what constitutes an arousal, as well as the various types of arousals. This heterogeneity makes it challenging to compare findings across studies and to establish standardized criteria for identifying and interpreting arousal events in sleep research. Studies showed that arousals can occur spontaneously or they can be elicited by an external stimuli. Besides, although AASM arousals are referred to as cortical arousals (Berry et al., 2012), subcortical arousals have also been described. These arousals reflect activation of the brainstem or subcortical arousal system without affecting the cortex (McNamara et al., 2002; Rees et al., 1995; Togo et al., 2006). These arousals are first introduced based on the evidence that somatosensory and auditory stimulation during sleep can produce alterations in cardiac, respiratory, and somatic measures without overt EEG desynchronization (Carley et al., 1997; Halasz, 1993; Winkelman, 1999).

*Functions of arousals*

Traditionally, arousals have been closely linked to disease, particularly in the context of sleep disorders such as sleep disordered breathing (SDB) or PLMD (Broughton, 1968; Mahowald & Schenck, 2005; Scoring, 1992). An excessive number of arousals is commonly observed in conditions like sleep apnea (Strollo Jr & Rogers, 1996), while too few have been associated with issues such as sudden infant death syndrome (SIDS) (Schechtman et al., 1992). However, more recent perspectives propose that spontaneous arousals are an integral component of normal sleep regulation. They may serve a crucial function in preserving the reversibility of sleep and without them, sleep would resemble a state of coma (Halasz & Bodizs, 2012). Additionally, arousals may help maintain a subtle level of environmental awareness, allowing the sleeper to maintain some level of alertness to potential external

threats (Halasz & Bodizs, 2012). For instance, in sleep apnea they act as an alerting response that reactivates the system to resume breathing and they have been interpreted as a vigilance mechanism. This suggests that arousals serve a monitoring function, ensuring the sleeper can respond to physiological disturbances when necessary (Halász et al., 2004).

A study showed that arousal index was not correlated with objective and subjective sleepiness. Arousal index was not correlated with performance in intelligence quotient (IQ) as well as attention, executive function and working memory. So they concluded that arousals are not associated with impairments in daytime function found in patients with the sleep apnoea/hypopnoea syndrome (Kingshott et al., 1998). However, the results on the association between arousals and cognition are mixed. For instance, one study that measured neurocognitive performance multiple times after wake to assess the correlation between arousals with different durations and mood and attention after mild sleep restriction in healthy individuals showed that subjective sleepiness was not significantly related to the number of arousals. However, more arousals, which lasted more than 5s were linked to lower positive affect upon waking. Arousals were also associated with selective, but not sustained, attention. Notably, only arousals ≥5 s or ≥7 s and not ≥3 s were correlated with selective attention. These associations were time-specific, with effects on positive affect at ~6:30 am (test time 1), and on selective attention at ~7:30 am (test time 3) and ~9:00 am (test time 6) (Zhai et al., 2024). This time-specificity implies that the circadian phase or time since waking may moderate the effects of arousal on mood and attention. Those findings are in contrast with the report of Duce et al. (Duce et al., 2021), who reported a link between arousals and sustained attention using the same task. This discrepancy may stem from sample differences: Duce et al.(Duce et

al., 2021) studied OSA patients with poor sleep and frequent microarousals, while the previous sample comprised healthy individuals with good sleep quality and fewer arousals (Zhai et al., 2024).

Furthermore, one study which categorized arousals based on their timing relative to changes in muscle tone and sleep stage transitions demonstrated that different types of sleep arousals have distinct associations with cognitive outcomes and brain amyloid burden. Specifically, they observed that arousals linked to sleep transitions but not muscle tone were associated with increased cortical Aβ accumulation in brain regions typically affected in the early stages of AD, indicating a relationship with sleep fragmentation and poorer brain health. In contrast, the more frequent arousals which accompanied my muscle tone and did not lead to stage transition, were found to be more frequent in individuals with lower Aβ deposition and better cognitive performance, especially in attention-related tasks. This type of arousal is thus linked to a more favorable cognitive and neurological profile (Chylinski et al., 2021). This study suggests that distinct types of arousals may play different roles in relation to Alzheimer's disease pathology and cognitive function.

*Arousals and aging*

A study on healthy subjects showed that elderly individuals exhibit significantly more arousals across total sleep time. This increase was especially notable in stage 1 NREM sleep, stage 2 NREM sleep, and across all NREM sleep stages combined. In contrast, teenagers showed a significantly higher number of arousals during stage 4 NREM sleep compared to other age groups. No significant age-related differences were found in stage 3 or REM sleep (Boselli et al., 1998). In this line, another study, which showed that

arousal frequencies increase significantly with age in healthy subjects and individuals with snoring and apnea (Mathur & Douglas, 1995).

Of note, although arousals tend to remain fairly consistent among healthy individuals within the same age group, it can still vary from night to night in the same person. The brain is never entirely disconnected from the external environment, and even under strictly controlled conditions, internal factors such as mental, motor, sensory, or autonomic processes can unexpectedly influence arousals (Boselli et al., 1998).

*How does LC regulate arousals?*

Recent findings suggest that infraslow fluctuations in NE serve as gatekeepers of sleep architecture in mice. Notably, these fluctuations appear to function as timers for arousals (Osorio-Forero et al., 2025). Arousals predominantly occur during periods of reduced sleep spindle activity (Antila et al., 2022; Cardis et al., 2021; Kjaerby et al., 2022) and coincide with surges in LC activity (Antila et al., 2022; Kjaerby et al., 2022; Osorio-Forero et al., 2021). Conversely, arousals are infrequent during troughs in LC activity, in the presence of sleep spindles, or during transitions into REM sleep (Osorio-Forero et al., 2025) (**Figure 2.6**). When global LC activity was chemogenetically suppressed, the number of baseline arousals decreased, suggesting that arousals originate from surges in LC activity (Antila et al., 2022). Interestingly, not all LC surges lead to an EEG/EMG-detectable arousal. Two studies reported approximately one arousal for every three LC surges (Kjaerby et al., 2022; Osorio-Forero et al., 2025), indicating an average frequency of about one arousal every 2–3 minutes (Lüthi & Nedergaard, 2025). The LC surges associate with arousals exhibiting higher amplitude and longer duration, leading to increased NE release (Kjaerby et al., 2022; Osorio-

Forero et al., 2025), which could explain a stronger and/or more global LC-dependent arousal effect that reaches the cortex (Lüthi et al., 2023). These more intense surges are typically preceded by a temporary drop in baseline LC activity, implying that arousals may result from rebound excitation of LC neurons (Kjaerby et al., 2022; Osorio-Forero et al., 2025). These studies reflect that arousals are marked by elevated NE levels, increased heart rate, and reduced spindle activity. This insight supports the idea that arousals may define discrete sleep episodes that play a role in maintaining homeostatic functions (Watson et al., 2016).

In humans, a recent study observed infraslow fluctuations in pupil diameter during NREM sleep and found these changes to be phase-locked with variations in sleep spindle density during stage N2. Arousals occurred more frequently when the pupils were dilated, indicating heightened autonomic arousal (Carro-Domínguez et al., 2023). Although pupil diameter provides an indirect measure of LC, this study provides important evidence for infraslow arousal dynamics in humans that resemble those observed in mice (Lecci et al., 2017; Lüthi & Nedergaard, 2025).

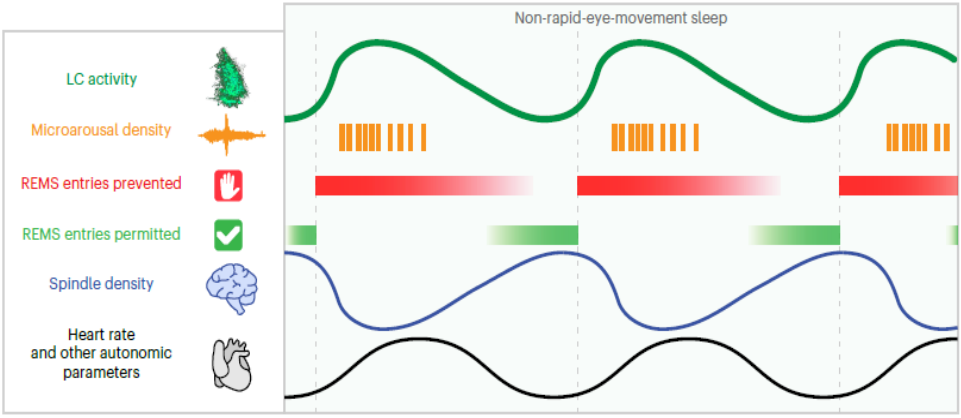


***Figure 2.6. A schematic representation LC activity causes arousals, transitions to REM sleep, sleep density, heart rate and other autonomic parameters*** *(Osorio-Forero et al., 2025)*

*An inverted U-shaped relationship between arousals and restorative sleep*

Arousals are temporally aligned with the spectral patterns of NREM sleep, particularly with sleep spindles. Sleep spindles tend to cluster during the descending phase of LC activity surges, while arousals typically occur at the ascending phase (Cardis et al., 2021; Kjaerby et al., 2022; Osorio-Forero et al., 2025). These spindle clusters are crucial for synaptic and circuit-level plasticity (Niethard et al., 2018; Seibt et al., 2017) and are also linked to behavioral outcomes (Antony et al., 2018). In addition, hippocampal ripple activity is synchronized with infraslow spindle clustering (Lecci et al., 2017). According to the active systems consolidation hypothesis, the precise timing of sleep spindles and hippocampal ripples enables the transfer of information from the hippocampus to the neocortex (Brodt et al., 2023). As such, NE-induced

suppression of spindles may disrupt or alter these processing windows. This disruption might serve an adaptive function by periodically increasing the brain's sensitivity to external stimuli, potentially to detect threats. Hence, arousals could enhance such responsiveness, as they elicit stronger global arousal compared to LC surges that do not lead to an arousal (Lüthi & Nedergaard, 2025; Osorio-Forero et al., 2025).

Arousals themselves could also play an active role in sleep-related plasticity. Arousals are linked to suppression of activity in dentate gyrus granule cells and mitral cells, which both exhibit infraslow fluctuations during NREM sleep (Turi et al., 2025) and participate in memory replay between the hippocampus and entorhinal cortex (De La Prida, 2020). Notably, this suppression seems to target neurons that are more active during NREM sleep than during wakefulness potentially helping to restore circuit balance during memory reactivation (Turi et al., 2025). Supporting this, field potential recordings have revealed increased coherence between hippocampal and cortical activity in conjunction with arousals (dos Santos Lima et al., 2019). Altogether, these findings suggest that arousals are part of a broader network of processes that support brain plasticity during sleep (Lüthi & Nedergaard, 2025).

The relationship between NE fluctuations, arousals frequency, and sleep's restorative functions may follow an inverted U-shaped pattern (**Figure 2.7**). It is proposed that arousals occurring approximately once every three infraslow NE cycles—roughly every 2–3 minutes—may offer an optimal neuromodulatory environment for sleep-dependent plasticity. This pattern would strike a balance: limiting sleep disruption while providing sufficient

windows for processes like synaptic plasticity and metabolic waste clearance (Lüthi & Nedergaard, 2025).

On the lower end of this inverted U-shaped curve, reduced LC activity and a narrowed dynamic range of NE release may be linked to neurodegenerative conditions such as AD. This is consistent with observed disruptions in sleep architecture and spectral features in AD, including altered spindle amplitude and frequency (Slutsky, 2024; Van Egroo et al., 2022). A decline in LC function has been identified as an early marker of preclinical AD (Van Egroo et al., 2022). Evidence from AD mouse models supports this, showing impaired NA signaling (Cankar et al., 2024). Pharmacological studies also confirm this idea. For instance, Zolpidem, a GABA receptor modulator and common sleep aid, has been shown to reduce NE levels and their fluctuations in the prefrontal cortex during mouse sleep (Hauglund et al., 2025). However, prolonged use of zolpidem has negative health outcomes (Edinoff et al., 2021), possibly due to the absence of essential restorative elements in pharmacologically induced sleep (Lüthi & Nedergaard, 2025).

At the upper end of the inverted U-shaped curve, pathologically elevated LC activity results in stronger NE surges and shorter troughs. Such dynamics have been observed in NREM sleep following stress exposure (Antila et al., 2022; Osorio-Forero et al., 2025). In these states, LC activity cycles at intervals shorter than 50 seconds, with insufficient trough duration to permit REM sleep. This leads to fragmented NREM sleep with excessive arousals (Osorio-Forero et al., 2025). Elevated LC activity is also seen in healthy aging (S.-B. Li et al., 2022) and in the early, prodromal stages of AD in animals and humans (Cassidy et al., 2022; Kelberman et al., 2023), which might have impact on impaired memory consolidation (Lüthi & Nedergaard, 2025). Monoamine

reuptake inhibitors, which increase NE availability and may reduce the variability of its fluctuations, have shown promise in mitigating cognitive decline in neurodegenerative diseases. However, these drugs are also associated with more fragmented sleep and a dominance of N2 stage sleep (Nicholson & Pascoe, 1986). Interestingly, they have also been found to enhance spindle activity and support certain memory functions, possibly by downregulating LC activity in response to elevated NE levels (Gais et al., 2011; Rasch et al., 2009).

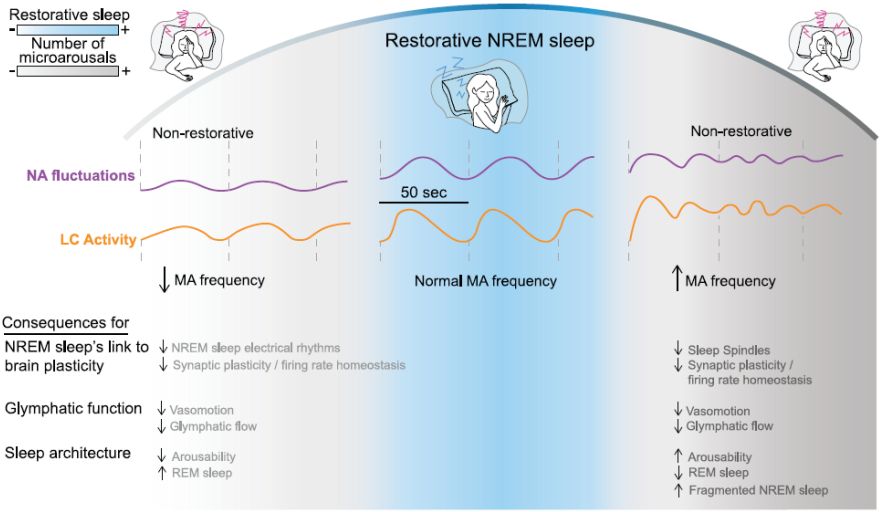


***Figure 2.7. Inverted U-shaped relationship between MAs, NA fluctuations, and key sleep benefits, such as sleep related brain plasticity, brain clearance, and NREM sleep continuity*** *(Lüthi & Nedergaard, 2025).*

*Arousals boost glymphatic clearance by NE*

The glymphatic system would use a network of perivascular spaces to allow cerebrospinal fluid (CSF) to flow through the brain, helping to remove metabolic waste and harmful substances like AB (Chong et al., 2022). In the mouse models, NE, which is a potent vasoconstrictor, regulates slow changes in arteriole diameter (vasomotor activity) at infraslow frequencies, particularly during NREM sleep (Lüthi & Nedergaard, 2025). These NE-driven vasomotor oscillations influence CSF flow via the glymphatic system: When arteries dilate, CSF is driven through the perivascular space; in contrast, vasoconstriction pulls CSF from the subarachnoid space into the perivascular compartment. A following dilation then propels the CSF forward once more (Holstein-Rønsbo et al., 2023; Rasmussen et al., 2022). Imaging studies show that NE levels and blood volume are strongly anticorrelated during NREM sleep, and optogenetic stimulation of the LC confirms NE's role in driving vasomotor activity. Notably, arousals amplify vasomotor fluctuations by triggering a short period of intensified vasoconstriction followed by vasodilation. This subsequent dilation compresses the perivascular space, promoting the entry of CSF into the brain parenchyma—driven by the infraslow vasomotor dynamics characteristic of NREM sleep (Bojarskaite et al., 2023; Lüthi & Nedergaard, 2025; Xie et al., 2013).

The link between NE fluctuations and vasomotion further reinforces the inverted U-shaped model (**Figure 2.7**). In neurodegenerative conditions, where the functional integrity of LC deteriorates, reduced NE fluctuations are likely to lead to diminished vasomotor activity and compromised glymphatic clearance. On the other hand, heightened NE fluctuations, such as those occurring during NREM sleep after stressful experiences, may result in excessive NE exposure that stiffens the vascular walls, thereby decreasing

vasomotion amplitude and slowing glymphatic flow through the perivascular space (Lüthi & Nedergaard, 2025).

*Locus coeruleus at the crossroad between sleep, aging and Alzheimer's disease*

Various dimensions of sleep including its timing, onset, continuity, and depth decline with age (Mander et al., 2017), and this deterioration is further intensified in neurodegenerative dementias, particularly in AD, the most prevalent form of dementia (Gagnon et al., 2019). In the context of aging with neurodegeneration, the LC has attracted significant attention, as it appears to be affected during the early stages of AD pathology (Lew et al., 2021). A growing body of evidence also suggests that sleep disturbances may accelerate the progression of AD-related changes (Wang & Holtzman, 2020). Consequently, investigating whether early LC dysfunction contributes to sleep disruptions holds promise for identifying prodromal stages of the disease. This prospect is further supported by recent findings indicating that in vivo structural assessments of LC integrity can be associated with early AD-related neurodegeneration and cognitive impairment (Jacobs et al., 2021; Osorio-Forero et al., 2022). Chemogenetic activation of the LC in a rat model of AD led to a recovery of spatial learning abilities; however, the extent and specific brain regions in which noradrenergic signaling was restored remain unclear (Rorabaugh et al., 2017).

A growing body of research over the past decade has highlighted sleep-wake dysregulation as a significant and modifiable factor capable of slowing the hallmark pathological processes of AD, namely the accumulation of beta-amyloid (Aβ), tau misfolded proteins, and neurodegeneration even during the preclinical stages (Van Egroo, Narbutas, Chylinski, Villar González, Maquet, et al., 2019). Notably, pivotal findings have shown that disrupted sleep and

wakefulness represent a fundamental mechanism in the early development of AD. The production and clearance of both Aβ and tau proteins are tightly regulated by the sleep-wake cycle. In turn, Aβ and tau pathologies have been shown to disrupt sleep architecture in AD mouse models, reinforcing the bidirectional relationship between sleep disturbances and AD-related neuropathology (Van Egroo et al., 2022; Van Egroo, Narbutas, Chylinski, Villar González, Maquet, et al., 2019).

Crucially, the LC is one of the earliest sites of tau pathology across the human lifespan (Braak & Del Tredici, 2012). Remarkably, by the age of 40, the majority of individuals already exhibit abnormally phosphorylated tau within the LC (Braak et al., 2011). Furthermore, the pathological consequences of tau accumulation in the LC appear to be particularly pronounced in AD compared to other tauopathies, such as progressive supranuclear palsy (PSP) and corticobasal degeneration. Specifically, significant LC neuronal loss has been observed as a hallmark uniquely associated with AD (Oh et al., 2019).

Together, these findings support a theoretical framework in which the LC-NE system acts as a critical link between sleep-wake disturbances and the early pathophysiological events of AD. In mouse models, both chronic sleep fragmentation and intermittent short sleep (three days per week over one month) were sufficient to induce significant structural changes in the LC-NE system, including marked reductions in LC-NE neuron numbers and axonal projections (Zhu et al., 2016). Furthermore, in a tauopathy mouse model, repeated episodes of sleep restriction accelerated tau accumulation within LC-NE neurons and promoted its spread to key brain regions such as the entorhinal cortex, hippocampus, and amygdala, while also advancing the emergence of neurobehavioral impairments (Zhu et al., 2018). Notably, these

changes were long-lasting: both structural degeneration of the LC-NE system and associated cognitive deficits persisted for up to a year following the sleep disruption protocol (Owen et al., 2021). Regarding LC neurodegeneration, a study in wild-type mice reported reduced neuronal density in the LC following prolonged wakefulness (Zhang et al., 2014). Notably, chronic sleep restriction resulted in LC neuronal loss that persisted despite a six-month period of recovery under normal sleep conditions, suggesting long-lasting or irreversible effects (Zhu et al., 2007). These animal studies suggest that early-life disturbances in sleep-wake regulation may initiate and perpetuate AD-related pathology through their impact on the LC-NE system (Van Egroo et al., 2022).

Besides, in human post-mortem studies, while early reports suggested that LC degeneration is a typical feature of aging, more recent investigations have shown that significant LC neuronal loss is not a characteristic of normal aging (Theofilas et al., 2017). Human studies have also shown that MRI-derived LC contrast, which indicates neuronal density and fiber projections, begins to gradually decline in cognitively healthy individuals starting in their fifth decade of life (Liu. et al., 2017); however, this reduction may be attributable to factors such as neuromelanin changes, neuronal shrinkage, or the loss of nearby dendrites, rather than actual neuronal death (Poe et al., 2020). With aging, noradrenergic modulation of plasticity at LC terminals diminishes, alongside a reduced influence of NE on synaptic plasticity in the hippocampus. These changes may underlie the reduced cognitive flexibility observed in healthy older adults, as well as the impaired ability to reorganize cortical circuits following brain injury in aging individuals (Poe et al., 2020). This LC decline becomes more pronounced in AD, where LC contrast is significantly reduced and associated with Aβ accumulation (Betts et al., 2019),

as well as with tau pathology in asymptomatic older adults (Jacobs et al., 2021; Van Egroo et al., 2022).

Sleep disruptions are also frequently observed in individuals with AD, often beginning in the preclinical phase as an exacerbation of typical age-related alterations in sleep-wake regulation (Van Egroo, Narbutas, Chylinski, Villar González, Maquet, et al., 2019). These disturbances intensify with disease progression, and are a major factor leading to patient institutionalization (Peter-Derex et al., 2015). Notably, many of these sleep-wake abnormalities—such as increased nighttime awakenings, insomnia, diminished REM sleep integrity, and reduced sleep spindles and slow-wave activity—have been linked to LC-NE dysfunction in animal models (Gagnon et al., 2019). Although histological studies have identified pronounced degeneration of the LC-NE system in AD brains, which may contribute to these sleep-wake disturbances (Oh et al., 2019), direct assessments of sleep-wake patterns were not included in these post-mortem investigations. Likewise, few in vivo studies have examined correlations between LC-NE activity and sleep-wake metrics in either healthy aging or AD populations, highlighting a critical gap in our understanding of how LC-NE system deterioration may drive sleep-wake dysregulation and AD-related pathology in humans (Van Egroo et al., 2022).

# Study objectives

As discussed in the previous sections, there is growing evidence that sleep alterations are not merely a consequence of aging and neurodegeneration, but may also actively contribute to the pathophysiological processes of AD. In parallel, a small brainstem nucleus, the LC, is gaining increasing attention for its dual relevance to both sleep regulation and AD pathogenesis. The LC is the brain's primary source of norepinephrine and is involved in modulating attention, arousal, and vigilance. Animal studies show that LC neurons exhibit distinct firing patterns across sleep–wake states and play a critical role in transitions between wakefulness, NREM sleep, and REM sleep. These dynamic fluctuations suggest that LC activity may have an integral influence on sleep architecture. Importantly, LC is also one of the first regions in the brain to accumulate tau pathology, decades before the clinical onset of AD symptoms. This raises the possibility that sleep disruption and AD pathology may converge, in part, through early changes in LC.

Despite strong preclinical evidence, the contribution of LC activity to sleep physiology, particularly in aging humans and in individuals with AD, remains poorly characterized. The overarching aim of this thesis is to first characterize how LC function and its connectivity relate to sleep architecture and aging in humans, using ultra-high-field neuroimaging and detailed sleep EEG analysis. The work then extends to investigate how LC-related sleep features may be linked to AD risk, using EEG measures and genetic risk profiling. Due to the challenges associated with directly recording LC activity during sleep such as the difficulty of sustained overnight fMRI scanning, we relied on carefully chosen proxies to assess LC function and its downstream effects on sleep. We measured LC activity indirectly during wakefulness using high-resolution 7-Tesla fMRI while participants performed tasks known to engage the LC. We hypothesized that the activity of the LC during wakefulness reflects its activity

during sleep. Then we tried to assess the association between LC activity during wakefulness and REM sleep measures. Then we expanded this work to see the association between effective connectivity between LC and hypothalamus (which is another important region for sleep- wake regulation) and REM sleep. In addition, the initial goal of this thesis was to measure LC activity using 7-Tesla fMRI in a large cohort of 100 participants and to investigate its association with genetic vulnerability to AD, as indexed by polygenic risk scores (PRS). However, due to time constraints, this larger data collection effort was not completed within the timeframe of this dissertation. As a result, we shifted our focus to a third proxy of LC function—spontaneous arousals during sleep, which have been shown to reflect LC activity in sleep (Osorio-Forero et al., 2025). In this thesis, we adopt these strategies to address a set of interrelated questions concerning the role of the LC activity and connectivity with hypothalamus in sleep regulation, and relevance of LC markers to AD risk across the adult lifespan.

To that end, this dissertation includes three empirical studies. In the first study (Chapter 3), we examined the relationship between LC activity, assessed during wakefulness through 2 distinct cognitive tasks that rely on different balances between phasic and tonic LC activity, and REM sleep features derived from polysomnographic recordings. Using a sample of younger and older adults, we tested how the balance between phasic and tonic modes of activity of the LC during wakefulness may be related to REM sleep. We hypothesized that LC activity across both tasks would primarily relate to the intensity of REM sleep. Additionally, we anticipated that these relationships would vary between the two tasks and be more prominent among older participants. To explore links with sleep features directly regulated by the LC, we also examined the association between LC activity and EEG sigma band

power preceding REM sleep episodes, as this measure has been shown to be causally linked to NE tone in rodent studies (Osorio-Forero et al., 2021).

In the second study (Chapter 4), we extended this line of inquiry by examining the effective connectivity between the LC and the hypothalamus. Using the same high-resolution fMRI data, we applied dynamic causal modeling (DCM) to assess directional influences between the LC and distinct hypothalamic subregions during task performance. We then related these connectivity patterns to electrophysiological sleep markers, focusing on REM sleep and age-related variability. The goal was to determine whether altered LC–hypothalamus interactions could explain some of the differences in REM sleep quality observed across the lifespan. We hypothesized that connectivity between the LC and one or more hypothalamic subregions specifically the anterior-superior, anterior-inferior, or posterior-lateral areas would serve as indicators of REM sleep quality. Furthermore, we hypothesized that these associations would be modulated by aging.

In the third study (Chapter 5), in a larger sample of 540 healthy individuals, we categorized arousals based on their EEG and electromyographic features and examined their relationship to polygenic risk for AD. We classified spontaneous arousals according to their association with muscular tone increase and sleep stage transition. This study aimed to establish a link between arousal dynamics, a LC-regulated sleep feature, and preclinical markers of AD risk. We hypothesized that certain types of arousals would be linked to higher genetic risk for AD, whereas others would be associated with lower genetic risk. Additionally, we anticipated that these distinct arousal patterns would show differential associations with cognitive performance and with cognitive decline over 2- and 7-year follow-up periods.

Through these studies, this dissertation wants to contribute to a more comprehensive understanding of how the LC and its related circuits shape sleep physiology in humans and how these dynamics may reflect the vulnerability to age-related neurodegenerative disease.

# Experimental results

## Chapter 3: REM sleep quality is associated with balanced tonic activity of the locus coeruleus during wakefulness

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**The results presented here were published *in Journal of Biomedical Science* (2025). Supplementary materials for this paper can be found in Appendix 1.**

**Abstract**

**Background**

Animal studies established that the locus coeruleus (LC) plays important roles in sleep and wakefulness regulation. Whether it contributes to sleep variability in humans is not yet established. Here, we investigated if the *in vivo* activity of the LC is related to the variability in the quality of Rapid Eye Movement (REM) sleep.

**Methods**

We assessed the LC activity of 34 healthy younger (~22y) and 18 older (~61y) individuals engaged in bottom-up and top-down cognitive tasks using 7-Tesla functional Magnetic Resonance Imaging (fMRI). We further recorded their sleep electroencephalogram (EEG) to evaluate associations between LC fMRI measures and REM sleep EEG metrics.

**Results**

Theta oscillation energy during REM sleep was positively associated with LC response in the top-down task. In contrast, REM sleep theta energy was negatively associated with LC activity in older individuals during the bottom-up task. Importantly, sigma oscillations power immediately preceding a REM sleep episode was positively associated with LC activity in the top-down task.

**Conclusions**

LC activity during wakefulness was related to REM sleep intensity and to a transient EEG change preceding REM sleep, a feature causally related to LC activity in animal studies. The associations depend on the cognitive task, suggesting that a balanced level of LC tonic activity during wakefulness is

required for optimal expression of REM sleep. The findings may have implications for the high prevalence of sleep complaints reported in aging and for disorders such as insomnia, Alzheimer's, and Parkinson's disease, for which the LC may play pivotal roles through sleep.

**Background**

Up to 35% of the general population report unsatisfying sleep (Morin et al., 2015) and one out of two adults aged over 50y complains about sleep or daytime sleepiness (Crowley, 2011). Insomnia is the obvious extreme form of these complaints and constitutes the second most prevalent psychiatric disorder (American Psychiatric Association & Association, 2013). Beyond the behaviors that do not favor appropriate sleep, some people are more vulnerable to poor sleep or more at risk of developing insomnia (Koshmanova et al., 2022; Van Someren, 2021). The biological origin of sleep variability is however not fully established (Poe et al., 2020).

The locus coeruleus (LC) is a small nucleus of the dorsal pons – cylinder-like shape of approximately 15 mm with ~2.5 mm diameter (Fernandes et al., 2012). It is responsible for producing norepinephrine (NE) as a part of the ascending arousal system and projects to the neocortex, hippocampus, amygdala, thalamus, and cerebellum (Lindvall et al., 1978). It plays a crucial role in the control of behavioral states, including alertness and attention (Maness et al., 2022), as well as in the regulation of sleep (Poe et al., 2020). LC-NE tone must decrease to allow sleep onset, while LC activity dynamically changes during sleep to shape the alternation between Rapid Eye Movement (REM) and non-REM sleep and some of the microstructure elements of sleep (Gompf et al., 2010; Osorio-Forero et al., 2022). A lot of our understanding of the role of the LC in sleep regulation arises from experiments conducted in rodents. Therefore translation to diurnal human beings, with a more developed cortex, is not straightforward (Poe et al., 2020). In addition, imaging the LC *in vivo* remains difficult because of its deep location and small size, as well as because of the difficulty to image human sleeping brains.

Recent advances in neuroimaging techniques have lifted part of these limitations (Van Egroo et al., 2022). For instance, degeneration of the LC was recently suggested to contribute to alteration in rest-activity patterns (Van Egroo et al., 2022). We further reported that a higher response of the LC to a salience detection task during wakefulness, which presumably reflects in part LC activity during sleep, was associated with lower intensity of REM sleep in healthy older individuals (Koshmanova et al., 2023). REM sleep intensity does not however solely depend on LC activity, and no association with sleep features shown to be under the direct control of the LC in animal were reported in the study.

LC neurons follow phasic and tonic activity patterns, which lead to distinct release of NE (Aston-Jones & Bloom, 1981). Phasic activity typically occurs in response to transient salient stimulation, while the tonic mode is mostly required for sustained processes. The dynamics of the tonic mode is considered to follow an inverted U-shape where low LC tonic discharge is associated with low arousal and poor performance, moderate LC tonic activity allowing pronounced phasic activity leads to optimal arousal and performance, and high tonic activity hinders phasic activity and leads to anxiety-like behavior and poorer performance (Aston-Jones et al., 1999). Likewise, during sleep the LC undergoes periods of higher or lower tonic activity (Gompf et al., 2010; Osorio-Forero et al., 2022).

How the balance between phasic and tonic modes of activity of the LC during wakefulness may be related to REM sleep is not known. In the present study, we used Ultra-High-Field (UHF) 7-Tesla (7T) Magnetic Resonance Imaging (MRI) to extract *in vivo* the LC activity in healthy younger and older adults during two cognitive tasks that rely on different tonic tones of the LC. We

related the LC activity during both tasks to electrophysiology metrics of REM sleep. We hypothesized that the LC activity in both tasks would be mainly associated with REM sleep intensity. Moreover, we expected that the associations would differ between two tasks, and would be more pronounced in older individuals. To test for associations with sleep features under direct control of the LC, we further tested the association between LC activity and the power of the sigma band of the EEG that preceded REM sleep episodes, because it is causally related to NE tone in rodents (Osorio-Forero et al., 2021).

**Methods**

This study was approved by the faculty-hospital ethics committee of ULiège. All participants provided written informed consent and received financial compensation. The study is part of a larger project that has led to previous publications (Berger et al., 2023; Koshmanova et al., 2023). Part of the results dealing with one cognitive task were reported in one of these publications (Koshmanova et al., 2023) and are included for comparisons purposes and to conduct additional analyses. Most of the methods were described in details in (Koshmanova et al., 2023). More details are also available in the **Supplementary Methods**.

*Participants*

Fifty-two healthy participants including 34 healthy young (22.2±3.1y, 28 women) and 18 late middle-aged (60.9±5.4y, 14 women) individuals, completed the study **(Table 3.1)**. The exclusion criteria were as follows: history of major neurologic/psychiatric diseases or stroke; recent history of depression/anxiety; sleep disorders; medication affecting the central nervous system; smoking, excessive alcohol (>14 units/week) or caffeine (>5 cups/day)

consumption; night shift work in the past 6 months; BMI ≤18 and ≥29 (for older individuals) and ≥25 (for younger individuals). All older participants had to show normal performance on the Mattis Dementia Rating Scale (score > 130/144).(Mattis, 1976)

**Table 3.1. Characteristics of the study sample**

| | Young individuals (n = 34) | | | | Late middle-aged individuals (n = 18) | | | | P-value |
|---|---|---|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Min** | **Max** | **Mean** | **SD** | **Min** | **Max** | |
| Age (years) | 22.20 | 3.17 | 18.00 | 29.00 | 60.88 | 5.41 | 53.00 | 70.00 | **<.0001** |
| BMI (kg/m2) | 21.90 | 2.74 | 17.20 | 28.40 | 24.75 | 3.40 | 19.40 | 30.90 | **0.0047** |
| Education (years) | 14.46 | 2.31 | 12.00 | 20.00 | 14.61 | 2.70 | 9.00 | 19.00 | 0.85 |
| Depression level (BDI) | 6.64 | 4.17 | 0 | 20.00 | 5.33 | 4.01 | 0 | 14.00 | 0.27 |
| Anxiety level (BAI) | 4.00 | 2.92 | 0 | 11.00 | 3.05 | 3.18 | 0 | 9.00 | 0.30 |
| TIV ($cm^3$) | 1402.1 | 136.6 | 1203.3 | 1946.7 | 1420.5 | 125.5 | 1167.6 | 1600.1 | 0.627 |
| Daytime sleepiness (ESS) | 6.93 | 3.90 | 0 | 14.00 | 5.77 | 3.76 | 0 | 13.00 | 0.30 |
| Sex (F–M) | 28 F – 6 M | | | | 12 F – 6 M | | | | 0.20 |
| Habitual Subjective sleep quality (PSQI) | 4.79 | 2.18 | 1.00 | 10.00 | 3.83 | 2.22 | 0 | 8.00 | 0.14 |
| TST (min) | 454.80 | 30.97 | 379.50 | 499.50 | 394.63 | 48.16 | 282.50 | 495.50 | **<.0001** |
| Theta power in REMS ($\mu V^2$) | 7281.5 | 5061.7 | 1162.4 | 22346.4 | 4789.5 | 3508.2 | 765.3 | 13051.8 | **0.0449** |
| REMS percentage (%) | 26.63 | 4.41 | 19.93 | 36.45 | 21.52 | 5.50 | 13.11 | 35.39 | **0.002** |
| REMS latency (min) | 103.0 | 47.834 | 0 | 213.5 | 81.944 | 56.806 | 38.000 | 295.5 | 0.190 |
| Sigma power prior to REMS ($\mu V^2$) | 15.7615 | 8.8957 | 5.0656 | 44.356 | 16.0544 | 6.795 | 3.654 | 27.856 | 0.896 |
| SWE ($\mu V^2$) | 175531 | 171295 | 14259.2 | 555453 | 126388 | 109290 | 23293.3 | 470116 | 0.2146 |

*The p-values shown in the table correspond to two-sample t-tests except for sex that were compared using a Chi-square test. BMI: Body Mass Index; BDI: Beck Depression Inventory; BAI: Beck Anxiety Inventory; TIV: Total Intracranial Volume; PSQI: Pittsburgh Sleep Quality Index; ESS: Epworth Sleepiness Scale; TST: Total Sleep Time; SWE: slow wave energy; REMS: rapid eye movement sleep. See supplementary methods for references of the questionnaires.*

*Protocol*

Participants' sleep was recorded in the lab twice. First, participants completed a night of sleep under polysomnography (called habituation night) to screen and exclude for sleep abnormalities (apnea hourly index and periodic leg movement >15; no parasomnia or REM behavioral disorder). All participants further completed a whole-brain structural MRI (sMRI) acquisition and a specific acquisition centered on the LC. Participants were then requested to sleep regularly for 7 days before the baseline night (±30min from their sleep schedule) based on their preferred schedule (compliance was verified using sleep diaries and wrist actigraphy – Actiwatch and AX3, AXIVITY LTD, Newcastle, UK). For the baseline night, participants first remained awake for 3h under dim light (<10 lux) then their habitual sleep was recorded in darkness (N7000 amplifier, EMBLA, Natus, Middleton, WI). Approximately 3h after wake-up time under dim light (<10 lux), participants completed a functional MRI (fMRI) session that included 3 tasks (**Figure 3.1A**). This paper is centered on the analyses of the first two tasks, the perceptual rivalry and auditory salience detection tasks.

Younger participants completed the fMRI session immediately following the baseline night but older participants were initially part of a different study(Narbutas et al., 2019; Van Egroo, Narbutas, Chylinski, Villar González, Ghaemmaghami, et al., 2019) and completed the MRI procedures in addition to their initial engagement, which included the baseline night recording. Consequently, the baseline nights of sleep and MRI sessions were completed about 1.25y apart (mean±SD: 15.5±5.3 months). Prior to the fMRI session, older participants slept regularly for 1 week and were maintained in dim light (<10 lux) for 45 minutes.

*Sleep EEG metrics*

Sleep was staged in 30s-epochs using an automatic algorithm (ASEEGA, PHYSIP, Paris) (Berthomier et al., 2007b) to provide REM sleep percentage and total sleep time (TST). Averaged power was computed per 30-min bin, adjusting for the proportion of rejected data and it was subsequently aggregated in a sum for REM (Skorucak et al., 2018) to provide REM theta energy (overnight cumulated 4.25-8Hz power). Sigma power (12.25–16 Hz) prior to REMS was calculated as the mean sigma power before REMS episodes, obtained by dividing the total sigma power in the 1-minute period preceding each REMS episode by the number of REMS episodes. Sigma power was computed as the weighted sum of 4s artifact-free windows (2s overlap per 30s epoch).

*Cognitive tasks*

*Visual perceptual rivalry task – high-tonic LC.* The task consisted of watching a 3D Necker cube, which can be perceived in two different orientations (**Figure 3.1B**), for 10 blocks of 1min separated by 10s of screen-center cross fixation (~12min). Participants were instructed to report switches between the two percepts through a button press. Given the top-down nature of the task, we considered that if the task recruited the LC, it would trigger a relatively lower phasic response over a relatively larger tonic tone (high-tonic LC).

*Auditory salience detection task – low-tonic LC.* The task consisted of an oddball paradigm requiring reports on the perception of rare deviant target tones (1,000Hz, 100ms, 20% of tones) that were pseudo-randomly interleaved within a stream of standard stimuli (500Hz, 100ms) through a

button press (**Figure 3.1C**). The task included 270 stimuli (54 targets; ~10min). Given the bottom-up nature of the task, we considered that if the task recruited the LC, it would trigger a relatively larger phasic response over a relatively lower tonic tone (low-tonic LC).

Pupil size was recorded (Eyelink-1000, SR Research, Osgoode, ON, Canada; sampling rate: 1000Hz) during both cognitive tasks only in a subset of participants due to technical difficulty in maintaining stable signal (in part because of the small window between receive and transmit parts of the MR head coils through which the eye is within camera view) leading to missing/corrupted data (> 25% of missing/corrupted). Analyses of pupil data of the perceptual rivalry and salience detection tasks respectively included 32 (23 women; 23 young individuals) and 27 (20 women; 19 young individuals) individuals with 22 participants common to both tasks (15 women; 15 young individuals).

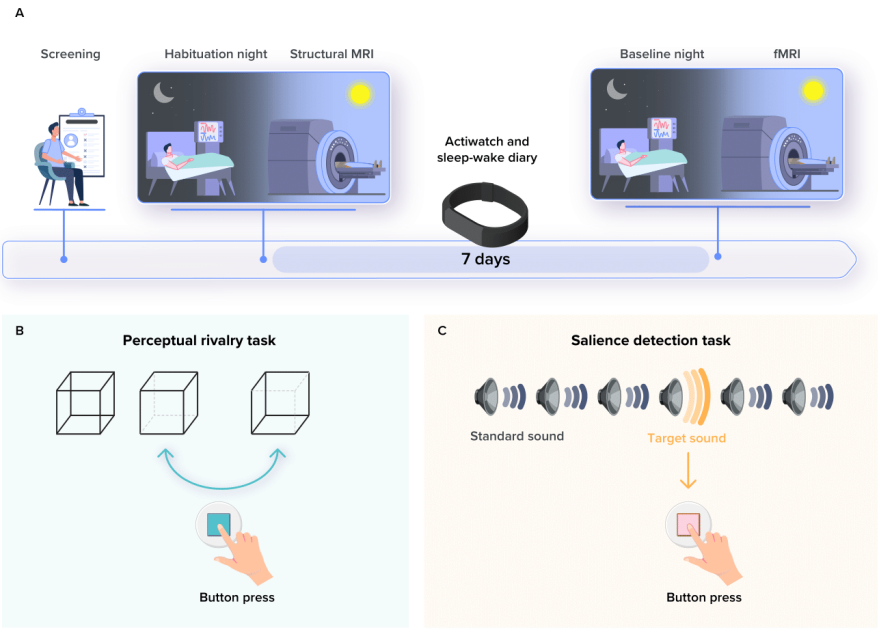


***Figure 3.1. Overview of the study protocol.***

*(A) After screening, participants completed an in-lab screening and habituation night under polysomnography to minimize the effect of the novel environment for the subsequent baseline night and to exclude volunteers with sleep disorders. They further completed a structural 7T MRI session including a whole-brain structural MRI and a LC-specific sequence. The latter was used to create individual LC masks in each participant's brain space. After 7 nights of regular sleep-wake time at home, which was confirmed by actigraphy data and/or sleep-wake diary, participants came to the lab three hours before their sleep time and were maintained in dim light (<10 lux) until sleep time. Participants' habitual baseline sleep were recorded overnight in-lab under EEG to extract our main sleep features of interest. All participants underwent an fMRI session approximately 3h after wake-up time (following ≥45min in dim light - < 10lux), during which they completed the visual perceptual rivalry task and the auditory salience detection task.*

(B) *The Visual perceptual rivalry task consisted of watching a 3D Necker cube, which can be perceived in two different orientations (blue arrow), for 10 blocks of 1min separated by 10s of screen-center cross fixation (total duration ~12min). Participants reported switches in perception through a button press. We considered that task would involve a relatively lower phasic response over a relatively larger tonic tone (high-tonic LC).*

(C) *The auditory salience detection task consisted of an oddball paradigm requiring button-press reports on the perception of rare deviant target tones (20% occurrence) within a stream of frequent tones (total duration ~10min). We considered that task would involve a relatively larger phasic response over a lower relatively tonic tone (low-tonic LC).*

*MRI data acquisition* and *preprocessing*

MRI data were acquired using a MAGNETOM Terra 7T MRI system (Siemens Healthineers, Erlangen, Germany). FMRI and sMRI data were preprocessed using SPM12, ANTs and SynthStrip brain extraction tool(Hoopes et al., 2022), as fully described previously (Koshmanova et al., 2023). The preprocessed data were resampled to a $1mm^3$ resolution. Individual statistical analyses included one regressor of interest consisting of a switch in perception (perceptual rivalry) or the occurrence of a target deviant tone (salience detection) modeled as an event (convolved with the canonical hemodynamic response function – HRF). Participant movement, respiration, and heart rate were used as covariates of no interest (physiological data of 4 volunteers were not available).

Individual LC masks were manually delineated by 2 experts based on LC-specific images (as in (Koshmanova et al., 2023)) to extract LC activity estimate or LC responses to the task (i.e., in arbitrary units – a.u. – as the mean value of the of the multiple regression fits over the LC mask) during

both tasks and to compute a LC probabilistic map in the group space for visualization. The T1 structural whole-brain image was used to extract individual total intracranial volume (TIV) using CAT12 toolbox (Gaser et al., 2022).

*Statistics*

Statistical analyses were performed in SAS 9.4 (SAS Institute, NC, USA) and consisted of Generalized Linear Mixed Models (GLMM) with 4 main sleep features of interest (REM sleep latency, REM sleep percentage, REM Theta energy, sigma power prior to each REM sleep episode) as the dependent variables (with adjusted distribution), LC activity estimate as an independent variable, as well as age group (younger and older individuals), sex, TST and TIV. Outliers among all variables lying beyond four times the standard deviation were removed from the analysis (maximum 2 data points were removed). The final number of individuals included in each analysis is reported in **table 3.2** and **table 3.3**. Partial $R^2$ ($R^{2*}$) values were computed as previously described (Koshmanova et al., 2023). We used Pearson correlations to assess the association between transient pupil dilation and estimates of LC activity and for visualization of the GLMM outputs. The analyses included 4 main dependent variables of interest: Benjamini and Hochberg False Discovery Rate (FDR) correction considering 4 independent tests was used to test for significant associations [p<.0125 (for rank ¼); p<.025 (for rank 2/4); p<.0375 (for rank ¾); p<.05 (for rank 4/4)]. All models included an interaction term between LC activity and age group. If the Bayesian Index Criterion (BIC) of the model without the interaction was better (i.e. lower) and the interaction term was not significant it was removed from the model.

Importantly, statistical analyses are slightly modified compared to (Koshmanova et al., 2023) with the inclusion of TIV as a covariate (rather than BMI) to take into account non-specific variation in EEG power computations. Statistical outcomes related to the auditory salience detection task may therefore slightly vary compared with our previous publication (Koshmanova et al., 2023).

We computed a prior sensitivity analysis to get an indication of the minimum detectable effect size in our main analyses given our sample size. According to G*Power 3 (version 3.1.9.4) (Faul et al., 2009), taking into account a power of .8, an error rate α=.05, and a sample size of 52, we could detect medium effect sizes $r$>.39 (2-sided; CI:.13–.6; $R^2$>.15, CI:.02–.36) within a linear multiple-regression framework including 2 tested predictor (LC activity, age group) and 2/3 covariates (sex, TST, TIV where relevant).

**Results**

Fifty-two healthy individuals aged 18 to 31y (N=34; 28 women) and 50 to 70y (N=18; 14 women) completed an fMRI protocol consisting of a top-down visual perceptual rivalry task (Kornmeier & Bach, 2005), which we considered could trigger a lower phasic response of the LC over a relatively larger tonic tone (high-tonic LC), and a bottom-up auditory salience detection task (P. R. Murphy et al., 2014), which we considered could trigger a larger phasic response of the LC over a relatively lower tonic tone (low-tonic LC task). Both tasks successfully triggered a phasic response of the left LC when inspecting the whole-brain statistical analyses over the entire sample (**Figure 3.2A and B**). The recruitment of the LC was further supported by the fact that, in a subset of participants (see methods), we successfully detected significant transient pupil dilation around the event of interest (**Figure 3.2C, D, E and G**).

The LC is indeed considered to drive part of the transient pupil dilation associated with the detection of salient stimuli or with changes in perception in a perceptual rivalry context (Einhauser et al., 2008; P. R. Murphy et al., 2014).

We therefore extracted the activity of the left LC in all individuals. We first found that transient pupil dilation during the salience detection task was significantly correlated to the activity estimate of the left LC (Pearson's r=.4, **p=.03**; **Figure 3.2H**), while it was not for the perceptual rivalry task (**Figure 3.2F**, Pearson's r=-.20, p=.29). Since pupil responses are associated with phasic responses of LC (Devilbiss & Waterhouse, 2011), the absence of significant correlation in the perceptual rivalry task is in line with our hypothesis that this task involved more tonic LC activity. We further found that LC responses during both tasks were not correlated (Figure 3.3A) (Pearson's r= .08, p=.54), indicating that the activity of LC in one task did not linearly predict its activity in the other task and supporting that both tasks probe different aspects of LC activity. We did find a significant positive Pearson correlation (whole sample: r=0.357; p=0.010; in the young only: r=0.415; p=0.018; in the older only: r=-0.457, p=0.056) between LC activity estimate during the salience detection task and squared LC activity estimate in the perceptual rivalry task. This suggests a U-shape relationship between LC activity estimate in two tasks and may support the idea that tonic and phasic activities interact in a nonlinear manner. This would deserve future investigations.

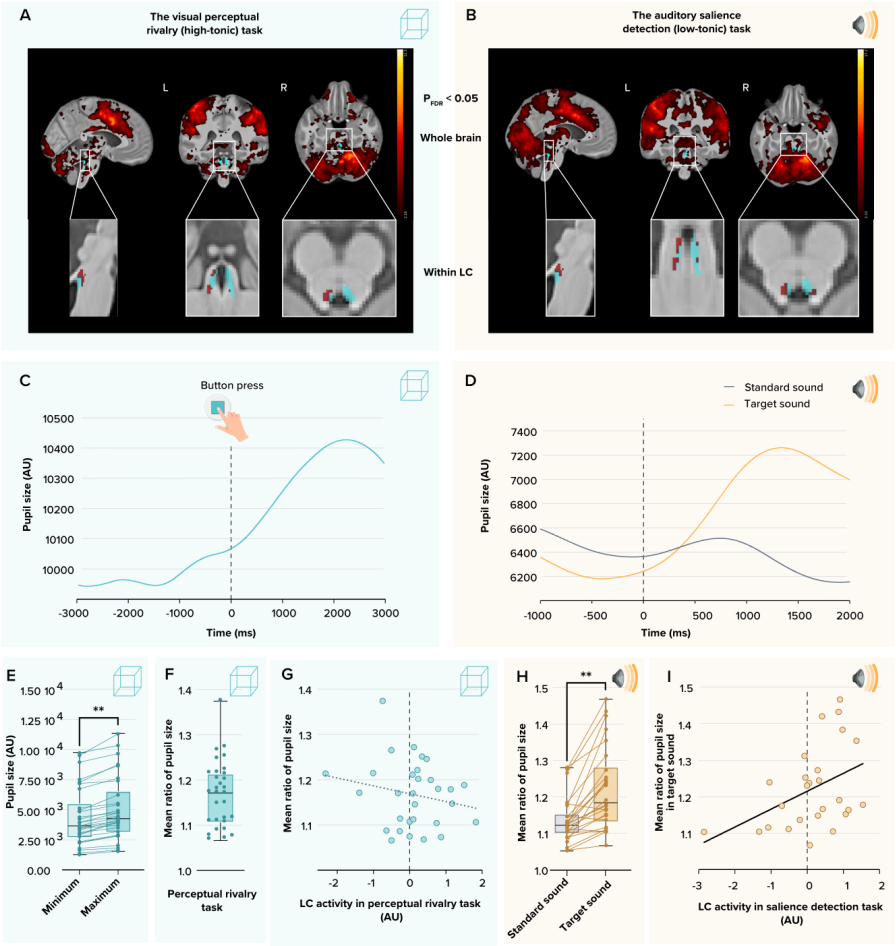


***Figure 3.2.*** ***(A)*** *Whole-brain and LC responses to the perceptual switches during the visual perceptual rivalry task. Sagittal, coronal, and axial views [MNI coordinates: (−4 −37 -21 mm)]. The images at the top show the whole-brain results using significance for a threshold of p< 0.05 FDR-corrected (t > 2.16) over the group average brain structural image coregistered to the MNI space. Insets at the bottom show the LC probabilistic template (blue) created based on individual LC masks and the significant activation detected within this mask (dark grey).* ***(B)*** *Whole-brain and LC responses to the target sound during the auditory salience detection task. Sagittal, coronal, and axial views [MNI coordinates: (−4 −34 -21 mm)]. The images at the top show the whole-brain results using significance for a threshold of p< 0.05 FDR-corrected (t > 2.33) over the group average brain structural image coregistered to the MNI space. Insets at the bottom show the LC probabilistic template (blue) and the significant activation detected within this mask (dark*

*grey). The legend shows the t-values associated with color maps.* ***(C)*** *A representative example of the pupil dilation in response to the perceptual switches during the visual perceptual rivalry task in one participant.* ***(D)*** *A representative example of the pupil dilation in response to the standard and target sounds during the auditory salience detection task in the same subject as in panel C.* ***I*** *Minimum and maximum pupil size before and after the perceptual switches during the visual perceptual rivalry task. Maximum pupil size was significantly higher than the minimum (N=32, p < 0.0001). Arbitrary units (AU) of the pupil size in the figures C, D and E are based on pixel number following proprietary algorithms application yet do not correspond exactly to pixel number.* ***(F)*** *Mean ratio of the pupil size in the perceptual rivalry task. The mean ratio of pupil size was computed as the change in the pupil diameter from before to after the perceptual switches.* ***(G)*** *Association between LC activity during visual perceptual rivalry task and mean ratio of pupil size in response to the perceptual switches. The association was not significant (Pearson's correlation r=-0.201, P=0.29).* ***(H)*** *Mean ratio of pupil size in response to standard sound and target sound during the auditory salience detection task. The mean ratio of pupil size was computed as the change in the pupil diameter from before to after the auditory stimulus presentation. The change in pupil size was significantly higher for target versus standard sound (N=27, p < 0.0001).* ***(I)*** *Association between LC activity during auditory salience detection task and mean ratio of pupil size in response to the target sound. We found a significant positive Pearson's correlation (r=0.4, P=0.03).*

We then targeted our main goal: testing relationships between LC activity and canonical characteristics of REM sleep estimated based on a night of sleep in the lab under EEG. Considering first the perceptual rivalry task, we found a significant positive association between REM theta energy and high-tonic LC activity (t=3.38; **p= .001**; **Figure 3.3B**) on top of the main effect of TST, while the other covariates were not significant (**Table 3.2)**. As previously reported,(Koshmanova et al., 2023) we then found a significant association between REM theta energy and the interaction between low-tonic LC activity during the salience detection task and age group (t=2.68; **p=.01; Table 3.3**) that came on top of a main effect of LC activity (t=-2.78; **p=.008**) (**Figure 3.3C**) while the other covariates were not significant (**Table 3.3**). Post hoc contrasts revealed that higher REM theta energy was associated with a lower response of the LC in older (t= -2.78; **p=.008**) but not in younger (t=.33; p=.74)

individuals, indicating that the main effect of LC activity was mainly driven by the older participants.

For both low-tonic and high-tonic LC activity the inclusion of REM sleep duration and/or REM sleep percentage instead of or on top of TST did not modify the statistical outputs of the models (data not shown). Furthermore, REM theta energy associations with the activity of the LC during both tasks remain similar when including them simultaneously in the same GLMM, i.e. a significant main effect of high-tonic LC activity ($F_{(1,41)}$=9.52, **p=.0036**) and a weak trend for interaction between low-tonic LC activity and age: $F_{(1,41)}$=2.72, p=.1), supporting that they explain different parts of the variance in REM theta energy (using squared values of LC activity estimates of each task separately or together estimates did not yield significant outputs – not shown).

**Table 3.2. Associations between REM sleep metrics and LC activity estimated via <u>the visual perceptual rivalry (high-tonic) task</u>.**

| Sleep metric (dependent variable) | LC activity | Age group | Sex | TIV | Total sleep time |
|---|---|---|---|---|---|
| REM Theta energy (N=51) | F(1,45)=11.39 **P=0.001*** **$R^{2*}$=0.201** | F(1,45)=0.14 P=0.709 | F(1,45)=0.22 P=0.645 | F(1,45)=0.08 P=0.781 | F(1,45)=5.15 **P=0.028** **$R^{2*}$=0.102** |
| Sigma power prior to REM (N=52) | F(1,46)=5.55 **P=0.023*** **$R^{2*}$=0.107** | F(1,46)=0.60 P=0.442 | F(1,46)=3.64 P=0.062 | F(1,46)=2.18 P=0.147 | |
| REMS percentage (N=52) | F(1,47)=2.05 P=0.159 | F(1,47)=13.27 **P=0.0007** **$R^{2*}$=0.220** | F(1,47)=1.66 P=0.204 | F(1,47)=3.15 P=0.082 | |
| REMS latency (N=52) | F(1,46)= 0.10 P= 0.750 | F(1,46)= 2.01 P= 0.163 | F(1,46)= 1.27 P= 0.265 | F(1,46)= 0.27 P= 0.602 | F(1,46)= 0.74 P= 0.392 |

*Prior to the analysis, we removed the outliers among all variables by excluding the samples lying beyond four times the standard deviation (the final number of individuals included in each analysis is reported below each dependent variable).*

** Significant following Hochberg False Discovery Rate (FDR) correction for 4 independent tests (see methods)*

*In all models the interaction between LC activity and age group was not significant. Goodness of fit metric (BIC) indicated that the interaction term should be removed. LC: locus coeruleus; TIV: total intracranial volume; REM: rapid eye movement; REMS: rapid eye movement sleep.*

No association was found when considering the other macroscopic REM sleep metrics (REM sleep onset latency, REM sleep percentage) (**Suppl. Figure S1, Table 3.2 and 3.3**). Further analyses indicated that the associations with REM theta energy were specific to this particular band as no significant association was uncovered between the energy of the other spectral bands of the EEG during REM sleep (except for a significant main effect of low tonic LC activity [t= -2.33; p=.025) and of an age-group-by-low tonic LC activity interaction (t= 2.14; p=.037) when using REM alpha energy as the dependent variable - **Suppl. Figure S2, Suppl. Table S1 and S2**]. The significant associations we detected were also specific to REM sleep as NREM slow wave energy (SWE), i.e. the cumulated overnight power over the delta band (.5-4Hz) typical of NREM sleep, was not significantly associated with either low-tonic and high-tonic LC activity (**Suppl. Figure S2, Suppl. Table S1 and S2**).

**Table 3.3. Associations between REM sleep metrics and LC activity estimated via the auditory salience detection (low-tonic) task.**

| Sleep metric (dependent variable) | LC activity | Age group | LC activity*age group | Sex | TIV | Total sleep time |
|---|---|---|---|---|---|---|
| **REM Theta energy (N=51)** | F(1,44)=6.74 **P=0.012*** **$R^{2*}$=0.132** | F(1,44)=0.54 P=0.468 | F(1,44)=7.19 **P=0.010*** **$R^{2*}$=0.140** | F(1,44)=0.14 P=0.707 | F(1,44)=0.10 P=0.750 | F(1,44)=3.48 P=0.068 |
| **Sigma power prior to REM (N=50)** | F(1,44)=1.90 P=0.175 | F(1,44)=0.26 P=0.611 | F(1,44)=2.35 P=0.132 | F(1,44)=8.01 **P=0.007** **$R^{2*}$=0.154** | F(1,44)=3.11 P=0.084 | |
| **REMS percentage (N=52)** | F(1,46)=1.38 P=0.245 | F(1,46)=15.60 **P=0.0003** **$R^{2*}$=0.253** | F(1,46)=2.07 P=0.157 | F(1,46)=0.23 P=0.635 | F(1,46)=5.56 **P=0.022** **$R^{2*}$=0.107** | |
| **REMS latency (N=52)** | F(1,45)= 2.88 P= 0.096 | F(1,45)= 0.75 P= 0.389 | F(1,45)=1.40 P=0.242 | F(1,45)= 2.07 P= 0.157 | F(1,45)= 0.88 P= 0.351 | F(1,45)= 0.96 P= 0.331 |

*Prior to the analysis, we removed the outliers among all variables by excluding the samples lying beyond four times the standard deviation (the final number of individuals included in each analysis is reported below each dependent variable).*

** Significant following Hochberg False Discovery Rate (FDR) correction for 4 independent tests (see methods)*

*In all models, the results without the interaction between LC activity and age group were not significant. Goodness of fit metric (BIC) indicated that the interaction term provides a better fitness.*

*LC: locus coeruleus; TIV: total intracranial volume; REM: rapid eye movement; REMS: rapid eye movement sleep.*

In the next steps, we turned towards sigma power prior to REM sleep as it has been causally related to LC activity during sleep in animal models.(Osorio-Forero et al., 2021) Statistical analyses yielded a significant positive main effect of high-tonic LC activity during the perceptual rivalry task (t=2.36; **p=0.022**; **Table 3.2; Figure 3.3D**) and no significant interaction between LC activity and age group. In contrast, no significant association with low-tonic LC activity during the salience detection task (p=0.17) and no significant interaction between LC activity and age group were detected (**Table 3.3;**

**Figure 3.3E**). In both analyses, we did not detect significant effects of the other covariates except for main effect of sex: a statistical trend in the perceptual rivalry task (t=-1.91; p= 0.062; Table 3.2) and a significant main effect in the salience detection task (t=-2.83; p= 0.007; Table 3.3), resulting from men presented less sigma power compared to women (Suppl. Figure S4). As it is not the main focus of our study, sex difference will not be discussed in detail. We note that the sex difference in sigma power prior to REM may be related to the reduced percentage of REM sleep sometimes found in other studies (Redline et al., 2004) (though REM sleep percentage did not differ between sexes in our sample: t = -0.63; p = 0.52).

Finally, when including the activity of both tasks in the same GLMM with sigma power prior to REM as the dependent variable, the analysis yielded a significant main effect of high-tonic LC activity ($F_{(1,42)}$=7.62,**p=.0085**) as well as a trending interaction between low-tonic LC activity and age ($F_{(1,42)}$=3.77, p=.058). As for REM theta energy, this result suggests that both types of LC activity estimates explain different parts of the variance in sigma power prior to REM.

For completeness, the final steps of our analyses consisted of exploratory analyses for associations between the activity of LC estimated during both tasks and other REM sleep metrics, i.e. REM sleep duration, REM bouts duration, and number of arousals during REM sleep. These led to no significant association (**Suppl. Figure S3, Suppl. Table S1 and S2**).

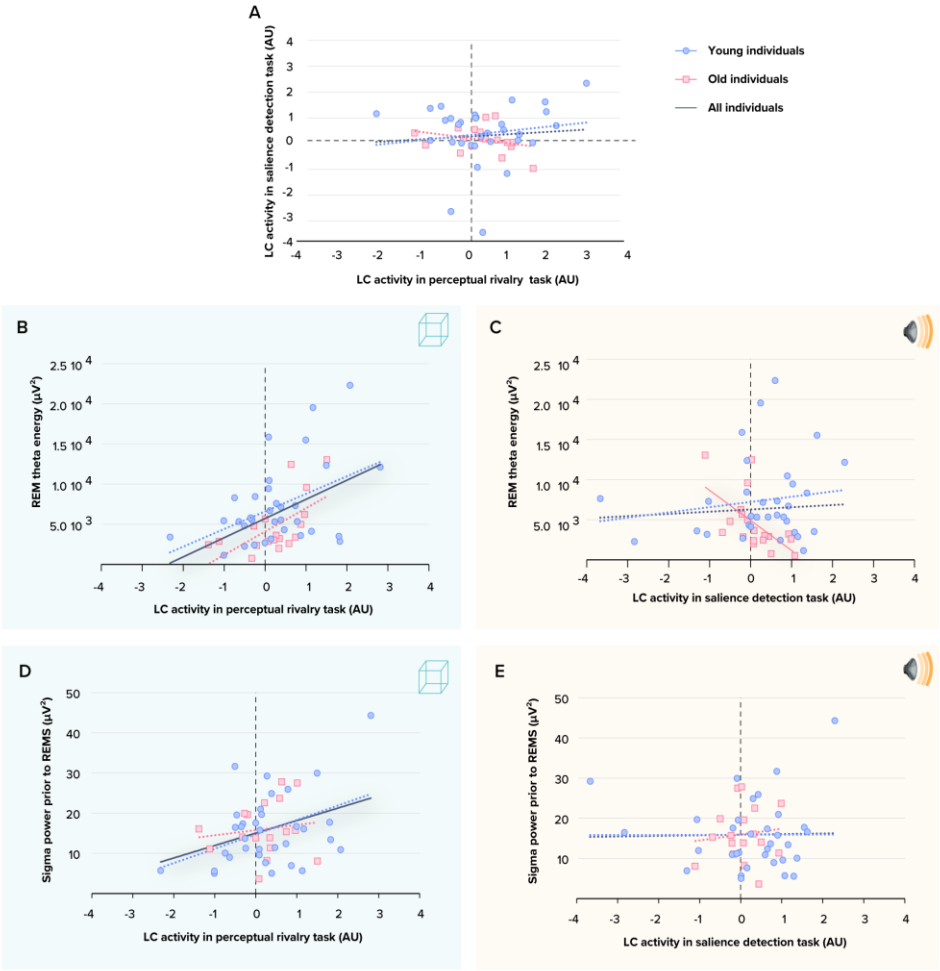


***Figure 3.3. (A)*** *LC activity estimates during the visual perceptual rivalry task and auditory salience detection task were not significantly correlated (p=0.54).* ***(B)*** *Association between REM theta energy and the LC activity estimates during the visual perceptual rivalry task. The GLMM yielded a significant main effect of LC activity (p=0.0015).* ***(C)*** *Association between REM theta energy and the LC activity estimates during the auditory salience detection task. The GLMM yielded a significant age group by LC activity interaction (p=0.0103), and post hoc analyses led to a significant association for the older (p=0.008) but not the young group (p=0.75).* ***(D)*** *Association between sigma power prior to REM sleep and the LC activity estimates during the*

*visual perceptual rivalry task. The GLMM yielded a significant main effect of LC activity (p=0.026).* ***(E)*** *Association between sigma power prior to REM sleep and the LC activity estimates during the auditory salience detection task. The GLMM showed neither a significant main effect of LC activity (p=0.859) nor a significant age group by LC activity interaction (p=0.132).*

*Simple regression lines are used for a visual display and do not substitute the GLMM outputs. The black line represents the regression irrespective of age groups (young + old, n = 52). Solid and dashed regression lines represent significant and non-significant outputs of the GLMM, respectively.*

## Discussion

Whether the variability in the functioning of human LC may contribute to the interindividual variability in sleep is not established. In the present cross-sectional study, we show that the activity of the LC, assessed during wakefulness through 2 distinct cognitive tasks, is related to the variability of REM sleep intensity, as reflected by the energy of the theta band of the EEG. We find that the associations depend on the task considered - presumably because they do not rely on the same balance between phasic and tonic LC activity, and on age - when considering LC activity during the low-tonic task. In addition, we find that the amount of sigma band oscillations immediately preceding REM sleep is associated with the activity of the LC assessed during the high-tonic task. These findings add to the existing view that LC function is essential for sleep regulation that was mainly brought by animal experiments. We show that the *in vivo* variability in the activity of the human LC may govern part of the variability in sleep quality assessed using electrophysiology. The findings may have implications for the neuropathology of several brain disorders such as insomnia, Alzheimer's disease (AD), and Parkinson's disease (PD) for which the LC may play a pivotal role through sleep.

LC cells discharge action potentials in both tonic and phasic modes during wakefulness. The tonic mode consists of irregular but constant baseline activity (1-6 spikes/s), while phasic activity is characterized by short (<300ms) bursts of high-frequency activity followed by a long period of sustained inhibition of spontaneous activity (Aston-Jones & Cohen, 2005). A balanced tonic activity is required for an optimal arousal level allowing the expression of phasic activity in response to salient stimuli or behavioral changes (Aston-Jones et al., 1999). While the LC was considered for long as a quiet region during sleep, recent animal studies revealed that the activity of the LC varies importantly during NREM sleep and may even transiently reach activity levels similar to wakefulness (Osorio-Forero et al., 2022). NE level, driven by LC neuron activity, fluctuates during NREM sleep between periods of higher and lower NE tone following an infraslow rhythm of ~50s (Osorio-Forero et al., 2022). This periodic decrease in NE tone must reach levels that are low enough to open the gate for REM sleep, which is considered to require sustained NE-free periods to allow, for instance, synaptic pruning (Van Someren, 2021). The periodic decrease of the LC activity is reflected in periodic increases in the expression of sleep spindles and in sigma power such that each REM episode is preceded by a pronounced transient increase in sigma power (Kjaerby et al., 2022) that is well known to somnologists. The overall picture emerging from this recent literature is that LC activity shapes the macro- and microstructure of sleep and that inappropriate fluctuations in LC tonic activity can disturb sleep and in particular REM sleep (Osorio-Forero et al., 2022; Van Someren, 2021).

We find that a higher phasic response of the LC during the top-down perceptual rivalry task, arguably characterized by a relatively high tonic activity of the LC is associated with a larger expression of theta oscillations

during REM sleep and of sigma power prior to REM sleep episodes. We interpret this finding as the reflection of a more appropriate lower tonic activity of the LC which allows a better expression of its phasic response during the task. In contrast, if tonic activity during the task is too high, it partly masks the phasic response of the LC. We detect this during wakefulness but surmise that it constitutes a trait such that the more appropriate tonic activity of the LC present during wakefulness would also be present during sleep. This would mean that, in the case of a low tonic LC activity allowing for a large dynamic range of phasic LC oscillation, NE tone reaches a lower level prior to a REM sleep episode, allowing a large expression of sigma oscillation and the initiation of a REM sleep episode and complete silencing during REM. This would in turn enhance the expression (intensity) of REM theta energy, the most typical oscillatory mode of REM sleep. Theta activity during REM sleep is considered to reflect hippocampus activity and plays key roles in memory consolidation during REM sleep (Boyce et al., 2016). The putative more appropriate tonic activity of the LC we report could therefore reflect a better expression of REM sleep functions.

In contrast, we find that a larger expression of the phasic response of the LC during the bottom-up salience detection task, which is arguably underlined by a lower tonic activity of the LC, is associated with lower expression of theta oscillations during REM sleep, particularly in older individuals. We link our results to the recent report in rodents that the so-called startle effect, which is triggered by abrupt sensory changes, is attenuated under higher vs. lower arousal levels as well as when LC activity is induced prior to the salient stimulation (Yang et al., 2021). This was in line with the established observation that elevated tonic firing of the LC-NE neurons makes the LC sensory-evoked response less pronounced (Aston-Jones & Cohen, 2005). This

would mean that in individuals with lower tonic activity of the LC during wakefulness, the dynamic range of the phasic response of the LC is potentially large. This trait-like phasic LC activity could become maladaptive and during sleep, perturb the activity of limbic areas, thereby resulting in a reduction in REM theta oscillations. This process becomes particularly manifest in aging which is characterized by increased sleep fragility and degradation of sleep quality (Carrier et al., 2011).

Overall, we interpret our findings as a reflection of the requirement for a balanced tonic LC activity for optimal REM sleep. This notion is supported, in our view, by the fact that our statistical outputs remain similar (either significant or as weak trends) when including concomitantly the activity of the LC as assessed in a low-tonic and high-tonic context. Our findings may further be related to the diversity in neuron types within the LC that is emerging in the literature, for instance, if both tasks were recruiting different proportions of LC neurons co-expressing or not dopamine or projecting to different brain territories (Poe et al., 2020) and were therefore characterizing the activity of these different neurons during sleep.

We stress that, although plausible, our interpretations remain putative and that measures of LC activity during sleep are required to confirm our hypotheses. Future studies could assess the connectivity of the LC with other brain structures in humans, as sleep and wakefulness regulation relies on a network of subcortical nuclei rather than on the LC alone (Scammell et al., 2017). Furthermore, while we interpret our finding in “LC centric” manner, i.e. the activity of the LC during wakefulness reflects its activity during sleep, it may also be that the activity of the LC during wakefulness shapes the activity of other brain areas during sleep. Sleep oscillations depend indeed in

prior wakefulness in a regional use-dependent manner (Huber et al., 2004) such that the impact of NE tone and LC activity on other regions, and particularly over cortical regions, may be reflected in a larger or lower expression of theta oscillation during REM.

Beyond the healthy population, our findings are in line with the view that inappropriate LC activity during both wakefulness and sleep can contribute to insomnia disorder (Van Someren, 2021). Insomnia is characterized by a hyperarousal state that is presumably due in part to inappropriately high tonic activity of the LC during wakefulness, contributing to delayed sleep onset, and during sleep, contributing to early and/or multiple awakenings. This would also lead to disturbed REM sleep and unresolved emotional distress (Van Someren, 2021). Our participants being healthy and devoid of sleep disorders, our finding could reflect the continuum between normal sleep and insomnia. They could also be indicative of the neuropathological mechanisms eventually leading to insomnia, i.e. a prolonged inappropriate LC tonic activity and REM sleep disturbance are consequences or contribute to the progressive changes leading to insomnia or maintaining the disorder. LC is also central to several neurodegenerative diseases characterized by preclinical alteration in sleep. The LC is indeed among the first sites showing abnormal tau and alpha-synuclein aggregates, hallmarks of AD and PD, respectively (Braak et al., 2011; Hawkes et al., 2010). Abnormal tau inclusions can even be detected post-mortem as early as during the second decade of life (Braak et al., 2011) and were suggested to be associated with larger cortical excitability in late middle-aged healthy individuals (Van Egroo et al., 2021). In addition, *post-mortem* degeneration of the human LC was recently associated with alteration in the rest-activity cycle over the ~7y preceding death, particularly in those with higher cortical AD pathology (Van Egroo et

al., 2024). Future research could try to link our findings to assessments of tau and alpha-synuclein burden within the LC, though the level of protein aggregate would go undetected *in vivo* in young individuals, and would remain challenging to assess over such a deep and small nuclei in older individuals.

As previously mentioned (Koshmanova et al., 2023), although our study provides new insights into the associations between LC activity and sleep variability in humans, it bears limitations. The baseline night of sleep was followed by the fMRI acquisition the next day in young individuals, while in the older group, there was a ~1y gap between the baseline night and the fMRI session. Although sleep changes over the lifespan (Zeitzer, 2013), it is stable over a short life period (e.g. a few years) (Tucker et al., 2007). We therefore consider that this important limitation is unlikely to fully explain our results, particularly given the fact that no age-related differences were found for the high-tonic perceptual rivalry task. Moreover, our sample was primarily composed of women. Although it was considered in our statistical analyses, this limits the generalizability of our results. In addition, although it represents a considerable data collection effort, the size of our sample remains modest, particularly for the older subsample. Replication in a larger sample is therefore warranted. Future studies could also apply individually tailored HRF to assess LC response. We used the canonical HRF to model activity over the entire brain to model average LC response, while individual LC responses can differ across individuals (Prokopiou et al., 2022). Likewise, we used multiple linear regression approaches for the statistical, mostly precluding isolation of non-linear relationships. Finally, the LC activity was measured using task-evoked fMRI BOLD signals, which reflect changes in neural activity relative to a baseline condition. Although, on average

responses were positive (cf. Figure 3.2 A-B), the negative values in LC activity detected in some participants (cf. Figure 3.2 G & I) represent instances where the BOLD signal in the LC during a task condition is lower than the baseline signal. The meaning of negative BOLD value is unclear. They may reflect differences in individual task engagement, arousal levels, or baseline LC activity. Further investigation is needed to better understand the underlying physiological or methodological factors contributing to this phenomenon.

**Conclusions**

In summary, we provide original evidence that, aside from its integrity, the functioning of the LC shapes part of the quality of sleep. Our data suggest that an optimal level of LC tonic activity is required for an optimal expression of REM sleep and its functions. This reinforces the view that the LC-NE is promising for interventions aiming at improving sleep quality, including preventing and/or delaying brain disorders. Our findings suggest that these interventions could aim at restoring optimal LC functioning during sleep as well as during wakefulness.

# Chapter 4: The Crosstalk Between the Anterior Hypothalamus and the Locus Coeruleus During Wakefulness Is Associated with Low-Frequency Oscillations Power During Sleep

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**Abstract**

Animal studies show that sleep regulation depends on subcortical networks, but whether the connectivity between subcortical areas contributes to human sleep variability remains unclear. We investigated whether the effective connectivity between the LC and hypothalamic subparts during wakefulness relates to sleep electrophysiology. Thirty-three younger (~22y, 27 women) and 18 late middle-aged (~61y, 14 women) healthy individuals underwent 7-Tesla functional MRI during wakefulness to assess LC-hypothalamus effective connectivity. Additionally, sleep EEG was recorded at night in the lab to examine the relationships between effective connectivity measures and REM sleep theta energy as well as sigma power prior to REM. Connectivity analyses revealed strong mutual positive influences between the LC and both the anterior-superior and posterior hypothalamus, consistent with animal studies. Aging was negatively associated with the connectivity from the anterior-superior hypothalamus (including the preoptic area) to the LC. In late middle-aged, but not younger adults, stronger effective connectivity from the anterior-superior hypothalamus to the LC was associated with lower REM theta energy. This association extended to other low-frequency bands during REMS and NREMS. These findings highlight the age-dependent modulation of LC-hypothalamus interactions and their potential roles in sleep regulation, providing new insights into neural mechanisms underlying age-related sleep changes.

**Introduction**

Sleep and wakefulness regulation and the fine-tuning of vigilance state are mostly regulated by a circuit of subcortical nuclei located in the basal forebrain, thalamus, hypothalamus and brainstem as part of the ascending activating system (Adamantidis & de Lecea, 2023; Scammell et al., 2017; Weber & Dan, 2016). The locus coeruleus (LC), located in the brainstem, is the main source of norepinephrine (NE) in the brain and sends widespread monosynaptic projections to nearly all brain regions (Lindvall et al., 1978; Poe et al., 2020). Research in animal models showed that its activity must decrease for transitioning from wakefulness to sleep. During sleep, the LC further shapes the switch between slow wave sleep (SWS) and rapid eye movement sleep (REMS) as well as some of the microstructure elements of sleep (Osorio-Forero et al., 2022). Investigations in humans indicated that the degeneration of the LC is likely driving part of the alteration in sleep-wake regulation commonly found in healthy and pathological aging (Van Egroo et al., 2022). Although indirect measures of the activity of the LC did not differ between healthy participants and insomnia patients (Frase et al., 2023), LC hyperactivity could contribute to a state of hyper arousal during wakefulness while it would either reduce REMS occurrence or REM bout stability during sleep, two phenomena associated with insomnia disorder (Van Someren, 2021). In line with the latter hypothesis, we recently reported that a balanced activity of the LC during wakefulness is associated with a more intense REMS, as indexed by the overnight energy over the most typical oscillatory mode of REMS (theta oscillations) (Koshmanova et al., 2023; Mortazavi et al., 2025). The activity of the LC during wakefulness was further associated with the sigma power immediately preceding REM sleep episode (Mortazavi et al.,

2025), a sleep feature that has been causally linked with the activity of the LC in animal models (Osorio-Forero et al., 2021).

Research in animal models also established that several nuclei of the hypothalamus are key elements of the circuit regulating sleep and wakefulness. The posterior part of the hypothalamus includes the lateral hypothalamus (LH) and the tuberomammillary nucleus (TMN) which produce orexin and histamine, respectively, two neuromodulators stimulating wakefulness, while the LH further produces melanin-concentrating hormone (MCH) which is considered to stabilize sleep and REMS in particular (Adamantidis & de Lecea, 2023). The LH is further known to exert an excitatory influence over the LC through glutamatergic afferents (Barcomb et al., 2022). The superior part of the anterior hypothalamus includes preoptic nuclei inhibiting the nuclei of the ascending activating system, including the LC, notably through the production of gamma-aminobutyric acid (GABA) and favor sleep initiation (Scammell et al., 2017). The inferior part of the anterior hypothalamus further includes the main circadian clock – in the suprachiasmatic nucleus - which organizes sleep and wakefulness in time of the 24h light-dark cycle mostly through vasoactive intestinal peptide (VIP) and GABA (Hastings et al., 2018), and notably through indirect projection to the LC (Aston-Jones et al., 2001). Similar to the LC, the nuclei of the hypothalamus undergo age-related changes, which can disrupt their regulatory role in sleep, potentially contributing to alterations of sleep patterns in healthy and pathological aging (Hajdarovic et al., 2022).

As for the LC, most of our understanding of the roles of the hypothalamus nuclei in sleep and wakefulness regulation arises from animal studies such that translations to human beings are needed if one wants to

develop efficient interventions geared toward the neuromodulator systems underlying vigilance state in healthy individuals and patients. Whether the crosstalk between the LC and the nuclei of the hypothalamus underlies variability in sleep electrophysiology and its age-related changes has not yet been investigated.

Similar to our initial reports of an association between LC activity and REM sleep, we hypothesized that the cross-talk between the LC and the hypothalamus during wakefulness would reflect a trait that would also be present during sleep (Koshmanova et al., 2023; Mortazavi et al., 2025). By using high-field 7 Tesla functional magnetic resonance imaging (7T fMRI) and stochastic Dynamic Causal Modeling (DCM) during wakefulness, we aimed to capture biologically plausible connectivity patterns that reflect enduring functional relationships between sleep-regulatory regions. These interactions may not be tied to task-specific modulation but could represent stable neuromodulatory dynamics relevant to sleep physiology. We used the same 7T fMRI dataset as our initial studies (Koshmanova et al., 2023; Mortazavi et al., 2025) to test whether the effective connectivity between the LC and subparts of the hypothalamus would be related to REM theta energy and sigma power prior to REM episodes (i.e. the sleep parameters related to wakefulness LC activity in our previous reports (Koshmanova et al., 2023; Mortazavi et al., 2025)). Based on functional and anatomical studies in animals, we anticipated that the connectivity between the LC and the anterior-superior, anterior-inferior or the posterior-lateral subpart of the hypothalamus would be associated with the quality of REMS. We further anticipated that the associations would be affected by aging.

**Results**

Fifty-one healthy individuals participated in the study, including 33 young adults (18–30y) and 18 late middle-aged adults (50–70y) **(Table 4.1)**. Participants first underwent an in-lab polysomnography night to rule out sleep disorders, followed by a baseline night of EEG sleep recording, out of which we computed our sleep metrics of interest, i.e. REM theta energy and sigma power prior REM sleep. Approximately three hours after waking from the baseline night (or following a comparable regular sleep period for late middle-aged participants), they completed a 7T fMRI session **(Figure 4.1A)** including a visual perceptual rivalry task **(Figure 4.1B)** and an auditory salience detection task **(Figure 4.1C)**. These tasks were chosen because they were expected to engage the LC (Einhauser et al., 2008; P. R. Murphy et al., 2014), and, as shown in our earlier work, both indeed elicited robust LC responses (Koshmanova et al., 2023; Mortazavi et al., 2025) **(Figure 4.1D and E)**. In the present work, we extended our focus to the hypothalamus using DCM which can estimate directional influences between regions (effective connectivity).

We applied an automated segmentation algorithm to divide the hypothalamus into five subparts—anterior-inferior, anterior-superior, posterior, inferior tubular, and superior tubular—each comprising several nuclei (Billot et al., 2020). We selected the hypothalamus subpart to include in our connectivity analyses in each task (see method). Specifically, we ran two GLMMs for each task, using REM theta energy and sigma power prior to REM episodes as dependent variables and hypothalamus subpart activity as the predictor. Only subparts showing at least a statistical trend ($p < 0.1$) with one of these sleep metrics were retained for the subsequent DCM analyses,

ensuring that connectivity analyses focused on hypothalamic regions most likely to be functionally relevant to our sleep measures of interest.

**Table 4.1. Characteristics of the study sample**

| | Young individuals (n = 33) | | Late middle-aged individuals (n = 18) | | P-value |
|---|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** | |
| **Age (years)** | 22.20 | 3.20 | 60.88 | 5.41 | **<.0001** |
| **BMI (kg/m2)** | 21.90 | 2.74 | 24.75 | 3.40 | **0.0047** |
| **Education (years)** | 14.46 | 2.31 | 14.61 | 2.70 | 0.85 |
| **Depression level (BDI)** | 6.64 | 4.17 | 5.33 | 4.01 | 0.27 |
| **Anxiety level (BAI)** | 4.00 | 2.92 | 3.05 | 3.18 | 0.30 |
| **TIV ($cm^3$)** | 1402.1 | 136.6 | 1420.5 | 125.5 | 0.627 |
| **Daytime sleepiness (ESS)** | 6.93 | 3.90 | 5.77 | 3.76 | 0.30 |
| **Sex (F–M)** | 28 F – 6 M | | 12 F – 6 M | | 0.20 |
| **Habitual Subjective sleep quality (PSQI)** | 4.79 | 2.18 | 3.83 | 2.22 | 0.14 |
| **Chronotype (Horne-Ostberg's Morningness-Eveningness)** | 43.41 | 7.22 | 50.50 | 7.78 | **0.001** |
| **TST (min)** | 454.80 | 30.97 | 394.63 | 48.16 | **<.0001** |
| **REMS duration** | 120.3 | 21.49 | 82.46 | 29.06 | **<.0001** |
| **Theta energy in REMS ($\mu V^2$)** | 7281.5 | 5061.7 | 4789.5 | 3508.2 | **0.0449** |
| **Sigma power prior to REMS ($\mu V^2$)** | 15.7615 | 8.8957 | 16.0544 | 6.795 | 0.896 |

*The p-values shown in the table correspond to two-sample t-tests except for sex that were compared using a Chi-square test. BMI: Body Mass Index; BDI: Beck Depression Inventory; BAI:*

*Beck Anxiety Inventory; TIV: Total Intracranial Volume; PSQI: Pittsburgh Sleep Quality Index; ESS: Epworth Sleepiness Scale; TST: Total Sleep Time; REMS: rapid eye movement sleep. See methods for references of the questionnaires.*

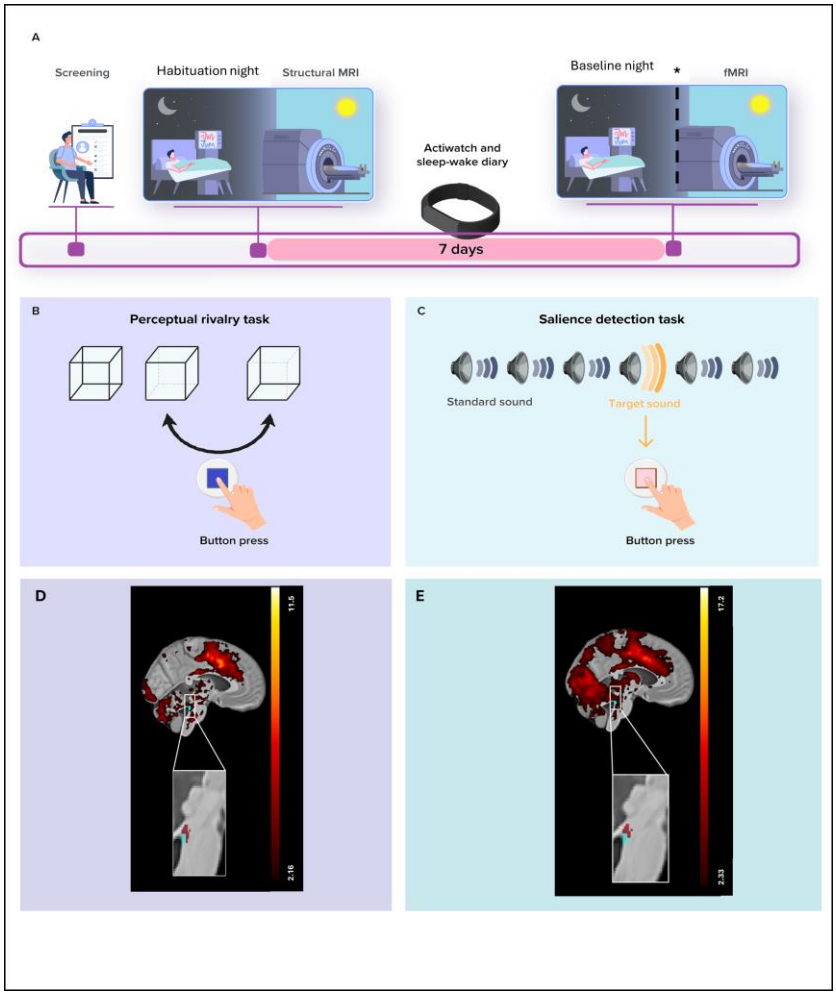


***Figure 4.1. Overview of the study protocol.***

*(A) After screening, participants completed an in-lab screening (i.e., habituation night) under polysomnography to minimize the effect of the novel environment for the subsequent baseline night and to exclude volunteers with sleep disorders. They further completed a structural 7T MRI session including a whole-brain structural MRI and a LC-specific sequence. After 7 nights of regular sleep-wake time at home, which was confirmed by actigraphy data and/or sleep-wake diary, participants came to the lab three hours before their sleep time and were maintained in dim light (<10 lux) until sleep time. Participants' habitual baseline sleep was recorded overnight in-lab under EEG to extract our main sleep features of interest. All participants underwent an fMRI session approximately 3h after wake-up time (following ≥45min in dim light - < 10lux), during which they completed the visual perceptual rivalry task.*

***** In late middle-aged participants, sMRI and fMRI sessions were completed about 1.25y later than the habituation and baseline nights (mean ±SD: 15.5 ±5.3 months). Prior to the fMRI session, late middle-aged participants slept regularly for 1 week (verified with a sleep diary). Late middle-aged participants were maintained in dim light (<10 lux) for 45min before the fMRI scanning. The sleep recording procedure was the same for both younger and late middle-aged participants.*

*(B) The Visual perceptual rivalry task consisted of watching a 3D Necker cube, which can be perceived in two different orientations (blue arrow), for 10 blocks of 1min separated by 10s of screen-center cross fixation (total duration ~12min). Participants reported switches in perception through a button press.*

*(C) The auditory salience detection task consisted of an oddball paradigm requiring button-press reports on the perception of rare deviant target tones (20% occurrence) within a stream of frequent tones (total duration ~10min).*

*(D) Whole-brain and LC responses to the perceptual switches during the visual perceptual rivalry task. [MNI coordinates: (−4 −37 -21 mm)]. The image at the top shows the whole-brain results using significance for a threshold of p< 0.05 FDR-corrected (t > 2.16) over the group average brain structural image coregistered to the MNI space. The inset at the bottom shows the LC probabilistic template (blue) created based on individual LC masks and the significant activation detected within this mask (red). The legend shows the t-values associated with color maps.*

*(E) Whole-brain and LC responses to the target sound during the auditory salience detection task [MNI coordinates: (−4 −34 -21 mm)]. The image at the top show the*

*whole-brain results using significance for a threshold of p< 0.05 FDR-corrected (t > 2.33) over the group average brain structural image coregistered to the MNI space. The inset at the bottom shows the LC probabilistic template (blue) and the significant activation detected within this mask (red). The legend shows the t-values associated with color maps.*

*This figure is adapted from* (Mortazavi et al., 2025).

Considering the perceptual rivalry task, we found a statistical trend for an association between REM theta energy and the interaction between hypothalamus activity and hypothalamus subparts (f=2.05; p=.08; **Table 4.2**). Post hoc contrasts revealed that REM theta energy shows a negative statistical trend with the response of the anterior-superior hypothalamus (t= -1.81; p=.07) and a positive nominally significant association with the response of the posterior hypothalamus (t=1.90; p=.05; **Figure 4.2A and B**). The other subparts did not yield statistical association with REM theta energy (t< -0.02 ;p> 0.18) (**Suppl. Figure S1, Suppl. Table S1**). The GLMM seeking potential associations between sigma power prior to REM and the activity of the different hypothalamus subparts during the perceptual rivalry task did not reveal any statistical meaningful associations **(Suppl. Figure S2, Table 4.2**, p = .86**)**. Considering the auditory salience detection task, the GLMMs including REMS theta energy or sigma power prior to REMS as dependent variable did not reveal any statistically significant association with hypothalamus subpart activity **(Suppl. Figure S3 and S4, Table 4.2)**.

**Table 4.2. Associations between the two sleep metrics of interest and the 5 hypothalamus subparts activity during the perceptual rivalry task and the salience detection task.**

| Task | Sleep metric (dependent variable) | hypothalamus activity | hypothalamus subpart | hypothalamus activity * hypothalamus subpart | Age group | Sex | TIV | Total sleep time |
|---|---|---|---|---|---|---|---|---|
| perceptual rivalry task | REM Theta energy (N=51) | F(1,239)= 0.62 P=0.432 | F(4,239)= 0.05 P=0.994 | F(4,239)=2.05 ***P=0.088*** ***$R^{2*}$=0.033*** | F(1,239)= 0.44 P=0.507 | F(1,239)= 0.94 P=0.334 | F(1,239)= 0.53 P=0.466 | F(1,239)= 28.5 **P<0.0001** **$R^{2*}$=0.106** |
| perceptual rivalry task | Sigma power prior to REMS (N=51) | F(1,239)= 0.58 P=0.446 | F(4,239)= 0.00 P=1.00 | F(4,239)=0.32 P=0.864 | F(1,239)= 2.74 P=0.098 | F(1,239)= 20.19 **P<0.0001** **$R^{2*}$=0.077** | F(1,239)= 15.03 **P=0.0001** **$R^{2*}$=0.059** | F(1,239)= 1.52 P=0.218 |
| salience detection task | REM Theta energy (N=51) | F(1,239)= 0.00 P=0.950 | F(4,239)= 0.01 P=0.999 | F(4,239)=0.22 P=0.927 | F(1,239)= 1.28 P=0.258 | F(1,239)= 1.70 P=0.193 | F(1,239)= 3.79 P=0.052 | F(1,239)= 33.00 **P<0.0001** **$R^{2*}$=0.121** |
| salience detection task | Sigma power prior to REMS (N=51) | F(1,234)= 5.00 **P=0.026** **$R^{2*}$=0.020** | F(4,234)= 0.01 P=0.999 | F(4,234)=0.65 P=0.628 | F(1,234)= 3.74 P=0.054 | F(1,234)= 24.94 **P<0.0001** **$R^{2*}$=0.096** | F(1,234)= 14.62 **P=0.0002** **$R^{2*}$=0.058** | F(1,234)= 2.28 P=0.132 |

*Prior to the analysis, we removed the outliers among connectivity and sleep metrics by excluding the samples lying beyond four times the standard deviation (the final number of individuals included in each analysis is reported below each dependent variable).*

*REM: rapid eye movement; REMS: rapid eye movement sleep.*

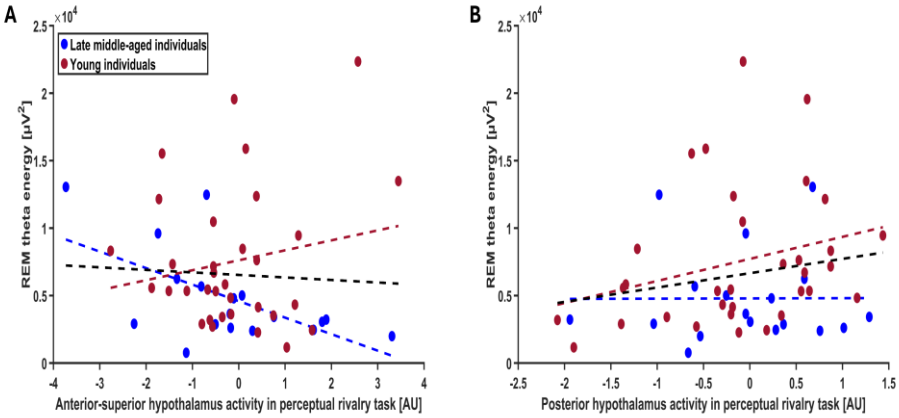


***Figure 4.2.*** *Association between REM theta energy and the anterior-superior and posterior hypothalamus activity during the perceptual rivalry task. The GLMM yielded a statistical trend for the hypothalamus activity by hypothalamus subpart interaction (p=0.08), and post hoc analyses led to a statistical trend for a negative association with the anterior-superior hypothalamus activity (p=0.07)* ***(A)*** *and a nominal significance for a positive association with the posterior hypothalamus activity (p=0.05)* ***(B)****.*

*Simple regression lines are used for a visual display and do not substitute the GLMM outputs (Table 4.2). The black line represents the regression irrespective of age groups (young + late middle-aged adults).*

*Non-meaningful associations (i.e. no statistical significance or trend) between REM theta energy or sigma power prior to REMS and the activity of all the subparts of hypothalamus during the perceptual rivalry task and salience detection task are displayed on Suppl. Figure S1- S4.*

Therefore, the DCM analysis concentrated on the connectivity between the LC and the anterior-superior or the posterior subparts of the hypothalamus during the perceptual rivalry task and sought relationship with REM theta energy, as it was the sole sleep metric that yielded a statistical trend with the activity of these hypothalamus subparts (**Figure 4.3A** illustrates the nuclei included in each subpart). As the task did not elicit a strong activation in the anterior-superior or the posterior subparts of the hypothalamus, we used stochastic DCM (Daunizeau et al., 2012), which can estimate directional influences between regions even without robust task-evoked responses. This method enables the detection of latent network dynamics and meaningful connectivity patterns that do not depend on pronounced BOLD activation in every region.

The DCM analyses first showed that there was strong evidence for reciprocal positive influence between LC and both subparts of hypothalamus (Pp=1.0), confirming that the connectivity anticipated based on animal model (Scammell et al., 2017), can be detected in humans using a stochastic DCM approach (**Figure 4.3B and C**). We computed separate GLMMs to determine whether each of the four connectivity parameters between the LC and hypothalamus nuclei (as dependent variable) varied between age groups, controlling for sex and TIV. The models yielded a significant difference between age groups only for the connectivity from the anterior-superior hypothalamus to the LC (t=-2.27; **p=0.027**), with late middle-aged individuals showing a reduced excitatory connectivity parameters compared with the younger individuals (**Figure 4.3D-G**, **Supplementary Table S2** for full statistical output of the GLMMs including each connectivity parameters).

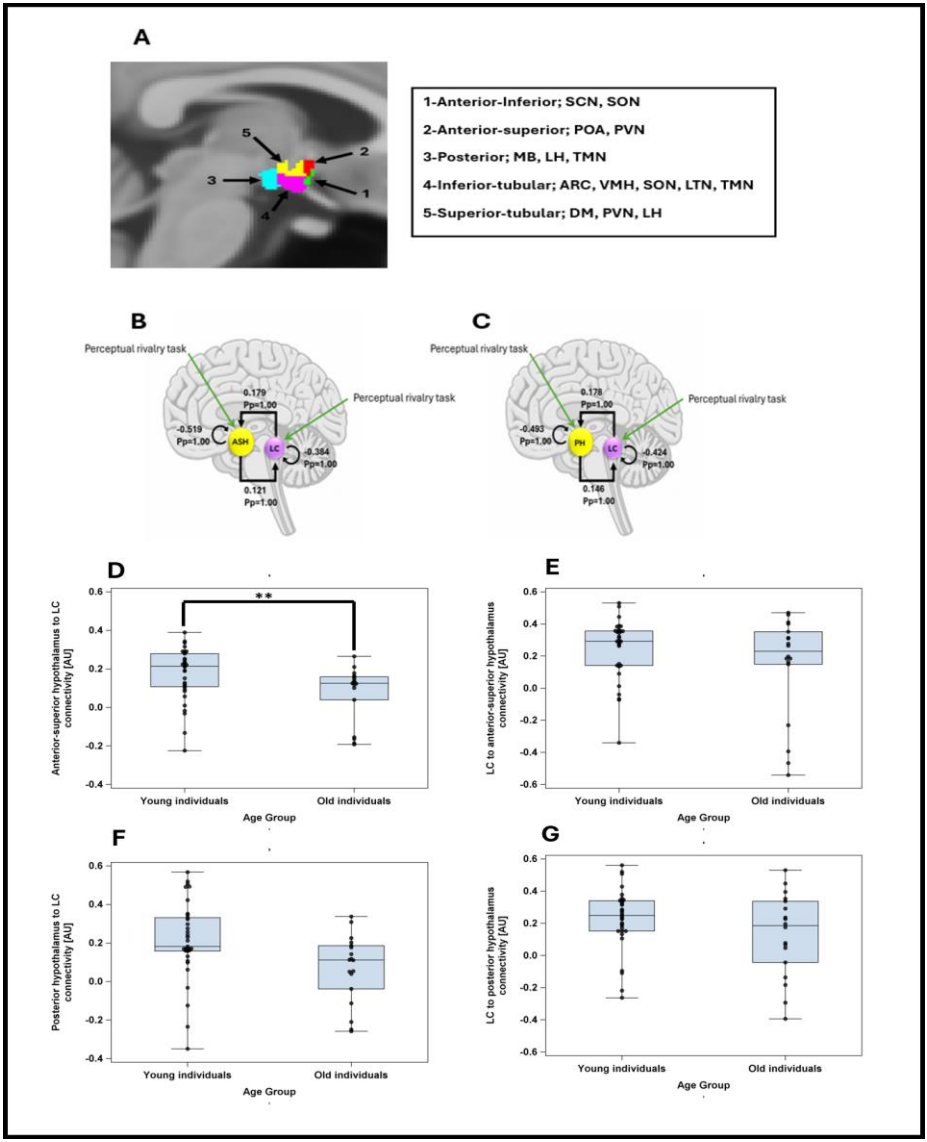
A
1-Anterior-Inferior; SCN, SON
2-Anterior-superior; POA, PVN
3-Posterior; MB, LH, TMN
4-Inferior-tubular; ARC, VMH, SON, LTN, TMN
5-Superior-tubular; DM, PVN, LH
B
Perceptual rivalry task
Perceptual rivalry task
0.179
Pp=1.00
-0.519
Pp=1.00
ASH
LC
-0.384
Pp=1.00
0.121
Pp=1.00
C
Perceptual rivalry task
Perceptual rivalry task
0.178
Pp=1.00
-0.493
Pp=1.00
PH
LC
-0.424
Pp=1.00
0.146
Pp=1.00
D
Anterior-superior hypothalamus to LC connectivity [AU]
**
Young individuals
Old individuals
Age Group
E
LC to anterior-superior hypothalamus connectivity [AU]
Young individuals
Old individuals
Age Group
F
Posterior hypothalamus to LC connectivity [AU]
Young individuals
Old individuals
Age Group
G
LC to posterior hypothalamus connectivity [AU]
Young individuals
Old individuals
Age Group

***Figure 4.3. (A)*** *Segmentation of the hypothalamus in five subparts in a representative participant. The nuclei encompassed by the different subparts are indicated in the right inset – according to* (Billot et al., 2020). *ARC: arcuate nucleus; DMH; dorsomedial nucleus; LH lateral hypothalamus; LTN: lateral tubular nucleus; MB: mamillary body; POA: preoptic area; PVN: paraventricular nucleus; PNH: posterior nucleus of the hypothalamus; SCN: suprachiasmatic nucleus; SON: supraoptic nucleus; TMN: tuberomammillary nucleus; VMN: ventromedial nucleus.* ***(B)*** *The DCM model, which included intrinsic connections between anterior superior hypothalamus and locus coeruleus, along with self-feedback gain control connections for both regions. Task inputs were further considered to reach both regions. The DCM analysis showed that there was very strong evidence (Pp=1.0) for reciprocal positive influence between LC and anterior superior hypothalamus as well as self-inhibition in both LC and anterior superior hypothalamus.* ***(C)*** *The DCM model, which included intrinsic connections between posterior hypothalamus and locus coeruleus, along with self-feedback gain control connections for both regions. Task inputs were further considered to reach both regions. The DCM analysis showed that there was very strong evidence (Pp=1.0) for reciprocal positive influence between LC and posterior hypothalamus as well as self-inhibition in both LC and posterior hypothalamus.* ***(D)*** *Anterior-superior hypothalamus to LC connectivity in young and late middle-aged groups. Younger individuals had significantly higher connectivity than late middle-aged individuals (t=-2.27; p=0.027).* ***(E)*** *LC to anterior-superior hypothalamus connectivity in young and late middle-aged groups. Connectivity strengths were not significantly different between two age groups (t=-1.32; p=0.193).* ***(F)*** *Posterior hypothalamus to LC connectivity in young and late middle-aged groups. Connectivity strengths were not significantly different between two age groups (t=-1.49; p=0.142).* ***(G)*** *LC to posterior hypothalamus connectivity in young and late middle-aged groups. Connectivity strengths were not significantly different between two age groups (t=-0.72; p=0473).*

To address our primary aim to test for potential associations between each connectivity parameter and REMS theta energy, we computed four separate GLMMs with REMS theta energy as dependent variable and the interaction between connectivity parameters and age group as independent variable, controlling for sex, total intracranial volume (TIV) and total sleep time. The model including the connectivity from anterior-superior subpart of hypothalamus to LC yielded a significant connectivity-by-age-group interaction (t=2.30; **p=0.026**), on top of a main effect of TST, while the other covariates were not significant **(Table 4.3)**. Post hoc contrasts highlighted that in the late middle-aged individuals, higher excitatory connectivity (i.e. positive connectivity values) was associated with lower REM theta energy, while stronger inhibitory connectivity (i.e. negative connectivity values) was associated with higher REM theta energy (t=-2.09; **p=0.042**). No such association was detected in the younger group (t=1.06; p=0.294) **(Figure 4.4A)**. In addition, if a participant putatively outlier for TIV is removed (SD=4.078), the connectivity-by-age-group interaction became more robust (t=2.37; p=0.022; i.e. below the multiple comparison correction $p < 0.025$ threshold) with post hoc contrasts identically showing the association between higher connectivity and lower REM theta energy in the late middle-aged group (t = -2.12, p = 0.039), but not in the younger group (t = 1.15, p = 0.255). We then considered the connectivity from LC to anterior-superior subpart of hypothalamus, but GLMM did not yield significant association with REM theta energy **(Table 4.3; Figure 4.4B)**. Likewise, when we considered the connectivity between the posterior hypothalamus subpart and the LC (i.e. both to and from the LC), GLMMs did not lead to any significant association with REM theta energy **(Table 4.3; Figure 4.4C and D)**. In summary, the only

connectivity parameters showing age related difference is also the only one showing and age-dependent association with REMS theta energy.

**Table 4.3. Associations between REM Theta energy and the connectivity between the anterior-superior hypothalamus and the LC and between the posterior hypothalamus and the LC.**

| Type of connectivity | Sleep metric (dependent variable) | connectivity | Age group | connectivity*age group | Sex | TIV | Total sleep time |
|---|---|---|---|---|---|---|---|
| From anterior-superior hypothalamus to LC | REM Theta energy (N=50) | F(1,43)=1.09 P=0.302 | F(1,43)= 0.62 P=0.436 | F(1,43)=5.29 **P=0.026[A]** **$R^{2*}$=0.109** | F(1,43)= 0.02 P=0.902 | F(1,43)= 0.03 P=0.854 | F(1,43)=4.74 **P=0.035** **$R^{2*}$=0.099** |
| From LC to anterior-superior hypothalamus | REM Theta energy (N=50) | F(1,43)=0.38 P=0.539 | F(1,43)= 0.17 P=0.686 | F(1,43)=2.21 P=0.144 | F(1,43)= 0.05 P=0.825 | F(1,43)= 0.00 P=0.981 | F(1,43)=4.73 **P=0.035** **$R^{2*}$=0.099** |
| From posterior hypothalamus to LC | REM Theta energy (N=50) | F(1,43)=0.00 P=0.994 | F(1,43)= 0.09 P=0.765 | F(1,43)=0.98 P=0.326 | F(1,43)= 0.58 P=0.451 | F(1,43)= 0.12 P=0.726 | F(1,43)=4.77 **P=0.034** **$R^{2*}$=0.099** |
| From LC to posterior hypothalamus | REM Theta energy (N=50) | F(1,43)=0.14 P=0.710 | F(1,43)= 0.01 P=0.911 | F(1,43)=0.61 P=0.439 | F(1,43)= 0.49 P=0.488 | F(1,43)= 0.08 P=0.776 | F(1,43)=4.40 **P=0.041** **$R^{2*}$=0.092** |

*Prior to the analysis, we removed the outliers among connectivity and sleep metrics by excluding the samples lying beyond four times the standard deviation (the final number of individuals included in each analysis is reported below each dependent variable).*

*[A] p = 0.022 and F(1,42)=5.61 after excluding one putative outlier in TIV (≥4 SD).*

*LC: locus coeruleus; TIV: total intracranial volume; REM: rapid eye movement; REMS: rapid eye movement sleep.*

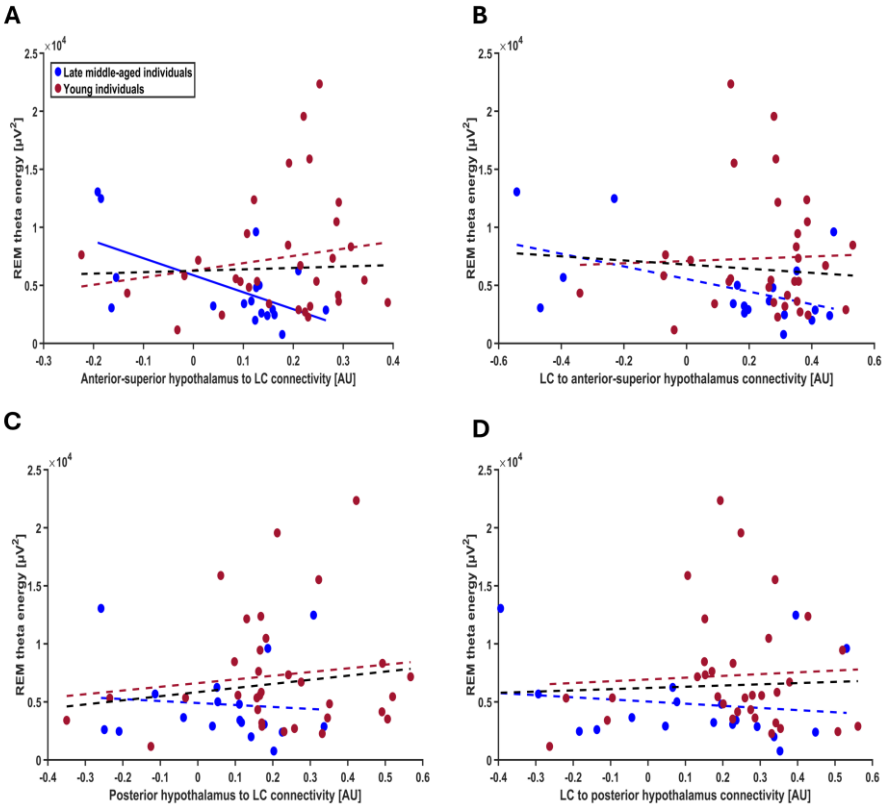


***Figure 4.4. Association between REM theta energy and the connectivity metrics between the LC and the hypothalamus nuclei. (A)*** *Connectivity from the anterior-superior hypothalamus to the LC. The GLMM yielded a significant age group by connectivity interaction (p=0.026), and post hoc analyses led to a significant association for the late middle-aged (p=0.042) but not the young group (p=0.294). After excluding one putative outlier in TIV (≥4 SD), the connectivity-by-age-group interaction became more robust (t=2.37; p=0.022).* ***(B)*** *Connectivity from the LC to the anterior-superior hypothalamus. The GLMM did not yield a significant age group by connectivity interaction.* ***(C)*** *Connectivity from posterior hypothalamus to the LC. The GLMM did not show a significant age group by connectivity interaction.* ***(D)*** *Connectivity from the LC to the*

*posterior hypothalamus. The GLMM did not show a significant age group by connectivity interaction.*

*Simple regression lines are used for a visual display and do not substitute the GLMM outputs (Table 4.3). The black line represents the regression irrespective of age groups. Solid and dashed regression lines represent significant and non-significant outputs of the GLMM, respectively.*

In the next steps, we verified the specificity of our finding for REM theta energy and turned toward the other frequency bands of the EEG during both REM and NREM and found that although association did not extend to all frequency bands, they were not restricted to REM theta energy. Separate GLMMs found that connectivity from the anterior-superior subpart of the hypothalamus to the LC by age group interaction was significantly associated with several lower frequency bands of both REM and NREM: interaction was significant for alpha energy in REMS (t=3.21; **p=0.0025**), delta energy in NREMS (t=2.51; **p=0.016**) theta energy in NREMS (t=2.91; **p=0.006**) and alpha energy in NREM (t=3.17; **p=0.0028**) but not for delta, sigma and beta energy in REM and sigma and beta energy in NREM; each time we observed a significant negative association between connectivity and frequency band energy in the late middle-aged group (REM alpha: t=-2.79; **p=0.008**; NREM delta: t=-2.04; **p=0.047**; NREM theta: t=-2.34; **p=0.024**; alpha NREM: t=-2.75; **p=0.009**) but not younger group (t≤1.76; p≥0.085 for the four frequency bands) **(Figure 4.5A, B, C and D, Table 4.4;** Non-significant associations between exploratory sleep metrics and the connectivity from anterior-superior hypothalamus to LC are presented in **Suppl. Table S3)**.

**Table 4.4. Significant associations between exploratory sleep metrics and the connectivity from anterior-superior hypothalamus to LC.**

| Sleep metric (dependent variable) | connectivity | Age group | connectivity*age group | Sex | TIV | Total sleep time |
|---|---|---|---|---|---|---|
| REM alpha energy (N=50) | F(1,43)=1.74 P=0.194 | F(1,43)=2.92 P=0.094 | F(1,43)=10.29 **P=0.002** **$R^{2*}$=0.193** | F(1,43)=0.04 P=0.851 | F(1,43)=0.10 P=0.757 | F(1,43)=5.43 **P=0.024** **$R^{2*}$=0.112** |
| NREM delta energy (N=51) | F(1,44)=0.59 P=0.445 | F(1,44)=2.02 P=0.162 | **F(1,44)=6.31** **P=0.015** **$R^{2*}$=0.125** | F(1,44)=0.13 P=0.722 | F(1,44)=1.30 P=0.260 | F(1,44)=0.22 P=0.642 |
| NREM theta energy (N=51) | F(1,44)=0.73 P=0.396 | F(1,44)=2.21 P=0.144 | F(1,44)=8.48 **P=0.005** **$R^{2*}$=0.161** | F(1,44)=0.14 P=0.714 | F(1,44)=0.57 P=0.452 | F(1,44)=1.33 P=0.254 |
| NREM alpha energy (N=51) | F(1,44)=0.83 P=0.368 | F(1,44)=4.36 **P=0.042** **$R^{2*}$=0.090** | F(1,44)=10.04 **P=0.002** **$R^{2*}$=0.185** | F(1,44)=0.09 P=0.765 | F(1,44)=0.00 P=0.973 | F(1,44)=0.37 P=0.546 |

*Prior to the analysis, we removed the outliers among connectivity and sleep metrics by excluding the samples lying beyond four times the standard deviation (the final number of individuals included in each analysis is reported below each dependent variable).*

*The table only includes results with $p < 0.05$; non-significant association are reported in Supplementary Table S3. P-values survive correction for multiple comparisons across the nine exploratory tests using the false discovery rate (FDR).*

*LC: locus coeruleus; TIV: total intracranial volume; REM: rapid eye movement; REMS: rapid eye movement sleep; NREM: non-rapid eye movement.*

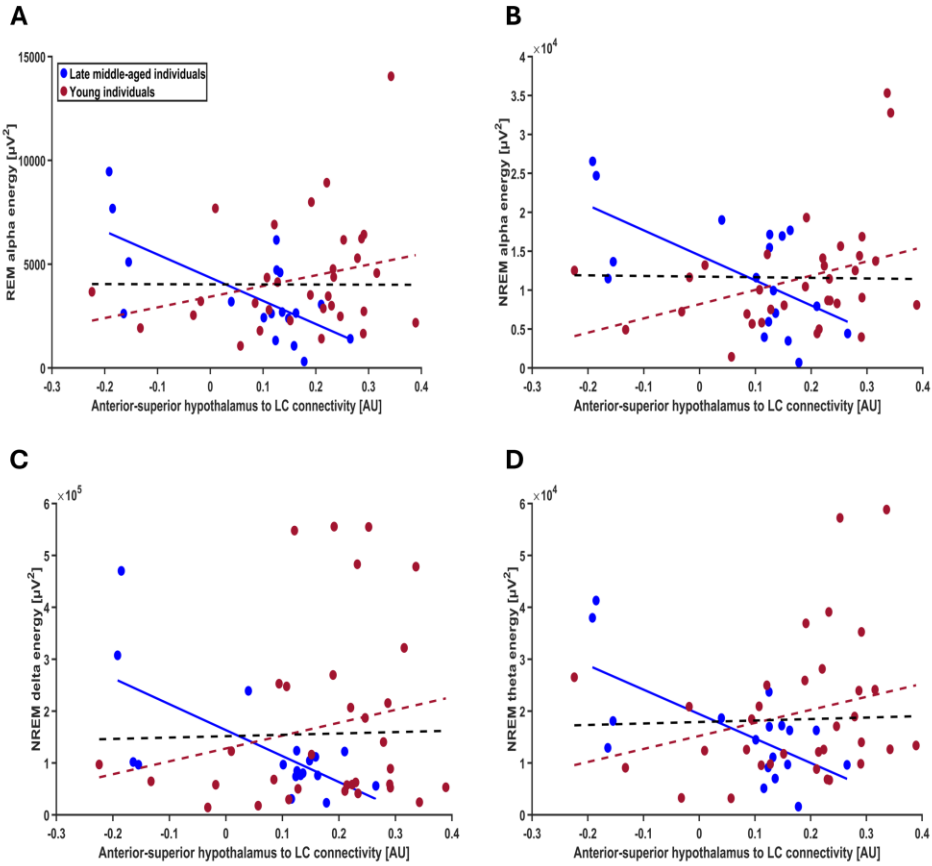


***Figure 4.5. Exploratory association between other frequency bands of the EEG during both REM and NREM sleep and the connectivity from the anterior-superior hypothalamus to the LC (A)*** *REM alpha energy; the GLMM yielded a significant age group by connectivity interaction (p=0.002), and post hoc analyses led to a significant association for the late middle-aged (p=0.007) but not the younger group (p=0.108).* ***(B)*** *NREM alpha energy; the GLMM yielded a significant age group by connectivity interaction (p=0.002), and post hoc analyses led to a significant association for the late middle-aged (p=0.0008) but not the younger group (p=0.099).*

***(C)*** *NREM delta energy; the GLMM yielded a significant age group by connectivity interaction (p=0.015), and post hoc analyses led to a significant association for the late middle-aged (p=0.047) but not the younger group (p=0.142).* ***(D)*** *NREM theta energy; the GLMM yielded a significant age group by connectivity interaction (p=0.005), and post hoc analyses led to a significant association for the late middle-aged (p=0.023) but not the younger group (p=0.085).*

*Simple regression lines are used for a visual display and do not substitute the GLMM outputs (Table 4.4). The black line represents the regression irrespective of age groups. Solid and dashed regression lines represent significant and non-significant outputs of the GLMM, respectively.*

*Non-significant associations between the energy of other frequency bands and the connectivity from anterior-superior hypothalamus to the LC are displayed on Suppl. Figure S5.*

## Discussion

The interactions between subcortical structures regulating sleep are not fully established in humans. Here, we used 7 Tesla fMRI to capture the crosstalk between the hypothalamus and the locus coeruleus and related it to the electrophysiology of REM sleep. We presumed that the connectivity between subparts of the hypothalamus and the LC during wakefulness would reflect in part their connectivity during sleep and would therefore be relevant to sleep physiology. We provide evidence that lower REM theta energy is associated with higher effective connectivity from the anterior-superior hypothalamus, which encompasses the preoptic area, to LC in the late middle-aged individuals of our sample. The association was not specific to REM theta energy and extended to other (but not all) lower frequency bands of both REM and NREM sleep. These findings constitute an original investigation of how a small network of subcortical areas may take part in sleep regulation in

humans and provide novel insights into the changes in sleep taking place over the healthy lifespan.

The tasks included in the protocol were geared toward ensuring a reliable recruitment of the LC (Koshmanova et al., 2023; Mortazavi et al., 2025) while they did not strongly recruit the hypothalamus. We therefore used an effective connectivity approach that was geared to such cases (i.e. stochastic DCM). We isolated candidate hypothalamus subparts that could be included in our connectivity models by seeking at least weak associations with our two sleep metrics of interest. We found weak indications that the activity of the posterior and anterior-superior subparts of the hypothalamus during the perceptual rivalry task were associated with REM theta energy, but not with sigma power prior to REM episodes. These indications were used to guide our connectivity analyses and will not be interpreted further although they warrant future investigations. We stress that the fact that we focused on specific subparts of the hypothalamus does not preclude the connectivity between the LC and other subparts of the hypothalamus that would be assessed in other contexts to be related to sleep electrophysiology, e.g. the anterior-inferior subpart encompassing the SCN (Aston-Jones et al., 2001) (i.e. if activity was assessed using different cognitive tasks, resting state fMRI, or a different vigilance state, etc.). Likewise, the connectivity of the LC with other parts of the brain in the perceptual rivalry as well as in the salience detection task may turn out to be related to sleep physiology in other analyses. The fact that we obtained high evidence (Pp = 1) that the posterior and anterior-superior subparts were part of a network with the LC demonstrates that one can grasp a meaningful part of the complex interplays between the LC and nuclei of the hypothalamus *in vivo* in humans following our approach. There was indeed no guarantee that the network we

constructed would be related to the fMRI signal we extracted for the hypothalamus and the LC. Our findings suggest that the mutual influence of the anterior-superior and posterior subparts of hypothalamus on the LC repeatedly demonstrated in animal (Giorgi et al., 2021; Szymusiak et al., 2007) can be isolated in humans. This important proof-of-concept paves the way for future investigations that could be built around other regions and more complex networks.

Following the extraction of the connectivity metrics from the 2 networks, respectively, composed of the posterior subpart of the hypothalamus and the LC and of the anterior-superior subparts of the hypothalamus and the LC, we find only one metric of the latter networks to be associated with REM theta energy. The anterior-superior subpart included the preoptic area, key to sleep regulation, but also the paraventricular nucleus (PVN) (Billot et al., 2020), which is typically related to food intake, energy balance (Qin et al., 2018), and vegetative regulation (Bon et al., 2025). Hence, we posit that the associations we observed were mostly driven by the preoptic area, which contains the ventrolateral (VLPO), lateral (LPO) and median (MPO) preoptic areas, which have all been involved in sleep regulation (Machado et al., 2022; Scammell et al., 2017; Yamagata et al., 2021). The neurons of the preoptic area promote sleep onset and sleep maintenance by inhibitory GABAergic modulation of multiple arousal systems such as LC (Giorgi et al., 2021; Szymusiak et al., 2007). The inhibitory action of the VLPO exerted on LC is considered as a requirement for sleep onset (Adamantidis & de Lecea, 2023).

Among the four connectivity parameters of the 2 DCM models, we find that only the connectivity from the anterior-superior subparts of the

hypothalamus to the LC decreases in the late middle-aged compared with the younger individuals. We further find that the stronger the excitatory connectivity from the anterior-superior hypothalamus to the LC during wakefulness (indicated by positive connectivity values), the lower REM theta energy in late middle-aged individuals, and the stronger the inhibitory connectivity from the anterior-superior hypothalamus to the LC during wakefulness (indicated by negative connectivity values), the higher REM theta energy in late middle-aged individuals. Theta oscillations consist of the most typical oscillatory mode of REM sleep. They are considered to be cortical correlates of the hippocampus ripple waves occurring during sleep and related to the memory function of REM sleep (Boyce et al., 2016). We interpret these as a reflection of REM sleep intensity. We previously reported that a larger expression of LC responses during the same task during wakefulness was associated with a better expression of REM sleep theta oscillations (Mortazavi et al., 2025). Our current finding may therefore indicate that a stronger connection between the preoptic nuclei and the LC prevents the LC from favoring REM sleep in late middle-aged individuals. The connectivity between the preoptic area and the LC, at least during wakefulness and potentially also during sleep, would decrease with aging and the extent of this decrease would contribute to REM sleep variability among late middle-aged individuals and also, potentially, to the decreased expression of REM associated with aging. These potential actions may be related to the overall alteration in the balance of the neural circuits previously reported in aging (Xia et al., 2024). They may also be linked to recent studies in rodents reporting that the preoptic nuclei regulates LC activity and prevent LC over reactivity that could become detrimental for the expression of REM sleep (Lu et al., 2002; Mondino et al., 2025). We find that the larger inhibitory

connectivity is associated with the larger REMS theta expression. How our findings captured during wakefulness in the diurnal human species, fit with the reports in animals will require further investigation, including using MRI recordings during sleep.

Interestingly, we found that the associations between anterior-superior hypothalamus to LC connectivity and neural oscillations extend beyond the theta energy in REMS. In late middle-aged individuals, its negative correlations with alpha energy in REMS and NREMS as well as delta and theta energy in NREMS suggest a potential broader role for this connectivity in influencing sleep electrophysiology. Delta energy in NREMS is recognized as a marker of sleep need as well as sleep maintenance, quality, and restorative processes (Dijk, 2009). Higher theta energy in NREMS is also linked to memory reactivation and memory consolidation (Schreiner & Rasch, 2017). In addition, alpha energy during REMS and NREMS is related to better cognitive performance as it is shown that individuals with cognitive impairment have lower alpha energy in REMS and NREMS compared to healthy individuals (Taillard et al., 2019). Consistent with our main finding involving REM theta energy, the observed negative association between the energy of these frequency bands and the strength of preoptic hypothalamus to LC connectivity in late middle-aged adults may reduce the beneficial effects of these oscillatory dynamics in sleep. It could also represent an adaptive mechanism in late middle-aged individuals to prevent LC overreaction. In any case, it shows that the connectivity from the anterior-superior subpart of the hypothalamus to the LC is associated with neuronal synchrony over the lower range of the EEG spectral bands during sleep, potentially affecting the mechanism related to this synchrony (e.g. memory consolidation, sleep

homeostasis). This could contribute to the previous findings on the aging brain's adaptive modifications in homeostatic sleep control (Scullin, 2017).

As noted earlier,(Koshmanova et al., 2023; Mortazavi et al., 2025) our study bears limitations. Young participants underwent fMRI scanning the day after their baseline night of sleep, while for the late middle-aged group, there was an approximately one-year interval between the baseline sleep night and the fMRI session. Although sleep changes during the lifetime (Zeitzer, 2013), it tends to remain stable over shorter periods (e.g. a few years) (Tucker et al., 2007). Therefore, we believe this significant limitation does not fully account for our findings. Additionally, while we posit that brain activity and connectivity in wakefulness partly reflects brain activity and connectivity relevant in sleep, this assumption has not been directly demonstrated. Moreover, despite the extensive data collection involved, the sample size, particularly for the late middle-aged cohort, is relatively small. Besides, our sample was predominantly female, a factor accounted for in our statistical analysis but still limiting the broad generalizability of the results. The absence of middle-aged individuals aged 30 to 50 years may have also obscured more gradual, age-related changes in LC-hypothalamus connectivity and sleep electrophysiology. Finally, although stochastic DCM allows modeling of effective connectivity without strong task-related activation, the observed LC-hypothalamus coupling could reflect baseline rather than task-specific modulation. Future studies should include recording of brain activity during wakefulness during other cognitive tasks and during sleep, they should include large and sex-balance sample of continuous age range.

## Materials and Methods

This study was approved by the faculty-hospital ethics committee of ULiège. All participants provided written informed consent and received financial compensation. The study is part of a larger project that has led to previous publications.(Berger et al., 2023; Koshmanova et al., 2023; Mortazavi et al., 2025) Most of the methods were described in details in(Koshmanova et al., 2023; Mortazavi et al., 2025).

*Participants*

Fifty-two healthy participants were included in the study. Due to technical issues, one subject was excluded and the final sample included 51 participants, with 33 healthy young (18-30y, 27 women) and 18 late middle-aged (50-70y, 14 women) individuals **(Table 4.1)**. The exclusion criteria were as follows: history of major neurologic/psychiatric diseases or stroke; recent history of depression/anxiety; sleep disorders; medication affecting the central nervous system; smoking, excessive alcohol (>14 units/week) or caffeine (>5 cups/day) consumption; night shift work in the past 6 months; BMI ≤18 and ≥29 (for late middle-aged individuals) and ≥25 (for younger individuals). All late middle-aged participants had to show normal performance on the Mattis Dementia Rating Scale (score > 130/144) (Mattis, 1976). Due to a miscalculation at screening, 1 late middle-aged participant had a BMI of 30.9 and one of the younger participants had a BMI of 28.4. Since their data do not deviate substantially from the rest of the sample these participants were included in the analyses (including BMI as a covariate in our statistical models did not modify our results).

*Protocol*

Participants' sleep was recorded in the lab twice. During the first session, participants completed a night of sleep under polysomnography to screen for sleep abnormalities (apnea hourly index and periodic leg movement >15; parasomnia or REM behavioral disorder). All participants further underwent a whole-brain structural MRI (sMRI) and a specific acquisition centered on the LC. Participants were then requested to sleep regularly for 7 days before the baseline night during the second session (±30min from their sleep schedule) based on their preferred schedule (compliance was verified using sleep diaries and wrist actigraphy - Actiwatch and AX3, AXIVITY LTD, Newcastle, UK). The evening before the baseline night, participants first completed questionnaires including Beck Depression Inventory (BDI)(Beck, Steer, et al., 1988), Beck Anxiety Inventory (BAI)(Beck, Epstein, et al., 1988), the Pittsburgh Sleep Quality Index (PSQI)(Buysse et al., 1989), Epworth sleepiness scale (ESS)(Johns, 1991) and Horne-Ostberg's Morningness-Eveningness scale(Horne & Ostberg, 1976) for assessing depression, anxiety, sleep quality, sleepiness, and chronotype respectively. They remained awake for 3h under dim light (<10 lux) for electrode placement and preparation to sleep prior to the recording of their habitual sleep in darkness under EEG. Approximately 3h after wake-up time under dim light (<10 lux), participants completed a functional MRI (fMRI) session that included 3 tasks (**Figure 4.1A**). This paper is centered on the analyses of the perceptual rivalry task and auditory salience detection task.

Younger participants completed the fMRI session immediately following the baseline night but late middle-aged participants were initially part of a different study (Narbutas et al., 2019; Van Egroo, Narbutas, Chylinski, Villar González, Ghaemmaghami, et al., 2019) and completed the sMRI and fMRI recordings in addition to their initial engagement. Late middle-aged

participants completed the habituation and baseline night EEG recordings as part of their initial study and the sMRI and fMRI sessions were completed about 1.25y later as part of the current study (mean ±SD: 15.5 ±5.3 months). Prior to the fMRI session, late middle-aged participants slept regularly for 1 week (verified with a sleep diary; based on our experience, actigraphy reports and sleep diaries do not deviate substantially in late middle-aged individuals). Late middle-aged participants were maintained in dim light (<10 lux) for 45min before the fMRI scanning. The sleep recording procedure was the same for both younger and late middle-aged participants. Both groups were allowed to have breakfast before the fMRI session but were instructed to avoid caffeine intake.

*Sleep EEG metrics*

Eleven channels were used for the baseline night (F3,z,4; C3,z,4; P3,z,4; O1,2) initially referenced to the left mastoid prior to re-referencing offline to the average of both mastoids (N7000 amplifier, EMBLA, Natus, Middleton, WI). Arousals and artefacts were detected automatically(Wallant et al., 2016) to provide the number of arousals during REM sleep, and excluded from the power spectral density analyses. Only frontal electrodes were considered in the analyses because the frontal region is commonly accepted as most sensitive to sleep homeostasis(Cajochen et al., 1999); focusing on the frontal electrodes may also facilitate interpretation of future large-scale studies using ambulatory EEG, often restricted to frontal electrodes.

Sleep was staged in 30s-epochs using an automatic algorithm (ASEEGA, PHYSIP, Paris) (Berthomier et al., 2007b) to provide total sleep time (TST). Averaged energy was computed for each 30-minute bin, adjusted for the proportion of rejected data. The adjusted values were then summed

across REM sleep(Skorucak et al., 2018) to provide REM theta energy (overnight cumulated 4.25-8Hz energy). Energy in the other typical bands of the sleep EEG were computed similarly during both REM and NREM for specificity assessments (Delta band: 0.5-4Hz; Theta: 4.25-8Hz; Alpha: 8.25-12HZ; Sigma: 12.25-16Hz; Beta: 16.25-30Hz). Sigma power (12.25-16Hz) was computed during the 1-min preceding each REM episode (if sleep stage was N2 and N3), as the weighted sum of 4s artefact-free window (2s overlap per 30s epoch), prior to averaging over the number of REM episodes.

*Cognitive tasks*

*Visual perceptual rivalry task.* The task (~12min total duration) consisted of watching a 3D Necker cube, which can be perceived in two different orientations (**Figure 4.1B**), for 10 blocks of 1min separated by 10s of screen-center cross fixation. Participants were instructed to report switches between the two percepts through a button press.

*Auditory salience detection task.* The task (~10min total duration) consisted of an oddball paradigm requiring reports on the perception of rare deviant target tones (1,000Hz, 100ms, 20% of tones) that were pseudo-randomly interleaved within a stream of standard stimuli (500Hz, 100ms) through a button press (**Figure 4.1C**). The task included 270 stimuli (54 targets).

These tasks were selected because they were thought to activate the LC (Einhauser et al., 2008; P. R. Murphy et al., 2014). As we previously reported, both tasks successfully triggered a response of the LC (Koshmanova et al., 2023; Mortazavi et al., 2025) **(Figure 4.1D and E)**.

*MRI data acquisition, preprocessing and univariate analyses*

MRI data were acquired using a MAGNETOM Terra 7T MRI system (Siemens Healthineers, Erlangen, Germany), with a single-channel transmit and 32-receiving channel head coil (1TX/32RX, Nova Medical, Forchheim, Germany). Blood-oxygen-level-dependent (BOLD) fMRI data were acquired using a multi-band (MB) gradient-recalled echo–echo-planar imaging (GRE-EPI) sequence (main parameters: repetition time = 2.340ms, flip angle = 90°, 86 axial 1.4 mm–thick slices, no interslice gap, matrix size = 160 × 160, voxel size = 1.4 × 1.4 × 1.4 $mm^3$ ,MB acceleration factor = 2, GeneRalized Autocalibrating Partial Parallel Acquisition (GRAPPA) acceleration factor = 3). The cardiac pulse and the respiratory movements were recorded concomitantly using, respectively, a pulse oximeter and a breathing belt (Siemens Healthineers). The fMRI acquisition was followed by a dual-echo 2D GRE field mapping sequence to assess B0 magnetic field inhomogeneities with the following parameters: TR = 5.2 ms, TEs = 2.26 ms and 3.28 ms, flip angle (FA) = 15°, bandwidth = 737 Hz/pixel, matrix size = 96 × 128, 96 axial slices with 2 mm thickness, voxel size = 2 × 2 × 2 $mm^3$, acquisition time = 1:38 minutes.

A Magnetization-Prepared with 2 RApid Gradient Echoes (MP2RAGE) sequence was used to acquire T1 anatomical images: TR = 4,300 ms, TE = 1.98 ms, FA = 5°/6°, TI = 940 ms/2,830 ms, bandwidth = 240 Hz/pixel, matrix size = 256 × 256, 224 axial 0.75 mm–thick slices, GeneRalized Autocalibrating Partial Parallel Acquisition (GRAPPA) acceleration factor = 3, voxel size = 0.75 × 0.75 × 0.75 $mm^3$, acquisition time = 9:03 minutes.(Marques & Gruetter, 2013)

The LC-specific sequence consisted of a 3D high-resolution magnetization transfer–weighted turbo-flash (MT-TFL) sequence with the following parameters(Priovoulos et al., 2018): TR = 400 ms, TE = 2.55 ms, FA = 8°, bandwidth = 300 Hz/pixel, matrix size = 480 × 480 × 60, number of

averages = 2, turbo factor = 54, magnetization transfer contrast (MTC) pulses = 20, MTC FA = 260°, MTC RF duration = 10,000 µs, MTC inter-RF delay = 4,000 µs, MTC offset = 2,000 Hz, voxel size = .4 × .4 × .5 $mm^3$, acquisition time = 8:13 minutes. Axial slices were acquired and centered for the acquisitions perpendicularly to the rhomboid fossa (i.e., the floor of the fourth ventricle located on the dorsal surface of the pons) (Priovoulos et al., 2018).

Functional and anatomical MRI data were preprocessed using SPM12, ANTs and SynthStrip brain extraction tool (Hoopes et al., 2022), as fully described previously (Koshmanova et al., 2023; Mortazavi et al., 2025). The preprocessed data were resampled to a $1mm^3$ resolution. Individual statistical analyses consisted of a general linear model (GLM) including one regressor of interest, consisting of a switch in perception (perceptual rivalry) or target tone (salience detection) modeled as an event (convolved with the canonical hemodynamic response function - HRF). Participant movement parameters, respiration, and heart rate were used as covariates of no interest (physiological data of 4 volunteers were not available and therefore not included in their individual design matrices). The T1 structural whole-brain image was used to extract individual total intracranial volume (TIV) using CAT12 toolbox (Gaser et al., 2022).

Individual LC masks were manually delineated by 2 experts based on LC-specific images (as in (Koshmanova et al., 2023)) and activity of left LC was extracted in each subpart (as LC responses were more prominent in the left LC in both tasks (Koshmanova et al., 2023; Mortazavi et al., 2025)). The different nuclei of the hypothalamus do not offer a lot of contrast in MRI images such that they cannot be segmented using current approaches. We therefore used an automated segmentation algorithm to parcellate the

hypothalamus into 5 subparts which encompass several nuclei - anterior-inferior, anterior-superior, posterior, inferior tubular and superior tubular (Billot et al., 2020) (see **Figure 4.3A** for the nuclei deemed to be included in each subpart). We then extracted the activity of these subparts within the left hypothalamus (i.e., mean value over each subpart of hypothalamus) during both tasks for each participant using the REX Toolbox (https://web.mit.edu/swg/software.htm). We selected subparts of the left hypothalamus because we were interested in the effective connectivity between the left hypothalamus and the left LC, where responses were more prominent.

*Effective Connectivity Analysis*

Our previous analysis of the fMRI data of both tasks indicated only weak hypothalamus activation (i.e. no responses associated to the events of interest were detected even when applying an uncorrected threshold of $p < .001$). This does not, in principle, prohibit effective connectivity from being computed and importantly to be associated with other features of interest, such as sleep electrophysiology metrics. To select the hypothalamus subpart to be considered in our connectivity analyses, we reasoned that the activity of a given hypothalamus subpart should at least be weakly associated with our sleep metrics of interest. We therefore determined whether the activity estimate of each subpart – separately for each task - was associated with either REM theta energy or sigma power prior to REM episodes. Left hypothalamus subpart activity was used in GLMM to seek correlation with REM theta energy and sigma power prior to REM sleep episodes (one GLMM per task and per sleep metric) (see statistic section below). Only the hypothalamus subparts that yielded at least a statistical trend ($p < 0.1$) with

either sleep metrics during a given task were considered for effective connectivity analyses.

DCM framework(K. J. Friston et al., 2003), implemented in SPM12, was used to compute the effective connectivity between the LC and each of the selected hypothalamus subparts during the selected task (i.e. the anterior-superior and posterior subparts during the Visual perceptual rivalry task – see results). BOLD signal time series associated with our event of interest (i.e. perceptual switch) were extracted from individually defined ROIs (i.e., over the LC and hypothalamus subparts masks), based on the individual statistical maps thresholded at $p<.05$ uncorrected. During the extraction of the BOLD signal time series, confounding effects such as head movement and physiological noise were regressed out, ensuring the resulting time series better reflected neural activity related to the event. We extracted the first principal component (eigenvariate) of the "adjusted" time series, which represents the time series after regressing out effects of no interest, using the approach outlined by (Zeidman, Jafarian, Corbin, et al., 2019).

Stochastic DCM was used because no significant task-related activations were detected in the selected hypothalamus subparts in the group-level whole-brain analysis across all participants (cf. above) (Daunizeau et al., 2012). We computed two DCM models based on animal evidence of reciprocal interaction between the LC and the anterior-superior subpart of hypothalamus as well as LC and posterior subpart of hypothalamus (Giorgi et al., 2021; Szymusiak et al., 2007) (i.e. intrinsic connections between the two regions), along with self-feedback gain control connections for LC and hypothalamus subpart (**Figure 4.3B and C**). Task inputs were further considered to reach both regions. Time series extracted from individual ROIs

were subjected to a first-level DCM analysis, where the model was estimated for each subject. To isolate the connectivity parameters that were contributing to the model and could therefore be used in a GLMM seeking associations with sleep metrics (see below), we performed a Parametric Empirical Bayes (PEB) analysis (K. Friston et al., 2015; Zeidman, Jafarian, Seghier, et al., 2019) over the first-level DCM parameter estimates. PEB is a hierarchical Bayesian model that evaluates commonalities and differences among subjects in the effective connectivity domain at the group level by performing Bayesian model reduction (BMR), which explores the space of DCM models and leads to a subset of models best explaining the data and Bayesian model averaging (BMA) of the parameters across models weighted by the evidence of each model. Subsequently, as there is no concept of significance in Bayesian analysis, we reported and used in the GLMM, the parameters contributed to the model evidence with a posterior probability (Pp) exceeding 0.90.

*Statistics*

GLMMs were performed in SAS 9.4 (SAS Institute, NC, USA) and were adjusted for the distribution of the dependent variables. Outliers among connectivity and sleep metrics lying beyond four times the standard deviation were removed from the analysis (maximum one data point was removed, the final number of individuals included in each analysis is reported in each table). The first GLMMs were meant to isolate which hypothalamus subparts would be included in DCM. They included the 2 sleep features of interest as dependent variables in each task separately and hypothalamus subpart, hypothalamus activity estimates and age-group, including sex, TST and TIV as covariates (i.e. 4 models in total, 1 per sleep metric and task). Our initial models included the

three-way interaction between hypothalamus activity, hypothalamus subpart and age group and all three simple two-way interaction terms. Non-significant three-way / two-way interactions were removed from the models based on Bayesian Index Criterion (BIC) for fit quality estimation such that final models only included the hypothalamus activity and hypothalamus subpart interaction. This yielded statistical trends with the activity of the anterior superior and posterior subparts during the perceptual rivalry task (see results) for REM theta energy so that DCM included these 2 subparts during the latter task.

The next set of GLMMs tested for associations between connectivity metrics and REM theta energy, again used as dependent variable, and including sex, TST and TIV as covariates. Following the same procedure described in the preceding paragraph, all final models included an interaction term between the connectivity metric and the age-group. Semi-partial $R^2$ ($R^{2*}$) values were computed to estimate the effect sizes of significant fixed effects and statistical trends in all GLMMs (Jaeger et al., 2017). Significance was determined following the Benjamini-Hochberg procedure for False discovery rate procedure [p<.025 (for rank 1/2); p<.05 (for rank 2/2)].

We computed a prior sensitivity analysis to get an indication of the minimum detectable effect size in our main analyses given our sample size. According to G*Power 3 (version 3.1.9.4) (Faul et al., 2009); taking into account a power of .8, an error rate $\alpha$=.025, and a sample size of 51, we could detect medium effect sizes $r$>.39 (2-sided; CI:.13–.6; $R^2$>.15, CI:.02–.36) within a linear multiple-regression framework including 2 tested predictor (connectivity, age group) and 2/3 covariates (sex, TIV, TST where relevant).

**Conclusions**

In summary, we show that the connectivity between key subcortical structures for sleep regulation assessed during wakefulness may reflect their crosstalk during sleep and contribute to the variability of sleep electrophysiology. Our main finding includes the dominant oscillatory mode of REM, as a potential reflection of REM intensity and amnesic function. The association is detected in participants aged between 50 and 70y and beyond REM theta rhythms, suggesting a more prominent impact in the fragile sleep found in aging and on neuronal synchrony over lower frequencies. These results underscore the age-dependent modulation of LC circuitry and its potential implications for sleep regulation and the age-related increase in sleep complaints.

# Chapter 5: Sleep arousals are associated with the polygenic risk for developing Alzheimer's disease and with cognitive decline in healthy late middle-aged individuals

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**The results presented here are submitted to *Sleep* (2025). Supplementary materials for this paper can be found in Appendix 3.**

**Abstract**

**Objective:** Sleep disturbances are increasingly recognized as early features of Alzheimer's disease (AD) neuropathology. In that context, spontaneous arousals during sleep, which have been linked to the activity of the locus coeruleus in rodents, have been associated with the burden of Amyloid beta in the brain of healthy late middle-aged individuals. Whether the heterogeneity of arousals during sleep may be related to the risk of developing AD in young adults is not established. Likewise, whether arousals may be associated with cognitive decline is not known. Here, we evaluated the association between arousals, the risk for developing AD and cognitive performance and cognitive decline in healthy young and late middle-aged individuals

**Methods:** We classified spontaneous arousals using in-lab EEG recordings of sleep in 453 younger individuals (22±2.7y; 49 women) and 87 late middle-aged individuals (59.3±5.3y; 59 women) based on their association with sleep stage transitions and changes in muscle tone. We examined the associations between arousal types and the polygenic risk scores (PRS) for AD, cognitive performance at baseline and, in late middle-aged individuals, cognitive decline over 2 and 7 years.

**Results:** The prevalence of arousals associated with sleep stage transition was higher in late middle-aged vs. younger individuals. Among these arousals, those with and without muscle tone increases were, respectively, associated with lower and higher PRS for AD in late middle-aged but not in younger individuals. In the late middle-aged individuals, transition arousals associated with and without muscle tone increases were correlated with better and

worse attentional performance at baseline, and lower and larger memory decline over 2 or 7 years.

**Conclusion:** The heterogeneity in spontaneous arousals during sleep may reflect their strength, or association with LC activity, and may indicate vulnerability to AD. The findings may contribute to identifying early markers of neurodegenerative risk.

**Introduction**

Individuals with Alzheimer's disease (AD) — who are widely known to experience cognitive deficits, particularly in memory — also show decreased total sleep time, sleep efficiency, N3 sleep and REM sleep, and increased number of awakenings compared with controls (Zhang et al., 2022) and the changes have been correlated with the severity of their cognitive impairment (Zhang et al., 2022). Alterations in sleep are also detected in relation to the hallmarks of AD pathogenesis (i.e., Amyloid-Beta (Aβ) and tau accumulation) in healthy individuals, prior to any clinical manifestations of the disease (Van Egroo, Narbutas, Chylinski, Villar González, Maquet, et al., 2019). In addition, sleep disturbances have been linked to the future risk of both cognitive decline and AD pathology (Shi et al., 2018). Notably, individuals at higher risk for AD exhibit more frequent nocturnal arousals – transient/abrupt shift of EEG frequency not associated with full awakenings– than those at lower risk (Tsai et al., 2022). In cognitively healthy late middle-aged individuals, sleep arousals were associated with early Aβ burden depending on whether arousals were linked to sleep stage transitions (T+/T–) and to detectable increase in muscle tone (M+/M-) (Chylinski et al., 2021). Specifically, T+M– arousals were associated with higher Aβ burden while T–M+ arousals were linked to lower Aβ accumulation and better cognitive performance, particularly in the attentional domain.

Recent studies in rodents indicate that the heterogeneity in arousals may be related to the activity of locus coeruleus (LC), which is among the first sites of tau protein aggregation in the brain (Braak & Del Tredici, 2012) and is likely to contribute to the alterations in sleep regulation found in preclinical AD (Van Egroo et al., 2024). Stronger activity of the LC during slow wave sleep

would be associated with increased arousal density while lower level of LC activation would lead to decreased arousal density (Osorio-Forero et al., 2022). The heterogeneity in sleep arousal appears therefore to be directly related to a key structure for sleep and for AD, and as a result, may constitute a marker of the vulnerability for AD. How early this vulnerability can be detected through arousals and whether it bears prediction for future cognitive decline is unknown.

Aβ protein accumulation in the brain typically begins to increase over the sixth decade in humans (Fleisher et al., 2013) and is therefore not a useful biomarker for assessing AD risk in younger individuals. In contrast, genetic approaches offer unique tools to assess the variability for the risk for complex diseases, including AD. AD is recognized to be polygenic (Escott-Price et al., 2015) and genome-wide association studies (GWAS) have identified over 70 genes which are associated with AD (Reitz et al., 2023). Due to the polygenic nature of AD, polygenic risk scores (PRS)—which aggregate the risks associated with common genetic variants— have proven to be an effective method for assessing AD risk. Studies have demonstrated that PRS can distinguish between AD cases and healthy controls, reaching a prediction accuracy up to between 75% and 84% (Escott-Price et al., 2015; Escott-Price et al., 2017). The PRS would be particularly useful for studying asymptomatic individuals of any age for studying AD risk long before classical pathological hallmarks are detectable (Baker & Escott-Price, 2020). Importantly, PRS may not grasp the same aspect of AD risk than Aβ assessment as reported in studies of cognitively healthy individuals where PRS was not associated with baseline Aβ burden but could predict future Aβ accumulation in longitudinal follow-up (Ge et al., 2018; Luckett et al., 2022; Xicota et al., 2022).

In this study, we examined the associations between PRS for AD, different types of sleep arousals—classified by the presence of muscle tone increase and sleep stage transitions— and longitudinal cognitive decline in a relatively large sample of cognitively unimpaired individuals (N=540). Our aim was to determine whether arousal types previously associated with Aβ accumulation were also linked to PRS for AD in late middle-aged individuals aged 50 to 70y and in much younger adults aged 18 to 31y. We anticipated that the more T+M- and T-M+ arousals would be linked, respectively, to higher and lower PRS values. We further assess their link to cognitive performance and, in late-middle aged individuals, with cognitive decline over 2y and 7y follow-up periods. We hypothesize that, in late middle-aged individuals, T-M+ arousals would be associated with better attentional performance in the baseline while they would be associated with reduced memory decline at follow-up.

## Methods

### Participants

A total of 540 healthy participants [453 younger individuals aged 18–31 years (22.04 ± 2.69y; 49 women;) and 87 late middle-aged individuals aged 50-69 years (59.28 ± 5.3y; 59 women)] part of different multi-modal studies were included in the present analyses **(Table 5.1)** (Chylinski et al., 2021; Muto et al., 2021). Data from young adults were collected across 6 different studies. Notably, one of these studies contributed the majority of the sample, including 357 young men. Because this study was originally designed as a genetic study, only Caucasian men within a narrow age range were included to maximize genetic uniformity. In contrast, data from late middle-aged individuals were collected as part of a single study. All studies collected

quantitative sleep parameters and blood samples to assess PRS for AD (**Figure 5.1**). Extensive cognitive evaluation was conducted in the largest studies of the younger dataset (N=357) and in all late middle-aged individuals, while the later part of the sample also completed follow-up cognitive assessment after 2 and 7 years.

**Table 5.1. Characteristics of the study sample**

| Characteristic | All Mean (SD) | Young Mean (SD) | Late middle-aged - Mean (SD) | p-value |
|---|---|---|---|---|
| Sample size (N) | 540 | 453 | 87 | - |
| Sex (Men) | 80.29% | 89.37% | 32.18% | <.0001 |
| Age (years) | 29.27 (15.07) | 22.04 (2.69) | 59.28 (5.35) | <.0001 |
| Body Mass Index (BMI) ($kg*m^{-2}$) | 22.67 (2.64) | 22.15 (2.31) | 24.83 (2.85) | <.0001 |
| Anxiety | 2.60 (2.92) | 2.56 (2.94) | 2.78 (2.86) | 0.5 |
| Depression | 3.46 (3.88) | 2.96 (3.53) | 5.52 (4.55) | <.0001 |
| Sleep Quality | 3.68 (1.99) | 3.46 (1.76) | 4.63 (2.59) | 0.0001 |
| Daytime sleepiness | 5.97 (3.63) | 5.94 (3.54) | 6.12 (4.00) | 0.67 |
| Chronotype | 50.80 (8.30) | 50.10 (8.26) | 53.68 (7.88) | 0.0003 |
| Total sleep time (min) | 439.9 (47.5) | 451.4 (41.53) | 392.3 (40.90) | <.0001 |
| T+M+ arousals number | 13.45 (5.88) | 12.92 (5.53) | 15.16 (6.57) | 0.004 |
| T+M- arousals number | 9.98 (5.79) | 9.50 (5.21) | 13.59 (7.29) | <.0001 |
| T-M+ arousals number | 96.12 (37.43) | 106.2 (33.12) | 55.60 (25.88) | <.0001 |
| T-M- arousals number | 118.64 (44.73) | 127.9 (45.59) | 95.71 (44.08) | <.0001 |
| T+M+ arousals density (number/h) | 1.82 (0.81) | 1.72 (0.72) | 2.33 (1.02) | <.0001 |
| T+M- arousals density (number/h) | 1.40 (0.87) | 1.27 (0.72) | 2.11 (1.19) | <.0001 |
| T-M+ arousals density (number/h) | 13.25 (4.62) | 14.16 (4.17) | 8.54 (3.98) | <.0001 |
| T-M- arousals density (number/h) | 16.70 (6.18) | 17.11 (6.07) | 14.59 (6.34) | 0.0005 |

*The p-values shown in the table correspond to two-sample t-tests except for sex that were compared using a Chi-square test.*

*Sleep quality was assessed by the Pittsburgh Sleep Quality index (PSQI) (Buysse et al., 1989). Daytime sleepiness was measured by the Epworth Sleepiness Scale (Johns, 1991), Chronotype was assessed by the Morningness-Eveningness Questionnaire (MEQ) (Horne & Ostberg, 1976). Anxiety was estimated by the Beck Anxiety Inventory (Beck, Epstein, et al., 1988) and Depression was estimated by the 21-item Beck Depression Inventory II (Beck, Steer, et al., 1988); Total sleep time was extracted from polysomnography recordings.*

In all studies, participants were excluded if they had a body mass index (BMI) less than 18 and greater than 27 kg/m² (greater than 29 kg/m² for late middle-aged individuals), a history of psychiatric disorders or severe brain injury, documented/diagnosed sleep pathologies such as insomnia and REM behavior disorder, substance addiction, chronic use of medication affecting the central nervous system, smoking, excessive alcohol consumption (more than 14 units per week), high caffeine intake (more than three cups per day for younger participants, or more than five cups per day for older participants), shift work within the past 6 months, trans-meridian travel within the preceding two months, moderate to severe subjective anxiety or depression, as indicated by a score greater than 16 on the Beck Anxiety Inventory (BAI) or greater than 19 on the Beck Depression Inventory-II (BDI-II). Poor sleep quality (Pittsburgh Sleep Quality Index score > 7), excessive daytime sleepiness (Epworth Sleepiness Scale score > 14), significant sleep apnea (apnea-hypopnea index > 15 events per hour), and parasomnia as determined during an in-laboratory screening night using polysomnography (PSG) and based on the 2017 American Academy of Sleep Medicine (AASM) criteria (version 2.4) (Berry et al., 2012), also led to exclusion. Old individuals were also excluded if they had clinical symptoms of cognitive impairment (dementia rating scale < 130; mini mental state examination < 27).

All study procedures were approved by the Ethics Committee of the Faculty of Medicine at the University of Liège (Belgium). Written informed consent was obtained from all participants prior to inclusion and participants received financial compensation for their participation. The study was conducted in accordance with the Declaration of Helsinki and the World Medical Association's International Code of Medical Ethics.

**Protocol**

Data from young adults were obtained from six separate studies, whereas data from late middle-aged individuals were derived from a single study (Chylinski et al., 2021; Koshmanova et al., 2022; Muto et al., 2021) and we detail here only those aspects that are relevant for the present study.

For the majority of young participants (N=357 young men), for three weeks prior to the in-lab experiment, participants adhered to a regular sleep schedule based on their usual sleep times (within ±30 minutes for the first two weeks and within ±15 minutes for the final week), verified through actigraphy data (Actiwatch 4, CamNtech, Cambridge, UK). Prior to the experiment, participants completed a urine drug screen (Multipanel Drug Test, SureScreen Diagnostics Ltd) and an adaptation night in the laboratory aligned with their habitual sleep-wake schedule. During this night, full PSG recordings were performed to screen for sleep-related breathing disorders and periodic limb movements. On the second day, participants left the lab in the morning and were instructed not to nap during the day, with adherence confirmed via actigraphy. They returned to the lab in the evening, 3.5 hours before their scheduled lights-off time, and had a baseline night of sleep recorded in complete darkness. The timing of this night was centered on the average sleep midpoint from the preceding week. Older participants and the

rest of young participants (N=96 young individuals) first completed an in-laboratory adaptation night to get familiar with the lab environment and allow for detection of any sleep disturbances. For the seven days before the baseline night, participants adhered to a regular sleep-wake schedule (within ±30 minutes) according to their habitual bed and wake-up times, verified using sleep diaries and wrist actigraphy (Actiwatch©, Cambridge Neurotechnology, UK). All participants were instructed to avoid daytime naps and to refrain from unusually intense physical activity during the final three days of the fixed sleep schedule. Their habitual sleep was then recorded in complete darkness using EEG. The current study focuses exclusively on the later baseline night of sleep in both younger and late middle-aged individuals.

**EEG acquisitions**

For both age groups, the PSG in the adaption night included EEG (Fz, Cz, C3, PZ, Oz electrodes), 2 bipolar electrooculograms (EOGs), 2 bipolar submental EMG electrodes, 2 bipolar electrocardiograms (ECGs), 2 sets of bipolar leg electrodes, thorax and abdominal belts, an oximeter, a nasal canula, and a snoring sensor. For young participants, polysomnographic sleep data were acquired using Vamp amplifiers (Brain Products, Germany) while for old participants sleep was recorded with N7000 amplifiers (EMBLA, Natus Medical Incorporated, Planegg, Germany). For young individuals, the electrode montage of the baseline night consisted of 9 EEG channels (F3, Fz, F4, C3, Cz, C4, Pz, O1, O2), while it inluded 11 EEG derivations in the late middle-aged (F3, Fz, F4; C3, Cz, C4; P3, Pz, P4; O1, O2 electrodes). All montages included 2 bipolar EOG, 2 bipolar EMG, and 2 bipolar ECG electrodes and all recordings were rereferenced to the mean of the 2 mastoids.

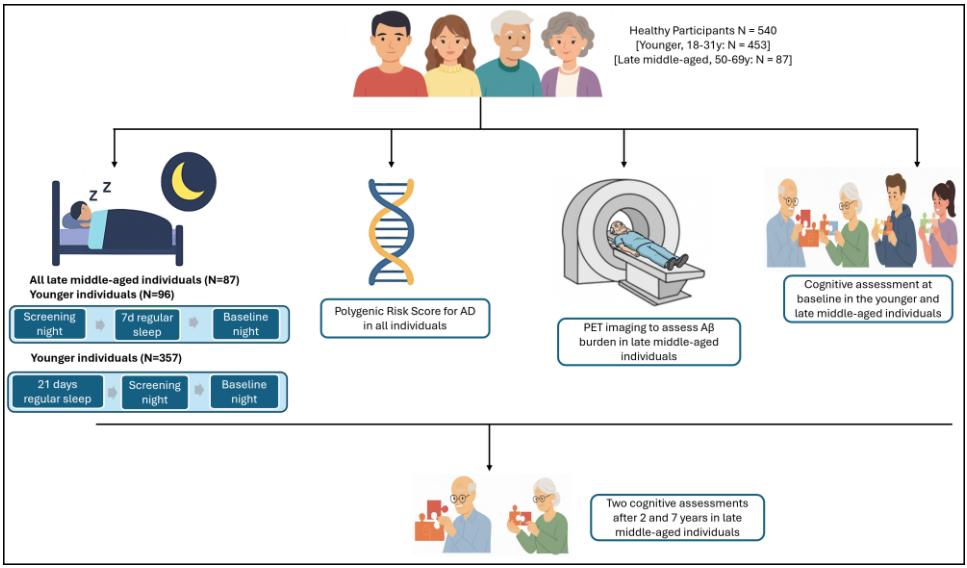


***Figure 5.1: Overview of the study design***

*The habitual sleep of young and late middle-aged adults was recorded in a laboratory setting to identify sleep arousals. Blood samples were then collected to measure Alzheimer's disease polygenic risk scores (PRS), and PET imaging was conducted in late middle-aged individuals to assess amyloid-beta (Aβ) burden. Participants subsequently completed a battery of cognitive tests. A subset of late middle-aged adults who initially participated in the study also returned for follow-up cognitive assessments after 2 and 7 years.*

## Arousal detection

Sleep stage scoring and arousal detection were carried out in separate steps by 2 independent algorithms. Sleep stage scoring was performed in 30-second windows using a validated algorithm (ASEEGA, Physip) (Berthomier et al., 2007a; Peter-Derex et al., 2021). Automatic arousal detection was then computed as it is objective and reproducible (Chylinski et al., 2020). We used an individually tailored validated algorithm based on the AASM definition

(Berry et al., 2012) 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 1-second epochs in 2 successive steps computed over the power in the broad-α (7–13 Hz), β (16–30 Hz), and lower-θ (3–7 Hz) frequency bands, excluding the σ band (11–16 Hz) — 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 1-second epoch with power in any of the 3 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 30-second window: all 1-second epochs without concomitant EMG tone increase are selected, as well as the first ten 1-second epochs without EMG increase before and after the 30-second window being evaluated; threshold of each frequency band consists in the median power over the selected 1-second epochs. Events composed of at least 3 consecutive 1-second epochs with changes in EEG frequencies higher than twice the local median and 1 median of the whole recording for that frequency band were considered as arousals. For detailed explanations on the method, see ref. (Chylinski et al., 2020).

As previously (Chylinski et al., 2021), we split arousal according to 2 criteria, which we considered as relevant in research settings, as well as clinical practice. The first criterion addressed whether arousals were associated with a sleep stage transition (T+) (when they occurred within 15 seconds 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 whether they did not (T–). The second criterion considered the concomitant increase in EMG tone (M+) or its absence (M–).

We used the absolute number of arousals rather than arousal density to capture the total arousal burden across the night, which was our primary variable of interest. Normalizing to total sleep time (TST) can obscure meaningful differences when TST varies, specially between young and late middle-aged individuals. By including TST as a covariate, we were able to assess the independent contribution of arousal events to genetic risk for AD and cognitive outcomes, focusing on whether a greater overall arousal load—regardless of sleep duration—was linked to neurodegenerative vulnerability.

**Genotyping, quality control and imputation**

Blood samples were collected and stored at -20ºC within few hours until DNA extraction. The genotyping was performed using the Illumina Infinium OmniExpress-24 BeadChip arrays (Illumina, San Diego, CA) based on Human Build 37 (GRCh37). All the study participants were European ancestry. Established quality control (QC) procedure was performed using PLINK (Purcell et al., 2007) (http://zzz.bwh.harvard.edu/plink/). In brief, the SNPs were excluded as follows: >10% missing genotypes, <95% call rate, minor allele frequency (MAF) below 0.01, out of Hardy-Weinberg equilibrium (p-value <10-4 for the Hardy-Weinberg test). SNPs on 23rd chromosome as well as ambiguous SNPs (A-T, T-A, C-G, G-C) were excluded as well. The data was matched for deviation with European ancestry using 1000 Genomes Project dataset (1KGP, https://www.internationalgenome.org). Imputation was conducted using the Sanger imputation server (https://imputation.sanger.ac.uk/) based on the Haplotype Reference

Consortium (r1.1) as reference panel and using Eagle2.4 pre-phasing algorithm. The detailed data processing and analysis for young and late middle-aged sub-sample is as described previously in (Koshmanova et al., 2022; Muto et al., 2021). We finally ended with 7,165,614 SNPs common to all participants.

**Polygenic risk score (PRS) calculation**

PRS analyses can be used to assess the genetic liability of an individual for a phenotype by calculating the weighted sum of risk alleles effect size identified in genome-wide association studies. In the current study, we calculated a PRS for AD for each participant using summary statistics from the recent GWAS meta-analysis of European ancestry (Wightman et al., 2021). This approach differed from the PRS calculation originally applied in part of the young individuals' dataset (Muto et al., 2021). The standardization and quality control of GWAS summary statistics was performed by MungeSumstats, a Bioconductor R package (A. E. Murphy et al., 2021). In the process, the summary statistics was pruned to align reference alleles to build GRCh37, remove multiallelic variants, and adjust weights for the appropriate reference alleles. The PRS was then generated using SBayesR algorithm implemented in GCTB software. The approach assumes that the SNP effects are drawn from mixtures of distributions with the key metrics defining these genetic architectures estimated through Bayesian frameworks. To derive PRSs from GWAS effect estimates of SNPs, SBayesR essentially uses Bayesian linear mixed model and the reference linkage disequilibrium (LD) correlation matrix. In our analysis, we used banded LD matrix to improve the prediction accuracy as recommended by the authors of GCTB. We used p-value thresholding

through PLINK to include only the SNPs reaching stringent GWAS significance (p-value <$1x10^{-8}$) to restrict the number of genetic markers to a minimum.

**MRI data**

MRI data were used in late middle-aged individuals in order to determine the region of interest used for extraction of Aβ burden value based on PET images, as fully described in (Chylinski et al., 2021). Quantitative multiparametric MRI acquisition was performed on a 3-Tesla MR scanner (Siemens MAGNETOM Prisma, Siemens Healthineers) to get a magnetization transfer–weighted (MT-weighted) contrast, based on multi-echo 3D fast low angle shot at 1 mm isotropic resolution (Weiskopf & Helms, 2008). MRI multiparameter maps were processed with the hMRI toolbox (Tabelow et al., 2019) (http://hmri.info) and SPM12 (Welcome Trust Centre for Neuroimaging, London, United Kingdom) to obtain a quantitative MT map and segmented images (gray matter, white matter, CSF), normalized to the standard MNI space using unified segmentation (Ashburner & Friston, 2005).

**PET scan**

Aβ PET imaging was performed only in late middle-aged individuals as fully described in (Chylinski et al., 2021). Younger individuals typically have no detectable Aβ accumulation such that it would be unethical to expose them to radiation. Aβ PET imaging was performed using [$^{18}$F]Flutemetamol, except for 3 volunteers for which [$^{18}$F]Florbetapir was used as fully described in (Chylinski et al., 2021). PET scans were performed on an ECAT EXACT+ HR scanner (Siemens). Individual PET average images were manually reoriented according to MT-weighted structural MRI volumes and coregistered to the individual space structural MT map. Flow-field deformation parameters

obtained from DARTEL spatial normalization of the MT maps were applied to averaged coregistered 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 labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002). Standardized uptake value ratio (SUVR) was computed using the whole cerebellum as a 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). Aβ burden was averaged over a composite mask covering the previously reported earliest aggregation sites for Aβ pathology (Grothe et al., 2017) — frontal medial cortex and basal part of temporal lobe (fusiform and inferior temporal gyri). According to the study by Krasny et al. (2024), the amyloid PET positivity threshold was set at Centiloid 20.

**Cognitive assessment**

Cognitive assessments were conducted at baseline in part of the younger sample (N=357, as part of the same unique study) while they were administered at baseline and at 2y (mean 767±54 days) and 7y (mean 2647±98 days) follow-up in the late middle-aged. Although cognitive assessments were conducted in both age groups, only data from late middle-aged participants were included in the analyses, as significant associations between arousals and PRS for AD were observed only in this group (see Results). The following description therefore focuses on the cognitive data from the late middle-aged participants. At the first time point, a cognitive battery of neuropsychological tasks was carried out in 2 sessions, while well rested. A first session of ~1 hour was performed in the afternoon prior to the

sleep assessment, approximately 7.5 hours before habitual bedtime, and a second session of ~1.5 hours was performed on another day (between 12 and 6 hours prior to habitual bedtime). From those 2 sessions, 3 domain-specific composites scores were computed for the memory, executive function, and attentional domains, and they consisted of the standardized (z- scores) sum of the standardized domain-specific scores, where higher values indicate better performance.

The first session comprised (a) mnemonic similarity task (MST) (Stark et al., 2013); (b) category verbal fluency (letter and animals) (Cardebat et al., 1990); (c) digit symbol substitution task (DSST) (Tulsky et al., 1997); and (d) visual N-back task (1and 3-back variants) (Kirchner, 1958). The second session of ~1.5 hours was performed on another day (between 12 and 6 hours prior to habitual bedtime) and comprised (a) inverse order digit span task (Tulsky et al., 1997); (b) free and cued selective reminding test (FCSRT) (Grober et al., 1988); (c) trail making test (TMT) (Bowie & Harvey, 2006), and (d) and logical memory from Wechsler memory test (MEM-III) (Wechsler, 2001). The memory score consisted of the FCSRT (sum of all 4 free recalls), the recognition memory score from the MST, and logical memory for delayed items from MEM-III. The executive function score included verbal fluency tests (letter and animals score for 2 minutes), inverse order digit span, TMT (part B minus part A), and N-back (3-back variant). The attentional score comprised the DSST, TMT (part A), and N-back (1-back variant).

The second assessment took place two years after the first, and the third was conducted seven years after the initial assessment. At these two time points, the same procedure and tests were used to compute the three composite scores. However, all individual did not participate in the follow up

assessments and only 66 old individuals participated in the second time point assessment and 64 old individuals participated in the third time point assessment (with 48 individuals common to both follow-ups). The substantial dropout observed at the second cognitive assessment was primarily due to the COVID-19 crisis. For each composite score, cognitive decline 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 time.

**Statistics**

Statistical analyses were performed in the R environment (version 4.1.3) (R Development Core Team, 2017) using generalized additive models for location scale and shape (GAMLSS) (Rigby & Stasinopoulos, 2005; D. M. Stasinopoulos & Rigby, 2008) adjusting for the distribution of the dependent variables. GAMLSS offer a wide variety of family of distributions for model fitting (Rigby et al., 2019; Rigby & Stasinopoulos, 2014; M. D. Stasinopoulos et al., 2017) and is considered more general cases than GLM or GAM approaches. Outliers among all the assessed metrics lying beyond four times the standard deviation were removed from the analysis (the final number of individuals included in each analysis is reported in each table). Our primary objective was assessed in the first GAMLSS including the total number of arousals as the dependent variable and a four-way interaction among PRS for AD, transition status, EMG status, and age group as the main predictor. Since it included all variables of interest in the same model, it was not corrected for multiple comparisons and significance was set at $p<0.05$. Additionally, all related three-way and two-way interaction terms were included. Sex and total sleep time (TST) were added as covariates in the model and subjects were

treated as random factors. This model yielded significant association between the four-way interaction and total number of arousals (see Results). Hence, a subsequent post-hoc GAMLSS was used to specify which type of arousal was associated with the PRS for AD and in which age group this association was observed. This second GAMLSS included the PRS for AD as the dependent variable and four two-way interactions between each type of arousal (T+M+, T+M-, T-M+, T-M- arousal) and age group as independent variables. Sex and TST were added as covariates.

The association between arousal and cognition in each time point was assessed in models including each composite score as a dependent variable together with two types of arousals related to the PRS for AD (T+M+ and T+M- arousals) as independent variables as well as age, sex, education and TST as covariates (i.e. 9 models in total, 3 per time point). As an exploratory analysis, in similar models, we also considered the association between recognition memory score of MST in all three time points and T+M+ and T+M- arousals as this memory task is highly sensitive to early signs of cognitive decline (Marks et al., 2017; Stark et al., 2013).

We computed a prior sensitivity analysis to get an indication of the minimum detectable effect size in our main analyses given our sample size. According to G*Power 3 (version 3.1.9.4) (Faul et al., 2009), taking into account a power of .8, an error rate α of .01 (corrected for 5 tests), a sample size of 540 allowed us to detect small effect sizes f2 >.035 (confidence interval: .016 –.086; $R^2$ > .034, $R^2$ confidence interval: .016 –.079) within a linear multiple regression framework including our tested predictors and 2 covariates (sex and TST).

We also computed a similar prior sensitivity analysis for the younger and late middle-aged groups, taking into account a power of .8, an error rate α of .05

(not correcting for multiple testing in posthoc tests), a sample size of 453 young individuals allowed us to detect small effect sizes $f^2$ =.032 (confidence interval: .02 –.09; $R^2$ > .031, $R^2$ confidence interval: .015 –.084); while a sample size of 87 allowed us to detect medium-large effect sizes $f^2$ =.187 (confidence interval: .09 –.56; $R^2$ > .157, $R^2$ confidence interval: .082 –.358). There is limited published data on the precise effect sizes for quantitative sleep metrics and genetic associations in late middle-aged adults. However, the recent studies have used similar sample size in the analysis (Tsapanou et al., 2020) providing further support to the validity of our study results.

**Results**

Comparing each type of arousals between the two age groups, we observe that the number of arousals associated with a sleep stage transition, i.e. T+M+ and T+M-, significantly increased in the late middle-aged individuals (**p<0.005**), while those not associated with a stage transition, i.e. T-M- and T-M+, decreased in this age group (**p<0.0001, Table 5.1**). Besides, age, body mass index (BMI), depression, sleep quality, and chronotype scores were significantly higher in late middle-aged group compared to the younger group, whereas the younger group had a significantly longer total sleep time. In addition, women were largely under-represented in the younger group, due to the selection criteria of one of the studies composing the sample (see methods), while they represent a large majority of the late-middle-aged group. This constitutes an inherent limitation of the present study, even if sex is included as covariates in all statistical analyses.

***Arousal heterogeneity reflects different associations with PRS for AD***

Prior to seeking association between PRS and sleep metrics, we first assessed whether early PET Aβ burden and PRS for AD were correlated in late middle-

aged individuals, since only this group underwent Aβ assessment. We did not find a significant association between Aβ levels and PRS for AD (**Figure 5.2A;** Sperman's r=-.05; p=.64, and Sperman's r=-.08; p=.43, when including the three Aβ-positive individuals of the sample, **Suppl. Fig. S1**), supporting that PRS for AD is relatively independent of Aβ accumulation and grasps, at least in part, a distinct aspects of the AD-related risk as previously suggested by others (Ge et al., 2018; Luckett et al., 2022; Xicota et al., 2022).

We then targeted our primary objective in a GAMLSS assessing whether arousal number, as dependent variable, was associated with PRS for AD across the different types of arousals (T+/T- & M+/M-), including age group, sex and total sleep time (TST) as covariates. The model yielded a significant 4-way interaction between PRS for AD, arousal transition and EMG statuses and age group (t= 2.52; **p=.01**), indicating that the association between arousals and PRS varied based on transition and EMG status as well as age group. Besides, the statistical model yielded significant main effects of transition status, age group, sex, TST, as well as significant two-way interactions between transition and EMG status, transition status and age group, EMG status and age group, and three-way interactions between PRS, and EMG and transition status, and between EMG and transition status and age groups (**Table 5.2**). Adding the variables that significantly differed between the two age groups (i.e., BMI, depression, sleep quality and chronotype) as a covariate to this primary GAMLSS did not change the statistical outputs, indicating that the observed associations were unlikely to be driven by these group differences (**Suppl. Table S1**).

**Table 5.2. Association between arousals number and PRS for AD (N=540).**

| Independent variable | t | df | p-value | $R^{2*}$ |
|---|---|---|---|---|
| **PRS** | -0.80 | 1801.64 | 0.42 | - |
| **Transition status** | 41.43 | 1801.64 | **<.0001** | **0.958** |
| **EMG status** | -1.00 | 1801.64 | 0.32 | - |
| **Age group** | -7.15 | 1801.64 | **<.0001** | **0.028** |
| **Sex** | 14.94 | 1801.64 | **<.0001** | **0.110** |
| **TST** | 38.02 | 1801.64 | **<.0001** | **0.445** |
| **PRS*Transition** | 1.61 | 1801.64 | 0.11 | - |
| **PRS*EMG** | 1.75 | 1801.64 | 0.08 | - |
| **Transition*EMG** | 13.48 | 1801.64 | **<.0001** | **0.091** |
| **PRS* age group** | 0.78 | 1801.64 | 0.44 | - |
| **Transition * age group** | 23.91 | 1801.64 | **<.0001** | **0.241** |
| **EMG*age group** | -5.83 | 1801.64 | **<.0001** | **0.019** |
| **PRS*Transition*EMG** | -2.54 | 1801.64 | **0.01** | **0.004** |
| **PRS*Transition*age group** | -1.73 | 1801.64 | 0.08 | - |
| **PRS*EMG*age group** | -1.24 | 1801.64 | 0.22 | - |
| **Transition*EMG*age group** | -2.27 | 1801.64 | **0.02** | **0.003** |
| **PRS*Transition*EMG *age group** | 2.52 | 1801.64 | **0.01** | **0.004** |

PRS: polygenic risk score; TST: total sleep time; EMG: Electromyography

To understand what was driving the 4-way interaction, we computed a post hoc GAMLSS with PRS, as a dependent variable, and the interaction between the number of each arousal type and age group as covariate, including their interactions (4 two-way interactions) regressing out TST and sex. The model yielded a significant link between PRS and the interaction between T+M- arousals and age group (t=-2.39; **p=.02**) as well as a nominally significant link with the interaction between T+M+ arousals and age group (t=1.96; **p=.05**) on top of the main effect of T+M+ and T+M- arousals number (**Figure 5.2B-C, Table 5.3**). The other associations, including between PRS and interaction between T- M- and T-M+ arousals and age group, were not significant (**Figure 5.2D-E, Table 5.3**). Post hoc contrasts showed that there is a negative association between PRS and T+M+ arousals (t=-2.57; **p=.01**) as well as a positive association between T+M- arousals (t=3.09; **p=.003**) in the late middle-aged group but not the younger group (t=.22; p=.83 and t=.29; p=.77 respectively; **Figure 5.2B-C**). Hence, the post hoc analysis indicates that the significant 4-way interaction found in the primary analysis is driven by the association between PRS and both T+M+ arousals and T+M- arousals in the late middle-aged group.

Given the sex imbalance of our samples (particularly in the young sample), we further assessed sex differences and could not find evidence that they were contributing to our finding. Besides, we could not find indications that the association between PRS and T+M+ arousals in late middle-aged individuals was driven by arousals detected during REM or NREM sleep (according to ASSM criteria, arousals must be associated with muscle tone increase during REM, such that all M- arousal were only detected during NREM) but rather found that it is the total number of T+M+ in REM and NREM that was associated with the PRS for AD.

**Table 5.3.Association between PRS for AD and the interaction between each arousal type and age group (N=540).**

| Independent variable | t | df | p-value | $R^{2*}$ |
|---|---|---|---|---|
| **T+M+ arousals number** | -1.93 | 520 | **0.05** | **0.007** |
| **T-M+ arousals number** | 1.28 | 520 | 0.20 | - |
| **T+M- arousals number** | 2.49 | 520 | **0.01** | **0.012** |
| **T-M- arousals number** | -1.19 | 520 | 0.23 | - |
| **T+M+ arousals number *group** | 1.96 | 520 | **0.05** | **0.007** |
| **T-M+ arousals number *group** | -1.50 | 520 | 0.14 | - |
| **T+M- arousals number *group** | -2.40 | 520 | **0.02** | **0.011** |
| **T-M- arousals number *group** | 1.52 | 520 | 0.13 | - |
| **group** | -0.22 | 520 | 0.83 | - |
| **sex** | -0.11 | 520 | 0.91 | - |
| **TST** | -0.29 | 520 | 0.77 | - |

*Prior to the analysis, we removed the outliers among all variables by excluding the samples lying beyond four times the standard deviation.*

*TST: total sleep time.*

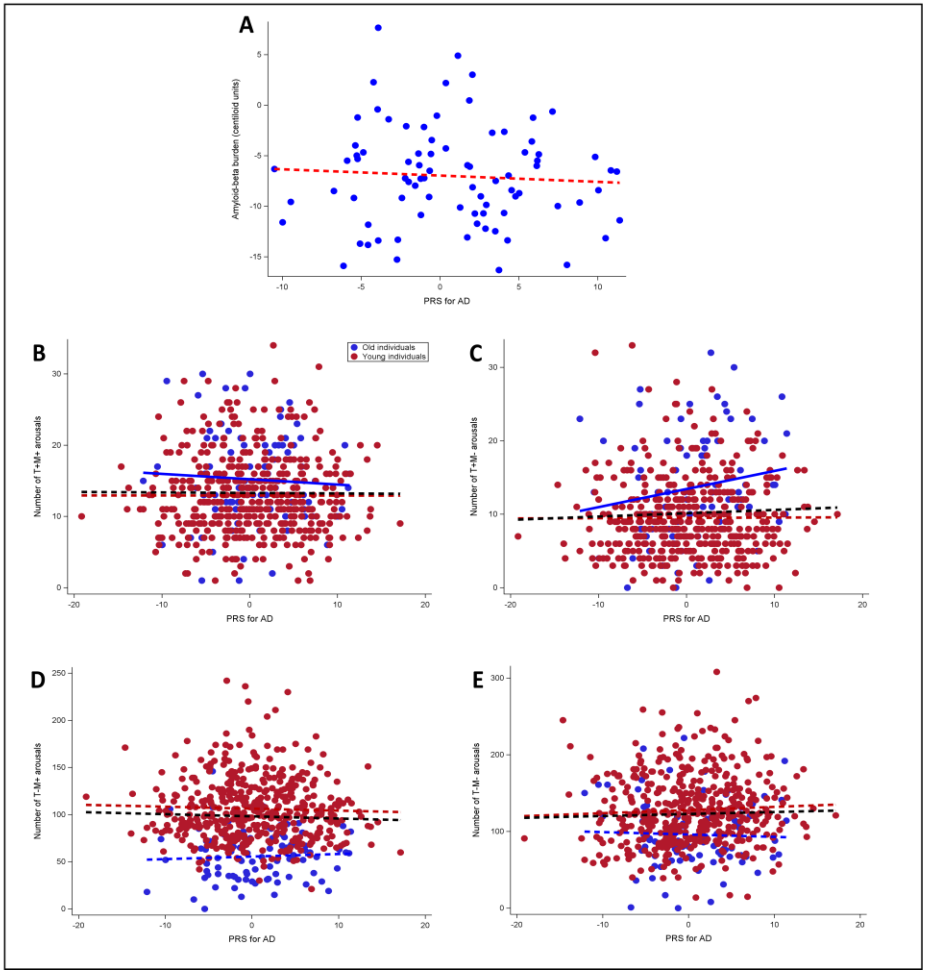


***Figure 5.2. Associations between PRS for AD and sleep arousals. (A)*** *Amyloid-beta burden and PRS for AD were not significantly correlated after excluding Aβ-positive individuals (p=0.64) (See Supplementary figure 1 for the association between PRS for AD and Aβ burden including Aβ-positive individuals).* ***(B)*** *Association between number of T+M+ arousals and the PRS for AD; age group by arousals interaction (p=0.05); post hoc analyses led to a significant negative association for the late middle-aged (p=0.01) but not the young group (p=0.83).* ***(C)*** *Association between number of T+M- arousals and the PRS for AD; age group by arousals interaction (p=.02); post hoc analyses led to a significant positive association for the late middle-aged (p=0.003) but not the*

*young group (p=0.77).* ***(D)*** *Association between number of T-M+ arousals and the PRS for AD; age group by arousals interaction (p=0.14).* ***(E)*** *Association between the number of T-M- arousals and the PRS for AD; age group by arousals interaction (p=0.13).*

*Simple regression lines are used for a visual display and do not substitute the GAMLSS outputs (see Table 5.3). The black line represents the regression irrespective of age groups (young + old). Solid and dashed regression lines represent significant and non-significant outputs of the GAMLSS, respectively.*

### *Arousals linked with PRS for AD are associated with cognitive performance and cognitive decline in late middle-aged individuals*

We then tested whether cognitive performance of the late middle-aged individuals was differentially associated with the 2 arousal types that showed opposite association with PRS for AD. The GAMLSSs for each time point included each cognitive domain as dependent variable (i.e. three GAMLSS at each time point) and T+M+ arousals and T+M-arousals as covariates together with sex, age, education and TST. Considering the baseline assessment, GAMLSSs yielded a negative association between attentional domain and T+M- arousals (t=-2.26; **p=.02**) and a positive association between attentional domain and T+M+ arousals (t=2.09; **p=.04**) on top of the effect of age and education (**Table 5.4**). There was no significant association between these arousals and the other cognitive domains (i.e. memory and executive functions; p>.10; **Table 5.4**, **Figure 5.3A-B, Suppl. Figure S2**). Adding PRS as a covariate to these models did not change the statistical outputs while PRS itself was not significantly associated with the cognitive performance (t<.003; p>.93).

We then focused on our first follow-up at 2 years that was completed in part of the late middle-aged individuals (N=66). The GAMLSSs led to a

significant negative association between T+M+ arousals and performance change over the memory domain, indicating that more T+M+ arousals at baseline are associated with reduced memory worsening over 2 years (t=-3.22; **p=.002**), on top of a main effect of age (**Figure 5.3C, Table 5.4**). The associations with the performance change in the other cognitive domains were not significant (p>.37; **Table 5.4**, **Suppl. Figure S3**). Adding PRS as a covariate to these models did not change the statistical output while PRS itself was not significant (t<.052; p>.09).

We further considered cognitive decline after 7 years that was assessed in a subset of the late middle-aged participants (N=64; 73% overlap with 2y follow up – see methods). GAMLSSs yielded a positive association between memory decline and T+M- arousals (t=2.33; **p=.02**), on top of the effect of education (**Table 5.4, Figure 5.3E**). Again, the associations with other cognitive domains were not significant (p>.37; **Table 5.4**, **Figure 5.3, Suppl. Figure S4**). Again also, adding PRS as a covariate to this model did not change the results while PRS itself was not significant (t<-.045; p>.16).

In a final step, we considered changes in the performance to the MST, as it is a memory test reported to be highly sensitive to early signs of cognitive decline (Marks et al., 2017). We found a significant negative association between MST performance decline after two years and T+M+ arousals (t=-2.42; **p=.02; Figure 5.3D, Table 5.4, Suppl. Figure S3**). Adding PRS as a covariate to this model did not change the results while PRS itself was not significant (t=-.001; p=.69). A subsequent GAMLSS also yielded a significant negative association between MST performance decline after seven years and T+M+ arousals (t=-2.90; **p=.01**) and a positive association between MST decline after seven years and T+M- arousals (t=2.11**p=.04**) on

top of a main effect of age (**Table 5.4**, **Figure 5.3F-G, Suppl. Figure S4**). Again, adding PRS as a covariate to this model did not change the results while PRS itself was not significant ($t<-.001$; $p>.70$).

Of note, none of the cognitive results survived false discovery rate (FDR) correction for the 18 tests performed (3 time points × 2 arousal metrics × 3 main cognitive domains), meaning these associations should be interpreted with caution.

**Table 5.4. Association between cognitive domains at each time point and T+M+ and T+M− arousals in late middle-aged individuals.**

| Cognitive assessment session | Cognitive domain (dependent variable) | T+M+ arousals number | T+M- arousals number | Age | Sex | Education | Total sleep time |
|---|---|---|---|---|---|---|---|
| **Baseline cognition** | **Attention (N=87)** | t(91)=2.09 **p=0.04** **$R^{2*}$= 0.046** | t(91)=-2.26 **p=0.026** **$R^{2*}$= 0.053** | t(91)=-2.18 **p=0.03** **$R^{2*}$= 0.050** | t(91)=1.24 p=0.22 | t(91)=2.14 **p=0.04** **$R^{2*}$= 0.048** | t(91)=1.07 p=0.29 |
| | **Memory (N=87)** | t(84)=-1.60 p=0.11 | t(84)=0.59 p=0.56 | t(84)=1.28 p=0.20 | t(84)=2.33 **p=0.02** **$R^{2*}$= 0.060** | t(84)=2.23 **p=0.03** **$R^{2*}$= 0.056** | t(84)=-0.41 p=0.68 |
| | **Executive function (N=87)** | t(89)=1.56 p=0.12 | t(89)=-0.69 p=0.49 | t(89)=-1.02 p=0.31 | t(89)=0.19 p=0.85 | t(89)=2.30 **p=0.02** **$R^{2*}$= 0.056** | t(89)=-0.04 p=0.97 |
| | **MST (N=87)** | t(89)=-1.85 p=0.07 | t(89)=1.28 p=0.20 | t(89)=-0.40 p=0.69 | t(89)=0.51 p=0.61 | t(89)=0.07 p=0.94 | t(89)=-0.06 p=0.95 |
| **Cognitive decline after 2 years** | **Attention (N=64)** | t(54)=0.06 p=0.95 | t(54)=-0.82 p=0.42 | t(54)=2.08 **p=0.04** **$R^{2*}$= 0.074** | t(54)=0.76 p=0.45 | t(54)=0.55 p=0.58 | t(54)=-0.62 p=0.54 |
| | **Memory (N=61)** | t(51)=-3.22 **p=0.002** **$R^{2*}$= 0.169** | t(51)=0.89 p=0.38 | t(51)=2.27 **p=0.03** **$R^{2*}$= 0.092** | t(51)=0.08 p=0.94 | t(51)=0.44 p=0.66 | t(51)=-0.40 p=0.69 |
| | **Executive function (N=65)** | t(55)=-0.27 p=0.79 | t(55)=0.12 p=0.91 | t(55)=-0.01 p=0.99 | t(55)=-1.11 p=0.27 | t(55)=-0.12 p=0.90 | t(55)=-1.36 p=0.18 |
| | **MST (N=64)** | t(56)=-2.42 **p=0.02** **$R^{2*}$= 0.094** | t(56)=1.29 p=0.20 | t(56)=-0.10 p=0.92 | t(56)=0.01 p=0.99 | t(56)=-0.07 p=0.95 | t(56)=1.33 p=0.19 |
| **Cognitive decline after 7 years** | **Attention (N=64)** | t(54)=0.46 p=0.65 | t(54)=-1.16 p=0.25 | t(54)=0.76 p=0.45 | t(54)=0.30 p=0.77 | t(54)=0.43 p=0.67 | t(54)=0.43 p=0.67 |
| | **Memory (N=60)** | t(50)=-0.85 p=0.40 | t(50)=2.33 **p=0.02** **$R^{2*}$= 0.098** | t(50)=1.89 p=0.06 | t(50)=2.03 p=0.05 | t(50)=2.67 **p=0.01** **$R^{2*}$= 0.125** | t(50)=-0.72 p=0.48 |
| | **Executive function (N=56)** | t(46)=-1.59 p=0.12 | t(46)=0.67 p=0.51 | t(46)=1.94 p=0.06 | t(46)=2.12 **p=0.04** **$R^{2*}$= 0.089** | t(46)=1.86 p=0.07 | t(46)=1.82 p=0.07 |
| | **MST (N=47)** | t(38)=-2.90 **p=0.01** **$R^{2*}$= 0.181** | t(38)=2.11 **p=0.04** **$R^{2*}$= 0.105** | t(38)=2.73 **p=0.01** **$R^{2*}$= 0.164** | t(38)=0.52 p=0.61 | t(38)=0.06 p=0.95 | t(38)=1.74 p=0.09 |

*Prior to the analysis, we removed the outliers among all variables by excluding the samples lying beyond four times the standard deviation (the final number of individuals included in each analysis is reported below each dependent variable).*

*MST: mnemonic similarity task.*

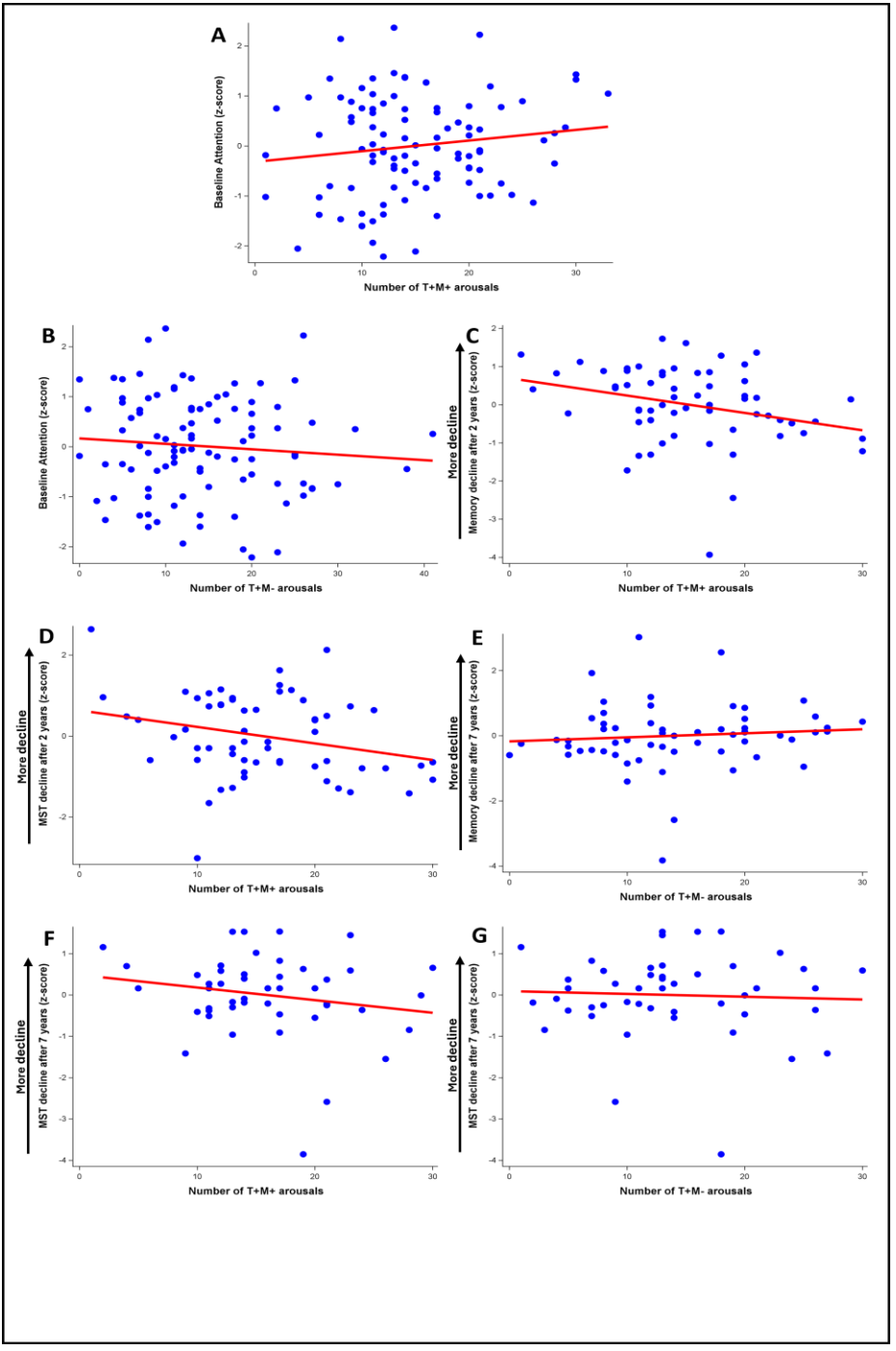
A
Baseline Attention (z-score)
Number of T+M+ arousals
B
Baseline Attention (z-score)
Number of T+M- arousals
C
More decline
Memory decline after 2 years (z-score)
Number of T+M+ arousals
D
More decline
MST decline after 2 years (z-score)
Number of T+M+ arousals
E
More decline
Memory decline after 7 years (z-score)
Number of T+M- arousals
F
More decline
MST decline after 7 years (z-score)
Number of T+M+ arousals
G
More decline
MST decline after 7 years (z-score)
Number of T+M- arousals

***Figure 5.3. Significant associations between sleep arousals and cognitive scores (A)*** *Significant positive association between the number of T+M+ arousals and baseline attention scores (p=0.03).* ***(B)*** *Significant negative association between the number of T+M- arousals and baseline attention scores (p=0.02).* ***(C)*** *Significant negative association between the number of T+M+ arousals and memory decline after 2 years (p=0.002).* ***(D)*** *Significant negative association between the number of T+M+ arousals and the MST decline after 2 years (p=0.01).* ***(E)*** *Significant positive association between the number of T+M- arousals and memory decline after 7 years (p=0.02).* ***(F)*** *Significant negative association between the number of T+M+ arousals and memory decline after 7 years (p=0.006).* ***(G)*** *Significant association between the number of T+M- arousals and MST decline after 7 years. Although the Spearman correlation was not significant and the positive association cannot be observed in the plot, after controlling for age, sex, education and total sleep time, the GAMLSS yielded a significant positive association between the number of T+M- arousals and MST decline after 7 years (p=0.04).*

*Simple regression lines are used for a visual display and do not substitute the GAMLSS outputs (Table 5.4). Solid regression line represent significant outputs of the GAMLSS.*

*See Supplementary figures S2-4 for the non-significant associations between sleep arousals and cognitive scores.*

## Discussion

Sleep arousals are commonly viewed as disruptive and leading to negative functional outcomes (Mahowald & Schenck, 2005). This likely arises in part from their increased prevalence with aging, their association with external disturbances (e.g. noise) or with hypopnea/apnea in patients with sleep disordered breathing (SDB). Arousals are also triggered spontaneously, i.e. without a concomitant disturbance, and these may serve beneficial purposes for brain functioning (Halasz & Bodizs, 2012). In addition, spontaneous arousal are heterogenous, likely in part because of differential activity of the LC (Foustoukos & Lüthi, 2025; Osorio-Forero et al., 2025), and this heterogeneity is associated with early Aβ burden (Chylinski et al., 2021). In

the present study, we determined whether spontaneous arousals are associated with the risk for developing AD and with cognition in healthy younger and late middle-aged individuals. We show that, depending on whether they are associated with a transition of sleep stage and with an increase in muscle tone, arousal can be associated with an increased or decreased polygenic risk for AD in late middle-aged, but not in younger adults. We further show that, in late middle-aged individuals, arousals heterogeneity is associated not only with current attentional performance but also bears predictive value for memory performance at 2 or 7 years. Although the effect sizes we observed were modest, if persistent, these small effects could influence lifelong health trajectories. These findings add to the growing literature showing that alterations in microstructural elements of sleep electrophysiology are associated with early AD neurobiology and precede the onset of AD symptoms (Chylinski et al., 2021, 2022; Ju et al., 2017; Sharon et al., 2025). They may contribute to establishing a marker of AD risk in otherwise healthy individuals.

We showed, in the same sample of late middle-aged individuals, that defining arousal based on stage transition and association with muscle tone was meaningful as arousal types differed in their oscillatory compositions, but – in absence of a younger group - could not report age related changes across the different types of arousals (Chylinski et al., 2021). Here, we report that, contrary to the general assumption, not all arousal types increase in the late middle-aged group. Only the number of arousals that are associated with sleep stage transition, and therefore most impinging on sleep continuity increase with age, while those not associated with these transitions become less common. This constitutes the first important observation that arousal heterogeneity is meaningful. In addition, it is precisely those arousals

increasing with age that we find associated with the PRS for AD. In other words, the extent of the age-related difference in T+ is linked to the genetic risk for developing AD.

We find that, in late middle-aged individuals, more T+M- arousals are associated with an increased PRS for AD, which is similar to the association between T+M- arousals and higher early amyloid burden we previously reported in the same sample (Chylinski et al., 2021). Arousals associated with sleep stage transition but not with a detectable muscle tone increase appear therefore to be related to more risk for developing AD both based on genetic and protein burden assessment. T+M- arousals may therefore reflect a chronic sleep disruption that contributes to, or is at least associated with, long-term neural vulnerability to AD-related processes. In line with this negative view, the amount of T+M- arousals is also associated with poorer attentional performance at baseline and memory decline at 7y (as shown using the global memory score and the exploration of the MST, a task sensitive to early hippocampal dysfunction (Marks et al., 2017), which supports the idea that attentional deficits may precede and potentially exacerbate memory deterioration over time. In contrast to T+M- arousals and still in the late middle-aged, we find that the amount of T+M+ arousals is associated with a reduced PRS for AD, better attentional performance at baseline and better memory performance at 2y and 7y (shown through both the global memory and MST scores at 2y and the MST score at 7y). Hence, these arousals would represent a better situation in terms of AD risk, associated with better attention processes which could favor subsequent better memory function.

According to our findings, the main feature differentiating negative and positive transition arousals is whether or not they are accompanied by

an increase in EMG tone. Since by definition an arousal that would exclusively consist of a muscle activation (without EEG activation) do not exist, arousals with muscle tone increase were proposed to represent stronger brain activation (Halász et al., 2004). Studies in mice showed that cortico-hippocampal coherence increases prior to and during arousals accompanied by EMG (dos Santos Lima et al., 2019). It is also shown that longer arousals that are accompanied by increased muscle tone are associated with the appearance of theta activity in the hippocampus (Jarosiewicz & Skaggs, 2004), while theta activity of hippocampus has been reported to be important for memory processing (Boyce et al., 2016).

Although multiple brain regions contribute to the regulation of arousal - for example an EEG-fMRI study in humans showed that the midbrain, thalamus, basal ganglia, and cerebellum, were activated during arousal while cortical regions were deactivated (Zou et al., 2020)-, the LC may be among the most important effector. Studies in animals showed that optogenetic activation of the LC, in the midbrain, causes arousals associated with EMG both during REM and NREM sleep, most often leading to full wakefulness (Carter et al., 2010). More importantly, spontaneous arousals in rodents were recently reported to align with variations in noradrenaline (arising from to LC activity) leading to two types of arousal (Foustoukos & Lüthi, 2025; Osorio-Forero et al., 2025). The LC appears to regulate arousal intensity, with most LC surges (70%) not leading to full wakefulness. Only stronger surges (30%) cause brief awakenings, while smaller ones induce partial arousal affecting the heart and thalamus but not the cortex (Osorio-Forero et al., 2025). The LC, and also potentially other subcortical structures, may underlie arousal heterogeneity, particularly those associated with EMG changes. According to our results these may be the most profitable, whether or not they are strong

enough to lead to a sleep stage transition (sleep stage being a somewhat arbitrary classification). In that respect T+M- arousal may represent incomplete arousal events in which the full arousal network is not recruited or arousal arising from a distinct set of brain regions and, according to our findings, would be associated with more deleterious outcomes.

We emphasize that in our previous work on arousal heterogeneity, we found that T-M+ arousals were associated with reduced Aβ burden and better attention performance (Chylinski et al., 2021), which is only partially in line with our current findings, where T+M+ are associated with lower PRS for AD and better attention. We may have lacked sensitivity to find positive associations with both T+M+ and T-M+ arousals across both analyses. The discrepancy may also arise from the fact that we used two different approaches that do not grasp exactly the same part of the risk for AD. Consistent with previous research (Leonenko et al., 2019), we indeed found no association between PRS for AD and Aβ burden. PRS may capture a broader genetic risk for AD and may be more predictive of disease progression than amyloid accumulation itself (Leonenko et al., 2019). In our earlier study, we interpreted the sleep-stage transition component as the key feature linking arousals to AD risk. Taken together, however, the two studies may suggest the following: while T– arousals may be associated with lower Aβ accumulation and T+ arousals with reduced genetic vulnerability, arousals accompanied by EMG activation—regardless of stage transition—consistently mark lower risk for future AD. This pattern supports the idea that motor-tone recruitment during arousals reflects a more complete and coordinated activation of the arousal system, which may have protective or compensatory value in maintaining cognitive function. One could speculate that M+ arousals offer recurring opportunities to transiently synchronize distant brain areas,

similarly to sleep spindles (Steriade, 2003). This warrant future investigation in a distinct, and ideally larger, sample of late middle-aged or older individuals.

Despite having a much larger sample, we could not isolate associations between arousal types and PRS in young adults. This could mean that it is only when brain function and sleep become more susceptible to challenges (Schmidt et al., 2012) that association between arousal and the biology of AD emerges in those that are more prone to develop AD. Sleep arousals would in turn not constitute a promising marker of AD risk in young adults. Yet in late middle-aged individuals, (T+M- and T+M+) arousals, and not the PRS for AD, were associated with cognitive changes over 2 or 7 years. Sleep microstructure could therefore constitute a promising early marker of future cognition and brain ageing trajectory that is more sensitive than risk assessment based on both genetics and amyloid beta burden assessments (Chylinski et al., 2021).

We acknowledge several limitations of our study. First, the number of participants who completed the follow-up cognitive assessment was relatively small (N= 66 and N = 64) and the number of participants that completed both follow-up assessment was even smaller (N = 48 – see methods). Our sensitivity at follow-ups was therefore lower and may have hindered other weaker significant associations such that our results have to be taken mostly in relative terms (stronger vs. weaker effect rather than presence vs. absence of effects). In addition, the memory composite and the MST scores were not consistently associated with T+M+ and T+M- arousals across the 2 follow-up assessments at 2 and 7y. It is rather the overall pattern of associations across both scores that is consistent with memory

performance changes being associated with arousal types. Whether this precise pattern reflects a lack of sensitivity or actual differences over different aspects of memory function is not known. Besides, the exclusion criteria applied were stringent and do not reflect the variability present in the general population. Although this guarantees that our findings are not biased by common age-related health issues, our participants exhibited minimal Aβ accumulation, with few individuals classified as Aβ-positive (N=3). In particular, since we did not included patients with SDB, our findings likely do not generalize to the perturbation-induced arousals, which have been associated with adverse behavioral (Aloia et al., 2004; Roehrs et al., 1994) and neurodegenerative outcomes (Ju et al., 2017). Furthermore, the predictive validity of PRS remains a subject of ongoing debate (Koch et al., 2023) and we cannot determine which participants will ultimately develop AD. Moreover, as already stated and even though it was controlled for, sex was not balanced within and across age groups while women exhibit distinct sleep characteristics (Eggert et al., 2021; J. Li et al., 2022), and are also more susceptible to AD (Andrew & Tierney, 2018). All these aspects limit the generalizability of our finding and warrants future investigations including larger and sex-balanced samples with more lenient exclusion criteria, e.g. including OSD and additional/more detailed memory performance assessments.

**Conclusion**

By leveraging EEG data and genotyping across a diverse age range, we provide novel insights into how subtle features of sleep architecture may reflect or interact with underlying genetic susceptibilities. We report that spontaneous arousals during sleep are not uniform but vary in their association with

genetic risk for AD and bears prediction value for future cognitive decline depending on their electrophysiological characteristics. These associations emerged only in older individuals, consistent with age-related vulnerability to subtle sleep disruptions. By distinguishing arousal subtypes, our study highlights sleep microstructure as a potential early marker of neurodegenerative risk during early aging. How arousal may interact with other features of sleep microstructure to contribute to AD risk remains to be assessed.

# Chapter 6: General discussion

## Preamble

Sleep is a fundamental biological process critical for cognitive, emotional, and physiological health. Beyond its restorative function, sleep plays an active role in memory consolidation, metabolic clearance, and neural plasticity (Assefa et al., 2015). The LC, the brain's primary source of norepinephrine, is emerging as a central modulator of sleep–wake dynamics. Besides, the LC is specifically vulnerable to AD pathology (Van Egroo et al., 2022). As early as during early adulthood, the LC is among the first brain region to accumulate hyperphosphorylated tau and with advancement of AD, it experiences significant neuronal degeneration (Braak et al., 2011; Jacobs et al., 2023). Age-related alterations in the activity of the LC and its connectivity with other subcortical regions, such as the hypothalamus, may underlie the variability in sleep features observed across the adult lifespan (Jones, 2020).

Despite the growing body of evidence implicating the role of the LC in sleep–wake regulation and AD in animals, human studies remain scarce, mostly because of the methodological challenges in measuring LC function and structure in vivo. Recent advances in ultra-high-field functional MRI (7T fMRI) and neuromelanin-sensitive imaging now permit unprecedented spatial resolution for quantifying LC activity and connectivity, offering new opportunities to bridge animal work with human sleep research (Priovoulos et al., 2018). Furthermore, the combination of imaging with electrophysiological and genetic measures allows researchers to explore how inter-individual differences in LC function and structure relate to sleep physiology and to the risk of developing AD.

The present thesis sought to address three complementary questions at the intersection of sleep, the LC, and AD vulnerability. First, we investigated

whether the activity of the LC during wakefulness predicts REM sleep features. Second, we explored the effective connectivity between the LC and hypothalamus, examining whether their connectivity relates to oscillatory characteristics of sleep. Finally, deviating from our initial plan to assess relationships between LC function and the polygenic risk of developing AD (due to insufficient sample size at the time of initiating the studies), we considered a large population-based dataset to determine whether sleep arousal subtypes—physiological markers mainly regulated by LC activity—are associated with polygenic risk scores for AD and with longitudinal cognitive decline.

By integrating high-resolution neuroimaging, polysomnography, and genetic data, this work aimed to clarify the role of LC in sleep architecture and AD risk across the adult lifespan. Importantly, these studies contribute to the growing recognition that sleep is not merely a passive state but a sensitive marker of brain health and a potential modifiable factor for delaying the onset of neurodegenerative diseases (Irwin & Vitiello, 2019). In this final chapter, we discuss the main findings from these three investigations, their methodological limitations, theoretical and clinical implications, and outline future directions for leveraging sleep as a biomarker and intervention target in aging and AD.

## Our main findings

### Integration of three experimental studies

Across the three experimental studies presented in this thesis, we build a stepwise case for the LC as a central hub linking LC wakeful neuromodulation, sleep regulation, and genetic vulnerability to AD.

First, we demonstrated that LC activity measured during wakefulness is predictive of specific sleep features in humans — notably REM theta energy and sigma power preceding REM onset. This provides direct evidence that LC reactivity during wake is related to sleep dynamics, aligning human neuroimaging data with decades of animal research, which showed the role of LC in sleep regulation (Aston-Jones & Bloom, 1981; Carter et al., 2010; Osorio-Forero et al., 2021). The observation that both positive and negative associations emerge, likely because of the variability in the tonic state of the LC suggests that an optimal LC tonic activity window exists for healthy REM expression, reinforcing an inverted-U model of LC impact on brain function, including during sleep.

Second, we report data to reinforce the view that the LC does not function in isolation but is most likely under control from other brain regions, notably the anterior-superior hypothalamus. In our second study, we showed that greater excitatory connectivity (positive connectivity values) was linked to reduced REM theta energy, whereas stronger inhibitory connectivity (negative connectivity values) was linked to increased REM theta energy in late middle-aged individuals, indicating a relationship between LC connectivity and sleep architecture that is affected by aging. This finding resonates with animal studies showing that the preoptic nuclei regulate LC activity and help to prevent LC overactivation, which could otherwise impair sleep (Lu et al., 2002; Mondino et al., 2025). Together, these data suggest that LC activity is not a static trait but is dynamically tuned by different inputs such as hypothalamic input, which could explain age related differences in sleep quality.

Finally, we showed that arousal characteristics — increasingly regarded as an indirect measure of LC function (Osorio-Forero et al., 2025)— are linked to

genetic risk for AD and to both cognitive performance and longitudinal cognitive decline. Specifically, arousals leading to sleep stage transition without motor activation (T+M–) were associated with higher AD genetic risk and lower attention and more longitudinal memory decline, whereas arousals leading to sleep stage transition with motor activation (T+M+) were related to lower AD risk and transiently higher attention and less memory decline over time in old individuals. These findings may suggest that the qualitative pattern of LC-mediated arousals may serve as an early biomarker of neuromodulatory health and AD vulnerability.

Taken together, these findings further position the LC as a crossroad between sleep and AD in humans, a role that has already been proposed in animal models (Martin et al., 2024). The LC is among the first brain regions to accumulate tau pathology (Braak et al., 2011) and its degeneration is hypothesized to be a key trigger of sleep–wake dysregulation that precedes cognitive decline (Van Egroo et al., 2022). Our work extends this framework by showing that LC activity and its network interactions, which can be measured in vivo with ultra-high-field MRI during wakefulness and also arousals in sleep, are meaningfully related to sleep oscillations and genetic risk for AD in humans. Figure 6.1 shows an integrated view from three studies.

A natural next step is to test whether interventions targeting LC activity or its hypothalamic control can normalize sleep microstructure and potentially reduce trajectories of cognitive decline. Future work should integrate LC-sensitive fMRI, detailed sleep phenotyping, and AD genetic risk assessment within the same cohort rather than in separate studies, to clarify whether LC dysfunction is a driver or a downstream consequence of sleep disruption and

neurodegenerative processes. Longitudinal, multimodal protocols — combining ultra-high-field MRI, home EEG monitoring, CSF or PET biomarkers, and genetic risk stratification — will be essential to map how LC activity, sleep physiology, and AD pathology interact across the adult lifespan.

Clinically, these findings highlight the potential of sleep-based biomarkers to serve as early indicators of LC dysfunction and AD vulnerability. Quantitative EEG markers—such as REM theta power, sigma power prior to REM, and specific microarousal patterns—could be incorporated into risk screening protocols for aging populations. Interventions aimed at optimizing REM quality, stabilizing arousal dynamics, and preserving LC integrity — through noradrenergic pharmacological modulation, non-invasive neuromodulation, or personalized behavioral strategies — may ultimately improve sleep health and delay neurodegenerative progression.

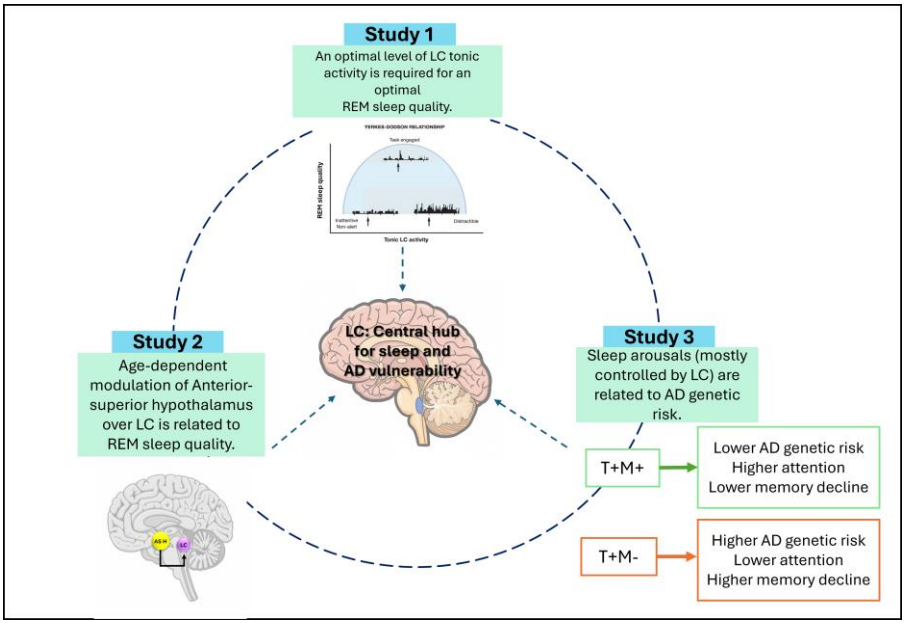

***Figure 6.1. The Locus coeruleus as a crossroad between sleep regulation and Alzheimer's disease risk: An integrated view from three studies***

*This figure summarizes the key findings from the three experimental chapters of this thesis and integrates them into a unified framework. Together, these findings suggest that the LC is not only a regulator of sleep–wake dynamics but it could be a potential mediator of the relationship between sleep disruption and AD risk.*

*LC: Locus coeruleus; AD: Alzheimer's disease; REM: Rapid eye movement sleep.*

## Study I – Balanced tonic activity of the locus coeruleus is associated to sleep

*Age-specific theta energy association in the low tonic task*

The finding that REM theta energy was associated with LC activity during the low tonic (bottom-up) task only in older individuals may reflect age-related changes in LC integrity. The LC is one of the earliest sites of tau pathology in aging and AD (Braak & Del Tredici, 2012). Although it is not yet established whether tau pathology impairs phasic or tonic LC activity directly, both animal and human data suggest that tau and Aβ may increase neuronal or cortical excitability (Targa Dias Anastacio et al., 2022). For example, one study showed that higher tau accumulation and neuroinflammation (the radioligand used was relatively unspecific) in the brainstem (including the LC) assessed by PET was associated with increased cortical excitability in healthy middle-aged adults (Van Egroo et al., 2021). Our results show that in older adults, tau accumulation could result in reduced flexibility of LC output, particularly in bottom-up contexts where tonic activity is naturally low. Consequently, the phasic LC activity in older individuals during low tonic tasks may disproportionately influence downstream sleep-related oscillations, such as theta power during REM, which is tightly linked to hippocampal function and

memory consolidation (Boyce et al., 2016). Younger individuals, with more intact LC circuitry, may maintain robust phasic responses regardless of baseline tonic levels, rendering the association less detectable.

Another key factor in this age-specific relationship may be galanin, a neuropeptide co-released with NE in the LC. Galanin is thought to be preferentially released during phasic bursting and provides a local inhibitory brake that reduces subsequent LC firing and NE tone (Weinshenker & Holmes, 2016). This negative feedback is critical for resetting LC activity and maintaining the ability to transition between sleep states (Weinshenker & Holmes, 2016). With aging, galanin expression and receptor function may decline (Murray et al., 2010), leading to impaired phasic-mediated inhibition of LC neurons. As a result, older individuals with higher LC phasic activity when tonic activity is low during wake may fail to achieve complete NE suppression during REM sleep because the system cannot effectively switch off, resulting in reduced REM theta power. In younger adults, robust galaninergic signaling may buffer phasic LC bursts and decouple waking LC activity from subsequent REM dynamics, thereby protecting REM theta expression. This interpretation highlights a potential role for galanin in maintaining healthy sleep–wake regulation across the lifespan. Future studies could confirm that reduced galaninergic tone contributes to age-related REM theta reduction.

*Broad theta energy association in the high tonic task*

In contrast, the association between LC activity and REM theta energy during the high tonic (top-down) task was observed across both age groups, suggesting that top-down tasks may engage LC circuitry in a way that preserves adaptive phasic responses. Importantly, phasic LC responses are

strongest when tonic activity is moderate rather than excessively high or low (Aston-Jones & Cohen, 2005). Our findings may therefore reflect that the high-tonic task engages the LC in this optimal regime, allowing robust phasic bursts that are linked to stronger REM theta power and potentially more effective memory-related processing during sleep. Experimental studies that directly manipulate LC activity will be needed to confirm whether such task engagement causally influences subsequent sleep oscillations.

*Sigma power prior to REMS in high tonic task*

We found that the association between sigma power prior to REM sleep and LC activity emerged only when LC activity was measured during the high-tonic (top-down) task, across the full sample. Sigma oscillations are thought to reflect sleep stability and gating mechanisms, and their enhancement may require a threshold level of LC activation (Weiner & Dang-Vu, 2016). Moreover, sigma power prior REM sleep facilitates smoother transitions into REM and supports the complete silencing of LC neurons during REM (Osorio-Forero et al., 2021)—a prerequisite for robust theta expression. Our results suggest that the feature of LC function captured in the high-tonic task is most predictive of this preparatory sigma activity. By contrast, LC activity measured during the low-tonic (bottom-up) task showed no such association, possibly reflecting different aspects of LC physiology that are less related to sleep spindle generation.

*An inverted U-shaped relationship*

We found a significant positive correlation between LC activity estimate during the salience detection task (low-tonic) and squared LC activity estimate in the perceptual rivalry (high-tonic) task. This suggests an additional

dimension worth considering, which is the possibility of non-linear, inverted U-shaped relationships between LC activity and REM theta energy. The LC-NE system shows an inverted-U relationship with attentional performance: very low tonic LC activity is associated with drowsiness and poor responsiveness, moderate tonic activity supports optimal performance through robust phasic responses to task-relevant stimuli, and very high tonic activity leads to hyperarousal and reduced phasic responsiveness, impairing performance (Aston-Jones et al., 1999). Applied to our data, this framework suggests that the LC trait reflected by a strong phasic response over a very low tonic baseline may indicate a dysregulated or less flexible LC profile. If such a profile also persists during sleep, it could hinder the complete silencing of LC neurons that is necessary for robust REM theta expression. In contrast, individuals showing higher LC activity in the high-tonic (top-down) context may have a more balanced tonic–phasic profile, which—if similarly expressed during sleep—could support well-timed LC quiescence and healthier REM theta generation. Thus, our results point toward a state-dependent and potentially non-linear association between LC tonic activity and REM sleep quality.

*Integrative Perspective*

These findings underscore the importance of contextual LC engagement in shaping sleep architecture. Aging appears to amplify the sensitivity of REM-related oscillations to LC activity, particularly in low-tonic contexts. The pattern observed across tasks suggests that a more appropriately tuned LC response may support the maintenance of REMS quality and partially offset age-related vulnerabilities. Future studies should explore whether tau burden within the LC modulates these associations, and whether task-based LC

activation could serve as a therapeutic lever to enhance sleep in aging and neurodegenerative conditions.

**Study II: Hypothalamus-locus coeruleus connectivity is related to sleep**

*LC-hypothalamus effective connectivity in humans*

In this study, we present novel evidence of the bidirectional influence between the anterior–superior and posterior subparts of the hypothalamus on the LC in humans. This connectivity has already been well-documented in animal studies (Giorgi et al., 2021; Szymusiak et al., 2007) but not in humans. Network-level analyses in humans are scarce, primarily due to the challenges inherent in non-invasive brain imaging. However, optogenetic techniques in animal models have provided insights into the LC's extensive projections. Our findings support the feasibility and relevance of investigating LC-hypothalamic interactions in humans.

This study represents an initial attempt to explore the LC-hypothalamic network in humans. The observed connectivity provides a compelling basis for further research using advanced neuroimaging techniques to map these interactions more comprehensively. Future studies should aim to replicate and extend these findings, employing methodologies such as fMRI and magnetoencephalography (MEG) to validate and refine our understanding of LC-hypothalamic connectivity.

*Task-specific association between hypothalamus activity and REM theta energy*

The reason for observing associations between the anterior-superior and posterior hypothalamus activity and REM theta energy during the perceptual rivalry task and not the salience detection task is likely due to the difference

in the nature of the two tasks. The perceptual rivalry task involves sustained top-down attentional engagement and perceptual ambiguity, requiring the brain to maintain tonic alertness and sustained cognitive processing over time. This type of processing may involve the hypothalamus's regulation of arousal and attentional states, including the anterior superior and posterior subparts (Szymusiak & McGinty, 2008). In contrast, the salience detection task is more phasic, characterized by bottom-up processing of salient stimuli and brief, transient responses to unexpected stimuli. This type of task likely involves more transient phasic engagement of systems like the LC-NE (Koshmanova et al., 2023; Mortazavi et al., 2025) and less sustained hypothalamic involvement compared to the other task. The association that we observed between REM theta energy and hypothalamic activity during the perceptual rivalry task, which requires more tonic activity is in line with animal studies showing that theta generation originates in tonic firing of reticular formation, which projects to hypothalamus. The hypothalamus may not only integrates this tonic input but also translates it into phasic output that then impinges on hippocampal regions, where the field oscillatory theta rhythm is recorded (McKenna et al., 2017). Our results highlight that REM theta energy is associated with LC activity during the top-down and high tonic task, which require hypothalamic contributions to maintain tonic arousal and resolve perceptual ambiguity.

*Association between hypothalamus subparts activity and REM theta energy*

We only observed statistical trends in the relationship between the anterior-superior and posterior hypothalamus activity during the perceptual rivalry task and REM theta energy. However, in this section of the general discussion,

we want to provide putative interpretations of these trends as they may provide “food for thoughts” for future investigations.

The negative association between REM theta energy and anterior superior hypothalamus activity may align with prior research, which indicates that the activation of neurons in the preoptic area promotes NREM sleep and not REM sleep (Zhang et al., 2015). If the activity of this region is also lower during REM sleep —when the drive to initiate NREM sleep is reduced—, it may allow higher theta power suggestive of more intense REM sleep. On the other hand, the potential positive correlation between REM theta energy and posterior hypothalamus activity may suggests a relationship driven by the activity of REM sleep-promoting MCH neurons (Adamantidis & de Lecea, 2023). In other words, the neurons responding to the task during wakefulness, whether orexinergic, histaminergic or MCH neurons, would be associated with a larger activity of the MCH neurons during sleep. This would in line with previous studies showing that MCH neurons in the lateral hypothalamus are involved in the generation of REMS theta rhythm (Adamantidis & de Lecea, 2023). On top of a potential weak recruitment by the task, the lack of statistical significance in the results may be due to the heterogeneous mix of neuron types in this region. Future studies should focus on isolating the MCH neuron population to test our assumption. The lack of association between REM theta energy and other subpart of hypothalamus suggest that the task we used may not have been suitable for assessing the activity of hypothalamus subparts. It could also suggest that although some of these regions are involved in circadian rhythm regulation and arousal (Saper & Lowell, 2014), their activity might not directly correlate with the REM theta energy.

*Integrative Perspective*

The age-related changes observed in this study, where stronger connectivity from the anterior hypothalamus to the LC was associated with reduced REM theta energy in older adults, may reflect a compensatory mechanism. This interplay underscores the complexity of sleep regulation, where multiple neural circuits interact to balance the need for arousal and sleep. Disruptions in this balance, such as those occurring with aging, could lead to sleep disturbances and cognitive decline.

**Study III: Sleep arousals are linked to genetic risk for AD and cognitive decline**

*Rethinking sleep arousals: Adaptive vs. maladaptive arousals*

Our results further challenge the conventional view of arousals as uniformly disruptive and opens the door to a more nuanced understanding of their role in brain health. T+M+ arousals, which were linked to lower genetic risk for AD and better cognitive trajectories, may reflect a more integrated connectivity pattern between the LC, thalamus, and cortex in a coordinated fashion. This could be beneficial for sleep and putatively facilitate memory consolidation, synaptic homeostasis, or glymphatic clearance during sleep. Conversely, T+M– arousals may represent incomplete or dysregulated arousal events, possibly reflecting impaired LC-noradrenergic signaling due to tau-related degeneration in the LC. Their association with higher AD genetic risk and long-term memory decline suggests that these arousals could be early indicators of neurodegenerative vulnerability.

*Arousals, Aβ burden and PRS for AD*

The lack of significant association between Aβ burden and PRS for AD may appear counterintuitive. We remind first that it is in fact consistent with

previous research (Leonenko et al., 2019) and supports that these two measures reflect distinct aspects of AD risk. In cognitively healthy individuals, PRS is not associated with baseline Aβ burden (Ge et al., 2018; Luckett et al., 2022; Xicota et al., 2022) but can predict Aβ accumulation in longitudinal studies (Luckett et al., 2022). Although Aβ is necessary for AD development,, it is not sufficient to cause clinical symptoms and has low specificity for predicting AD (Musiek & Holtzman, 2015), which may explain why some clinically normal older adults show high Aβ levels without signs of AD (Dubois et al., 2016). This discrepancy has led researchers to propose that AD may unfold in two distinct stages: amyloid dependent and amyloid independent. APOE affects amyloid deposition and that the PRS affects conversion from amyloid positivity to AD. Therefore, in the context of the amyloid cascade hypothesis, APOE acts prior to amyloid deposition and the remaining genetic risk factors identified through GWASs act between amyloid deposition and clinical onset of AD (Ge et al., 2018; Leonenko et al., 2019; Mishra et al., 2018). Hence, PRS predict progression to AD over and above APOE (Leonenko et al., 2019). Moreover, changes in Aβ accumulation over time tend to be minimal when baseline Aβ levels are low and individuals are Aβ-negative. In contrast, greater changes are typically observed in individuals who are Aβ-positive, indicating that most accumulation occurs above the threshold for Aβ positivity (Jack Jr et al., 2013; Leonenko et al., 2019). Therefore, in a sample like ours—composed of cognitively unimpaired individuals, with only three classified as Aβ-positive— (Chylinski et al., 2021) Aβ is unlikely to accumulate significantly over time.

Our group previously studied the association between the same types of arousals and Aβ burden in the same group of older participants we analyzed (Chylinski et al., 2021). Our finding regarding the positive association between

T+M- arousals and genetic risk for AD is in line with the previous study showing positive link between this type of arousal and Aβ accumulation in old individuals (Chylinski et al., 2021). Together, these results suggest that T+M– arousals may represent a physiological phenotype reflecting early LC–NE system dysregulation that contributes both to Aβ accumulation and to a genetic vulnerability pathway that promotes conversion from amyloid positivity to clinical disease. By contrast, while the previous study (Chylinski et al., 2021) found an inverse association between T–M+ arousals and Aβ burden, we observed no link between this arousal type and PRS for AD, suggesting that their relationship with AD pathology may be limited to the amyloid deposition phase and not to progression beyond amyloid positivity. Alternatively, we may have lacked the sensitivity to detect positive associations with both T+M+ and T–M+ arousals in either analysis. Interestingly, we found that T+M+ arousals were associated with lower genetic risk for AD, raising the possibility that more robust arousal responses, involving both thalamocortical and motor activation, may represent a protective or compensatory mechanism that maintains sleep–wake stability in the face of emerging neuropathology. It is worth noting, however, that arousals classified as M– in our study may not be entirely devoid of muscle activity, as subtle tone changes in muscles not captured by our EMG montage could still occur. This highlights the importance of carefully considering EMG detection sensitivity when interpreting the physiological significance of arousal subtypes.

*Integrated perspective*

In summary, our findings suggest that sleep arousals are not uniformly pathological but may represent distinct physiological signatures of

vulnerability or resilience to AD. These results highlight the importance of differentiating arousal subtypes when assessing sleep health in aging and support their potential as early biomarkers and targets for interventions aimed at preserving cognitive function.

## Methodological limitations

Aside from the limitations that are already mentioned in the experimental studies chapters, there are some other limitations to consider.

The selection of participants and the criteria for their exclusion are points of concern in this study. As with many studies, selection bias poses a significant challenge. A number of potential participants may have opted out, possibly due to the recruitment methods, which included word of mouth, university advertisements, and city-organized fairs, such as those in Liège. Our samples involved individuals, who had at least a secondary school diploma. This indicates that individuals with lower educational attainment are underrepresented. Furthermore, the study protocol required considerable commitment, which meant participants needed to be relatively fit and available. It is well established that individuals with lower socio-economic status are often harder to recruit and typically constitute only a small fraction of research samples (Henrich et al., 2010).

Additionally, participants were largely free from many established risk factors for cognitive decline and AD, such as hypertension, midlife obesity, or smoking (Norton et al., 2014). This approach aimed at controlling variables that could influence the relationships between our metrics of interest. However, because our participants represent only a small, selective segment of the population, the generalizability of our findings to the wider population

is limited. Replication of these results in a more inclusive sample would therefore be necessary. Nonetheless, observing these relationships in a sample with relatively low variability is noteworthy. It is possible that the microstructural features we examined may have been different in the general population.

The cross-sectional design of our studies is an inherent limitation. Longitudinal assessments were only done for cognitive data in experimental chapter 3. However, longitudinal assessment of sleep characteristics, as well as LC function and its connectivity with other sleep-related brain regions, would be particularly valuable, as changes in sleep or LC may precede alterations in each other. Moreover, the cross-sectional nature of our data prevents us from drawing conclusions about how these features evolve across the lifespan. Future longitudinal studies with repeated sleep EEG, MRI, and PET measurements are needed to clarify how sleep and LC interact, how they may influence AD, and how changes in these measures might affect cognition over time.

Importantly, in Chapter 5 we focused on cognitive decline. Although this follow-up covers the main cognitive domains, it should be noted that the interval between the neuropsychological evaluations was relatively short (2 years and 7 years), especially for healthy individuals in late midlife, who are expected to show only minimal changes compared to baseline. Moreover, it is not possible to predict whether individuals in our cohort will develop AD. Hence, Extending the longitudinal assessment over a longer period would therefore be highly valuable.

Considering that tau pathology is thought to emerge very early in the LC (Braak et al., 2011), an important aspect we did not investigate in the present

study is the potential relationship between sleep and the development of tau neurofibrillary tangles (NFTs). Future studies combining longitudinal sleep assessment with quantitative measures of LC activity and connectivity with tau burden would help clarify whether sleep disturbances we observed in this study are simply a consequence of early tau accumulation in the LC or not.

Beyond these points, several additional limitations deserve mention. First, although we used neuromelanin-sensitive structural imaging and pupillometry in combination with ultra-high-field (7T) fMRI to maximize LC specificity, the LC remains a very small brainstem nucleus. Its proximity to the fourth ventricle further increases susceptibility to noise, including partial-volume effects, physiological artifacts, and cerebrospinal fluid pulsations. Variability in brainstem vascular dynamics, cardiac and respiratory artifacts, or BOLD hemodynamic responses may still introduce measurement error and could obscure subtle associations. Future studies could benefit from physiological noise correction pipelines optimized for the brainstem to further enhance signal specificity.

Second, while our interpretation of arousals as reflecting LC dynamics is grounded in prior animal work (Osorio-Forero et al., 2025), these measures are still indirect. Micro-arousals are influenced by multiple neuromodulatory systems, including cholinergic and serotonergic (Halász et al., 2004; Kaur et al., 2020) pathways, as well as midbrain, thalamus, basal ganglia, and cerebellum activation and cortical regions deactivation (Zou et al., 2020), which we did not measure directly. Future multimodal studies combining EEG, LC-fMRI, and simultaneous autonomic measures — or ideally PET imaging of multiple neurotransmitter systems — could help disentangle the unique contribution of LC–NE activity from other arousal-promoting circuits.

Finally, our studies focused primarily on REM and NREM oscillations power and micro-arousal phenotypes. Other sleep features such as K-complex dynamics, autonomic indices (e.g., heart rate variability), and other sleep related features were not examined, even though they may also be modulated by LC activity and could provide complementary insight. Including these markers in future studies would allow for a more comprehensive mapping of LC influence across the sleep–wake cycle.

## Future directions and clinical implications

Although this thesis examined the association between sleep and LC function separately from the association between LC-regulated arousals during sleep and AD risk, future work in our laboratory will integrate these approaches. Specifically, my study (ASLEEP) will be combined with the larger IRONSLEEP project to provide a more comprehensive dataset. This integration will allow to investigate, in a larger and more statistically powerful sample, how AD genetic risk, LC function, and sleep architecture interact with each other in the same study.

Most research on the LC in animals and humans has examined its activation as a whole, rather than distinguishing between its left and right sides (e.g., (P. R. Murphy et al., 2014; Vazey et al., 2018)). In Chapter 3, we present in vivo evidence of lateralized LC activation, with stronger activity observed in the left LC. While these findings may indicate a lateralization effect, it is important to consider that such differences could also arise from methodological factors, such as coil sensitivity or hardware bias, which can disproportionately affect small regions like the LC. Nevertheless, these results highlight the potential importance of analyzing the left and right LC separately in future studies. Only a few studies have examined LC lateralization in humans or

animals. For example, a recent study shows that neuronal density along the caudal–rostral axis of the LC is asymmetric in individuals with AD pathology (Beckers et al., 2024), emphasizing the potential relevance of lateralization for both basic and clinical research.

Future studies would benefit from combining in vivo and ex vivo examinations in both healthy individuals and those with AD to establish a comprehensive picture of LC involvement across the disease continuum. Longitudinal assessments of LC structure and function, together with repeated measures of sleep, could clarify whether deviations in LC activity precede changes in sleep microarchitecture and predict later cognitive decline. Multimodal protocols integrating 7-Tesla neuromelanin-sensitive MRI, functional LC activation measures, polysomnography, and tau- and amyloid-PET imaging would be particularly valuable for determining whether LC-related sleep alterations occur prior to detectable neurodegenerative pathology. Following participants' deaths, these longitudinal in vivo data could be correlated with post-mortem LC neuronal density and tau burden, providing a direct histopathological link between lifetime LC activity, sleep physiology, and neurodegeneration.

In parallel, it would be informative to compare microarousal characteristics and LC imaging biomarkers between individuals with AD and cognitively healthy controls. Such comparisons could clarify whether the LC signatures and arousal patterns observed in healthy participants represent early biomarkers or downstream effects of pathology. From a clinical perspective, combining LC structural and functional measures with sleep microarchitecture could yield a multimodal biomarker framework for early diagnosis, risk stratification, and patient selection for clinical trials.

Importantly, the second study revealed that effective connectivity between the anterior hypothalamus and LC during wakefulness is associated with variations in REM and NREM sleep oscillatory dynamics, particularly in older adults. Future work should investigate whether other regions within the sleep-regulating network, in addition to the hypothalamus–LC pathway, contribute to these oscillatory dynamics.

In Chapters 3 and 4, LC activity and connectivity were measured during morning wakefulness, and their associations with sleep at night were assessed under the assumption that daytime LC activity reflects its activity during sleep. Future studies could improve on this design by using simultaneous EEG and MRI recordings at night to directly investigate whether the associations observed in this study during wakefulness are also present during sleep. Although earlier studies at 3T by using simultaneous EEG and fMRI have reported sleep-related activity in the pontine tegmentum—including the LC (Dang-Vu et al., 2008; Schabus et al., 2007)—the limited spatial resolution of conventional 3T imaging precludes precise localization to the LC. Ultra-high-field MRI (7T) combined with sleep EEG would allow more accurate mapping of LC activity and its temporal coupling to sleep oscillations, thereby providing a stronger mechanistic link between LC function and sleep microstructure.

Dysfunction of the LC-NE system and sleep is a feature of AD and age-related cognitive decline. Developing non-invasive interventions that manipulate sleep-wake regulation or modulate LC-NE activityrepresents a crucial step toward establishing effective therapeutic strategies for AD. For instance, transcutaneous vagus nerve stimulation has been suggested to enhance LC-NE function and improve subjective sleep quality in older adults (Bretherton

et al., 2019). However, assessments of its long-term impact on sleep-wake patterns and potential synergy with other interventions—such as sleep hygiene education or cognitive behavioral therapy for insomnia—are still lacking.

In chapter 5, the identification of specific microarousal subtypes linked to genetic risk for AD and cognitive decline points toward a novel electrophysiological signature of early neurophysiological disruption. Future studies should examine whether modifying arousal dynamics—through interventions such as auditory closed-loop stimulation, autonomic regulation techniques, or orexin system modulation—can alter cognitive trajectories in at-risk individuals (De Luca et al., 2022). Furthermore, integrating polygenic risk scores, LC functional measures, and sleep microarchitecture into predictive models could yield highly sensitive, multimodal biomarkers for preclinical AD detection. Moreover, integrating arousal metrics into wearable sleep technologies could enable real-time monitoring of brain health, offering a scalable tool for early detection and intervention in AD.

In the context of AD, further animal studies investigating how glymphatic clearance of toxic proteins interacts with fluctuations in LC-NE activity across the sleep-wake cycle could shed light on mechanisms connecting LC-NE dysfunction to AD pathogenesis via sleep-wake disturbances. Supporting this notion, in mice models, it has been demonstrated that elevated norepinephrine levels impede glymphatic function by reducing interstitial space volume (Xie et al., 2013), although LC-NE activity was not directly assessed in that study. Likewise, existing optogenetic studies that manipulate LC-NE activity across the sleep-wake cycle have not examined its effects on glymphatic clearance or the long-term consequences of chronic LC-NE

photoactivation or photoinhibition on Aβ or tau accumulation in AD mouse models. In humans, robust evidence and reliable methods for measuring a sleep-dependent glymphatic clearance system are still lacking, though a recent study provided support for a temporally ordered sequence of events during sleep linking slow-wave activity to cerebrospinal fluid flow mediated by hemodynamic oscillations (Fultz et al., 2019).

## Conclusion

This thesis brought me to the intersection of sleep physiology, LC, and AD risk—a scientific crossroad that I have found endlessly fascinating. Working on this project has been both challenging and inspiring: it required bridging complex neuroimaging, electrophysiology, and genetics, but in doing so, it revealed how tightly interconnected sleep and brain health truly are. One of the most exciting aspects of this journey was the opportunity to study the human LC in vivo and to show that its functional state during wakefulness is meaningfully linked to sleep microstructure. The realization that this small brainstem nucleus may sit at the heart of a network connecting arousal, sleep, and pathology has been a constant source of motivation.

Looking back, this work has deepened my appreciation for the complexity of sleep as a window into brain function. The LC, once thought of mainly as a stress or arousal hub, now appears to be a subtle regulator whose balance may help protect cognitive health across the lifespan. Conducting this research has strengthened my belief that sleep is not only a biomarker but also could be a potential therapeutic lever for neurodegenerative disease prevention.

On a personal level, this project has been a formative scientific experience—demanding rigorous thinking, technical innovation, and collaboration across disciplines. It has been a privilege to contribute to a field that is still in its early days and to help pave the way toward a more integrated understanding of sleep, arousal, and brain aging. Unraveling the mysteries of the LC and its role in sleep will remain one of the most exciting frontiers in neuroscience, and I am eager to see where future studies will take us.

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# Chapter 7: Appendices

# Appendix 1: Supplementary material for the paper presented in Chapter 3

## Supplementary Methods

*Participant*

Due to a miscalculation at screening, 1 older participant had a BMI of 30.9 and one of the younger participants had a BMI of 28.4. Since their data do not deviate substantially from the rest of the sample these participants were included in the analyses (including BMI as a covariate in our statistical models did not modify our results).

*Protocol*

The evening before the baseline night, participants arrived at the laboratory 3 hours before their habitual bedtime, completed questionnaires including Beck Depression Inventory (BDI)(Beck, Steer, et al., 1988), Beck Anxiety Inventory (BAI)(Beck, Epstein, et al., 1988), the Pittsburgh Sleep Quality Index (PSQI)(Buysse et al., 1989), and Epworth sleepiness scale (ESS)(Johns, 1991) for assessing depression, anxiety, sleep quality, and sleepiness, respectively.

The procedures for the baseline night recordings were identical in older and younger participants. Prior to the fMRI session, older participants slept regularly for 1 week (verified with a sleep diary; based on our experience, actigraphy reports and sleep diaries do not deviate substantially in older individuals). Older participants were maintained in dim light (<10 lux) for 45min before the fMRI scanning.

*Sleep EEG metrics*

Eleven channels were used for the baseline night (F3,z,4; C3,z,4; P3,z,4; O1,2) initially referenced to the left mastoid prior to rereferencing offline to the average of both mastoids. Arousals and artefacts were detected automatically(Wallant et al., 2016) to provide the number of arousals during REM sleep, and excluded from the power spectral density analyses. Only frontal electrodes were considered because the frontal region is most sensitive to sleep pressure manipulations;(Cajochen et al., 1999) focusing on the frontal electrodes may also facilitate interpretation of future large-scale studies using ambulatory EEG, often restricted to frontal electrodes.

*Cognitive tasks*

*Auditory salience detection task – low-tonic LC.* The recording started with a setting of the volume to ensure an optimal auditory perception.

*MRI data acquisitions and preprocessing*

MRI data were acquired using a MAGNETOM Terra 7T MRI system (Siemens Healthineers), with a single-channel transmit and 32-receiving channel head coil (1TX/32RX, Nova Medical, Forchheim, Germany). Blood-oxygen-level-dependent (BOLD) fMRI data were acquired using a multi-band gradient-recalled echo–echo-planar imaging (GRE-EPI) sequence (main parameters: repetition time=2.340ms, flip angle=90°, matrix size = 160 × 160, 86 axial 1.4 mm–thick slices, MB acceleration factor = 2, GeneRalized Autocalibrating Partial Parallel Acquisition (GRAPPA) acceleration factor = 3, voxel size = 1.4 × 1.4 × 1.4 $mm^3$). The cardiac pulse and the respiratory movements were recorded concomitantly using, respectively, a pulse oximeter and a breathing belt (Siemens Healthineers). The fMRI acquisition was followed by a 2D GRE field mapping sequence to assess B0 magnetic field inhomogeneities with the

following parameters: TR = 5.2 ms, TEs = 2.26 ms and 3.28 ms, flip angle (FA) = 15°, bandwidth = 737 Hz/pixel, matrix size = 96 × 128, 96 axial slices, voxel size = 2 × 2 × 2 mm $^3$, acquisition time = 1:38 minutes.

A Magnetization-Prepared with 2 RApid Gradient Echoes (MP2RAGE) sequence was used to acquire T1 anatomical images: TR = 4,300 ms, TE = 1.98 ms, FA = 5°/6°, TI = 940 ms/2,830 ms, bandwidth = 240 Hz/pixel, matrix size = 256 × 256, 224 axial 0.75 mm–thick slices, GRAPPA acceleration factor = 3, voxel size = 0.75 × 0.75 × 0.75 mm$^3$, acquisition time = 9:03 minutes.(Marques & Gruetter, 2013) The LC-specific sequence consisted of a 3D high-resolution magnetization transfer–weighted turbo-flash (MT-TFL) sequence with the following parameters: TR = 400 ms, TE = 2.55 ms, FA = 8°, bandwidth = 300 Hz/pixel, matrix size = 480 × 480, number of averages = 2, turbo factor = 54, magnetization transfer contrast (MTC) pulses = 20, MTC FA = 260°, MTC RF duration = 10,000 μs, MTC inter-RF delay = 4,000 μs, MTC offset = 2,000 Hz, voxel size = (.4 × .4 × .5)mm$^3$, acquisition time = 8:13 minutes. Sixty axial slices were acquired and centered for the acquisitions perpendicularly to the rhomboid fossa (i.e., the floor of the fourth ventricle located on the dorsal surface of the pons).(Priovoulos et al., 2018)

The LC appearing hyperintense on the MT-TFL images was manually delineated by 2 expert raters (as in(Koshmanova et al., 2023)), and the intersection of their masks was computed as the final LC mask for each individual. The masks were used for extracting the LC activity during each task in the participant brain space using the REX Toolbox (https://web.mit.edu/swg/software.htm) (average over all mask voxels per hemisphere) and to compute a LC probabilistic map in the MNI space for group level visualization.

Visualization of whole-brain results over the entire sample was completed following normalization of fMRI and sMRI data to the Montreal Neurological Institute (MNI) space. Due to the small size of the nucleus, LC activation was not expected to survive stringent whole-brain family-wise error (FWE) correction for multiple comparisons. Therefore, a false discovery rate (FDR) correction was conducted using SPM12 to detect voxel-level $p<.05$ results within the LC mask.

*Eye tracking data*

Pupil data were processed as described in(Campbell et al., 2023). Transient pupil dilation in response to target and standard stimuli (salience detection) consisted of the maximum value detected over the 1.5s following stimulus onset relative to the min pupil diameter over -1000ms to -50ms window preceding stimuli onset. Individual values consisted of the mean of these dilation values per stimulus type. Because of the likely intra- and inter-individual variability in the temporal association between the motor response and the perceptual switch, transient pupil dilation associated with perceptual switches in the perceptual rivalry task was computed differently. Baseline pre-switch pupil size consisted of the median value over a 500ms window centered around the minimum pupil value detected over a window of 3000 ms preceding each button press. The pupil size associated to the switch consisted of the median value over a 500ms window centered around the maximum pupil size value detected over the 3000ms following the baseline minimum pupil values. Individual values consisted of the mean of these trial values. Pupil size associated with the switch were compared to baseline pre-switch pupil size to test for pupil dilation.

**Supplementary Table S1. Exploratory analysis on the associations between sleep metrics and LC activity estimated via the visual perceptual rivalry (high-tonic) task.**

| Sleep metric (dependent variable) | LC activity | Age group | Sex | TIV | Total sleep time |
|---|---|---|---|---|---|
| **REM Delta energy (N=52)** | F(1,46)=3.81 P=0.057 | F(1,46)=0.14 P=0.713 | F(1,46)=0.55 P=0.461 | F(1,46)=0.01 P=0.940 | F(1,46)=0.69 P=0.410 |
| **REM Sigma energy (N=52)** | F(1,46)=1.73 P=0.194 | F(1,46)=1.27 P=0.265 | F(1,46)=0.59 P=0.445 | F(1,46)=0.46 P=0.502 | F(1,46)=5.67 **P=0.021** **$R^{2*}$=0.109** |
| **REM Alpha energy (N=51)** | F(1,45)=3.06 P=0.087 | F(1,45)=0.32 P=0.576 | F(1,45)=0.00 P=0.944 | F(1,45)=1.04 P=0.313 | F(1,45)=6.80 **P=0.012** **$R^{2*}$=0.131** |
| **REM Beta energy (N=51)** | F(1,45)=0.28 P=0.596 | F(1,45)=0.04 P=0.850 | F(1,45)=3.64 P=0.062 | F(1,45)=3.04 P=0.088 | F(1,45)=9.87 **P=0.003** **$R^{2*}$=0.179** |
| **NREM SWE (N=52)** | F(1,46)=2.83 P=0.099 | F(1,46)=0.00 P=0.9634 | F(1,46)=0.02 P=0.882 | F(1,46)=0.35 P=0.556 | F(1,46)=0.14 P=0.709 |
| **REMS duration (N=52)** | F(1,46)=1.56 P=0.217 | F(1,46)=4.76 **P=0.034** **$R^{2*}$=0.093** | F(1,46)=1.48 P=0.229 | F(1,46)=3.70 P=0.060 | F(1,46)=19.85 **P<0.0001** **$R^{2*}$=0.301** |
| **REM bouts duration (N=52)** | F(1,46)=0.51 P=0.478 | F(1,46)=13.24 **P=0.0007** **$R^{2*}$=0.223** | F(1,46)=1.43 P=0.237 | F(1,46)=0.94 P=0.336 | F(1,46)=0.17 P=0. 0.686 |
| **REM bouts number (N=52)** | F(1,46)=0.11 P=0.736 | F(1,46)=3.27 P=0.076 | F(1,46)=0.04 P=0.844 | F(1,46)=0.01 P=0.915 | F(1,46)=6.65 **P=0.013** **$R^{2*}$=0.126** |
| **Number of arousals in REMS (N=52)** | F(1,46)=0.60 P=0.443 | F(1,46)=0.38 P=0.539 | F(1,46)=5.55 **P=0.022** **$R^{2*}$=0.107** | F(1,46)=4.31 **P=0.043** **$R^{2*}$=0.085** | F(1,46)=1.82 P=0.184 |

*Prior to the analysis, we removed the outliers among all variables by excluding the samples lying beyond four times the standard deviation (the final number of individuals included in each analysis is reported below each dependent variable).*

*In all models the interaction between left LC activity and age group was not significant. Goodness of fit metric (BIC) indicated that the interaction term should be removed.*

*LC: locus coeruleus; TIV: total intracranial volume; REM: rapid eye movement; NREM: Non-rapid eye movement; SWE: slow wave energy; REMS: rapid eye movement sleep.*

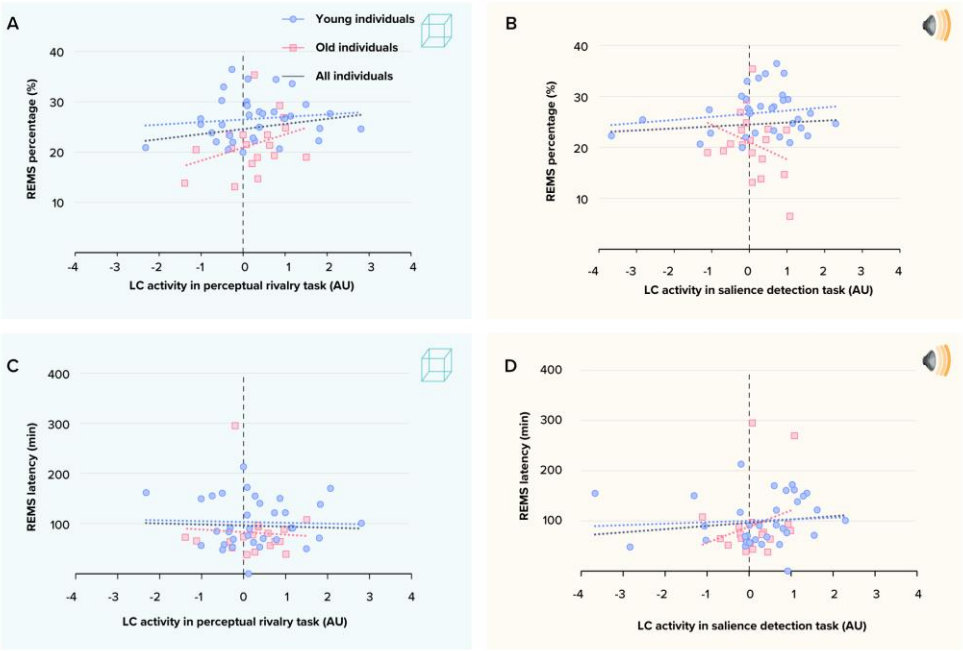
**Supplementary Table S2. Exploratory analysis on the associations between REM sleep metrics and LC activity estimated via the auditory salience detection (low-tonic) task.**

| Sleep metric (dependent variable) | LC activity | Age group | LC activity*age group | Sex | TIV | Total sleep time |
|---|---|---|---|---|---|---|
| **REM Delta energy (N=52)** | F(1,45)=1.35 P=0.251 | F(1,45)=0.29 P=0.592 | F(1,45)=1.79 P=0.187 | F(1,45)=0.00 P=0.945 | F(1,45)=0.16 P=0.688 | F(1,45)=1.01 P=0.320 |
| **REM Sigma energy (N=52)** | F(1,45)=0.32 P=0.572 | F(1,45)=1.87 P=0.177 | F(1,45)=0.54 P=0.466 | F(1,45)=0.13 P=0.722 | F(1,45)=0.39 P=0.536 | F(1,45)=5.8 **P=0.020** **$R^{2*}$=0.114** |
| **REM Alpha energy (N=51)** | F(1,44)=5.35 **P=0.025** **$R^{2*}$=0.108** | F(1,44)=0.01 P=0.925 | F(1,44)=4.59 **P=0.037** **$R^{2*}$=0.094** | F(1,44)=0.72 P=0.400 | F(1,44)=0.91 P=0.346 | F(1,44)=4.97 **P=0.031** **$R^{2*}$=0.101** |
| **REM Beta energy (N=51)** | F(1,44)=1.46 P=0.233 | F(1,44)=0.04 P=0.849 | F(1,44)=0.02 P=0.881 | F(1,44)=3.36 P=0.073 | F(1,44)=4.06 **P=0.050** **$R^{2*}$=0.084** | F(1,44)=11.39 **P=0.001** **$R^{2*}$=0.205** |
| **NREM SWE (N=52)** | F(1,45)=0.65 P=0.424 | F(1,45)=0.00 P=0.954 | F(1,45)=0.80 P=0.377 | F(1,45)=0.05 P=0.827 | F(1,45)=0.20 P=0.658 | F(1,45)=0.19 P=0.664 |
| **REMS duration (N=52)** | F(1,45)=0.29 P=0.594 | F(1,45)=5.69 **P=0.021** **$R^{2*}$=0.112** | F(1,45)=0.50 P=0.482 | F(1,45)=0.36 P=0.550 | F(1,45)=6.09 **P=0.017** **$R^{2*}$=0.119** | F(1,45)=17.29 **P=0.0001** **$R^{2*}$=0.277** |
| **REM bouts duration (N=52)** | F(1,45)=1.40 P=0.243 | F(1,45)=11.04 **P=0.001** **$R^{2*}$=0.197** | F(1,45)=0.19 P=0.666 | F(1,45)=0.84 P=0.364 | F(1,45)=0.97 P=0.331 | F(1,45)=0.00 P=0.981 |
| **REM bouts number (N=52)** | F(1,45)=3.11 P=0.084 | F(1,45)=1.80 P=0.186 | F(1,45)=1.75 P=0.192 | F(1,45)=0.23 P=0.632 | F(1,45)=0.01 P=0.943 | F(1,45)=5.37 **P=0.025** **$R^{2*}$=0.106** |
| **Number of arousals in REMS (N=52)** | F(1,45)=0.85 P=0.360 | F(1,45)=0.32 P=0.573 | F(1,45)=2.24 P=0.141 | F(1,45)=2.18 P=0.146 | F(1,45)=4.45 **P=0.040** **$R^{2*}$=0.089** | F(1,45)=2.16 P=0.148 |

*Prior to the analysis, we removed the outliers among all variables by excluding the samples lying beyond four times the standard deviation (the final number of individuals included in each analysis is reported below each dependent variable). In all models, the results without the interaction between LC activity and age group were not significant. Goodness of fit metric (BIC) indicated that the interaction term provides a better fitness.*

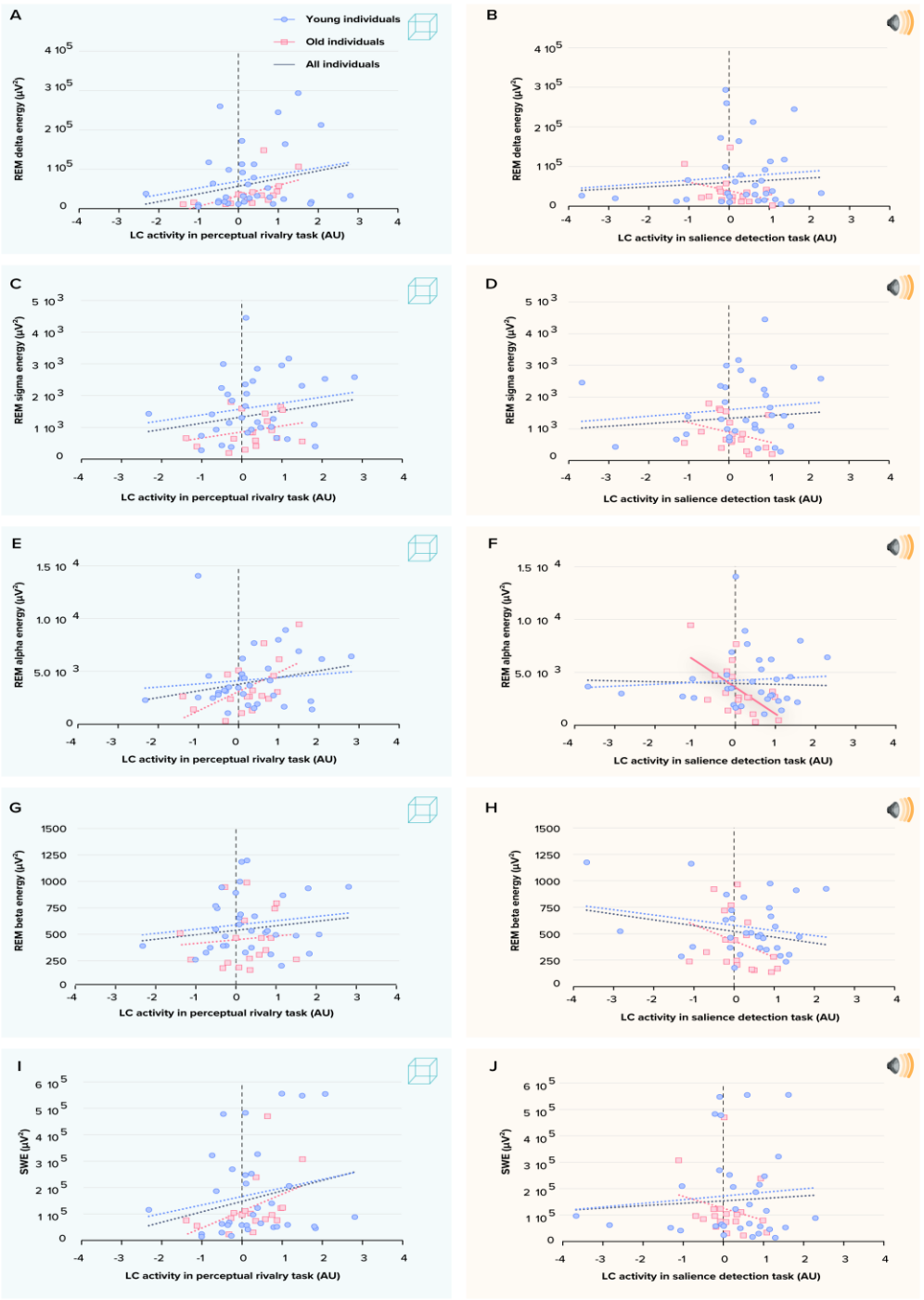
*The significant main effect of REM alpha energy may consist of prolongation of the effect detected in the theta band. Since the associations with the other bands were not significant, exploratory specificity analyses suggest that the association is specific to theta oscillation and potentially the surrounding alpha band.*

*LC: locus coeruleus; TIV: total intracranial volume; REM: rapid eye movement; NREM: Non-rapid eye movement; SWE: slow wave energy; REMS: rapid eye movement sleep.*




***Supplementary Figure S1. Non-significant associations between the LC activity estimates and main sleep metrics of interest. (A)*** *Association between REM sleep percentage and the LC activity estimates during the perceptual rivalry task. The GLMM showed neither a significant main effect of LC activity (p=0.159) nor a significant age group by LC activity interaction (p=0.164).* ***(B)*** *Association between REM sleep percentage and the LC activity estimates during the salience detection task. The GLMM showed neither a significant main effect of LC activity (p=0.245) nor a significant age group by LC activity interaction (p=0.157).* ***(C)*** *Association between REM sleep latency and the LC activity estimates during the perceptual rivalry task. The GLMM showed neither a significant main effect of LC activity (p=0.750) nor a significant age group by LC activity interaction (p=0.816).* ***(D)*** *Association between REM sleep latency and the LC activity estimates during the salience detection task. The GLMM showed neither a significant main effect of LC activity (p=0.218) nor a significant age group by LC activity interaction (p=0.242).*

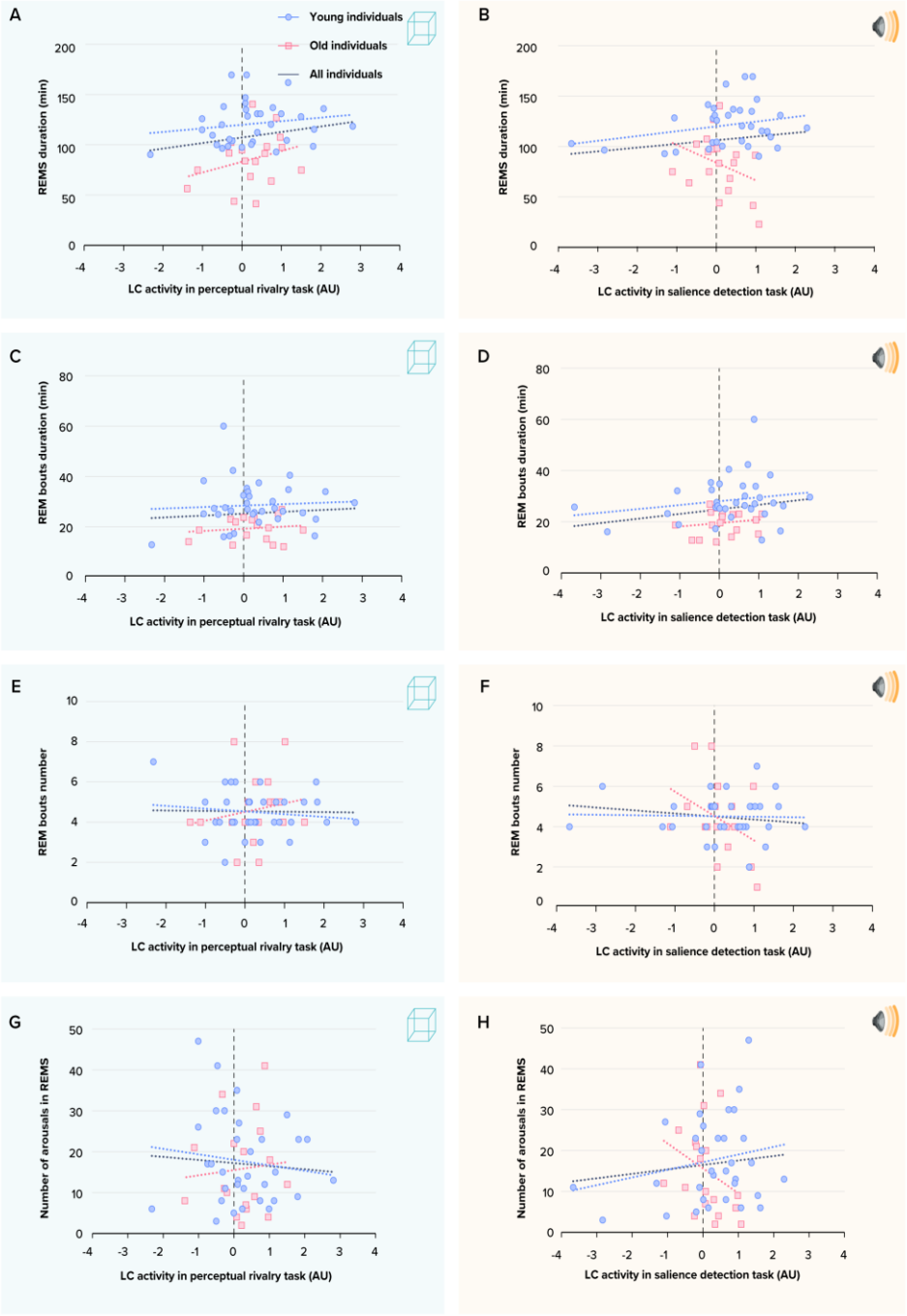
*Simple regression lines are used for a visual display and do not substitute the GLMM outputs. The black line represents the regression irrespective of age groups (young + old, n = 52). Solid and dashed regression lines represent significant and non-significant outputs of the GLMM, respectively.*


A
Young individuals
Old individuals
All individuals
REM delta energy (µV²)
LC activity in perceptual rivalry task (AU)
B
REM delta energy (µV²)
LC activity in salience detection task (AU)
C
REM sigma energy (µV²)
LC activity in perceptual rivalry task (AU)
D
REM sigma energy (µV²)
LC activity in salience detection task (AU)
E
REM alpha energy (µV²)
LC activity in perceptual rivalry task (AU)
F
REM alpha energy (µV²)
LC activity in salience detection task (AU)
G
REM beta energy (µV²)
LC activity in perceptual rivalry task (AU)
H
REM beta energy (µV²)
LC activity in salience detection task (AU)
I
SWE (µV²)
LC activity in perceptual rivalry task (AU)
J
SWE (µV²)
LC activity in salience detection task (AU)

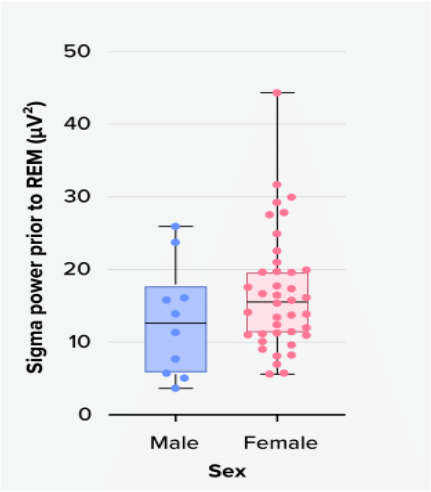
***Supplementary Figure S2. Associations between the LC activity estimates and sleep metrics of interest to test the specificity. (A)*** *Association between REM delta energy and the LC activity estimates during the perceptual rivalry task.* ***(B)*** *Association between REM delta energy and the LC activity estimates during the salience detection task.* ***(C)*** *Association between REM sigma energy and the LC activity estimates during the perceptual rivalry task.* ***(D)*** *Association between REM sigma energy and the LC activity estimates during the salience detection task.* ***(E)*** *Association between REM alpha energy and the LC activity estimates during the perceptual rivalry task.* ***(F)*** *Association between REM alpha energy and the LC activity estimates during the salience detection task.* ***(G)*** *Association between REM beta energy and the LC activity estimates during the perceptual rivalry task.* ***(H)*** *Association between REM beta energy and the LC activity estimates during the salience detection task.* ***(I)*** *Association between NREM slow wave energy and the LC activity estimates during the perceptual rivalry task.* ***(J)*** *Association between NREM slow wave energy and the LC activity estimates during the salience detection task.*

*None of the associations were significant (p>0.057) except for a significant main effect of LC activity during the salience detection task (p=.025) and age-group by LC activity interaction (p=.037) when using REM alpha energy as the dependent variable.*

*Simple regression lines are used for a visual display and do not substitute the GLMM outputs. The black line represents the regression irrespective of age groups (young + old, n = 52). Solid and dashed regression lines represent significant and non-significant outputs of the GLMM, respectively.*


A
Young individuals
Old individuals
All individuals
REMS duration (min)
LC activity in perceptual rivalry task (AU)
B
REMS duration (min)
LC activity in salience detection task (AU)
C
REM bouts duration (min)
LC activity in perceptual rivalry task (AU)
D
REM bouts duration (min)
LC activity in salience detection task (AU)
E
REM bouts number
LC activity in perceptual rivalry task (AU)
F
REM bouts number
LC activity in salience detection task (AU)
G
Number of arousals in REMS
LC activity in perceptual rivalry task (AU)
H
Number of arousals in REMS
LC activity in salience detection task (AU)

***Supplementary Figure S3. Associations between the LC activity estimates and sleep metrics of interest for exploratory analysis. (A)*** *Association between REM sleep duration and the LC activity estimates during the perceptual rivalry task.* ***(B)*** *Association between REM sleep duration and the LC activity estimates during the salience detection task.* ***(C)*** *Association between REM bouts duration and the LC activity estimates during the perceptual rivalry task.* ***(D)*** *Association between REM bouts duration and the LC activity estimates during the salience detection task.* ***(E)*** *Association between REM bouts number and the LC activity estimates during the perceptual rivalry task.* ***(F)*** *Association between REM bouts number and the LC activity estimates during the salience detection task.* ***(G)*** *Association between number of arousals in REM sleep and the LC activity estimates during the perceptual rivalry task.* ***(H)*** *Association between number of arousals in REM sleep and the LC activity estimates during the salience detection task.*
*None of the associations were significant (P>0.141).*
*Simple regression lines are used for a visual display and do not substitute the GLMM outputs. The black line represents the regression irrespective of age groups (young + old, n = 52). Solid and dashed regression lines represent significant and non-significant outputs of the GLMM, respectively.*




***Supplementary Figure S4. Sigma power prior to REM sleep in males and females.*** *Men have significantly less sigma power prior to REM sleep compared to women*

## Appendix 2: Supplementary material for the paper presented in Chapter 4

**Supplementary Table S1. Post hoc contrast on the associations between REMS theta energy and hypothalamus subparts activity estimated via the visual perceptual rivalry task.**

| Hypothalamus subpart | Estimate | DF | t value | P |
|---|---|---|---|---|
| **Interior-inferior hypothalamus** | -0.001 | 239 | -0.02 | 0.983 |
| **Anterior-superior hypothalamus** | -0.121 | 239 | -1.81 | **0.071** |
| **Posterior hypothalamus** | 0.194 | 239 | 1.90 | **0.058** |
| **Inferior-tubular hypothalamus** | -0.116 | 239 | -1.15 | 0.252 |
| **Superior-tubular hypothalamus** | -0.104 | 239 | -1.32 | 0.187 |

**Supplementary Table S2. Associations between age and the connectivity between the anterior-superior hypothalamus and the LC as well as between the posterior hypothalamus and the LC.**

| Type of connectivity | Age | Sex | TIV |
|---|---|---|---|
| From anterior-superior hypothalamus to LC | F(1,47)=5.17<br>**P=0.027**<br>**$R^{2*}$=0.099** | F(1,47)=0.69<br>P=0.410 | F(1,47)=0.07<br>P=0.799 |
| From LC to anterior-superior hypothalamus | F(1,47)=1.74<br>P=0.199 | F(1,47)=0.25<br>P=0.617 | F(1,47)=0.00<br>P=0.962 |
| From posterior hypothalamus to LC | F(1,47)=2.23<br>P=0.142 | F(1,47)=0.40<br>P=0.529 | F(1,47)=0.23<br>P=0.633 |
| From LC to posterior hypothalamus | F(1,47)=0.52<br>P=0.473 | F(1,47)=1.33<br>P=0.254 | F(1,47)=0.06<br>P=0.812 |

*LC: locus coeruleus; TIV: total intracranial volume.*

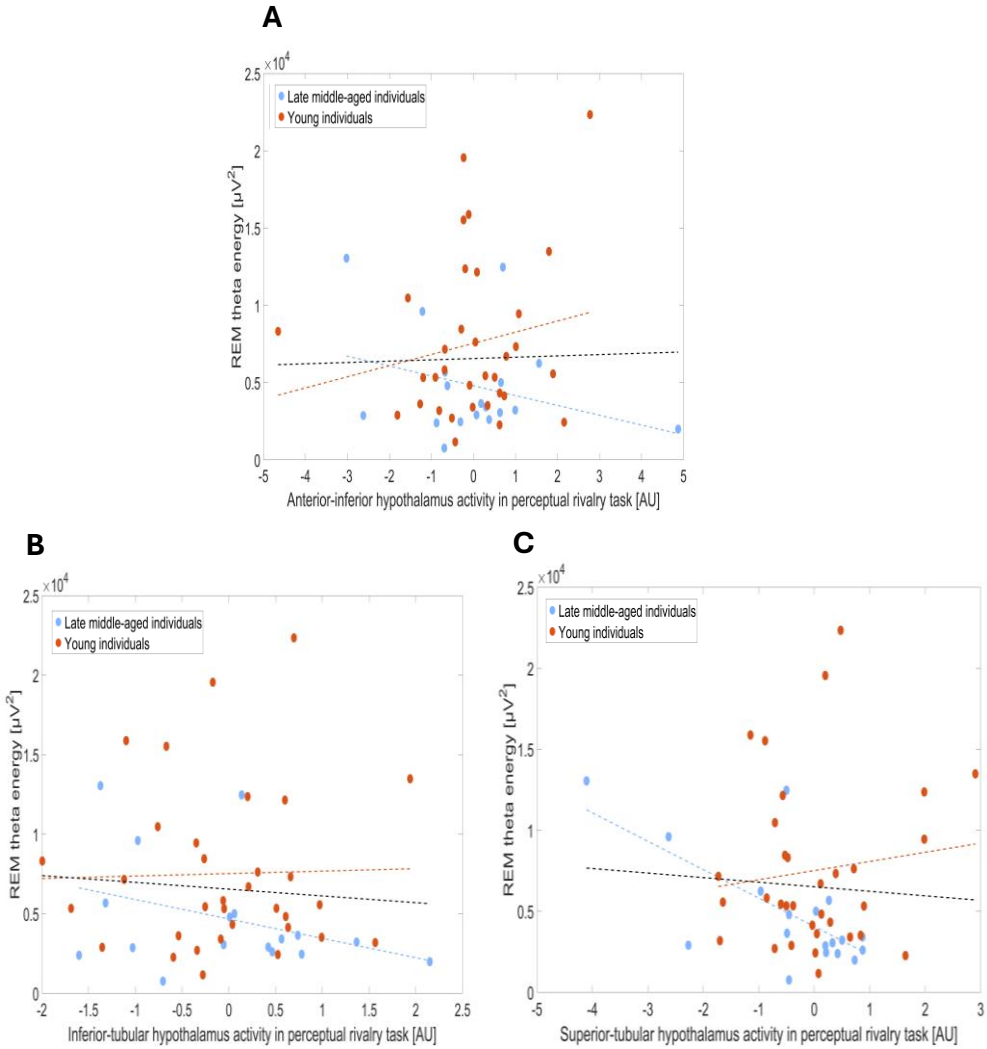
**Supplementary Table S3. Non-significant associations between exploratory sleep metrics and the connectivity from anterior-superior hypothalamus to LC.**

| Sleep metric (dependent variable) | connectivity | Age group | connectivity*age group | Sex | TIV | Total sleep time |
|---|---|---|---|---|---|---|
| REM delta energy (N=51) | F(1,44)=0.49<br>P=0.487 | F(1,44)=0.66<br>P=0.420 | F(1,44)=4.11<br>P=0.048*<br>$R^{2*}$=0.085 | F(1,44)=0.11<br>P=0.745 | F(1,44)=0.13<br>P=0.718 | F(1,44)=0.86<br>P=0.358 |
| REM sigma energy (N=51) | F(1,44)=0.00<br>P=0.963 | F(1,44)=0.83<br>P=0.366 | F(1,44)=0.07<br>P=0.795 | F(1,44)=0.46<br>P=0.503 | F(1,44)=0.26<br>P=0.611 | F(1,44)=6.08<br>P=0.017<br>$R^{2*}$=0.121 |
| REM beta energy (N=50) | F(1,43)=0.15<br>P=0.703 | F(1,43)=0.11<br>P=0.743 | F(1,43)=0.82<br>P=0.369 | F(1,43)=5.50<br>P=0.023<br>$R^{2*}$=0.113 | F(1,43)=4.19<br>P=0.046<br>$R^{2*}$=0.088 | F(1,43)=12.02<br>P=0.001<br>$R^{2*}$=0.221 |
| NREM sigma energy (N=51) | F(1,44)=0.35<br>P=0.559 | F(1,44)=0.00<br>P=0.953 | F(1,44)=0.32<br>P=0.575 | F(1,44)=0.02<br>P=0.899 | F(1,44)=0.13<br>P=0.717 | F(1,44)=3.79<br>P=0.058 |
| NREM beta energy (N=51) | F(1,44)=0.17<br>P=0.680 | F(1,44)=0.75<br>P=0.390 | F(1,44)=0.36<br>P=0.550 | F(1,44)=0.06<br>P=0.804 | F(1,44)=0.20<br>P=0.657 | F(1,44)=4.16<br>P=0.047<br>$R^{2*}$=0.086 |

*Prior to the analysis, we removed the outliers among connectivity and sleep metrics by excluding the samples lying beyond four times the standard deviation (the final number of individuals included in each analysis is reported below each dependent variable).*

**post hoc analysis showed that the significant association between REM delta energy and age group by connectivity interaction considering the connectivity from anterior-superior hypothalamus to LC (p=0.048) is driven by the association between REM delta energy and the difference of connectivity values between two age groups and not each group separately.*

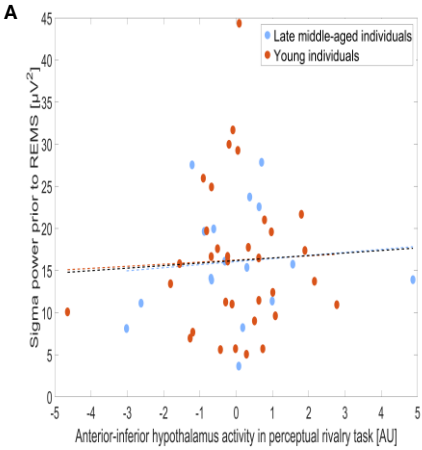
*LC: locus coeruleus; TIV: total intracranial volume; REM: rapid eye movement; REMS: rapid eye movement sleep; NREM: non-rapid eye movement.*

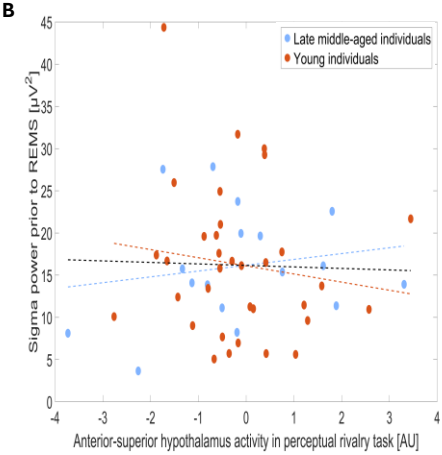


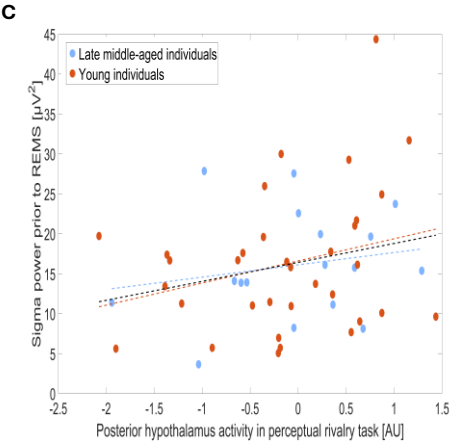

***Supplementary Figure S1. Non-significant associations between hypothalamus subparts activity estimates during the perceptual rivalry task and REM theta energy. (A)*** *Association between the interior-inferior hypothalamus activity estimates during the perceptual rivalry task and REM theta energy.* ***(B)*** *Association between the inferior-tubular hypothalamus activity estimates during the perceptual rivalry task and REM theta energy.* ***(C)*** *Association between the*

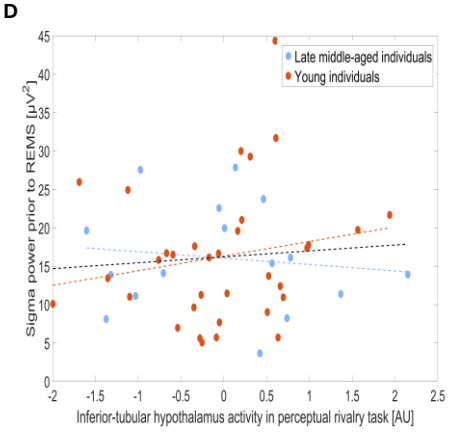
*superior-tubular hypothalamus activity estimates during the perceptual rivalry task and REM theta energy.*

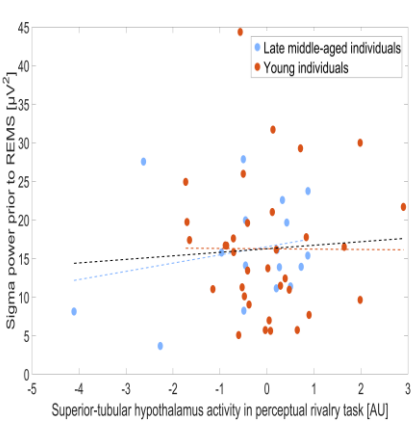
*Although the GLMM yielded to a statistical trend for the hypothalamus activity by hypothalamus subpart interaction (p=0.8), post hoc analyses did not show a statistical trend for any of these hypothalamus subparts (p>0.18).*
*Simple regression lines are used for a visual display and do not substitute the GLMM outputs. The black line represents the regression irrespective of age groups (young + old). Dashed regression lines represent non-significant outputs of the GLMM.*


A
Late middle-aged individuals
Young individuals
Sigma power prior to REMS [μV²]
Anterior-inferior hypothalamus activity in perceptual rivalry task [AU]

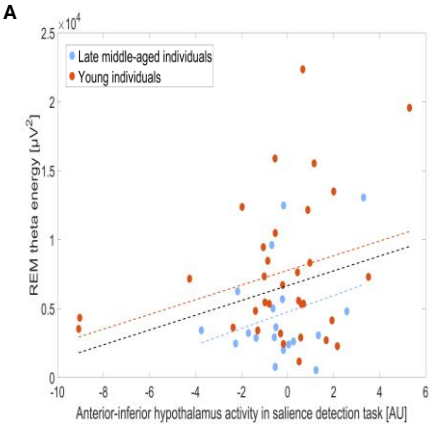

B
Late middle-aged individuals
Young individuals
Sigma power prior to REMS [μV²]
Anterior-superior hypothalamus activity in perceptual rivalry task [AU]

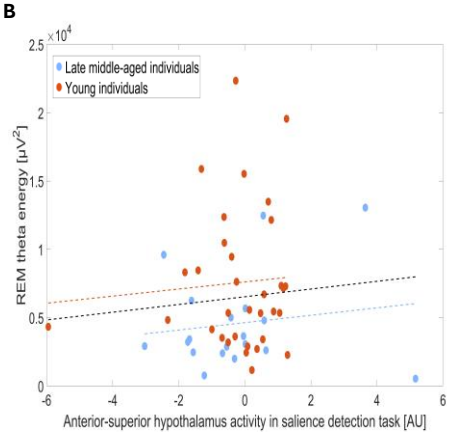

C
Late middle-aged individuals
Young individuals
Sigma power prior to REMS [μV²]
Posterior hypothalamus activity in perceptual rivalry task [AU]

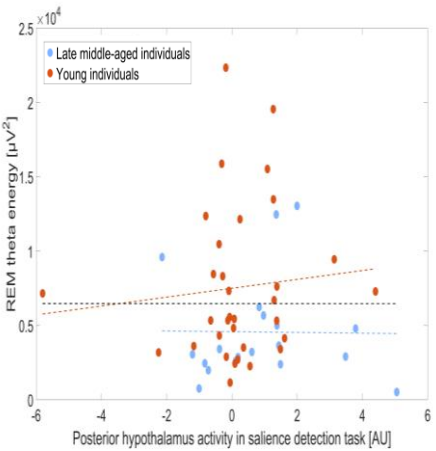

D
Late middle-aged individuals
Young individuals
Sigma power prior to REMS [μV²]
Inferior-tubular hypothalamus activity in perceptual rivalry task [AU]

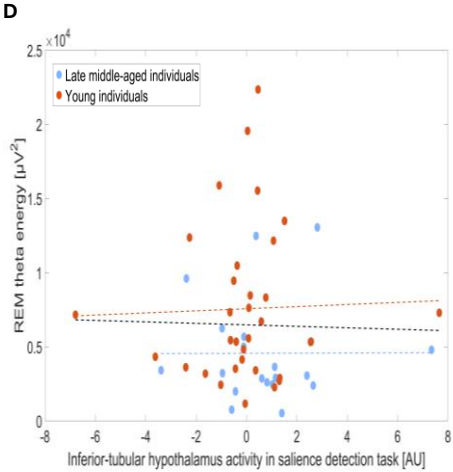

E
Late middle-aged individuals
Young individuals
Sigma power prior to REMS [μV²]
Superior-tubular hypothalamus activity in perceptual rivalry task [AU]

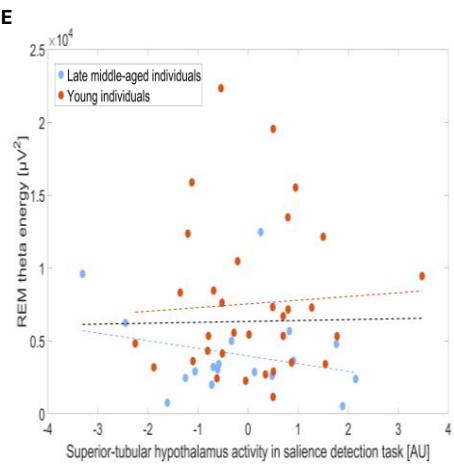
***Supplementary Figure S2. Non-significant associations between hypothalamus subparts activity estimates during the perceptual rivalry task and sigma power prior to REMS. (A)*** *Association between the interior-inferior hypothalamus activity estimates during the perceptual rivalry task and sigma power prior to REMS.* ***(B)*** *Association between the interior-superior hypothalamus activity estimates during the perceptual rivalry task and sigma power prior to REMS.* ***(C)*** *Association between the posterior hypothalamus activity estimates during the perceptual rivalry task and sigma power prior to REMS.* ***(D)*** *Association between the inferior-tubular hypothalamus activity estimates during the perceptual rivalry task and sigma power prior to REMS.* ***(E)*** *Association between the superior-tubular hypothalamus activity estimates during the perceptual rivalry task and sigma power prior to REMS.*
*The GLMM did not yield a statistical trend for the hypothalamus activity by hypothalamus subpart interaction (p=0.8).*
*Simple regression lines are used for a visual display and do not substitute the GLMM outputs. The black line represents the regression irrespective of age groups (young + old). Dashed regression lines represent non-significant outputs of the GLMM.*


A
×10^4
Late middle-aged individuals
Young individuals
REM theta energy [μV^2]
Anterior-inferior hypothalamus activity in salience detection task [AU]


B
×10^4
Late middle-aged individuals
Young individuals
REM theta energy [μV^2]
Anterior-superior hypothalamus activity in salience detection task [AU]


C
×10^4
Late middle-aged individuals
Young individuals
REM theta energy [μV^2]
Posterior hypothalamus activity in salience detection task [AU]


D
×10^4
Late middle-aged individuals
Young individuals
REM theta energy [μV^2]
Inferior-tubular hypothalamus activity in salience detection task [AU]

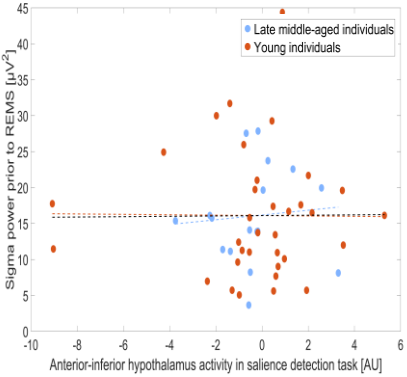

E
×10^4
Late middle-aged individuals
Young individuals
REM theta energy [μV^2]
Superior-tubular hypothalamus activity in salience detection task [AU]

***Supplementary Figure S3. Non-significant associations between hypothalamus subparts activity estimates during the salience detection task and REM theta energy. (A)*** *Association between the interior-inferior hypothalamus activity estimates during the salience detection task and REM theta energy.* ***(B)*** *Association between the interior-superior hypothalamus activity estimates during the salience detection task and REM theta energy.* ***(C)*** *Association between the posterior hypothalamus activity estimates during the salience detection task and REM theta energy.* ***(D)*** *Association between the inferior-tubular hypothalamus activity estimates during the salience detection task and REM theta energy.* ***(E)*** *Association between the superior-tubular hypothalamus activity estimates during the salience detection task and REM theta energy.*

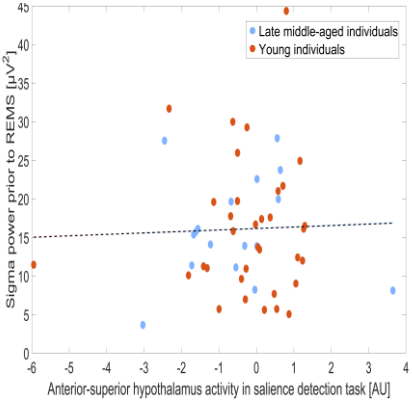
*The GLMM did not yield a statistical trend for the hypothalamus activity by hypothalamus subpart interaction (p=0.9).*

*Simple regression lines are used for a visual display and do not substitute the GLMM outputs. The black line represents the regression irrespective of age groups (young + old). Dashed regression lines represent non-significant outputs of the GLMM.*

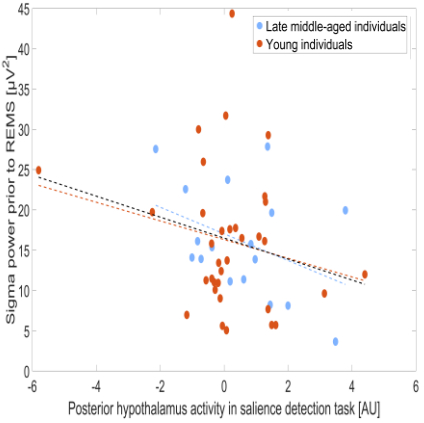
A


Late middle-aged individuals
Young individuals
Sigma power prior to REMS [μV²]
Anterior-inferior hypothalamus activity in salience detection task [AU]

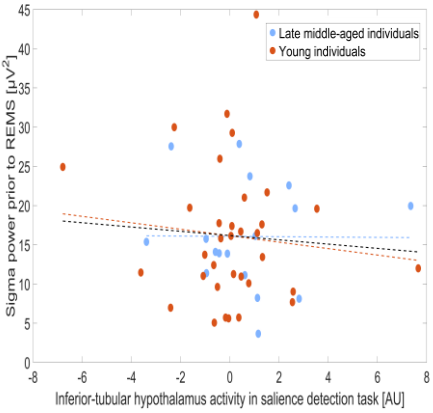
B


Late middle-aged individuals
Young individuals
Sigma power prior to REMS [μV²]
Anterior-superior hypothalamus activity in salience detection task [AU]

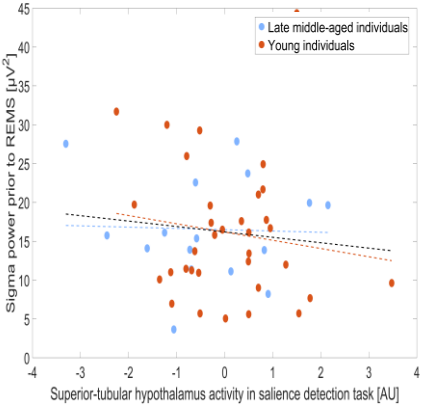
C


Late middle-aged individuals
Young individuals
Sigma power prior to REMS [μV²]
Posterior hypothalamus activity in salience detection task [AU]

D


Late middle-aged individuals
Young individuals
Sigma power prior to REMS [μV²]
Inferior-tubular hypothalamus activity in salience detection task [AU]

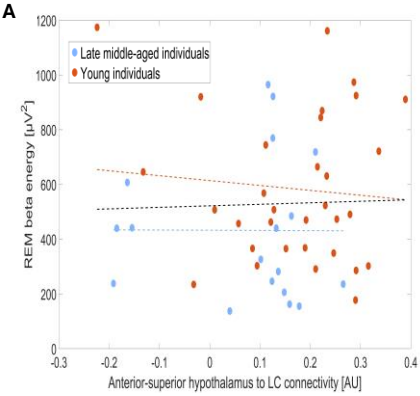
E

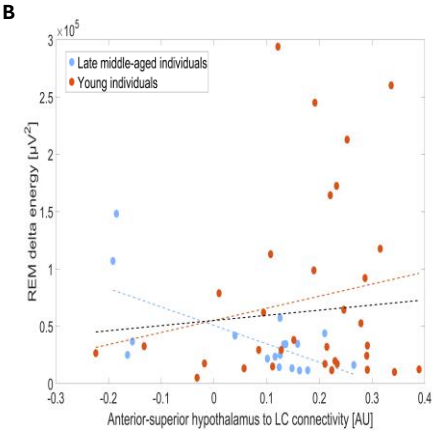

Late middle-aged individuals
Young individuals
Sigma power prior to REMS [μV²]
Superior-tubular hypothalamus activity in salience detection task [AU]

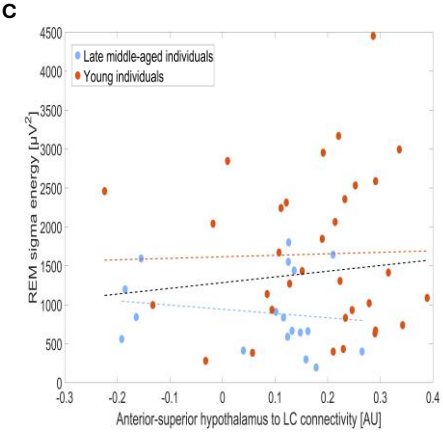
***Supplementary Figure S4. Non-significant associations between hypothalamus subparts activity estimates during the salience detection task and sigma power prior to REMS. (A)*** *Association between the interior-inferior hypothalamus activity estimates during the salience detection task and sigma power prior to REMS.* ***(B)*** *Association between the interior-superior hypothalamus activity estimates during the salience detection task and sigma power prior to REMS.* ***(C)*** *Association between the posterior hypothalamus activity estimates during the salience detection task and sigma power prior to REMS.* ***(D)*** *Association between the inferior-tubular hypothalamus activity estimates during the salience detection task and sigma power prior to REMS.* ***(E)*** *Association between the superior-tubular hypothalamus activity estimates during the salience detection task and sigma power prior to REMS.*

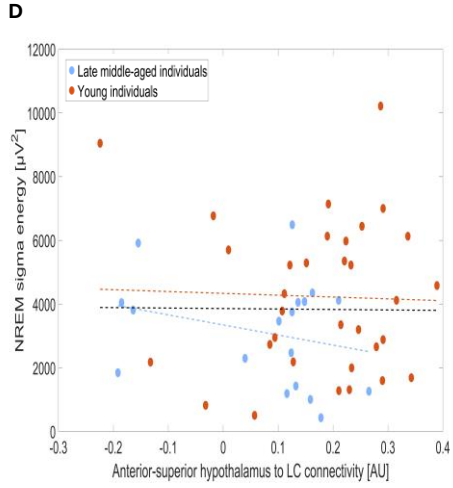
*The GLMM did not yield a statistical trend for the hypothalamus activity by hypothalamus subpart interaction (p=0.6).*

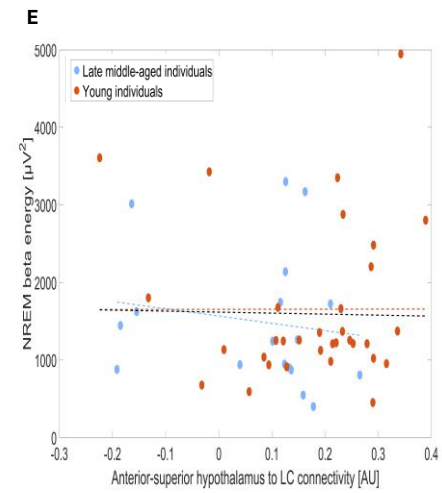
*Simple regression lines are used for a visual display and do not substitute the GLMM outputs. The black line represents the regression irrespective of age groups (young + old). Dashed regression lines represent non-significant outputs of the GLMM.*


A
Late middle-aged individuals
Young individuals
REM beta energy [μV²]
Anterior-superior hypothalamus to LC connectivity [AU]


B
×10⁵
Late middle-aged individuals
Young individuals
REM delta energy [μV²]
Anterior-superior hypothalamus to LC connectivity [AU]


C
Late middle-aged individuals
Young individuals
REM sigma energy [μV²]
Anterior-superior hypothalamus to LC connectivity [AU]


D
Late middle-aged individuals
Young individuals
NREM sigma energy [μV²]
Anterior-superior hypothalamus to LC connectivity [AU]


E
Late middle-aged individuals
Young individuals
NREM beta energy [μV²]
Anterior-superior hypothalamus to LC connectivity [AU]

***Supplementary Figure S5. Non-significant associations between sleep metrics of interest and the connectivity from anterior-superior hypothalamus to LC to test the specificity. (A)*** *Association between REM beta energy and the anterior-superior hypothalamus to LC connectivity.* ***(B)*** *Association between REM delta energy and the anterior-superior hypothalamus to LC connectivity.* ***(C)*** *Association between REM sigma energy and the anterior-superior hypothalamus to LC connectivity.* ***(D)*** *Association between NREM sigma energy and the anterior-superior hypothalamus to LC connectivity.* ***(E)*** *Association between NREM beta energy and the anterior-superior hypothalamus to LC connectivity.*

*None of the associations were significant (p>0.051).*

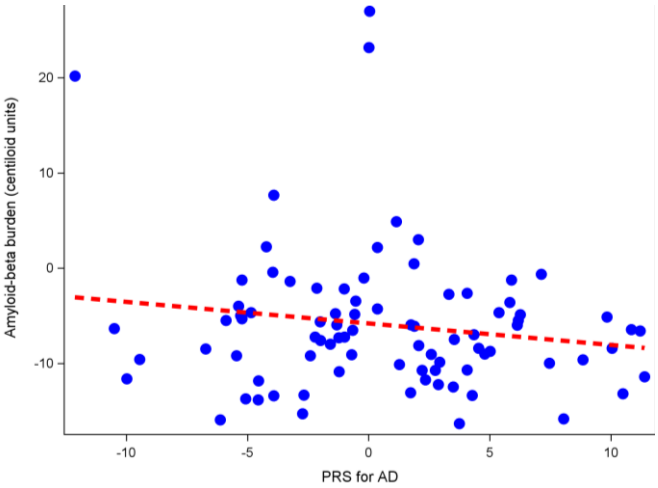
*Simple regression lines are used for a visual display and do not substitute the GLMM outputs. The black line represents the regression irrespective of age groups (young + old). Dashed regression lines represent non-significant outputs of the GLMM.*

# Appendix 3: Supplementary material for the paper presented in Chapter 5.

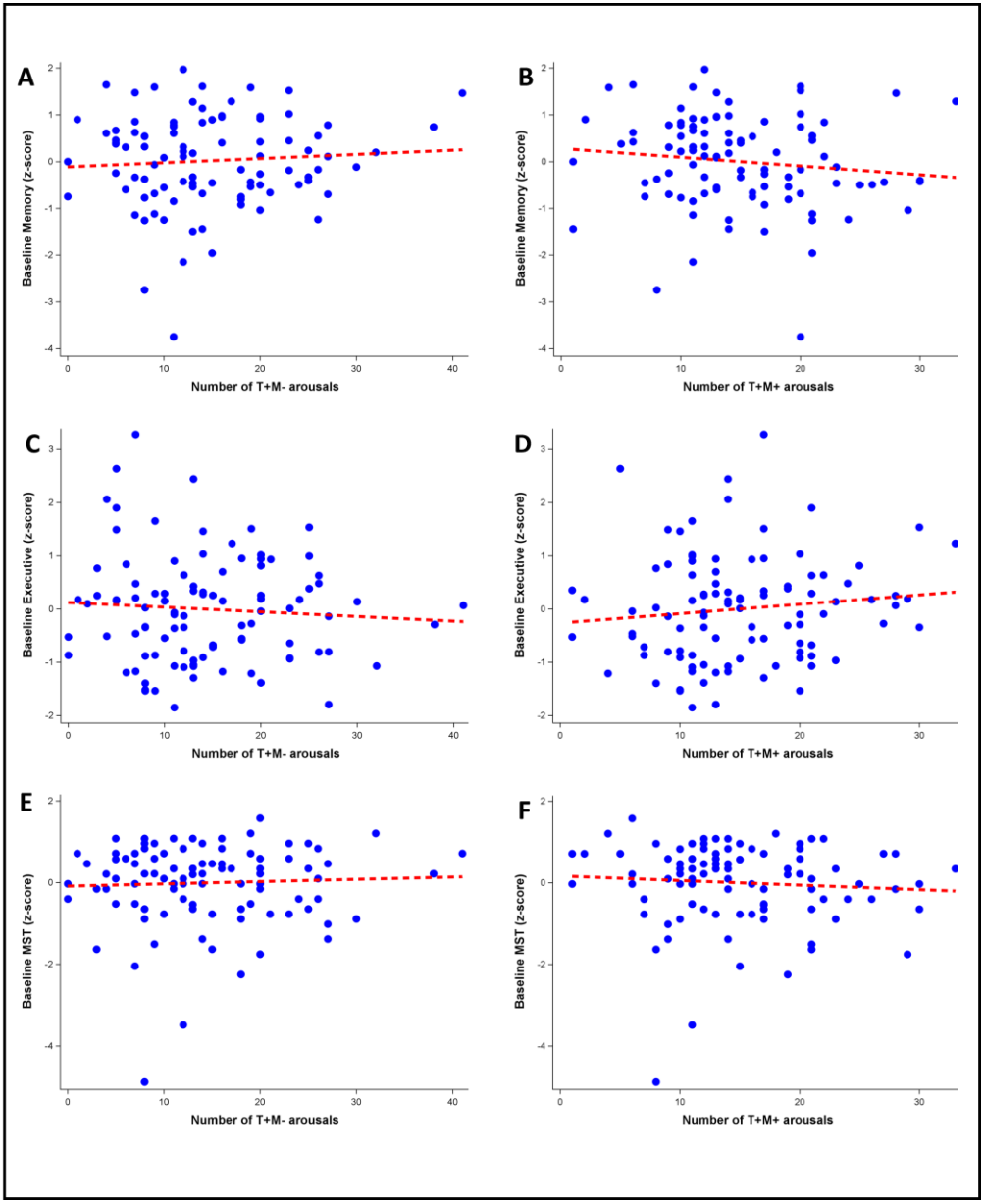
**Supplementary Table S1. Association between arousals number and PRS for AD after adding the variables that significantly differed between the two age groups (i.e., BMI, depression, sleep quality and chronotype) as covariates.**

| Independent variable | t | df | p-value | $R^{2*}$ |
|---|---|---|---|---|
| **PRS** | -1.05 | 1447.663 | 0.29 | - |
| **Transition status** | 41.14 | 1447.663 | **<.0001** | **0.921** |
| **EMG status** | -0.83 | 1447.663 | 0.41 | - |
| **Age group** | -8.27 | 1447.663 | **<.0001** | **0.045** |
| **Sex** | 2.68 | 1447.663 | **<.0001** | **0.005** |
| **TST** | 38.64 | 1447.663 | **<.0001** | **0.911** |
| **BMI** | -7.90 | 1447.663 | **<.0001** | **0.041** |
| **Depression** | -5.32 | 1447.663 | **<.0001** | **0.019** |
| **Chronotype** | 6.13 | 1447.663 | **<.0001** | **0.025** |
| **Sleep quality** | 2.36 | 1447.663 | **<.0001** | **0.004** |
| **PRS*Transition** | 1.66 | 1447.663 | 0.10 | - |
| **PRS*EMG** | 1.69 | 1447.663 | 0.09 | - |
| **Transition*EMG** | 13.16 | 1447.663 | **<.0001** | **0.107** |
| **PRS* age group** | 0.79 | 1447.663 | 0.43 | - |
| **Transition * age group** | 22.58 | 1447.663 | **<.0001** | **0.261** |
| **EMG*age group** | -6.91 | 1447.663 | **<.0001** | **0.032** |
| **PRS*Transition*EMG** | -2.40 | 1447.663 | **<.0001** | **0.004** |
| **PRS*Transition*age group** | -1.54 | 1447.663 | 0.12 | - |
| **PRS*EMG*age group** | -1.36 | 1447.663 | 0.17 | - |
| **Transition*EMG*age group** | -1.41 | 1447.663 | 0.16 | - |
| **PRS*Transition*EMG* age group** | 2.57 | 1447.663 | **<.0001** | **0.005** |

*BMI: Body mass index; PRS: polygenic risk score; TST: total sleep time; EMG: Electromyography.*



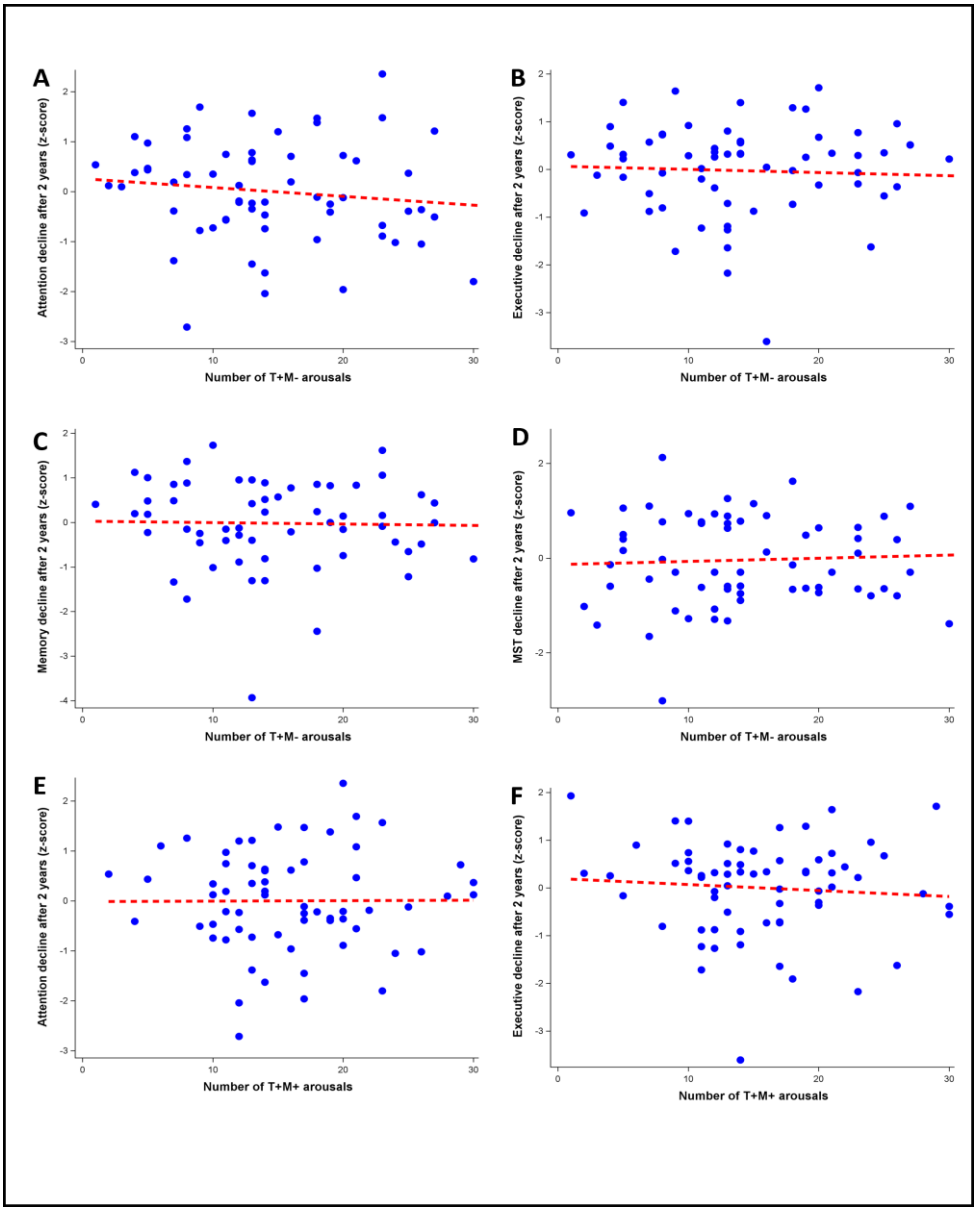

***Supplementary Figure S1.*** *Association between the polygenic risk score (PRS) for Alzheimer's disease and amyloid-beta (Aβ) burden including Aβ-positive individuals. Positivity was set at Aβ centiloid > 20* (Krasny et al., 2024) . *Sperman's r=-.08; p=.43 . The figure is complementary to Figure 2A.*




***Supplementary Figure S2. Non-significant associations between baseline cognitive scores and T+M+ and T+M- arousals. (A)*** *Association between the number of T+M- arousals and baseline*

*memory scores.* ***(B)*** *Association between the number of T+M+ arousals and baseline memory scores.* ***(C)*** *Association between the number of T+M- arousals and baseline executive scores.* ***(D)*** *Association between the number of T+M+ arousals and baseline executive scores.* ***(E)*** *Association between the number of T+M- arousals and baseline MST scores.* ***(F)*** *Association between the number of T+M+ arousals and baseline MST scores.*

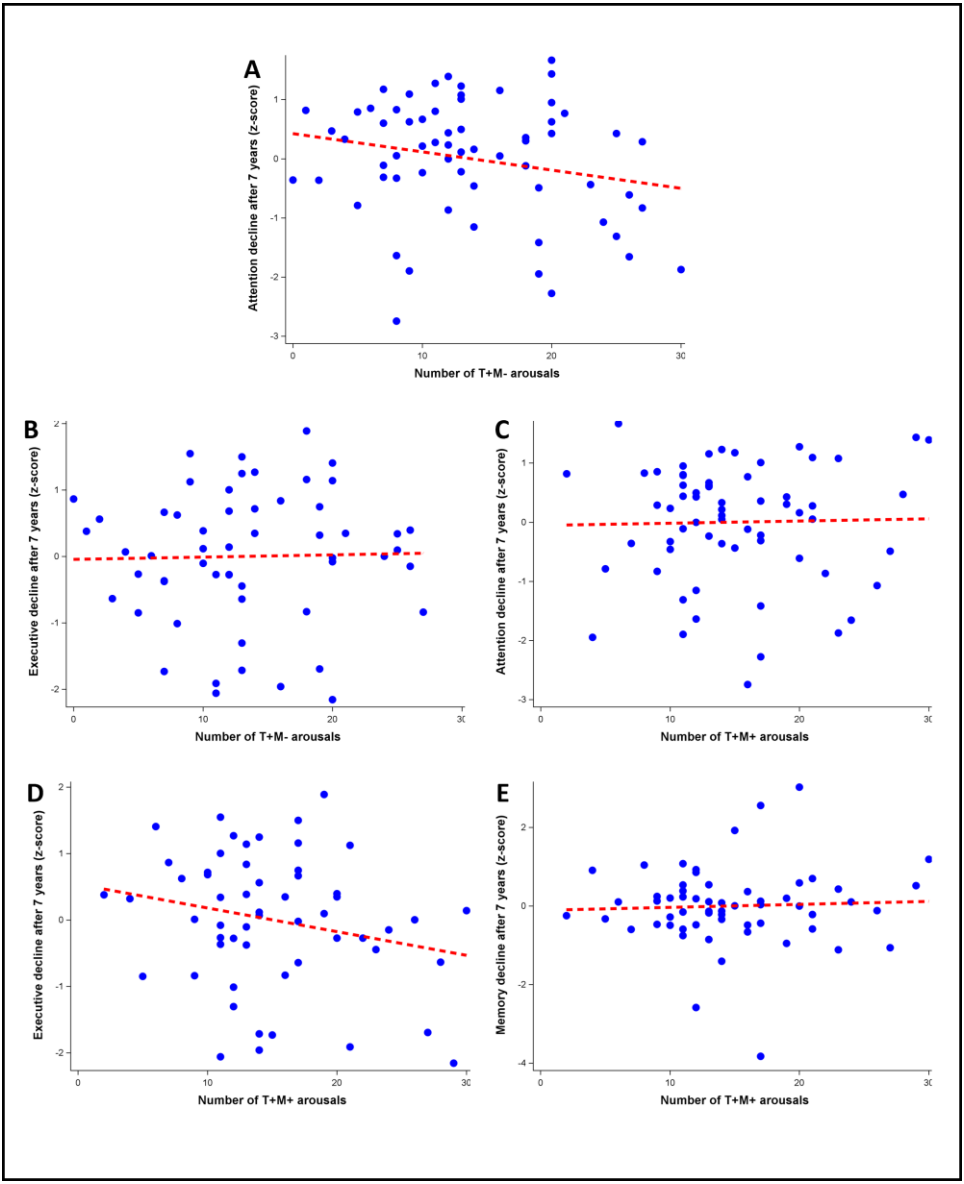
*None of the associations were significant (p>0.06).*




**Supplementary Figure S3. Non-significant associations between cognitive decline after two years and T+M+ and T+M- arousals. (A)** Association between the number of T+M- arousals and

attention decline after 2 years. **(B)** Association between the number of T+M- arousals and executive decline after 2 years. **(C)** Association between the number of T+M- arousals and memory decline after 2 years. **(D)** Association between the number of T+M- arousals and MST decline after 2 years. **(E)** Association between the number of T+M+ arousals and attention decline after 2 years. **(F)** Association between the number of T+M+ arousals and executive decline after 2 years.

None of the associations were significant ($p>0.20$). Refer to Table 4 for detailed statistical outputs.




***Supplementary Figure S4. Non-significant associations between cognitive decline after seven years and T+M+ and T+M- arousals. (A)*** *Association between the number of T+M- arousals and attention decline after 7 years.* ***(B)*** *Association between the number of T+M- arousals and executive decline after 7 years.* ***(C)*** *Association between the number of T+M+ arousals and*

*attention decline after 7 years.* ***(D)*** *Association between the number of T+M+ arousals and executive decline after 7 years.* ***(E)*** *Association between the number of T+M+ arousals and memory decline after 7 years.*

*None of the associations were significant (p>0.10). Refer to Table 4 for detailed statistical outputs.*