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Investigations of the links between locus coeruleus and basal forebrain activity during an oddball auditory task and characteristics of sleep spindles

Master thesis conducted by

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Under the supervision of
Gilles VANDEWALLE

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Abstract (English)

This master thesis entitled “Investigation of the links between locus coeruleus and basal forebrain activity during oddball auditory task and characteristics of sleep spindles” written by Emilie Seret has been realized during academic year 2024-2025 in the GIGA-CRC In Vivo Imaging laboratory in Gilles Vandewalle’s team, promoter of this master thesis.

This work aims to highlight potential links between sleep spindles and two brain regions of interest, the locus coeruleus (LC) and the basal forebrain (BF). Sleep spindles represent brief bursts of 11-16 Hz lasting < 1 to 3 seconds, occurring during N2 and N3 stages of slow waves sleep, as a result of thalamic reticular nucleus activity (TRN). This nucleus is innervated by several brain regions, among which the LC and the BF, which exert inhibitory effects on TRN neurons. Both the LC and BF may underlie part of the reported age- and sex-related differences in sleep spindle characteristics. Most of what is known about spindle regulation stems, however, from animal research. The translation to humans is incomplete, despite the increasing number of studies allowed by technological advancements in neuroscience, including high resolution 7 Tesla Magnetic Resonance Imaging.

This cross-sectional study aims to investigate the potential links between spindle characteristics (their number, density and spectral power) and the activity of the LC and the cholinergic BF assessed during a cognitive task. Data were collected using 7T MRI in 92 individuals of both sexes aged 18 to 75y (38.6±19.8; 61 women), including structural approaches to isolate the LC and the BF, on top of the functional MRI recording.

The analyses first suggested an age-related decrease in the number and density of spindles and confirmed a higher number and density of spindles in women compared to men. Importantly analyses further reveal that a higher response of the LC during the task is significantly and positively associated with the number and density of spindles, with a stronger link in men, while no significant interactions with age were detected. Likewise, the analyses did not yield any significant association between LC activity and spindle spectral power, as well as between cholinergic BF activity and spindle characteristics, potentially due to the limited number of participants that could be included in the analyses.

The findings suggest that LC activity during wakefulness may favor sleep spindle in men, potentially though sex-specific LC-TRN interactions. The results may have implications for the understanding of sex differences in the prevalence of certain brain disorders to which sleep is considered to contribute.

Abstract (French)

Ce mémoire intitulé « Investigation des liens entre l'activité du locus coeruleus et du cerveau antérieur basal durant une tâche auditive de oddball et les caractéristiques des fuseaux du sommeil » écrit par Emilie Seret a été réalisé durant l'année académique 2024-2025 dans le laboratoire GIGA-CRC In Vivo Imaging au sein de l'équipe de Gilles Vandewalle, promoteur de ce mémoire.

Ce travail vise à mettre en évidence les liens potentiels entre les fuseaux du sommeil et deux régions cérébrales d'intérêt, le locus coeruleus (LC) et le cerveau antérieur basal (BF). Les fuseaux du sommeil sont de brèves salves de 11 à 16 Hz d'une durée de < 1 à 3 secondes, apparaissant durant les stades N2 et N3 du sommeil à ondes lentes, résultant de l'activité du noyau réticulaire thalamique (TRN). Ce noyau est innervé par plusieurs régions cérébrales, dont le LC et le BF, qui exercent des effets inhibiteurs sur les neurones du TRN. Tous deux pourraient contribuer aux différences liées à l'âge et au sexe qui ont été rapportées dans les caractéristiques des fuseaux du sommeil. Toutefois, la plupart des connaissances proviennent de modèles animaux, et la transposition à l'humain reste incomplète malgré les progrès récents des neurosciences, notamment l'Imagerie par Résonance Magnétique à haute résolution 7 Tesla.

Cette étude transversale explore les liens potentiels entre les caractéristiques des fuseaux (leur nombre, leur densité et leur puissance spectrale) et l'activité du LC et du BF cholinergique évaluée pendant une tâche cognitive. Les données ont été recueillies à l'aide d'une IRM 7T chez 92 individus des deux sexes âgés de 18 à 75 ans (38,6 ± 19,8; 61 femmes), incluant des approches structurelles pour isoler le LC et le BF ainsi que de l'IRM fonctionnelle.

Les analyses indiquent une diminution du nombre et de la densité des fuseaux avec l'âge, et confirment leur plus grande abondance chez les femmes. Elles révèlent aussi qu'une réponse plus élevée du LC durant la tâche est significativement et positivement associée au nombre et à la densité des fuseaux, avec un lien plus fort chez les hommes, tandis qu'aucune interaction significative avec l'âge n'a été détectée. De même, les analyses n'ont pas mis en évidence d'association significative entre l'activité LC et la puissance spectrale des fuseaux, ni entre la réponse du BF cholinergique pendant la tâche cognitive et les caractéristiques des fuseaux.

Cette découverte suggère que l'activité du LC pendant l'éveil pourrait favoriser les fuseaux du sommeil chez les hommes, potentiellement via des interactions LC-TRN spécifiques selon le sexe. Ces résultats pourraient éclairer la compréhension des différences entre les sexes dans la prévalence de certains troubles cérébraux auxquels le sommeil serait associé.

Acknowledgments

Before discussing this master thesis, I would like to express my gratitude to all the people that have contributed directly or indirectly to the realization of this work. It would not have been possible without their help.

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Contents

List of abbreviations

ACh	Acetylcholine
AD	Adenosine
ARAS	Ascending reticular activating system
BAI	Beck Anxiety Inventory
BDI	Beck Depression Inventory
BF	Basal forebrain
BMI	Body Mass Index
BOLD	Blood Oxygen Level-Dependent
ChBF	Cholinergic basal forebrain
CNS	Central Nervous System
ECG	Electrocardiogram
EEG	Electroencephalogram
EMG	Electromyogram
EOG	Electrooculogram
ESS	Epworth Sleepiness Scale
GABA	γ-aminobutyric acid
GLM	Generalized Linear Model
GLMM	Generalized Linear Mixed Models
GLUT	Glutamatergic
HDB	Horizontal diagonal band nucleus
HRF	Hemodynamic Response Function
KSS	Karolinska Sleepiness Scale
LC	Locus coeruleus
LDT	Laterodorsal tegmental nucleus
LH	Lateral hypothalamus
LVFA	Low-Voltage Fast Activity
MCH	Melanin-concentrating hormone
MCPO	Magnocellular preoptic nucleus
MP2RAGE	Magnetization Prepared 2 Rapid Acquisition Gradient Echo
MPM	Multiparameter Mapping

MRI	Magnetic Resonance Imaging
MS	Medial septal nucleus
NBM	Nucleus basalis magnocellularis
NE	Norepinephrine
NREM	Non-Rapid Eye Movement
NODDI	Neurite Orientation Dispersion and Density Imaging
PAG	Periaqueductal gray
PB	Parabrachial nucleus
PPT	Pedunculopontine nucleus
PSQI	Pittsburgh Sleep Quality Index
PV	Parvalbumin
PVT	Psychomotor Vigilance Task
QSM	Quantitative Susceptibility Mapping
REM	Rapid Eye Movement
SCN	Suprachiasmatic nucleus
SO	Slow oscillation
SOM	Somatostatin
SW	Slow wave
SWA	Slow wave activity
TC	Thalamocortical
TMN	Tuberomammillary nucleus
TRN	Thalamic reticular nucleus
TST	Total Sleep Time
VAS	Visual Analogue Scale
VDB	Vertical diagonal band nucleus
VLPO	Ventrolateral preoptic nucleus

I. Introduction

In this work, we will study one of the many aspects of sleep physiology. This work begins with generalities about sleep and its regulation. Afterwards, we detail an EEG element, the spindles, and their regulation, which will be the subject of this thesis work. The structure, functions and activity of two brain structures, the locus coeruleus and the basal forebrain, are described, in relation with spindles. We explain the methods employed to conduct my research and the results, before discussing these results and replacing them in the context of the literature.

1. Sleep

1.1. Generalities

Sleep is hard to define as it can take different forms in the animal kingdom. It ranges from sleep-like quiescent state in several invertebrates such as nematodes or insects (as they do not have encephalization with a brain similar to the mammalian one), to complex sleep divided in two well-described stages in mammals and birds. Intermediate forms are found in fish and reptiles. Certain species even show particular sleep patterns, such as unihemispheric sleep in dolphins and whales, or drastic sleep decrease in migratory birds (Jha & Jha, 2020).

It is widely known that sleep plays a central role in the life of our species, *Homo sapiens*. Indeed, a third of our existence is spent in this altered state of consciousness. Its presence in all the taxa, and its prevalence in our daily life indicate its importance in survival and welfare of all animal species (R. Zielinski et al., 2016).

1.2. Definition

Sleep is described as a reversible altered state of consciousness characterized by a modified architecture of cerebral waves. The term unconsciousness is not used to describe this state, because the brain still shows activity and treats information, although the activity is different compared to wakefulness (Scammell et al., 2017). Sleep is also characterized by a reduction of the muscular tone and the perception of the external environment, as well as a decrease of respiratory and cardiac rhythm, just as thermoregulation (Jha & Jha, 2020; Saper et al., 2005). It reveals that not only brain activity is impacted, but also many physiological processes. In humans (and in vertebrates in general), sleep is

defined based on electrophysiology, as it is a reliable marker of sleep (see section 1.4) (Adamantidis et al., 2019).

1.3. Functions

It is common knowledge that sleep is essential to survival due to its numerous roles. In mammals, sleep modulates diverse cognitive processes, such as decision making and reaction time, and is essential for memory consolidation (Brown et al., 2012). Several physiological processes are also regulated by/during sleep, for instance the immune system and the glymphatic waste clearance system in the brain. The metabolism also shows alterations after sleep loss that are restored after sleep (R. Zielinski et al., 2016). Furthermore, the reorganization of neuronal circuitry, in addition to neurogenesis, occurs during sleep (Jha & Jha, 2020). Sleep is thus indispensable to proper brain function, regulation of cognitive processes and in the preservation and restoration of overall health.
Consequently, insufficient sleep or poor sleep quality leads to diverse matters. It increases risks of mental health disorders and neurodegeneration, and enhances the prevalence of certain diseases such as diabetes and cardiovascular diseases (Anothaisintawee et al., 2016; Bjorvatn et al., 2007). It also affects cognitive performances, including attention, emotional reactivity, decision making and memory formation (Zavecz et al., 2020).

1.4. Architecture

Sleep is divided in several cycles. A cycle is composed of 2 different states, rapid eye movement (REM) and non-rapid eye movement (NREM) sleep, composed of 3 stages. A cycle always begins by an episode of NREM sleep and ends with an episode of REM sleep. A cycle lasts approximately 90 minutes, though its duration varies substantially across individuals while being relatively stable within an individual. NREM sleep represents 75 to 80% of this time while REM sleep constitutes the remaining 20 to 25% (Dauvilliers & Billiard, 2004). The proportion of NREM sleep decreases through the night in favour of the proportion of REM sleep (Figure 1). Indeed, the sleep pressure is directly associated with the length of NREM sleep and is at its highest at the beginning of the night (Brown et al., 2012). During the night, this pressure is reduced so is the time spent in this stage throughout successive cycles (Achermann & Borbély, 2017).

REM and NREM sleep can be differentiated by their electrical activity measured with an EEG, their muscular tonus measured with an electromyogram (EMG), and the eye movement detected with an electrooculogram (EOG) (Figure 2) (Berry et al., 2017). All these measures are recorded using electrodes placed on the scalp or on the face. Sleep recording can be extended to other signal for what is called a polysomnography. Cardiac rhythm can also be recorded using electrocardiography (ECG), as well as respiratory parameters, but they are not used as criteria to distinguish different sleep stages (Dauvilliers & Billiard, 2004).

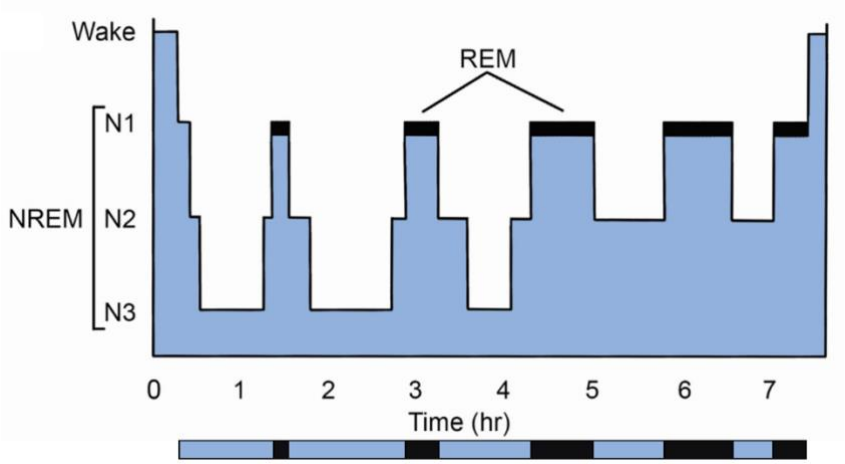

Figure 1. Sleep cycles through the night (adapted from Scammell et al., 2017). REM, rapid eye movement (sleep); NREM, non-rapid eye movement (sleep).

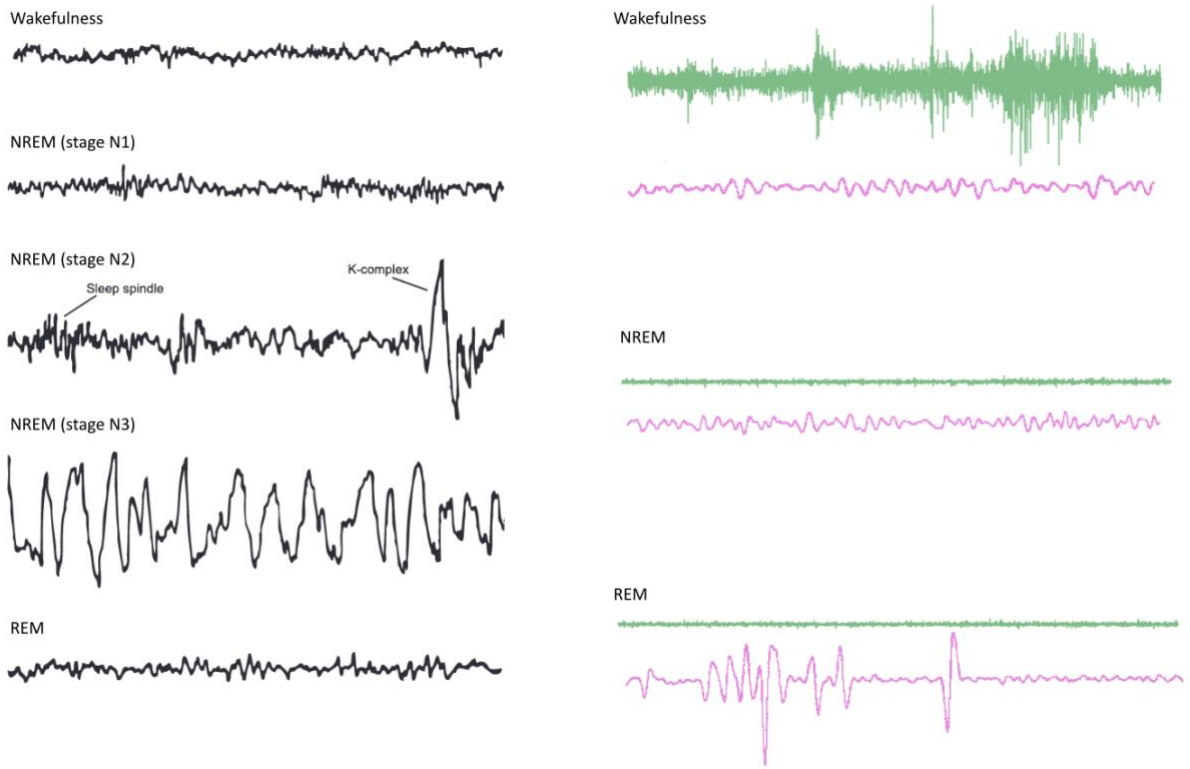

Figure 2. EEG (black), EMG (green) and EOG (pink) signals of wakefulness, NREM and REM sleep (adapted from Brown et al., 2012). REM, rapid eye movement (sleep); NREM, non-rapid eye movement sleep.

1.5. Electroencephalogram (EEG)

An electroencephalogram measures electrical activity of the cerebral cortex from the surface of the head, making it a non-invasive method. Electrodes are placed on the scalp at specific places (most often following the so-called 10-20 convention), with reference electrodes placed on the mastoid (behind both left and right ears) (Berry et al., 2017). The potential difference between the reference and each electrode is measured and mainly represents the post-synaptic currents from the apical dendrites of pyramidal neurons. However, other parameters can contribute to this signal, such as intrinsic membrane properties and neuronal firing, and even some type of glial activity (Fields, 2008; R. Zielinski et al., 2016).

The signal obtained is a complex waveform, which is different for wakefulness, NREM and REM sleep. It can be divided into five bands as defined according to previous studies (Aboalayon et al., 2016; Buzsáki & Draguhn, 2004), while most recent work defines six bands. This division relies on the frequency, separating the signal in delta, theta, alpha, sigma, beta and gamma bands (Adamantidis et al., 2019; Brown et al., 2012; Fernandez & Lüthi, 2020; Pignatelli et al., 2012; R. Zielinski et al., 2016):

- *Delta rhythms* are characterized by a frequency between 0.5 and 4 Hz. They are detected mostly during sleep and correspond to the frequency of the slow waves (SWs), and their intensity is a marker of sleep intensity. Slow waves activity (SWA) increases with sleep deprivation, is higher at the beginning of the sleep and decreases during the sleep period. Slow waves are a hallmark of deep sleep, corresponding to NREM sleep N3 stage. Slow waves are generated by the alternating up and down states. Up state corresponds to depolarized resting membrane potential and down state corresponds to hyperpolarized resting membrane potential. These two states alternate in a synchronized way in cortical neurons and are at the origin of SWs detected in the EEG. These oscillations are in part regulated by the thalamus.
  The frequencies between 0.5 and 1 Hz are more specifically called slow oscillations (SOs). SOs derivate from K-complex, which are slow-high amplitude EEG waveform distinctive of NREM sleep N2 stage, composed of a brief negative sharp followed by a positive component. The SOs are known for grouping other types of activity such as spindles and delta waves.

- At higher frequency are the *theta rhythms*, between 4 and 8 Hz. They arise mostly from hippocampal activity, and are detected indirectly through cortical EEG, due to functional

synchronicity between those regions influencing cortical activity. Theta activity can also arise directly from cortical sources. This band correlates with the sleep drive during wakefulness and increases with sleep deprivation. During REM sleep, theta waves are coupled with gamma waves, and they become more salient upon transition between NREM and REM sleep. Besides, these rhythms are detectable during attention or memory tasks during wakefulness.

- Between 8 and 12 Hz, the activity is called *alpha rhythms*. They are detected during relaxed wakefulness, with eyes closed, and are suppressed by eye opening or sleep. Alpha waves result from an interaction between thalamic and neocortical neurons.

- *Sigma rhythms*, comprised between 12 and 16 Hz, are mostly detected during sleep and correspond to frequency of the sleep spindles. Those are brief bursts of < 1 to 3 seconds taking the shape of a spindle on the EEG. They are the most significant feature of NREM sleep N2 stage, with the K-complex. They are also present in N3 stage, but less easily detected. Spindles are associated with the up state of slow waves. They are the result of thalamic activity. This oscillation will be the main interest of this work and will be describe in detail below.

- *Beta rhythms* correspond to EEG activity between 16 and 30 Hz. It is detected during quiet waking and is enhanced in particular cortical area after presentation of a sensory stimuli. A role of these beta waves is to synchronize activity of distant cerebral regions.

- The frequencies of ≥30 Hz, extending up to approximately 120 Hz, characterize *gamma rhythms*. It results from synchronization of local cortical network of fast-spiking interneurons. Gamma waves are recorded in the cortex and a lot of subcortical structures. They are detected during REM sleep and quiet waking and enhanced with theta rhythms.

1.5.1. Wakefulness

Wakefulness can be divided in two types: quiet or active. Quiet waking with eyes closed displays dominant alpha activity, variable muscular tone and no particular eye movement. Active waking shows a difference in the EEG activity called low-voltage fast activity (LVFA) and in the eye movements (Dauvilliers & Billiard, 2004). This activity mostly includes beta rhythms and gamma rhythms, as well as theta rhythms during certain cognitive tasks (Brown et al., 2012).

1.5.2. NREM sleep

NREM is the first stage of the sleep cycle. This sleep is characterized by slower brain activity, high amplitude slow waves, and low muscular tone (Brown et al., 2012).

In the past, the first sleep scoring systems distinguished 4 stages in NREM sleep (S1-S4), but it has been simplified since by the American Academy of Sleep Medicine (AASM) into the 3 stages N1, N2 and N3. By agreement, sleep is scored by 30 seconds epochs. If two stages coexist in an epoch, the stage comprising the greater part of the epoch defines this epoch (Berry et al., 2017).

N1 and N2 stages define light sleep while N3 defines deep sleep (Jha & Jha, 2020; Kales et al., 1968). Those 3 stages are also distinguished by specific electrical activity.

N1 stage is characterized by alpha activity shortly followed by theta activity (Adamantidis et al., 2019). Slow eye movements are also present. This stage is short and lasts only a few minutes. An epoch is defined as N1 stage if at least 50% of its activity is theta rhythm. Following, the apparition of K-complex and sleep spindles defines N2 stage, lasting 10 to 25 minutes (Dauvilliers & Billiard, 2004; R. Zielinski et al., 2016). An epoch is scored as N2 stage if any of these 2 characteristics occur during the first half of this epoch. The eye movements are suppressed.

N3 stage, or deep sleep, is hallmarked by slow delta waves replacing theta activity for a duration of 20 to 40 minutes, taking precedence over spindles still present during this stage (Brown et al., 2012). Slow wave activity has to be present in more than 20% of the epoch to score it N3 stage. This stage is also referred as slow wave sleep.

1.5.3. REM sleep

The REM sleep is characterized by a cerebral activity similar to the wakefulness phase, for this reason this type is also called paradoxical sleep. This phase shows LVFA, corresponding to low amplitude waves (gamma activity), associated with theta rhythms (Dauvilliers & Billiard, 2004). Another type of activity has been found in cats and rodents at first and detected later in humans, and is called ponto-geniculo-occipital waves, or P waves in rodents (Datta, 1996; Peigneux et al., 2001). They appear during REM sleep in bursts of low amplitude and also right before the onset of this phase at higher amplitude (Steriade et al., 1989).

As its name suggests, the hallmark of this stage is stereotyped eye movement, occurring under closed eyelids. Muscular tone is abolished but disturbed by twitches, which are transient muscular discharges (Brown et al., 2012). Typically, the first episode of REM sleep lasts 4 to 8 minutes and lengthens through the night.

An epoch is defined as REM sleep if it shows LVFA without K complex or spindles, rapid eye movements and low chin EMG tone (Berry et al., 2017).
REM sleep is not divided into different stages like NREM sleep but shows two different types of features: tonic and phasic activities. Tonic activity is lasting, including LVFA and muscle atonia, while phasic activity is transient, instantaneous and includes the rapid eye movements and twitches. REM sleep is associated with dreams, although several studies have shown that dreaming can occur during NREM sleep as well (Siclari et al., 2018).

1.6. Regulation

The regulation of such a complex process requires a lot of precision and coordination. The different modalities of sleep, the timing, length and deepness, are driven by external and internal factors. The first is the circadian rhythm, which coordinates physiology over the day, and the second is the homeostatic pressure, which depends on the duration of the prior awakening (Borbély, 2022).
Several brain structures are involved and constitute networks of feedback and modulation essentials for sleep onset, but also sleep offset and wakefulness.

1.6.1. Sleep homeostasis

Sleep homeostasis corresponds to the dynamic regulation of sleep pressure, which increase during the wake period. The longer this period is, the longer and deeper sleep has to be to dissipate the high homeostatic pressure.
NREM follows wake and is promoted by substances, called somnogens according to their function, accumulating during the wakefulness period, such as adenosine, prostaglandins and cytokines (Scammell et al., 2017).
Sleep pressure is multifactorial and not only due to these somnogens. During wake, changes in brain physiology and connectivity, such as synaptic potentiation, contribute to this pressure (Sawada et al., 2024).
Adenosine (AD) is the best understood of the somnogens. It originates from neuronal and glial activity, as well as energy utilization. AD levels increase in the cortex, hypothalamus and basal forebrain through prolonged awakening. The extracellular level declines during sleep, as well as slow wave activity which is a marker of sleep pressure, thus being higher at the beginning of the night (Schmitt et al., 2012; Sulaman et al., 2023).

AD promotes sleep by inhibiting wake-promoting neurons and activating sleep-promoting neurons (Scammell et al., 2017). A blocking of this neurochemical factor prevents NREM rebound (Halassa et al., 2009).

1.6.2. Circadian rhythm

Circadian rhythm is driven by an autonomously rhythmic system, organized by the principal biological clock of the body, being the suprachiasmatic nucleus (SCN) of the hypothalamus. This pair of nuclei in the hypothalamus is capable of self-sustained oscillations. That is supported by specific activity of a variety of neuropeptides-expressing network of neurons (Colwell, 2011).

Circadian is from Latin *circa diem*, meaning about a day. Indeed, daily oscillations in the internal state follows the 24h rotation of the Earth. External timing cues synchronize the SCN, itself providing temporal organization of physiological processes as sleep and metabolism, as well as endocrine signalling. The external cues are called zeitgebers. The main one is light, which is detected mainly through specialized photoreceptors of the retina that express the photopigment melanopsin and are more sensitive to blue wavelength light. This information is transferred to the SCN through the retinohypothalamic tract (Saper et al., 2005; Scammell et al., 2017).

Another signal involved in circadian regulation is the hormone melatonin. This hormone is secreted in the pineal gland and released during dark period, acting as a biological signal of this period. It is under the control of circadian clock and acts on the SCN, thus forming a feedback loop. Its effect is to help regularizing sleep onset and to contribute to entraining circadian rhythms (Scammell et al., 2017).

SCN synchronizes sleep-wake cycles with the light-dark cycle. It does so through its anatomical connections and the release of secreted factors, but those mechanisms have not been fully investigated yet. SCN firing rate variates during this cycle, and is the highest during light period (Scammell et al., 2017).

SCN projects to other hypothalamic nuclei. These nuclei communicate and send glutamatergic projections to wake-promoting regions and GABAergic projections to sleep-promoting regions (described below). This network integrates signals from the SCN, then promoting wake (Scammell et al., 2017).

1.7. Cerebral networks

1.7.1. Wakefulness

Wakefulness is supported by a system of interconnected regions called the ascending reticular activating system (ARAS). Cortical and subcortical structures are involved in this system, comprising the thalamus, hypothalamus, brainstem and basal forebrain. They all play different roles but are engaged in arousal (Brown et al., 2012). This system has a ventral and a dorsal pathway projecting to different areas, but having a same final function: maintaining cerebral activation needed for wake (Figure 3) (Saper et al., 2005).

The ventral pathway includes the cholinergic parabrachial nucleus (PB), the noradrenergic locus coeruleus (LC), the serotoninergic raphe, the dopaminergic periaqueductal gray (PAG) and the histaminergic tuberomammillary nucleus (TMN). These structures project to the cholinergic basal forebrain (BF), the lateral hypothalamus (LH) and the cortex. The dorsal pathway arises from the cholinergic pedunculopontine (PPT) and laterodorsal tegmental (LDT) nuclei, as well as the cholinergic PB, and projects to the thalamus, and to the cortex via glutamatergic thalamocortical projections.

They all receive excitatory projections from orexins neurons of the LH, themselves triggered through the activation of SCN pathway during light period. Those particular neurons are important to maintain long period of wake.

ARAS regulates sleep-wake cycle, promotes wake or arousal, cortical activation and functions through the release of various neurotransmitters by their widespread projections (Scammell et al., 2017).

1.7.2. Sleep

Wake-promoting regions discussed above need to be inhibited to allow sleep onset. It is the role of regions in the preoptic area, called the ventrolateral preoptic nucleus (VLPO) and median preoptic nucleus (MnPO) (Saper & Fuller, 2017). ARAS structures innervate back VLPO, and possibly MnPO, and inhibit its activity. This mutually inhibitory interaction is resumed in the name of flip-flop model. When activated by adenosine accumulating during wakefulness, VLPO neurons inhibit ARAS and promote sleep. When ARAS is activated, its structures inhibit VLPO neurons and promote wake. This creates a dynamic interaction enabling swift switches between wakefulness and sleep. Orexin and melanin-concentrating hormone (MCH) secretion from LH neurons stabilizes this flip-flop switch. Orexin reinforces the arousal system while MCH reinforces REM sleep (Saper et al., 2005; Scammell et al., 2017).

In some wake-promoting regions, such as BF or brainstem, are found populations of sleep-promoting neurons. These neurons projects to the cortex and other populations of wake-active neurons and inhibits them to promote sleep (Figure 4) (Scammell et al., 2017).

The alternations between NREM and REM sleep mainly depends on pons activity. Subregions of the pons including PPT, LDT and sublaterodorsal tegmental nucleus play various role in REM sleep, such as contributing to muscle atonia, regulating transition between NREM and REM sleep and promoting typical brain activity of REM sleep. They act through local projections and more distant projections to other brain regions as thalamus and BF (Figure 5) (Sulaman et al., 2023).
Extended VLPO is also involved in REM sleep by its inhibitory action on wake-active regions and activation of REM sleep-promoting regions (Lu et al., 2002).

As discussed in the previous section, the LC is a part of the ARAS. It then has role as REM sleep-suppressing nucleus, by inhibiting REM sleep-promoting regions mentioned above. Its activity is nearly suppressed during REM sleep, allowing the generation of specific characteristics of this stage. The PAG and the raphe act the same way as the LC, as REM sleep-suppressing nuclei (Scammell et al., 2017).

1.8. Sex- and age-related differences

Sleep micro- and macro-structures vary across individuals, as well as within individuals, and are strongly influenced by age and sex. Ageing is associated with a decline in all aspects of sleep function. During ageing, sleep becomes more fragmented, absolute power density diminish, sleep efficiency lowers and slow wave sleep decreases (Dijk et al., 2010; Schwarz et al., 2017). These effects of ageing are distinct between males and females, and sex difference are well documented at various ages. Indeed, women reports more subjective sleep disturbances, while objectives sleep measures shows more sleep-wake disruption in males (Mander et al., 2017). There is also hormonal influence in sleep architecture, that impact sleep physiology globally as well, and may modulate brain oscillations (Carrier et al., 2017). Previous researches have highlighted such age- and sex-related differences (Koshmanova et al., 2023; Mortazavi et al., 2025; Van Egroo et al., 2024).

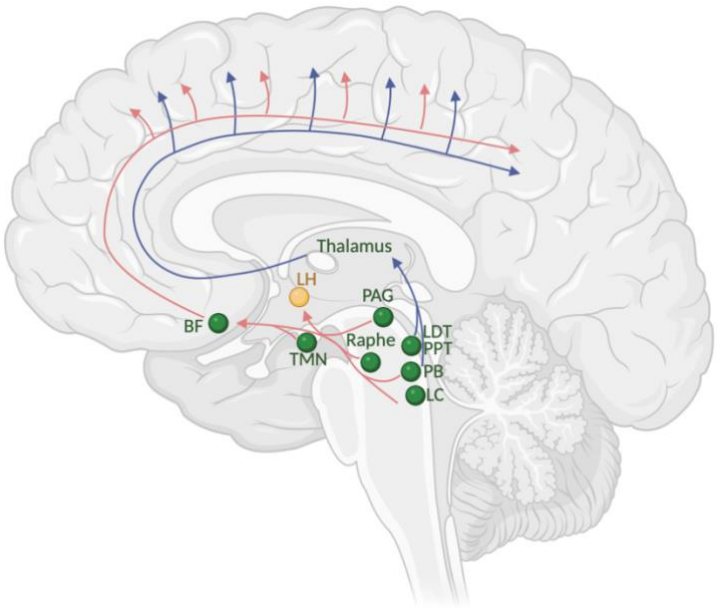

Figure 3. Key components of the dorsal (in blue) and ventral (in pink) pathways of the ascending reticular activating system (adapted from Saper et al., 2005; Scammel et al., 2017). Lateral hypothalamus (LH; in yellow) sends orexin projections (not represented) to all structures represented in the figure, activating them. BF, basal forebrain; LC, locus coeruleus; LDT, laterodorsal tegmental nucleus; PAG, periaqueductal gray; PB, parabrachial nucleus; PPT, pedunculopontine nucleus; TMN, tuberomammillary nucleus.

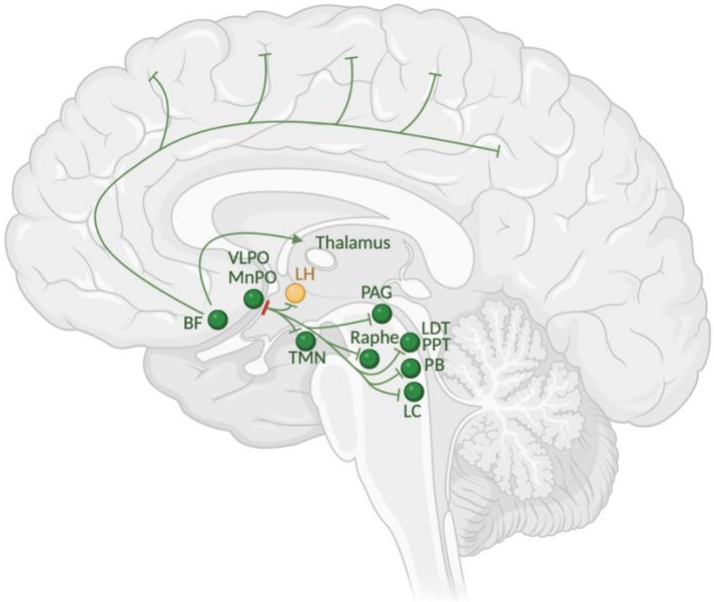

Figure 4. Key components of the NREM sleep-activating pathway (in green) (adapted from Saper et al., 2005; Scammel et al., 2017). The red line represents the innervation of the ventrolateral preoptic nucleus (VLPO) and the median preoptic nucleus (MnPO) by wake-promoting regions, building the flip-flop switch.

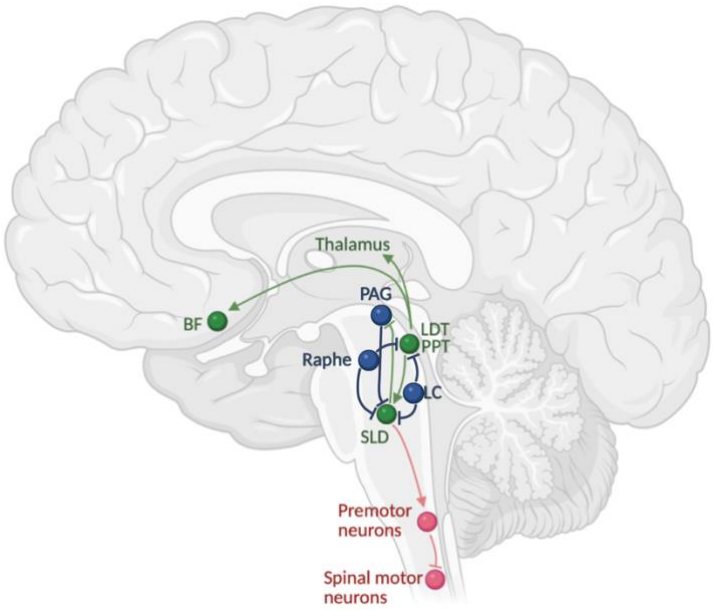

Figure 5. Key components of the REM sleep-activating pathway (in green) and the REM sleep-suppressing pathway (in blue) (adapted from Saper et al., 2005; Scammel et al., 2017). The pink pathway promotes muscle atonia.

2. Spindles

As mentioned above, this work focuses on one aspect of sleep physiology, sleep spindles. They will be first described, with their role, their generation and their regulation.

2.1. Definition

A sleep spindle is defined by the American Academy of Sleep Medicine as "A train of distinct sinusoidal waves with frequency 11-16 Hz (most commonly 12-14 Hz) with a duration ≥ 0.5 seconds, usually maximal in amplitude in the central derivations". It is a hallmark of the NREM sleep N2 stage, generated in the GABAergic neurons of the thalamic reticular nucleus (TRN) and detected in the cortex. As mentioned above, they are also present in NREM sleep N3 stage, but less prominent, and they are more difficult to detect visually as the signal is dominated by delta waves (De Gennaro & Ferrara, 2003; Fernandez & Lüthi, 2020).
They are also observed in more frequently during the few tens of seconds preceding the NREM-to-REM transitions (Osorio-Forero et al., 2021).

It was first observed 90 years ago by Loomis and colleagues, but only fully understood 40 years ago by M Steriade, who dedicated most of his research on this oscillation (Loomis et al., 1935; Steriade et al., 1985, 1987).

A spindle lasts 0.5 to 3 seconds and is followed by a refractory period of 5 to 10 seconds, caused by a combination of intrathalamic, cortical and brainstem mechanisms, particularly the locus coeruleus-norepinephrine system (LC-NE; described later). These mechanisms are transient, and when their activity fades away, another cycle of spindle is possible (Bal & McCormick, 1996).

The frequencies used to define spindles varies between studies, the lower limit fluctuates between 10 and 12 Hz while the upper one does between 14 and 16 Hz. They are divided in slow and fast spindles according to the frequency threshold of 13 Hz. These two types of spindles are detected preferentially in different cortical areas during NREM sleep N2 and N3 stages (De Gennaro & Ferrara, 2003; Fernandez & Lüthi, 2020).

The frequencies used to define spindles varies between studies, the lower limit fluctuates between 10 and 12 Hz while the upper one does between 14 and 16 Hz. They are commonly divided in slow and

fast spindles according to the frequency threshold of 13 Hz, although this remains a debate in the literature ((Zerouali et al., 2014)). These two type of spindles are detected preferentially in different cortical areas during NREM sleep N2 and N3 stages (De Gennaro & Ferrara, 2003; Fernandez & Lüthi, 2020).

On a longer timescale, it has been observed that sigma band power, mostly populated by spindles, fluctuates on a ~50 seconds timing both in rodents and in humans. Spindles cluster, creating spindle-rich period, called continuity period, and spindle-poor period, called fragility period, occurring both in the ~50 seconds period (Figure 6). During the fragility period, the susceptibility to arousal is higher, hence its name (Fernandez & Lüthi, 2020; Osorio-Forero et al., 2021).

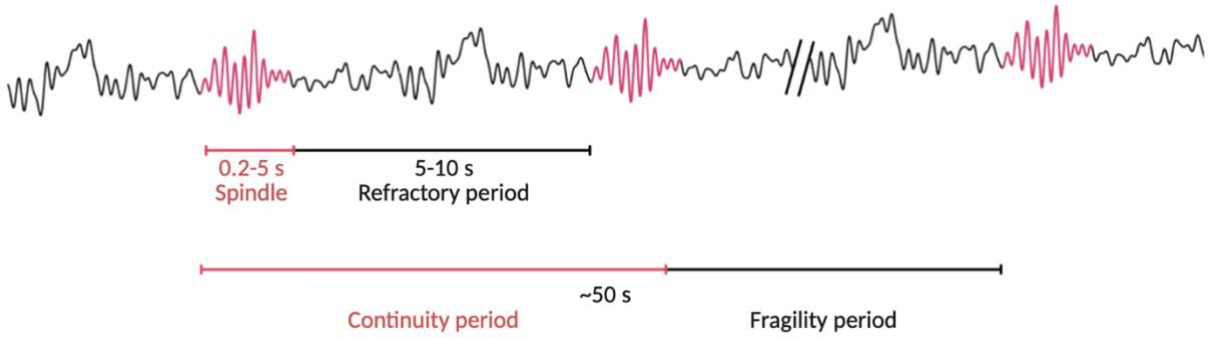

Figure 6. Temporal structure of spindles in EEG signal.

2.2. Functions

Every oscillation represents a mode of communication between neurons. This communication is essential to many cognitive processes and overall health. That is supported by the timing and characteristics of the different sleep oscillations (Brown et al., 2012; Jha & Jha, 2020).

Spindles, as other dominant sleep rhythm, are involved in memory consolidation. Memory consolidation is composed of 4 steps: creation of a memory trace in neurons, transmission, storage in a retrievable format and then retrieval of the trace. As mentioned above, the timing of oscillations is important. Indeed, the process of memory consolidation is optimal when the spindles are time locked to other oscillations. They are coupled to SOs and hippocampal sharp wave-ripples (brief, high-frequency discharges of 80-100 Hz). This coupling optimizes essential neuronal conditions for memory consolidation, generated by spindles (Fernandez & Lüthi, 2020; Rasch & Born, 2013).

Sleep spindles would also be linked with some intellectual abilities. Several studies have observed that various spindle characteristics, such as number, amplitude and duration, might be markers for IQ (Fogel & Smith, 2011).

Then, disrupted spindle activity is found in several sleep disorders and neurological diseases. They are not yet described as a cause but could be markers of many disruptions such as epilepsy, insomnia, schizophrenia or even Alzheimer's and Parkinson's diseases (Fernandez & Lüthi, 2020).

2.3. The thalamic reticular nucleus

The thalamic reticular nucleus is a sheet of GABAergic neurons enveloping the anterior and lateral parts of the thalamus (Halassa, 2022). It receives glutamatergic inputs from the collaterals of the thalamocortical neurons and from the corticothalamic neurons. Besides, it receives inputs from the brainstem and the BF modulating its activation. TRN sends back dense axonal innervations to the thalamus alone, and innervates each of its nuclei (Steriade, 2003). It forms a feedback loop essential to the production of sleep oscillations (Figure 7).

TRN neurons show 2 types of firing mode, either tonic or phasic/bursting. The tonic mode is typical of wakefulness, while the burst mode is responsible for spindle generation during NREM sleep N2 stage (Halassa & Acsády, 2016).

Therefore, spindles are generated in the thalamus by the interaction between inhibitory GABAergic neurons of the TRN and thalamocortical cells from the corresponding thalamic zones, but detected over widespread cortical territories due to thalamocortical projections (Bal & McCormick, 1996).

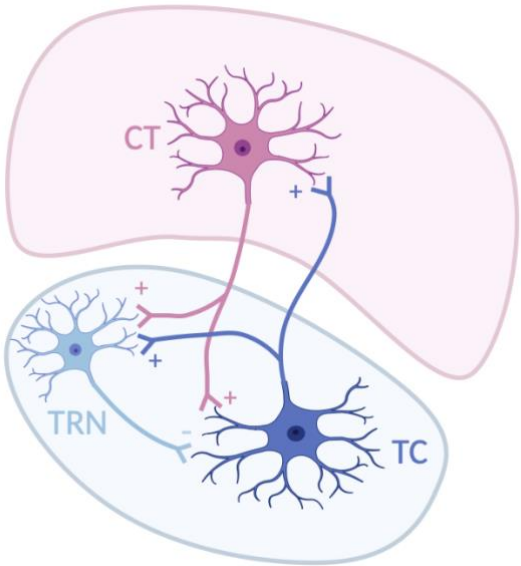

Figure 7. Feedback loop for spindle generation (adapted from Steriade, 2003). In blue, the thalamus with inhibitory TRN neuron and excitatory thalamocortical (TC) neuron. In pink, the cortex with excitatory corticothalamic neuron

2.3.1. Spindle generation

The bursts responsible for spindle oscillations are generated by low-threshold (T-type) Ca^{2+} channels present in the TRN neurons and in TC neurons (Cueni et al., 2008). These channels are recruited and induce bursts of 2 to 10 action potentials. Release of GABA by TRN neurons results in an inhibition of thalamocortical neurons, essential for spindles. Indeed, this recruits again T-type channels in TC neurons, resulting in a rebound burst in those cells (Fernandez & Lüthi, 2020; Halassa & Acsády, 2016). Glutamate is released from TC neuron collaterals in TC-TRN synapses, and drives another TRN burst which initiates a new cycle/supports the spindle-like rhythm. Spindles are also sent to the cortex via these thalamocortical projections.

2.3.2. Regulation

The TRN is innervated by several brain regions, including the cortex, essential to the feedback loop of the spindle regulation, but also subcortical areas, mainly the BF and several nuclei of the brainstem, such as the LC. During wakefulness, LC and BF projections excite TRN neurons, which inhibit TRN bursting activity. This excitation is supported only by BF during REM sleep. Different neurotransmitters are involved, acetylcholine (ACh), norepinephrine (NE), serotonin and GABA (Brown et al., 2012).

The two modes of activation of TRN neurons are modulated by the LC-NE system. NE release induces a switch of TRN activity from burst mode to tonic/single-spike mode. This is mediated through α1-receptors as well as β-receptors (Osorio-Forero et al., 2021). TRN neurons need hyperpolarization to recruit T-type calcium channels essentials to produce spindles, and NE depolarizes those neurons, preventing those oscillations during wakefulness. This system not only has a role in inhibition of TRN activity, but also in the regulation of spindles during NREM sleep (Osorio-Forero et al., 2025; Sulaman et al., 2023). This will be discussed in the following sections.

Basal forebrain is involved in the regulation of TRN via ACh and GABA. This area is activated during both wakefulness and REM sleep as part of ARAS, as explained above. GABAergic and cholinergic projections thus inhibit spindles (Parent et al., 1988). However, it has been shown that these projections have also an activating action (Ni et al., 2016).

3. Locus coeruleus

3.1. Generalities

The locus coeruleus, or blue spot, has been first described in 1876 by Félix Vicq-d'Azyr. This structure is localized profoundly in the pons nearby the fourth ventricle floor, which makes it difficult to observe (Keren et al., 2009). With the new technological advances in the 60s, the monoaminergic nature of most of its neurons was discovered, and more precisely the synthesis of NE. LC is the main source of NE in the central nervous system (CNS), but other peptide transmitters are found in LC neurons (Counts & Mufson, 2012). The pigmented polymer, called neuromelanin, responsible of its blue colour is a result of the oxidation of the NE in the neurons, giving this nucleus its name (Wakamatsu et al., 2015). LC sends monosynaptic projections to almost all cerebral regions, including the cortex, forebrain, hippocampus, amygdala, cerebellum, thalamus and spinal cord, suggesting a prominent role of NE in the CNS (Fernandes et al., 2012; Poe et al., 2020).

3.2. Connectivity

LC receives several cortical and subcortical afferent inputs, including prefrontal cortex, amygdala, hypothalamus, spinal cord, basal forebrain and brainstem. These structures modulate the activity of the LC through different neurotransmitters via their action on several receptors (Counts & Mufson, 2012).

Despite its small size, the LC projects its noradrenergic axons to almost all brain regions (Poe et al., 2020). This efferent system is split in two: the large dorsal tegmental bundle and the smaller rostral limb of the periventricular bundle. The first one innervates the forebrain and spinal cord, as well as the amygdala, cortex and hippocampus. The second system sends projections to diencephalon, comprising thalamus and hypothalamus, and to brainstem sensory structures (Counts & Mufson, 2012). This enables specific modulation of the different functions affected by this nucleus, with some neurons projecting to a single brain region, while others project to different brain regions via collaterals, these regions generally being functionally related (Chandler et al., 2019).

LC actions are mediated by four major classes of G protein-coupled adrenoreceptors, one of them being inhibitory ($\alpha 2$) and the three others being excitatory ($\alpha 1$, $\beta 1$, $\beta 2$). There is variation in the distribution and the molecular mechanisms of those receptors according to their localization in the brain, and they can be postsynaptic or presynaptic (Counts & Mufson, 2012). Indeed, the LC autoregulates its functions via presynaptic $\alpha 2$-adrenoreceptors (Wagner-Altendorf et al., 2019). This variety explains the diversity of the response to norepinephrine observed in different brain regions.

3.3. Functions

LC-NE system has many roles in the brain, largely associated with sensory information, whether to maintain the cerebral state needed to collect or to process it (Berridge & Waterhouse, 2003). This makes the LC essential to interact and process with the complex surrounding environment. This nucleus promotes arousal, as a part of the ARAS together with other brainstem nuclei. It regulates sleep and wakefulness through its connection with the circadian system and is involved in diverse cognitive functions such as attention, memory and perception (G. S. Aston-Jones et al., 2007; Berridge & Waterhouse, 2003). LC is also involved in salience and novelty detection. It plays a role in emotion such as stress and anxiety (Keren et al., 2009). Furthermore, LC has a role in pupil control and this feature is widely used as marker of LC activity (Poe et al., 2020). Because of its involvement in all these functions, an alteration of this nucleus is considered to contribute to several pathological processes, including Parkinson and Alzheimer disease, insomnia, REM sleep behavioural disorders and schizophrenia (Counts & Mufson, 2012; García-Lorenzo et al., 2013; Mäki-Marttunen, 2020; Van Someren, 2021).

3.4. Activity

Alike the TRN, LC neurons fire in two distinct modes: tonic or phasic. The tonic mode is characterized by a sustained, regular discharge pattern, with low frequencies of 2-3 Hz when awake, and < 1 Hz during NREM sleep. This activity is absent during REM sleep (Counts & Mufson, 2012; Foote et al., 1980). Superimposed to this, there is the phasic mode. It consists of bursts of 2-3 action potentials followed by a sustained period of inactivity. This occurs in response to salient stimulation and is consequently involved in vigilance. A transient increase in NE is associated with these bursts (Berridge & Waterhouse, 2003).

During sleep, LC alternates between different states. It shows diminished discharge during transition from wake to NREM sleep and from NREM sleep to REM sleep. On the other hand, burst of impulse accompanies transition from NREM sleep to wake. As aforementioned, variations of tonic activity are observed between stages, this type of discharge being the highest during active wakefulness, slower during NREM sleep and absent during REM sleep (Aston-Jones & Bloom, 1981). Indeed, REM sleep state is enabled by this cessation of discharge, as LC-NE system maintains arousal through its modulation on different brain regions. Despite that, NE levels can reach higher levels during NREM

sleep than during quiet wakefulness, although at minor rates. This level is needed because NE modulates some features of NREM sleep, such as spindles.

The LC activity during NREM sleep present two distinct rhythms: one that is faster, and one that is infraslow.

LC faster activity is associated to one spindle as follows: its activity is reduced the second preceding spindle, increases progressively during the spindle thus leading to spindle termination, and decreases again the second following the spindle (G. Aston-Jones & Bloom, 1981; Swift et al., 2018).

LC coordinates the infraslow rhythm in spindles generation defining the continuity periods, which are spindle-rich, and the fragility period, which are spindle-poor. LC activity increases progressively during the fragility period, NE levels increase consequently in TRN and it suppresses sleep spindles. Then it diminishes rapidly and enables spindle generation during the continuity period, clustering these in this period of ~50 seconds (Osorio-Forero et al., 2021, 2025).

4. Basal forebrain

4.1. Generalities

The basal forebrain (BF) is a large structure, positioned in the rostral telencephalon near the ventral surface. This structure is divided into 6 subregions: vertical diagonal band nucleus (VDB), medial septal nucleus (MS), horizontal diagonal band nucleus (HDB), magnocellular preoptic nucleus, substantia innominata and nucleus basalis of Meynert (NBM). Those different subregions innervate specific brain areas and play different roles in the regulation of the sleep-wake cycle (Rye et al., 1984; Wu et al., 2020) The BF is composed of 3 main neuronal types: cholinergic, glutamatergic (GLUT) and GABAergic, divided in parvalbumin(PV)-containing or somatostatin(SOM)-expressing neurons (Yang et al., 2017). At first, only the cholinergic neurons were considered playing a role in the functions assigned to the BF. Then, with the recent technical advances, the involvement of the noncholinergic populations was discovered (Lin et al., 2015).

4.2. Connectivity

BF projects mainly to the cortex, but also to other areas such as hippocampus, amygdala and thalamus, and receives inputs from ARAS. BF serves as a relay between ARAS and the cortex. Its projection exhibits a high degree of specificity. Furthermore, an overlap between these different populations, following connection between cortical regions targeted, enables parallel modulation of these interconnected cortical regions (Zaborszky et al., 2015).

Internally to the BF, ACh neurons project to cortically-projecting GABAergic neurons, exciting PV-containing GABAergic neurons projecting directly to the cortex. GLUT neurons send excitatory projections to all BF neurons, and directly to the cortex. These three types support wakefulness by acting together, GLUT projections exciting both ACh and PV-containing GABAergic neurons which promote wake. These GLUT neurons receive inhibitory feedback projections from ACh and PV-containing GABAergic neurons. SOM-expressing GABAergic neurons inhibit all BF neuron populations, in accordance with their role in sleep-promotion, and receive both inhibitory ad excitatory projections from ACh neurons (Figure 8)(Xu et al., 2015).

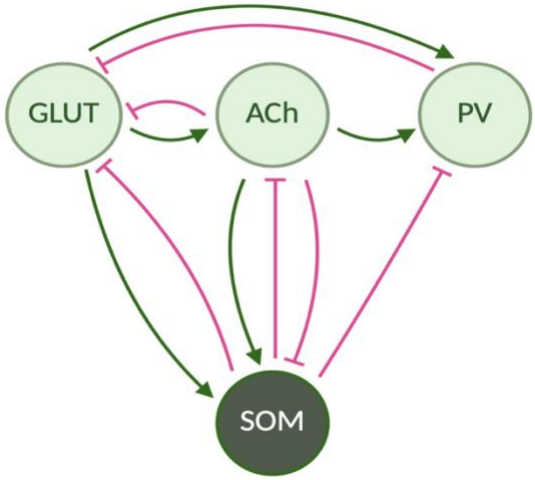

Figure 8. Basal forebrain local circuit. The light green circles represent the wake-promoting neurons, and the dark green circle represent the sleep-promoting neurons. The green arrows indicate excitatory projections, and the pink lines indicate inhibitory projections (adapted from Xu et al., 2015).

4.3. Functions

The basal forebrain, via its various projections, modulates different cognitive functions and regulates sleep-wake cycle. Indeed, through its connexions with the hippocampus and amygdala, BF plays a role in certain aspects of emotional and motivational behaviour (Zahn et al., 2020). It is also involved in attention and memory with hippocampal and cortical connexions (Ananth et al., 2023). Then, BF projections regulate sleep-wake cycle mostly via their cortical projections, and as a target of the ARAS (Deurveilher & Semba, 2011; Wu et al., 2020). This role will be of interest in this work and will be further described.

BF degeneration is a reliable biomarker in Alzheimer's disease and other forms of dementia. Its impairment is also associated with cognitive dysfunctions and disorders of attention, as well as disruptions of sleep-wake cycle (Anaclet et al., 2015; Lin et al., 2015).

4.4. Activity

During sleep, BF cholinergic system activity varies between stages, being active during wakefulness and REM sleep but mostly silent during NREM sleep. During wake and REM sleep, they are stimulated by ARAS and their activity favours a desynchronization in the cortex, characterized by LVFA typical of these stages (Jones, 2005). Cholinergic neurons activity increases during the transition from NREM to REM sleep, switching the activity of TRN neurons from phasic to tonic activity (Maness et al., 2022).

Interestingly, TRN neurons are also stimulated by cholinergic projections from the basal forebrain, more precisely, the NMB cholinergic neurons (as shown by Ni et al. 2016, with the optogenetic activation of those neurons inducing mice to fall asleep). These cholinergic projections activate TRN GABAergic neurons via nicotinic receptors when the acetylcholine level is low enough during NREM sleep. Those nicotinic receptors are found in moderate quantity on the GABAergic neurons (Ni et al., 2016).

It also has a role in the peak of spindles observed before the transition NREM-REM sleep, when ACh activity occurs at the same time as LC-NE activity (Osorio-Forero et al., 2021).

PV-containing GABAergic and glutamatergic neurons of BF are also wake-promoting, as mentioned before, but their activity is less described than the one of cholinergic neurons. Regardless, the interaction between those 3 types of neurons, specified above, is known and essential for triggering and maintaining wake (Xu et al., 2015).

SOM-expressing GABAergic neurons are more active during NREM sleep. They play an inhibitory role on the others BF neurons, ceasing their wake-promoting action (Xu et al., 2015).

II. Objectives

Most of what is known about spindle regulation stems from animal research, particularly in rodents like rats, mice and ferrets, as well as in cats and primates. The translation to humans remains incomplete, despite the increasing number of studies allowed by technological advancements in neuroscience.

In light of the importance of spindles in many aspects of sleep, a comprehensive understanding of their regulation, variability and associated mechanisms in our species is essential to envisage interventions aiming to improve sleep functions. Given, for instance, their likely implication in brain diseases, with spindle disruptions reported in Alzheimer's and Parkinson's disease, schizophrenia, epilepsy and insomnia (Counts & Mufson, 2012; García-Lorenzo et al., 2013; Mäki-Marttunen, 2020; Van Someren, 2021), it would be a great interest to determine whether they can be used as marker of disease risk or as intervention target.

As described in the introduction, the main generator of spindles is the TRN (Steriade et al., 1985). The first idea of this MSc thesis was therefore to focus on the link between this structure and spindle characteristics. Unfortunately, due to technical limitation (delay in the set-up of the algorithm allowing to precisely delineate the TRN by collaborators of G. Vandewalle), we had to change our focus to those structures influencing spindle generation through their connection to the TRN. Several studies have highlighted the influence of the LC and the BF on the activity of the TRN, and in turns on spindle dynamics (G. Aston-Jones & Bloom, 1981; Maness et al., 2022; Osorio-Forero et al., 2021; Swift et al., 2018). Although both of these structures have other roles in sleep and other processes, here the main focus is their influence on spindles via the TRN (Brown et al., 2012). The link with age and sex will also be investigated since both influence sleep micro and macrostructure as described in 1.8.

This thesis aims to assess the links between 3 electrophysiological markers related to spindles and the activity of the LC and the cholinergic BF (ChBF) during a cognitive task. Only the cholinergic nuclei of the latter are examined, i.e. the MS (Ch1), the VDB (Ch2), HDB (Ch3) and the NBM (Ch4) (Geula et al., 2025), and neither the GABAergic nor the glutamatergic nuclei, again due to technical limitations (aka for the TRN).

To investigate these links, we will use EEG data of the sleep of healthy participants recorded in the sleep labs of the GIGA-In Vivo imaging (GIGA-IVI) platform. We will extract the number and density of spindles as well as the cumulated sigma power, corresponding to the power of the brain electrical

activity in the sigma band (12-16Hz) during sleep which is closely associated with the amplitude of the spindles. The use of these metrics allows a complementary view on spindle dynamics. The activity of LC and ChBF will be assessed using fMRI during an auditory task. These two structures are small and difficult to isolate. For this study, we will use the 7T MRI scanner of the GIGA-IVI platform as it provides high-resolution images allowing better LC and BF isolation in vivo.

Given that EEG recordings inside an MRI apparatus are technically complicated (i.e. an MRI-compatible EEG device is needed), and because it is relatively difficult to sleep in an MRI scan (which is both noisy and uncomfortable), we chose to study those two structures first during wakefulness during an auditory cognitive task that is known to recruit the LC (Berger et al., 2023; Murphy et al., 2014), and that may also recruit the ChBF to some extent. We therefore posit that the activity of the LC and of the BF during wakefulness reflects or shapes, at least in part, their activity during sleep. Since spindle generation in the TRN is associated with a decrease of NE (LC activity) (Osorio-Forero et al., 2021), our main hypothesis related to the LC is that a higher response of the LC during the task would be associated with alteration spindles during sleep, resulting in a reduced number and density of spindles and a lower mean sigma power.
Likewise, regarding ChBF, given that its main action is to inhibit TRN phasic activity (Maness et al., 2022), our hypothesis is that higher activity in the ChBF during wake could also desynchronize TRN and alter spindle dynamic during sleep, resulting in a reduced number and density of spindles. We will not consider sigma power in correlation with ChBF activity since the LC has a more direct modulation on spindles than the ChBF, which has been shown to modulate NREM sleep and not specifically spindles (Maness et al., 2022; Osorio-Forero et al., 2021; Swift et al., 2018).

Considering sex and age, we hypothesized that spindle number and density, as well as sigma power, will decrease with aging. We expect to see sex-related difference in the effect of LC and BF activity on the three markers of interest.

In a second step, only for the spindles-related parameters associated with both nuclei, we will consider both ChBF and LC activities combined. We hypothesize that an imbalance/asynchrony between their activity could lead to a desynchronization of the TRN and a disruption of spindle generation, resulting in a reduced number and low cumulated sigma power. We will therefore include the activity estimates of both nuclei in the same model.

III. Methods

1. Subjects and recruitment

The MRI and EEG data of 92 participants between 18 and 75 years old (mean age: 38.4 ±19.8; sex: 32 males, 61 females) were used in this thesis (Table 1). 52 participants were recruited in the context of a project called ASLEEP between the beginning of 2020 and the end of 2022. The 40 others were recruited between 2023 and mid-2025, in the context of a project called IRONSLEEP. Those two projects were launched at the GIGA-CRC-Human Imaging using the tools of the GIGA-In Vivo Imaging platform, University of Liège.

The first step was a semi-structured interview with several questionnaires to evaluate the exclusion criteria. These criteria included the presence of ferromagnetic metal parts in the body, history of neurodegenerative or neurological diseases, stroke or major psychiatric disorders, body mass index < 18 or > 29, consumption of alcohol (>14 units of alcohol/week, > 21 units of alcohol/week for older participants) or caffeine (> 3 cups of caffeine/day, > 5 cups of caffeine/day for older participants), high anxiety score (Beck Anxiety Inventory – BAI (Beck et al., 1988); score > 16) or depression score (Beck Depression Inventory – BDI (Beck et al., 1961); score > 19), sleep disorder (apnea or periodic movements of the limbs, that are also checked during the first night at the laboratory, as explained below), irregular sleep caused by transmeridian travel to more than one time zone in the previous month, or night shift work in the previous 6 months, as well as claustrophobia and pregnancy, known or suspected.

During this first interview, the goals of the study and its conduct were explained as well as the potential risks, and the participants signed an informed consent form. They were informed of the compensation for their participation, and of their freedom to leave at any moment without consequences.
The data of every participant were anonymized in accordance with the European recommendations in terms of collection and sharing of data (EU General Data Protection Regulation) and confidential. Both ASLEEP and IRONSLEEP projects were approved by the Hospital-faculty Ethic Committee of the Université de Liège.

Table 1. Characteristics of the study sample

	Mean	SD
Age (years)	38.63	19.84
Sex	31 M – 61 F	
BMI (kg/m^2)	23.55	3.30
Education (years)	15.28	2.83
Depression level (BDI)	5.41	4.14
Anxiety level (BAI)	3.34	3.11
Daytime sleepiness (ESS)	6.56	3.98
Habitual subjective sleep quality (PSQI)	4.13	2.08
TST	426.01	56.25
Cumulated sigma power (µV^2)	3347.63	2135.42
Spindle number	6305.54	1937.63
Spindle density (/h)	882.05	232.28

BAI: Beck Anxiety Inventory; BDI: Beck Depression Inventory; ESS: Epworth Sleepiness Scale; PSQI: Pittsburgh Sleep Quality Index; TST: Total Sleep Time.

2. Protocol

The participants followed a succession of steps as described below (Figure 9). They slept a first night at the laboratory. This night is called habituation night, and several recordings are made, using EEG, EOG, EMG and ECG recordings (N7000 amplifier, *EMBLA, Natus, Middleton, WI*), nasal canula, snoring sensor, thoracic and abdominal belt, oximetry and legs movements. This night allows habituation of the participant to the equipment, as well as detection of possible sleep disorders, such as apnea or legs movements (indicative of restless legs syndrome), that are considered problematic from 15 occurrences/h (Epstein et al., 2009; Gossard et al., 2021). It also allowed to exclude parasomnias and REM sleep behavioral disorder. This night was followed in the morning by a high-resolution structural MRI (sMRI), using Ultra High Field (7T) MAGNETOM Terra MRI system

(Siemens Healthineers). The structural data were only used for localization purposes in the present MSc work while other members of the lab are using them for their respective research questions.

The following week, the participants wore an Actiwatch (Actiwatch and AX3, *AXIVITY LTD, Newcastle, UK*), measuring their activity-rest rhythm, and had to follow a constant sleep schedule, with regular sleep and wake time (± 1 hour) based on their preferred schedule. They filled a sleep dairy during this week. The participants were requested not to drink alcohol and caffeine/theine 3 days before the second night.

The second night is called baseline night and was the night used in our analyses. It was scheduled right at the end of the week of wearing the Actiwatch. During the montage of electrodes, participants filled questionnaires about sleep quality (Pittsburgh Sleep Quality Index - PQSI) (Buysse et al., 1989), depression (BDI), anxiety (BAI) and sports. The light was dimed (<10 lux) from their arrival (4 hours before bedtime) until bedtime, and from wake until the scan of the next day. Sleep was recorded in complete darkness, under EEG, EOG, EMG and ECG.

Two hours after wake time, a functional MRI (fMRI) was completed, still under dim lights. Participants had to wear a pair of glasses that reduce the light intensity during the displacement from the room to the MRI. Before the scan, participants were asked to fill the LEEDS sleep evaluation questionnaire about sleep quality during the preceding night (Parrott & Hindmarch, 1978). Before and after the scan, participants were also asked to fill other questionnaires allowing to compare the level of drowsiness, attention and emotional state before and after the functional part of the study. The three questionnaires used were the Karolinska Sleepiness Scale (KSS) (Akerstedt & Gillberg, 1990) that evaluates the subjective drowsiness level, the Visual Analogue Scale (VAS) that is about the emotional state and the Psychomotor Vigilance Task (PVT) (Dinges & Powell, 1985) that tests the reaction time. The KSS was also realized in the scanner, at the beginning and at the end of the scan.

While in the scanner, participants had to complete 2 tasks: an auditory (oddball task) and a visual (cube task), using an MRI compatible response box. These two tasks were explained and practiced on a computer before the scan. During both tasks, pupil size was also recorded (Eyelink-1000*, SR Research, Osgoode, ON, Canada*; sampling rate: 1000Hz).

A blood sample is taken after the first scan for genetics research, and cognitive tests were realized to evaluate memory, attention and cognition. After this, the participants still had to wear an Actiwatch for 3 weeks and fill a sleep dairy, without any restrictions this time. These three aspects will not be used in the context of this thesis.

The old participants of ASLEEP project (18 participants) completed sMRI and fMRI scans a year after the sleep recordings, because they participated in another research project. As the other ASLEEP and

IRONSLEEP subjects, they slept regularly during a week before the fMRI scan and were maintained in dim light 45 minutes before the scan, 2 hours after waking.

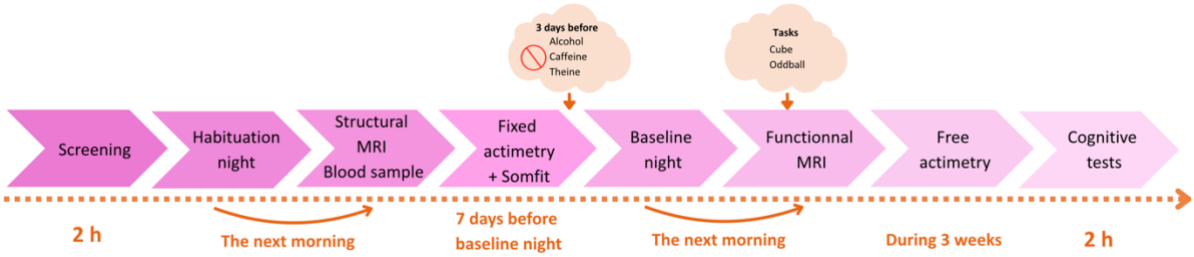

Figure 9. Timeline of the different steps of the protocol.

3. Sleep measurement

Sleep was recorded through electroencephalography using electrodes placed on the scalp according to the 10-20 convention (Figure 10). The positions of interest were cleaned using Nupred ® and the electrodes were stuck using an electro-conductive paste EC2+ ® and a compress placed above. The impedance of each electrode was kept < 5 kOmhs. The signal of each electrode was checked with a reference electrode placed on the mastoid, behind the ear. All the electrodes were plugged in the EMBLA N7000 amplifier (*Natus, Germany*), allowing an amplification of electrical signal and a conversion of this signal on a trace on the computer. The EEG recording was started using RemLogic software.

Automatized procedures were used to detect and score the different stages of sleep, and to remove artefacts (Berthomier et al., 2007). These procedures have the advantages of saving time and allowing a better reproducibility than manual detection.

In this thesis, the variable of interest will be the spindle number and density, and the cumulated sigma power.

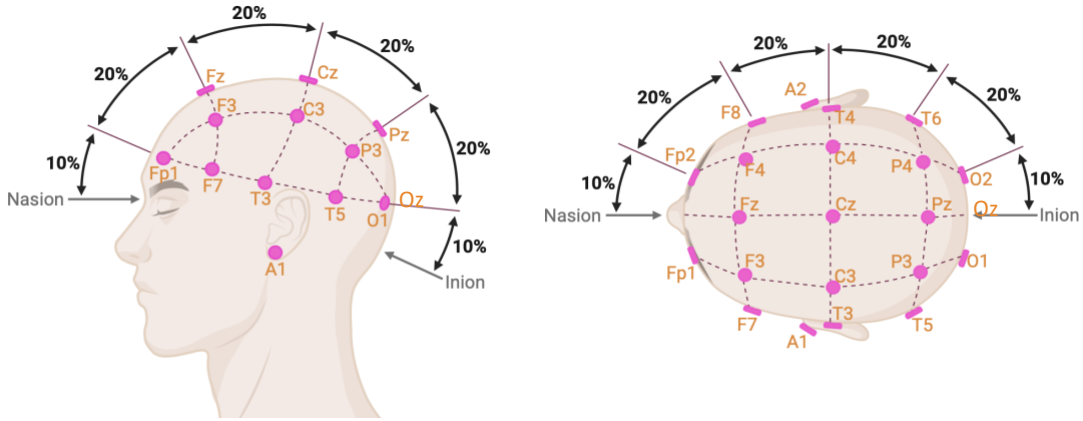

Figure 10. 10-20 convention for the placement of the electrodes on the scalp. The letter z indicates the median line of the scalp. Odd numbers always indicate the placement of electrodes at the left of this line while even numbers indicate the placement of electrodes at the right. Letter F means frontal, C means central, P means parietal, O means occipital, and T means temporal. The letter A represents reference electrodes

3.1. Habituation night

For this first night, electrodes were placed on the scalp at the frontal (Fz), central (Cz, C3), parietal (Pz) and occipital (Oz) level. Reference electrodes (A1, A2) were placed behind both ears, on the mastoid bone; EOG electrodes were placed laterally and above and below the left and right eye, EMG electrodes were placed on both sides of the chin; the ground electrode as well as the second reference electrode specific to the EEG the system of the laboratory, called X1, were placed on the forehead. To measure legs movement, two electrodes were placed on each leg, at the anterior tibialis muscle level. Finally, two electrodes were placed aligned so that the heart is in the middle, to record the cardiac rhythm.

Other equipment was placed, like thoracic and abdominal belts for respiratory rhythm, nasal canula for nasal flux, oximeter for blood oxygen saturation and snoring sensor.

3.2. Baseline night

For this second night, no other equipment than the scalp, face and cardiac electrodes described above were used. On the scalp, more electrodes were placed for a total of 11 channels, at the frontal (Fz, F3, F4), central (Cz, C3, C4), parietal (Pz, P3, P4) and occipital level (O1, O2).

3.3. Variables extraction

Sleep spindles were automatically detected over N2 and N3 stages of slow wave sleep using a previously published method of collaborators of Dr. Vandewalle (Gaudreault et al., 2018; Lafortune et al., 2014; Martin et al., 2013). The EEG signal was first filtered using linear phase FIR filter (-3 dB at 10 and 16 Hz), to keep the frequencies between 10 and 16 Hz. The instantaneous amplitude of the band-limited signal, obtained via the Hilbert transform, was smoothed. Then, a threshold was set at the 75^{th} percentile. Only the events of 0.3 to 3 seconds long were selected as spindles.

Considering sigma power, only frontal electrodes were considered to facilitate potential interpretations of future largescale studies using headband EEG, often restricted to frontal electrodes. Averaged power was computed in 30-min bins, adjusted for the proportion of data rejected due to artifacts or arousals. It was subsequently summed separately for REM and NREM sleep, and sigma-cumulated power was computed in the sigma frequency band (12-16 Hz) during SWS, a measure closely related to spindle density and amplitude.

4. MRI

MRI is a non-invasive medical imagery technique that allows to obtain inside-body images in cross sections. In this study, we target only the brain.

MRI principle is based on magnetic properties of hydrogen atom, that is found in each body tissue. It works on the base of a magnetic field that is produced by a powerful magnet. This magnetic field stimulates the protons of hydrogen atoms and orients them all in the same direction. Another magnetic field is periodically superimposed to the first one. This field is composed of radio frequency waves and makes the hydrogen atoms resonate. When the excitation is done, hydrogen atoms go back to their basal state by restoring energy accumulated. This produces a signal that is measured and localized in the brain. The different body tissues do not contain the same quantity of hydrogen atom, then the amount of energy restored is heterogenous and this allows the visualization of different contrasts according to the tissue composition (Sadleir & Minhas, 2022).

The MRI scanner used during this study is an ultra-high field (7T) MAGNETOM Terra scanner, developed by Siemens Healthineers (*Erlangen, Germany*), located within the GIGA-In Vivo technological platform of the University of Liège.

Before each scanning session, the participants had to fill up a metal check questionnaire to confirm the absence of any non-MRI-compatible element in or on their body. After each scanning session, they were also asked to fill a feedback form of their experience in the MRI scanner.

4.1. Structural MRI

Different structural images were acquired during this scanning session. It lasted about 90 minutes and participants wore earplugs to mitigate the sound of the scanner. Several sequences were realized in succession on the whole brain, as well as focused on the LC. This included NODDI sequences (Neurite Orientation Dispersion and Density Imaging) (1.4 x 1.4 x1.4 mm^3 voxel size) and multiparameter mapping (MPM) protocol over the whole brain leading to quantitative MRI maps (T1 weighted, PD weighted, MT weighted; 0.6 x 0.6 x 0.6 mm^3 voxel size) that will not be used as part of this MSc work. The session further included Magnetization Prepared 2 Rapid Acquisition Gradient Echo (MP2RAGE) sequence (Marques et al., 2010) (0.6 x 0.6 x 0.6 mm^3 voxel size) used for the localization of the activation, multi-echo GRE acquisition used to segment the BF, and a magnetization transfer-weighted turbo flash centered around the LC (0.4 x0.4 x 0.5 mm^3 voxel size), as it is highly sensitive to neuromelanin found in quantity in the LC (Clewett et al., 2016) that allowed the precise delineation of the LC.

4.2. Functional MRI

fMRI allows to visualize brain activity indirectly, by measuring blood flow variation locally when the zone of interest is stimulated during a task or rest. This is due to the blood oxygen level dependent (BOLD) effect, describing signal changes due to variations in blood oxygenation levels. This signal is related to the magnetic properties of hemoglobin, that are different depending on whether it is oxygenated or not. When a certain brain area is activated, oxygenated blood flows to this zone. The local relative variations in oxygen quantity in blood are detected and form the BOLD signal (Glover, 2011).

The participants were installed in the scanner with several types of equipment: a respiratory belt for breathing measurement, an oximeter for cardiac rhythm measurement, and earphones and a response box essential for the tasks. Pupil measurements were also realized but won't be used here. Two tasks are realized using the Open Sesame software: a visual task and an auditory task. The visual task is called the "cube task". The participants have to press a button on the response box when they perceive a change of perspective of a cube, that can be in depth or in relief. The auditory task is called the oddball task.

During this scan, structural images are acquired at rest, as well as functional images during the task. The analysis of this thesis will be based on these functional data.

4.2.1. Oddball auditory task

During this task, standard sounds (80%) are presented randomly with deviant sounds (20%). Participants have to detect the deviant sounds. This task mimics novelty and is thus known to recruit the LC, and then allows to see its activity (Krebs et al., 2018; Murphy et al., 2014).

It lasts about 10 minutes. During this time, a series of sounds is played, and the participants have to press a button on the response box, as fast as possible, when the target sound (1000 Hz) is heard in opposite to standard sounds (500 Hz). The stimuli last 100 ms while the interstimulus interval lasts 2000 ms.

The task began with a volume check during which the participant was asked to adjust the volume to ensure an optimal perception of the sounds.

5. Analysis

The data acquired were treated and analyzed using MATLAB and SPM12 (*Statistical Parametric Mapping, Wellcome Centre for Human Neuroimaging, Institute of Neurology, University College London*). SPM is a software used to preprocess functional and structural MRI data further allowing to detect differences in brain activity recorded during tasks. It is based on statistical parametric maps, composed of voxels that possess a value according to their BOLD signal. The voxels considered as statistically significative are represented on a structural image of the brain to localize and visualize the activity at the whole brain level. The statistical analysis is based on Generalized Linear Model (GLM). First, the structural MP2RAGE data underwent several preprocessing steps, using SPM12. The background noise was removed, and the images were automatically reoriented, then corrected for intensity inhomogeneities that might appear in MRI images due to scanner-related artifacts. They were then segmented into 3 classes of tissues (gray matter, white matter and cerebrospinal fluid) and the brain was extracted from the surrounding non-brain tissues using Advanced Normalization Tools (ANTs; *Penn Image Computing and Science Laboratory (PICSL), University of Pennsylvania*). The last step was smoothing to increase the signal-to-noise ratio using a Gaussian filter. A whole-brain T1 group template was created using ANTS, based on the preprocessed MP2RAGE images of all participants. The functional EPI images also underwent several steps of preprocessing, including motion and distortion correction using SPM12, followed by brain extraction. The resulting images were smoothed with a Gaussian filter.

5.1. First-level analysis

After the whole preprocessing and coregistration part, a subject-level (fixed effect) analysis was performed in the participant brain space using a GLM. The timing vector of the target tones was convolved with the hemodynamic response function (HRF) to model the event-related response. Physiological data consisting of the breathing measurement and cardiac rhythm were computed and merged with realignment parameters that were all included as covariates of no-interest in the statistical models. The model further included high-pass filters with a 128-second cutoff to remove slow signal drift. The GLM adjustment was computed to estimate the regression coefficient (β) for each regressors and each voxel. A β value near 1 corresponds to a regressor explaining a great part of the BOLD signal, while near 0 corresponds to a regressor explaining a little part of the BOLD signal.

Then, one contrast of main interest was defined, being the effect associated with detection of the target sounds. This allows highlighting brain regions specifically activated in a context of salient stimuli and

novelty detection. A voxel-wise unilateral t-test was applied to obtain statistical maps of the individual brains with a t value for each voxel that can be used to determine whether the voxel shows a significant association with the contrast of main interest. Then, each statistical map is registered to the group template space and then to the MNI space for the second-level analysis (using MNI152 template). This normalization procedure reduces inter-individual variability in brain anatomy and allow accurate comparison between subjects.

5.2. Second-level analysis

This second part of the analysis was performed at the group level, in MNI space, with the aim of assessing the consistency of the effect on the whole group. The contrast used was the same than during the first-level analysis, so the brain activation associated with detection of the target sounds. A voxel wise unilateral t-test was then also performed on the statistical maps of every subject analysed in the first level. This allowed to identify brain regions showing significant activation at the group-level. This analysis was conducted with the aim of visually checking the presence of activity in the LC and the ChBF. It is however, the data from first-level analysis will be used to extract betas needed for statistical analysis.

5.3. Brain segmentation

MASSP (Multi-Atlas Segmentation with Shape Priors, *Version 2.0*; Bazin et al., 2020), an automatic segmentation technique, was used to extract the ChBF. This algorithm segments 35 structures, based on multi-atlas registration. Multi-echo GRE images acquired during structural scan were used to compute quantitative susceptibility maps (QSM), which provided sufficient contrast for brain segmentation using MASSP algorithm. ChBF mask for each subject was extracted from this segmentation using SPM12. Lastly, the structural image of structural scan was coregistered to the structural image of functional scan using ANTs, to extract the transformation matrix. This matrix was applied to the mask to align it to the structural space of functional scan.

For the LC, masks were realized manually for each subject using ITK-SNAP. To do so, LC slab acquired during structural scan were coregistered to the structural MP2RAGE images acquired during functional scan, after up-sampling the latter by a factor 2 to avoid losing in-plane resolution using SPM12. This coregistration was performed in two steps: an approximate manual registration to extract the parameters for an initial transformation using UTK-SNAP, and an automatic affine registration based on the initial transformation parameters using ANTS. Then, a voxel-wise mask was created by

two different raters, selecting voxel based on anatomical references and tissue contrast from the MRI sequence. The two masks were computed in a final mask for each subject.

The ChBF masks and LC masks were then used to extract the activity estimates (beta) of ROIs during oddball task for each subject. The REX (Region of interest Extraction) toolbox in SPM was used for this step. It allowed BOLD signal extraction from ROIs.

Each mask was also normalized to the structural group template and then to the MNI space using ANTs, and summed to create a ChBF and a LC group-wise probability template. These masks were used to visually assess the selective activations of the two regions. This visualization of the group-level activation was realized in MNI space using Fsleyes (*University of Oxford, Oxford, UK)* and using SPM12 for LC-restricted activations.

5.4. Statistical analysis

The statistical analyses were carried out using GLMMs (Generalized Linear Mixed Models) on RStudio (*Version 2025.05.1+513, Posit Software, PBC*). The dependent variables used in this subject were spindle number and spindle density, as well as cumulated sigma power. LC or ChBF beta values were used as explicative, dependent variables. The sex, age, total sleep time and BMI were included as covariates. The models were adjusted according to the distribution of these variables, and according to the explicative variables, with a significance set at $p < 0.05$.

For each brain region, the analysis started with the three-way interaction between LC betas, sex and age. New GLMMs were recomputed without the non-significative interactions to ensure that they were not masking other effects. Post hoc analyses were conducted on variables showing significant effects in the GLMMs, using emtrends from the emmeans package to estimate and test specific trends. Data and results visualization were performed using the R package ggplot2.

IV. Results

In this chapter we will first show the cerebral activation linked with oddball task detected during MRI scans. Then, we will highlight the potential links between spindle characteristics and LC and ChBF activation.

1. Cerebral activation in response to auditory stimuli (oddball task)

In the group-level analysis, 92 participants were used to create a mean image of cerebral activation with the aim of verifying the presence of activity in the two ROI, i.e. the LC and the BF. Several activations were significant after stringent correction for multiple comparison over the entire brain (Family Wise Error - FWE - method). The list of these activations can be found in Suppl. Table S2. Given the small size of the LC, we did not expect activation to survive such stringent correction and therefore also considered the activations for uncorrected p-value < 0.001 (Figure 11). Group-wise LC mask was created with the masks of 71 participants, while group-wise BF mask was created with the masks of 26 participants, as described above, in MNI space. These masks were added to the group-level activation maps including all participants displayed in Figure 11.

The group-level analysis shows that the task appeared to successfully recruited the LC as well as the BF, as the masks are covered with the activation contrast in Figure 11 (LC masks were superimposed to the activation contrast as, in view of LC high activation, the masks would not have been visible under it).

In view of the high activation of the LC observed on the whole brain analysis, we focused on LC specific activation by applying the group-wise LC mask in SPM12. We found significant activations in this region ($P_{FWE} < 0.05$), with a local maximum of activation located within the mask, supporting LC activation during oddball task (Figure 12). This led me to extract bilateral LC activity betas and relate it to spindle characteristics of interest.

Even though the BF activation did not pass the FWE correction within the BF mask, we found activation for uncorrected p-value. In light of this activation, we extracted bilateral BF activity betas in the same manner as for the LC.

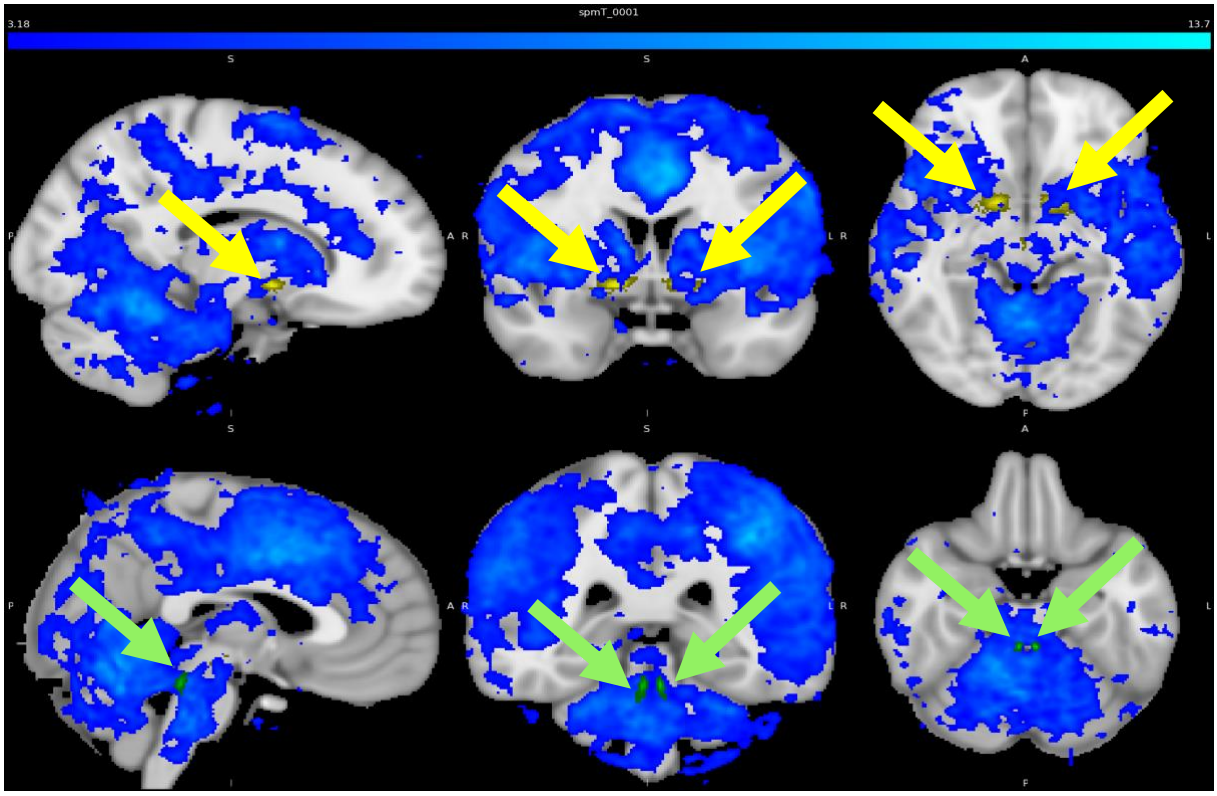

Figure 11. Whole brain response during oddball task, in sagittal, coronal and axial views. They show whole brain results using significance for a threshold of p < 0.001 uncorrected over the MNI template. Images at the top show group-wise ChBF mask in yellow [MNI coordinates: (3 -35 -22)], while images at the bottom show group-wise LC mask in green (MNI coordinates: (14 1 -9)]. The color scale represents the degree of activation.

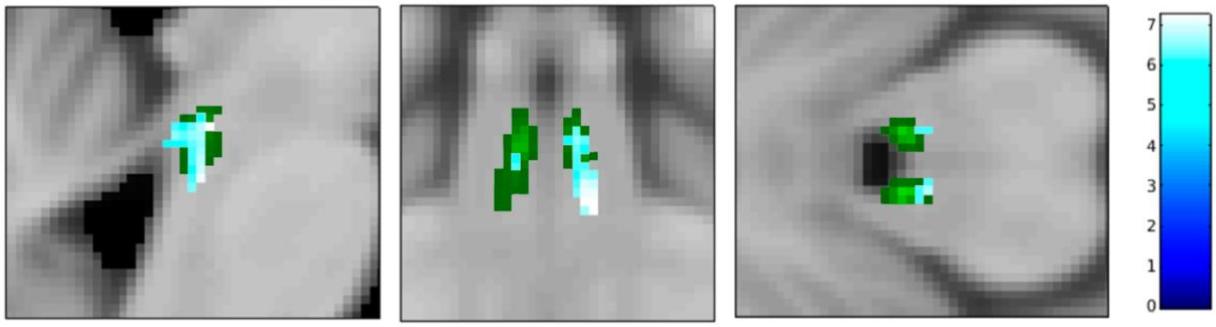

Figure 12. Zoom on the pons at the level of the LC. It shows LC-restraint results of the activation of the LC during oddball task in sagittal, coronal and axial views [MNI coordinates: (4 -34 -24)], for a threshold of p < 0.05 FEW-corrected over the MNI template, using group-wise LC mask (in green). The color scale represents the degree of activation.

2. Exploration of spindle characteristics and LC and BF activity

Sleep data were available in 92 individuals. We first considered sleep characteristics with respect to sex and age. Spindle number was significantly larger in women, and spindle density shows a statistical tendency, while sigma power did not differ significantly between sexes (Table 2 and Figure 13A,C,E).

Furthermore, spindle number and density as well as total sleep time (p= 0.01) significantly decreased with age in our sample (Table 2 and Figure 13B,D).

Table 2. Characteristics of the full study sample, and according to sex

	Female (n=61)		Male (n=31)		P-value	Full (n=92)
	Mean	**SD**	**Mean**	**SD**		**Mean**
Age (years)	37.82	19.60	40.23	20.53	0.592	38.63
BMI (kg/m2)	23.27	3.34	24.09	3.19	0.256	23.55
TST	433.81	46.25	409.21	71.52	0.117	426.01*
Cumulated sigma power (μV^2)	3611.36	2156.19	2767.43	2009.83	0.0952	3347.63
Spindle number	6698.07	1716.24	5441.96	2144.97	**0.0139**	6305.54*
Spindle density (/h)	918.83	202.45	801.13	274.70	0.0629	882.05*

The p-values shown in the table corresponds to two-sample t-tests. The asterisk indicates that the effect of the age is significant. BMI: Body Mass Index; TST: Total Sleep Time.

Beta values associated to target sound detection were extracted for the two regions for each participant. They represent the estimate of the activity of the LC and the BF associated with the detection of these sounds. Using GLMMs, we tested if the three spindle characteristics of interest, i.e. spindle number, spindle density and cumulated sigma power, varied with LC activity during the oddball task, sex and age, while controlling for TST (except for spindle density) and BMI. Then, we computed the same models with BF activity during oddball task rather than LC activity. We further computed the combination of both activities only on models that were significant. 71 subjects were used in the models including LC activity, while 27 subjects were used when including BF activity (See Suppl. Table S2).

Considering spindle number, we found significant positive association with LC activity (p= 0.010), with sex (women having more spindles than men; p=0.002; Figure 13A) and with TST (p=0.002). Spindle number showed a negative tendency with age, even if it was not significant (Figure 13B). We

then found a significant association of spindle number with the interaction between LC activity and sex (p=0.013). Post hoc contrast revealed that higher spindle number was associated with higher LC activity during oddball task in males (z= 2.586; p= 0.010) but not in females (z= -0.244; p= 0.807) (Table 3; Figure 14A).
For spindle density, we found positive significant association with LC activity (p=0.040), sex (p=0.012; Figure 13C) and the interaction between both (p=0.046). Spindle density showed the same non-significant negative tendency with age (Figure 13D). These results confirmed the results of the previous model. As for the spindle number, higher spindle density was associated with higher LC activity in males (t= 2.092; p= 0.040), but not in females (t= -0.321; p= 0.749) (Table 4; Figure 14B). In contrast, for the cumulated sigma power, the model showed no significant association other than with the TST (p=0.021) (Table 5; Figure 13E,F; Figure 14C).

We also investigated the relationships between BF activity and spindle number and density using GLMMs adjusted for sex, age, BMI and TST (except for spindle density), in the same manner as with the LC. The statistical analysis showed a significant positive association between TST and spindle number. For spindle density, the model showed negative tendency with age and BMI. No other tendency was revealed between spindle characteristics and the variables (Figure 15; Table 6).

Ultimately, we considered the effect of both LC and BF activity combined on spindle number and spindle density, since these two models had significant results with LC alone. BF activity was added as covariable on these models. For these models, only 18 subjects were used (i.e. those with both a LC and BF mask available). The model for spindle density led to no significant associations (Table 7).
Considering spindle number, the model showed similar results as the model without BF, with positively significant association with LC activity (p= 0.002), sex (p=0.010) and TST (p= 0.001), as well as a significant association with the interaction between LC activity and sex (0.007) (Table 7).

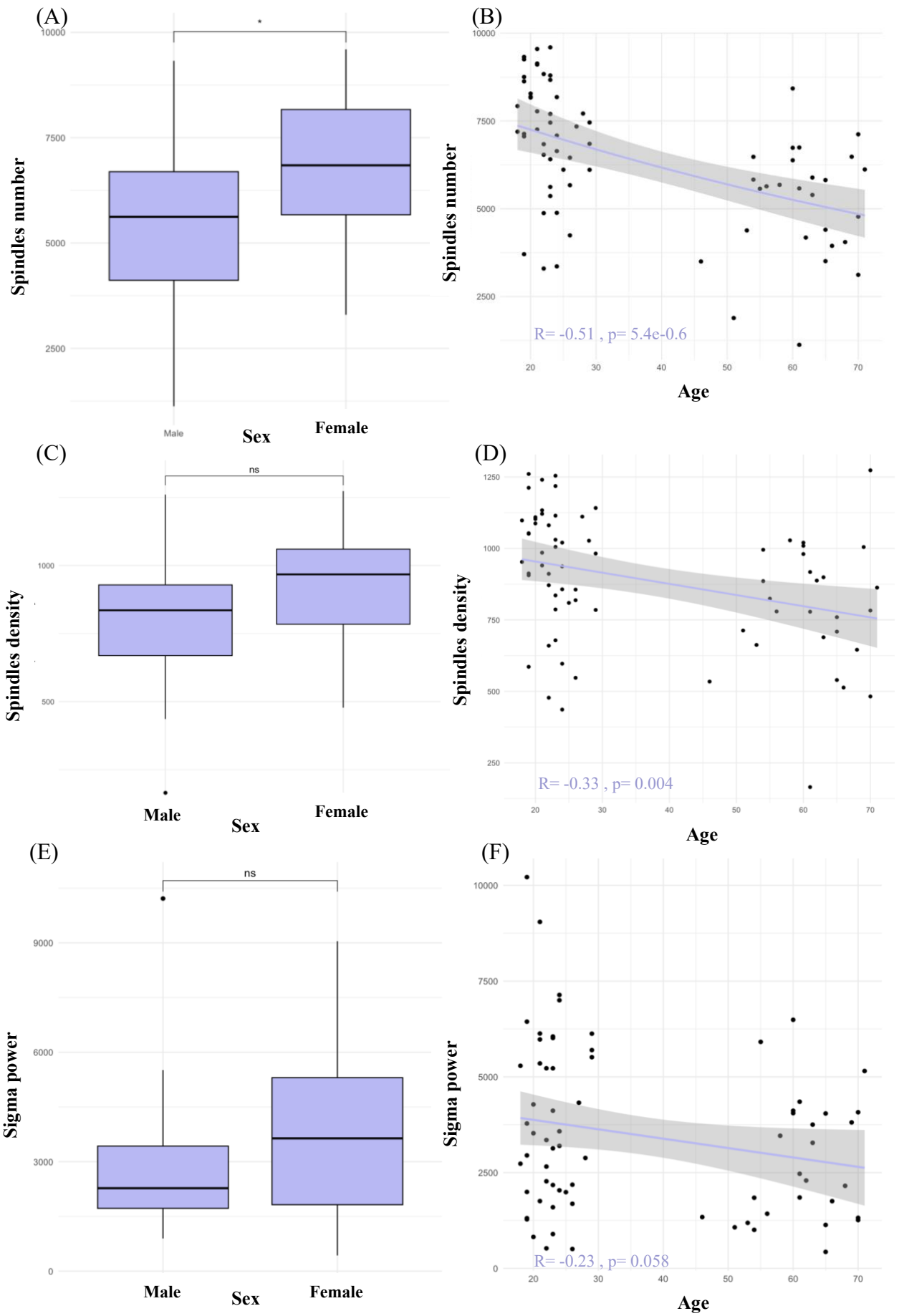

Figure 13. Associations between spindles characteristics and sex (A)(C)(E) and age (B)(D)(F).

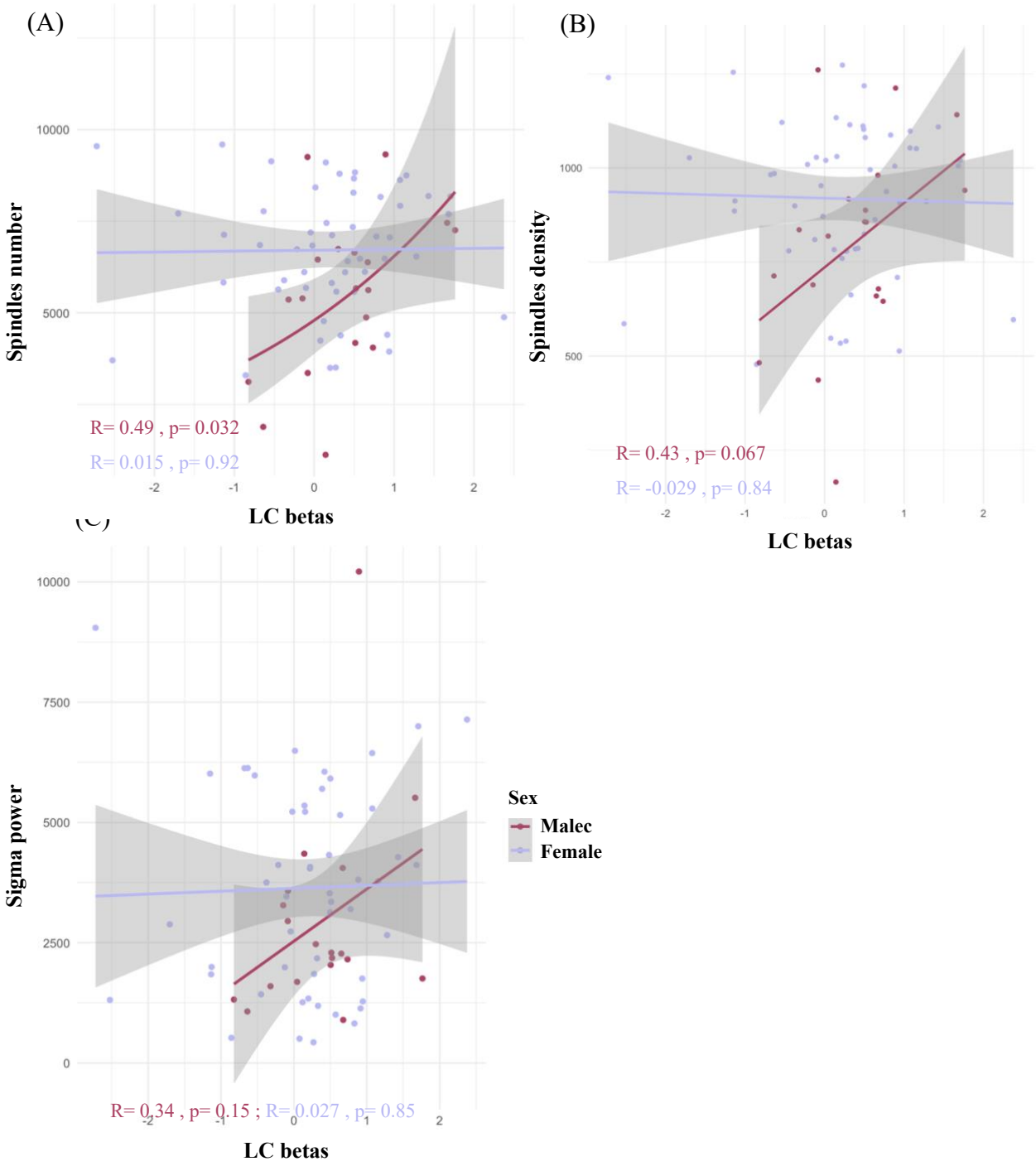

Figure 14. Associations between spindles characteristics and LC activity during oddball task (LC betas), according to sex (men in red and female in purple). Associations between spindles number and LC betas in men is significant (p=0.032). ‹

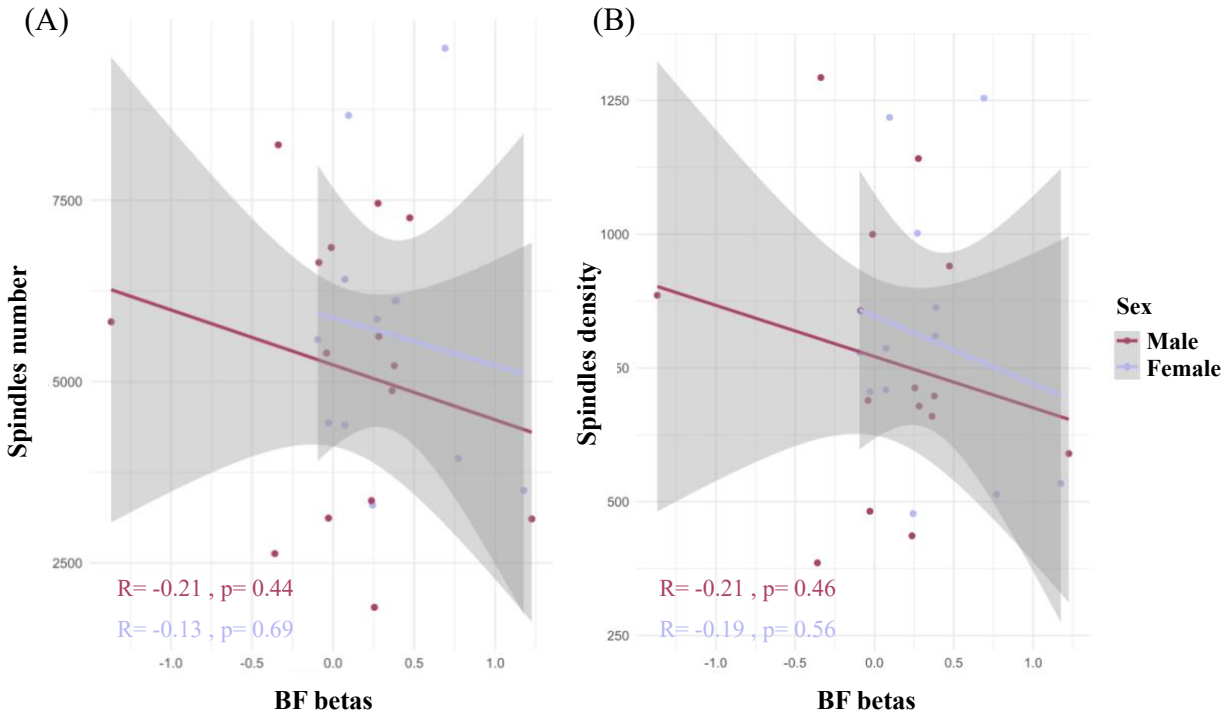

Figure 15. Associations between spindle characteristics and BF activity during oddball task (BF betas), according to sex (men in red and females in purple). No association is significant.

Table 3. Association between spindle number, LC activity, age and sex.

	Estimate	SD	Test Statistic	P value
LC activity	0.251	0.097	2.586	**0.010**
Sex	0.254	0.082	3.099	**0.002**
LC activity*sex	-0.261	0.105	-2.478	**0.013**
Age	-0.004	0.002	-1.857	0.063
TST	0.002	0.001	3.048	**0.002**
BMI	-0.012	0.011	-1.109	0.267

*Results of the GLMM testing a link between spindle number and LC activity during oddball task, sex, age, TST and BMI. The model is Spindle_number~LC_betas*sex + age + TST + BMI.*

Table 4. Association between spindle density, LC activity, age and sex.

	Estimate	SD	Test Statistic	P value
LC activity	159.926	76.455	2.092	**0.040**
Sex	163.225	63.147	2.585	**0.012**
LC activity*sex	-169.818	83.549	-2.033	**0.046**
Age	-2.731	1.386	-1.970	0.053
BMI	-10.512	8.492	-1.238	0.220

*Results of the GLMM testing a link between spindle density and LC activity during oddball task, sex, age, TST and BMI. The model is Spindle_density~LC_betas*sex + age + TST + BMI .*

Table 5. Association between sigma power, LC activity, age and sex.

	Estimate	SD	Test Statistic	P value
LC activity	0.033	0.080	0.412	0.682
Sex	0.120	0.162	0.738	0.463
Age	-0.002	0.004	-0.479	0.634
TST	0.004	0.002	2.363	**0.021**
BMI	0.005	0.0228	0.208	0.836

Results of the GLMM testing a link between cumulated sigma power and LC activity during oddball task, sex, age, TST and BMI. The model is Sigma_power~LC_betas + sex + age + TST + BMI.

Table 6. Association between spindle number and BF activity, age and sex, and between spindle density, BF activity age and sex.

	Spindles number	Spindles density
BF activity	0.418	0.607
Sex	0.251	0.347
Age	0.062	0.059
TST	**0.002**	/
BMI	0.098	0.052

Results (p-values) of the GLMMs testing a link between spindle characteristics and BF activity during oddball task, sex, age, TST and BMI. The models are Spindle_number~BF_betas + sex + age + TST + BMI, Spindle_density~BF_betas + sex + age + BMI.

Table 7. Association between spindle number, LC activity, BF activity, age and sex, and between spindle density, LC activity, BF activity, age and sex.

	Spindle number	Spindle density
LC activity	**0.002**	0.073
BF activity	0.640	0.978
Sex	**0.010**	0.171
LC activity*sex	**0.007**	0.137
Age	0.897	0.828
TST	**0.001**	/
BMI	0.054	0.104

*Results (p-values) of the GLMMs testing a link between spindle characteristics and BF and LC activities combined during oddball task, sex, age, TST and BMI. The models are Spindle_number~LC_betas*sex + BF_betas + age + TST + BMI, Spindle_density~LC_betas*sex + BF_betas + age + TST + BMI.*

V. Discussion

This master thesis aimed to investigate the links between LC and BF activity evaluated via oddball auditory task using 7T MRI, and sleep spindle characteristics extracted from EEG recordings. Given their central role in sleep and their alterations observed in several neurodegenerative diseases, sleep spindles represent a promising candidate for potential markers of risk or as target for sleep intervention. Before discussing our results related to spindle characteristics, we will address the activation maps related to the auditory task.

1. Cerebral activation related to oddball task

During an auditory oddball task, it is expected to observe activations in cerebral regions related to the different aspects of the task, being regions involved in auditory processing, attention modulation, sensory and motor control, as described in Kiehl et al. study on fMRI auditory target detection (Kiehl et al., 2005). Globally, the results obtained in our activation maps for uncorrected p-value showed similar activated regions. This supports that the task was well completed by the participants and that our analyses were well conducted.

The whole-brain maps were computed to verify that, on average, the task was triggering activation in the LC and the BF. The LC did show its expected activation, confirming that the oddball auditory task induces a robust response in this region as that the LC is involved in novelty detection (Berridge & Waterhouse, 2003). Activation in this region persisted after the application of FWE-corrected thresholds ($p < 0.05$) over the average LC mask.
The BF was also activated, although to my knowledge no prior evidence in the literature indicated that this region is recruited during the task. Such activation could be explained by the role of the BF in top-down attention and memory processes, both relevant to the cognitive demands imposed by the task (Ananth et al., 2023). In view of the implication of BF and LC in the ARAS, BF could also be activated in consequence of LC activation (Brown et al., 2012).

2. Links between sleep spindles and LC activity

Considering sleep spindles, we found the expected age-related decrease in spindle number and density (Fernandez & Lüthi, 2020; Poirson et al., 2024) as well as the reduced number and density of spindles

in men compared to women (Carrier et al., 2001; Huupponen et al., 2002; Purcell et al., 2017). This supports that the spindle detection method and the sigma power extraction were well computed.

Spindle generation in the TRN is associated with decrease in LC input on two distinct rhythms: at the spindle scale, LC activity decreases just before a spindle, increases progressively until termination and drops again afterwards, while on a slower timescale, it clusters spindles in continuity periods (~50 s) alternating with spindle-free fragility periods (Osorio-Forero et al., 2021; Swift et al., 2018). This led us to postulate that higher response of the LC during the task would be associated with a reduced number and density of spindles and a lower sigma power. Contrary to this expectation, we found that LC activity showed a significant positive association with spindle number and density ($p=0.010$). This could be an indication that higher LC activity favor spindle generation. These results could be coherent with previous work of our team (Koshmanova et al., 2023) which described the link between LC activity and REM theta power, a marker of REM sleep intensity, as well as subjective sleep quality. They found that, in older participants, higher LC activity was associated with worse subjective sleep quality and lower theta power during REM. This could be complementary to the results we found, in the sense that if higher LC activity negatively impacts REM sleep may lead to more NREM sleep and therefore more spindles.

The model for cumulated sigma power showed no significant association. This may appear counter intuitive at first as more spindles could be associated with more power over the spindle frequency. One possible explanation would be that spindles may not be the only oscillations contributing to this EEG band, and that other oscillatory components in this band would prevent any direct association with LC activity, in contrast to direct measure as spindle number and density (Cox et al., 2017; Palepu et al., 2023). Another hypothesis is that spindles would exhibit compensatory mechanism: when they are less numerous, spindles would become larger in amplitude and maintain sigma power. Further studies assessing spindle amplitude could help clarify the specific contribution of spindles to sigma power.

A consistency is observed between the results of the model for spindle number and for spindle density. This is not surprising given the close relationship between the two variables. We nonetheless considered both variables, because as total sleep time vary considerably between individuals, participants with a similar spindle count may display very different spindle densities. This highlights the relevance of using both measures in the analysis and supports the consistency of the finding.

As anticipated, we further found a significant link between sex and spindle number and spindle density. Post-hoc analyses revealed that in men, LC activity showed a significant positive association, whereas in women, the association was not significant. This would mean that the main effects between spindle

number and density were mainly driven by the men of our sample and that the interpretation of the findings proposed above may mainly pertain to men. Findings about sex-related difference in LC structures and regulation may explain this result. While such differences have been documented in rodents model, research on human are still at an early stage of exploring sex-specific variations in LC anatomy and regulation (Bangasser & Valentino, 2014; Bennett et al., 2024; Jacobs et al., 2021). Studies in rodents notably show that these variations are likely influenced by hormones. Translating that to humans, fluctuations in hormonal factors in women may modulate LC activity in comparison to men that have more stable hormonal levels. Indeed, across the menstrual cycle, the level of estrogens and progesterone varies, causing the LC to be more or less reactive according to the phase of the cycle, as shown in rodents (Bangasser & Valentino, 2014; Helena et al., 2006). Such dynamic modulation might alter the association between LC activity and spindles when examined at the group-level in women.

3. Links between sleep spindles and BF activity

It was hypothesized that higher activity in the ChBF during wake could also desynchronize TRN and alter spindle dynamic during sleep, resulting in a reduced number and density of spindles. Yet, no significant associations were observed with BF activity. Several factors could explain this result. First, the analysis only included 26 subjects, while 71 subjects were analyzed for the LC. This reduced statistical power and the likelihood of detecting significant effects. Second, BF has more ambivalent effect on the TRN than the LC: ACh from BF neurons can both inhibit or facilitate spindle generation (Jones, 2005; Maness et al., 2022; Ni et al., 2016). Third, the oddball task used may not have been optimal for engaging BF circuits. While the task is known for recruiting the LC, there is no prior evidence in the literature that it recruits the BF and our evidence for its recruitment was weaker (cf above). The analyses of the entire sample are warranted to test under better statistical conditions whether the activity of the BF may be associated with sleep spindles.

Importantly, this absence of significant result in the BF can be seen as a control for the effect observed in the LC. The fact that no similar association was found in a different brain location supports that this effect was unlikely to be a random finding. This assumption is further supported by my final statistical model incorporating both the activity of the LC and BF as it did not fundamentally alter the association between the LC and spindle number and density, even though it only included 18 participants (though the association between LC activity and spindle density became a statistical trend – potentially because of the small sample size).

4. Limitations

As in any research, our study bears several limitations. First, the sex-ratio was unbalanced in favor of women, and the restriction to Caucasian participants limited the generalization of the results to the general population. Then, as mentioned above, for some models the number of participants included was restricted, which lowered statistical power and reduced the likelihood of detecting significant results. Finally, our sample included only few participants between 30 and 55 years old. Since age was treated as continuous variable, this underrepresentation in the age range may have reduced the ability to observe potential non-linear effect. A trend was still observed between spindles and age. Hence, further analysis might benefit from considering age as categorical variable (age group), to better capture potential non-linear effect of ageing, as previous studies have explored maybe (Koshmanova et al., 2023; Mortazavi et al., 2025).

As this study measured all variables at one time point (one night and one scan), the association observed cannot be taken as evidence of causality. A longitudinal approach would be required to confirm these associations.

The protocol also differed for some of the participants. In particular, some older participants (N=18) completed the functional MRI sessions ~1 year after the sleep recordings instead of a week later like the others. They did not sleep at the laboratory the night before, nor did wore an Actiwatch the week preceding the scan. However, their sleep schedule of the three days prior to the session were reported. Although sleep changes over the lifetime, it is relatively stable over a shorter period of life (e.g. a few years) (Tucker et al., 2007; Zeitzer, 2013). We therefore consider it unlikely to fully explain the significant effects observed in our study.

VI. Perspectives and conclusions

Our results provide support to the idea that LC activity during sleep influences sleep spindles and contributes to modulating microstructure of sleep. Specifically, a more reactive LC during wake would lead to more spindles during sleep. This effect could extend to overall optimized sleep, in line with previous studies showing that excessive LC activity would reduce REM expression, whereas a balanced tonic-phasic activity of the LC is required for optimal REM (Koshmanova et al., 2023; Mortazavi et al., 2025). Investigating the two modes of LC activity though different tasks, as in Mortazavi et al., could provide insight about how this balance affects spindles and how it might relate to REM results, and how it could potentially generalize to overall sleep quality. Individual variations of the LC could explain interindividual differences in spindles, and more generally sleep quality.
We further found that the association between LC activity and spindle number appears to be higher in men. In light of this sex-specific result, and of the scarce research on sex-related difference in LC regulation and structure, it would be of great interest to consider sex and hormonal status when examining the role of the LC in sleep regulation.

Given the alteration in spindles observed in several disorders as well as normal aging, the association between LC activity and spindle expression could open perspectives for intervention aiming to increase spindle number. This would limit the effects of ageing and disorders and improve spindles-related functions, as well as slow wave sleep. Since spindles play a key role in memory consolidation, notably through their coupling to the ascending phase of slow oscillation, LC-targeted intervention could have positive impacts on memory. Potential approaches include non-invasive brain stimulations, as previously explored in the literature with transcranial stimulation for connectivity between cortical area and for modulation of brain function in deep cortical areas (Gann et al., 2025; Yaakub et al., 2023). A second potential intervention would be medication acting on the noradrenergic system. Evidence from the literature supports this approach, for example, GHB (gamma-hydroxybutyrate) has been shown to enhance slow wave sleep and strengthen functional connectivity in healthy men (Dornbierer et al., 2019). Another study on chemogenetic activation of the LC indicate that activating the LC can improve cognitive deficits (Rorabaugh et al., 2017). These researches suggest that pharmacological approaches could improve sleep quality and that the LC-NE system could be a promising therapeutic target. According to our results, this could especially be relevant for men. Acting on the LC could potentially slow down spindles decrease, in particular in Parkinson's disease, epilepsy and schizophrenia, which are brain disorder prevalent in men (Moisan et al., 2016; Ochoa et al., 2012).

The results show that BF activity do not seem to have an impact on sleep spindles, or that this impact might be hidden by lower number participants and inadequate task. Exploring BF engagement in oddball task or other cognitive task would help determine which condition faithfully reflects its functional activity. More importantly, the measurement of BF and LC activity during sleep would likely provide a more accurate understanding of their roles.

In summary, we provided evidence that the LC shapes sleep microstructures, including spindles, in addition to what other members of the lab showed previously with respect to REM sleep quality and infraslow oscillation of sigma power. This reinforces the view that focusing on the LC and the spindles could be particularly relevant for intervention aimed at improving sleep, potentially in the context of neurodegenerative diseases and other brain disorders as insomnia, schizophrenia and epilepsy.

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Appendices

Table S1. Statistic values and coordinates of significant voxel-level activation peaks

Coordinates			$p_{FWE\text{-}corr}$	Z_E
x	y	z		
-49	-25	41	0.000	Inf
-2	5	36	0.000	Inf
49	-22	12	0.000	Inf
10	-52	-21	0.000	Inf
10	6	2	0.000	7.71
-12	-46	50	0.000	7.08
-4	-12	28	0.000	7.05
43	-33	-1	0.000	6.78
21	31	0	0.000	6.75
15	21	-3	0.000	6.46
-13	-41	-38	0.000	6.45
-38	6	17	0.000	6.44
43	-41	19	0.000	6.4
52	-27	-8	0.000	6.4
-7	-18	-5	0.000	6.39
-9	-23	-13	0.000	6.34
0	-59	-34	0.000	6.31
-3	-28	-22	0.000	6.3
15	-14	9	0.000	6.25
12	38	10	0.001	6.21
15	19	5	0.001	6.2
-65	-46	9	0.001	6.18
42	-5	-16	0.001	6.14
20	-19	-33	0.001	6.11
-3	-52	45	0.001	6.09
27	50	30	0.001	6.07
48	10	47	0.001	6.02
-39	2	-20	0.002	6
-14	-17	6	0.002	5.98
-10	-76	-26	0.002	5.97
-11	5	67	0.002	5.97
9	-65	13	0.002	5.97
-51	-47	2	0.002	5.95
-13	-50	-33	0.003	5.93
45	29	38	0.003	5.92
-1	-74	-1	0.003	5.89
-54	19	5	0.003	5.89
-27	-52	-27	0.003	5.88
-14	-78	5	0.003	5.88
-11	-28	-39	0.004	5.86
-3	-73	14	0.004	5.85
56	22	21	0.004	5.83
-8	32	44	0.004	5.82

-13	-65	-27	0.005	5.81
22	20	-2	0.005	5.8
-25	40	29	0.006	5.76
41	2	56	0.007	5.74
-26	4	62	0.007	5.73
27	-14	5	0.008	5.71
-2	-13	64	0.008	5.71
23	-43	-38	0.008	5.7
-12	-68	30	0.009	5.7
-60	11	7	0.009	5.7
33	39	31	0.009	5.69
21	-39	66	0.009	5.68
-9	41	38	0.01	5.66
62	-40	5	0.011	5.65
61	-15	-4	0.012	5.63
-51	-39	3	0.016	5.57
18	-32	38	0.016	5.57

Values of p and Z are reported for voxels showing significant activation after FWE ($p < 0.05$) correction with a cluster extent threshold of 15 voxels across the whole brain. Inf: Infinity.

Table S2. Characteristics of the study sample for the LC subgroup and the BF subgroup

	LC		**BF**	
	Mean	**SD**	**Mean**	**SD**
Age (years)	37.32	19.52	41.44	20.45
Sex	19 M – 52 F		15 M – 12 F	
BMI (kg/m2)	23.15	2.3.22	23.77	2.85
Education (years)	15.14	2.82	16.15	3.39
Depression level (BDI)	5.54	4.23	3.96	3.80
Anxiety level (BAI)	3.38	2.97	2.96	2.59
Daytime sleepiness (ESS)	6.04	3.90	5.37	3.94

Habitual subjective sleep quality (PSQI)	4.15	2.05	3.82	2.11
TST	429.94	55.01	413.11	66.77
Cumulated sigma power (μV^2)	3451.18	2121.43	2458.84	1710.58
Spindle number	6384.07	1912.22	5386.04	1934.96
Spindle density (/h)	886.53	227.87	781.50	250.75

BAI: Beck Anxiety Inventory; BDI: Beck Depression Inventory; ESS: Epworth Sleepiness Scale; PSQI: Pittsburgh Sleep Quality Index; TST: Total Sleep Time.