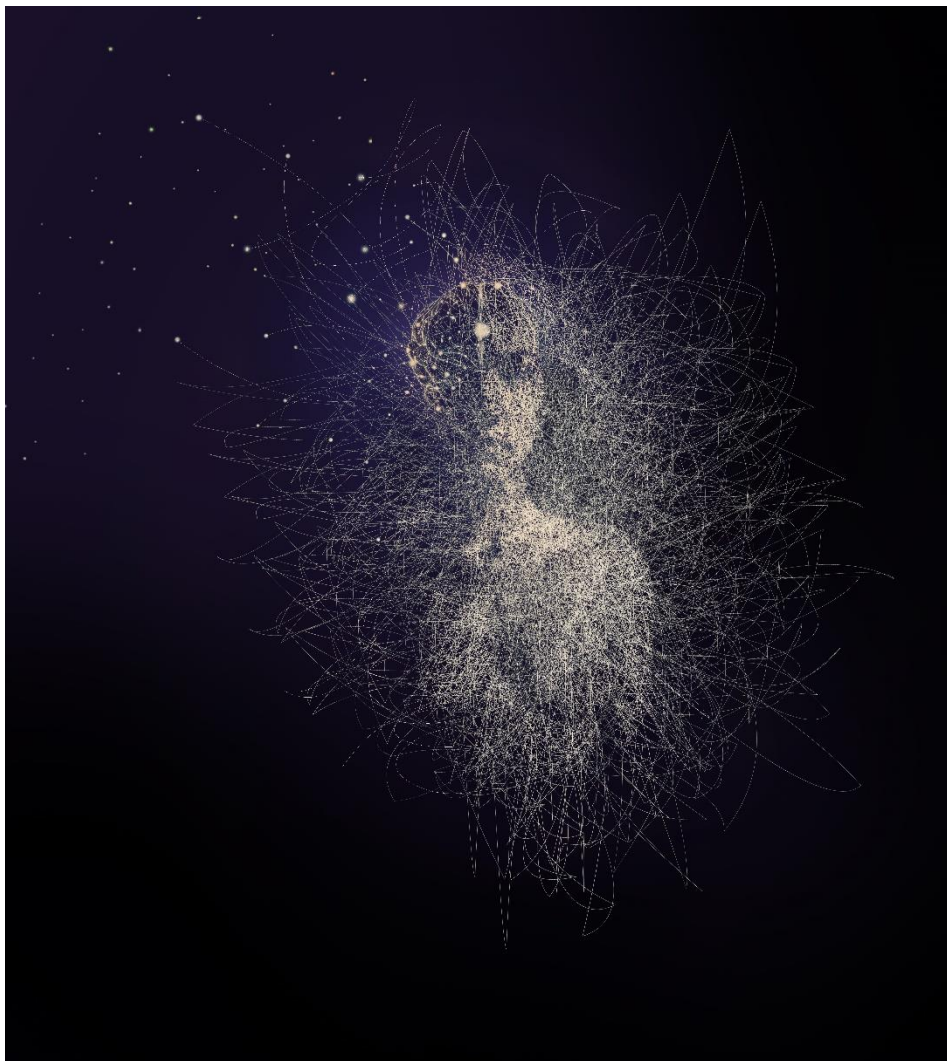# Dynamics of the cortical responsiveness during extended wakefulness, in young and older participants

Giulia Gaggioni

Dissertation submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Biomedical and Pharmaceutical Sciences

Liège, October 2020

Jury members of the private and public defence.

Prof. Julie Duqué, CoActions Lab, Université Catholique Louvain

Prof. Christin Cajochen, Centre for Chronobiology, University of Basel

Dr. Daniel Abásolo, Centre for Biomedical Engineering, University of Surrey

Prof. Fabienne Collette, Centre de Recherches du Cyclotron, Université de Liège

Dr. Christophe Phillips, Centre de Recherches du Cyclotron, Université de Liège

Prof. Pierre Maquet, Centre de Recherches du Cyclotron, Université de Liège

Dr. Gilles Vandewalle, Centre de Recherches du Cyclotron, Université de Liège, Supervisor

Cover : Bruno Vergauwen

Summary

Abstract

Although it has been established that human brain physiology and cognition are under the joint effect of the sleep homeostasis and the circadian alerting signal, the detrimental effect of sleep deprivation is still mostly seen as merely a consequence of a lack of sleep. While this approach is valuable, in order to develop a complete understanding, a circadian perspective needs to be integrated. However, a major difficulty of measuring circadian rhythmicity stems from the complexity of assessing it, because confounders such as light, activity, meals etc. could mask the underlying circadian regulation. Here, we performed two constant routine studies that allow us to measure the interaction between sleep homeostasis and the circadian processes at the cortical level. During the studies, three complementary aspects of the cortical function were investigated, as well as their associations with behavioural performance, and age-related changes of the cortical dynamics. In phase I of the study, the dynamics of cortical excitability, and of response scattering and complexity were described during a 28 hour wake extension protocol in young participants (18-30 y). In phase II, the dynamics of cortical excitability and response complexity were investigated during a 34 hour wake extension in young (18-30 y) and older (50-70 y) participants in order to address lifetime changes. Overall, the results of this thesis demonstrated an age-dependent homeostatic and circadian regulation of basic cortical function. That was especially evident at the local level, when focusing on cortical excitability profile: young participants showed a clear circadian rhythmicity and sleep homeostasis regulation, the dynamic of which was dampened in the older participants. At the global level, cortical response scattering and complexity changed with time spent awake, i.e. according to the circadian phase Furthermore, cortical complexity response was higher in the older group, showing a simple age effect, but the dynamic did not differ between the two age groups. Preliminary analyses demonstrated that these cortical dynamics sustain part of the profile of behavioural performance across the circadian cycle. Importantly, older people with higher cortical excitability, particularly during the biological night, were performing better at higher order tasks, possibly indicating that older people that maintain

a degree of sensitivity toward sleep homeostasis and circadian processes perform better. Understanding the principal forces that regulate the dynamics of cortical neurophysiology in two age groups –and their impact on cognition– is of uppermost importance for our ageing society, in which sleep deprivation and circadian misalignment are commonplace.

Résumé

Il est actuellement reconnu que la physiologie cérébrale et la cognition chez l'homme sont sous l'effet conjoint de l'homéostat du sommeil et du signal d'alerte circadien, mais les effets délétères de la privation de sommeil restent essentiellement vus comme la simple conséquence d'un manque de sommeil. Bien que cette approche soit intéressante, une perspective circadienne devrait être intégrée dans le but d'en obtenir une compréhension plus complète. Une des principales difficultés dans la mesure de la rythmicité circadienne provient toutefois de la difficulté à l'évaluer. Des variables confondantes telles que la lumière, l'activité, les repas, etc. pourraient en effet masquer la régulation circadienne sous-jacente. Deux études en routine constante ont été menées dans le but de mesurer l'interaction entre l'homéostat de sommeil et le processus circadien au niveau cortical. Trois aspects complémentaires de la fonction corticale et leurs associations avec les performances comportementales et les changements de la dynamique corticale liés à l'âge ont pu être explorés au sein de ces études. Dans la phase I de l'étude, les dynamiques de l'excitabilité corticale, ainsi que la propagation et la complexité de la réponse ont été décrites durant un protocole d'éveil prolongé de 28 heures chez des participants jeunes (18-30 ans). Dans la phase II, dont le but était d'explorer les changements au cours de la vie, les dynamiques de l'excitabilité corticale et la complexité de la réponse ont été étudiées chez des participants jeunes (18-30 ans) et âgés (50-70 ans) durant un éveil prolongé de 34 heures. Dans l'ensemble, les résultats de cette thèse démontrent une régulation circadienne et homéostatique de la fonction corticale de base dépendante de l'âge. Ceci était particulièrement évident au niveau local pour le profil de l'excitabilité corticale : une claire régulation circadienne et homéostatique était mesurable chez les jeunes, mais cette dynamique était aplatie chez le group des personnes plus âgées. Au niveau global, la diffusion et la complexité de la réponse corticale changeaient avec le temps passé éveil, c'est-à-dire en fonctionne de la phase circadienne. En outre, la complexité de la réponse corticale était plus haut chez le personne plus âgées (effet de l'âge), mais la dynamique ne changeait pas entre les deux groups. Des analyses préliminaires ont

démontré que les dynamiques corticales soutiennent le profil de performances comportementales au travers du cycle circadien. A noter que les personnes âgées avec un niveau d'excitabilité corticale plus haut, particulièrement pendant la nuit biologique (c'est-à-dire qui maintient une certain dégrée de flexibilité corticale) étaient celles qui performaient mieux à des tests complexes. Dans notre société vieillissante au sein de laquelle la privation de sommeil et le décalage circadien sont fréquentes, il est primordial de comprendre les principales forces qui régulent les dynamiques de la neurophysiologie corticale et leurs effets sur la cognition dans ces deux groupes d'âge.

<u>Introduction</u>

<u>I. Circadian periodicity</u>

The first section will mainly focus on some ubiquitous key aspects of circadian periodicity and its intimate relation to the Earth, and will end with a brief exploration of the sense of physiological timing in humans. Thoughts are based on the book *Circadian Rhythms: A very Short Introduction*, by Russell G. Foster & Leon Kreitzman, Oxford University Press, as well as the course of *Biological Rhythms* (BMS3066), module co-ordinator Prof. D.J. Skene, University of Surrey.

Day outside: diurnal rhythm

> *Il y a une vérité fondamentale que j'aimerais exprimer, mais les mots me manquent. Le Ciel et la Terre ne parlent pas, ni les quatre saisons et pourtant, ils nous enseignent tellement mieux que des paroles.* –Fabienne Verdier, Passagère du silence

About 4.5 billions years ago, an explosion formed the Earth, our home planet, the only one where life is known to exist. The Earth does a revolution around the Sun in 365.25 days, while rotating around its axis in 23 hours, 56 minutes and 4 seconds: that generates what we call a year and a sidereal day (while a mean solar day is of 24 hours). The axis of the Earth is tilted at an angle of 23.5 degrees: Sun's rays hit different parts of the planet more directly depending on the time of year, generating seasons. Earth is a rhythmic environment, and life on Earth has been ruled by the constant, predictable, rhythmic change of day and night (i.e. nyctohemeral rhythm, 24 hours). Since Earth's rotation causes profound but repetitive changes in light, temperature, living beings must find the optimal balance between foraging, predation risk and reproduction, by maximizing the orchestration of timing of these behaviours across the day. A signature of life is the existence of circadian rhythms (from the Latin *about a day*, i.e. approximately 24 hours), found in almost all living beings, from prokaryotes to higher organisms, including algae, fungi, plants, and animals. The ubiquity of

circadian rhythms speaks to their importance: natural selection seems to have favoured organisms with biology and behaviour spanned over a circadian cycle, which is intimately intertwined with the Earth's solar day. Thus, understanding these biological processes tells us much about ourselves and the world in which we live.

Day within: circadian rhythm

Definition

Based on the previous section, it is evident that circadian rhythms convey notions of biology and astronomy. It was indeed a French astronomer, Jean-Jacques d'Ortous de Mairan, to prove for the first time the existence of a circadian rhythm back in 1729. He noticed the leaves of the Mimosa plant (*Mimosa pudica*) would droop at dusk and rise during the day (i.e. daily rhythm). So he stored the plant in a cupboard to keep it in a dark environment free of time cues. De Mairan noticed that the leaves still opened and closed rhythmically, like if the plant would have its own representation of day and night (i.e. subjective day and night). This finding suggested that the movements represented something more than a simple response to the Sun and were controlled by an internal clock, proving the self-generated nature of the circadian rhythms. This simple yet comprehensive experiment highlights an essential caveat: circadian rhythms are endogenous, and can be defined as it if they persist in constant conditions (constant light or darkness), whereas daily rhythms are passive response to the changes in the environment (exogenous response). Circadian rhythms that are expressed in the absence of any 24 hours signals from the external environment are called free running. This means that the rhythm is not synchronized by any cyclic change in the physical environment. Strictly speaking, a diurnal rhythm should not be called circadian until it has been shown to persist under constant environmental conditions, and thereby can be distinguished from those rhythms that are simply a response to 24-hour environmental changes (Vitaterna et al., 2017). For example, the rooster of crowing (a.k.a. cock-a-doodle-doo), a symbol of the break of dawn, has been proved in constant

environmental conditions to be under the control of an internal circadian clock, instead of being a passive response to external changes (Shimmura and Yoshimura, 2013). Activity and rest cycle studies conducted in the 1950/60s on circadian rhythmicity in fruit flies by Colin Pittendrigh (Pittendrigh, 1950), and in humans by Jurgen Aschoff (Aschoff, 1965) can be considered the foundation of the field (Daan, 2000) (**Fig.1**).
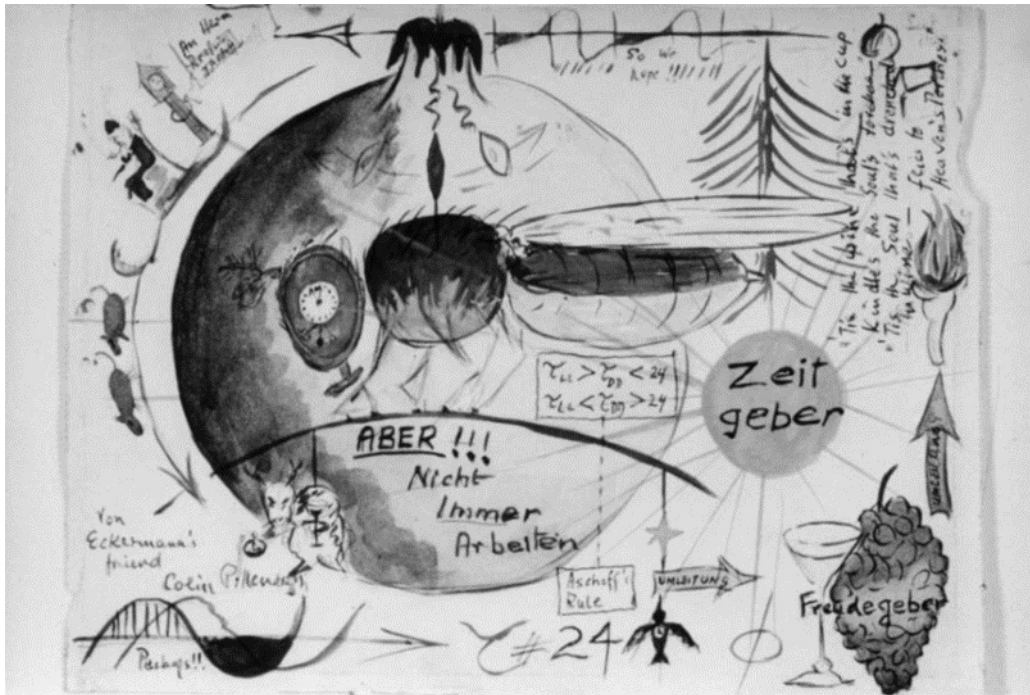
Figure 1: The drawing done by Pittendrigh after visiting Aschoff in 1959 (Daan, 2000).

They first defined the essential features of circadian rhythms:

(i)   they are endogenous generated rhythms that show near 24-hour rhythms (with a mean period ranging from 23-h in *Apis mellifera* (honey bee) to 26-h in *Glyphiulus cavernicolus* (cave-dwelling millipede)). The seeming imprecision is an important feature of rhythmicity, however: the deviation from a 24-hour cycle actually provides a margin for the internal time-keeping system to be continuously aligned by and aligned with the light-dark environment.

(ii)   they persist under constant conditions for several cycles.

(iii)   they are entrained to the solar day via *zeitgebers* (time givers, i.e. synchronisers, in primis light, known as photoentrainment, but also temperature, social interactions, exercise, food). While light is probably the dominant zeitgeber for most animals, temperature can also entrain rhythms, though its importance as a zeitgeber depends on clock hierarchy and whether the animal is poikilothermic or homoeothermic by possibly altering cellular humoral signalling (for review see (Rensing and Ruoff, 2002)). Properly speaking, a true zeitgeber must have different effects at different times of day by eliciting a phase delay or phase advance in the timing of the rhythm. This difference in responses can be represented by a phase-response curve. Light exposure around dusk and during the first half of the night causes a phase delay in activity, while light during the second half of the night and around dawn generates an advance (Duffy and Wright, 2005).

(iv)   they show temperature compensation[1] (to avoid changes in the period of the oscillation with changes in temperature the Q10 is close to 1). Thus, a change in temperature can affect the phase of a cycle without substantially altering the rate of cycling.

Purposes

Endogenously generated circadian rhythms are important to life organisms because they provide an internal temporal order of a range of physiological and behavioural processes (e.g. core body temperature, digestive processes, hormone release, blood pressure, rest-activity patterns etc.) that repeat on a regular ~24-hour basis ((Mure et al., 2018) although this paper presents daily rhythmicity). Furthermore, biological processes under circadian control anticipate the recurring changes generated by the Earth's rotation, thus supporting the idea that must be generated by a self-sustained timing

---

[1]When the British carpenter and clockmaker John Harrison built his mechanical clock back in the 1700s, he paid attention to the last point: indeed, the clock did not speed up or slow down when carried on a ship anchored in London or in the Caribbean. Harrison's timepieces are still visible at the Royal Greenwich Observatory.

mechanism (Pittendrigh, 1993). Organisms that use circadian rhythms to predict conditions in the near future are thought to have a major advantage over competitors and predators: they optimize physiology and behaviour in advance, instead of merely respond to changes of the solar day and night, allowing immediately exploit of the new situation (e.g. in humans melatonin production and excretion *before* falling asleep, and cortisol production and excretion *before* waking-up (Brown, 1994)), supporting this idea that fact that survival comparisons of rhythmic and arrhythmic animals in a natural setting clearly showed effects of circadian rhythm disruption on fitness (DeCoursey et al., 2000). Finally, to be biological useful for life functioning, circadian rhythms must also be synchronized to the external world by anticipatory signal of day and night (mainly the change of light around dawn and dusk). Overall, the net result is that biological processes under circadian rhythmicity occur not all day long, but in a logical sequence, by maximizing the timing orchestration of these processes across the day. For the sake of survival, and in an ecological perspective, that has forced species to become specialist instead of generalist, by causing the evolution of diurnal, nocturnal, crepuscular, but also cathemeral species (i.e. species-specific temporal niche). Interestingly, slightly different temporal patterns may be exhibited within the same group, both in animals (e.g. morning and evening typology in humans (Horne and Östberg, 1976)) and in plants (**Fig.2**).
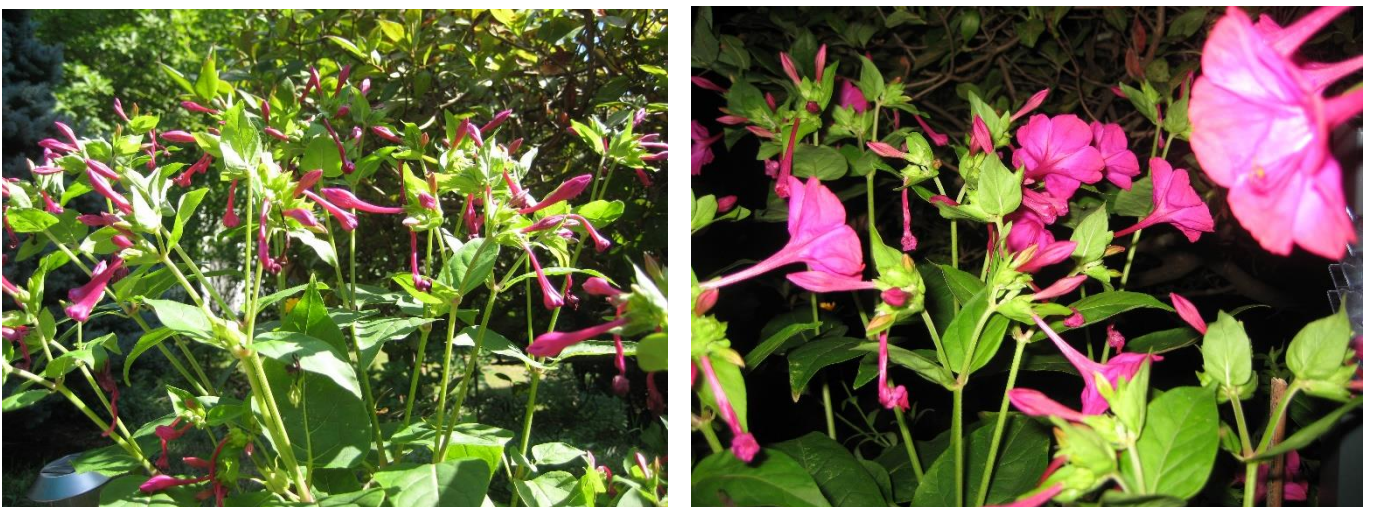

Figure 2: Four o'clock flowers (Mirabilis jalapa) open at dusk and close at dawn during Summer, thus reducing the direct competition for pollinators. Their sweet-smelling fragrance throughout the night attracts nocturnal pollinators.

Circadian resonance hypothesis

Another important pioneer contribution of Pittendrigh has been the circadian resonance hypothesis: circadian rhythms with an endogenous period close to or equal to the natural light-dark cycle are considered evolutionarily adaptive (Pittendrigh and Minis, 1972), and increase the fitness of the organisms. In recent years, Spoelstra and colleagues have tested this idea in eukaryotic organisms in semi-natural conditions. Wild-type, heterozygous, and homozygous mice with a short-period mutation (*tau* mutation) were tracked over the course of 14 months in outdoor experimental enclosures. Over several generations, survival was reduced in the homozygote mutant mice, revealing strong selection against the mice with the accelerating free-running circadian period. Thus, circadian rhythms with an abnormal period compared to the natural ~24-hour cycle are predicted to have negative consequences for fitness (Spoelstra et al., 2016). In this perspective, humans with their free-running average period of 24.18 hours (Czeisler et al., 1999) are well suited for life on Earth, but significant variations of the human circadian phenotypes exist. In a hypothetical colonisation of Mars, people who have longer circadian rhythms are probably better suited to become Martian, because Mars completes a rotation in 1 day, 37 minutes (i.e. a sol). Thus, instead of circadian, 'circasol' rhythms would be preferred. However, Mars revolves around the Sun in 686.93 days and gets less amount of light compared to the Earth. In the eventuality, a Darian calendar has already been prepared, allowing to keep time on Mars.

Locking the day within with the day outside: the entrainment

This section has been narrowed down to the mammalian species. As previously mentioned, to be of any value, circadian rhythms must synchronise to the external environment. For this purpose, the change in the light at dawn and dusk has been the main zeitgeber that allows entrainment (Roenneberg and Foster, 1997). This important light signals are perceived, in mammals, through the eyes: thus eyes provide us with a sense of time. However, the detection of the dawn and dusk signal is not based

on rods and cones, but on intrinsically photosensitive retinal ganglion cells (ipRGCs) that express the blue light-sensitive photopigment melanopsin (maximum sensitivity at 480 nm) (Hattar et al., 2002; Lucas et al., 1999). Interestingly, mammals do not have extra-retinal photoreceptors like other vertebrates (despite some disproved publications e.g. (Campbell and Murphy, 1998)). One explication could be that mammals arose from nocturnal ancestor and switched to daytime after dinosaurs' disappearance (Maor et al., 2017), thus they lost extra-retinal photoreceptors with the evolutionary path (e.g. birds have pineal organ that is directly receiving light and is sensitive to it). Signals detected by ipRGCs are then fed via the retino-hypothalamic tract (RHT) directly to the suprachiasmatic nucleus (SCN), a pair of small nuclei located at the base of the anterior hypothalamus, above the optic chiasm, at the base of the third ventricle. The pivotal role of the SCN was demonstrated by a series of discoveries in the early 1970s: lesion of the SCN in rats resulted in abolished endocrine and behavioural circadian rhythms (Moore and Eichler, 1972; Stephan and Zucker, 1972). Furthermore, transplantation of the SCN between mutant hamsters with different periods transfers the donor period to the recipient animal (Ralph et al., 1990). These experiments established the SCN as the master circadian pacemaker of mammals. Further works established that the SCN displays anatomical heterogeneity but functional homogeneity: SCN is made up of autonomous neurons with independent firing rhythms (Welsh et al., 1995) but, when incorporated into the SCN, they all fire in synchrony, providing a coherent ~24-hour signal, due to extensive synaptic coupling and gap junctions (Liu et al., 2007; Yamaguchi et al., 2003). SNC neurons exhibit a circadian rhythm of spontaneous action potentials that has higher frequency during daytime, in both diurnal and nocturnal mammals (Herzog et al., 2017). SCN communicates with the rest of the brain mainly via indirect neural pathways –mainly by relays through the subparaventricular zone–, which dictate the circadian rhythmicity of wake-sleep cycle, locomotion, feeding, and corticosteroid secretion (for a review (Saper, 2013)). SCN also controls the circadian rhythmicity of the melatonin secretion (known as the sleep hormone), via a multisynaptic pathway that includes the parventricular nucleus, the intermediolateral column of the spinal cord, and then the superior cervical ganglia to

reach the pineal gland. As night approaches, melatonin is secreted into blood circulation, in both nocturnal and diurnal species (Arendt and Skene, 2005). The SCN maintains circadian synchrony in the body via its control over behaviour, neuroendocrine pathways and the autonomic nervous system. However, in vitro experiments showed that circadian rhythms could persist in various peripheral tissues that were not under the control of the SCN (Yamazaki et al., 2000), proving that there are clocks within each cell of every organ and tissue examined so far (Balsalobre et al., 1998; Yoo et al., 2004). However, without the influence of the SNC, peripheral clocks of a given organ would continue to tick (Stokkan et al., 2001), but all at different phases so that an overall 24-hour rhythm within the organ would be lost, impacting local physiology and gene expression. Like Greenwich Mean Time, the SCN serves as a reference point for the peripheral clocks, so that they can run in sync (**Fig.3**). However, during jet lag, the SCN resets to local time in around one day based on light signals, but peripheral oscillators can take more than a week to adjust (Yamazaki et al., 2000). Peripheral clocks are also interconnected themselves, providing further coherence, and they also sent feedback signals back to the SCN, allowing the whole body to function in synchrony with the varying demands of the 24-hour ligh/dark cycle (Johnston et al., 2009).
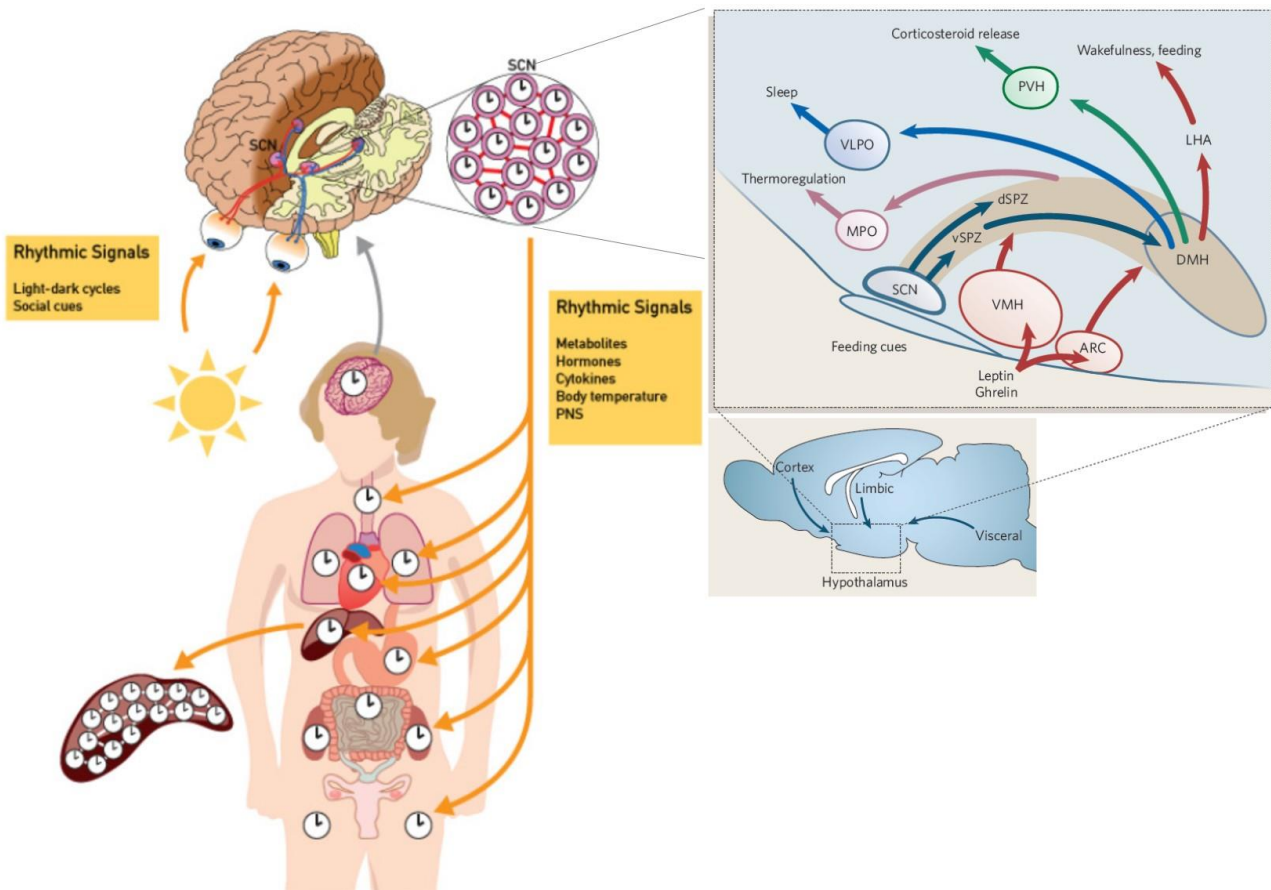
Figure 3: Left: simplified organisation of the mammalian circadian system. The principal circadian clock, i.e. the suprachiasmatic nucleus (SCN), is responsible for all overt rhythms in behaviour and physiology. SCN provides the peripheral clocks of the body with coherent daily timer (i.e. global synchronisation), via multiple and complementary downstream connections. External light inputs entrain the phase of the SCN to the external solar day, via retina receptors (Bollinger and Schibler, 2014). Right, upper panel: a schematic diagram showing the different brain targets of the SCN. The principal outputs consist of ventral and dorsal subparaventricular zone (vSPZ and dSPZ), and the dorsomedial nucleus of the hypothalamus (DHM). The vSPZ-DHM axis drives circadian cycles of sleep, activity, feeding, and corticosteroid secretion, by projecting to the ventrolateral preoptic nucleus (VLPO), lateral hypothalamic area (LHA), and paraventricular nucleus of the hypothalamus (PVH). dSPZ neurons are responsible for regulating the rhythms of body temperature, via the medial preoptic area (MPO). Right, lower panel: circadian rhythms regulation adapts to environmental stimuli, such as food availability (via the ventromedial (VMH) and arcuate (ARC) nuclei), as well as visceral sensory inputs, cognitive influence from the prefrontal cortex and emotional inputs from the limbic system (Saper et al., 2005).

Molecular circadian oscillator

During the 1970s, Benzer and his student Konopka asked whether it would be possible to identify genes that control the circadian rhythm in fruit flies. They demonstrated that mutations in an unknown gene disrupted the circadian cycle of flies, and they named this gene period (*per*) (Konopka and Benzer, 1971). A trio of American researchers, Hall, Rosbash, and Young, aimed to further identify

the underlying mechanisms that control fruit flies' internal circadian cycles. They succeeded in isolating the period gene, and they discovered that the encoded PER protein accumulated in the cytoplasm during the day, peaked in the early subjective night, and was degraded during the night (Bargiello et al., 1984; Hardin et al., 1990; Zehring et al., 1984), suggesting cyclic changes in the expression of certain genes as a possible mechanism underlying the cellular internal pacemaker. The importance of these findings has been recognised in 2017 with the Nobel Prize in Medicine or Physiology. Finally, in the 1990s, researchers described the first mouse circadian mutation, called Clock (King et al., 1997; King et al., 1997; Vitaterna et al., 1994). This and subsequent work established that endogenous molecular clocks consist of a transcription–translation feedback loop (TTFL) (**Fig.4**), a classic negative feedback loop that oscillates every 24 hours and generated at the cellular level, in unicellular organisms as well as in highly complex mammals (for a review (King and Takahashi, 2000; Lowrey and Takahashi, 2000; Wager-Smith and Kay, 2000)). Although the specific clock genes are not evolutionarily conserved across distinct phyla, the principle is the same in almost all forms of life: the forward limb of the clock involves a set of transcriptional activators that induce the transcription of a set of repressors. The latter comprise the negative limb, which feeds back to inhibit the forward limb. This cycle repeats itself every 24 hours, and the mRNA is used to generate the protein products encoded by the circadian clock genes (Bass and Takahashi, 2011). However, the presence of circadian rhythms in cells without nuclei indicate that circadian patterns of peroxiredoxin oxidation of human blood cells persist even in the absence of gene transcription (O-Neill and Reddy, 2011). Together, these mechanisms result in the circadian expression of 10%–30% of all transcriptome (Panda et al., 2002; Reddy et al., n.d.; Storch et al., 2002).
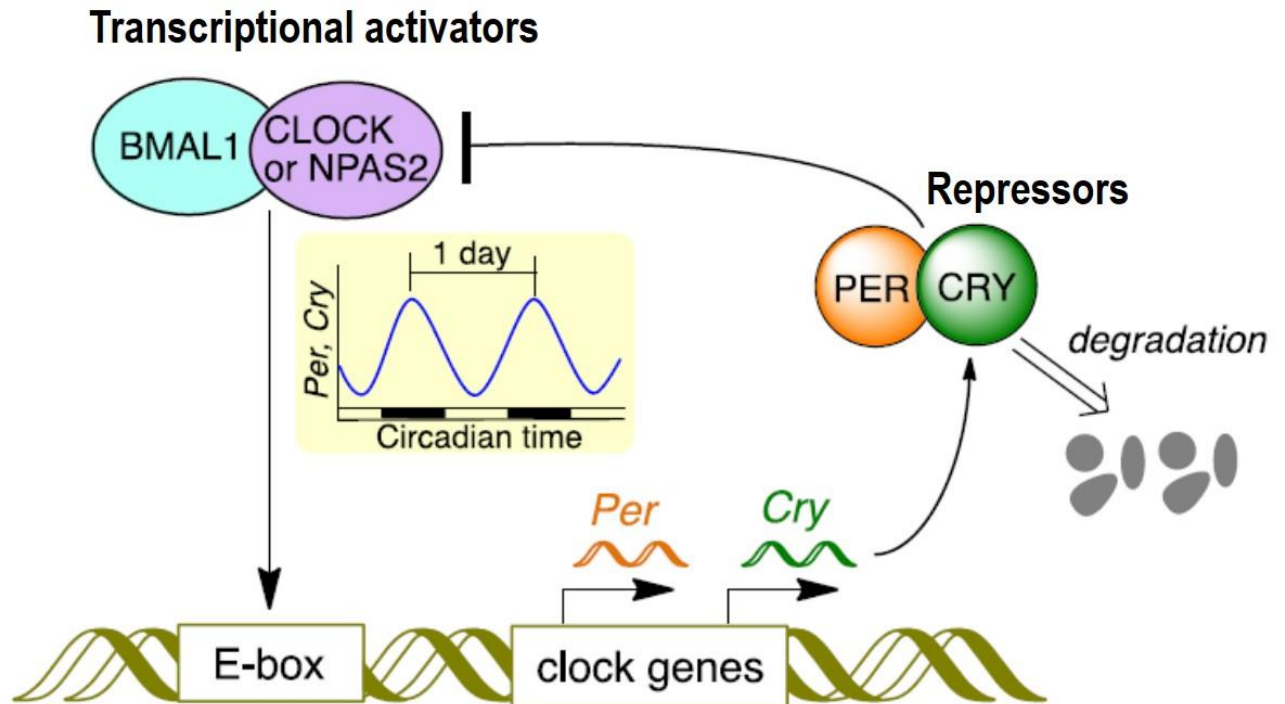
Figure 4: Overview of the transcription–translation feedback loop: a set of transcriptional activators (BMAL1, CLOCK) induces the transcription of a set of repressors (PER, CRY), which feeds back to inhibit the forward limb. Proteins of repressors accumulate in the cytoplasm during the day, peak in the early subjective night, and are degraded during the night, suggesting that the cycle repeats itself every 24 hours (adapted from (Minegishi et al., 2018)).

Intermezzo: humans' time-tracking

What is time? Ask me not, and I know. Ask me, and I cannot tell you. –St. Augustine

In the last paragraph of this section, I would like to put forward a few considerations about the evolution of humans' time-tracking. In this sentence above, St. Augustine[2] well expressed this perennial riddle: although each human has an internal master clock, the timing information does not reach awareness. The Japanese kanji time 時 responds to this riddle: time is a Sun rising over a Buddhist temple, each day. We need indeed to consider our relationship to the Sun not only in terms of main zeitgeber, but also through a cultural lens: the awareness of its changing positions was key to our understanding of time, and shaped our culture and religion. Cave art and stone carvings

---

[2]This section is dedicated to two Augustine: the first one, with his >80 revolutions around the Sun, taught me how to read sundials and the star clock, while the second little one has just started to rotate around the Sun.

revealed early humans' time-tracking sophistication based on the observation of the Sun movement (dawn and dusk, Winter and Summer solstice, Spring and Fall equinox), Moon phase and other stars, which have been conducive to the computation of time and seasons. Until the recent past, the changes in seasons influence human biology (like birds and other mammals still do), and was important for activities, a matter of life and death for these societies. With sedentary societies based on agriculture, there was a further need to divide the day into hours: sundials were elaborated to measure the length of a day (*horas non numero nisi serenas*, loosely translated as I only count the sunny hours). The most natural sundials were those that divided the day in 24 hours from the sunset (**Fig.5**), indicating how many hours were left until the next sundown: in Summer hours were long, while in Winter short.
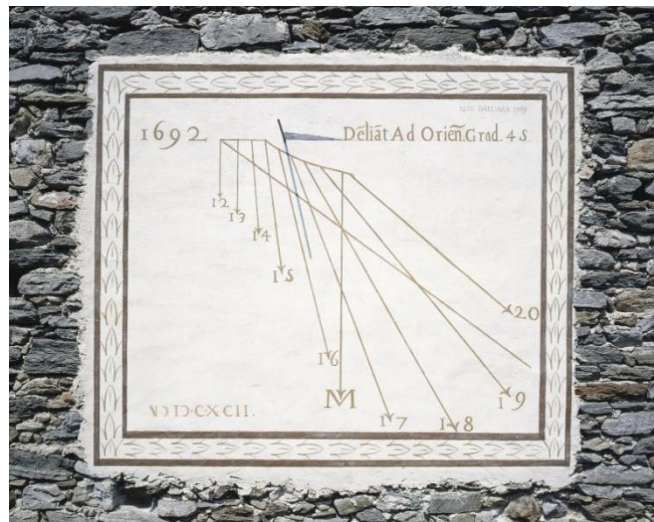
Figure 5: Sundial on the wall of a church. It indicates how many hours are left until the sunset, local meridian is also depicted.

Since we lived by patterns driven by the solar day, being able to track time was an invaluable skill to plan activities outdoor. Indeed, for most of our evolution, humans lived outdoors in the natural environment. However, at the beginning of XIV century in Europe, early mechanical clocks allowed humans to progressively detach from nature: time was divided in 24 hours of equal length throughout the year, and the measurement was more infinitesimal. Further modern needs required to standardise time between cities to facilitating communication, transport etc. We started to live our lives according

to pattern based on the clock and independent from natural rhythms. Within recent history, following the invention of electricity, estrangement from natural sources of light became even more pronounced, with repercussions on our biological clock, physiology and health (this point will be further approached in the Discussion). Thus, how we compute the time is the result of human conventions that have changed with human evolution, leading to a progressive detachment from nature. However, the sky clock still regulates some of our celebrations: Easter is celebrated on the Sunday after the full moon on or after 21 March (Spring Equinox, in northern hemisphere), the Persian New Year (Nowruz) coincides with the Spring equinox, while the Chinese New Year is the first new Moon after the Sun enters the Aquarius constellation, attesting that a link with the Sun/Moon is still strongly embedded in our culture (**Fig.6**).

Figure 6: A person is cheering sunrise on Summer solstice, when Sun is centred between these standing stones (*menhir*).

II. Sleep-wake cycle

In this second section, the focus will be on one of the most obvious 24-h circadian behaviour: the sleep and wake cycle, its definition, regulation and changes during healthy ageing. Considerations regarding sleep are based on research conducted in birds and mammals, with particular emphasis on

humans. A final paragraph will briefly summarise homeostatic and circadian processes at the human brain level, which led the work of this PhD thesis.

The circadian rest-activity cycle and the appearance of sleep homeostasis

Nothing in biology makes sense except in light of evolution. –Theodosius Dobzhansky

If circadian activity-rest cycle is ubiquitous, sleep possibly required a further evolutionary step (Allada and Siegel, 2008). Sleep is also part of the activity–rest cycle and, in ideal conditions, the daily recurrence of sleep coincides to a large extent with the circadian rest-activity rhythm. Therefore, rest and sleep should provide the organism with a similar primary function: a 'trivial function' of sleep. The evolutionary advantage of developing an intensity dimension of sleep, provided sleep with a relative independence from the circadian clock, which regulates the timing of sleep, allowing organisms a more flexible adaptation to changes in sleep-wake pattern (Tobler and Achermann, 2007). Thus, homeostatic regulation of sleep is believed to be modern in front of the circadian one. Rial and colleagues (Rial et al., 2007) presented a provocative hypothesis: the circadian rhythm of poikilotherms is thought to be sufficient to enforce rest at night, whereas true sleep with homeostatic regulation evolved only in homeotherms to ensure periods of inactivity. However, mammal and bird sleep shows a number of additional traits that go beyond a mere trivial function (e.g. two phases of sleep, changes in the activity of discrete central nervous regions, changes in psychological efficiency etc.), suggesting that sleep serves also a restorative function (Rattenborg et al., 2007). Sleep and its trivial and restorative functions have probably evolved as a species-specific response to a 24-hour world in which light, temperature, and food availability changes dramatically, to ensure an optimal internal environment for processes occurring during the circadian resting phase. However, there is an incredible variation in sleep expression. For example, within mammals, the pocket mouse (*Perognathuslongimembris*) sleeps 20 hours, whereas the horse (*Equuscaballus*) sleeps only 3 hours, despite the fact they both have a similar mean circadian period.

Definition of sleep and wakefulness

Sleeping is no mean art: for its sake one must stay awake all day. –Friedrich Nietzsche

This sentence of Nietzsche highlights how sleep and wakefulness are intertwined: the value of sleep is inseparably linked to the one of waking. Both states have distinctive features in term of behaviour, vigilance and brain electrical activity. On the behavioural level, wakefulness has been characterised by consciousness, awareness and activity, allowing coherent cognitive and behavioural responses to the external world. On the brain level, the electroencephalogram (EEG) is defined by a low amplitude, fast frequency (i.e. desynchronised EEG). Electrooculogram (EOG) and electromyogram (EMG) also show high activity. Sleep instead is mainly characterized by specific sleeping site, typical body posture, physical quiescence, reduced responsiveness to external stimuli, rapid reversibility, and regulatory capacity (i.e. compensation after sleep loss) (Tobler, 1995). In mammals and birds, sleep is divided into non-rapid eye movement (NREM) sleep, characterized by higher amplitudes and slower frequency (i.e. synchronized EEG), gradual rolling eye movements, and low or minimal muscle activity. NREM alternates with rapid eye movement (REM) sleep, with higher EEG activity similar to waking EEG, rapid bursts of eye movements, and atonia (Deboer, 2013). Sleep and wake are complementary albeit may occur simultaneously: local sleep-like activity in awake animals (Vyazovskiy et al., 2011), and local wake-like activity in sleeping patients (Nir et al., 2011; Nobili et al., 2011) were recently demonstrated. Vyazovskiy and colleagues raised the intriguing question of whether sleep-like off states in awake animals reflect an adaptive or a maladaptive response to sleep loss. Local sleep could be adaptive if it allows some beneficial sleep-related processes to occur while the animal continues to engage in adaptive waking behaviours. However, a global regulator of sleep-wake may be needed to prevent maladaptive mixed behavioural states, and maintain the behavioural shutdown that may be needed to ensure sleep related functions.

Functions of sleep

> *If sleep does not serve an absolute vital function, then it is the biggest mistake the evolutionary process ever made.* –Rechtschaffen

Multiple, nonexclusive functions of sleep have been proposed, despite the fact that sleep entrains some costs: during sleep, animals are typically immobile and less aware of the local environment, making them vulnerable and unable to perform other essential tasks. That suggests that sleep must serve essential functions favoured by natural selection: (*i*) behavioural function, such as sleep as an immobilizer that prevents animals from being active during unfavourable times of day (Siegel, 2009), (*ii*) maintenance and recovery (housekeeping) functions, such as brain thermoregulation (McGinty and Szymusiak, 1990), energy conservation (Berger and Phillips, 1995), brain waste clearance (Xie et al., 2013), tissue restoration (Adam and Oswald, 1977), maintenance of the immune system (Besedovsky et al., 2012), and (*iii*) long-term maintenance of cerebral integrity, such as memory consolidation (Diekelmann and Born, 2010; Maquet, 2001), and synaptic homeostasis function (de Vivo et al., 2017; Tononi and Cirelli, 2006). The synaptic homeostasis hypothesis (SHY), formulated by Tononi and Cirelli, proposes that sleep is the price the brain pays for plasticity. From this perspective, the role of sleep would be to downscale synaptic strength to a baseline level that is energetically sustainable, i.e. homeostatic regulation of the total synaptic weight impinging on neurons (but also see (Frank, 2013)).

The two-process model of sleep-wake regulation

The following text has been adapted from the review (Gaggioni et al., 2014). The concept of homeostasis was first developed in the 1800s by Bernard, and was further elaborated by Cannon, who coined the term. As defined by the Commission for Thermal Physiology of the International Union of Physiological Sciences, homeostasis characterises 'the relative constancy of physicochemical

properties of the internal environment of an organism as being maintained by regulation', and is one of the major concepts in physiology. Rhythmicity instead is characterized by the deviation from a stable baseline. Almost 40 years ago, the two-process model by Borbély and colleagues (Borbély, 1982; Borbély et al., 2016; Daan et al., 1984) conceptualized sleep-wake regulation in humans, by the interaction of a circadian and a homeostatic process. Sleep homeostasis regulates the balance between sleep and waking: homeostatic mechanisms counteract deviations from an average reference species-specific level of sleep (Borbély, 1980). Sleep homeostasis is characterized by an exponential increase or dissipation of sleep pressure, as wakefulness extends or sleep progresses, respectively, and is almost exclusively dependent on sleep-wake behaviour. The mechanisms underlying this hourglass-like process are still debated, but animal research suggests that it arises from a use-dependent local augmentation of sleep-promoting substances (adenosine (Basheer et al., 2004; Porkka-Heiskanen et al., 1997) and cytokines (Krueger, 2008)), from an increase in extracellular glutamate level (Dash et al., 2009), and/or from an experience-dependent increase of average brain synaptic strength, excitability and size during wakefulness (Bushey et al., 2011; Vyazovskiy et al., 2008). Other molecular markers of sleep loss have been identified in rodents (Franken and Dijk, 2009), while human polymorphisms have been associated with difference in sleep regulation (e.g. PERIOD3 (PER3) (Viola et al., 2007), Adenosine Deaminase (ADA), Adenosin A2a receptor (ADORA2A), Brain Derived Neurotrophic Factor (BDNF), Catechol-O-Methyltransferase (COMT), human leukocyte antigen (HLA) (Goel and Dinges, 2011), dopamine transporter (DAT) (Valomon et al., 2014), ABCC9 (Allebrandt et al., 2013); for review see (Landolt, 2011)). EEG provides the best established markers of sleep need and intensity: slow wave activity (SWA; approximately 0.5-4.0 or 4.5 Hz) during Non-Rapid Eye Movement (NREM) sleep (Dijk, 1995; Dijk et al., 1990, 1987; Werth et al., 1996), and theta activity (4-8 Hz) during wakefulness (Aeschbach et al., 1997; Cajochen et al., 2002). Such increases are particularly marked over frontal EEG derivations, the frontal cortex being particularly sensitive to the sleep pressure (Cajochen et al., 1999). Besides global increase, SWA changes are also detected locally in areas most implicated in the task previously performed during

wakefulness (Kattler et al., 1994), likely reflecting synaptic changes (Huber et al., 2004; Hung et al., 2013). Behaviourally, increased sleep pressure is associated with a deterioration of cognitive performance, a decrease in alertness and an increase in sleepiness (Dijk et al., 1992; Wyatt et al., 1999). However, cognitive performance and its associated brain activity do not linearly decrease with increasing amount of time spent awake. This shows the existence of a second, circadian regulation process that impinges on cognition. As previously explained, the circadian signal is defined as a near-24-h endogenous, self-sustained oscillator, which determines the timing of the rest-activity cycle and of most physiological processes in synchrony with the environmental light-dark cycle. It is controlled by the suprachiasmatic nucleus (SCN), located in the anterior hypothalamus, also known as the circadian master clock (Moore, 2007). The circadian alerting signal increasingly promotes wakefulness during the day, opposing the progressive accumulation of sleep pressure. The minimum circadian alerting signal is in the early morning hours, and the maximum is reached in the early evening, in the so called wake-maintenance zone (~2-3 hours before one's habitual bedtime), preventing us from falling asleep despite the high need for sleep (Dijk and Czeisler, 1994; Dijk and Czeisler, 1995). As the evening progresses, the SCN alerting pulses start to weaken, melatonin production in the pineal gland increases (also under the direction of the SCN), the 'sleep gate' (also known as the primary sleepiness zone or sleep onset zone) opens, and the urge to sleep increases dramatically. Thus, once passing into the biological night, the circadian signal turns into a sleep-promoting signal, which increasingly opposes the dissipation of homeostatic sleep pressure during sleep, allowing a consolidated ~8-hour sleep episode. Although still putative, a sense of the circadian sleep-promoting signal can be found in the regulation of rapid eye movement (REM) sleep and sleep spindles, which are most prominent at the end of the night (Dijk and Czeisler, 1995). In humans, core body temperature (CBT) circadian profile is probably the closest to the dynamics of the circadian alerting signal. Core body temperature progressively increases during the day to peak in the early evening, before initiating a progressive decrease with nadir in the very early morning hours (Dijk and Czeisler, 1995). Other gold-standard markers are melatonin and cortisol levels (Czeisler et al., 1999).

As previously mentioned, the late evening onset of melatonin secretion, a hormone signalling the biological night, coincides with the end of the wake-maintenance zone and CBT maximum. Melatonin secretion peaks in the middle of the night, around CBT minimum. The well-known increase in cortisol upon awaking is considered as a marker of the end of the putative sleep-promoting zone and, being activating, has been suggested to provide a gate for the transition between sleep and wakefulness (Czeisler and Gooley, 2007). In summary, sleep-wake transition is coordinated by the homeostatic mechanism that regulates sleep intensity (*how*), and by the circadian rhythmicity that regulates the timing of sleep (*when*), and the two-process model illustrate this interaction (**Fig.7**). However, sleep-wake transition involves a highly complex set of interaction between multiple neural circuits (i.e. sleep- and wake-promoting brain areas), neurotransmitter, and hormones, generating a 'flip flop' switch that produces changes in behavioural and consciousness states (Saper et al., 2010, 2001; Scammell et al., 2017; Sehgal and Mignot, 2011; Weber and Dan, 2016). These aspects will not be considered in further detail here.
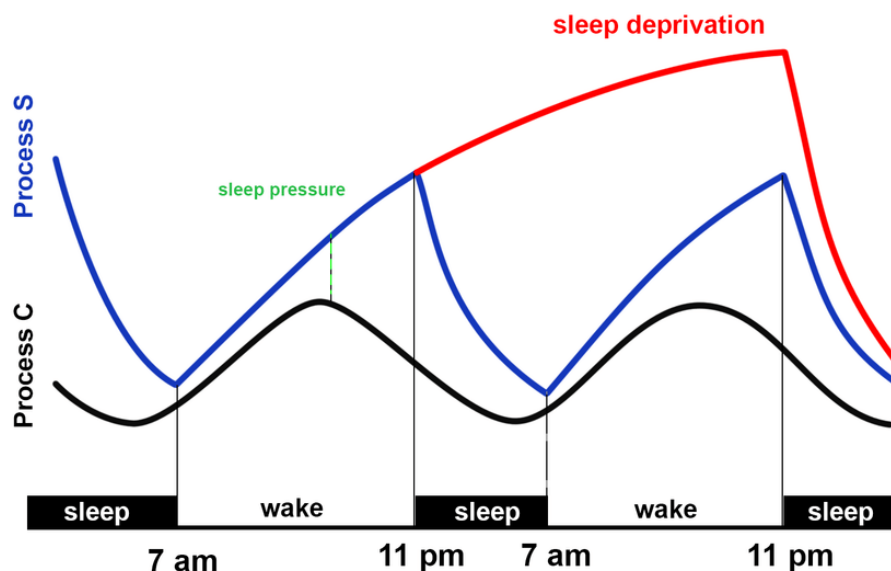
Figure 7: The conceptual two-process model: interaction between a homeostatic process (S), which increases as a function of the duration of wakefulness and dissipate during sleep, and a circadian process (C), which determines the timing of sleep and wakefulness. This interaction result in a monophasic bout of 8-hour sleep and 16-hour period of wakefulness. The peak of the process C coincides with the maximal circadian drive for wakefulness (just before the nocturnal increase in melatonin secretion), while the trough coincides with the maximal circadian drive for sleep (figure retrieved from the ResearchGate page of Dr. A. Patanaik, DO – 10.13140/RG.2.1.1201.9923).

Finally, it has been demonstrated that SCN lesions clearly disrupt the pattern of sleep and wakefulness in rats, but have only minimal effects on the animals' amount of sleep or sleep need (Mistlberger et al., 1987). This evidences the existence of two separated forces, i.e. a homeostatic and a circadian process. However, studies in mice carrying mutations in genes influencing cellular circadian rhythmicity, such as *dbp* and *clock*, also produced changes in sleep regulation (Franken et al., 2000; Naylor et al., 2000), raising the possibility that the homeostatic and circadian processes may be more interrelated than expected.

The two-process model of sleep and wake regulation and its repercussions on performance

The earliest scientific evidence of a link between sleep and performance dates back to the early 1930s, when Nathaniel Kleitman, one of the most significant figures in the field of sleep medicine, discovered a daily pattern in the speed and accuracy of cognitive performance. He showed that, even in well-rested individuals, there was a decrease in the level of individual performance that occurred in the early morning and late at night (Kleitman, 1949). Subsequently, numerous laboratory studies have demonstrated circadian rhythmicity in cognitive performance, which increases from morning to late afternoon or early evening (around the time of the peak of the core body temperature and the wake maintenance zone) (Johnson et al., 1992), independently from task complexity (Van Eekelen and Kerkhof, 2003). During a normal waking day, the increase in homeostatic sleep pressure and deterioration of brain activity are counteracted by the circadian alerting signal. However, when wakefulness is extended into the biological night, the circadian system no longer opposes the high need for sleep, and cognitive performance is jeopardized, most strongly at the end of the night when the circadian signal maximally favours sleep (Dijk and Archer, 2010). Thus, the interplay between the circadian and homeostatic processes not only determines sleepiness and alertness levels, but also affects higher order cognitive functions (Dijk et al., 1992; Wright et al., 2012). Following chronic sleep restriction, which is common nowadays, the circadian signal cannot efficiently oppose

abnormally high sleep pressure and maintain adequate performance already during the day. If wakefulness is extended into the biological night following chronic sleep restriction, the negative impact of acute sleep deprivation on cognitive performance is exacerbated (Lo et al., 2012). Overall, these findings stress the importance of a temporal harmony between the sleep homeostatic and circadian processes. To note however that most people, if there is something important or interesting to do –for example taking care of a baby, finishing a task before a deadline, or watching an entertaining movie– can perform normally despite having accumulated an important sleep pressure, and being awake and functional during the biological night. Environmental and motivational drives compete with homeostatic and circadian processes to decide whether we sleep or engage in other important or interesting activities (Aulsebrook et al., 2016). This point will be largely addressed in the discussion.

Ageing, sleep homeostasis and the circadian timing system

Naturally occurring inter-individual variations in sleep-wake cycle provide an opportunity to gain insight into the regulation and functions of sleep and wakefulness. Sleep-wake cycle changes occur over the lifespan, a living process from childhood to ageing (Roenneberg et al., 2004). Healthy ageing is characterized by a series of changes at the cognitive, behavioural, and physiological levels. With advanced age, sleep undergoes modifications, such as more frequent nocturnal awakenings and reduced slow waves sleep during non-rapid eye movement (NREM) sleep (Cajochen et al., 2006; Dijk et al., 1999; Klerman and Dijk, 2008). This attenuated NREM sleep consolidation, together with the advance in circadian rhythms observed as one ages, mirror an attenuation of the homeostatic and/or circadian drive for sleep in older people. Furthermore, older people exhibit shallower dissipation of sleep pressure, as indexed by reduced dynamics of slow-wave activity during the night (Landolt et al., 1996), but these changes are not systematically accompanied by increased daytime sleepiness (Klerman and Dijk, 2008). In fact, sleep need and its build-up during wakefulness decrease

as one gets older (Landolt et al., 2012; Schmidt et al., 2012a). With respect to circadian rhythmicity, ageing is usually linked to reduced circadian amplitude of core body temperature (CBT) (but no changes in the circadian period), and together with a phase advance of CBT and melatonin rhythm (Dijk et al., 1999; Duffy et al., 2002; Kondratova and Kondratov, 2012; Münch et al., 2005), are possibly responsible for the earlier wake-up time. However, circadian markers, such as CBT, melatonin and cortisol, may not necessarily change with advanced age (Zeitzer et al., 1999). Age-related changes in sleep homeostasis and circadian rhythmicity underlie changes in cognition, such that older people experience a linear decline over 36 hours of wakefulness for several domains of cognitive performance (Schmidt et al., 2007): even though they may achieve overall lower performance than young adults, older individuals suffer relatively less during a night without sleep (Adam et al., 2006), at least over several cognitive domains, including vigilant attention, executive function (inhibitory motor control) and mental arithmetic. Age-related changes in sleep-wake regulation could be determined by disruption of the circadian timing system (Farajnia et al., 2012), reduced entrainment to light (lens yellowing), reduced amplitude of clock gene expression and desynchronization of physiological rhythms, or even by diminished social constraints. Transplantation of the SCN from young mice into aged mice restored youthful rhythmicity, and increased life duration (Hurd and Ralph, 1998), suggesting an adaptive advantage of robust circadian timing.

Interaction of sleep homeostasis and circadian system at the cerebral level

At the cerebral level, the conceptual two-process model has been validated with multichannel electroencephalography (EEG) and functional magnetic resonance imaging (fMRI), by observing temporal correlations between brain function and behaviour/cognition: data have been interpreted as an interaction between sleep homeostatic mechanisms and the circadian clock (Dijk and Archer, 2010; Schmidt et al., 2012a). Regarding fMRI, pioneering studies have been conducted aiming at

characterizing the brain correlates of non-visual response to light exposure in humans, which is known to influence the circadian alerting signal (Vandewalle et al., 2011, 2006). Further work was based on contrasting chronotypes (morning vs evening) that differ in sleep-wake regulation, allowing to investigating differences in task-related brain activity driven by inter-individual differences in the expression of circadian and homeostatic signals (Schmidt et al., 2012b, 2009). A more recent functional neuroimaging study under constant environmental conditions enabled to translate the interaction of homeostatic and circadian processes at the brain level, demonstrating primarily circadian effect and primarily time awake effect on distinct brain regions (Muto et al., 2016). Based on the same protocol, seasonal variations of brain activity were also demonstrated (Meyer et al., 2016). Other studies were able to further disentangle the singular impact of circadian phase and sleep pressure (high vs low) at the brain level (Maire et al., 2018, 2015; Reichert et al., 2017). Overall, these functional neuroimaging studies confirmed that the interaction of the circadian clock and sleep homeostasis is rooted in measurable changes in different cortical and subcortical brain regions. Peaks of brain responsiveness were consistent with the wake maintenance zone, whereas troughs were found in the early morning hours, thus paralleling the well-known ups and downs of human performance (Czeisler, 2016).

Recently, transcranial magnetic stimulation (TMS) has been used as a novel method to directly and non-invasively stimulate the cerebral cortex by electromagnetic induction, and thus causally investigate the neurophysiology of the cortical activity. TMS, by interacting with neural circuits, can lead to causal inferences that bridge human, nonhuman primate, and other model system studies. (Burke et al., 2019). Interesting measures can be derived from EEG recordings of TMS-evoked responses, such as excitability (i.e. the initial local response), scattering (i.e. the global spread of the stimulation), and complexity (i.e. the information content of the EEG response that is time-locked with TMS); a more detailed presentation of these measures will follow in section III of the Method. Cortical excitability evoked by TMS has been correlated with synaptic strength using intracranial

recordings in rodents (Vyazovskiy et al., 2008), and is thought to quantitatively assess local average synaptic potentiation in humans (Huber et al., 2013), the putative cellular correlate of local sleep pressure. Subsequent cortical scattering and complexity might depend also on neuronal firing state (Vyazovskiy et al., 2009, 2011), which determines the fate of the propagation of the initial stimulation, and its information-content, over the cortex. During normal wakefulness, TMS evokes an initial local cortical activation (i.e. cortical excitability), which invariably engages distant cortical areas in a complex and differentiated way for about 300 ms (i.e. cortical scattering) (Massimini et al., 2005). The exactly same stimulation during deep sleep triggers a larger, low-frequency initial wave, which does not propagate to connected brain regions: it remains localized, dissipates rapidly, lacks high-frequency components, and is stereotypical regardless of stimulation site (Massimini et al., 2005). Increasing intensities of stimulation during NREM deep sleep might result in long-range bursts of cortical scattering, but always associated to simple stereotypical and non-specific responses (Massimini et al., 2007). Thus, during slow-wave sleep, the cortical response triggered by TMS either breaks down in causally independent modules, or bursts into an explosive and non-specific response. Interestingly, during transition into light sleep (stage 1 of NREM sleep), TMS-evoked early response is higher but spreads less than wakefulness. During REM sleep instead, TMS triggers a smaller first response that propagates in a widespread and differentiated way, resembling that observed in wakefulness (Massimini et al., 2010) (**Fig.8a-b**). Regarding cortical complexity, Casali and colleagues (Casali et al., 2013) calculated the spatiotemporal complexity of the cortical activity evoked by TMS, and called it the Perturbational Complexity Index (PCI), which is high only if many regions of the cerebral cortex react to the initial perturbation in different ways. High PCI values are found during alert wakefulness, when consciousness is unambiguously present, and low PCI values during sleep, when consciousness is clearly reduced; that is independent of the stimulation parameters. Moreover, complexity gradually decreases from wakefulness to light to deep NREM sleep, and rises to wakefulness levels during REM sleep (Casali et al., 2013; Tosun et al., 2019) (**Fig.8c**). Compared to young individuals, complexity of the sleep EEG increases with ageing (Tosun

et al., 2019). However, the dynamics of cortical complexity during sleep deprivation remains unknown. Two studies in rodents found no statistically significant change in complexity during partial sleep deprivation, based on local field potential (Abásolo et al., 2015), or EEG scalp recordings (Tosun et al., 2017). Additionally, both studies found significantly lower complexity values in recovery NREM sleep following sleep deprivation compared to baseline, suggesting that emergence of new activity patterns in the EEG is reduced after sleep deprivation, impacting the Lempel-Ziv counter. How cortical responsiveness changes during sleep deprivation is a currently open and intriguing research question. It has been showed that cortical excitability progressively increases with time awake during sleep deprivation and is rebalanced during sleep (Huber et al., 2013) (**Fig.8 d**).
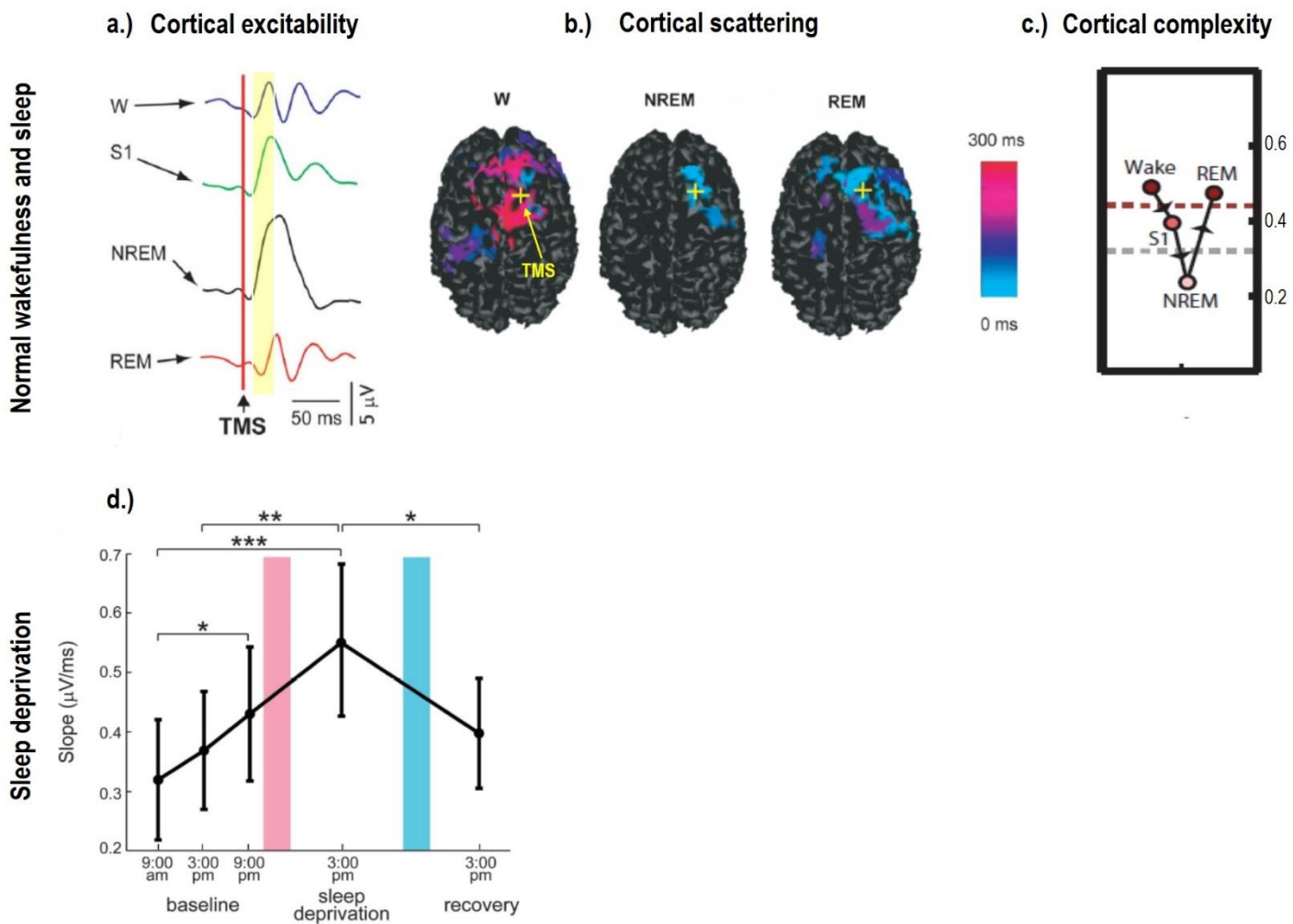

Figure 8: Dynamics of cortical excitability, response scattering and complexity evoked by TMS in healthy humans during wakefulness, prolonged wakefulness and sleep. **a.-b.-c.)** Compared to normal wakefulness, cortical excitability increases during NREM deep sleep, while cortical response scattering and complexity decrease. Interestingly, the opposite happens

during REM compared to NREM sleep, reaching a situation close to normal wakefulness. **d.)** Cortical excitability increases during sleep deprivation compared to a normal baseline day, and recovers after a recovery night. Significant changes in cortical scattering and complexity have not been reported during sleep deprivation, in humans (adapted from (Casali et al., 2013; Huber et al., 2013; Massimini et al., 2010)).

General objectives and hypotheses of the PhD thesis

Overall, these studies suggest that TMS-related measures may be used to differentiate between consciousness and vigilance levels, which accompany the progressive transition between alert wakefulness, drowsiness, light, deep and REM sleep.

Critically, we stress that the changes in cortical responsiveness across the sleep-wake cycle have been almost solely investigated from a sleep homeostasis perspective. However, they may not be simply a function of previous wake duration, and may also depend on individual internal circadian time. Whether homeostatic and circadian interactions are rooted in measurable changes of cortical responsiveness, and across the lifespan, remains essentially unknown, as well as their subsequent impact on vigilance states and cognitive functions. Overall, the studies presented here aim at investigating the relationship between circadian phase and sleep pressure on vigilance, cognition and basic cortical functions –i.e. excitability, response scattering and complexity of the cortex– in young (*phase I*) and older individuals (*phase II*). Our ultimate goal is to gain an integrative understanding of the underlying cortical mechanisms that sustain vigilance states and cognitive functions, during prolonged wakefulness and aligned with individual internal circadian time (state-like effect), and the evolution across ageing (trait-like effect).

In essence, the main objectives (O) and hypotheses (HP) are as follows:

O1: to investigate the temporal dynamics of cortical responsiveness (i.e. cortical excitability, scattering, and response complexity) during a wakefulness extension in the young. In particular, we investigate if significant changes are detectable during windows of circadian interest, i.e. in the early

evening (around the wake maintaining zone), in the late night / early morning (around the sleep promoting zone), and during the following circadian day.

HP1: At the local level, we expect that cortical excitability will decrease during the wake maintenance zone, i.e. in the early evening, because of the strongest effect of the circadian alerting signal over this part of the circadian cycle. Cortical excitability will then increase during the biological night, when high sleep pressure is no longer counteracted by the circadian alerting signal, which turns into a sleep promoting signal instead. Finally, we anticipate that cortical excitability will decrease the following circadian morning, when the circadian signal promotes again wakefulness and opposes to the high sleep pressure (refer to Phase I, Paper 1).

HP2: At the global level, we expect at least that cortical response scattering will decrease during the biological night, possibly reflecting reduced cross-talk between cortical regions (the subtler changes around the wake maintenance zone and sleep promoting zone could be overridden by the increase noise in the data when going globally at the scalp level). Regarding cortical response complexity, we expect a decrease during the biological night, reflecting a reduced neuronal information content that mirror the increased amount of slower and regular oscillations (refer to Phase I, Paper 2).

O2: to determine if the dynamics of the cortical responsiveness correlate with the peaks and troughs of the cognitive performance.

HP3: Since vigilance impairments are typically observed during sleep deprivation and circadian misalignment, we hypothesise that higher level of cortical excitability will be correlated with worse vigilance performance during this time window, when high sleep pressure and circadian misalignment coincide. Furthermore, we expect that a circadian-related decrease of cortical excitability in the early evening will be associated with a stable cognitive performance during a

normal waking day. Conversely, lower cortical response scattering (reduced cross-talk between brain regions) and complexity (more regular neuronal information content) will be associated with worse vigilance performance (refer to Phase I, Paper 1 and Paper 2).

O3: to investigate if the modifications of the sleep homeostasis and the circadian alerting signal during healthy ageing have an effect also on the dynamics of cortical responsiveness and its relation with cognition.

    HP4: We postulate that cortical excitability profile will be dampened in older participants, reflecting reduced modulation by sleep homeostasis and circadian processes. We further anticipate that an increase in cortical excitability in older participants will be associated with better cognitive performance and this independently of the circadian phase or neurobehavioural task: in other words, that synaptic plasticity-related changes will always be related to better cognitive performance in older people (i.e. linear relationship). Furthermore, we expect that older people displaying a higher degree of modulation of the cortical excitability during wakefulness extension will be those performing better during a normal waking day (refer to Phase II, Paper 3).

    HP5: Regarding cortical complexity response, since EEG brain activity during both sleep and wakefulness undergoes a relative shift towards higher frequency and oscillation power in ageing, we further anticipate that cortical response complexity will be higher in the older group over the entire protocol compared to young participants, while its profile during prolonged wakefulness will be dampened compared to the young. Finally, we postulate that higher cortical complexity response will reflect increased randomness in the time series and be associated with worse vigilance performance (refer to Phase II, Paper 4).

Methods

In this section, I will first explain how circadian rhythmicity can be measured in humans by providing a short overview of the main protocols. Then, I will explain the basic principles of the TMS combined with EEG. Finally, I will present briefly the three measures of cortical responsiveness (excitability, scattering, and complexity) that were applied. Thus, I will further develop only those aspects that are central to this thesis and cannot find a space in the articles. I will not detail all the additional methods used in the studies (e.g. cognitive test battery, wakefulness EEG, habituation and baseline nights, data processing), which can be found in the *Materials and Methods* section of each article.

I. Protocols to measure circadian rhythmicity in humans

Constant routine (CR) protocol

Since the goal of these studies was to measure the interaction of sleep homeostatic and circadian components on cortical responsiveness, constant routine protocols were adopted. This approach was initially proposed by Mills, Minors and Watherhouse (1978), and developed to characterise circadian rhythmicity of physiologic variables, over one or two days, in the absence of periodic changes in behaviour. Using a modification of this technique (Czeisler et al., 1985), it was demonstrated that, even when subjects remain continuously awake, at constant bed rest, in constant indoor room light, for extended (40-70 hours) periods, with meals and activity distributed uniformly throughout the 24-hour day, near-24-hour rhythms persist in a number of different physiologic and cognitive variables (Czeisler and Dijk, 2001). Thus, CR protocol is characterized by the following constant routine conditions:

- continuous wakefulness over at least 24 hours, i.e. in the absence of a sleep-wake cycle (continuous sleep deprivation) – or evenly spread periods of sleep and waking (see below)

- isolated environment (no time cues)

- constant dim light

- constant temperature

- constant body position (semi-recumbent posture in bed)

- regular food intake (hourly iso-caloric snacks)

Consequently, masking factors –environmental and behavioural confounders that interfere with circadian rhythmicity– are either abolished or evenly distributed through the circadian cycle. This allows the measurements of unbiased/unmasked physiological and/or behavioural variables of interest at difference circadian phases (i.e. time points aligned to the internal circadian clock), typically at regular time intervals, while tracking the prior wakefulness duration and concomitant change in the circadian cycle. Circadian phase can be indexed by measuring core body temperature or melatonin rhythms. While the most accurate way to measure core body temperature is via rectal thermometer, melatonin can be assed in blood, saliva and urine (6-sulphatoxymelatonin (aMT6s) metabolite), making the circadian rhythm of melatonin the most reliable indirect marker of the SCN, mainly because of its stability and robustness in the absence of sleep (Czeisler et al., 1990), confirming that it is generated by an endogenous circadian oscillatory process. However, to measure the tempo of the internal pacemaker, a series of time points have to be acquired across at least one circadian cycle, even though recent efforts aim at reducing this endeavour (Laing et al., 2017). As already said, although the CR protocol was initially applied to unmask endogenous circadian rhythms usually embedded within the sleep-wake cycle, it allows also to measure the effect of the interaction of homeostatic and circadian components on the variables of interest. Furthermore, if the protocol lasts more than 24 hours, it enables to assess variables of interest at the same circadian phase under different sleep pressure (**Fig.9**). However, the CR protocol does not allow to disentangle the single

contribution of the homeostatic and circadian processes, and there is also the potential confound that continuous sleep deprivation can generate a stressful situation for the body.
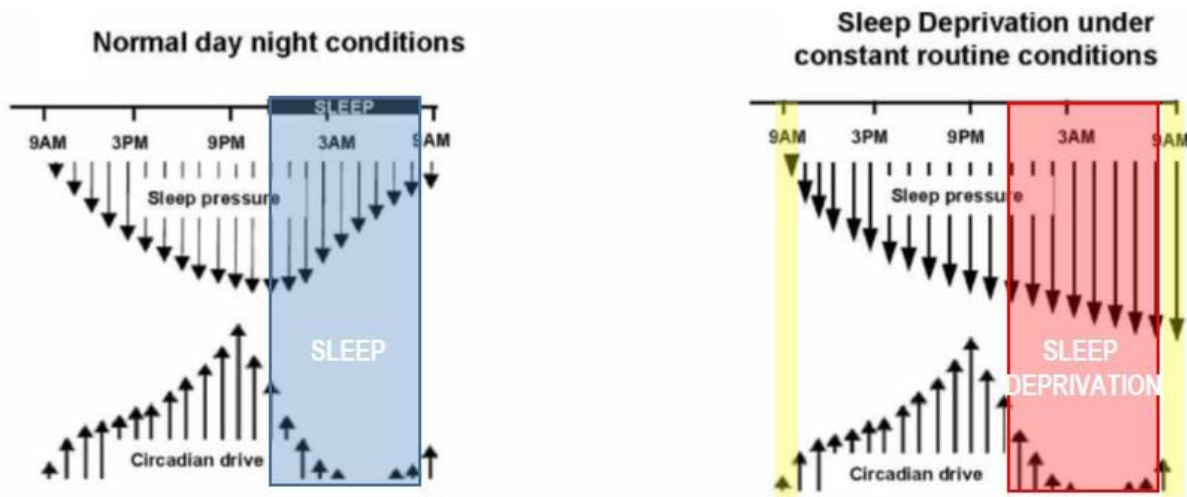

Figure 9: A normal day-night conditions is compared with a constant routine protocol (on the left). During a sleep deprivation under constant routine conditions, a linearly increasing sleep pressure interacts with the circadian alerting signal: during the biological night, the increasing sleep pressure is no longer counteracted by the circadian signal. The yellow bars indicate that, when the protocol lasts > 24-h, the same circadian phase can be assed under different sleep pressure (high versus low) (modified from (Schmidt et al., 2007)).

Forced desynchrony (FD) protocol

The most sophisticated (and demanding) design that overcomes the limitation of the CR, and allows the separation of the homeostatic and circadian components, is the forced desynchrony (FD) protocol. In such a study, participants are separated from the natural environment for 3-4 weeks, and scheduled on a specific sleep-wake cycle. Even though the ratio of sleep to wake remains of approximately 1:2, the artificial day varies with a period significantly shorter (20 hours) or longer (28 hours) than the normal 24-hour day (e.g. in (Dijk and Czeisler, 1994)). This will result in a progressive desynchronization of the artificial sleep–wake cycle from the endogenous circadian cycle, because the period of the artificial day is outside the range of entrainment of the endogenous circadian pacemaker (**Fig.10a**). When the experiment is of sufficient length, sleep and wakefulness are scheduled to occur at virtually all circadian phases of the biological day or night, and following all

prior duration of wakefulness. The influence of differential sleep pressure levels can thus be assessed at virtually all circadian phases, or conversely, circadian influences can be measured under differential sleep pressure conditions (**Fig.10b**). Consequently, a forced desynchrony protocol allows to investigate the interaction between circadian and sleep homeostatic processes, and to quantify their separate contribution in the variables of interest (Czeisler and Dijk, 2001).
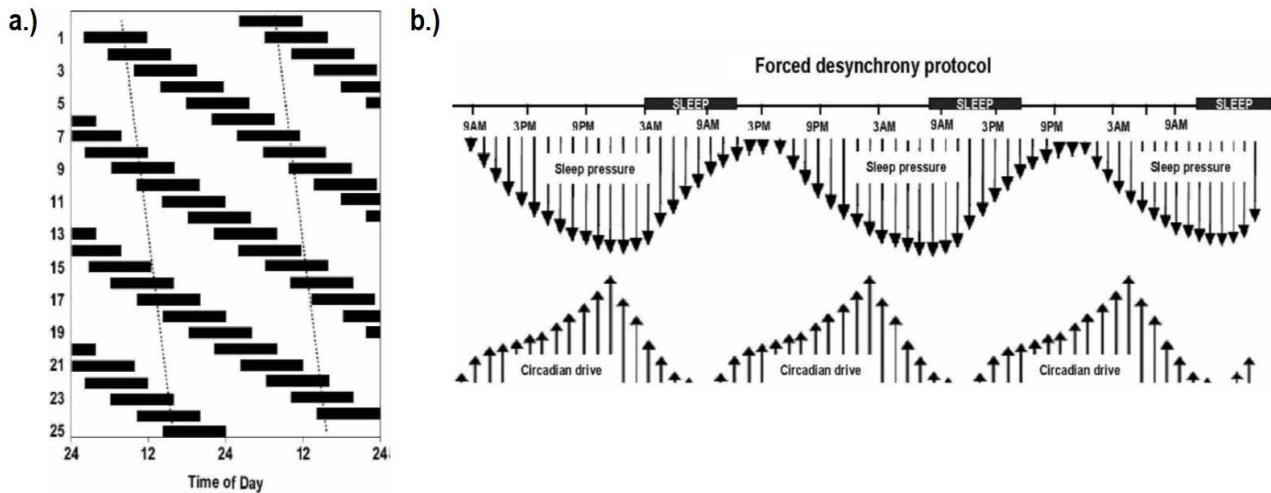

Figure 10: a.) Plot of a 25-day forced desynchrony protocol. Subjects are placed on an artificial 28-h day with a sleep wake cycle of 9.3-h and 18.7-h, respectively. The black bars indicate the sleep episodes. **b.)** If the protocol lasts enough weeks, the influence of differential sleep pressure levels can be assessed at virtually all circadian phases (Schmidt et al., 2007).

However, the disadvantage is that FD protocol is long and demands large research staff, since subjects have to be kept for weeks in a room that is strictly isolated from the usual external circadian zeitgebers (daily light and temperature variations), but also from culturally and socially related cues (alarm clocks, mealtimes) (Schmidt et al., 2007).

Nap protocol (NP)

Less time-consuming than the FD, the nap protocol allows the partial separation of the homeostatic and circadian processes: a condition in which subjects are continuously sleep-deprived (high sleep pressure) is compared to a condition in which they are allowed to nap regularly, thereby keeping homeostatic sleep pressure at a low level (**Fig.11**) (e.g. in (Cajochen et al., 2001).
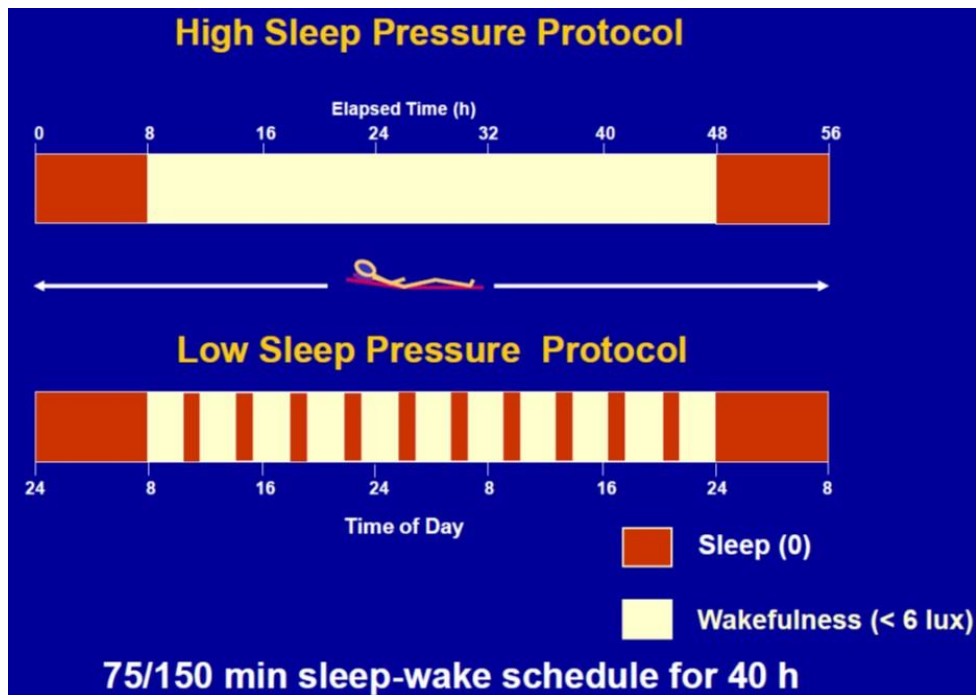
Figure 11: Nap protocol: a sleep deprivation of 40-h under constant conditions (high sleep pressure, upper panel) is compared with a multiple nap condition with short sleep-wake cycle paradigm (75/150 minutes) (low sleep pressure, lower panel) (from the course materials *Biological Rhythms*, University of Surrey; courtesy of Prof. Cajochen).

Besides the main protocols presented above, self-assessment questionnaires are also frequently used to gather further information to better understand the group of participants: e.g. the morningness-eveningness questionnaire (MEQ) (Horne and Östberg, 1976) to assess diurnal preference, and the Munich Chronotype Questionnaire (MCTQ) (Roenneberg et al., 2003) to assess individual phase of entrainment on work and work-free days.

II. Transcranial Magnetic Stimulation (TMS)

The basic principle

The idea that nerves of muscles and brains can be electrically stimulated date back to 1790s, with the pioneering work of Luigi Galvani, Alessandro Volta and Giovanni Aldini (**Fig.12**).

Figure 12: Pioneering work of electrical stimulation of nerves. Giovanni Aldini, 1804, Wellcome Library, London (from Gehirn und Geist, Spektrum, Nr.6/2015).
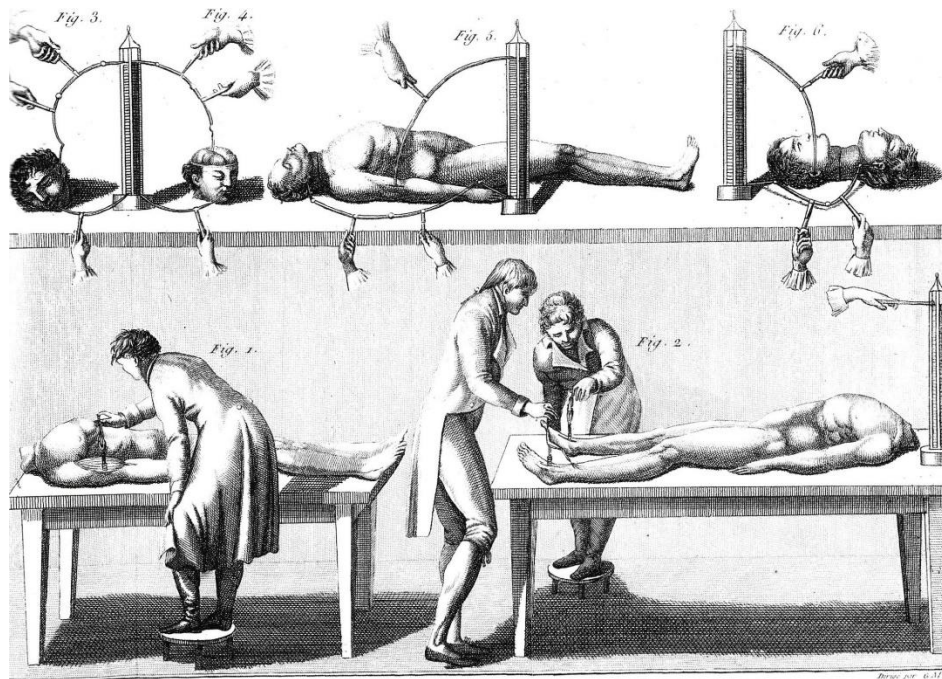
However, injecting electrical currents into the body via electrical stimulation (surface or implanted electrodes, needles) presents some obvious limitations. A new solution to this old problem came in 1985, when Anthony Barker and colleagues in England demonstrated the application of magnetic stimulation on the cortex, by introducing a novel non-invasive technique of neural stimulation – transcranial magnetic stimulation (TMS) (Barker et al., 1985). TMS relies on the principle of electromagnetic induction, which is based on the laws originally described by Faraday in 1831: a time-varying magnetic field will induce an electric field in any volume through which it passes, irrespective of the conductivity of that volume. During TMS, a brief pulse of electric current is passed through a coil placed next to the subject's head, creating a magnetic field fluctuation, which in turn induces electric currents in the brain area below the coil (Barker, 1994). If electrical currents (electrical charges) are of the right intensity, duration, and orientation to nerves of interest (Casarotto et al., 2010), they will stimulate superficial neurons, by causing electrical membrane depolarization and the initiation of an action potential that then propagates (**Fig.13**). Thus, magnetic stimulation is

merely a way of getting electrical current into the tissue to cause nerve depolarization, and the term magnetic stimulation is almost a misnomer, as the stimulation at the neuronal level is actually electrical (Barker and Shields, 2017). The main advantages of TMS is that bypasses sensory pathways and subcortical structures to probe directly and non-invasively the cerebral cortical system. Therefore, TMS does not depend on the integrity of sensory and motor systems to evoke a response, and can access the brain of any participant/patient mimicking normal cortical signal processing. Furthermore, since TMS does not require the subject to be involved in a task, subject's willingness and performance cannot influence the TMS-evoked activations (Rosanova et al., 2012). However, TMS is inducing also non-specific responses in the brain, such as auditory evoked responses (due to the coil click occurring concurrently with discharge of the magnetic stimulator), muscular responses (eye blink reflexes, muscular contractions due to the stimulation). To prevent the occurrence of such events, it is a good practice to inspect for muscle twitch, insert earplugs continuously playing a masking noise, and use a thin layer of foam placed between coil and scalp to attenuate bone conduction (Rosanova et al., 2012).
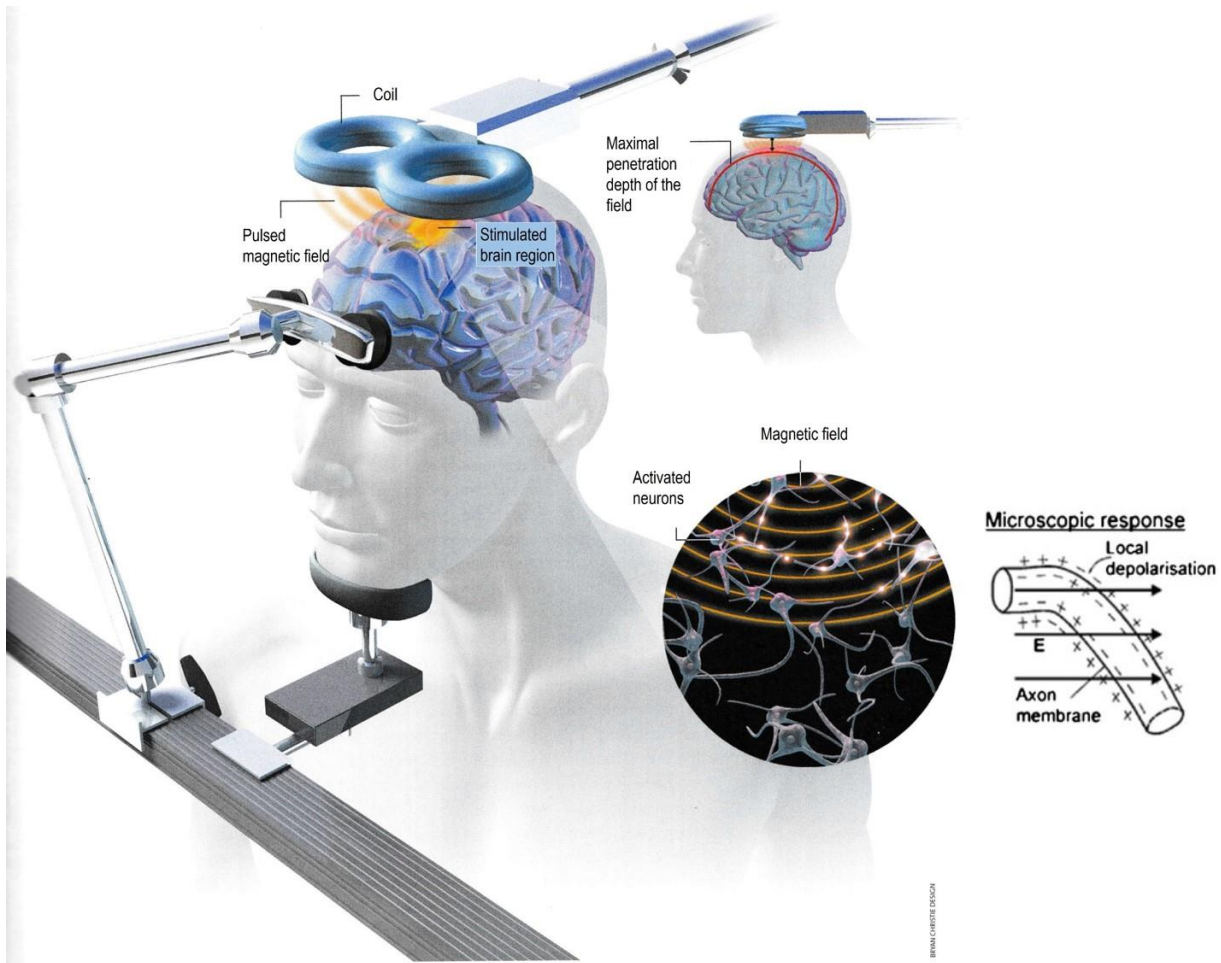
Figure 13: Local stimulation of the surface of the brain cortex by TMS (Gehirn und Geist, Spektrum, Nr.6/2015). Microscopic response: the flow of electrical charges through the axon membrane generates a local depolarisation that can result in an action potential (Mario Rosanova et al., 2012).

A main disadvantage of TMS compared with electrical stimulation is that the site of stimulation is not well defined at the cortical surface. A magnetic field cannot be focused to a point as can light with a lens; instead of precise stimulation, one thinks of areas where stimulation is likely to occur. A figure-of-eight coil, in which two coils are placed beside each other, wired such that the stimulator current rotates in opposite directions in the two coils, allowing more focal stimuli to be delivered to the target area (**Fig.14**). Typically, in a figure-eight-shape coil, the maximum magnetically induced field occurs on a circle of approximately the same mean diameter as the coil, and stimulation can, in principle, occur at any point on this circle (Barker, 1991): that means a circle up to 12 cm^2 (80 up to 100% of the electrical field) on the cortical surface (20 mm below the coil), with the area directly

under the centre of the side-by-side coils experiencing approximately 98% of the maximum stimulating electric field (i.e. focal area of stimulation or hot spot, 0.68 cm2) (Krings et al., 1997). The electric field induced by TMS rapidly decays with distance, and thus the maximal effect of stimulation is limited to the surface of the cortex (Dayan et al., 2013). To note that the size of the cortical patch in which neurons will fire as a direct result of TMS always depends on the exact position of the coil relative to the head, coil characteristics, skull anatomy and stimulation intensity (Komssi and Kähkönen, 2006).
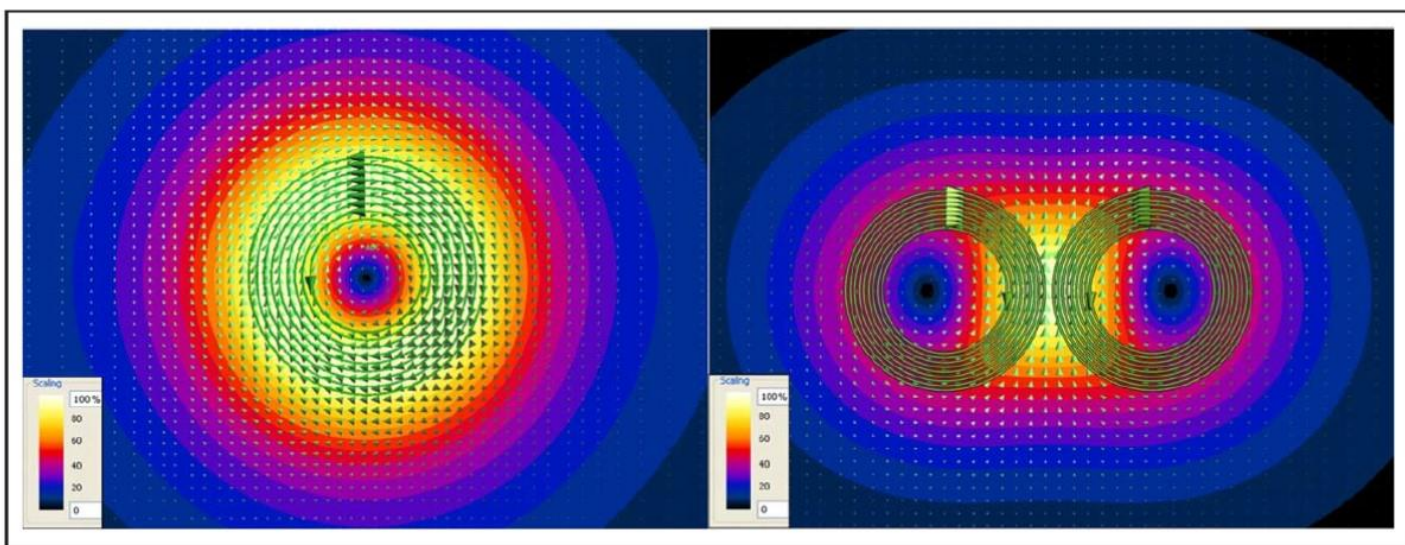
Figure 14: Comparison between the electric field induced by a circular (left) vs a figure-of-eight coil (right); the gradient bar depicts the scaling. Green arrows show the direction of the electric field (Barker and Shields, 2017).

Neurobiological effects

Microscopically, there is limited knowledge about the neurobiological effects of TMS, particularly in humans. The human cerebral cortex is organized in a six-layered structure, with specific cell types and connections in each sheet. TMS depolarises mainly the axons of perpendicular glutamatergic pyramidal neurons (excitatory neurons, estimated to be the main build blocks of the cerebral cortex), and parallel GABAergic interneurons (inhibitory neurons) (Murphy et al., 2016; Rogasch and Fitzgerald, 2013; Siebner et al., 2009). Unlike sensory stimulation, TMS acts simultaneously on a rather large cortical volume containing both inhibitory and excitatory fibres, generating excitatory

and inhibitory post-synaptic potentials (i.e. EPSPs and IPSPs), related to the state of cortical synapses, and generating the EEG signal when summated (Ziemann, 2011). TMS is commonly applied either in single pulses (stimulus intervals randomised), or repetitively (rTMS, applied in low or high frequencies. Low-frequency rTMS (< 1Hz) inhibits the cortical firing of the target brain area, which persist past the period of stimulation, while high-frequency rTMS (1-10 Hz) typically induces facilitation, through mechanisms that possibly modify synaptic efficacy based on long-term potentiation (LTP) and long-term depression (LTD) (Dayan et al., 2013). At the EEG frequency scale, it has been shown –in healthy and awake subjects– that TMS perturbs the ongoing brain activity, likely resulting in a resetting of the intrinsic oscillation (Paus et al., 2001). TMS elicits early γ components (0-20 ms), immediately followed by prominent α band (8-13 Hz) oscillations after occipital stimulation, β band (13-30 Hz) oscillations after parietal stimulation, and fast β/γ oscillations (13-30 Hz; 30-50 Hz) after frontal stimulation, during the first 200 ms post-TMS (Event Related Spectral Perturbation (ERSP), (Rosanova et al., 2009)).

Neuronavigation and compatible EEG system

To target cortical regions with higher accuracy (< 3 mm mismatch), a neuronavigation system based on individual structural MRI image is required (**Fig.15**). The system employs a 3D infrared Tracking Position Sensor Unit to map the positions of TMS coil and subject's head within the reference space of individual structural MRI, by the optimal alignment between MRI fiducials and digitised scalp landmarks (nasion, left and right ear tragus). At the end of each session, a pen visible to the infrared camera can be used to register the EEG electrode positions on the subject's head, allowing to perform off-line, accurate source modelling of the TMS-evoked responses recorded with the EEG. Indeed, electroencephalographic responses measured simultaneously with TMS allow to measure cortical excitability of virtually any cortical area, and map specific patterns of effective connectivity with a millisecond time scale (Ilmoniemi et al., 1997). The Nexstim eXimia TMS-compatible 60-channel

amplifier gates the TMS artefact (induced by the magnetic field fluctuations), and prevents saturation by means of a proprietary sample-and-hold circuit that keeps the output of the amplifier constant between 100 μs before and 2 ms after the stimulus (Virtanen et al., 1999). This device guarantees total absence of TMS-induced magnetic artefacts in most EEG recordings, and artefact-free EEG recordings from 8 ms after stimulus in all cases (N.B.: parameters and mean to remove magnetic artefact may vary depending on the device).
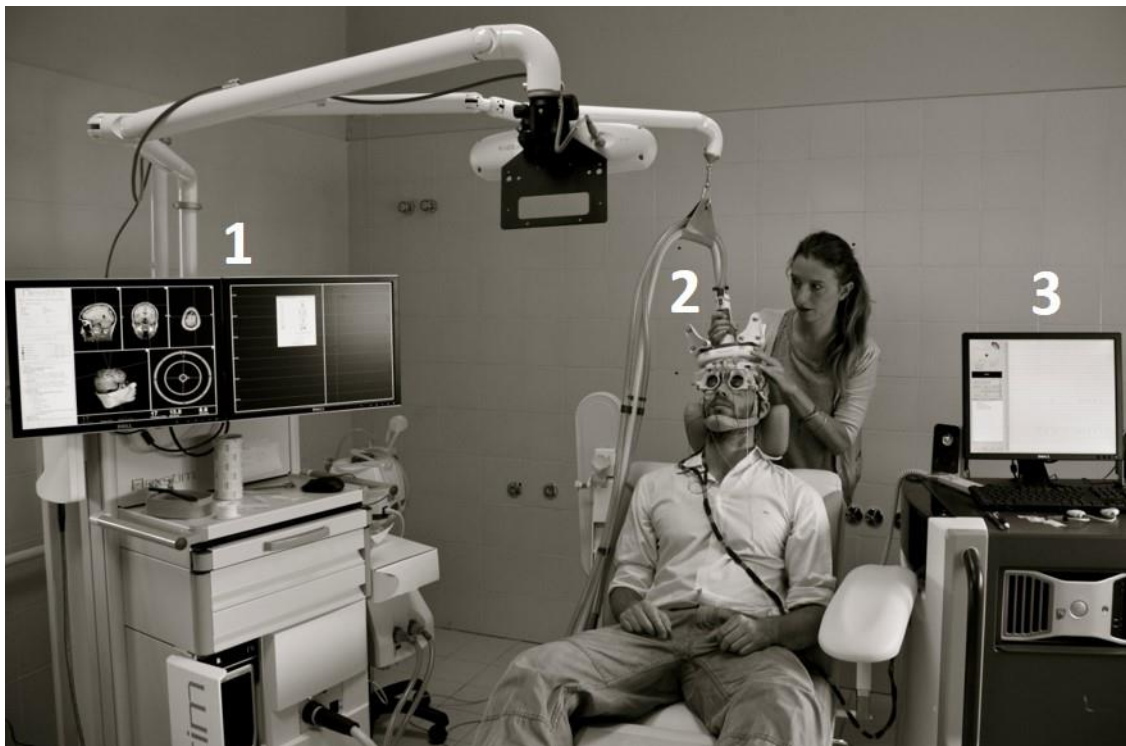
Figure 15: Illustration of the different components. **1)** Neuronavigation: to precisely target the cortical are of interest based on individual structural MRI image. **2)** TMS: magnetic perturbation of the neuronal activity. **3)** compatible 60-channel EEG system: to record TMS evoked potentials over the scalp, with a millisecond time scale. TMS-evoked potentials are quantifiable markers of the cerebral neurophysiological state in behaviourally silent areas, like motor evoked potentials are markers of the state of the motor system, recorded from muscles after TMS over motor cortex (picture courtesy of Dr. Bodart).

III. Measures of cortical responsiveness

The initial part of TMS-evoked potentials (TEPs), recorded immediately from the EEG electrodes underneath the stimulating coil, very likely results from the direct activation of the stimulated brain

area, whereas the later deflections of the TEPs are generated by postsynaptic activity that propagates along cortico-cortical and thalamo-cortical projections for about 300 ms post stimulus (Ziemann, 2011). The effect of TMS on cerebral cortex can be analysed by different measurements, aimed at capturing complementary properties of cortical responsiveness: local and immediate effects, as well as global and longer-term effects. In the studies included in the present thesis, three measurements of cortical responsiveness, based on the "perturb and measure" approach, were adopted and defined as follows (**Fig.16**):

- _Cortical excitability_: local and immediate slope (or amplitude) of the stimulated cortical area (Huber et al., 2013).

- _Cortical scattering_: spread of the initial response as a measure of global effective connectivity from the stimulated area to other cortical regions, within an interval of 300 ms post-TMS (Massimini et al., 2005).

- _Cortical complexity_: global information content of the EEG signal, over 300 ms post-TMS interval (Casali et al., 2013).
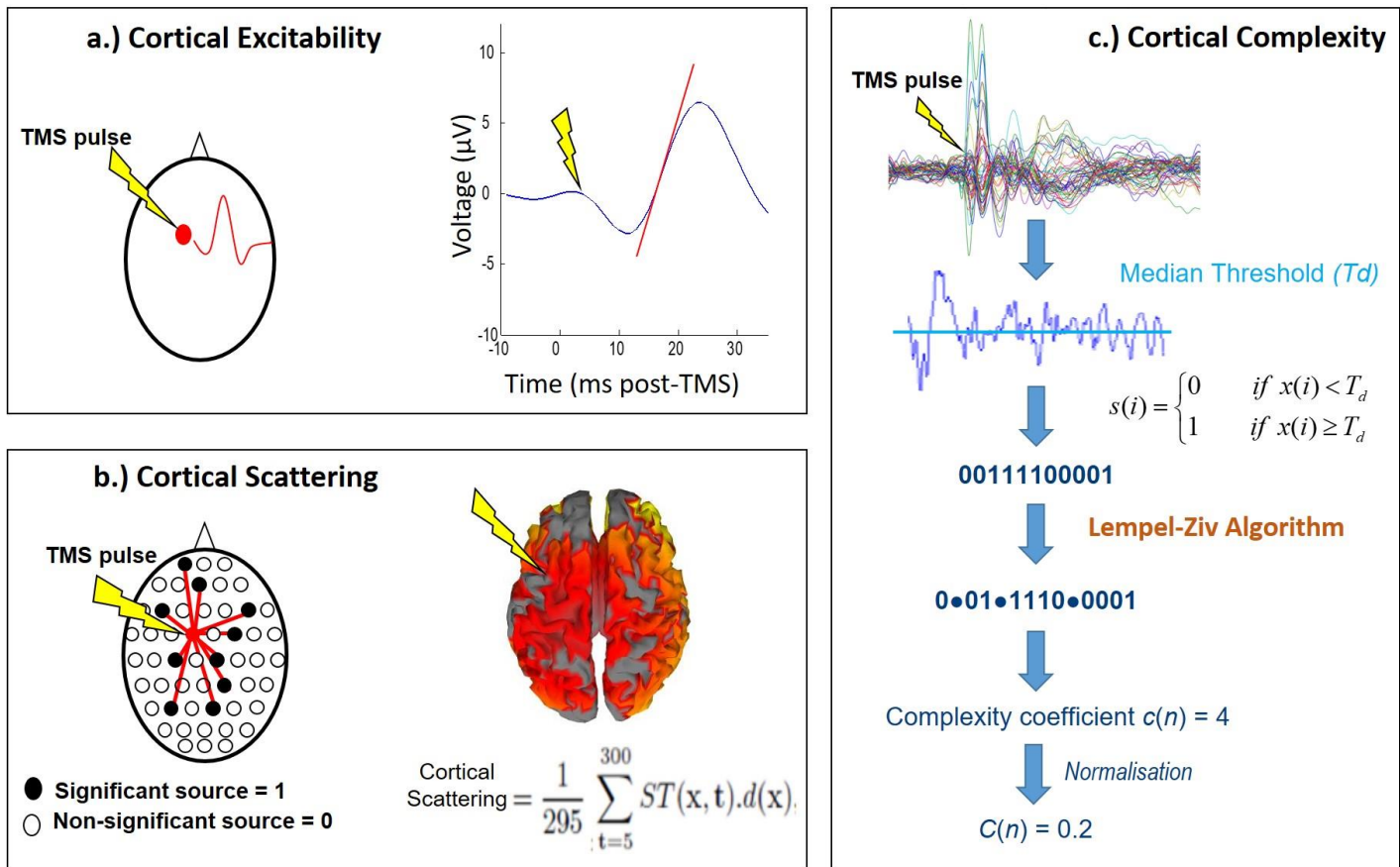
Figure 16: Panel depicting the three measurements of cortical responsiveness used in the present thesis. **a.)** Cortical excitability: slope of the early response of the TMS-evoked potential. **b.)** Cortical scattering: sum, from 5 to 300 ms, of the geodesic distances [d(x)] between the TMS hotspot and all significant sources of the binary spatio-temporal matrix [ST(x,t))], averaged over 295 ms of the 5–300 ms post-TMS period. **c.)** Cortical complexity: for each of the EEG channels of the butterfly response evoked by TMS, the coarse-graining approach first converts the original signal into 0-1 sequence, through comparison of the amplitude values with a given threshold (Td). The median value of the amplitude values will be chosen as Td. Then, the Lempel-Ziv algorithm counts the number of different patterns in the sequence. The final complexity measure is normalised (the complexity panel has been adapted from a slide of Dr. Abásolo).

Cortical excitability

In neurophysiology, the term "excitability" usually refers to the amplitude of the immediate neural response to a perturbation. Cortical excitability is dependent on the intensity of the stimulus (Komssi and Kähkönen, 2006): in these studies, the stimulation intensity was defined as the minimum stimulation intensity eliciting a reliable cortical response of the target cortical area while well-rested, with a negative deflection ~10 ms, a positive deflection at ~20 ms, and with ~7-8 μV of amplitude (Huber et al., 2013). The stimulation intensity was set for each participant during a so-called "pretest"

session, and kept throughout the study. Originally, cortical excitability has been defined as the minimum stimulation intensity eliciting a reliable motor response of the target muscles (Rossini et al., 1994). However, this approach, although practical and reliable, inevitably confines the assessment of excitability to cortico-spinal circuits. Cortical excitability depends on the physiological state of the neurons in the stimulated cortex (state-dependency), as well as on the type of cortex stimulated (morphology-dependency). Cortical excitability possibly reflects the initial trans-synaptic activation of the neuronal population located under the coil, thus reflecting changes in excitability related to the strengthening or weakening of cortical synapses (i.e. cortical plasticity) (Huber et al., 2013).

Cortical scattering

TMS-induced effective connectivity is the effect of the activation of a neuronal group on another set of neurons (Friston, 2011), i.e. the ability of a set of neuronal elements to causally affect the firing of other anatomically connected, lasting for around 300 ms post-stimulus (Virtanen et al., 1999). It has been shown that cortical areas ipsilateral to the stimulation site are responding first, while contralateral areas are responding with longer latencies (Ilmoniemi et al., 1997). This cortico-cortical pattern contributes to elucidate the functioning of cortical circuits, i.e. the ability of the brain to integrate information. Effective connectivity differs from functional connectivity, a pragmatic concept that simply refers to any type of correlation between time series of brain activity. In order to study non-invasively cortical effective connectivity, TMS coupled to EEG and combined with source reconstruction provides the appropriate spatial and temporal resolution. Multiple steps are required in source reconstruction in order to estimate the electrical activity of current dipoles (equivalent to neuronal sources) from the scalp potential measured by sensors (Litvak et al., 2011). First the head model is built based on: (*i*) individual structural scan, (*ii*) coregistration, linking the coordinate system of sensor positions to the coordinate system of structural MRI image, through at least three fiducials (typically nasion, and both pre-auricular points, i.e. *Rosetta stone*). Then, the forward model is

computed –based on Maxwell's equations and theoretical assumptions about the conductive properties of the head (e.g. isotropic conductivity and concentric compartments in the "Boundary Element Method", BEM) (**Fig.17a**)– by calculating for each of the dipoles on the cortical mesh the effect it would have on the sensors. The result is a NxM matrix, where N is the number of sensors and M is the number of sources considered, thus the matrix relates the dipole currents to the sensor measurements. Each column in this matrix is a so called "lead field", corresponding to one cortical source. The inverse problem is ill-defined, since there is no unique solution when one tries to estimate the sources of the signal measured at the scalp level. With a linear forward model, as described by a set of lead fields, the number M of cortical current sources far exceeds the number N of EEG sensors. Therefore, the inverse problem has to be constrained (e.g. using "multiple sparse priors", MSP (Friston et al., 2008)) to reach a unique estimation of the sources location from the activity detected by the sensors. Once the sources have been estimated, the cortical scattering can be calculated as the sum of the geodesic distance between the sources and the TMS hotspot (**Fig.17b**) (Casali et al., 2010).
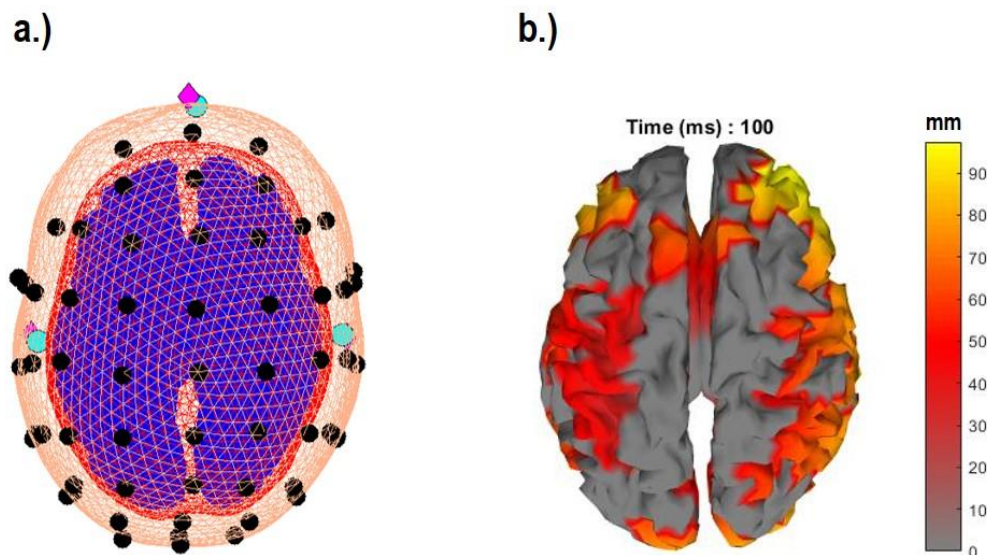
Figure 17: a.) Head model (based on BEM): cortical mesh (blue), sensors (black), tissue (brain, skull, scalp) compartment interfaces (orange) and fiducials (light blue and magenta) depicted. **b.)** Visualisation of the results of inverse reconstruction (based on MSP approach): cortical sources that give rise to the electrical activity measured with EEG outside the head at 100 ms. Colour code corresponds to the distance between TMS hotspot (in this case superior frontal gyrus) and source.

Cortical complexity

Linear approaches are normally used to analyse the EEG, e.g. the classical spectral analysis (Fast Fourier Transform, FFT), resulting in the frequency content of the signal. In a linear-system, there is a linear relation between causes and effects (small causes have small effects), whereas in a non-linear system, small causes may have large effects. Realistic biological systems, such as the neural dynamics of the brain, are likely to be non-linear, non-stationary, and noisy (Stam, 2005). Although several improvements have made possible to use linear approaches in non-linear physiological phenomena, complementary non-linear approaches have been also applied recently to mine deeper into the nature of biosignal analysis, including the TMS-EEG signal (Casali et al., 2013). Lempel–Ziv Complexity (LZC) is a complexity estimator introduced by Lempel and Ziv to evaluate the randomness of finite time series (Lempel and Ziv, 1976). LZC is a non-linear, simple-to-calculate, coarse-graining measure that provides the rate of appearance of new patterns in time series (Lempel and Ziv, 1976). Thus, this measure approximates the amount of non-redundant information contained in a string by estimating the minimal size of the "vocabulary" necessary to describe the string. In the EEG analysis, LZC measures the capacity of information in the signal fragment, and then reflects the underlying activeness of the neurons (i.e. state dependent) (Hu and Zhang, 2019). Before calculating LZC, the signal must be transformed into a symbolic time series, normally a binary (0–1) sequence, although in few studies, the signal has been transformed into a three symbols sequence (Abásolo et al., 2006). However, previous studies have shown that 0–1 conversion is adequate to estimate the LZ complexity in biomedical signals (Aboy et al., 2006). Usually, the median is used as the threshold for the conversion of the raw time series, because of its robustness to outliers. In order to compute LZ complexity, the binary sequence is scanned from left to right and the complexity counter [$c(n)$] is increased by one-unit every time a new subsequence of consecutive characters is encountered. To obtain a complexity measure that is independent of the sequence length, it must be normalized [$C(n)$],

resulting in a scalar metric ranging between 0 and 1: the lower limit shows a stationary signal with no varying dynamics, while the upper limit shows a very complex signal with multiple complex dynamics (Aboy et al., 2006). The advantage of the method is the simplicity (does not require any inputs selection such as complexity measure based on permutation), the robustness to noise, the computational efficiency, and that it can be calculated even for short data segments (i.e. milliseconds range) (Zhang et al., 1999). The disadvantage is that LZC mostly reflects changes in the spectral composition, i.e. it is sensitive to signal amplitudes, whereas complexity measured with permutation approaches considers the order relation within the values, but not the absolute amplitude values (Tosun et al., 2019). However, studies have suggested that LZC captures the change of patterns within a time series, which does not merely correspond to changes in the spectral content of the EEG, providing additional information that cannot be captured with conventional linear analysis methods (Stam, 2005).

Results

The main objective of this thesis work was to investigate cortical response dynamics during prolonged wakefulness in young (18-30 y) and older (50-70 y) participants. The protocol included an overnight sleep deprivation (28 hrs and 34 hrs), to track the joint effect of the sleep homeostasis and the circadian alerting signals. In order to assess non-invasively but directly cortical response, we employed TMS coupled with high-density EEG. Simultaneously to TMS response recording, participants performed a vigilance task. Furthermore, in between TMS sessions, participants completed cognitive test batteries, including basic sustained attention and higher-order executive tasks. Adding a cognitive dimension allowed to explore statistical correlations between cortical response state and behaviour performance in humans. Spontaneous wake EEG recordings preceded each TMS session to gain insights into the frequency content of the EEG without ongoing TMS perturbation. Subjective sleepiness tests were performed hourly. All data were reported with respect to individual's internal circadian clock (expressed in degrees, with 0° being the DLMO; 15° = 1h), instead of the external clock time.

Overall, my Ph.D. project aimed at investigating three different measures of the cortical state to gain an integrative understanding of the underlying mechanisms that likely sustain cognitive functions during prolonged wakefulness (*phase I*), and their evolution across ageing (*phase II*). In the next pages, four research articles are presented. At the beginning of each article, I briefly mention the main research hypothesis supporting the rationale of the study and the main scientific contribution that can be drawn.

<u>**Phase I, Paper 1: Circadian regulation of human cortical excitability**</u>

Ly JQM[*], Gaggioni G[*], Chellappa SL[*], Papachilleos S, Brzozowski A, Borsu C, Rosanova M, Sarasso S, Middleton B, Luxen A, Archer SN, Phillips C, Dijk DJ, Maquet P, Massimini M, Vandewalle G. *Nature Communications 2016 – * shared first authorship*

In 2013, Huber et al. reported that cortical excitability, a candidate marker of synaptic strength, increases linearly throughout a sleep deprivation and decreases after a night of sleep (Huber et al., 2013). In the discussion section of the paper, authors acknowledged a possible contribution of the circadian system in modulating the profile of cortical excitability, which however would have required a forced desynchrony protocol to come to light. Thus, the main objective of this paper is to demonstrate that the dynamic of cortical excitability over 24 hrs shows a circadian regulation, besides a sleep homeostasis effect, in a group of healthy young participants (18-30 y). In order to achieve that, a constant routine protocol is adopted. As already stated, our hypothesis is that cortical excitability will decrease during the wake maintenance zone, i.e. in the early evening, because of the strongest effect of the circadian alerting signal. Cortical excitability will then increase during the biological night, when high sleep pressure is no longer counteracted by the circadian alerting signal, which turns into a sleep promoting signal instead. Finally, cortical excitability will decrease the following circadian morning, when the circadian signal promotes again wakefulness and opposes to the high sleep pressure. Since vigilance impairments are typically observed during sleep deprivation and circadian misalignment, we hypothesise that higher level of cortical excitability will be correlated with worse vigilance performance. The results indicate indeed that cortical excitability is under the synergetic influence of sleep homeostasis and circadian rhythmicity. Regarding behaviour, higher cortical excitability is associated with worse vigilance performance, and that independently of the circadian phase.

ARTICLE

Circadian regulation of human cortical excitability

Julien Q.M. Ly[1,2,3,*], Giulia Gaggioni[1,2,*], Sarah L. Chellappa[1,2,*], Soterios Papachilleos[1,2], Alexandre Brzozowski[1,2], Chloé Borsu[1,2], Mario Rosanova[4,5], Simone Sarasso[4], Benita Middleton[6], André Luxen[1,2], Simon N. Archer[6], Christophe Phillips[1,2], Derk-Jan Dijk[6], Pierre Maquet[1,2,3], Marcello Massimini[4] & Gilles Vandewalle[1,2]

Prolonged wakefulness alters cortical excitability, which is essential for proper brain function and cognition. However, besides prior wakefulness, brain function and cognition are also affected by circadian rhythmicity. Whether the regulation of cognition involves a circadian impact on cortical excitability is unknown. Here, we assessed cortical excitability from scalp electroencephalography (EEG) responses to transcranial magnetic stimulation in 22 participants during 29 h of wakefulness under constant conditions. Data reveal robust circadian dynamics of cortical excitability that are strongest in those individuals with highest endocrine markers of circadian amplitude. In addition, the time course of cortical excitability correlates with changes in EEG synchronization and cognitive performance. These results demonstrate that the crucial factor for cortical excitability, and basic brain function in general, is the balance between circadian rhythmicity and sleep need, rather than sleep homoeostasis alone. These findings have implications for clinical applications such as non-invasive brain stimulation in neurorehabilitation.

[1] GIGA-Research, Cyclotron Research Centre–In Vivo Imaging Unit, University of Liège, 8 allée du 6 août, B30, B-4000 Liège, Belgium. [2] Walloon excellence in life sciences and biotechnology (WELBIO), Avenue de l'Hôpital, 1B 4000 Liège, Belgium. [3] Department of Neurology, Centre Hospitalier Universitaire de Liège, Domaine Universitaire du Sart Tilman, Bâtiment B 35, B-4000 Liège, Belgium. [4] Department of Biomedical and Clinical Sciences "L.Sacco", Universit degli Studi di Milano, via G. B. Grassi 74, 20157 Milano, Italy. [5] Fondazione Europea Di Ricerca Biomedica, Ferb Onlus, 16, V. Stresa, 20125 Milano, Italy. [6] Surrey Sleep Research Centre, University of Surrey, Egerton Road, Guildford GU2 7XP, UK. * These authors contributed equally to this work. Correspondence and requests for materials should be addressed to G.V. (email: gilles.vandewalle@ulg.ac.be).

Wakefulness is associated with molecular, cellular and systemic changes in human brain function, which are deemed to negatively impact on cognition[1,2]. Deterioration of performance is, however, not a simple linear function of prior wakefulness duration. During the first ∼16 h of a normal waking day, human cognitive performance remains stable despite the concurrent build-up of sleep homoeostatic pressure. However, if wakefulness is extended into the biological night, cognitive performance deteriorates abruptly[3,4]. This reflects the influence of the circadian timing system, which counters the detrimental effect of sustained wakefulness during the day, until the end of the so-called evening 'wake-maintenance zone' (WMZ)[5,6]. Subsequently, at night, the circadian system switches to a sleep promoting signal which favours sleep continuity, and opposes the progressive tendency to wake-up due to sleep pressure dissipation during sleep, up to the end of the early morning 'sleep-promoting zone' (SPZ)[5]. Behavioural, neural and molecular correlates of the impact of the circadian timing system are being established[7,8]. However, its neuronal bases remain elusive[9].

Cortical excitability, here defined as the strength of the response of cortical neurons to a given stimulation, reflects neuron reactivity and response specificity and is therefore a fundamental aspect of human brain function. It has been reported to increase with time awake in humans[10]. This may underlie performance decrements and greater seizure[11] or hallucination[12] propensity with sleep deprivation. Changes in human cortical excitability have been related to rodent data showing a linear increase with time awake in the firing rate and synchronization of cortical neurons[13] and in the amplitude and slope of the local field potential evoked by electrical cortical stimulation[14].

Synaptic function and structure have however also been reported to undergo marked circadian dependency[9,15–17]. Circadian variations in neuronal excitability have in fact been clearly established in invertebrates[18]. In humans, TMS-inferred corticospinal excitability (that is, TMS-evoked motor responses) was reported to depend on chronotype[19] and to undergo a time-of-day influence, which appeared independent of sleep[20]. Sleep deprivation has been reported to have no effect[21] or to decrease[22] human corticospinal excitability, while it increased somato-sensory cortex excitability[23]. It is therefore controversial, or it has at least not been conclusively established, whether, cortical excitability, similar to other aspects of human brain function, is modulated by both elapsed time awake and circadian phase.

Here we addressed this issue and investigated whether the circadian timing system impacts on human cortical excitability. We further investigated whether this potential circadian modulations of cortical excitability would correlate with the established circadian fluctuations in cortical synchrony across neuronal populations[14,24] and behaviour[1]. We used transcranial magnetic stimulation coupled to high-density electroencephalography (TMS/EEG), as a non-invasive tool to gauge, in vivo, the time course of human cortical excitability during prolonged wakefulness. We hypothesized a circadian influence on cortical excitability to be most evident near the WMZ and SPZ and that individual variability in circadian signal strength as derived from endocrine markers (cortisol) to be related to the dynamics of cortical excitability. We further postulated cortical excitability to be associated with spontaneous waking EEG measures and performance assessments. Results confirm these hypotheses and reveal a robust circadian modulation of cortical excitability which correlates with changes in EEG synchronization and cognitive performance. The findings demonstrate that the balance between circadian rhythmicity and sleep need, rather than sleep homoeostasis alone, is crucial for cortical excitability regulation, and basic brain function in general.

Results

Following an 8-h nocturnal baseline sleep episode quantified by polysomnography, 22 healthy young men (22 years old ± 2.61; Supplementary Table 1 for participants characteristics), underwent eight TMS/EEG recordings during ∼29-h of continuous wakefulness. This sleep deprivation was conducted under strictly controlled behavioural and environmental conditions (constant routine protocol) to minimize external and internal factors masking circadian rhythmicity[25] (Fig. 1). The frontal cortex supplementary motor area was chosen as stimulation target because it is highly sensitive to sleep deprivation[26], as previously investigated using TMS/EEG[10]. TMS sessions were scheduled to adequately detect any predicted changes near the putative WMZ and SPZ. During TMS/EEG recordings, participants performed a simple visual vigilance task to assess performance as well as to exclude TMS/EEG segments during vigilance lapses from the analyses[10]. For the analyses all data were aligned to circadian phase as determined from individual melatonin profiles[27]. Participants were not provided with any information about time of day or the frequency and timing of assessments during the entire protocol to prevent any bias related to expectations on how one's brain state should change in relation to these variables (for example, it is 23:00, I must be sleepy).

Each TMS/EEG acquisition was preceded by a 2-min eyes open spontaneous waking EEG recording to extract theta frequency
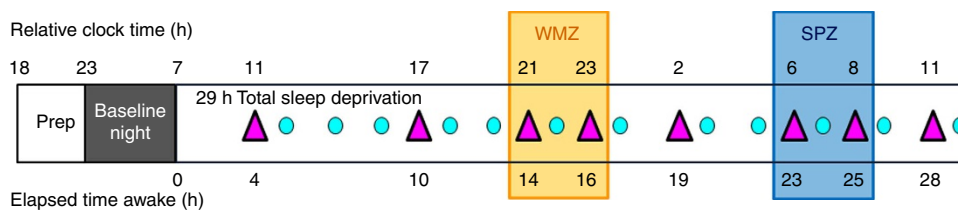

Figure 1 | Experimental protocol. Participants underwent a 29 h sustained wakefulness protocol under constant routine conditions (no time-of-day information, constant dim light (<5 lux), external temperature and semi-recumbent posture, regular liquid isocaloric intake, sound proofed rooms). TMS-evoked EEG potential (TEP) were recorded eight times (>250 trials per session; violet triangles ▲) and test batteries including the psychomotor vigilance task (PVT; turquoise circle ◯) were completed 12 times. TMS/EEG sessions were scheduled throughout the 29-h period with higher frequency around the wake-maintenance (WMZ) and sleep-promoting zones (SPZ), the timing of which was predicted based on habitual sleep times (data realigned a posteriori). During TMS/EEG sessions, participants performed a visual vigilance task consisting in maintaining a constantly moving cursor in the centre of a computer screen to assess simultaneous performance and exclude vigilance lapses. Saliva samples were collected hourly for melatonin and cortisol assays, together with subjective sleepiness and affect measures. Relative clock time displayed is for a participant with a 23:00–07:00 sleep–wake schedule. Prep: 5 preparatory hours, including test battery task practice (<5 lux). Baseline night: 8 h night of sleep in darkness at habitual sleep times and under EEG recording.

band power (4.5–7.5 Hz), an established marker of alertness and sleep need[24]. In between TMS/EEG sessions, participants also completed an auditory psychomotor vigilance task (PVT)[28], used to monitor sustained attention. Subjective sleepiness and affect dimensions were assessed hourly. All these classical alertness-related measures exhibited typical and statistically significant variations during the protocol, with relatively stable values during the normal waking day period, followed by decrements during the biological night and subsequent partial recovery during the next day[3] (PROC MIXED; $n = 22$; $P < 0.002$) (Supplementary Fig. 1).

Non-linear cortical excitability change with wakefulness. Cortical excitability was inferred from the amplitude and slope of the first component of the TMS-evoked EEG potential (TEP; 0–30 ms post-TMS)[10], measured at the electrode closest to the maximally stimulated brain location (hotspot). Both TEP amplitude and slope significantly changed with time awake (PROC MIXED; $n = 22$; $P < 0.0001$) (Fig. 2; see Supplementary

Fig. 2 for non-standardized values). *Post hoc* analysis showed that cortical excitability increased globally from the first to the last session of the protocol ($n = 22$; amplitude: $P_{corr} = 0.025$; slope: $P_{corr} = 0.064$). [All *post hoc* analyses for PROC MIXED were performed with Kenward-Roger's multiple comparison correction]. However, the dynamics of this increase was not linear. A marked significant local decrease was observed from the afternoon session (S2) to the evening session (S3), close to the onset of melatonin secretion, in the WMZ (amplitude: $P_{corr} = 0.037$; slope: $P_{corr} = 0.058$). Both amplitude and slope then significantly increased up to the seventh session (S7) around the maximum of cortisol secretion (Fig. 3d), at the end of the putative early morning SPZ ($n = 22$; $P_{corr} < 0.0001$). This sharp increase appeared to subsequently cease 3 h later, in the last session (S8) of the protocol, which was no longer significantly different from the previous one ($n = 22$; $P_{corr} > 0.8$).

Importantly, changes in estimated cortical excitability followed a similar pattern when inferring amplitude and slope of the TEP first component from a dipole computed at the hotspot, following EEG source reconstruction, that is, based on separate analyzes using signals from all available EEG electrodes (Supplementary Fig. 3).

These results confirm that human cortical excitability varies with extended wakefulness[10], but reveal local non-linear variations compatible with a strong influence of the circadian timing system, in addition to a linear trend likely related to sleep homoeostasis.

Cortical excitability correlates with circadian/sleep need markers. To further investigate this dual influence, we compared the predictive value of two different models to explain the observed time course of cortical excitability. The first fit consisted of a linear function representing the progressive build-up of sleep pressure[29]. The second fit comprised a 24 h period sine-wave function, centred on individual melatonin secretion onset, aimed at modelling the circadian signal[30] (Fig. 3a). Both fits turned out to be good predictors of observed data, as indexed by low error indices.

In a next step, we related cortical excitability to independent individual standard measures of sleep homoeostasis and circadian rhythmicity. We first associated cortical excitability to a
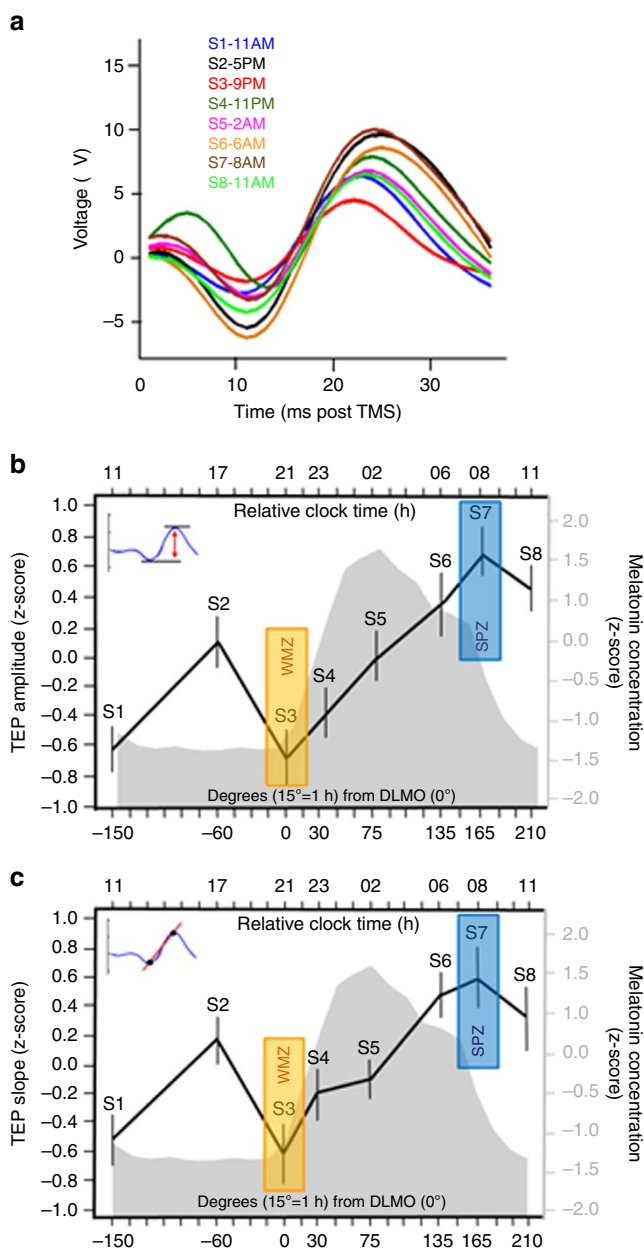

Figure 2 | Non-linear changes in cortical excitability with wakefulness extension. (**a**) TMS-evoked potentials (TEP; 0–30 ms post TMS) measured at the electrode closest to the hotspot, averaged in each of the eight TMS/EEG sessions, in a representative participant (habitual sleep time: 23:00–07:00). Hotspot location was provided by the neuronavigation system. Time course of TEP amplitude (**b**) and slope (**c**) with respect to the circadian cycle. Data were averaged (mean ± s.d.) after standardization (z-score) and realignment to individual circadian phase ($n = 22$; melatonin secretion onset = 0°). Mean z-scored melatonin profile is displayed in grey with respect to circadian phase (bottom X axis). The top x axis indicates relative clock time for a participant with a 23:00–07:00 sleep–wake schedule. Both TEP amplitude and slope significantly changed across the 29 h of sustained wakefulness (PROC MIXED; $n = 22$; main effect of circadian phase: amplitude $F_{(7,128)} = 8.17$, $P < 0.0001$; slope: $F_{(7,/129)} = 5.91$, $P < 0.0001$). *Post hoc* analysis revealed (1) a significant increase from the first to the last session ($n = 22$; S1 versus S8: amplitude: $P_{corr} = 0.0025$; slope: $P_{corr} = 0.0635$], (2) a local decrease from the second afternoon session (S2) to the third evening session (S3) in the hypothetical WMZ ($n = 22$; S2 versus S3: amplitude: $P_{corr} = 0.037$; slope: $P_{corr} = 0.058$), (3) a sharp increase during the biological night ($n = 22$; S3 versus S7: amplitude and slope: $P_{corr} < 0.0001$), (4) ceasing after the seventh session, at the end of the theoretical SPZ ($n = 22$; S7 versus S8: amplitude and slope: $P_{corr} > 0.8$).
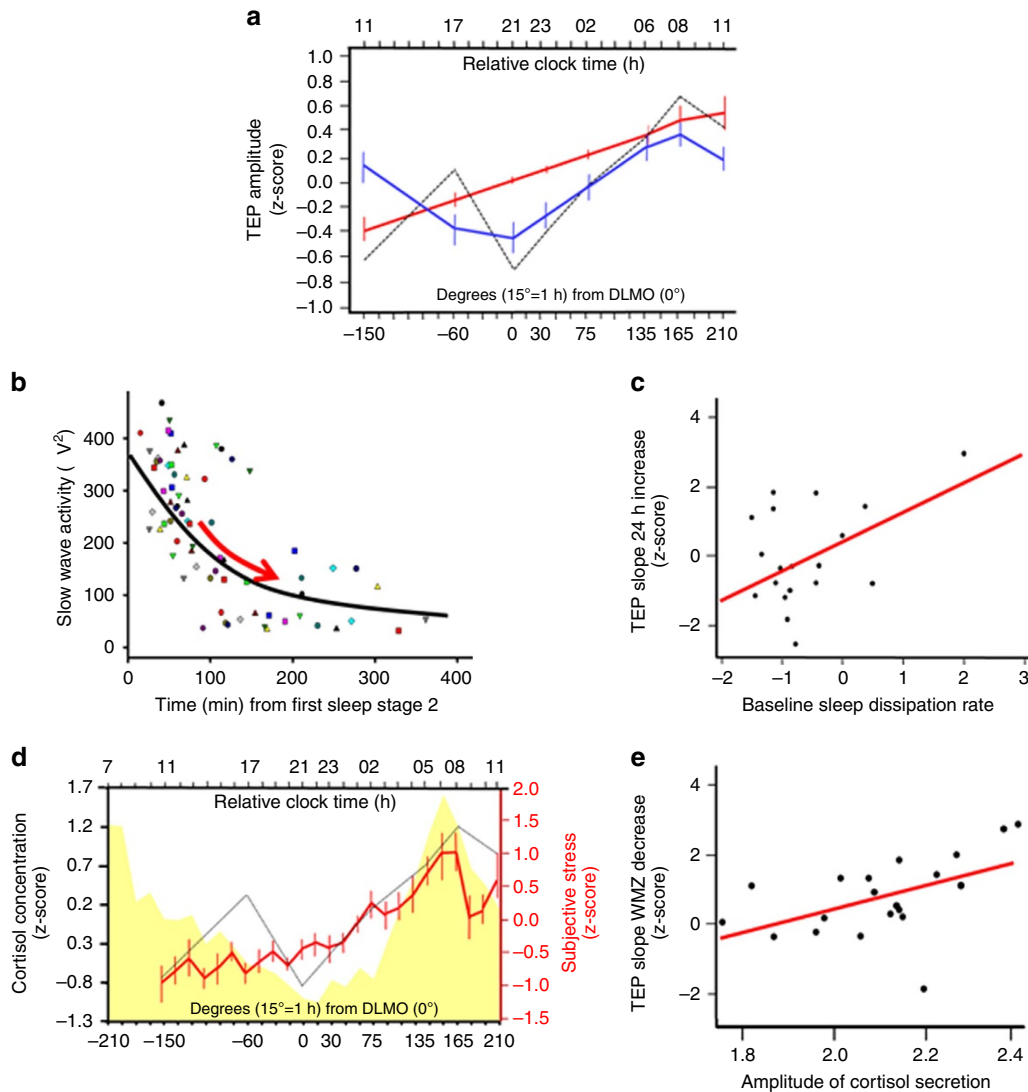
Figure 3 | The circadian system modulates cortical excitability. (**a**) Individual cortical excitability measured by TEP amplitude (dashed line represents average z-scored TEP amplitude) was fitted with linear (**red**) and 24 h period sine-wave (**blue**) functions to mimic sleep pressure build-up and the circadian signal respectively. Error sum of squares (ESS) was <10 for both indices (amplitude: ESS linear fit = 4.9, $P < 0.0001$; ESS sine fit = 4.1, $P < 0.0001$; slope: ESS linear fit = 5.19, $P < 0.0001$; ESS sine fit = 4.24, $P < 0.0001$). (**b**) Slow wave activity across the first four cycles of sleep baseline night was fitted to compute individual dissipation rate (schematically shown by **red arrow**). Each dot represents SWA of an individual sleep cycle (four identical symbols per participant). (**c**) Regression analysis showed that individual dissipation rate was positively correlated with the increase in cortical excitability from first to last session, recorded 24 h apart, at the same circadian phase, following a normal night of sleep and after sleep deprivation ($n = 18$; amplitude: $P = 0.044$; $r^2 = .23$; slope: $P = 0.036$, $r^2 = 0.25$). (**d**) Cortisol (**yellow**) and subjective stress (**red**) levels. Salivary cortisol concentration was not significantly different between the first and the last protocol samples, collected 24 h apart, at the same circadian phase, following a normal night of sleep and after sleep deprivation ($n = 22$; $F_{(28,482)} = 13.44$; $P < 0.0001$). Dashed line: shape of TEP z-scored amplitude dynamics. (**e**) Regression analysis revealed that individual fitted amplitude of cortisol secretion over the protocol was positively associated with the decrease in cortical excitability measured around the wake-maintenance zone ($n = 20$; amplitude: $P = 0.017$; $r^2 = 0.24$; slope: $P = 0.023$, $r^2 = 0.21$).

well-established marker of sleep pressure: NREM sleep slow wave activity (SWA)[2]. Individual dissipation rate of SWA reflects individual sleep homoeostasis efficacy in eliminating sleep pressure[31]. In our protocol, The first and last session were recorded 24 h apart at the same circadian phase (11:00 for an individual waking up at 07:00), such that their comparison should exclusively reflect the impact of time awake, that is, the build-up of sleep pressure. Regression analysis showed that SWA dissipation rate during the baseline night before sleep deprivation was positively associated with the build-up in cortical excitability in this interval (PROC REG; $n = 18$; $r^2 > 0.22$; $P < 0.037$) (Fig. 3b,c).

Cortisol rhythm is characterized by declining values during the biological day, with a nadir near the WMZ, and rising values during the biological night with a peak at habitual wake time[32]. This is in contrast to the melatonin rhythm which is characterized by an on–off time course with very low levels during the day and high values during the night. We therefore evaluated a possible link between cortisol and cortical excitability dynamics. We found that cortisol levels covaried positively with increased TEP amplitude and slope over the entire protocol (Fig. 3d; analysis of covariance (ANCOVA); $n = 22$; $r^2 > 0.24$, $P < 0.0001$). As it has been hypothesized that the amplitude of the cortisol rhythm may reflect the strength of a circadian signal[27], we then

investigated whether the amplitude of the cortisol rhythm is related to the non-linear change in cortical excitability. Regression analysis revealed a significant positive association between individual estimates of cortisol amplitude during the protocol and the decrease in cortical excitability from the afternoon session to the evening WMZ session (PROC REG; $n = 20$; $r^2 > 0.21$; $P < 0.023$) (Fig. 3e).

Collectively, these findings speak to a critical role for sleep homeostasis on the dynamics of cortical excitability but they also indicate a relationship with the variation of a classical 'hand-of-the clock' endocrine marker which putatively reflects individual circadian strength.

Cortical excitability correlates with theta power and behaviour. Finally, we investigated whether the dynamics in cortical excitability, which arguably reflect a circadian influence, could constitute the neuronal bases for variations in individual brain system-level and behavioural measures, for which a circadian influence is widely accepted[1,3]. We found that TEP amplitude and slope significantly covaried with theta power over the frontal region across the 29 h of sustained wakefulness, with high cortical excitability associated with high theta power (ANCOVA; $n = 22$; amplitude: $r^2 = 0.69$, $P < 0.0001$; slope: $r^2 = 0.69$, $P < 0.0001$) (Fig. 4a–c). This association was specific to theta power and was not observed for delta (0.75–4 Hz; ANCOVA; $n = 22$; $r^2 \leq 0.05$; $P \geq 0.95$), alpha (8–12 Hz; ANCOVA; $n = 22$; $r^2 \leq 0.07$; $P \geq 0.77$), sigma (12.5–18 Hz; ANCOVA; $n = 22$; $r^2 \leq 0.13$; $P \geq 0.13$) and beta powers (18.5–30 Hz; ANCOVA; $n = 22$; $r^2 = 0.08$; $P \geq 0.66$) (Supplementary Fig. 4).

We then focused on the vigilance task which was performed simultaneously with the TMS/EEG recordings. Task performance showed non-linear changes across the protocol (PROC MIXED; $n = 22$; main effect of circadian phase: $F_{(7,122)} = 13.78$; $P < 0.0001$) and was significantly linked to cortical excitability dynamics such that higher indices of cortical excitability associated with worst performance (ANCOVA; $n = 22$; $r^2 = 0.23$, $P < 0.03$) (Fig. 4d,e). Dynamics of cortical excitability also appeared to translate to the dynamics of subjective feelings. A last set of analyses showed that increases in subjective sleepiness (Fig. 4a,b) and negative affect (anxiety, stress and fatigue) and reductions in positive affect (mood, motivation and sociability) were related to increases in TEP amplitude and slope (ANCOVA; $n = 22$; $r^2 > 0.4$, $P < 0.0001$). Altogether, these findings point towards a direct relationship between cortical excitability profiles and brain system-level or behavioural measure dynamics.

Discussion

Our study confirms that cortical excitability, defined as the electrical reactivity of cortical neurons to a direct perturbation (TMS in the present case), is affected by the duration of wakefulness[2,9,10], but it also demonstrates that cortical excitability is significantly modulated by circadian phase. An exclusive dependency on wakefulness duration would have led to a monotonic increase in cortical excitability with time awake. Our data show, however, that the initial increase in cortical excitability during a normal waking day returns to baseline value around the evening WMZ. In the context of our protocol, this evening excitability reduction can only be explained through an endogenous circadian influence independent of sleep, because the participants did not nap, had no direct knowledge of clock time and all environmental and behavioural conditions were kept constant. Reduction of cortical excitability would therefore represent a previously unappreciated marker of the circadian mechanisms by which performance is maintained at the end of a normal waking day, when sleep pressure is high.

Our results provide indeed a strong link between cortical reactivity, system-level measures of brain function (spontaneous waking EEG theta power) and behaviour (vigilance task, subjective feelings). Hence, the well-recognized non-linear variation in cognitive performance and subjective feeling during extended wakefulness[3] appears to be related to basic aspect of neuronal function, that is, cortical excitability. During the biological night, cortical excitability exhibited a marked increase which coincided with decrements in performance, subjective feelings and objective EEG measure of alertness. Our data also suggest that the typical recovery observed in the morning of the second day of sustained wakefulness, as indexed by spontaneous waking EEG and behavioural measures, is mirrored by a decrease or at least a stabilization of cortical excitability. Further support for this statement would, however, require the demonstration of a significant reduction in cortical excitability following more extreme sleep deprivation.

Altogether, these findings strongly suggest that sleep is not the only process that regulates and restores neuronal function, as previously pointed out[9]. It has been suggested that mammals with weak circadian rhythms (for example, endotherm versus ectotherm) do not show evident circadian variations in synaptic function over the sleep–wake cycle[18]. This could explain in part why most previous studies have associated synaptic changes mostly with the sleep–wake rather than the circadian cycle[18]. Here we show that when vigilance state is kept constant, that is, participants remain awake in a constant routine protocol, circadian variations in neuronal and synaptic function become evident also in humans. The full separation and quantification of sleep homoeostasis and circadian influence is not possible using a constant routine protocol, during which wakefulness extension is always accompanied by concomitant changes in sleep pressure and circadian phase, and would require a forced desynchrony paradigm[5]. Our data show nevertheless that variations in cortical excitability are most obvious in individuals with strongest variations in spontaneous EEG activity, performance and subjective feeling as well as in those that have the largest amplitude in cortisol secretion, hypothesized to relate to the strength of the circadian wake promoting signal[27]. Cortical excitability also covaried with cortisol level which has been reported to rapidly affect synaptic function[33,34]. As a strongly circadian-driven signal, cortisol secretion could therefore mediate in part circadian variations in cortical excitability[18,35]. Cortisol co-variation with excitability could also reflect that they are both strongly influenced by the circadian system without a direct causal effect of cortisol. Core body temperature variation, also under circadian control, could equally contribute to the effect we report, as previously suggested[9,18]. However, the frequency specific effects of the circadian modulation of the wake EEG as assessed in a forced desynchrony protocol make it unlikely that all of the circadian effects can be attributed to temperature[24].

In addition to its tonic circadian secretion, cortisol level also varies phasically with stress exposure. This phasic secretion has been suggested to mediate in part the effect of sleep deprivation in rodents[36]. We consider however that stress and stress-induced cortisol secretion are unlikely to have contributed significantly to cortical excitability dynamics in our protocol. First, subjective stress levels were relatively low in our sample, even though they did show previously reported significant circadian-related variations[37] ($P < 0.0001$) (Fig. 3d). Second, salivary cortisol levels of our participants did not exceed laboratory norms[38]. And finally, cortisol followed its typical circadian secretion profile[32] in our sample, and cortisol levels at the end of the protocol were not significantly different from the beginning of the protocol, that is, at same circadian phase but 24 h apart (cf. Fig. 3; $P_{corr} = 1$).
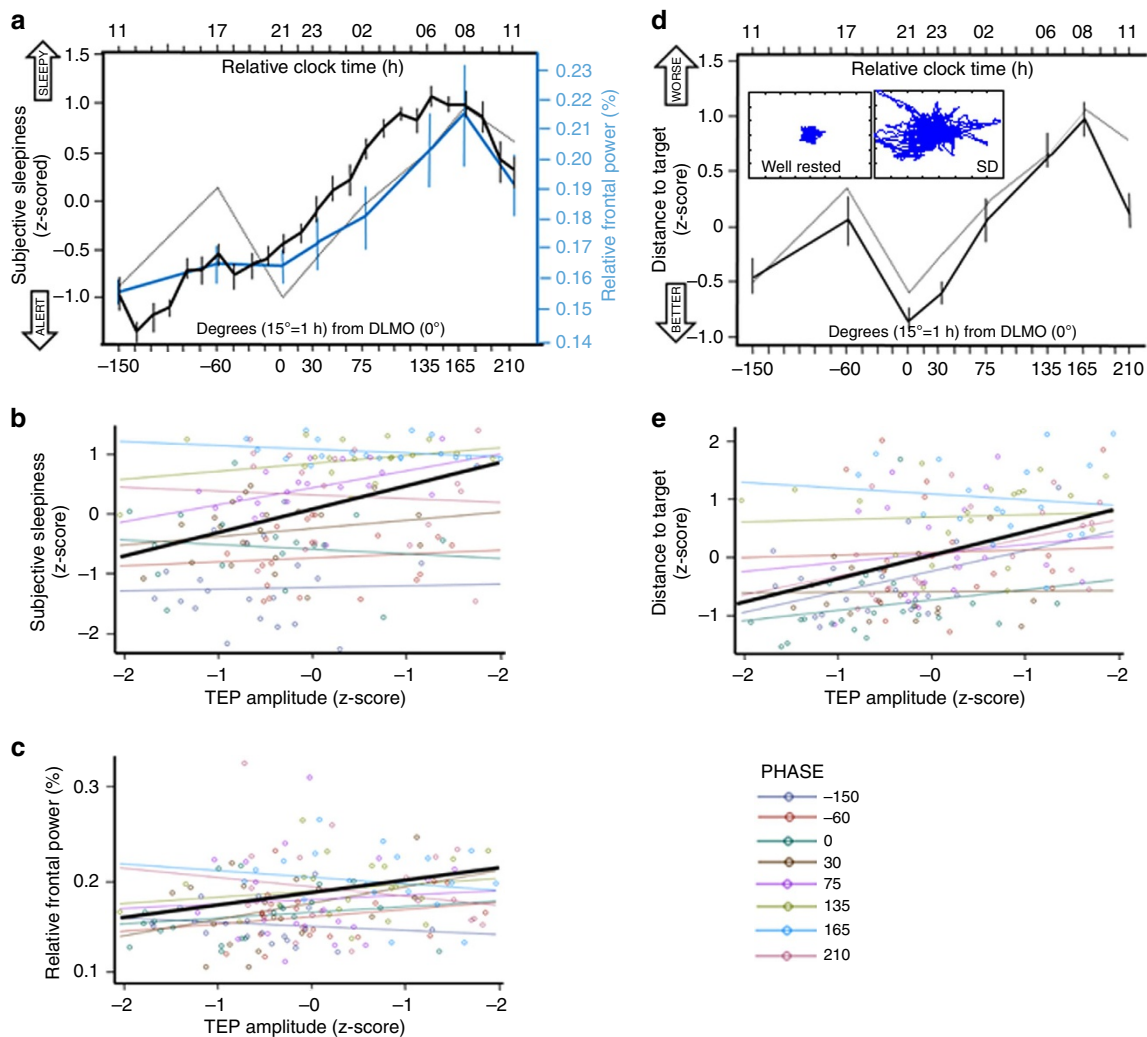
Figure 4 | Cortical excitability dynamics is associated with changes in system-level brain function measures and in behaviour. (**a**) Time course of relative theta (4.5–7.5 Hz) power (%) in spontaneous waking EEG (**blue**) and subjective sleepiness (**black**) (mean ± s.d.). Both variables showed significant variation over the sleep deprivation protocol (PROC MIXED; $n = 22$; main effect of circadian phase: $P < 0.001$; Supplementary Fig. 1 for details). Dashed line: shape of TEP z-scored amplitude dynamics. (**b,c**) ANCOVAs showed that relative theta power (**b**) ($n = 22$; amplitude: $r^2 = 0.19$, $P = 0.004$) and subjective sleepiness (**c**) ($n = 22$; amplitude: $r^2 = 0.69$, $P < 0.0001$) were significantly and positively associated with both indices of cortical excitability. Amplitude × circadian phase interactions was not significant ($P > 0.28$). (**d**) Time course of performance to the vigilance task performed simultaneously to TMS/EEG recordings (mean ± s.d.). The task consisted of maintaining a constantly moving cursor in the centre of a computer screen. Small inset depicts a representative well-rested and sleep-deprived (SD) session. Task performance (average distance kept from the screen center) significantly changed with time awake (PROC MIXED; $n = 22$; main effect of circadian phase: $F_{(7,122)} = 13.78$; $P < 0.0001$). (**e**) An ANCOVA revealed that vigilance task performance impairment was associated to TEP amplitude/slope increase ($n = 22$; amplitude: $r^2 = 0.44$, $P < 0.0001$; slope: $r^2 = 0.43$, $P < 0.0001$). Amplitude/slope × circadian phase interaction was not significant ($P > 0.69$).

Importantly, our results do not preclude a previously reported influence of sleep and sleep homoeostasis on synaptic function[10]. In our data, the overall build-up in cortical excitability, from the morning after a normal night of sleep to 24 h later following continuous wakefulness, is related to the individual differences in the dissipation of slow wave activity during sleep. This dissipation is mainly related to sleep homoeostasis, although for this variable, circadian influences are becoming evident[5,39]. Our findings supports a link between cortical excitability build-up during wakefulness and sleep-induced excitability reduction, at least when considering time points ∼ 24 h apart during extended wakefulness, that is, in the absence of a circadian confound.

Methodological differences are likely to explain the absence of circadian modulation of cortical excitability in previous studies[21–23], including a study of ours[10]. In those studies time resolution was poorer (less assessments included over 24 h) and constant routine conditions were not implemented such that food intake, light exposure and physical activity for instance may have masked circadian rhythmicity[25]. In addition, in previous studies, the knowledge of time of day and of the number of assessments may have induced phasic motivation or engagement during experimental recordings[40]. Constant routine conditions, although strictly controlled should, however, not be considered as impoverished. Demanding test batteries are regularly performed, social interactions with researchers occur and participants engage in quiet activities between tests (reading, watching video, drawing, and so on—low light and acoustic levels). Therefore, we do not consider constant routine to have had a major impact on wake and use-dependent aspects of sleep

homoeostasis, as participants' activities were intellectually demanding, resembled daily activity and included learning of novel information[2]. Finally, in prior studies, prior sleep–wake history was also not as carefully controlled as in the present study and data were not realigned to the onset of melatonin secretion, as a marker of circadian phase. This implies that in previous studies prior chronic sleep restriction may have not been fully dissipated before the experiment and that a 21:00 assessment in a given study[10] could in fact represent a very different combination of sleep pressure and circadian phase than an assessment at 14 h of wakefulness in the present experiment, which also occurred at around 21:00 (for a participant waking up at 07:00).

The amplitude and particularly the slope of an EEG signal are considered to reflect neuronal synchrony and synaptic strength at the cortical level[14]. The variations in TMS-evoked EEG responses and their sharp overnight increase could therefore reflect a loss of discrimination or specificity of individual neurons and the impoverishment of firing repertoires of neuronal populations, which would jeopardize performance. Furthermore, global and local dynamics in neuronal synchrony have been demonstrated both during wakefulness and sleep[41,42]. As we stimulated a single brain area, we can only speculate about this global/local aspect. We delivered TMS over the frontal cortex because this region shows the most pronounced impact of sleep–wake history based on lower EEG frequency power variations[3,39]. The increase in these lower frequencies associated with wakefulness extension is global but also follows a fronto-occipital gradient[3]. This pleads for similar variations in cortical excitability over the entire brain that would be attenuated towards the occiput. Cortical excitability shows, however, region specific characteristics in the main frequency of a TMS-evoked EEG response in human[43]. Both gradual and maybe quite focal brain variations in the dynamics of cortical excitability are therefore likely and their extent deserves further investigation.

Modifications in cortical excitability imply changes in excitation/inhibition balance across subpopulations of neurons. This balance would therefore be under strong circadian influence, possibly through circadian changes in synaptic structure which is evident in many species other than humans[9,18], through change in the extracellular milieu[44], via a glial contribution, or through changes in the influence of brainstem and mesencephalic structure of the ascending arousal system[45].

Cortical excitability increases have been associated with chronic insomnia[46] and epilepsy[47] and reductions have been observed in stroke[48] and disorders of consciousness[49]. Combinations of increases and decreases have been reported in neurodegeneration[50,51], depression[52,53], possibly depending of the type and the stage of the disorder, as well as on time of day. Whether these abnormalities are sustained over the entire 24 h sleep–wake cycle or are only transient is unclear. Likewise, whether the dynamics of cortical excitability over the circadian cycle is altered in those pathological conditions is also not known.

Circadian disruption is, for instance, very common in Alzheimer disease and is deemed to contribute to cognitive impairment in those patients[54]. A time-of-day variation in the occurrence of seizures is also well established in certain forms of epilepsy[55]. Our data also imply that there may be optimal times of day for neurorehabilitation approaches which attempt to restore normal cortical activity in neurological conditions, either through cognitive intervention programs[56] or non-invasive neurostimulation[57]. A circadian influence on cortical excitability may explain for instance why neurostimulation using TMS or transcranial electric stimulation (TES) fails to induce consistent improvement across clinical studies in Alzheimer's disease or stroke patients[57,58]. A full characterization of the temporal profile of cortical excitability in clinical populations may contribute to the development of TMS or TES neurorehabilitation strategies.

As a whole, our study, based on a relatively large sample and on repeated assessments over the 24 h day–night cycle, provides novel insights in the regulation of neuronal and synaptic function in healthy individuals and demonstrates that cortical excitability dynamics is strongly influenced by circadian rhythmicity. Its full characterization holds promise for cognitive enhancement in healthy and clinical brains[58,59].

Methods

Participants. The study was approved by the Ethics Committee of the Medicine Faculty of the University of Liège. Participants gave their written informed consent after the nature and possible consequences of the studies were explained and received a financial compensation. Twenty-four healthy Caucasian men (18–30 years) were enroled. Women were excluded from the study as changes in ovarian hormones may influence cortical excitability in humans[60]. Other exclusion criteria included: (1) BMI ≤ 18 and ≥ 25; (2) psychiatric history, severe trauma, sleep disorders; (3) addiction, chronic medication; (4) smokers, excessive alcohol (>14 doses per week) or caffeine (>3 cups per day) consumption; (5) night shift workers during the last year; (6) transmeridian travel during the past 2 months; (7) anxiety or depression; (8) poor-sleep quality; (9) excessive self-reported daytime sleepiness. One participant was excluded due to a melatonin phase-delay >6 h compared with the remainder of the sample, and one due to low EEG recording quality. Thus, data presented here include 22 participants. Supplementary Table 1 summarizes the demographic characteristics of the final study sample. Participants were recruited based on a polymorphism in *PERIOD3* (*PER3* variable number of tandem repeat, with 4 or 5 repeats)[61], but genotype was ignored in the analysis given the limited sample size of PER3$^{5/5}$ genotype (7 PER3$^{5/5}$ for 15 PER3$^{4/4}$).

Experimental protocol. Participants first completed a 'pretest' TMS/EEG session to determine the optimal TMS parameters providing artefact-free EEG recordings. The left or right supplementary motor area (SMA) was set as stimulation target for right or left handed, respectively. This brain area was identical to[10] and was chosen for the following reasons: (1) similar to the entire frontal lobe, the SMA is exquisitely sensitive to sleep pressure, including at the neuronal level, as indicated in a previous EEG-TMS experiment[10]; (2) it plays a key role in cognitive performance, and is heavily connected to the prefrontal cortex[62]; (3) its stimulation does not trigger muscle activation, sources of EEG signal contamination.

The second step consisted of a laboratory polysomnographically monitored habituation night to exclude potential sleep disorders. During the 7 days preceding the study, participants kept a regular sleep–wake schedule of 8 h sleep duration (± 15 min). Compliance was verified using wrist actigraphy (Actiwatch, Cambridge Neurotechnology, UK) and sleep diaries (Supplementary Table 1). Participants were requested to abstain from all caffeine- and alcohol-containing beverages and from intense physical activity for 3 days preceding the study.

For the experiment *per se*, participants arrived at the laboratory ∼6 h before their habitual sleep time. They were maintained in dim-light from there on (5<lux, except for sleep episode in complete darkness) and trained twice on the behavioural test battery. They then slept for an 8 h sleep baseline episode starting at their habitual bedtimes (Supplementary Table 2). The TMS-compatible electrode cap was placed upon awaking before the 29 h of sustained wakefulness period (sleep deprivation) under constant routine conditions (that is, light ca. 5 lux, temperature ca. 19 °C, regular isocaloric liquid meals and water, semi-recumbent position and no time-of-day information, sound proofed rooms) during which they did not interact with other participants but could engage conversation with research staff (outside test periods). These conditions aim to minimize external and internal factors masking circadian rhythmicity[25].

Spontaneous quiet waking EEG and TMS-evoked EEG potentials (TEP) were recorded eight times during sleep deprivation to cover the entire near-24 h circadian cycle, with higher session frequency around the circadian WMZ and SPZ[5] (11:00, 17:00, 21:00, 23:00, 02:00, 06:00, 08:00, 11:00, for a subject sleeping from 23:00 to 07:00; Fig. 1). Behavioural test batteries were carried out 12 times during the sleep deprivation period in between EEG sessions (12:00, 14:00, 16:00, 18:, 20:00, 22:00, 00:00, 03:00, 05:00, 07:00, 09:00, 00:00). Subjective sleepiness and affect dimensions were assessed hourly by the Karolinska Sleepiness Scale (KSS) and a Visual Analogical Scale (VAS), respectively. Saliva samples for melatonin and cortisol assays were also collected hourly.

TMS-evoked EEG responses acquisition. TMS pulses were generated by a Focal Bipulse 8-Coil (Eximia; Nexstim, Helsinki, Finland). Stimulation target (SMA) was located on individual structural MRI by means of a neuronavigation system (Navigated Brain Stimulation; Nexstim). This device allows for reproducible evoked EEG responses[63] and precise target location (FDA approval for presurgery). Each session included between 250 and 300 trials. Interstimulus interval was randomly jittered between 1,900 and 2,200 ms. Coil recharge was set at 900 ms post-TMS. Total number of stimulations of the eight EEG/TMS sessions was well below safety recommendations[64].

TMS responses were recorded with a 60-channel TMS-compatible EEG amplifier (Eximia; Nexstim), equipped with a proprietary sample-and-hold circuit equipment guaranteeing TMS artifact-free data 8 ms post TMS[65]. Electrooculogram was recorded with two additional bipolar electrodes. Participants wore the EEG cap during the entire constant routine protocol and electrodes impedance was kept below 5 kΩ. Signal was band-pass-filtered between 0.1 and 500 Hz and sampled at 1,450 Hz. Each EEG/TMS session ended with a neuronavigated digitization of the location of each electrode.

Auditory EEG potentials (AEP) evoked by the TMS and bone conduction were minimized by diffusing a continuous loud white masking noise through earplugs and applying a thin foam layer between the EEG cap and the TMS coil, respectively[63]. Each session was followed by a 'sham' session consisting in 30–40 TMS pulses delivered parallel to the scalp while white noise was diffused with the same level. Absence of AEP was checked online on Cz between 0 and 300 ms post TMS.

Spontaneous waking and sleep EEG acquisition. Spontaneous quiet waking EEG was recorded prior to each TMS session using the same 60-channel TMS-compatible EEG (+ 2 EOG) amplifier (Eximia; Nexstim). Participants were instructed to fix a black dot during 2 min while relaxing and suppressing blinking.

Sleep EEG data were recorded using a V-Amp 16 amplifier (Brain Products GmbH, Gilching, Germany) according to 10/20 system). The habituation night montage consisted of a full PSG with five EEG channels (Fz, Cz, Pz, Oz, C3) referenced to left mastoid (A1), two bipolar electrooculogram (EOG), two bipolar electrocardiogram channels, two bipolar electrodes placed on a leg to check for periodic movements and an oximeter for sleep related breathing disorder detection. Baseline night montage consisted of 11 EEG channels (F3, Fz, F4, C3, Cz, C4, P3, Pz, P4, O1 and O2) referenced to left and right mastoids (A1 and A2), two bipolar EOG and two bipolar electromyogram (EMG) channels. EEG data were digitized at a sampling rate of 500 Hz.

TMS vigilance task. While recording TMS-evoked EEG responses, participants performed a visual task (CTT) to monitor their vigilance level[10]. The task consisted of keeping a constantly randomly moving cursor on a target located in the centre of a computer screen, using a trackball device. Performance to the task was computed as the average distance, in pixels, between the cursor and the target during EEG/TMS recording (normalized according to the duration of the session). Transitory lapses of vigilance resulted in temporary increases of the target—cursor distance which could be automatically detected offline. A lapse was defined as a time when the cursor was located outside of a central 200 by 200 pixel box surrounding target following > 500 ms from the last trackball movement. The lapse period included the period between the last trackball movement and the lapse detection. TMS-evoked responses occurring during and < 1 s from a lapse were discarded from the analyses.

Psychomotor vigilance task. Participants were required to press a computer space bar as soon as an auditory signal, presented at a random interval of 3–7 s, occurred. The PVT lasted 5 min. Session performance was inferred from the median reaction time following removal of lapses (> 500 ms), anticipation (< 100 ms) and error (> 3,000 ms)[28].

Saliva collection for melatonin and cortisol assays. Saliva samples were first placed at 4 °C, prior centrifugation and congelation at − 20 °C within 12 h. Salivary melatonin and cortisol were measured by radioimmunoassay (Stockgrand Ltd, Guildford, UK), as previously described[66]. Of a total of 624 samples, 546 were analysed in duplicate for melatonin concentration. The limit of detection of the assay for melatonin was 0.8 ± 0.2 pg ml^{−1} using 500 μl volumes. Of a total of 631 samples, 631 were analysed in duplicate for cortisol concentration. The limit of detection of the assay for cortisol was 0.37 ± 0.05 nmol l^{−1} using 500 μl volumes[67].

TMS/EEG data analysis. TMS/EEG data pre-processing was computed using Statistical Parametric Mapping 12 (SMP12, http://www.fil.ion.ucl.ac.uk/spm/) implemented in Matlab 2011a (The Mathworks Inc, Natick, MA). Continuous EEG recordings were successively re-referenced to the average of all good channels, low-pass filtered at 80 Hz, downsampled from 1,450 to 1,000 Hz, and high-pass filtered at 1 Hz, split into epochs between –101 and 300 ms around TMS pulses, and baseline corrected -101 to − 1.5 ms pre-TMS periods. Robust averaging was applied to compute the mean evoked response of each session[68].

Cortical excitability was inferred from the amplitude and slope of the first EEG component (0–30 ms) of the TEP measured at the artifact-free electrode closest from the hotspot (that is, brain location with highest TMS-induced electrical field estimated by the neuronavigation system). The latter electrode was always located in the stimulated brain hemisphere. It could vary across participants but remained constant at the individual level. TEP amplitude and slope were also extracted from a reconstructed signal at the hotspot using localization of equivalent current dipole.

Spontaneous waking and sleep EEG analyses. Waking EEG data were analysed with MATLAB (2011a, The Mathworks Inc, Natick, MA). Data pre-processing was performed using Statistical Parametric Mapping 12 (SPM12, http://www.fil.ion.ucl.ac.uk/spm). Artefacted channels were rejected after visual inspection.

Continuous EEG recordings were downsampled from 1,450 to 500 Hz. Data were then manually and visually scored offline for artefacts (eye blinks, body movements, and slow eye movements). Power spectral densities were computed using a fast Fourier transform on artifact-free 4-s, overlapping by 2 s, using the Welch's method (pwelch function in MATLAB 7.5.0). EEG activity was computed over frontal region (FP1, FPz, FP2, AF1, AFz, AF2, F7, F3, F1, Fz, F2, F4 and F8) for delta (0.75–4 Hz), theta (4.5–7.5 Hz), alpha (8–12 Hz), sigma (12.5–18 Hz) and beta (18.5–30 Hz) frequency bands over the entire 2-min recording.

Sleep EEG recordings were re-referenced to the average of both mastoids and band-pass filtered between 0.5 and 25 Hz. Data were visually inspected for artefact and manually scored for sleep stages on a 30-s epoch basis using FASST (an SPM compatible toolbox[69]), according to AASM criteria[65]. One baseline night was excluded from analyses because of poor quality of the recording ($n = 21$). NREM-REM sleep cycles were determined according to Feinberg and Floyd. Power spectra were computed using a fast Fourier transform on successive 4-s epochs, overlapping by 2 s and weighted by a Hanning window.

Statistics. All statistical analyses were performed with SAS version 9.3 (SAS Institute, Cary, NC, USA). For TEP amplitude and slope, cortisol level, KSS and PVT measures, standardization was provided by a z-score at individual level. TMS vigilance task was normalized by dividing performance to the duration of task and then z-scored. Frontal waking theta activity was normalized by dividing theta power by the sum of frequencies within 0.75 and 20 Hz over the same region. The time course of cortical excitability (that is, TEP amplitude and slope) was examined with mixed-model analyses of variance for repeated measures (PROC MIXED), with within-subject factor 'circadian phase'. Contrasts were assessed with Difference of Least Square Means statement. TEP amplitude and slope were realigned, at the individual level, to dim-light melatonin onset (DLMO).

Estimation of circadian phase (where 0° = individual DLMO) was determined based on raw values. The four first samples were disregarded and maximum secretion level was set as the median of the three highest concentrations during the constant routine. Baseline level was set to be the median of the values collected from wake-up time + 5 h to wake-up time + 10 h. DLMOn was computed as time at which melatonin level reach 20% of the baseline to maximum difference (following linear interpolation).

ANCOVA were performed to estimate how TEP amplitude and slope were associated to theta EEG activity, subjective sleepiness and effects, cortisol level, and TMS vigilance task behavioural responses. To investigate the influence of sleep homoeostasis and circadian rhythmicity on cortical excitability, TEP amplitude and slope were fitted with, respectively, linear and sine-wave functions:

Linear function: $\text{Var} = (C + L \times \text{time})$, where C corresponds to initial constant and L is the linear increment across time[27].

Sine-wave function: $\text{Var} = \text{Mesor} + \text{Amplitude} \times \sin((\text{sample} \times \text{ti-time})/24.2)$, where mesor, amplitude, and time are free parameters, ti represents clock time i at which a sample is collected[25].

Estimated fitted cortisol secretion profile was obtained using this same sine-wave function. The amplitude of cortisol estimated secretion, as a proxy of the circadian signal strength, was derived from the difference between the maximal and minimal cortisol predicted values.

An exponential decay function (PROC NLIN, SAS 9.3) was fitted to sleep delta data power (0.75–4 Hz) of the first four sleep cycles[70] and derived from the frontal derivations, known to be more sensitive to sleep deprivation: $\text{SWA}(t) = \text{SWA0} \times \exp(- r \times \text{epi})^{3,70}$. The amount of initial slow wave activity (SWA0) and its dissipation rate (r) were derived.

Regression (PROC REG) were also performed between individual estimated cortisol amplitude and the TEP amplitude and slope decrease from session 2 to session 3 (two participants were excluded from this latter analysis because one showed a cortisol amplitude more than four standard deviations below the sample mean and another because the TMS responses of session 2 were of poor quality); 2) between individual estimated slow wave activity dissipation rate (r) and the TEP amplitude and slope increase from the first to the last session (four participants were excluded from this latter analysis because two showed dissipation more than three standard deviations above the sample mean and two had a TMS response during first or last session of poor quality).

Data availability. The authors declare that the data that support the findings of this study are available from the corresponding author upon request.

References

1. Schmidt, C., Collette, F., Cajochen, C. & Peigneux, P. A time to think: circadian rhythms in human cognition. *Cogn. Neuropsychol.* **24**, 755–789 (2007).
2. Tononi, G. & Cirelli, C. Sleep and the price of plasticity: from synaptic and cellular homeostasis to memory consolidation and integration. *Neuron* **81**, 12–34 (2014).
3. Cajochen, C., Foy, R. & Dijk, D. J. Frontal predominance of a relative increase in sleep delta and theta EEG activity after sleep loss in humans. *Sleep Res. Online* **2**, 65–69 (1999).

4. Dijk, D. J., Duffy, J. F. & Czeisler, C. A. Circadian and sleep/wake dependent aspects of subjective alertness and cognitive performance. *J. Sleep Res.* **1,** 112–117 (1992).

5. Dijk, D. J. & Czeisler, C. A. Contribution of the circadian pacemaker and the sleep homeostat to sleep propensity, sleep structure, electroencephalographic slow waves, and sleep spindle activity in humans. *J. Neurosci.* **15,** 3526–3538 (1995).

6. Strogatz, S. H., Kronauer, R. E. & Czeisler, C. A. Circadian pacemaker interferes with sleep onset at specific times each day: role in insomnia. *Am. J. Physiol.* **253,** R172–R178 (1987).

7. Curie, T. *et al.* Homeostatic and circadian contribution to EEG and molecular state variables of sleep regulation. *Sleep* **36,** 311–323 (2013).

8. Gaggioni, G., Maquet, P., Schmidt, C., Dijk, D.-J. & Vandewalle, G. Neuroimaging, cognition, light and circadian rhythms. *Front. Syst. Neurosci.* **8,** 126 (2014).

9. Frank, M. G. & Cantera, R. Sleep, clocks, and synaptic plasticity. *Trends Neurosci.* **37,** 491–501 (2014).

10. Huber, R. *et al.* Human cortical excitability increases with time awake. *Cereb. Cortex* **23,** 332–338 (2013).

11. Gastaut, H. & Tassinari, C. A. Triggering mechanisms in epilepsy. The electroclinical point of view. *Epilepsia* **7,** 85–138 (1966).

12. Babkoff, H., Sing, H. C., Thorne, D. R., Genser, S. G. & Hegge, F. W. Perceptual distortions and hallucinations reported during the course of sleep deprivation. *Percept. Mot. Skills* **68,** 787–798 (1989).

13. Vyazovskiy, V. V. *et al.* Cortical firing and sleep homeostasis. *Neuron* **63,** 865–878 (2009).

14. Vyazovskiy, V. V., Cirelli, C., Pfister-Genskow, M., Faraguna, U. & Tononi, G. Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. *Nat. Neurosci.* **11,** 200–208 (2008).

15. Appelbaum, L. *et al.* Circadian and homeostatic regulation of structural synaptic plasticity in hypocretin neurons. *Neuron* **68,** 87–98 (2010).

16. Vollrath, L. & Spiwoks-Becker, I. Plasticity of retinal ribbon synapses. *Microsc. Res. Tech.* **35,** 472–487 (1996).

17. Weber, P., Kula-Eversole, E. & Pyza, E. Circadian control of dendrite morphology in the visual system of *Drosophila melanogaster*. *PLoS ONE* **4,** e4290 (2009).

18. Frank, M. G. Erasing synapses in sleep: is it time to be SHY? *Neural Plast.* **2012,** 264378 (2012).

19. Tamm, A. S., Lagerquist, O., Ley, A. L. & Collins, D. F. Chronotype influences diurnal variations in the excitability of the human motor cortex and the ability to generate torque during a maximum voluntary contraction. *J. Biol. Rhythm* **24,** 211–224 (2009).

20. Lang, N. *et al.* Circadian modulation of GABA-mediated cortical inhibition. *Cereb. Cortex* **21,** 2299–2306 (2011).

21. Doeltgen, S. H. & Ridding, M. C. Behavioural exposure and sleep do not modify corticospinal and intracortical excitability in the human motor system. *Clin. Neurophysiol.* **121,** 448–452 (2010).

22. De Gennaro, L. *et al.* Neurophysiological correlates of sleepiness: a combined TMS and EEG study. *Neuroimage* **36,** 1277–1287 (2007).

23. Gorgoni, M. *et al.* Sleep deprivation affects somatosensory cortex excitability as tested through median nerve stimulation. *Brain Stimul.* **7,** 732–739 (2014).

24. Cajochen, C. & Dijk, D.-J. Electroencephalographic activity during wakefulness, rapid eye movement and non-rapid eye movement sleep in humans: comparison of their circadian and homeostatic modulation. *Sleep Biol. Rhythms* **1,** 85–95 (2003).

25. Duffy, J. F. & Dijk, D. J. Getting through to circadian oscillators: why use constant routines? *J. Biol. Rhythm* **17,** 4–13 (2002).

26. Cajochen, C., Wyatt, J. K., Czeisler, C. A. & Dijk, D. J. Separation of circadian and wake duration-dependent modulation of EEG activation during wakefulness. *Neuroscience* **114,** 1047–1060 (2002).

27. Dijk, D. J. *et al.* Amplitude reduction and phase shifts of melatonin, cortisol and other circadian rhythms after a gradual advance of sleep and light exposure in humans. *PLoS ONE* **7,** e30037 (2012).

28. Dinges, D. F. & Powell, J. W. Microcomputer analyses of performance on a portable, simple visual RT task during sustained operations. *Behav. Res. Meth Instr Comput* **17,** 625–655 (1985).

29. Dijk, D. J., Beersma, D. G. & Daan, S. EEG power density during nap sleep: reflection of an hourglass measuring the duration of prior wakefulness. *J. Biol. Rhythm* **2,** 207–219 (1987).

30. Viola, A. U. *et al.* Interindividual differences in circadian rhythmicity and sleep homeostasis in older people: effect of a PER3 polymorphism. *Neurobiol. Aging* **33,** 1010 e17–1010 e27 (2012).

31. Tarokh, L., Carskadon, M. A. & Achermann, P. Dissipation of sleep pressure is stable across adolescence. *Neuroscience* **216,** 167–177 (2012).

32. Morris, C. J., Aeschbach, D. & Scheer, F. A. J. L. Circadian system, sleep and endocrinology. *Mol. Cell. Endocrinol.* **349,** 91–104 (2012).

33. Liston, C. *et al.* Circadian glucocorticoid oscillations promote learning-dependent synapse formation and maintenance. *Nat. Neurosci.* **16,** 698–705 (2013).

34. Yuen, E. Y. *et al.* Acute stress enhances glutamatergic transmission in prefrontal cortex and facilitates working memory. *Proc. Natl Acad. Sci. USA* **106,** 14075–14079 (2009).

35. Tononi, G. & Cirelli, C. Time to be SHY? Some comments on sleep and synaptic homeostasis. *Neural Plast.* **2012,** 415250 (2012).

36. Mongrain, V. *et al.* Separating the contribution of glucocorticoids and wakefulness to the molecular and electrophysiological correlates of sleep homeostasis. *Sleep* **33,** 1147–1157 (2010).

37. Boivin, D. B. *et al.* Complex interaction of the sleep-wake cycle and circadian phase modulates mood in healthy subjects. *Arch. Gen. Psychiatry* **54,** 145–152 (1997).

38. Aardal, E. & Holm, A. C. Cortisol in saliva--reference ranges and relation to cortisol in serum. *Eur. J. Clin. Chem. Clin. Biochem.* **33,** 927–932 (1995).

39. Lazar, A. S., Lazar, Z. I. & Dijk, D. J. Circadian regulation of slow waves in human sleep: topographical aspects. *Neuroimage* **116,** 123–134 (2015).

40. Hull, J. T., Wright, Jr. K. P. & Czeisler, C. A. The influence of subjective alertness and motivation on human performance independent of circadian and homeostatic regulation. *J. Biol. Rhythm* **18,** 329–338 (2003).

41. Vyazovskiy, V. V. *et al.* Local sleep in awake rats. *Nature* **472,** 443–447 (2011).

42. Sarasso, S. *et al.* Fluid boundaries between wake and sleep: experimental evidence from Stereo-EEG recordings. *Arch. Ital. Biol.* **152,** 169–177 (2014).

43. Rosanova, M. *et al.* Natural frequencies of human corticothalamic circuits. *J. Neurosci.* **29,** 7679–7685 (2009).

44. Dash, M. B., Douglas, C. L., Vyazovskiy, V. V., Cirelli, C. & Tononi, G. Long-term homeostasis of extracellular glutamate in the rat cerebral cortex across sleep and waking states. *J. Neurosci.* **29,** 620–629 (2009).

45. Saper, C. B., Fuller, P. M., Pedersen, N. P., Lu, J. & Scammell, T. E. Sleep state switching. *Neuron* **68,** 1023–1042 (2010).

46. van der Werf, Y. D. *et al.* Is disturbed intracortical excitability a stable trait of chronic insomnia? A study using transcranial magnetic stimulation before and after multimodal sleep therapy. *Biol. Psychiatry* **68,** 950–955 (2010).

47. Kimiskidis, V. K. *et al.* Transcranial magnetic stimulation combined with EEG reveals covert states of elevated excitability in the human epileptic brain. *Int. J. Neural Syst.* **25,** 1550018 (2015).

48. Huynh, W., Vucic, S., Krishnan, A. V., Lin, C. S. & Kiernan, M. C. Exploring the evolution of cortical excitability following acute stroke. *Neurorehabil. Neural Repair* **30,** 244–257 (2015).

49. Rosanova, M. *et al.* Recovery of cortical effective connectivity and recovery of consciousness in vegetative patients. *Brain* **135,** 1308–1320 (2012).

50. Casarotto, S. *et al.* Transcranial magnetic stimulation-evoked EEG/cortical potentials in physiological and pathological aging. *Neuroreport* **22,** 592–597 (2011).

51. Pennisi, G. *et al.* Transcranial magnetic stimulation in Alzheimer's disease: a neurophysiological marker of cortical hyperexcitability. *J. Neural Transm.* **118,** 587–598 (2011).

52. Bunse, T. *et al.* Motor cortical excitability assessed by transcranial magnetic stimulation in psychiatric disorders: a systematic review. *Brain Stimul.* **7,** 158–169 (2014).

53. Canali, P. *et al.* Changes of cortical excitability as markers of antidepressant response in bipolar depression: preliminary data obtained by combining transcranial magnetic stimulation (TMS) and electroencephalography (EEG). *Bipolar Disord.* **16,** 809–819 (2014).

54. Musiek, E. S. *et al.* Circadian clock proteins regulate neuronal redox homeostasis and neurodegeneration. *J. Clin. Invest.* **123,** 5389–5400 (2013).

55. Mirzoev, A. *et al.* Circadian profiles of focal epileptic seizures: a need for reappraisal. *Seizure* **21,** 412–416 (2012).

56. Jean, L., Bergeron, M. E., Thivierge, S. & Simard, M. Cognitive intervention programs for individuals with mild cognitive impairment: systematic review of the literature. *Am. J. Geriatr. Psychiatry* **18,** 281–296 (2010).

57. Di Pino, G. *et al.* Modulation of brain plasticity in stroke: a novel model for neurorehabilitation. *Nat. Rev. Neurol.* **10,** 597–608 (2014).

58. Kadosh, R. C. Using transcranial electrical stimulation to enhance cognitive functions in the typical and atypical brain. *Transl. Neurosci.* **4,** 20–33 (2013).

59. Luber, B. & Lisanby, S. H. Enhancement of human cognitive performance using transcranial magnetic stimulation (TMS). *Neuroimage* **85,** 961–970 (2014).

60. Smith, M. J., Adams, L. F., Schmidt, P. J., Rubinow, D. R. & Wassermann, E. M. Effects of ovarian hormones on human cortical excitability. *Ann. Neurol.* **51,** 599–603 (2002).

61. Dijk, D. J. & Archer, S. N. PERIOD3, circadian phenotypes, and sleep homeostasis. *Sleep Med. Rev.* **14,** 151–160 (2010).

62. Bonini, F. *et al.* Action monitoring and medial frontal cortex: leading role of supplementary motor area. *Science* **343,** 888–891 (2014).

63. Rosanova, M. *et al.* Combining transcranial magnetic stimulation with electroencephalography to study human cortical excitability and effective connectivity. *Neuromethods* **67,** 435–467 (2012).

64. Rossi, S., Hallett, M., Rossini, P. M. & Pascual-Leone, A. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin. Neurophysiol.* **120,** 2008–2039 (2009).

65. Virtanen, J., Ruohonen, J., Naatanen, R. & Ilmoniemi, R. J. Instrumentation for the measurement of electric brain responses to transcranial magnetic stimulation. *Med. Biol. Eng. Comput.* **37,** 322–326 (1999).

66. English, J., Middleton, B. A., Arendt, J. & Wirz-Justice, A. Rapid direct measurement of melatonin in saliva using an iodinated tracer and solid phase second antibody. *Ann. Clin. Biochem.* **30,** 415–416 (1993).

67. Read, G. F., Fahmy, D. R. & Walker, R. F. Determination of cortisol in human plasma by radioimmunoassay. *Ann. Clin. Biochem.* **14,** 343–349 (1977).

68. Leonowicz, Z., Karvanen, J. & Shishkin, S. L. Trimmed estimators for robust averaging of event-related potentials. *J. Neurosci. Methods* **142,** 17–26 (2005).

69. Leclercq, Y., Schrouff, J., Noirhomme, Q., Maquet, P. & Phillips, C. fMRI artefact rejection and sleep scoring toolbox. *Comput. Intell. Neurosci.* **2011,** 598206 (2011).

70. Mongrain, V., Carrier, J. & Dumont, M. Difference in sleep regulation between morning and evening circadian types as indexed by antero-posterior analyses of the sleep EEG. *Eur. J. Neurosci.* **23,** 497–504 (2006).

Author contributions

J.Q.M.L. acquired and analysed the data, provided technical expertise on the TMS/EEG and wrote the paper. G.G. and S.L.C. acquired and analysed the data and wrote the paper. S.P., C.B. and A.B. acquired the data. M.R. and S.S. provided expertise for TMS/EEG acquisitions. B.M. performed melatonin/cortisol assays. D.J.D. provided expertise for the statistical analysis and study design and wrote the paper. A.L. and S.N.A. provided expertise for the statistical analysis. C.P. provided expertise for EEG data analyzes. M.M. designed the experiment, provided expertise for TMS/EEG acquisitions, and wrote the paper. P.M. designed the experiment and wrote the paper. G.V. designed the experiment, acquired and analysed the data, and wrote the paper.

Additional information

Phase I, Paper 2: Human fronto-parietal response scattering subserves vigilance at night

Gaggioni G[*], Ly JQM[*], Chellappa SL[*], Coppieters 't Wallant D, Rosanova M, Sarasso S, Luxen A, Salmon E, Middleton B, Massimini M, Schmidt C, Casali A, Phillips C[§], Vandewalle G [§].
*NeuroImage 2018 – * shared first authorship*

After having investigated the local TMS-related effects at the brain cortical level in young participants (18-30 y), the next step is to investigate global TMS-related effects on the same group of volunteers. In this paper, we investigate the profile of cortical scattering to the initial TMS, and we do a first attempt to measure the cortical response complexity. We are interested in unravelling global cortical modifications during a night without sleep, when the vigilance performance is clearly impaired, because a high sleep pressure is no longer counteracted by the circadian alerting signal. We hypothesise that both fronto-parietal response scattering and complexity will change with time spent awake and according to the internal circadian clock. During a night without sleep, we expect a decrease of cortical response scattering –possibly reflecting reduced cross-talk between cortical regions– and a decrease of cortical response complexity –reflecting a reduced neuronal information content. These changes at the cortical level will correlate with the vigilance impairment typically observed during this night-time window. Results disclose that fronto-parietal response scattering significantly changes with time spent awake, while –contrary to our expectations– response complexity does not. Data further suggest that fronto-parietal response scattering tends to decrease during night-time wakefulness: lower night-time level is correlated with worse vigilance performance and lower alertness. These results reiterate that cortico-cortical transmission varies during prolonged wakefulness, which may contribute to explain why vigilance performance is affected during a night without sleep.

Human fronto-parietal response scattering subserves vigilance at night

Giulia Gaggioni [a,b,1], Julien Q.M. Ly [a,b,c,1], Sarah L. Chellappa [a,b,2,1],
Dorothée Coppieters 't Wallant [a,b,d], Mario Rosanova [e,f], Simone Sarasso [e], André Luxen [a],
Eric Salmon [a], Benita Middleton [g], Marcello Massimini [e], Christina Schmidt [a,b,h],
Adenauer Casali [i], Christophe Phillips [a,b,d,*,3], Gilles Vandewalle [a,b,**,3]

[a] GIGA-Research, Cyclotron Research Centre/In Vivo Imaging Unit, University of Liège, Liège, Belgium
[b] Walloon Excellence in Life Sciences and Biotechnology (WELBIO, Belgium), Belgium
[c] Department of Neurology, CHU de Liège, Liège, Belgium
[d] GIGA-In Silico Medicine, University of Liège, Liège, Belgium
[e] Department of Biomedical and Clinical Sciences "L.Sacco", Universit degli Studi di Milano, Milan, Italy
[f] Fondazione Europea Di Ricerca Biomedica, Ferb Onlus, Milan, Italy
[g] Surrey Sleep Research Centre, University of Surrey, Guildford, UK
[h] Psychology and Neuroscience of Cognition Research Unit, Faculty of Psychology and Educational Sciences, University of Liège, Liège, Belgium
[i] Institute of Science and Technology, University of Sao Paolo, Brazil

ARTICLE INFO

ABSTRACT

Lack of sleep has a considerable impact on vigilance: we perform worse, we make more errors, particularly at night, when we should be sleeping. Measures of brain functional connectivity suggest that decrease in vigilance during sleep loss is associated with an impaired cross-talk within the fronto-parietal cortex. However, fronto-parietal effective connectivity, which is more closely related to the causal cross-talk between brain regions, remains unexplored during prolonged wakefulness. In addition, no study has simultaneously investigated brain effective connectivity and wake-related changes in vigilance, preventing the concurrent incorporation of the two aspects. Here, we used electroencephalography (EEG) to record responses evoked by Transcranial Magnetic Stimulation (TMS) applied over the frontal lobe in 23 healthy young men (18–30 yr.), while they simultaneously performed a vigilance task, during 8 sessions spread over 29 h of sustained wakefulness. We assessed Response Scattering (ReSc), an estimate of effective connectivity, as the propagation of TMS-evoked EEG responses over the fronto-parietal cortex. Results disclose a significant change in fronto-parietal ReSc with time spent awake. When focusing on the night-time period, when one should be sleeping, participants with lower fronto-parietal ReSc performed worse on the vigilance task. Conversely, no association was detected during the well-rested, daytime period. Night-time fronto-parietal ReSc also correlated with objective EEG measures of sleepiness and alertness. These changes were not accompanied by variations in fronto-parietal response complexity. These results suggest that decreased brain response propagation within the fronto-parietal cortex is associated to increased vigilance failure during night-time prolonged wakefulness. This study reveals a novel facet of the detrimental effect on brain function of extended night-time waking hours, which is increasingly common in our societies.

Introduction

Modern lifestyle is associated with longer waking hours that perturb circadian rhythmicity and reduce sleep time. This negatively affects vigilance (Durmer and Dinges, 2005; Krause et al., 2017), a basic neuropsychological feature, yet essential to complex cognitive processes. It is defined as the ability to sustain attention on a series of stimuli over prolonged periods of time, or a state of readiness to detect and respond to certain small changes occurring at random time intervals in the environment (Oken et al., 2006; Posner and Petersen, 1990). Vigilance has been reported to suffer the most from insufficient sleep compared with complex cognitive aspects (Lim and Dinges, 2008; Lo et al., 2012). This is particularly the case when wakefulness is extended during the night, because the circadian timing system no longer opposes high sleep pressure, but rather promotes sleep, thereby amplifying the deleterious effects of high sleep pressure on waking performance (Cajochen et al., 1999). In fact, the misalignment between sleep-wake behavior and endogenous circadian time is a major cause of the night-time peak in errors and accidents (e.g. medical errors and car accidents) (Lim and Dinges, 2008; Philip and Akerstedt, 2006). Electrophysiology studies demonstrated that the drop of vigilance at night parallels a wake-dependent increase in the power of slow delta (0.75–4 Hz) and theta (4.5–7.5 Hz) frequency bands of the waking EEG, and a decrease in the power of faster alpha frequency band (8–12 Hz) (Aeschbach et al., 2001; Cajochen et al., 2002, 1999).

The brain substrates of vigilance encompass mainly frontal and parietal areas at the cortical level, and the thalamus at the subcortical level (Corbetta et al., 1993; Coull et al., 2004; Culham et al., 1998). Reduced fronto-parietal response to a vigilance task has been observed following a night of sleep deprivation (Poudel et al., 2013; Tomasi et al., 2009). Moreover, vigilance failures (lapses) were associated with reduced activation within these cortical and subcortical areas (Chee et al., 2008; Chee and Tan, 2010). Beyond abnormal brain activations, vigilance decline during sleep deprivation appears to be related to modifications in the cross-talk between brain areas. This is indicated by changes in spontaneous (i.e. task free) functional connectivity, showing reduced within-network connectivity (or integration) in the default mode and dorsal/ventral attention networks (including many frontal and parietal regions), and reduced segregation between these networks (De Havas et al., 2012; Sämann et al., 2010; Tüshaus et al., 2017; Yeo et al., 2015).

Functional connectivity is based on temporal correlations in brain activity, whereas effective connectivity refers to the ability of a set of neuronal elements to causally affect the firing of other neuronal groups within a system (Friston, 2011). Effective connectivity is therefore more directly related to the cross-talk between brain regions. Brain effective connectivity has been reported to change during sleep deprivation: Granger Causality measures of effective connectivity within the cingulate cortex decreased during task-free recordings of brain activity following sleep deprivation (Piantoni et al., 2013). Similarly to functional connectivity measures, the magnitude of this decrease in cingulate effective connectivity predicted vigilance performance, assessed after brain activity recording. However, variations in effective connectivity within the fronto-parietal cortex have not yet been assessed during prolonged wakefulness. Likewise, performance to a vigilance task has not yet been assessed *simultaneously* to effective connectivity measures.

Compared to wakefulness, effective connectivity sharply decreased during sleep, as probed by the propagation of a direct Transcranial Magnetic Stimulation (TMS) perturbation of neuronal activity (Massimini et al., 2005). This decrease in effective connectivity was associated with a reduction in the complexity of the cortical response: neuronal activity was less variable, more regular, i.e. neurons information content was impoverish during sleep compared to wakefulness (Casali et al., 2013). To our knowledge, a single study addressed variations of complexity driven by sleep loss (Abásolo et al., 2015), and found no

significant changes. This study was however conducted on rat, and following 4 h of partial sleep deprivation. Whether effective connectivity changes induced by a full night of sleep deprivation in human fronto-parietal are accompanied by changes in response complexity remains unknown.

Here, we assessed variations in fronto-parietal Response Scattering (ReSc), based on TMS response propagation, during prolonged wakefulness while participants simultaneously performed a vigilance task. We hypothesized that wakefulness extension into the night is associated with a decrease in fronto-parietal ReSc, and consequently with a decrease in Response Complexity (ReC). Furthermore, since vigilance failures are more prominent at night, we anticipated that lower ReSc and ReC at night would be related to lower vigilance performance and lower markers of alertness.

Material and methods

Except for TMS Response Scattering (ReSc) and Response Complexity (ReC), data analyses are as in (Chellappa et al., 2016; Ly et al., 2016). The following section details nevertheless all aspects of the protocol and analyses.

Participants

The study was approved by the Ethics Committee of the Medicine Faculty of the University of Liège. Participants gave their written informed consent. Twenty four healthy Caucasian men (18–30 yr.) were enrolled. Women were excluded from the study as changes in ovarian hormones may influence cortical excitability in humans (Smith et al., 2002). Other exclusion criteria included: 1) Body Mass Index (BMI) ≤ 18 and ≥ 25; 2) psychiatric history, severe trauma, sleep disorders; 3) addiction, chronic medication; 4) smokers, excessive alcohol (>14 doses/week) or caffeine (>3 cups/day) consumption; 5) night shift workers during the last year; 6) transmeridian travel during the last two months; 7) anxiety or depression; 8) poor sleep quality; 9) excessive self-reported daytime sleepiness. One participant was excluded due to melatonin phase-delay > 6 h compared to the remainder of the sample. Thus, data presented here include 23 participants. Table 1 summarizes the demographic characteristics of the final study sample.

Anxiety was measured by the 21 item Beck Anxiety Inventory (BAI ≤ 14) (Beck et al., 1988); mood by the 21 items Beck Depression Inventory II (BDI-II ≤ 14) (Steer et al., 1997); sleep quality by the Pittsburgh Sleep Quality Index Questionnaire (PSQI ≤ 7) (Buysse et al., 1989); daytime propensity to fall asleep in non-stimulating situations by the Epworth Sleepiness Scale (ESS ≤ 11) (Johns, 1991); chronotype by the Horne-Östberg Questionnaire (lower than 42: evening types; 42–58: intermediate types; higher than 58: morning types) (Horne and Östberg, 1976).

Table 1
Sample demographics, questionnaires scores (mean $\pm$ SD).

N	23
Age (yr.)	22.74 (2.58)
Ethnicity	Caucasian
BMI (kg/m^2)	22.13 (2.07)
Right handed	17/23
Anxiety level (BAI)	1.17 (1.90)
Mood (BDI-II)	1.61 (2.10)
Caffeine (cups/day)	0.39 (0.50)
Alcohol (doses/week)	3.26 (3.21)
Sleep quality (PSQI)	4.10 (1.12)
Daytime propensity to fall asleep (ESS)	3.57 (2.78)
Chronotype (HO)	52.35 (4.92)
Sleep time (hh:min, sleep diary)	23:23 (47 min)
Wake time (hh:min, sleep diary)	7:24 (47 min)
Sleep time (hh:min, actigraphy)	23:29 (47 min)
Wake time (hh:min, actigraphy)	7:27 (46 min)

Experimental protocol

Participants first completed a pretest TMS-EEG session to determine optimal TMS parameters. The left or right supplementary motor area (SMA) was set as stimulation target for right or left-handed individuals, respectively. This brain area was chosen for the following reasons: 1) similar to the entire frontal lobe, the SMA is sensitive to sleep pressure (Huber et al., 2013; Ly et al., 2016); 2) it has extensive fronto-parietal cortico-cortical connections, making this area interesting for studying effective connectivity (Massimini et al., 2010, 2005; Rosanova et al., 2012); 3) it is engaged in continuous visuomotor vigilance task (Maquet et al., 2003; Poudel et al., 2013); 4) its stimulation does not trigger muscle activation artefact.

Participants completed a screening night of sleep to exclude major sleep disorders. During the 7 days preceding the study, they kept a regular 8 h sleep-wake schedule (±15 min; verified using wrist actigraphy -Actiwatch, Cambridge Neurotechnology, UK- and sleep diaries). Participants were requested to abstain from all caffeine and alcohol-containing beverages for 3 days preceding the study.

For the experiment *per se*, participants were maintained in dim-light for 6 h (<5 lux), prior to sleeping for 8 h at their habitual bedtime (in complete darkness). The TMS-compatible electrode cap was placed upon awaking prior to the 29 h of sustained wakefulness period under constant routine conditions (i.e. light <5 lux, temperature ~19 °C, regular isocaloric liquid meals and water, semi-recumbent position, no time-of-day information, sound proofed rooms). These conditions aim at minimizing external and internal factors masking circadian rhythmicity (Duffy and Dijk, 2002). Spontaneous quiet eyes-open waking EEG and TMS-evoked EEG potentials (TEP) were recorded 8 times during the prolonged wakefulness period to cover the entire near-24 h circadian cycle, with higher session frequency around the circadian wake maintenance (WMZ) and sleep promoting zones (SPZ) (Dijk and Czeisler, 1995) (1100, 1700, 2100, 2300, 0200, 0600, 0800, 1100, for a subject sleeping from 2400 to 0800; Fig. 1). Behavioral test batteries, including the psychomotor vigilance task (PVT), were carried out 12 times during the protocol in between TMS-EEG sessions (1200, 1400, 1600, 1800, 2000, 2200, 2400, 0300, 0500, 0700, 0900, 1200). Subjective sleepiness was assessed hourly using the Karolinska Sleepiness Scale (KSS) (Åkerstedt et al., 2014). Saliva samples were collected hourly for melatonin assays.

TMS-evoked EEG response acquisitions

TMS pulses were generated by a Focal Bipulse 8-coil (Eximia; Nexstim, Helsinki, Finland). Stimulation target (SMA) was located on individual structural MRI by means of a neuronavigation system (Navigated

Brain Stimulation; Nexstim). This device allows for reproducible evoked EEG responses (Rosanova et al., 2012) and precise target location (FDA approval for presurgery). Each session included 250–300 trials. Inter-stimulus intervals were randomly jittered between 1900 and 2200 ms. TMS responses were recorded with a 60-channel TMS-compatible EEG amplifier (Eximia; Nexstim), equipped with a proprietary sample-and-hold circuit that provides TMS artifact free data from 5 ms post-TMS (Virtanen et al., 1999). Electrooculogram was recorded with two additional bipolar electrodes. Participants wore the EEG cap during the entire constant routine protocol, and electrodes impedance was set below 5 kΩ prior to each recording session. Signal was band-pass-filtered between 0.1 and 500 Hz and sampled at 1450 Hz. Each TMS-EEG session ended with a neuronavigated digitization of the location of each electrode. Auditory EEG potentials (AEP) evoked by TMS and bone conductance were minimized by diffusing a continuous loud white masking noise through earplugs, and applying a thin foam layer between the EEG cap and the TMS coil (Rosanova et al., 2012). Each session was followed by a sham session consisting in 30–40 TMS pulses delivered parallel to the scalp while white noise was diffused at the same level. Absence of AEP was checked online on Cz between 0 and 500 ms post-TMS (all sessions were AEP-free). Data of sham sessions were not considered any further in ReSc and ReC analyses.

Spontaneous waking EEG acquisition

Spontaneous quiet eyes-open waking EEG (WEEG) was recorded prior to each TMS session using the same 60-channel TMS-compatible EEG (+2 electro-occulogram - EOG - channels) amplifier (Eximia; Nexstim). Participants were instructed to fix a black dot during 2 min while relaxing and suppressing blinking.

Visuomotor vigilance task

While recording TMS-evoked EEG responses, participants performed a visuomotor compensatory tracking task (CTT) to monitor their vigilance as in (Huber et al., 2013). The task consisted of keeping a constantly randomly moving cursor on a target located in the center of a computer screen, using a trackball device. This task recruits the fronto-parietal cortex (Poudel et al., 2013) and was used for correlations with ReSc as a measure of vigilance level. The task was preferred to the PVT (see 2.6.) during TMS-EEG recording because it requires continuous smooth and limited movement of a single finger, compared to the burst-like muscular activity engaged by PVT that could be time-locked to TMS evoked EEG responses. Performance was computed as the average distance (in pixels) between the cursor and the target during TMS-EEG recordings. If signs of drowsiness were detected while performing the
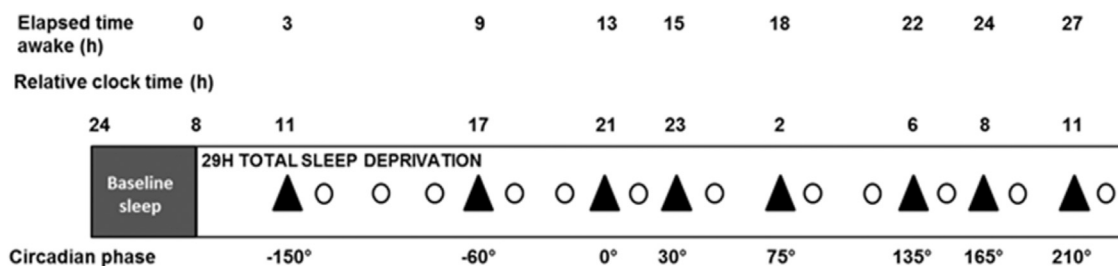
Fig. 1. Experimental protocol.
After an 8 h baseline night of sleep under polysomnographic recording, participants (N = 23) underwent 29 h of sustained wakefulness under constant routine conditions (no time-of-day information, dim light < 5 lux, temperature 19 °C, regular isocaloric liquid intake, semi-recumbent posture, sound proofed rooms). TMS-evoked EEG potentials (TEP) were recorded 8 times (~250 trials per session; black triangles) over the frontal cortex contralateral to the dominant hand (i.e. mostly left frontal cortex), while participants performed a visuomotor vigilance compensatory tracking task (CTT). Each TMS-EEG session was preceded by 2 min quiet eyes-open wake EEG recording (WEEG). In-between TMS-EEG sessions, 12 behavioural test batteries were administered (circles) - including the psychomotor vigilance task (PVT). Saliva samples were collected hourly for melatonin assays, allowing a posteriori data realignment based on individual endogenous circadian timing. Subjective sleepiness measures were collected hourly. Time is expressed in circadian phase (°) and equivalent elapsed time awake (h). Relative clock time displayed here is for a participant with a 2400–0800 sleep-wake schedule. For simplicity, elapsed time awake and clock time are given as integer values but 0.5 h should be added.

task during TMS-EEG sessions, the experimenter briefly touched the participant. Transitory lapses of vigilance resulted in temporary increases of the target-cursor distance, and could be automatically detected offline. A lapse was identified when the cursor was located outside a central 200 by 200 pixel box surrounding the target for >500 ms from the last trackball movement. The lapse period ranged from the last trackball movement until the lapse detection. TMS evoked responses occurring during and <1 s from a lapse period were discarded from analyses.

Psychomotor Vigilance Task (PVT)

In between TMS-EEG assessments, participants were required to press a computer space bar as soon as an auditory signal occurred (presented at a random interval of 3–7 s (Graw et al., 2004)). We opted for an auditory version, because it would lead to fewer lapses of vigilance and potential micro-sleep episodes, which would have biased our results (Jung et al., 2011). The PVT lasted 5 min (Roach et al., 2006). Performance was inferred from the median reaction time following removal of lapses (>500 ms), anticipation (<100 ms) and error (>3000 ms) (Dinges and Powell, 1985).

Melatonin

Saliva samples were first placed at 4 °C, prior centrifugation and congelation at −20 °C within 12 h. Salivary melatonin was measured by radioimmunoassay (Stockgrand Ltd, Guildford, UK), as previously described (English et al., 1993). The limit of detection of the assay for melatonin was 0.8 ± 0.2 pg/ml using 500 μL volumes.

TMS-EEG data pre-processing and source reconstruction

TMS-EEG data were processed using SPM12 software package (Statistical Parametric Mapping 12, http://www.fil.ion.ucl.ac.uk/spm/) implemented in Matlab 2011a (The Mathworks Inc, Natick, MA). Continuous EEG recordings were re-referenced to the average of all good channels, low-pass filtered at 80 Hz, resampled from 1450 to 1000 Hz, and high-pass filtered at 1 Hz, split into epochs between −100 and 300 ms around TMS pulses, and baseline corrected (−100 to −1 ms pre-TMS). Robust averaging was applied to compute the mean evoked response of each session (Leonowicz et al., 2005). Source reconstruction was computed on the averaged TMS-evoked EEG response of each recording session (up to 300 ms post-TMS), to obtain a spatio-temporal history of the significant cortical sources responsible for the observed EEG pattern. Sensor and fiducial positions were used for realistic head model based on individual structural image (conductive head volume model based on Boundary Element Method (BEM), i.e. 3 compartments of fixed conductivities: scalp, skull, brain). All analyses were performed within the individual subject space. The cerebral cortex was modeled using 5124 dipoles oriented normally to the cortical surface. For the inverse reconstruction, "Multiple Sparse Prior" (MSP) was adopted, because (i) it produces more accurate localizations (Friston et al., 2008), and (ii) model comparison (computed as the difference of log model evidence (Mattout et al., 2006)) indicated that "MSP" was more appropriate than the "Loreta" approach for our dataset (i.e. difference in log evidence higher than 3). MSP source reconstruction resulted in patches of currents that were transformed in a binary spatio-temporal distribution of statistically significant sources over the 300 ms post-TMS. To determine source electrical activity that was "truly" induced by TMS, standardization was performed. A source electrical activity higher than 4 standard deviation from the mean TMS baseline activity was considered as significant and allocated 1 (0 if non-significant). The cut-off of 4 Z-Score allows a false positive rate of less than 0.01% (i.e. $p < 0.0001$) (Casali et al., 2010). This procedure was applied to each source and to each time bin. The resulting binary spatio-temporal matrix allowed the identification of statistically significant sources over the 300 ms post-TMS. The matrix was then masked with a fronto-parietal 3D mask (WFU PickAtlas; http://fmri. wfubmc.edu/software/PickAtlas, based on Talairach Daemon database, implemented in SPM12), i.e. all significant sources outside the fronto-parietal 3D mask were set to zero. This approach did not include the thalamus and focused solely on the cortical mantle.

During TMS acquisitions, participants were performing a continuous visuomotor vigilance task, recruiting fronto-parietal regions (Poudel et al., 2013) as well as the occipital cortex (Chee et al., 2011). However, TMS by-passes afferent sensory systems to trigger brain responses, and TMS-evoked responses were not time-locked to any particular event related to the task. Our protocol allowed therefore to focus only on core vigilance cortical regions -i.e. the fronto-parietal cortex- without considering areas involved in sensory processing. For completeness, we computed a supplementary analysis including the occipital areas, i.e. fronto-parietal-occipital (FPO) mask (refer to Supplementary Fig. 1).

Synthetic indices of Response Scattering (ReSc) and Response Complexity (ReC)

Indices were computed based on the binary spatio-temporal source matrix ST (x,t) (Fig. 2). For computation of both indices the first 5 ms were discarded to avoid possible artefacts contamination. *Response Scattering (ReSc)* was measured based on the scattering of significant current, i.e. the spatial spreading of the significant electrical activity elicited by TMS pulses. ReSc is a measure of effective connectivity: it originates at the stimulation hotspot and propagates over an ensemble comprised within the fronto-parietal cortex. It is however distinct from other types of effective connectivity measures between specific brain regions (Moran et al., 2013). ReSc was computed as the sum of the geodesic distance between any significant sources within the fronto-parietal cortex and the TMS target, averaged either over the entire 5–300 ms period post-TMS, or in 50 ms bins over the 300 ms (first bin: 5–50 ms post-TMS). A higher ReSc index means that the initial perturbation reaches more sources and/or more distant sources over the cortical brain surface. *Responses Complexity (ReC)* (first conceptualized by (Tononi et al., 1994)) was derived by applying the Lempel-Ziv compression algorithm on the fronto-parietal binary matrix of significant sources followed by normalization, as in (Casali et al., 2013). It is therefore a proxy of the neuronal information content following stimulation (Aboy et al., 2006). A lower ReC means that the brain response is more stereotypical, less variable over time and space, but is not directly related to the scattering of the response. A large and widespread response could still contain little pattern variations and have low ReC. Importantly, in the current study, source reconstruction model (MSP instead of Loreta) and significant sources determination (ST (x,t) matrix), which precede ReSc and ReC computation, were different than the original publications (Casali et al., 2013, 2010): direct comparison of absolute values between studies is therefore not pertinent.

Spontaneous waking EEG analyses

Data preprocessing was performed using SPM12. Continuous EEG recordings were band-pass filtered between 0.1 and 500 Hz and resampled from 1450 to 500 Hz. Data were then manually and visually scored offline for artefacts and microsleep episodes (eye blinks, body movements, and slow eye movements), using FASST toolbox (http://www. montefiore.ulg.ac.be/~phillips/FASST.html). Power spectral densities were computed using a fast Fourier transform on artifact-free 4 s windows, overlapping by 2 s, using the Welch's method (pwelch function in MATLAB 2011a) (Welch, 1967). EEG activity was computed over frontal-parietal regions for delta (0.75–4 Hz), theta (4.5–7.5 Hz), alpha (8–12 Hz), sigma (12.5–18 Hz) and beta (18.5–30 Hz) frequency bands over the entire 2 min recording.

Statistics

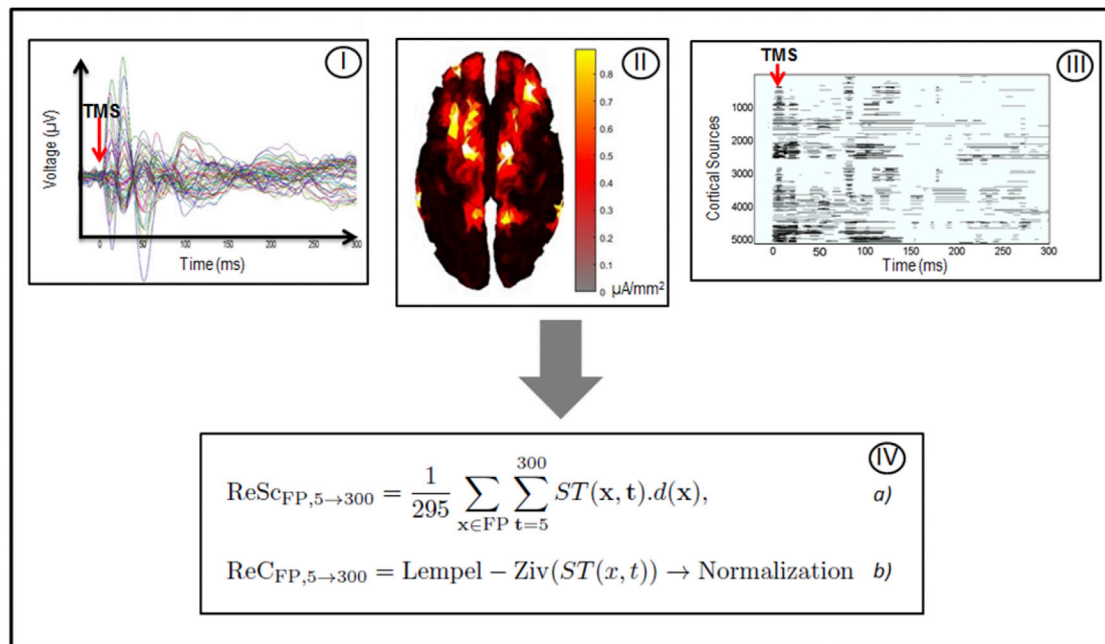Statistical analyses were performed with SAS version 9.3 (SAS

Fig. 2. TMS-EEG data processing.
I.) Butterfly plot of the average TMS-evoked response in all 60 EEG channels over 300 ms post TMS. **II.)** EEG source reconstruction showing the inferred spatio-temporal history of the electrical activity over the cortical surface. **III.)** The spatio-temporal history is transformed into a binary spatio-temporal matrix ST (x,t): for each time bin, significant fronto-parietal sources were allocated 1 (resp. 0 when non-significant/outside fronto-parietal cortex). **IV.)** Equations underlying the synthetic indices of cortical responsiveness. *a) Response Scattering (ReSc)*, where ST (x,t) is the binary spatio-temporal matrix, with x = [1:5124] indexing the cortical source dipoles and t = [0:300] the post-TMS interval in ms, and d(x) being the geodesic distance between the TMS hotspot and source (x). ReSc is the sum, from 5 to 300 ms, of the geodesic distances between all significant fronto-parietal sources ($x \epsilon FP \wedge x = 1$) and the TMS stimulation area, averaged over 295 ms of the 5–300 ms post-TMS period. *b) Response Complexity (ReC):* derived by applying the Lempel-Ziv algorithm to the binary matrix ST (x,t), followed by normalization.

Institute, Cary, NC, USA). Fronto-parietal ReSc, ReC, PVT and KSS values were standardized by computing z-scores at the individual level across circadian phases (PROC STANDARD). CTT was normalized by dividing the performance to the task duration, and then z-scored (technical issues prevented CTT to be properly recorded for one participant that was discarded, $N_{CTT} = 22$). WEEG activity was averaged across channels within the fronto-parietal region, and normalized by dividing each power band by the sum of all frequencies within 0.75 and 30 Hz (i.e. relative activity). The time-course of all variables was examined with mixed-model analyses of variance for repeated measures (PROC MIXED), with "circadian phase" as fixed factor and "subject" as random factor. For two within-subject factors, i.e. "circadian phase" and "bin" a general linear model was used (PROC GLM, predictors based on type III SS). Differences between circadian phases were assessed with LSMEANS statement. All P-values were based on Kenward-Roger's corrected degrees of freedom and were adjusted for multiple testing with Tukey's procedure.

All data were realigned to individual dim light melatonin onset (DLMO). Estimation of circadian phase (where $0° =$ individual DLMO) was determined based on raw values. The 4 first samples were disregarded and maximum secretion level was set as the median of the 3 highest concentrations. Baseline level was set to be the median of the values collected from "wake-up time +5 h" to "wake-up time +10 h". DLMO was computed as the time at which melatonin level reached 20% of the baseline to maximum level (linear interpolation). All data points were grouped in the following circadian phase bins: −150° [-180° to −130°], −60° [-105° to −15°], 0° [-15°–15°], 30° [15°–45°], 75° [45°–105°], 135° [120°–150°], 165° [150°–180°], 210° [180°–240°] (i.e. each data point was attributed to its closest bin).

Pearson's Correlations (PROC CORR) were performed between ReSc, WEEG markers and CTT performance. Values distribution was checked for normality by visual inspection and based on Shapiro-Wilk test. Non-parametric equivalent tests were used for non-normally distributed variables (Spearman's rank correlations). Correlations were first considered only for night-time data points, when one should be asleep, i.e. the first circadian point that belongs to the night (75°) until the end of it (165°, around wake up time). The difference between the last (165°) and first (75°) night-time data point was also computed to assess the night-time decline in ReSc, and CTT performance. Six participants (missing data for one of these circadian phases) and one outlier (+3 SD) were discarded. Correlation analyses for night-time decline included 16 participants. Similar correlations were also computed using daytime values (circadian phases: −150°, −60°, 0°) to assess whether the significant correlations were specific to the night-time period.

Results

Time course of objective and subjective measures of sleepiness and vigilance performance

In all analyses data were realigned according to the circadian phase 0°, which corresponds to the onset of melatonin secretion.

As expected, EEG recordings of spontaneous waking fronto-parietal activity showed that relative theta (4.5–7.5 Hz) and alpha power (8–12 Hz) -objective physiological markers of sleepiness and alertness (Strijkstra et al., 2003)- significantly varied during the protocol (Fig. 3B–C) (n = 23; PROC MIXED main effect of circadian phase; theta: $F_{(7, 118)} = 5.99$; P < 0.0001; alpha: $F_{(7, 117)} = 5.45$; P < 0.0001). Likewise, relative fronto-parietal delta power (0.75–4 Hz) displayed a trend (Fig. 3A) (n = 23; PROC MIXED, main effect of circadian phase; $F_{(7, 119)} = 2.03$; P = 0.057). Hourly subjective sleepiness scores (KSS) significantly varied with circadian phase (Fig. 3D) (n = 23; PROC MIXED, main effect of circadian phase; $F_{(25, 437)} = 6.57$; P < 0.0001). The time course of these subjective and objective measures of sleepiness and alertness reflects the expected dual influence of sleep homeostasis and circadian phase (Dijk and Czeisler, 1995): a fairly stable profile during the day compared to the night (−150°, −60°, 0°, or 30° vs. 135° or 165°:

$P_{corr} < 0.048$). Importantly, within the night-time period (75° vs. 165°), Tukey post-hoc test revealed a significant increase in relative theta power ($P_{corr} = 0.049$), and decrease in relative alpha power ($P_{corr} = 0.044$), as well as a tendency for an increase in relative delta power ($P_{corr} = 0.09$).

As expected also, the progressive intrusion of WEEG slow frequencies during the night-time period was accompanied by a drop in PVT performance, assessed *in between* TMS-EEG recordings (Fig. 3E) (n = 23; PROC MIXED, main effect of circadian phase; F (11, 224) = 6.24;

$P < 0.0001$). Vigilance was also assessed *simultaneously* to TMS-EEG recordings using a visuomotor vigilance task (compensatory tracking task, CTT). CTT displayed a pattern similar to PVT (Fig. 3F) (n = 22; PROC MIXED, main effect of circadian phase; F (7, 122) = 13.78; $P < 0.0001$): good performance during day compared to night time (−150°, −60°, 0°, or 30° vs. 135° or 165°: $P_{corr} < 0.003$), contrasting with a sharp overnight decrement (75° vs. 165°: $P_{corr} = 0.003$), and a partial recovery the subsequent morning (165° vs. 210°: $P_{corr} = 0.0025$).
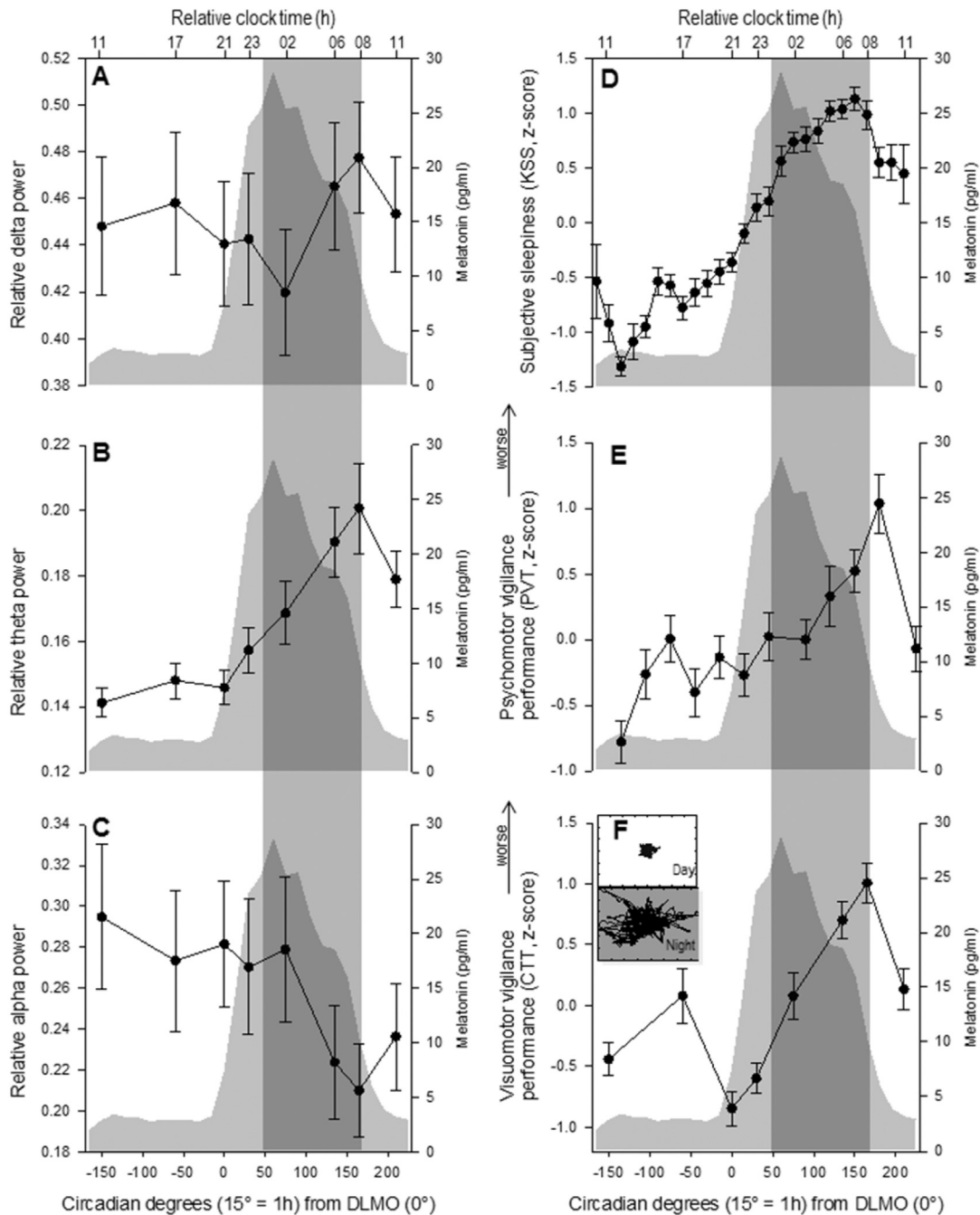
Fig. 3. Time courses of objective and subjective measures of sleepiness and vigilance performance (means and standard errors). **A.** Relative fronto-parietal delta power (0.75–4 Hz) (n = 23; PROC MIXED, main effect of circadian phase; F (7, 119) = 2.03; P = 0.057). **B.** Relative theta power (4.5–7.5 Hz) (n = 23; PROC MIXED, main effect of circadian phase; F (7, 118) = 5.99; P < 0.0001). **C.** Relative alpha power (8–12 Hz) (n = 23; PROC MIXED, main effect of circadian phase; F (7, 117) = 5.45; P < 0.0001). **D.** Subjective sleepiness (n = 23; PROC MIXED, main effect of circadian phase; F (25, 437) = 6.57; P < 0.0001). **E.** PVT performance (median reaction times; n = 23; PROC MIXED, main effect of circadian phase; F (11, 224) = 6.24; P < 0.0001). **F.** Compensatory tracking task performance (CTT; n = 22; PROC MIXED, main effect of circadian phase; F (7, 122) = 13.78; P < 0.0001). Insets: representative performance to the task; cursor remains close to target (screen centre) during the day, while it deviates during night-time wakefulness. The light gray area represents the average melatonin profile (0° = dim light melatonin onset (DLMO)). All variables are plotted in degree (15° = 1h) relative to DLMO. The dark gray bars indicate night-time period for a participant with 2400–0800 sleep-wake schedule.

Fronto-parietal response scattering varies during prolonged wakefulness but not cortical response complexity

Following these first analyses, we turned to fronto-parietal ReSc, our main focus of interest. The scattering of the electrical current significantly varied with circadian phase (Fig. 4A) (n = 23; PROC MIXED, main effect of circadian phase; F (7, 135) = 2.09; P = 0.049). Tukey post-hoc tests between the different circadian phases were non-significant following correction for multiple comparisons ($P_{corr} > 0.05$). However, qualitative inspection of the data, as well as uncorrected post-hoc tests, suggest that ReSc increased from day to evening and early night (−150° or −60° vs. 0°, 30° or 75°: $P_{uncorr} < 0.04$), and from the first morning

session up to the last morning session (i.e. 24 h later; −150° vs. 210: $P_{uncorr} = 0.03$). During the night, a visual decline was perceptible (75° vs 135° or 165°: $P_{uncorr} > 0.05$). A similar pattern was observed when considering the occipital cortex within the fronto-parietal-occipital mask (Supplementary Fig. 1).

Since ReSc varied with circadian phases, we also investigated whether the within session temporal dynamics differed according to the circadian phase. We decomposed ReSc in six bins of 50 ms over the 300 ms post-TMS period. This additional analysis showed that fronto-parietal ReSc significantly varied from bin to bin and between circadian phases, but without a bin x circadian phase interaction (Fig. 4B) (n = 23; PROC GLM, model: F (47) = 2.79, P < 0.0001, $R^2 = 0.11$; circadian phase: F
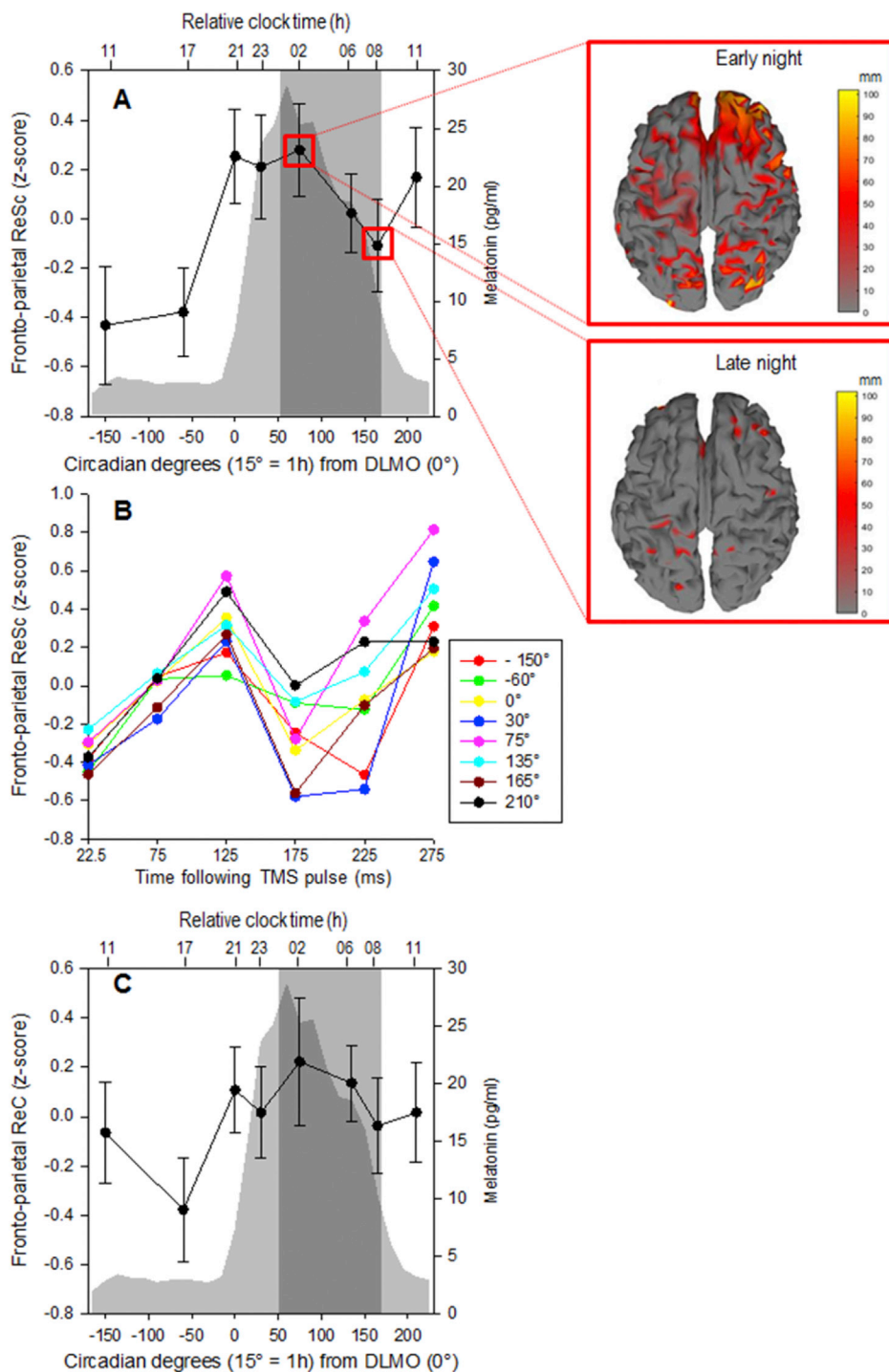
Fig. 4. Response Scattering (ReSc) and Response Complexity (ReC) during 29 h of sustained wakefulness (means and standard errors, z-scores). **A.** Fronto-parietal ReSc significantly varied during the protocol (n = 23; PROC MIXED, main effect of circadian phase; F (7, 135) = 2.09; P = 0.049). The right panels are displayed as representative examples of ReSc in the early and late night for a representative subject. Colour code corresponds to the distance from the supplementary motor area (SMA) stimulated by TMS. **B.** When divided in six bins of 50 ms over the 300 ms post-TMS (midpoint of bin plotted; first bin: 5–50 ms post-TMS), fronto-parietal ReSc significantly varied from bin to bin and between circadian phases, but the bin x circadian phase interaction was not significant (n = 23; PROC GLM, model: F (47) = 2.79, P < 0.0001, $R^2 = 0.11$; circadian phase: F (7) = 2.21, P = 0.03; bin: F (5) = 18.54, P < 0.0001; bin*circadian phase: F (35) = 0.69, P = 0.9). Standard errors were omitted for clarity. **C.** Fronto-parietal ReC did not significantly vary during prolonged wakefulness (n = 23; PROC MIXED, main effect of circadian phase; F (7, 137) = 0.83; P = 0.56). The light gray area represents the average melatonin profile (0° = dim light melatonin onset (DLMO)). All variables are plotted in degree (15° = 1h) relative to DLMO. The dark gray bars indicate night time period for a participant with 2400–0800 sleep-wake schedule.

(7) = 2.21, P = 0.03; bin: F (5) = 18.54, P < 0.0001; bin*circadian phase: F (35) = 0.69, P = 0.9). The within session temporal dynamics appears therefore to remain similar across sessions.

Given that the power in slower spontaneous waking EEG oscillations and ReSc significantly varied during the protocol, we further asked whether fronto-parietal response complexity (ReC) would change as well. Contrary to our hypothesis, ReC did not vary significantly during prolonged wakefulness (Fig. 4C) (n = 23; PROC MIXED, main effect of circadian phase; F (7, 137) = 0.83; P = 0.56).

Worse vigilance correlates with lower fronto-parietal response scattering at night

Our analyses then focused on how fronto-parietal ReSc translated to *simultaneous* vigilance performance during night-time period, when vigilance is mostly affected by prolonged wakefulness. A significant correlation between ReSc and performance at the visuomotor vigilance task was found (Fig. 5A) (rs (54) = −0.35, P = 0.01), suggesting that lower fronto-parietal ReSc was associated with worse vigilance performance
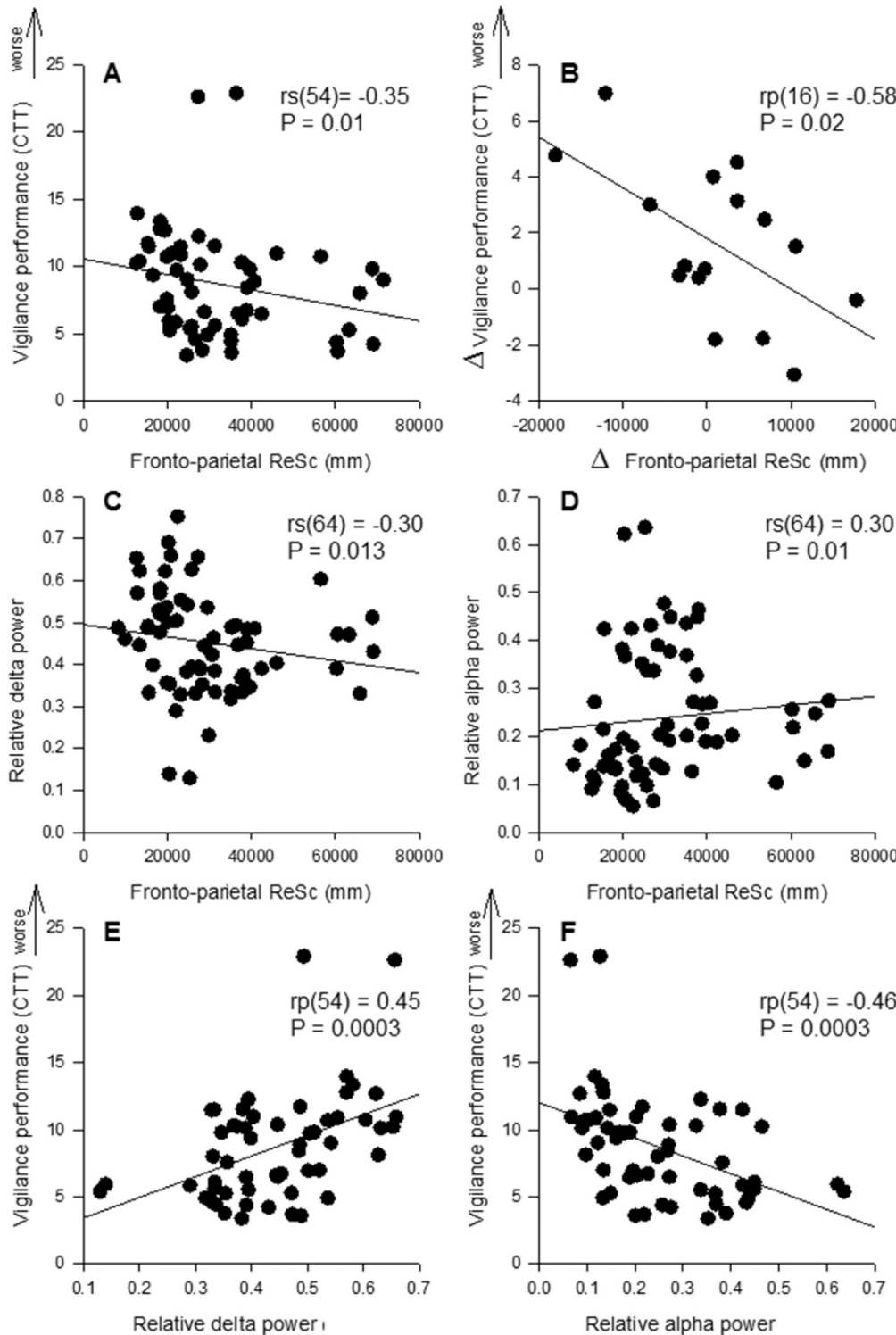
Fig. 5. Associations between fronto-parietal response scattering, vigilance performance and relative wake EEG power spectra at night.

The following correlations include data collected at 75°, 135°, 165°, which were considered together irrespective of circadian phase. For each plot, the correlation coefficient [Pearson: rp; Spearman: rs; (degree of freedom)] and the corresponding P-value are reported. A. Significant correlation between fronto-parietal ReSc and CTT performance at night. Higher ReSc is associated to better vigilance performance. B. Significant correlation between decrease in fronto-parietal ReSc and decrement in CTT performance from the beginning to the end of the night-time period (Δ = 165°–75°). C. Significant negative correlation between fronto-parietal ReSc and relative delta power (0.75–4 Hz) of the spontaneous WEEG recordings. D. Significant positive correlation between fronto-parietal ReSc and relative alpha power (8–12 Hz) of the spontaneous WEEG recordings. E. Significant positive correlation between relative delta power and CTT performance. F. Significant negative correlation between relative alpha power and CTT performance.

during night-time wakefulness. Importantly, ReSc did not correlate with CTT performance during a normal waking day ($-150°–30°$; rs (74) $= -0.09$, P $= 0.45$).

In a next step, we computed the difference between the first and last point within our night-time window ($\Delta = 165°–75°$). The night-time period is indeed a heterogeneous window: important neurobehavioral impairments start at the end of a normal waking day and reach the peak at the end of the night, around the circadian sleep promoting zone (SPZ) (Wright et al., 2012). A marked decrease in ReSc was significantly associated with a marked decline in CTT performance over this time window (Fig. 5B) (rp (16) $= -0.58$, P $= 0.02$). This indicates that participants maintaining or increasing ReSc overnight were those having better vigilance performance.

ReSc measure was also significantly and negatively associated with relative delta (Fig. 5C) (rs (64) $= -0.30$, P $= 0.013$), but not theta power (rs (64) $= 0.17$, P $= 0.16$) of the spontaneous night-time waking EEG recordings, while it was significantly and positively correlated with relative alpha power (Fig. 5D) (rs (64) $= 0.3$, P $= 0.01$). These latter results suggest that higher fronto-parietal ReSc at night is associated with lower sleepiness and higher alertness. Further correlations showed that higher delta and lower alpha power were associated with worse performance to the visuomotor vigilance task at night (Fig. 5E–F) (delta: rp (54) $= 0.45$, P $= 0.0003$; theta: rp (54) $= 0.25$, P $= 0.06$; alpha: rp (54) $= -0.46$, P $= 0.0003$).

Discussion

In this study, we tested whether a reduction of fronto-parietal response scattering (ReSc) and response complexity (ReC) could contribute to the vigilance impairment typically observed during night-time wakefulness. We perturbed brain activity with TMS and recorded the propagation of the triggered response over the cortical surface with EEG. Fronto-parietal TMS response scattering, as assessed following EEG source reconstruction, significantly changed with circadian phase, while, contrary to our expectations, response complexity did not. Data further suggest that ReSc tended to decrease during night-time wakefulness and, in line with our prediction, lower night-time level was correlated with worse vigilance performance and lower alertness. Furthermore, the extent of the night-time decrease in ReSc was correlated to the decline of vigilance performance.

The fronto-parietal cortex includes many polymodal associative areas and is very active during wakefulness. Beyond vigilance regulation, it is heavily involved in higher cognitive processes and in the top-down control of attention (Chee and Tan, 2010). This region shows substantial variations in the amount of slow activity rhythms during both wakefulness and sleep, indicating that it is a site of important homeostatic sleep pressure accumulation and dissipation (Cajochen et al., 1999). Although variations between circadian phases did not reach post-hoc statistical significance, fronto-parietal ReSc profile seemed to change non-linearly as a function of prolonged wakefulness, suggesting a dual influence of sleep homeostasis together with the circadian timing system (Borbély et al., 2016). Moreover, fronto-parietal ReSc seemed to increase from the first recording in the morning after sleep to the last recording in the morning after a night without sleep (24 h later). A period of night-time sleep appears therefore necessary to bring back ReSc to baseline level. Thus, although this should be formally tested, we assume that ReSc after recovery sleep would be similar to the first session. After the night time period, ReSc seems to increase again, i.e. it increased accross the last 2 sessions of the protocol (i.e. from 0800 to 1100). This might suggest a circadian influence that switches from sleep to wake promotion around that period (Dijk and Czeisler, 1994).

Our data suggest that fronto-parietal ReSc increased during a normal waking day, which is reminiscent of a previous study reporting significant MRI-based functional connectivity alterations from morning to evening of a normal waking day (Kaufmann et al., 2016). This daytime variation does not seem detrimental for the visuomotor vigilance

performance, which is typically good and stable during a normal waking day (Gaggioni et al., 2014). It is only at night that visuomotor vigilance performance drops, when both sleep homeostatic and circadian processes greatly challenge cognitive abilities. In line with our hypothesis, we found a significant correlation between night-time fronto-parietal ReSc and simultaneous measures of vigilance, indicating that a relative reduction in fronto-parietal ReSc at night is associated with worse vigilance performance. In addition, individuals showing marked vigilance impairment over the night-time period had a more important decline in ReSc. Importantly, even resilient participants at night had lower CTT performance compared to daytime, reminding that sleep is necessary for assuring optimal performance. Nocturnal modifications in ReSc were accompanied by the intrusion of slow brain activity rhythms, typical of sleepiness and lower alertness level (Cajochen et al., 1999; Slater et al., 2017): we found that the relative decrease in ReSc at night was related respectively to increase of slower (delta), and decrease of faster (alpha) brain activity rhythms.

A relative reduction of ReSc at night suggests that fronto-parietal areas, sustaining vigilance, are less connected, or are less integrated when compared to the end of a normal waking day. It also implies that night-time integration level directly affect vigilance. This observation is similar to the previously reported link between increased spontaneous eyelid closures following sleep deprivation, as proxy for sleepiness level, and reduced functional connectivity within the default mode and dorsal/ventral attention networks (Wang et al., 2016). Using resting state functional MRI, a recent study (Ben Simon et al., 2017) also reported a reduced functional connectivity of the brain following sleep deprivation based on Graph modularity measures, and subsequent behavioral impairment. Our finding recalls the link between the decrease in effective connectivity within the cingulate cortex following sleep deprivation, and subsequent worse vigilance performance (Piantoni et al., 2013). Here we confirm and extend the latter observation to the fronto-parietal cortex and simultaneous vigilance assessment.

Effective connectivity is close to the intuitive notion of a connection (Büchel and Friston, 1997). Changes in effective connectivity may therefore reflect changes in structural brain connectivity. A day of wakefulness was associated with widespread increases in white matter fractional anisotropy ((FA), reflecting changes in axonal microstructure), whereas sleep deprivation triggered widespread FA decreases (Elvså-shagen et al., 2015), reminiscent of the ReSc variations we observed. In addition, higher FA within the fronto-parietal cortex while well rested was associated with better PVT performance during sleep deprivation (Cui et al., 2015). In contrast, participants with lower FA values within multiple brain regions while well rested had worse performance to a visuomotor task after sleep deprivation (Rocklage et al., 2009).

If effective connectivity allows insight about cortico-cortical interaction, cortical excitability informs about the responsiveness of the cortex. We previously showed that local cortical reactivity (i.e. measured at the electrode closest to the area stimulated by the TMS pulse) was stable during a normal waking day prior to increasing sharply during overnight wakefulness, and was correlated to CTT performance (Ly et al., 2016). With the present results, it seems that, following the initial responses, the degree of effective connectivity of the fronto-parietal cortex is also important for night-time vigilance performance. Thus, these results bring together different facets of the changes in neuronal response triggered by extended wakefulness: spatio-temporal changes in local excitability and in global fronto-parietal effective connectivity negatively affect behavior at night, and may thus represent a form of "neuronal tiredness" (Fisher and Vyazovskiy, 2014). During extended wake (beyond normal sleep time), neurons can undergo off periods similar to sleep, although the EEG shows signals typical of wakefulness (Vyazovskiy et al., 2011). Neuronal activity is therefore more synchronous. In our data, this is confirmed by an increase in the prevalence of slower EEG rhythms, which are associated with poorer performance. Delivering TMS pulses during sleep deprivation results in an increased local excitability (Huber et al., 2013; Ly et al., 2016), either because neurons reply more synchronously or

because more neurons respond to an external perturbation. Our results suggest that effective connectivity, as indexed by ReSc, increases first during the day, before local excitability increases, and then seems to show a relative decrease at night. Our results are in line with a recent paper showing that short and long range signal characteristics can differ importantly: the intrusion of off-period can compromise long range signal propagation during sleep deprivation (Meisel et al., 2017). One could therefore posit that the increased prevalence of off-line periods contribute in part to this disruption of long range response scattering. Likewise, since the thalamus plays an important role in vigilance regulation (Killgore et al., 2015), changes in thalamo-cortical loops could contribute to effective connectivity variation during prolonged wakefulness.

Sleep is characterized by a sharp reduction in effective connectivity (Massimini et al., 2005), and TMS-evoked response complexity (Casali et al., 2013). Given that our data showed changes in ReSc and in slow oscillation power, we expected changes in cortical response complexity: a simplification of the neuronal information content, concomitant with the night-time vigilance state instability. However, we did not find a significant difference, suggesting that cortical response complexity does not change during prolonged wakefulness. This result is in line with another study showing no significant variation of complexity during a partial sleep deprivation in rats (Abásolo et al., 2015).

Conclusions

Overall, our study shows that TMS applied over the frontal lobe triggers responses within the fronto-parietal cortex that vary as a function of wakefulness duration. It reinvigorates the concept that cortico-cortical transmission varies during prolonged wakefulness (Piantoni et al., 2013; Verweij et al., 2014; Yeo et al., 2015), and that lower effective connectivity is linked to worse vigilance performance and lower alertness level at night.

Acknowledgements

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.neuroimage.2018.03.055.

Authors' contribution

GG acquired and analyzed the data, wrote the paper. JQLM and SLC acquired and analyzed the data. MR and SS provided expertise for TMS-EEG acquisitions. MM designed the experiment, provided expertise for TMS-EEG acquisitions. BM performed melatonin assays. CS, AL and ES provided expertise for statistical analysis. DC, AC provided expertise for EEG data analyses. CP provided expertise and analysed EEG data and wrote the paper. GV designed the experiment, acquired and analyzed the data, wrote the paper. All authors edited the manuscript.

Disclosure statement

The authors declare no competing financial interests.

References

Abásolo, D., Simons, S., Morgado da Silva, R., Tononi, G., Vyazovskiy, V.V., 2015. Lempel-Ziv complexity of cortical activity during sleep and waking in rats. J. Neurophysiol. 113, 2742–2752. https://doi.org/10.1152/jn.00575.2014.

Aboy, M., Hornero, R., Abásolo, D., Álvarez, D., 2006. Interpretation of the lempel-ziv complexity measure in the context of biomedical signal analysis. IEEE Trans. Biomed. Eng. 53 https://doi.org/10.1109/TBME.2006.883696.

Aeschbach, D., Postolache, T.T., Sher, L., Matthews, J.R., Jackson, M.A., Wehr, T.A., 2001. Evidence from the waking electroencephalogram that short sleepers live under higher homeostatic sleep pressure than long sleepers. Neuroscience 102, 493–502. https://doi.org/10.1016/S0306-4522(00)00518-2.

Åkerstedt, T., Anund, A., Axelsson, J., Kecklund, G., 2014. Subjective sleepiness is a sensitive indicator of insufficient sleep and impaired waking function. J. Sleep. Res. 23, 242–254. https://doi.org/10.1111/jsr.12158.

Beck, A.T., Epstein, N., Brown, G., Steer, R.A., 1988. An inventory for measuring clinical anxiety: psychometric properties. J. Consult Clin. Psychol. 56, 893–897.

Ben Simon, E., Maron-Katz, A., Lahav, N., Shamir, R., Hendler, T., 2017. Tired and misconnected: a breakdown of brain modularity following sleep deprivation. Hum. Brain Mapp. 38, 3300–3314. https://doi.org/10.1002/hbm.23596.

Borbély, A.A., Daan, S., Wirz-Justice, A., Deboer, T., 2016. The two-process model of sleep regulation: a reappraisal. J. Sleep. Res. 25, 131–143. https://doi.org/10.1111/jsr.12371.

Büchel, C., Friston, K.J., 1997. Effective Connectivity and Neuroimaging. SPMcourse, Short Course Notes, pp. 1–18.

Buysse, D.J., Reynolds 3rd, C.F., Monk, T.H., Berman, S.R., Kupfer, D.J., 1989. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 28, 193–213.

Cajochen, C., Khalsa, S.B., Wyatt, J.K., Czeisler, C.A., Dijk, D.J., 1999. EEG and ocular correlates of circadian melatonin phase and human performance decrements during sleep loss. Am. J. Physiol. 277, R640–R649.

Cajochen, C., Wyatt, J.K., Czeisler, C.A., Dijk, D.J., 2002. Separation of circadian and wake duration-dependent modulation of EEG activation during wakefulness. Neuroscience 114, 1047–1060.

Casali, A.G., Casarotto, S., Rosanova, M., Mariotti, M., Massimini, M., 2010. General indices to characterize the electrical response of the cerebral cortex to TMS. Neuroimage 49, 1459–1468. https://doi.org/10.1016/j.neuroimage.2009.09.026.

Casali, A.G., Gosseries, O., Rosanova, M., Boly, M., Sarasso, S., Casali, K.R., Casarotto, S., Bruno, M.-A., Laureys, S., Tononi, G., Massimini, M., 2013. A theoretically based index of consciousness independent of sensory processing and behavior. Sci. Transl. Med. 5, 198ra105. https://doi.org/10.1126/scitranslmed.3006294.

Chee, M.W., Tan, J.C., Zheng, H., Parimal, S., Weissman, D.H., Zagorodnov, V., Dinges, D.F., 2008. Lapsing during sleep deprivation is associated with distributed changes in brain activation. J. Neurosci. 28, 5519–5528. https://doi.org/10.1523/JNEUROSCI.0733-08.2008.

Chee, M.W.L., Goh, C.S.F., Namburi, P., Parimal, S., Seidl, K.N., Kastner, S., 2011. Effects of sleep deprivation on cortical activation during directed attention in the absence and presence of visual stimuli. Neuroimage 58, 595–604. https://doi.org/10.1016/j.neuroimage.2011.06.058.

Chee, M.W.L., Tan, J.C., 2010. Lapsing when sleep deprived: neural activation characteristics of resistant and vulnerable individuals. Neuroimage 51, 835–843. https://doi.org/10.1016/j.neuroimage.2010.02.031.

Chellappa, S.L., Gaggioni, G., Ly, J.Q.M., Papachilleos, S., Borsu, C., Brzozowski, A., Rosanova, M., Sarasso, S., Luxen, A., Middleton, B., Archer, S.N., Dijk, D.-J., Massimini, M., Maquet, P., Phillips, C., Moran, R.J., Vandewalle, G., 2016. Circadian dynamics in measures of cortical excitation and inhibition balance. Sci. Rep. 6, 33661. https://doi.org/10.1038/srep33661.

Corbetta, M., Miezin, F.M., Shulman, G.L., Petersen, S.E., 1993. A PET study of visuospatial attention. J. Neurosci. 13, 1202–1226.

Coull, J.T., Jones, M.E., Egan, T.D., Frith, C.D., Maze, M., 2004. Attentional effects of noradrenaline vary with arousal level: selective activation of thalamic pulvinar in humans. Neuroimage 22, 315–322. https://doi.org/10.1016/j.neuroimage.2003.12.022.

Cui, J., Tkachenko, O., Gogel, H., Kipman, M., Preer, L.A., Weber, M., Divatia, S.C., Demers, L.A., Olson, E.A., Buchholz, J.L., Bark, J.S., Rosso, I.M., Rauch, S.L., Killgore, W.D.S., 2015. Microstructure of frontoparietal connections predicts individual resistance to sleep deprivation. Neuroimage 106, 123–133. https://doi.org/10.1016/j.neuroimage.2014.11.035.

Culham, J.C., Brandt, S.A., Cavanagh, P., Kanwisher, N.G., Dale, A.M., Tootell, R.B.H., Jody, C., Brandt, S.A., Cavanagh, P., Kanwisher, N.G., Dale, A.M., Tootell, R.B.H., Nov 1998. Cortical FMRI Activation Produced by Attentive Tracking of Moving Targets. J. Neurophysiol, 80 (5), 2657–2670.

De Havas, J.A., Parimal, S., Soon, C.S., Chee, M.W.L., 2012. Sleep deprivation reduces default mode network connectivity and anti-correlation during rest and task performance. Neuroimage 59, 1745–1751. https://doi.org/10.1016/j.neuroimage.2011.08.026.

Dijk, D.J., Czeisler, C.A., 1995. Contribution of the circadian pacemaker and the sleep homeostat to sleep propensity, sleep structure, electroencephalographic slow waves, and sleep spindle activity in humans. J. Neurosci. 15, 3526–3538.

Dijk, D.J., Czeisler, C.A., 1994. Paradoxical timing of the circadian rhythm of sleep propensity serves to consolidate sleep and wakefulness in humans. Neurosci. Lett. 166, 63–68. https://doi.org/10.1016/0304-3940(94)90841-9.

Dinges, D.F., Powell, J.W., 1985. Microcomputer analyses of performance on a portable, simple visual RT task during sustained operations. Behav. Res. Methods, Instrum. Comput. 17, 625–655.

Duffy, J.F., Dijk, D.J., 2002. Getting through to circadian oscillators: why use constant routines? J. Biol. Rhythm 17, 4–13.

Durmer, J.S., Dinges, D.F., 2005. Neurocognitive consequences of sleep deprivation. Semin. Neurol. 25, 117–129. https://doi.org/10.1055/s-2005-867080.

Elvsåshagen, T., Norbom, L.B., Pedersen, P.Ø., Quraishi, S.H., Bjørnerud, A., Malt, U.F., Groote, I.R., Westlye, L.T., 2015. Widespread changes in white matter microstructure after a day of waking and sleep deprivation. PLoS One 10. https://doi.org/10.1371/journal.pone.0127351 e0127351.

English, J., Middleton, B.A., Arendt, J., Wirz-Justice, A., 1993. Rapid direct measurement of melatonin in saliva using an iodinated tracer and solid phase second antibody. Ann. Clin. Biochem. 30 (Pt 4), 415–416.

Fisher, S.P., Vyazovskiy, V.V., 2014. Local sleep taking care of high-maintenance cortical circuits under sleep restriction. Sleep 37, 1727–1730. https://doi.org/10.5665/sleep.4156.

Friston, K., Harrison, L., Daunizeau, J., Kiebel, S., Phillips, C., Trujillo-Barreto, N., Henson, R., Flandin, G., Mattout, J., 2008. Multiple sparse priors for the M/EEG inverse problem. Neuroimage 39, 1104–1120. https://doi.org/10.1016/j.neuroimage.2007.09.048.

Friston, K.J., 2011. Functional and effective connectivity: a review. Brain Connect. 1, 13–36. https://doi.org/10.1089/brain.2011.0008.

Gaggioni, G., Maquet, P., Schmidt, C., Dijk, D., Vandewalle, G., 2014. Neuroimaging, cognition, light and circadian rhythms. Front. Syst. Neurosci. 8, 126. https://doi.org/10.3389/fnsys.2014.00126.

Graw, P., Kräuchi, K., Knoblauch, V., Wirz-Justice, A., Cajochen, C., 2004. Circadian and wake-dependent modulation of fastest and slowest reaction times during the psychomotor vigilance task. Physiol. Behav. 80, 695–701. https://doi.org/10.1016/j.physbeh.2003.12.004.

Horne, J.A., Östberg, O., 1976. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int. J. Chronobiol. 4, 97–110.

Huber, R., Maki, H., Rosanova, M., Casarotto, S., Canali, P., Casali, A.G., Tononi, G., Massimini, M., 2013. Human cortical excitability increases with time awake. Cereb. Cortex 23, 332–338. https://doi.org/10.1093/cercor/bhs014.

Johns, M.W., 1991. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 14, 540–545.

Jung, C.M., Ronda, J.M., Czeisler, C.A., Wright Jr., K.P., 2011. Comparison of sustained attention assessed by auditory and visual psychomotor vigilance tasks prior to and during sleep deprivation. J. Sleep. Res. 20, 348–355. https://doi.org/10.1111/j.1365-2869.2010.00877.x.

Kaufmann, T., Elvsåshagen, T., Alnæs, D., Zak, N., Pedersen, P., Norbom, L.B., Quraishi, S.H., Tagliazucchi, E., Laufs, H., Bjørnerud, A., Malt, U.F., Andreassen, O.A., Roussos, E., Duff, E.P., Smith, S.M., Groote, I.R., Westlye, L.T., 2016. The brain functional connectome is robustly altered by lack of sleep. Neuroimage 127, 324–332. https://doi.org/10.1016/j.neuroimage.2015.12.028.

Killgore, W.D.S., Vanuk, J.R., Knight, S.A., Markowski, S.M., Pisner, D., Shane, B., Fridman, A., Alkozei, A., 2015. Daytime sleepiness is associated with altered resting thalamocortical connectivity. Neuroreport 26, 779–784. https://doi.org/10.1097/WNR.0000000000000418.

Krause, A.J., Simon, E. Ben, Mander, B.A., Greer, S.M., Saletin, J.M., Goldstein-Piekarski, A.N., Walker, M.P., 2017. The sleep-deprived human brain. Nat. Rev. Neurosci. https://doi.org/10.1038/nrn.2017.55.

Leonowicz, Z., Karvanen, J., Shishkin, S.L., 2005. Trimmed estimators for robust averaging of event-related potentials. J. Neurosci. Methods 142, 17–26. https://doi.org/10.1016/j.jneumeth.2004.07.008.

Lim, J., Dinges, D.F., 2008. Sleep deprivation and vigilant attention. Ann. N. Y. Acad. Sci. 1129, 305–322. https://doi.org/10.1196/annals.1417.002.

Lo, J.C., Groeger, J.A., Santhi, N., Arbon, E.L., Lazar, A.S., Hasan, S., von Schantz, M., Archer, S.N., Dijk, D.-J., 2012. Effects of partial and acute total sleep deprivation on performance across cognitive domains, individuals and circadian phase. PLoS One 7, e45987. https://doi.org/10.1371/journal.pone.0045987.

Ly, J.Q.M., Gaggioni, G., Chellappa, S.L., Papachilleos, S., Brzozowski, A., Borsu, C., Rosanova, M., Sarasso, S., Middleton, B., Luxen, A., Archer, S.N., Phillips, C., Dijk, D.-J., Maquet, P., Massimini, M., Vandewalle, G., 2016. Circadian regulation of human cortical excitability. Nat. Commun. 7, 11828. https://doi.org/10.1038/ncomms11828.

Maquet, P., Schwartz, S., Passingham, R., Frith, C., 2003. Sleep-related consolidation of a visuomotor skill: brain mechanisms as assessed by functional magnetic resonance imaging. J. Neurosci. 23, 1432–1440.

Massimini, M., Ferrarelli, F., Huber, R., Esser, S.K., Singh, H., Tononi, G., 2005. Breakdown of cortical effective connectivity during sleep. Science 309 (80-), 2228–2232. https://doi.org/10.1126/science.1117256.

Massimini, M., Ferrarelli, F., Murphy, M.J., Huber, R., Riedner, B.A., Casarotto, S., Tononi, G., 2010. Cortical reactivity and effective connectivity during REM sleep in humans. Cogn. Neurosci. 1, 176–183. https://doi.org/10.1080/17588921003731578.

Mattout, J., Phillips, C., Penny, W.D., Rugg, M.D., Friston, K.J., 2006. MEG source localization under multiple constraints: an extended Bayesian framework. Neuroimage 30, 753–767. https://doi.org/10.1016/j.neuroimage.2005.10.037.

Meisel, C., Klaus, A., Vyazovskiy, V.V., Plenz, D., 2017. The interplay between long- and short-range temporal correlations shapes cortex dynamics across vigilance states. J. Neurosci. 37, 10114–10124. https://doi.org/10.1523/JNEUROSCI.0448-17.2017.

Moran, R., Pinotsis, D.A., Friston, K., 2013. Neural masses and fields in dynamic causal modeling. Front. Comput. Neurosci. 7, 57. https://doi.org/10.3389/fncom.2013.00057.

Oken, B.S., Salinsky, M.C., Elsas, S.M., 2006. Vigilance, alertness, or sustained attention: physiological basis and measurement. Clin. Neurophysiol. 117, 1885–1901. https://doi.org/10.1016/j.clinph.2006.01.017.

Philip, P., Akerstedt, T., 2006. Transport and industrial safety, how are they affected by sleepiness and sleep restriction? Sleep. Med. Rev. 10, 347–356. https://doi.org/10.1016/j.smrv.2006.04.002.

Piantoni, G., Cheung, B.L.P., Van Veen, B.D., Romeijn, N., Riedner, B.A., Tononi, G., Van Der Werf, Y.D., Van Someren, E.J.W., 2013. Disrupted directed connectivity along the cingulate cortex determines vigilance after sleep deprivation. Neuroimage 79, 213–222. https://doi.org/10.1016/j.neuroimage.2013.04.103.

Posner, M.I., Petersen, S.E., 1990. The attention system of the human brain. Annu. Rev. Neurosci. 13, 25–42. https://doi.org/10.1146/annurev.ne.13.030190.000325.

Poudel, G.R., Innes, C.R.H., Jones, R.D., 2013. Distinct neural correlates of time-on-task and transient errors during a visuomotor tracking task after sleep restriction. Neuroimage 77, 105–113. https://doi.org/10.1016/j.neuroimage.2013.03.054.

Roach, G.D., Dawson, D., Lamond, N., 2006. Can a shorter psychomotor vigilance task be used as a reasonable substitute for the ten-minute psychomotor vigilance task? Chronobiology Int. 1379–1387. https://doi.org/10.1080/07420520601067931.

Rocklage, M., Williams, V., Pacheco, J., Schnyer, D.M., 2009. White matter differences predict cognitive vulnerability to sleep deprivation. Sleep 32, 1100–1103.

Rosanova, M., Casarotto, S., Pigorini, A., Canali, P., Casali, A.G., Massimini, M., 2012. Combining transcranial magnetic stimulation with electroencephalography to study human cortical excitability and effective connectivity. Neuromethods 67, 435–467.

Sämann, P.G., Tully, C., Spoormaker, V.I., Wetter, T.C., Holsboer, F., Wehrle, R., Czisch, M., 2010. Increased sleep pressure reduces resting state functional connectivity. Magn. Reson. Mat. Phys. Biol. Med. 23, 375–389. https://doi.org/10.1007/s10334-010-0213-z.

Slater, J.D., Chelaru, M.I., Hansen, B.J., Beaman, C., Kalamangalam, G., Tandon, N., Dragoi, V., 2017. Focal changes to human electrocorticography with drowsiness: a novel measure of local sleep. J. Neuropsychiatry Clin. Neurosci. appi.neuropsych. https://doi.org/10.1176/appi.neuropsych.16060120.

Smith, M.J., Adams, L.F., Schmidt, P.J., Rubinow, D.R., Wassermann, E.M., 2002. Effects of ovarian hormones on human cortical excitability. Ann. Neurol. 51, 599–603. https://doi.org/10.1002/ana.10180.

Steer, R.A., Ball, R., Ranieri, W.F., Beck, A.T., 1997. Further evidence for the construct validity of the Beck depression Inventory-II with psychiatric outpatients. Psychol. Rep. 80, 443–446.

Strijkstra, A.M., Beersma, D.G.M., Drayer, B., Halbesma, N., Daan, S., 2003. Subjective sleepiness correlates negatively with global alpha (8-12 Hz) and positively with central frontal theta (4-8 Hz) frequencies in the human resting awake electroencephalogram. Neurosci. Lett. 340, 17–20.

Tomasi, D., Wang, R.L., Telang, F., Boronikolas, V., Jayne, M.C., Wang, G.J., Fowler, J.S., Volkow, N.D., 2009. Impairment of attentional networks after 1 night of sleep deprivation. Cereb. Cortex 19, 233–240. https://doi.org/10.1093/cercor/bhn073.

Tononi, G., Sporns, O., Edelman, G.M., 1994. A measure for brain complexity: relating functional segregation and integration in the nervous system. Neurobiology 91, 5033–5037.

Tüshaus, L., Balsters, J.H., Schläpfer, A., Brandeis, D., O'Gorman Tuura, R., Achermann, P., 2017. Resisting sleep pressure: impact on resting state functional network connectivity. Brain Topogr. 30, 757–773. https://doi.org/10.1007/s10548-017-0575-x.

Verweij, I.M., Romeijn, N., Smit, D.J., Piantoni, G., Van Someren, E.J., van der Werf, Y.D., 2014. Sleep deprivation leads to a loss of functional connectivity in frontal brain regions. BMC Neurosci. 15, 88. https://doi.org/10.1186/1471-2202-15-88.

Virtanen, J., Ruohonen, J., Naatanen, R., Ilmoniemi, R.J., 1999. Instrumentation for the measurement of electric brain responses to transcranial magnetic stimulation. Med. Biol. Eng. Comput. 37, 322–326.

Vyazovskiy, V.V., Olcese, U., Hanlon, E.C., Nir, Y., Cirelli, C., Tononi, G., 2011. Local sleep in awake rats. Nature 472, 443–447. https://doi.org/10.1038/nature10009.

Wang, C., Ong, J.L., Patanaik, A., Zhou, J., Chee, M.W.L., 2016. Spontaneous eyelid closures link vigilance fluctuation with fMRI dynamic connectivity states. Proc. Natl. Acad. Sci. U. S. A. 113, 9653–9658. https://doi.org/10.1073/pnas.1523980113.

Welch, P.D., 1967. The use of fast fourier transform for the estimation of power spectra: a method based on time averaging over short, modified periodograms. IEEE Trans. Audio Electroacoust 15, 70–73. https://doi.org/10.1109/TAU.1967.1161901.

Wright, K.P., Lowry, C.A., Lebourgeois, M.K., 2012. Circadian and wakefulness-sleep modulation of cognition in humans. Front. Mol. Neurosci. 5, 50. https://doi.org/10.3389/fnmol.2012.00050.

Yeo, B.T.T., Tandi, J., Chee, M.W.L., 2015. Functional connectivity during rested wakefulness predicts vulnerability to sleep deprivation. Neuroimage 111, 147–158. https://doi.org/10.1016/j.neuroimage.2015.02.018.

Phase II, Paper 3: Age-related decrease in cortical excitability circadian variations during sleep loss and its links with cognition

Gaggioni G, Ly JQM, Muto V, Chellappa SL, Jaspar M, Meyer C, Delfosse T, Vanvinckenroye A, Dumont R, Coppieters 't Wallant D, Berthomier C, Narbutas J, Van Egroo M, Luxen A, Salmon E, Collette F, Phillips C, Schmidt C, Vandewalle G. *Neurobiology of Aging 2019*

As previously mentioned, important changes in the sleep homeostatic and the circadian processes occur during healthy ageing, as well as in the cognitive domain (Schmidt et al., 2012a). In *phase I*, we show that cortical excitability –essential for proper brain function and cognition– is regulated by both sleep homeostasis and circadian processes, in a group of young people (18-30 y). In *phase II*, we investigate the dynamics of cortical excitability with time spent awake and according to the circadian clock, in young (18-30 yo) and older participants (50-70 yo). The hypothesis is that the cortical excitability profile will be dampened in older participants, reflecting reduced synaptic plasticity modulation at the brain level, and that it will be linked to worse cognitive performance, especially during daytime. This hypothesis is indeed confirmed, with older participants having a flattened cortical excitability profile over a circadian cycle. Regarding behaviour, we further anticipate that higher level of cortical excitability in older participants will be associated with better cognitive performance and that independently of the circadian phase or neurobehavioural task: in other words, that increased synaptic plasticity will be always related to better cognitive performance in older people (i.e. linear relationship). Furthermore, older people displaying a degree of modulation of the cortical excitability will be those performing better during a normal waking day. Results show that older participants with higher cortical excitability are associated with better executive performance, but no significant association between overnight cortical excitability profile and diurnal cognitive performance is detected in our small sample. Future works should confirm whether maintaining cortical excitability dynamics can counteract age-related cognitive decline.

Check for updates
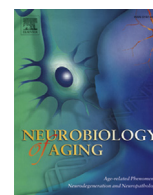
Age-related decrease in cortical excitability circadian variations during sleep loss and its links with cognition

Giulia Gaggioni [a,b], Julien Q.M. Ly [a,b,c], Vincenzo Muto [a,b], Sarah L. Chellappa [a,b,1], Mathieu Jaspar [a,b,d], Christelle Meyer [a,b], Tillo Delfosse [a], Amaury Vanvinckenroye [a], Romain Dumont [a], Dorothée Coppieters 't Wallant [a], Christian Berthomier [e], Justinas Narbutas [a], Maxime Van Egroo [a], Andé Luxen [a], Eric Salmon [a,c,d], Fabienne Collette [a,d], Christophe Phillips [a,f], Christina Schmidt [a,d], Gilles Vandewalle [a,b,*]

[a] GIGA-Institute, Cyclotron Research Center/In Vivo Imaging, Sleep and Chronobiology Lab, University of Liège, Liège, Belgium
[b] Walloon Excellence in Life sciences and Biotechnology (WELBIO), Belgium
[c] Department of Neurology, CHU Liège, Liège, Belgium
[d] Psychology and Neuroscience of Cognition Research Unit, Faculty of Psychology and Educational Sciences, University of Liège, Liège, Belgium
[e] Physip, Paris, France
[f] GIGA- Institute, In silico Medicine, University of Liège, Liège, Belgium

ARTICLE INFO

ABSTRACT

Cortical excitability depends on sleep-wake regulation, is central to cognition, and has been implicated in age-related cognitive decline. The dynamics of cortical excitability during prolonged wakefulness in aging are unknown, however. Here, we repeatedly probed cortical excitability of the frontal cortex using transcranial magnetic stimulation and electroencephalography in 13 young and 12 older healthy participants during sleep deprivation. Although overall cortical excitability did not differ between age groups, the magnitude of cortical excitability variations during prolonged wakefulness was dampened in older individuals. This age-related dampening was associated with mitigated neurobehavioral consequences of sleep loss on executive functions. Furthermore, higher cortical excitability was potentially associated with better and lower executive performance, respectively, in older and younger adults. The dampening of cortical excitability dynamics found in older participants likely arises from a reduced impact of sleep homeostasis and circadian processes. It may reflect reduced brain adaptability underlying reduced cognitive flexibility in aging. Future research should confirm preliminary associations between cortical excitability and behavior and address whether maintaining cortical excitability dynamics can counteract age-related cognitive decline.

© 2019 Elsevier Inc. All rights reserved.

1. Introduction

The intrinsic excitability, or reactivity, of cortical neuronal cells is a basic, yet essential, feature of brain function (Rizzo et al., 2015). Cortical excitability reflects inherent cellular properties of neurons that arise from the combined impacts of multiple parameters (e.g., ion concentration in the intracellular and extracellular milieus, neuromodulator actions, membrane potential, action potential threshold) (Bushey et al., 2015; Frank and Cantera, 2014; Meisel et al., 2015; Rizzo et al., 2015; Tononi and Cirelli, 2014). Cortical excitability is grounded in the responsiveness and response selectivity of cortical neurons which determines, at least in part, how an input is processed by the brain and is therefore central to cognition. In fact, a decrease in neuron excitability has been implicated in the cognitive decline found in normal and pathological aging (Chang et al., 2005; Rizzo et al., 2015). Critically, cortical excitability was recently demonstrated to vary substantially during wakefulness and following sleep (Huber et al., 2013; Ly et al., 2016). Yet, the regulation of sleep and wakefulness profoundly change in aging (Schmidt et al., 2012). Whether these age-related changes affect cortical excitability is unknown.

* Corresponding author at: GIGA-Institute, Cyclotron Research Center/In Vivo Imaging, Bâtiment B30, Allée du VI Août, 8, 4000 Liege, Belgium. Tel.: +3243662316; fax: +3243662946.

E-mail address: gilles.vandewalle@uliege.be (G. Vandewalle).
[1] Current address: Medical Chronobiology Program, Division of Sleep and Circadian Disorders, Departments of Medicine and Neurology, Brigham and Women's Hospital, Boston, MA 02115, USA.

Two fundamental mechanisms regulate sleep and wakefulness and their associated cognitive functions: sleep homeostasis and the circadian system (Dijk and Czeisler, 1995; Schmidt et al., 2012). During the day, the circadian signal opposes the homeostatic buildup of sleep needs to maintain wakefulness and cognition, up to the evening, shortly before habitual sleep onset (Dijk and Czeisler, 1995). At night, the circadian system promotes sleep to favor sleep continuity, up to the end of the biological night, shortly before habitual wake-up time (Dijk and Czeisler, 1995). Any disturbance in this fine-tuned interplay is detrimental for cognition (Lo et al., 2012; Schmidt et al., 2012). An extreme disruption consists in prolonging wakefulness overnight: cognition is greatly compromised because the circadian system promotes sleep at a time of high sleep need (Lo et al., 2012; Schmidt et al., 2012). If wakefulness is further prolonged the next day, the wake-promoting signal of the circadian system rescues in part cognition (Lo et al., 2012). Thus, because of the interplay between the homeostatic and circadian processes, all periods of prolonged wakefulness are not equivalent or linearly related to one another. Likewise, all aspects of cognition are also not equally affected by sleep loss: the magnitude of the detrimental impact of insufficient sleep and prolonged wakefulness during the biological night has been most repeatedly observed and showed strongest effect sizes for monotonous tasks with high attentional demands, at least in young adults (Lo et al., 2012). At the level of cortical excitability, the interplay between sleep homeostasis and the circadian system is reflected in young individuals in an overall increase in excitability after 24 hours of continuous wakefulness—attributed to the build-up of sleep need (Huber et al., 2013; Ly et al., 2016)—and in more local variations around the evening and early morning—attributed to the influence of the circadian system (Ly et al., 2016).

Even in the absence of clinically significant sleep disorders, aging is characterized by deterioration in sleep-wake regulation. In healthy older individuals, sleep intensity, duration and continuity decrease (Dijk et al., 1999; Klerman and Dijk, 2008; Schmidt et al., 2012; Van Cauter, 2000), but these changes are not systematically accompanied by increased daytime sleepiness (Klerman and Dijk, 2008). In fact, sleep need and its buildup during wakefulness decrease as one gets older (Landolt et al., 2012; Schmidt et al., 2012). Concomitantly, the timing of the circadian system is advanced and the strength of the circadian signal has been suggested to decrease (Dijk et al., 1999; Kondratova and Kondratov, 2012; Münch et al., 2005). Overall, these combined changes lead to changes in cognition. The acute detrimental cognitive effect of sleep loss is reduced in aging (Landolt et al., 2012; Sagaspe et al., 2012; Schmidt et al., 2012): although they may achieve overall lower performance than young adults, older individuals suffer relatively less during a night without sleep, at least over several cognitive domains, including vigilant attention, executive function (inhibitory motor control), and mental arithmetic. Whether these changes in cognition regulation during wakefulness may arise from alterations in the impact of sleep homeostasis and of the circadian system on cortical excitability is unknown, however. This question is important because long-term age-related sleep-wake changes lead to a fragmentation of the normal waking-rest cycle—for example, more wakefulness during night-time sleep—that is associated with an overall decline of cognitive abilities in older individuals (Lim et al., 2013; Oosterman et al., 2009).

Here, we repeatedly probed cortical excitability in healthy older and younger individuals during prolonged wakefulness. We used transcranial magnetic stimulation (TMS) coupled to electroencephalogram (EEG) to record direct perturbations of cortical neuron activity—bypassing sensory systems—using identical stimulations delivered over the exact same brain location. Because frontal brain regions are particularly prone to both aging (Reuter-Lorenz and Park, 2014) and the interplay between circadian and homeostatic processes (Landolt et al., 2012; Schmidt et al., 2012), cortical excitability was assessed over the frontal cortex. We hypothesized that fluctuations in cortical excitability during prolonged wakefulness would be reduced in older participants, particularly at critical time points for the interplay between the circadian alerting signal and the homeostatic sleep pressure, that is, in the evening and the end of the biological night—when the circadian signal maximally/minimally opposes high sleep pressure, respectively. Our protocol also included repeated cognitive test batteries, spanning executive and attentional domains. We therefore explored whether a lower but stable cortical excitability profile in older individuals during wake extension would be associated with reduced performance impairment during sleep loss.

2. Material and methods

2.1. Participants

The study was approved by the Ethics Committee of the Medicine Faculty of the University of Liège. Participants gave their written informed consent and received a financial compensation. Twenty-six healthy participants were enrolled, 13 older adults (62.6 years ± 3.8; 7 women) and 13 young (22.8 years ± 2.9; 5 women). Exclusion criteria included (1) body mass index (BMI) < 18 and >28; (2) recent psychiatric history, severe trauma, sleep disorders; (3) addiction, chronic medication; (4) smokers, excessive alcohol (>14 doses/week) or caffeine (>3 cups/day) consumption; (5) night shift workers during the last year; (6) transmeridian travel during the last 2 months; (7) anxiety or depression; (8) poor sleep quality; (9) excessive self-reported daytime sleepiness; (10) early signs of dementia (in older participants). Anxiety was measured by the 21-item Beck Anxiety Inventory (BAI ≤14) (Beck et al., 1988); mood by the 21-item Beck Depression Inventory II (BDI-II ≤ 14) (Steer et al., 1997); sleep quality by the Pittsburgh Sleep Quality Index Questionnaire (PSQI ≤ 7) (Buysse et al., 1989); daytime sleepiness by the Epworth Sleepiness Scale (ESS ≤ 11) (Johns, 1991); and early signs of dementia using Mattis scale (Mattis, 1988). Chronotype was also assessed using the Horne-Östberg Questionnaire (Horne and Östberg, 1976). One older participant was removed because his performance was 3 interquartile ranges above or below the 25th and 75th percentile of the older participant sample across all cognitive tasks. Table 1 summarizes the demographic characteristics of the final study sample.

2.2. Experimental protocol

At least a week before the experiment, participants completed a preparatory TMS-EEG session to determine optimal TMS parameters for artifact-free recordings. As in the studies by Huber et al. (2013) and Ly et al. (2016), the left or right superior frontal gyrus was set as the stimulation target for right- or left-handed, respectively. Participants also completed a screening night of sleep to exclude major sleep disorders (periodic leg movement; apnea-hypopnea index >15/h). During the 7 days preceding the study, they kept a regular sleep-wake schedule (±15 minutes; verified using wrist actigraphy—actiwatch, Cambridge Neurotechnology, UK—and sleep diaries). Schedule and duration were based on at least 10 days of unconstrained actimetry recordings and/or self-reported sleep times and duration. Participants were requested to abstain from all caffeine and alcohol-containing beverages for 3 days preceding the study.

The experiment consisted in a constant routine (i.e., light <5 lux, temperature ∼19 °C, regular isocaloric liquid meals and water, semi-recumbent position, no time-of-day information, sound

Table 1
Sample characteristics (mean ± SD)

Age group	Younger (18–30 y)	Older (50–70 y)	p Value
N	13	12	–
Women	5	6	0.96
Age (y)	22.8 ± 2.9	62.3 ± 3.7	–
Right handed	10	11	0.32
BMI (kg/m^2)	22.3 ± 3	24.8 ± 2.3	**0.03**
Anxiety level	2.6 ± 3.9	3 ± 3.8	0.8
Mood	2.8 ± 2.7	3.2 ± 2.8	0.77
Caffeine (cups/d)	1.1 ± 1.9	2.2 ± 1.3	0.12
Alcohol (doses/wk)	2.7 ± 3.2	4.7 ± 5	0.23
Subjective sleep quality	3.2 ± 1	5.3 ± 2.8	**0.03**
Subjective daytime sleepiness	3.6 ± 2.8	4.8 ± 4.3	0.41
Chronotype	56 ± 6.1	59.6 ± 7.2	0.58
Clock time of dim light melatonin onset (hh:min)	21:34 ± 01:11	21:43 ± 00:38	0.71
Clock time of dim light melatonin offset (hh:min)	08:21 ± 01:01	07:55 ± 01:05	0.31
In-lab baseline total time in bed (min, EEG)	509 ± 19	502 ± 18	0.21
In-lab baseline sleep duration (min, EEG)	456 ± 45	405 ± 67	**0.01**
In-lab baseline sleep efficiency (%, EEG)	90 ± 9	81 ± 13	**0.01**
Baseline sleep time (hh:min)	23:20 ± 00:48	23:21 ± 00:30	
Baseline wake time (hh:min)	07:48 ± 00:52	07:37 ± 00:33	
Sleep duration for 7 preceding d (min, actigraphy)	511 ± 30	490 ± 32	0.18
Sleep time for 7 preceding d (hh:min, actigraphy)	23:28 ± 00:43	23:35 ± 00:28	
Wake time for 7 preceding d (hh:min, actigraphy)	08:04 ± 00:53	07:48 ± 00:44	
Intensity of TMS pulses (%)	54.2 ± 4.5	55.2 ± 5.2	0.66
Estimated electric field of TMS pulses (V/m) [a]	108.5 ± 16	116.2 ± 16.6	0.91
Distance from coil (scalp) and cortical hotspot (mm) [a]	17.9 ± 2.2	17.5 ± 2.2	0.87
Mean distance between individual hotspot location and group average hotspot location (MNI space, mm) [b]	7.3 ± 4.3	10.94 ± 6.93	0.18

N.B.: Sample of in-lab baseline sleep EEG: N_{young} = 10 (due to artifacted signal); N_{older} = 12.
Bolded values represent the significant p value ($p < 0.05$).
Key: TMS, transcranial magnetic stimulation; EEG, electroencephalogram; BMI, body mass index.
Anxiety was measured by the 21-item Beck Anxiety Inventory (BAI ≤14) (Beck et al., 1988); mood by the 21-item Beck Depression Inventory II (BDI-II ≤ 14) (Steer et al., 1997); sleep quality by the Pittsburgh Sleep Quality Index Questionnaire (PSQI ≤7) (Buysse et al., 1989); daytime sleepiness by the Epworth Sleepiness Scale (ESS ≤11) (Johns, 1991); chronotype by the Horne-Östberg Questionnaire (<42: evening types; 42–58: intermediate types; > 58: morning types) (Horne and Östberg, 1976).
[a] As provided by the TMS-EEG system.
[b] See section 2.3 for more details.

proofed rooms) sleep deprivation protocol, which has repeatedly been a successful means to assess in-lab interindividual differences in sleep homeostatic and circadian interplay (Duffy and Dijk, 2002). Participants were maintained in dim light for 5.5 hours (<5 lux), during which they were trained to the cognitive test batteries, before sleeping at their habitual bedtime, for their habitual duration (in complete darkness) (Fig. 1A). The TMS-compatible electrode cap was placed on awaking before sustained wakefulness period under 34 hours of constant routine conditions. TMS-evoked EEG potentials were recorded 9 times (1000, 1600, 2000, 2200, 0100, 0500, 0700, 1000, and 1600 for a subject sleeping from 2300 to 0700). Cognitive test batteries were carried out 13 times during the protocol in between TMS-EEG sessions (1100, 1500, 1700, 1900, 2100, 2300, 0200, 0400, 0600, 0800, 1100, 1300, 1500). Overall, the study included 1500 protocol hours with multiple measures including 225 TMS-EEG sessions derived from 13 young and 12 older participants.

2.3. TMS-evoked EEG response acquisitions and processing

Stimulation target was located in the superior frontal cortex on individual structural MRI by means of a neuronavigation system (Navigated Brain Stimulation; Nexstim) (Fig. 1B). This device allows for reproducible evoked EEG responses and precise target location (FDA approval for presurgery). TMS pulses were generated by a Focal Bipulse 8-coil (Nexstim, Helsinki, Finland). Each TMS-EEG session included 250–300 trials. Interstimulus intervals were randomly jittered between 1900 and 2200 ms. TMS responses were recorded with a 60-channel TMS-compatible EEG amplifier (Eximia; Nexstim), equipped with a proprietary sample-and-hold

circuit that provides TMS artifact free data from ∼5 ms post-TMS (Virtanen et al., 1999). Electrooculogram (EOG) was recorded with 2 additional bipolar electrodes. Participants wore the EEG cap during the entire constant routine protocol, and electrodes impedance was set below 5 kΩ before each recording session. Signal was band pass–filtered between 0.1 and 500 Hz and sampled at 1450 Hz. Each TMS-EEG session ended with a neuronavigated digitization of the location of each electrode. Auditory EEG potentials (AEPs) evoked by TMS and bone conductance were minimized by diffusing a continuous loud white masking noise through earplugs and applying a thin foam layer between the EEG cap and the TMS coil. Each session was followed by a sham session consisting in 30–40 TMS pulses delivered parallel to the scalp while white noise was diffused at the same level. Absence of AEP was checked online on Cz between 0 and 500 ms after TMS (all sessions were AEP free). Data of sham sessions were not considered any further.

EEG data were processed using SPM12 (Statistical Parametric Mapping 12, http://www.fil.ion.ucl.ac.uk/spm/) implemented in MATLAB (2015) (The Mathworks Inc, Natick, MA). Processing included the following: visual rejection of artifact, rereferencing to average of good channels, low-pass filtering at 80 Hz, resampling from 1450 to 1000 Hz, high-pass filtering at 1 Hz, epoching between −100 and 300 ms around TMS pulses, baseline correcting (−100 to −1 ms pre-TMS), and robust averaging. Cortical excitability was inferred from the slope of the first EEG component (0–35 ms) of the TMS-evoked potential (∼ 250 trials per session), measured at the artifact-free electrode closest from the frontal hotspot (i.e., the brain location with highest TMS-induced electrical field estimated by the neuronavigation system) (Fig. 1B). This electrode was always located in the stimulated brain hemisphere. It
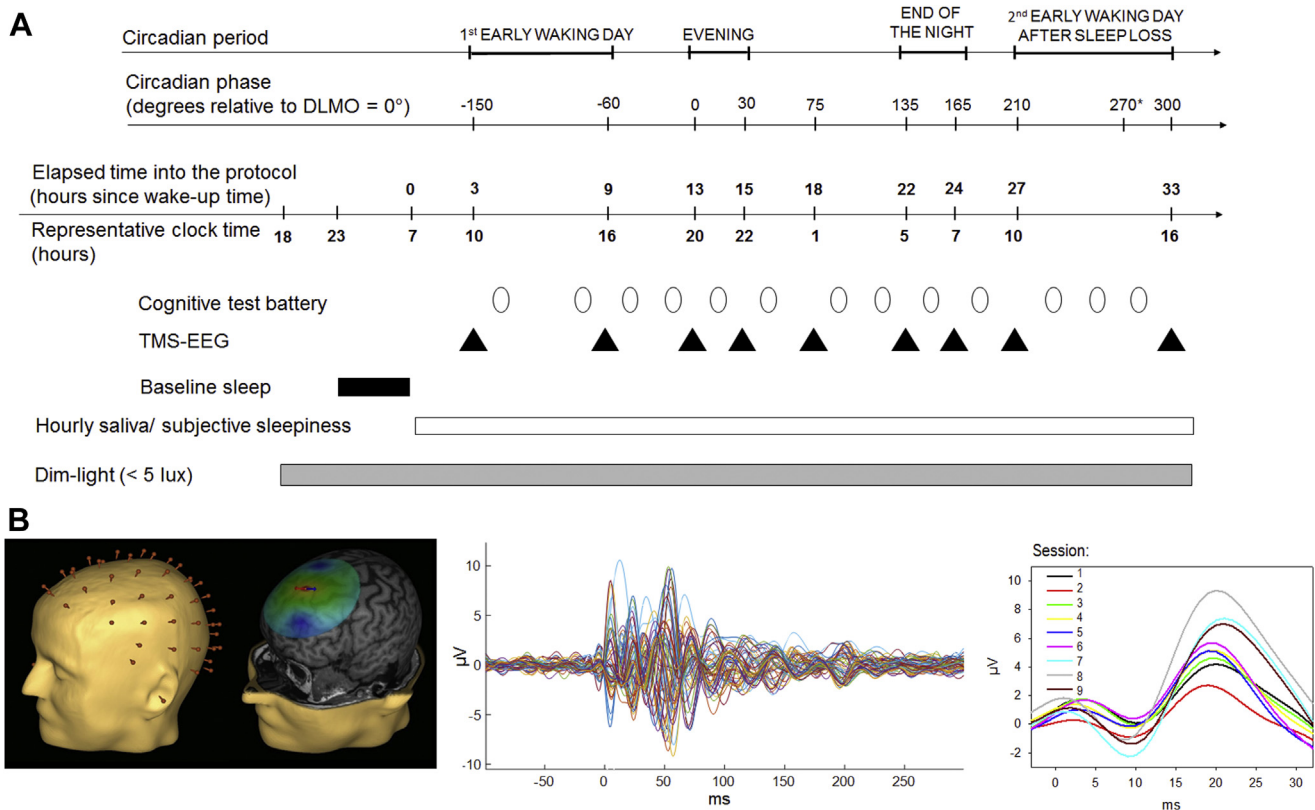
Fig. 1. Experimental protocol and TMS-evoked potentials. (A) After a baseline night of sleep, 12 older and 13 young healthy participants underwent 34 hours of sustained wakefulness under constant routine conditions. Cortical excitability was assessed 9 times using TMS-EEG (▲), over the first early waking day, evening, biological night, and second early waking day after sleep loss. During TMS-EEG sessions, a visuomotor compensatory tracking task (CTT) was administered. In-between, 13 behavioral test batteries were administered (○)—including the psychomotor vigilance task (PVT) and executive tasks (2-back, 3-back, GO/NO-GO). Saliva samples were collected hourly for melatonin and cortisol assays, allowing a posteriori data realignment and interpolation based on individual endogenous circadian timing (inferred based on dim light melatonin onset [DLMO]). Time is expressed in circadian phase (degrees-°; 15° = 1h) and equivalent elapsed time awake (h). Representative clock time is for a participant with a 2300−0700 sleep-wake schedule.* Data were not extrapolated >15° from the last recording: resampling at 300° could not be carried out in most participants and was done at 270° instead. (B) Left panel: MRI-based head reconstruction together with the neuronavigated position of the electrodes. Representative location of a TMS hotspot over the superior frontal gyrus as provided by the neuronavigation system. The arrows represent the direction of the generated electric field. Middle panel: A butterfly plot of all electrodes of a representative TMS-evoked potential. Right panel: Representative average TMS-evoked potentials measured at the electrode closest to the hotspot (−2 to 32 ms post-TMS) in each of the 9 sessions of the protocol. Abbreviations: TMS, transcranial magnetic stimulation; EEG, electroencephalogram; MRI, magnetic resonance imaging.

could vary across participants but remained constant at the individual level.

The neuronavigation system ensured that hotspot location remained constant across sessions within an individual (±2 mm). Across individuals, hotspot location varied. The mean coordinates (x, y, z ± SD; MNI space) of the hotspot across all subjects was [−6.6 ± 3.2, 10.1 ± 9.8, 71 ± 4.3], whereas across young or older individuals only, it was [−6.1 ± 3.6, 11.8 ± 7.5, 70 ± 2.8] and [−7.1 ± 2.9, 8.3 ± 11.9, 72.1 ± 5.5], respectively [nb: coordinates of the right hemisphere (case of 3 volunteers) were transpose to the homolog location in the left hemisphere, for average location computation]. Averages in each group are therefore <1.8 mm in either direction from the overall average, indicating that the area of the superior frontal cortex stimulated was similar in each group. To further assess whether hotspot location could contribute to potential group differences, we computed the distance between individual hotspot (median location across all TMS sessions) and average location within each group. Statistical analyses (Wilcoxon rank-sum test) revealed no significant difference between both groups (Table 1).

2.4. Cognitive test batteries

Cognitive test batteries placed in between TMS-EEG recordings were administered in the same following order to all participants:

2.4.1. GO/NO-GO task

This task probes motor inhibition (Sagaspe et al., 2012) and requires to press a keypress as quickly as possible for the frequent letter "M" and to refrain from responding for the target "W" (320 trials; 20% of NO-GO targets; ∼ 8.5 minutes). Letters were displayed for 200 ms and stimulus onset asynchrony randomly varied between 1500 and 1900 ms. Our main performance measure consisted in the number of false alarm (i.e., commission error rate of NO-GO trials, keyboard response).

2.4.2. N-back tasks

These tasks require continuous updating of presented information (Lo et al., 2012). Participants were instructed to state whether or not the current letter was identical to the consonant presented 2 and 3 stimuli earlier, respectively, for the 2-back and 3-back tasks, by pressing one of two possible keys of the keyboard (75 trials per task; 30% of targets; 2.5 minutes). Stimulus onset asynchrony was 2 seconds, and letter was displayed for the entire 2 seconds. D-prime—a response discriminability index [i.e., a measure of sensitivity, following the signal detection theory (Ingleby, 1967)]—was computed for both versions of the task. The n-back task is sensitive to aging (De Beni and Palladino, 2004) and is a difficult task for older individuals, particularly the 3-back version. Although comprehension of the instructions and accuracy was verified

during the training before baseline sleep, 3 older subjects did not apply the instructions correctly (e.g., they only responded every 2 or 3 items or less) or did not do the task at all, as indicated by a D-prime value close to zero. These subjects were removed from the analyses leaving, for this analysis, 13 young individuals and 9 older individuals. Thus, associations between cortical excitability and behavior are to be considered as preliminary results.

2.4.3. Psychomotor vigilance task

This task probes vigilant attention (Basner and Dinges, 2011) and requires participants to press a computer space bar as soon as a chronometer pseudorandomly starts on the screen (random interval of 2–10 seconds; 48 trials per task; 5 minutes). Performance was inferred from the mean reaction time after removal of anticipation (<100 ms) and lapses (>500 ms) [and error (>3000 ms)].

2.4.4. Visuomotor vigilance compensatory tracking task (CTT)

This task also probes vigilant attention and was performed during the TMS-EEG recordings [as in (Huber et al., 2013; Ly et al., 2016)]. It consists of keeping a constantly randomly moving cursor on a target located in the center of a computer screen, using a trackball device. The task was preferred to psychomotor vigilance task (PVT) during TMS-EEG recordings because it only requires continuous smooth and limited movement of a single finger and allows for continuous vigilance monitoring. Performance was computed as the average distance (in pixels) between the cursor and the target during TMS-EEG recordings, after removal of lapses. If signs of drowsiness were detected while performing the task during TMS-EEG sessions, the experimenter briefly touched the participant. Transitory lapses of vigilance resulted in temporary increases of the target-cursor distance and could be automatically detected offline. A lapse was identified when the cursor was located outside a central 200-by-200 pixel box surrounding the target for >500 ms from the last trackball movement. The lapse period ranged from the last trackball movement until the lapse detection. TMS evoked responses occurring during and <1 second from a lapse period were discarded from analyses.

2.5. Salivary melatonin and cortisol samples

Salivary melatonin and cortisol samples were first placed at 4 °C, before centrifugation and congelation at −20 °C within 12 hours. Salivary melatonin and cortisol were measured by radioimmuno-assay (Stockgrand Ltd, Guildford, UK), as previously described (English et al., 1993). Most samples were analyzed in duplicate. The limit of detection of the assay for melatonin was 0.8 ± 0.2 pg/mL using 500 μL volumes, whereas it was 0.37 ± 0.05 nmol/L using 500 μL volumes (Read et al., 1977). Estimation of individual's dim light melatonin onset (DLMO = phase 0°) was determined based on raw values. The 4 first samples were disregarded and maximum secretion level was set as the median of the 3 highest concentrations. Baseline level was set to be the median of the values collected from "wake-up time +5 hours" to "wake-up time +10 hours." DLMO was computed as the time at which melatonin level reached 20% of the baseline to maximum level (linear interpolation).

2.6. Sleep EEG

Sleep EEG data were recorded using M7000 amplifiers (EMBLA, NATUS, Planegg, Germany) according to the 10/20 system. The habituation night montage consisted of a full polysomnography with 5 EEG channels (Fz, Cz, Pz, Oz, C3) referenced to left and right mastoids (A1, A2), 2 bipolar EOG, 2 bipolar electrocardiogram channels, 2 bipolar electrodes place on the chin (electromyogram), 2 bipolar electrodes placed on a leg to check for periodic movements, thoracic and stomach respiratory belts, nasal cannula, and an oximeter for sleep-related breathing disorder detection. Baseline night montage consisted of 11 EEG channels (F3, Fz, F4, C3, Cz, C4, P3, Pz, P4, O1, O2) referenced to left and right mastoids (A1, A2), 2 bipolar EOG, and 2 bipolar electromyogram channels. EEG data were digitized at a sampling rate of 200 Hz. Sleep EEG recordings were automatically scored using a validated algorithm (ASEEGA, PHYSIP, Paris, France), including artifact rejection (Berthomier et al., 2007). Three recordings of young participants were rejected because of artifacted signal. Total time spent in bed (TIB), total sleep time, sleep efficiency (the ratio between total sleep time and TIB in %) are reported in Table 1. The other aspects related to sleep will be reported elsewhere.

2.7. Statistics

The circadian phase of all data points was estimated relative to individual DLMO (i.e., phase 0°, 15° = 1 hour). All data points were resampled after linear interpolation at the theoretical phases of the TMS-EEG sessions in the protocol (Fig. 1A): −150°, −60°, 0°, 30°, 75°, 135°, 165°, 210°, and 270°. Data were not extrapolated beyond 15° (i.e., 1 hour), such that resampling at 300° could not be carried out for most participants and was advanced at 270° instead. For analyses only including cognitive test batteries, data were resampled every 30°, after linear interpolation, from −135° to 255°. Data points situated 3 interquartile ranges above or below the 25th and 75th percentile were defined as extreme outliers and removed (up to 2 data points were removed per analyses, i.e., 1%–2% data points per analyze).

Statistical analyses were performed with SAS version 9.3 (SAS Institute, Cary, NC, USA). T-test on independent samples compared group characteristics (χ^2 test for proportion comparisons; Table 1). Wilcoxon rank-sum test compared sleep, melatonin, cortisol, and relative distance mean values by group (non-normal distribution). Generalized linear mixed models (GLMMs) (PROC GLIMMIX) were applied to compute all statistics after determination of the dependent variable distribution (using Allfitdist MATLAB function). Subject (intercept) effect was included as a random factor. Circadian phase was included as the repeated measure together with an autoregressive estimation of autocorrelation of order 1 [AR (1)], and the covariance structure specified both the subject and group effect. In all GLMMs, degrees of freedom were estimated using Kenward-Roger's correction (they are reported between brackets for each test). If an interaction term was significant, simple effects were assessed using post hoc contrasts (difference of least square means) adjusted for multiple testing with Tukey's procedure. Betas (i.e., regression coefficient) were derived by applying the ESTIMATE statement. Differences of beta between age groups were not corrected for multiple comparisons. Regressions were used for visual display only and not as a substitute of the full GLMM statistics.

When analyzing the time course of a given variable (i.e., cortical, behavioral and endocrine measures), GLMM included circadian phase, age group, and their interaction. When seeking for associations between cortical excitability (slope of the first TMS evoked EEG response) and behavior, GLMM included cortical excitability, the 4 circadian periods of the protocol (first early waking day, evening, end of the biological night, second early waking day after sleep loss), age group and all double/triple interactions. Each circadian period gathered 2 circadian phases [phase 75° was excluded to provide a clear distinction as in (Shekleton et al., 2013)] to identify over what part of the circadian cycle associations were detected—rather than specific phase—and to increase statistical power. Circadian phase was included as the repeated measure (i.e., the smallest experimental unit), and an interaction between subject and circadian period was included in the covariance structure to

specify that measures from the same subject should be correlated within the same circadian period. Betas in each group are only reported for completeness as the age group difference in beta was considered for statistics. *T*-tests on beta coefficients were performed when seeking for group differences in the link between cortical excitability and performance. The association between cortical excitability and 2-back performance significantly diverged across age groups, irrespective of circadian period, in a 2-tailed *t*-test on beta coefficients; this finding was then used as prior for subsequent tests of beta group difference (one-tailed *t*-test).

Semipartial R^2 (R^2_{Sp}) was reported for each significant effect of interest as described in the study by Jaeger et al., 2017. Generalization of the R^2 statistic to GLMMs remains an unresolved problem, with several method proposed (Jaeger et al., 2017; Nakagawa and Schielzeth, 2013). We opted for the approach proposed and validated in the study by Jaeger et al., 2017 because it allows for a simple computation of semipartial R^2 as [Sum of Squares/(1+Sum of Square)], with [Sum of Squares = NumDF * FValue/DenDF] (NumDF: numerator degrees of freedom (DF); DenDF: denominator DF), provided that DF are estimated using Kenward-Roger's methods.

3. Results

3.1. Endocrine and sleepiness measures in older and young participants

The sleep deprivation protocol was performed under strictly controlled constant environmental conditions to detect both the influence of sleep homeostasis and of the circadian system on our measures of interest (Duffy and Dijk, 2002). Melatonin levels were assayed in hourly saliva samples, and all data were subsequently realigned relative to the onset of melatonin secretion [DLMO = circadian phase 0°], a gold standard marker of endogenous circadian phase (Pevet and Challet, 2011). Thus, all data are reported with respect to individual's internal circadian clock (and expressed in degrees; 15° = 1h), instead of the external clock time. Statistical analyses sought for effects of circadian phase, age group, and their interaction on the measures of interest through GLMMs.

Before the wakefulness extension, participants slept in the laboratory under polysomnography (Fig. 1A). TIB did not differ between age groups (Wilcoxon rank-sum test: $Z = 0.79$, $p = 0.21$; Table 1) but, as expected (Klerman and Dijk, 2008), sleep efficiency

was significantly lower in older compared to young participants (Wilcoxon rank-sum test: $Z = 2.47$, $p = 0.01$; Table 1). Also as expected (Sagaspe et al., 2007), during the following 34 hours of prolonged wakefulness, older participants did not feel sleepier than younger participants [main effect of age group, $F(1, 21.51) = 0.46$, $p = 0.5$; main effect of circadian phase, $F(30, 583.5) = 11.72$, $p < 0.0001$; age group × circadian phase interaction, $F(30, 583.5) = 1.10$, $p = 0.33$; Fig. 2C]. In addition, melatonin showed its typical night time secretion profile in both age groups (Fig. 2A), but levels tended to be lower in the older versus younger group (area under the curve, Wilcoxon rank-sum test: $Z = -1.55$, $p = 0.06$). This may reflect the previously reported reduction in the strength of the circadian signal (Münch et al., 2005). Hourly saliva samples were also assayed for cortisol, which is under strong circadian control as well (Fig. 2B). Cortisol level was significantly higher in older compared with younger individuals (area under the curve, Wilcoxon rank-sum test: $Z = 3.4$, $p < 0.0007$), in line with previous findings (Van Cauter, 2000). Our sample of younger and older healthy individuals appears therefore in line with previous studies on the impact of prolonged wakefulness in aging.

3.2. Age-related dampening of the dynamics in cortical excitability during prolonged wakefulness

When focusing on cortical excitability measures (i.e., the slope of the earliest EEG response evoked by the TMS pulses), GLMM analyses revealed that its modulation across circadian phases differed between older and young participants [circadian phase × age group interaction, $F(8,128.1) = 2.09$, $p = 0.04$; Fig. 3]. A significant simple effect of circadian phase was also detected [$F(8,128.1) = 2.37$, $p = 0.02$]. Subsequent post hoc comparisons indicated that cortical excitability was lower in the evening and first part of the biological night when compared to the end of the biological night in young individuals (0°, 30°, 75° < 135°, $p < 0.015$), whereas in older, cortical excitability was void of any robust changes over the protocol ($p > 0.05$ for all comparisons). Furthermore, cortical excitability was higher in younger versus older individuals at the end of the biological night (young > older: 135°, $p = 0.02$; 165°, $p = 0.06$), when the circadian signal does not counter high sleep pressure, suggesting that high sleep homeostatic and circadian misalignment do not impact equally cortical excitability of older and young participants. No significant simple effect of age group was found (i.e., irrespective of circadian phase, $F(1,24) = 1.56$, $p = 0.22$). Analyses of the amplitude of the earliest EEG response evoked by the TMS
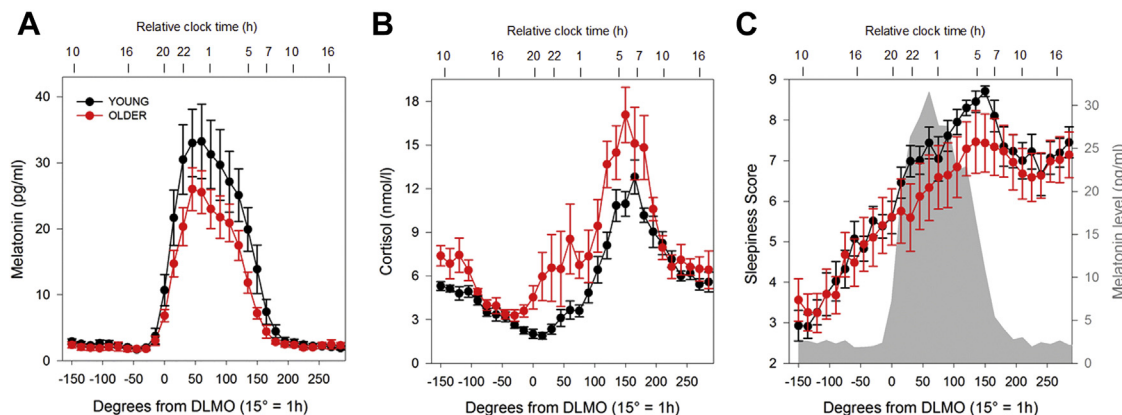
Fig. 2. Endocrine and sleepiness time course during 34 hours of prolonged wakefulness in young and older adults (mean ± SE). (A−C) Time course of melatonin, cortisol, and subjective sleepiness (mean ± SE; $N_{young} = 13$; $N_{older} = 12$) relative to individual melatonin onset (phase 0°; 15° = 1h). Average melatonin profile is displayed in gray on panel C. Refer to main text for differences between circadian phases. Abbreviation: DLMO, dim light melatonin onset.
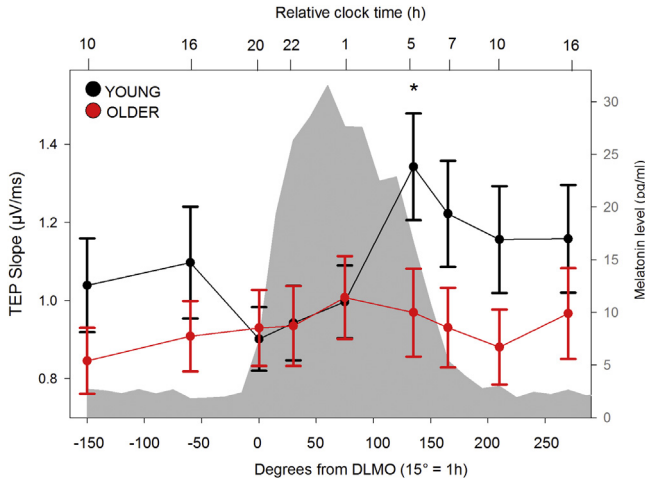
Fig. 3. Cortical excitability dynamics during 34 hours of prolonged wakefulness in young and older adults (mean ± SE). Time course of cortical excitability (slope of the first TMS-evoked EEG response; N_{young} = 13; N_{older} = 12): a circadian profile is visible in young, whereas is dampened in older participants. Time course is expressed relative to individual melatonin onset (DLMO = phase 0°; 15° = 1h). Average melatonin profile is displayed in gray. * significant group differences (p = 0.04) at circadian phase 135°, that is, around the end of the biological night. Abbreviations: TMS, transcranial magnetic stimulation; DLMO, dim light melatonin onset; EEG, electroencephalogram; TEP, TMS-evoked potential.

pulses, as an alternative measure of cortical excitability (Ly et al., 2016), led to similar statistical outcomes (Fig. S1). Importantly, these differences were detected while intensity of TMS pulses, estimated electric field generated by TMS, and the distance between the TMS coil and cortical hotspot did not differ between age groups (Table 1).

3.3. No significant association between cortical excitability and performance to vigilant attention tasks

We then switched to exploratory analyses including measures of cognitive performance to gain insight into the potential impact of cortical excitability dynamics on the outputs of brain function. We first considered the "simpler" tasks of the protocol, which probed vigilant attention. The PVT (Basner and Dinges, 2011) was administered 13 times during the protocol in-between TMS-EEG recordings, whereas the visuomotor compensatory tracking task (CTT) (Ly et al., 2016) was administered 9 times during TMS-EEG recordings (Fig. 1A). PVT performance significantly changed across circadian phases (main effect of circadian phase, $F_{(13,240.7)}$ = 6.97, $p < 0.0001$; Fig. 4A): it remained stable during a normal waking day and then sharply deteriorated (i.e., reaction time increased) during the biological night and early morning hours (75°–210° > −135°–0°, 270°, $p < 0.05$). Although qualitative inspection of data may suggest that older individuals suffered less from night time prolonged wakefulness, no significant age group difference nor any circadian phase by group interaction was detected [as in (Buysse et al., 2005), but see (Sagaspe et al., 2012)]. Compensatory tracking task performance yielded a circadian phase × age-group interaction ($F_{(8,131.9)}$ = 1.99, p = 0.05; Fig. 4B). Group differences were detected at all circadian phases except the last 3 assessments (young < older; −150°–135°, $p < 0.05$; 165°–270°, $p > 0.05$), indicating a differential response to sleep loss, leading to less pronounced differences in performance between age groups toward the end of the protocol. An overall simple effect of circadian phase was also found [$F_{(8, 131.9)}$ = 9.64, $p < 0.0001$], with worse performance at the end of the biological night as compared to the first and second circadian day (−150°–0°, 210°, 270° < 135°, 165°, $p <$

Table 2
Association between cortical excitability (measured as the slope of the first TMS-evoked potentials) and cognitive performance

GLMM predictor factors	PVT performance (mean reaction times[a])	CTT performance (distance from target)	2-Back performance (D-prime)	3-Back performance (D-prime)	GO/NO-GO performance (commission error rate)
Cortical excitability	$F_{(1,146.1)}$ = 0.28 p = 0.59	$F_{(1,122.6)}$ = 0.17 p = 0.68	$F_{(1,92.63)}$ = 1.32 p = 0.25	$F_{(1,101.7)}$ = 0.10 p = 0.75	**$F_{(1,138.3)}$ = 3.90 p = 0.051 R^2_{sp} = 0.03**
Circadian period	**$F_{(3,82.82)}$ = 5.32 p = 0.002 R^2_{sp} = 0.16**	$F_{(3,78.78)}$ = 2.06 p = 0.11	$F_{(3,55.4)}$ = 1.16 p = 0.33	$F_{(3,62.69)}$ = 0.39 p = 0.76	$F_{(3,72.72)}$ = 1.00 p = 0.40
Age group	$F_{(1,66.67)}$ = 0.82 p = 0.37	$F_{(1,82.93)}$ = 1.07 p = 0.30	**$F_{(1,73.2)}$ = 11.67 p = 0.001 R^2_{sp} = 0.14**	$F_{(1,75.06)}$ = 2.65 p = 0.11	$F_{(1,79.54)}$ = 1.44 p = 0.23
Cortical excitability × age group	$F_{(1,146.1)}$ = 1.06 p = 0.30	$F_{(1,122.6)}$ = 0.01 p = 0.93	**$F_{(1,92.63)}$ = 5.67 p = 0.02 R^2_{sp} = 0.06**	$F_{(1,101.7)}$ = 0.03 p = 0.86	$F_{(1,138.3)}$ = 0.02 p = 0.89
Cortical excitability × circadian period	$F_{(3,79.35)}$ = 0.43 p = 0.73	$F_{(3,74.66)}$ = 0.50 p = 0.68	$F_{(3,52.74)}$ = 0.26 p = 0.85	$F_{(3,59.99)}$ = 0.68 p = 0.57	$F_{(3,75.37)}$ = 0.40 p = 0.75
Age group × circadian period	$F_{(3,82.82)}$ = 0.78 p = 0.51	**$F_{(3,78.78)}$ = 2.66 p = 0.09 R^2_{sp} = 0.05**	$F_{(3,55.4)}$ = 0.07 p = 0.98	$F_{(3,62.96)}$ = 0.72 p = 0.54	**$F_{(3,72.72)}$ = 3.25 p = 0.03 R^2_{sp} = 0.12**
Cortical excitability × age group × circadian period	$F_{(3,79.35)}$ = 0.89 p = 0.45	$F_{(3,74.66)}$ = 0.91 p = 0.44	**$F_{(3,59.99)}$ = 2.87 p = 0.04 R^2_{sp} = 0.13**	**$F_{(3,75.35)}$ = 3.89 R^2_{sp} = 0.13**	$F_{(3,75.35)}$ = 3.89 p = 0.13

Factors including cortical excitability are in italic. Statistically significant results are in bold.
GLMMs including first row variable as dependent variables and left column variable as predictors. Degrees of freedom are indicated between brackets and were estimated using Kenward-Roger's correction.
Dependent variable sample: PVT, CTT, GO/NO-GO tasks: N_{young} = 13; N_{older} = 12. 2-back, 3-back tasks: N_{young} = 13; N_{older} = 9 (refer to Methods for details).
Key: TMS, transcranial magnetic stimulation; PVT, psychomotor vigilance task; CTT, compensatory tracking task; GLMM, Generalized linear mixed model.
[a] All statistical outcomes are identical when considering other metrics of the PVT such as 10% slowest/fastest/median reaction times or lapses (Basner and Dinges, 2011) (not shown).
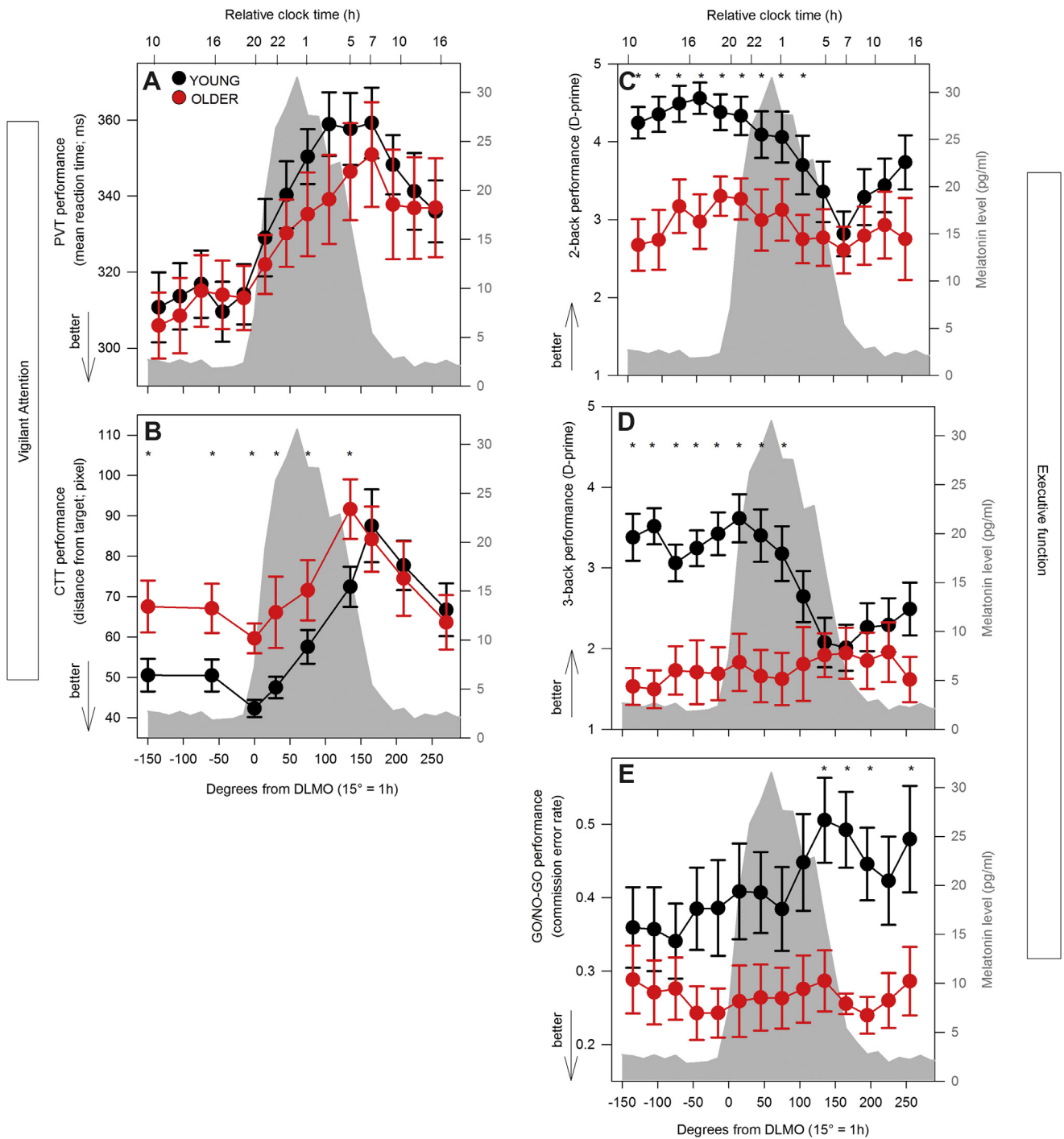
Fig. 4. Cognitive performance dynamics during 34 hours of prolonged wakefulness in young and older adults (mean ± SE). (A, B) Time course of vigilant attention performance [psychomotor Vigilance Task (PVT), mean reaction times; visuomotor compensatory tracking task (CTT), distance from target; $N_{young} = 13$; $N_{older} = 12$]. (C–E) Time course of executive performance (2-back and 3-back task, D-prime (Ingleby, 1967): $N_{young} = 13$; $N_{older} = 9$; GO/NO-GO task, commission error rate: $N_{young} = 13$; $N_{older} = 12$). Time course of all measures is expressed relative to individual melatonin onset (DLMO = phase 0°; 15° = 1h). Average melatonin profile is displayed in gray. Vertical black arrows indicate the direction of performance improvement. * significant group differences ($p < 0.05$). Refer to main text for differences between circadian phases. Abbreviation: DLMO, dim light melatonin onset.

0.05). A trend for an age group difference was found [young < older, $F(1, 23.92) = 3.74$, $p = 0.07$].

We asked whether variations in performance to each vigilant attention task were significantly associated with cortical excitability changes during the protocol. Associations between cortical excitability and vigilant attention measures were investigated

over 4 broad circadian periods of the protocol (instead of single circadian phase), known to be critical for the interplay between the sleep homeostasis and the circadian timing system (Dijk and Czeisler, 1995), that is, the first early waking day, the evening period, the end of the biological night, and the second early waking day after sleep loss (Fig. 1A; see 2.7 Statistics). GLMM

statistical outcomes are reported in Table 2. These analyzes did not reveal any significant association (Supplementary Fig. S2). In our sample, cortical excitability is therefore not significantly associated with performance to tasks relying primarily on vigilant attention.

3.4. Significant association between the dynamics of cortical excitability and executive performance during prolonged wakefulness

Our focus then switched to the cognitive tasks with a higher executive load: the 2-back and 3-back versions of the n-back task and the GO/NO-GO task, which were administered during the cognitive test batteries (Fig. 1A; right before the PVT). The 2- and 3-back tasks are more resource-demanding than the GO/NO-GO, such that 3 older individuals were removed from the n-back analyses because task instructions were not applied correctly (De Beni and Palladino, 2004) (see 2.4.2 N-back tasks). The 2- and 3-back tasks showed overall similar performance profiles (Fig. 4C−D). Performance to the 2-back task changed across circadian phases [$F_{(13,191.7)} = 2.30$, $p = 0.007$], and according to the age group [young > older, $F_{(1,20.27)} = 8.01$, $p = 0.01$], but without a circadian phase × age group interaction [$F_{(13,191.7)} = 1$, $p = 0.45$]. Performance to the 3-back task showed a significant circadian phase × age group interaction [$F_{(13,221.1)} = 3.29$, $p = 0.0001$], a simple effect of age [$F_{(1,19.96)} = 11.96$, $p = 0.03$], but no simple effect of circadian phase [$F_{(13,221.1)} = 1.43$, $p = 0.15$]. For both tasks, post hoc comparisons revealed that young individuals performed significantly better than older adults from the beginning of the protocol to the middle of the night (2-back: young > older, $-135°-105°$, $p ≤ 0.05$; 3-back: young > older, $-135°-75°$, $p ≤ 0.05$). In addition, in young individuals, performance was significantly worse during the end of the biological night and early morning after sleep loss compared with all prior measurements (2-back: young, $-135°-75° > 165°$, $-75°$ to $-15° > 195°$, $-75°$ to $-45°$ > $135°$, $p < 0.05$; 3-back: young, $-135°-75° > 105°-225°$, $p < 0.05$), whereas no differences between circadian phases were detected in older individuals ($p > 0.05$ for all comparisons). GO/NO-GO performance (Fig. 4E) yielded a significant main effect of circadian phase [$F_{(13,234.8)} = 1.84$, $p = 0.04$], a trend for a main effect of age group [$F_{(1,23.21)} = 3.99$, $p = 0.057$], with higher commission error rate in younger individuals but no circadian phase × age group interaction [$F_{(13,234.8)} = 0.79$, $p = 0.67$]. Post hoc contrasts yielded significant differences between age groups, with better performance in the older group from the end of the biological night until the end of the protocol (older < younger: $135°-195°$, $255°$, $p < 0.05$).

These results show that overall performance to an n-back task is lower in older individuals, whereas it is higher for the GO/NO-GO, as in the study by Sagaspe et al., 2012. Better age-related performance to the GO/NO-GO may arise from a speed-accuracy trade-off (Staub et al., 2015) (Supplementary Fig. S3D). The results further confirm that, for both types of executive tasks, older individuals suffer relatively less from sleep loss as compared with the younger group (Sagaspe et al., 2012), a pattern that is reminiscent of the dynamics in the underlying cortical excitability. To formally test this
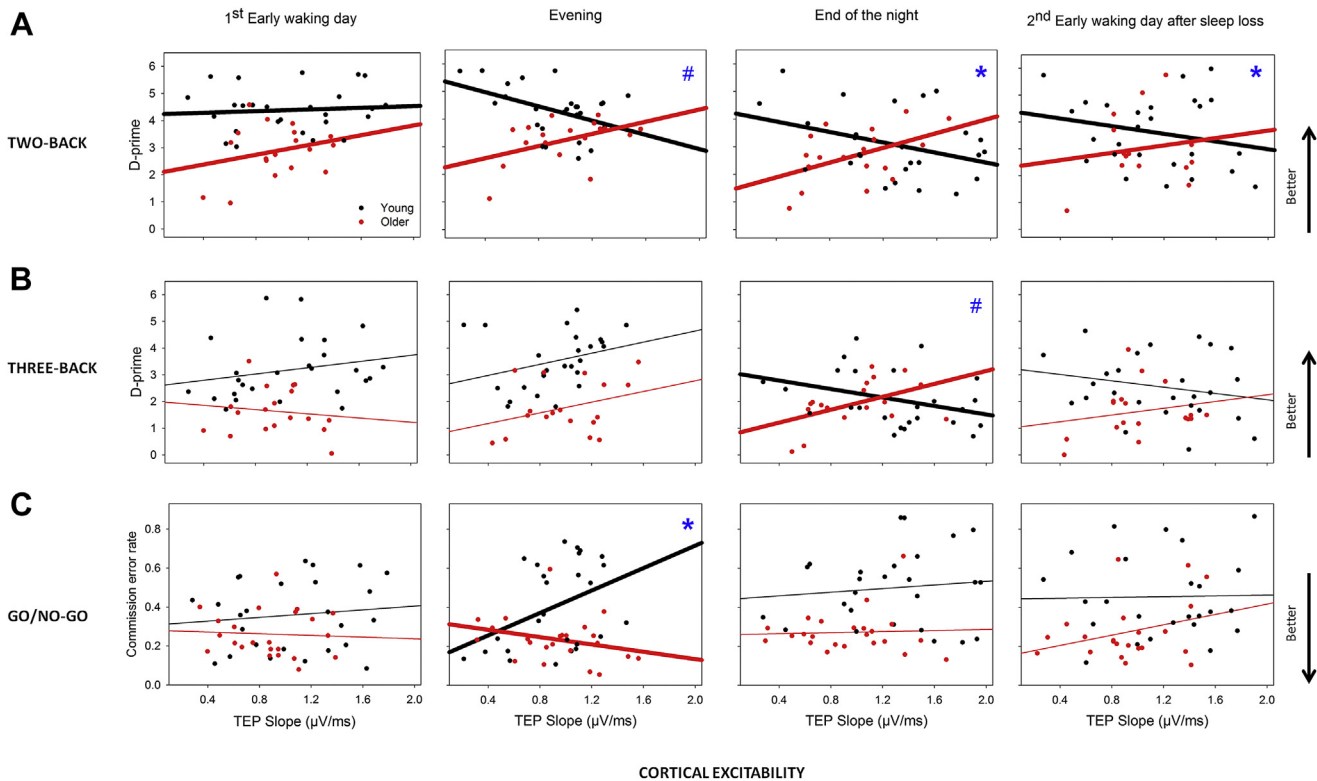
Fig. 5. Associations between executive performance and cortical excitability in young and older individuals during prolonged wakefulness. Regression display between executive performance measures to the 2-back ($N_{young} = 13$; $N_{older} = 9$) (A), 3-back ($N_{young} = 13$; $N_{older} = 9$), (B) and GO/NO-GO ($N_{young} = 13$; $N_{older} = 12$), (C) tasks and cortical excitability (measured as the slope of the first TMS-evoked response), across the 4 circadian periods of the protocol (i.e., first early waking day, evening, end of the biological night and second early waking day after sleep loss). Vertical black arrows indicate the direction of performance improvement. Thicker regression lines highlight the significant associations found in the GLMM analyses; * age group difference of beta, $p ≤ 0.05$; # trend for age group difference of beta, $p ≤ 0.07$. Regressions were used for visual display only and not as a substitute of the full GLMM statistics presented in Table 2. For consistency, cortical excitability and 2-back association was also displayed across all circadian periods; refer to Supplementary Fig. S3 for associations between executive performance and cortical excitability irrespective of circadian period. Abbreviations: GLMM, Generalized linear mixed model; TEP, TMS-evoked potential.

similarity, we computed GLMMs to address whether executive task performance was associated with cortical excitability over the 4 circadian periods of the protocol (first early waking day, evening, end of the biological night, second early waking day after sleep loss). Statistical outcomes are reported in Table 2.

We found that the direction of the association between executive performance and cortical excitability differed between age groups. For the 2-back, this association was irrespective of the circadian period (significant cortical activity × age group interaction; Table 2). Higher cortical excitability was associated with better performance in the older group, whereas the inverse was true for young adults (beta young = −0.41; beta older = 1.17; young vs. older, p = 0.02; Fig. 5A and Supplementary Fig. S3A). Analyses yielded similar results when considering the 3-back and GO/NO-GO tasks but at specific critical circadian periods (significant cortical excitability × age group × circadian period interaction; Table 2). For the 3-back, higher cortical excitability was associated with poorer and better performance, respectively, in the young and older groups at the end of the biological night, when the circadian signal maximally promotes sleep at a time of very high sleep need (Dijk and Czeisler, 1995) (beta young = −0.36; beta older = 0.6; young vs. older, p = 0.07; Fig. 5B). Considering the GO/NO-GO task, higher cortical excitability was associated with poorer and better performance, respectively, in the young and older groups during the evening, when the circadian alerting signal maximally counteracts the need for sleep (Dijk and Czeisler, 1994) (beta young = 0.73; beta older = −0.19; young vs. older, p = 0.02; Fig. 5C). GO/NO-GO performance was also positively related to cortical excitability, irrespective of age group and circadian period [main effect of cortical excitability, $F(1,138.3)$ = 3.90, p = 0.05; Table 2].

4. Discussion

Elucidating the bases of age-related changes in brain function is a crucial scientific challenge. Here we focused on cortical excitability, an essential aspect of basic brain function previously implicated in age-related cognitive decline (Rizzo et al., 2015). The data reveal that cortical excitability dynamics during prolonged wakefulness dampens in aging, with only minor variations during the protocol. The age-related decrease in the buildup of sleep pressure and in the amplitude of the circadian signal, previously detected in EEG synchrony, behavior, and endocrine measures (Dijk et al., 1999; Landolt et al., 2012; Münch et al., 2005; Schmidt et al., 2012), are therefore also reflected in the dynamics of a basic aspect of brain function, making cortical excitability of older adults less susceptible to sleep loss and circadian misalignment. This finding alone may have implications for neurostimulation and neurorehabilitation, which are therapies commonly provided for age-related neurological disorder (Di Pino et al., 2014).

There are several potential mechanisms underlying the progressive change in cortical excitability dynamics in aging, and we are not in a position to isolate them. Recent mouse data indicate that the repertoire of single neuron activity during wakefulness and sleep in the motor cortex is stable in aging, suggesting that single neuron functional characteristics change very little over the lifespan (McKillop et al., 2018). Change in threshold and amplitude of action potentials, as well as in their frequency, has, however, been reported in aging (Rizzo et al., 2015). Similarly, ion channel function and neuromodulator concentrations are progressively altered over the lifespan (Mather and Harley, 2016; Raz and Rodrigue, 2006; Rizzo et al., 2015). In addition, age-related reduction in clock gene expression (Chen et al., 2016; Kondratov et al., 2006) or alterations in homeostatic sleep-dependent gliotransmission regulation (Meyer et al., 2007) were detected. Interestingly, neuronal desynchrony in the aged suprachiasmatic nucleus, that is, the circadian

master clock in mammals, was found in an animal model, resulting in an overall dampening of suprachiasmatic nucleus activity fluctuation over the circadian cycle (Farajnia et al., 2012). Our findings suggest that reduced circadian variation in neuronal function also takes place within the frontal cortex, that is, outside the master circadian clock.

Cortical excitability may ultimately be related to synaptic strength (Rossini and Rossi, 2007). If true, we could infer that, in young individuals, extended wakefulness during the biological night prevent sleep-dependent synaptic downscaling (Tononi and Cirelli, 2006) and increases overall synaptic strength (de Vivo et al., 2017), concomitantly to a strong circadian modulation. In older individuals, we barely detected any changes in cortical excitability when wakefulness was prolonged from one day to the next day (cf. Fig.3, −150° vs. 210° or −60° and 270°). This could be due to age-related synaptic changes (Morrison and Baxter, 2012), which would lead to overall reduced experience-dependent synaptic modification so that sleep would be less required for maintaining synaptic function in aging. This is in line with the age-related reduction in sleep need buildup (Klerman and Dijk, 2008; Shiromani et al., 2000). In vitro research suggests that TMS triggers responses mainly arising from neuron somas (Pashut et al., 2014), such that age-related changes in cortical excitability may also be driven, at least in part, by neuron cell body.

Importantly, we do not find significant difference between age groups irrespective of circadian phase. This is in line with another study (Casarotto et al., 2011) but is contradicting other previous indications of a reduced cortical and neuronal excitability in aging (Ferreri et al., 2017). Discrepancies between studies may in fact reside, at least in part, in the differential impact of sleep need and circadian phase on cortical excitability as one gets older (if prior sleep-wake history or time-of-day were not properly controlled for). Although we do not demonstrate that physiological aging has no impact on overall cortical excitability, our results strongly suggest that, in comparison, the age-related changes in the dynamics of cortical excitability during prolonged wakefulness are more important.

Change in cortical excitability represents part of one's capacity to adapt to daily challenges. We confirm that, in young individuals, this adaptation takes the form of a nonlinear circadian modulation of cortical excitability (i.e., significant difference between the evening vs. early morning) likely reflecting combined circadian and sleep homeostasis influences (Huber et al., 2013; Ly et al., 2016). The dampening of cortical excitability dynamics during prolonged wakefulness in older participants might therefore reflect less adaptable brain underlying reduced cognitive flexibility in aging. In other words, the flexibility in cortical excitability and behavior seen in young during prolonged wakefulness might be a positive allostatic response to acute disruption of the sleep-wake cycle and ultimately an indicator of cognitive fitness.

Exploratory analyses show that cortical excitability may be differentially related to different aspects of cognition, as in our data set, it was significantly related to performance to executive tasks but not to vigilant attention tasks. Using a larger sample of younger individuals, we did find, however, an association between cortical excitability dynamics during sleep loss and vigilant attention (Ly et al., 2016). Our data further suggest that the direction of the association between cortical excitability and executive performance may change across the age groups: in our data set, older individuals' increased cortical excitability is associated with better performance, whereas in young adults, it is associated with worse performance. This may again be related to specific and relatively subtle synaptic alterations which are associated with impairments in cognitive function rather than to merely loss of neurons in the neocortex (Morrison and Baxter, 2012). This preliminary finding

may also indicate that older participants displaying a margin ability in increasing cortical excitability (i.e., cortical resilience) perform better in task requiring a high degree of cognitive flexibility, such as executive function (Gajewski and Falkenstein, 2018). It is important to stress, however, that no causal link can be drawn for the present study. Our findings may point toward a role for the dynamics of cortical excitability during prolonged wakefulness in driving age-related variations in cognitive performance, at least for executive processes. We surmise that this link would follow two different trajectories depending on age: an inverted U-shape for the young, with an optimal level of cortical excitability beyond which performance would be negatively related to higher cortical excitability. In young individuals, cortical excitability would be close to this optimal level during the circadian day while well rested, as indicated by mostly high and stable performance, but the significant rise in cortical excitability found during the biological night would be detrimental for cognition. In contrast, in older individuals, the link between cortical excitability and performance would be linear. Modifications of cortical excitability, through changes in the circadian system and in the buildup of the need for sleep, are reduced or compromised in older individuals: the optimal level beyond which the association becomes negative is not reached. Because the association between cortical excitability and executive performance was positive in older adults, it may imply that cognition could be improved in aging by acting on neuron excitability, but this remains to be formally tested with a large sample size. Herein, we observed an association between cortical excitability and executive performance at specific circadian periods for two of the three executive tasks. Future investigations, in larger sample size, are required to confirm these preliminary findings and address notably whether the association between cortical excitability and executive performance is specific to certain circadian periods or is present at all circadian phases with variable strength.

The reason for the unequal association between cortical excitability and different cognitive domains may reside in part on the distinct brain regions sustaining them: executive function rely heavily (but not exclusively) on the frontal cortex, the region probed with TMS in the present study, while the cortical substrates of attentional processes are more posterior and depend more substantially on the parietal cortex and on subcortical areas (Fan et al., 2005; Schmidt et al., 2009). Furthermore, evidence suggest that early age-specific and subtle neural changes are nested primarily in the frontal cortex areas (Daigneault et al., 1992; Masliah et al., 1993), sustaining high order abilities (Wang et al., 2011) so that executive functions are among those most vulnerable to the aging process. Our cortical measure may have caught these subtle age-related differences in measures of executive performance, especially when considering early stages of cognitive decline (our age sample was ∼60 years old).

5. Conclusions

Herein, we tested whether sleep-wake regulation of basic cortical function changed across young adults (<30 years) to late middle-aged individuals (50—70 years). We demonstrate that the dynamics of cortical excitability during prolonged wakefulness dampens in older individuals, presumable because of the age-related changes in the interplay between circadian rhythmicity and sleep homeostasis (Schmidt et al., 2012). We further provide preliminary evidence that the lessened clockwork of the circadian and sleep homeostasis processes in aging may act on cognition through a reduction of cortical excitability during extended wakefulness. It is likely that this process does not suddenly change at the age range of 60 years but gradually abate from the middle year of life (Carrier et al., 2001). The current results provide a framework for future studies that should address whether preserved cortical excitability dynamics during sustained wakefulness may not only counteract cognitive decline into advanced age but also protect against neurodegenerative diseases, such as Alzheimer's disease.

Disclosure

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neurobiolaging.2019.02.004.

References

Basner, M., Dinges, D.F., 2011. Maximizing sensitivity of the psychomotor vigilance test (PVT) to sleep loss. Sleep 34, 581—591.

Beck, A.T., Brown, G., Epstein, N., Steer, R.A., 1988. An inventory for measuring clinical anxiety: psychometric properties. J. Consult. Clin. Psychol. 56, 893—897.

Berthomier, C., Drouot, X., Herman-Stoïca, M., Berthomier, P., Prado, J., Bokar-Thire, D., Benoit, O., Mattout, J., D'Ortho, M.-P., 2007. Automatic analysis of single-channel sleep EEG: validation in healthy individuals. Sleep 30, 1587—1595.

Bushey, D., Tononi, G., Cirelli, C., 2015. Sleep- and wake-dependent changes in neuronal activity and reactivity demonstrated in fly neurons using in vivo calcium imaging. Proc. Natl. Acad. Sci. U S A 112, 4785—4790.

Buysse, D.J., Monk, T.H., Carrier, J., Begley, A., 2005. Circadian patterns of sleep, sleepiness, and performance in older and younger adults. Sleep 28, 1365—1376.

Buysse, D.J., Reynolds, C.F., Monk, T.H., Berman, S.R., Kupfer, D.J., 1989. The Pittsburgh sleep quality index: A new instrument for psychiatric practice and research. Psychiatry. Res. 28, 193—213.

Carrier, J., Land, S., Buysse, D.J., Kupfer, D.J., Monk, T.H., 2001. The effects of age and gender on sleep EEG power spectral density in the middle years of life (ages 20-60 years old). Psychophysiology 38, 232—242.

Casarotto, S., Mä Ä Ttä, S., Herukka, S.-K., Pigorini, A., Napolitani, M., Gosseries, O., Niskanen, E., Kö Nö Nen, M., Mervaala, E., Rosanova, M., Soininen, H., Massimini, M., 2011. Transcranial magnetic stimulation-evoked EEG/cortical potentials in physiological and pathological aging. Neuroreport 22.

Chang, Y.-M., Rosene, D.L., Killiany, R.J., Mangiamele, L.A., Luebke, J.I., 2005. Increased action potential firing rates of layer 2/3 pyramidal cells in the prefrontal cortex are significantly related to cognitive performance in aged monkeys. Cereb. Cortex. 15, 409—418.

Chen, C.-Y., Logan, R.W., Ma, T., Lewis, D.A., Tseng, G.C., Sibille, E., McClung, C.A., 2016. Effects of aging on circadian patterns of gene expression in the human prefrontal cortex. Proc. Natl. Acad. Sci. U S A 113, 206—211.

Daigneault, S., Braun, C.M.J., Whitaker, H.A., 1992. Early effects of normal aging on perseverative and non-perseverative prefrontal measures. Dev. Neuropsychol. 8, 99–114.

De Beni, R., Palladino, P., 2004. Decline in working memory updating through ageing: intrusion error analyses. Memory 12, 75–89.

de Vivo, L., Bellesi, M., Marshall, W., Bushong, E.A., Ellisman, M.H., Tononi, G., Cirelli, C., 2017. Ultrastructural evidence for synaptic scaling across the wake/sleep cycle. Science 355, 507–510.

Di Pino, G., Pellegrino, G., Assenza, G., Capone, F., Ferreri, F., Formica, D., Ranieri, F., Tombini, M., Ziemann, U., Rothwell, J.C., Di Lazzaro, V., 2014. Modulation of brain plasticity in stroke: a novel model for neurorehabilitation. Nat. Rev. Neurol. 10, 597–608.

Dijk, D., Duffy, J.F., Riel, E., Shanahan, T.L., Czeisler, C.A., 1999. Ageing and the circadian and homeostatic regulation of human sleep during forced desynchrony of rest, melatonin and temperature rhythms. J. Physiol. 516, 611–627.

Dijk, D.J., Czeisler, C.A., 1995. Contribution of the circadian pacemaker and the sleep homeostat to sleep propensity, sleep structure, electroencephalographic slow waves, and sleep spindle activity in humans. J. Neurosci. 15, 3526–3538.

Dijk, D.J., Czeisler, C.A., 1994. Paradoxical timing of the circadian rhythm of sleep propensity serves to consolidate sleep and wakefulness in humans. Neurosci. Lett. 166, 63–68.

Duffy, J.F., Dijk, D.J., 2002. Getting through to circadian oscillators: why use constant routines? J. Biol. Rhythms. 17, 4–13.

English, J., Middleton, B.A., Arendt, J., Wirz-Justice, A., 1993. Rapid direct measurement of melatonin in saliva using an iodinated tracer and solid phase second antibody. Ann. Clin. Biochem. 30 (Pt 4), 415–416.

Fan, J., McCandliss, B.D., Fossella, J., Flombaum, J.I., Posner, M.I., 2005. The activation of attentional networks. Neuroimage 26, 471–479.

Farajnia, S., Michel, S., Deboer, T., vanderLeest, H.T., Houben, T., Rohling, J.H.T., Ramkisoensing, A., Yasenkov, R., Meijer, J.H., 2012. Evidence for neuronal desynchrony in the aged suprachiasmatic nucleus clock. J. Neurosci. 32, 5891–5899.

Ferreri, F., Vecchio, F., Guerra, A., Miraglia, F., Ponzo, D., Vollero, L., Iannello, G., Maatta, S., Mervaala, E., Rossini, P.M., Di Lazzaro, V., 2017. Age related differences in functional synchronization of EEG activity as evaluated by means of TMS-EEG coregistrations. Neurosci. Lett. 647, 141–146.

Frank, M.G., Cantera, R., 2014. Sleep, clocks, and synaptic plasticity. Trends. Neurosci. 37, 491–501.

Gajewski, P.D., Falkenstein, M., 2018. ERP and behavioral effects of physical and cognitive training on working memory in aging: a randomized controlled study. Neural. Plast. 2018, 3454835.

Horne, J.A., Östberg, O., 1976. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int. J. Chronobiol. 4, 97–110.

Huber, R., Mäki, H., Rosanova, M., Casarotto, S., Canali, P., Casali, A.G., Tononi, G., Massimini, M., 2013. Human cortical excitability increases with time awake. Cereb. Cortex. 23, 332–338.

Ingleby, J.D., 1967. Signal detection theory and psychophysics, Journal of Sound and Vibration. Wiley, New-York.

Jaeger, B.C., Edwards, L.J., Das, K., Sen, P.K., 2017. An R 2 statistic for fixed effects in the generalized linear mixed model. J. Appl. Stat. 44, 1086–1105.

Johns, M.W., 1991. A new method for measuring daytime sleepiness: the epworth sleepiness scale. Sleep 14, 540–545.

Klerman, E.B., Dijk, D.J., 2008. Age-related reduction in the maximal capacity for sleep-implications for insomnia. Curr. Biol. 18, 1118–1123.

Kondratov, R.V., Kondratova, A.A., Gorbacheva, V.Y., Vykhovanets, O.V., Antoch, M.P., 2006. Early aging and age-related pathologies in mice deficient in BMAL1, the core componentof the circadian clock. Genes. Dev. 20, 1868–1873.

Kondratova, A. a, Kondratov, R.V., 2012. The circadian clock and pathology of the ageing brain. Nat. Rev. Neurosci. 13, 325–335.

Landolt, H.P., Rétey, J.V., Adam, M., 2012. Reduced neurobehavioral impairment from sleep deprivation in older adults: Contribution of adenosinergic mechanisms. Front. Neurol. 3, 62.

Lim, A.S.P., Kowgier, M., Yu, L., Buchman, A.S., Bennett, D.A., 2013. Sleep fragmentation and the risk of incident alzheimer's disease and cognitive decline in older persons. Sleep 36, 1027–1032.

Lo, J.C., Groeger, J.A., Santhi, N., Arbon, E.L., Lazar, A.S., Hasan, S., von Schantz, M., Archer, S.N., Dijk, D.-J., 2012. Effects of partial and acute total sleep deprivation on performance across cognitive domains, individuals and circadian phase. PLoS One 7, e45987.

Ly, J.Q.M., Gaggioni, G., Chellappa, S.L., Papachilleos, S., Brzozowski, A., Borsu, C., Rosanova, M., Sarasso, S., Middleton, B., Luxen, A., Archer, S.N., Phillips, C., Dijk, D.-J., Maquet, P., Massimini, M., Vandewalle, G., 2016. Circadian regulation of human cortical excitability. Nat. Commun. 7, 11828.

Masliah, E., Mallory, M., Hansen, L., DeTeresa, R., Terry, R.D., 1993. Quantitative synaptic alterations in the human neocortex during normal aging. Neurology 43, 192–197.

Mather, M., Harley, C.W., 2016. The locus coeruleus: essential for maintaining cognitive function and the aging brain. Trends. Cogn. Sci. 20, 214–226.

Mattis, S., 1988. Dementia Rating Scale: Professional Manual. Psychological Assessment Resources, Odessa, FL.

McKillop, L.E., Fisher, S.P., Cui, N., Peirson, S.N., Foster, R.G., Wafford, K.A., Vyazovskiy, V.V., 2018. Effects of ageing on cortical neural dynamics and local sleep homeostasis in mice. J. Neurosci. 38, 2513–2517.

Meisel, C., Schulze-Bonhage, A., Freestone, D., Cook, M.J., Achermann, P., Plenz, D., 2015. Intrinsic excitability measures track antiepileptic drug action and uncover increasing/decreasing excitability over the wake/sleep cycle. Proc. Natl. Acad. Sci. 112, 14694–14699.

Meyer, P.T., Elmenhorst, D., Boy, C., Winz, O., Matusch, A., Zilles, K., Bauer, A., 2007. Effect of aging on cerebral A1 adenosine receptors: A [18F]CPFPX PET study in humans. Neurobiol. Aging. 28, 1914–1924.

Morrison, J.H., Baxter, M.G., 2012. The ageing cortical synapse: hallmarks and implications for cognitive decline. Nat. Rev. Neurosci. 13, 240–250.

Münch, M., Knoblauch, V., Blatter, K., Schröder, C., Schnitzler, C., Kräuchi, K., Wirz-Justice, A., Cajochen, C., 2005. Age-related attenuation of the evening circadian arousal signal in humans. Neurobiol. Aging. 26, 1307–1319.

Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods. Ecol. Evol. 4, 133–142.

Oosterman, J.M., Van Someren, E.J.W., Vogels, R.L.C., Van Harten, B., Scherder, E.J.A., 2009. Fragmentation of the rest-activity rhythm correlates with age-related cognitive deficits. J. Sleep. Res. 18, 129–135.

Pashut, T., Magidov, D., Ben-Porat, H., Wolfus, S., Friedman, A., Perel, E., Lavidor, M., Bar-Gad, I., Yeshurun, Y., Korngreen, A., 2014. Patch-clamp recordings of rat neurons from acute brain slices of the somatosensory cortex during magnetic stimulation. Front. Cell. Neurosci. 8, 145.

Pevet, P., Challet, E., 2011. Melatonin: Both master clock output and internal time-giver in the circadian clocks network. J. Physiol. 105, 170–182.

Raz, N., Rodrigue, K.M., 2006. Differential aging of the brain: Patterns, cognitive correlates and modifiers. Neurosci. Biobehav. Rev. 30, 730–748.

Read, G.F., Fahmy, D.R., Walker, R.F., 1977. Determination of cortisol in human plasma by radioimmunoassay. use of the 125I-labelled radioligand. Ann. Clin. Biochem. 14, 343–349.

Reuter-Lorenz, P.A., Park, D.C., 2014. How does it STAC up? revisiting the scaffolding theory of aging and cognition. Neuropsychol. Rev. 24, 355–370.

Rizzo, V., Richman, J., Puthanveettil, S.V., 2015. Dissecting mechanisms of brain aging by studying the intrinsic excitability of neurons. Front. Aging. Neurosci. 6, 337.

Rossini, P.M., Rossi, S., 2007. Transcranial magnetic stimulation: diagnostic, therapeutic, and research potential. Neurology 68, 484–488.

Sagaspe, P., Taillard, J., Amiéva, H., Beck, A., Rascol, O., Dartigues, J.F., Capelli, A., Philip, P., 2012. Influence of age, circadian and homeostatic processes on inhibitory motor control: a Go/Nogo task study. PLoS One 7.

Sagaspe, P., Taillard, J., Chaumet, G., Moore, N., Bioulac, B., Philip, P., 2007. Aging and nocturnal driving: better with coffee or a nap? a randomized study. Sleep 30, 1808–1813.

Schmidt, C., Collette, F., Leclercq, Y., Sterpenich, V., Vandewalle, G., Berthomier, P., Berthomier, C., Phillips, C., Tinguely, G., Darsaud, A., Gais, S., Schabus, M., Desseilles, M., Dang-Vu, T.T., Salmon, E., Balteau, E., Degueldre, C., Luxen, A., Maquet, P., Cajochen, C., Peigneux, P., 2009. Homeostatic sleep pressure and responses to sustained attention in the suprachiasmatic area. Science 324, 516–519.

Schmidt, C., Peigneux, P., Cajochen, C., 2012. Age-related changes in sleep and circadian rhythms: impact on cognitive performance and underlying neuroanatomical networks. Front. Neurol. 3, 118.

Shekleton, J.A., Rajaratnam, S.M.W., Gooley, J.J., Van Reen, E., Czeisler, C.A., Lockley, S.W., 2013. Improved neurobehavioral performance during the wake maintenance zone. J. Clin. Sleep. Med. 9, 353–362.

Shiromani, P.J., Lu, J., Wagner, D., Thakkar, J., Greco, M.A., Basheer, R., Thakkar, M., 2000. Compensatory sleep response to 12 h wakefulness in young and old rats. Am. J. Physiol. Integr. Comp. Physiol. 278, R125–R133.

Staub, B., Doignon-Camus, N., Marques-Carneiro, J.E., Bacon, É., Bonnefond, A., 2015. Age-related differences in the use of automatic and controlled processes in a situation of sustained attention. Neuropsychologia 75, 607–616.

Steer, R.A., Ball, R., Raneeri, W.F., Beck, A.T., 1997. Further evidence for the construct validity of the beck depression inventory-ii with psychiatric outpatients. Psychol. Rep. 80, 443–446.

Tononi, G., Cirelli, C., 2014. Sleep and the price of plasticity: from synaptic and cellular homeostasis to memory consolidation and integration. Neuron 81, 12–34.

Tononi, G., Cirelli, C., 2006. Sleep function and synaptic homeostasis. Sleep. Med. Rev. 10, 49–62.

Van Cauter, E., 2000. Age-related changes in slow wave sleep and rem sleep and relationship with growth hormone and cortisol levels in healthy men. JAMA 284, 861–868.

Virtanen, J., Ruohonen, J., Naatanen, R., Ilmoniemi, R.J., 1999. Instrumentation for the measurement of electric brain responses to transcranial magnetic stimulation. Med. Biol. Eng. Comput. 37, 322–326.

Wang, M., Gamo, N.J., Yang, Y., Jin, L.E., Wang, X.-J., Laubach, M., Mazer, J.A., Lee, D., Arnsten, A.F.T., 2011. Neuronal basis of age-related working memory decline. Nature 476, 210–213.

Phase II, Paper 4: Dynamics of cortical response complexity during sleep deprivation and circadian misalignment in young and older healthy individuals and association with vigilance performance

Gaggioni G., Shumbayawonda E., Montanaro U., Ly JQM., Phillips C., Vandewalle G.[§], Abasolo D.

[§] *In prep.*

Since EEG complexity has been shown to change between sleep and alert wakefulness vigilance states, as well as according to age (both during sleep and wakefulness) (Casali et al., 2013; Tosun et al., 2019), and to be related to cognitive performance (Stam, 2005), we decided to take an alternative approach this time to re-investigate cortical complexity response dynamic during prolonged wakefulness and, in addition, to assess whether it changes with age. In *phase I* indeed, a first attempt to measure cortical response complexity was done at the source level, in a group of young participants (18-30 y). In *phase II*, cortical complexity response is computed at the sensor level, both locally (closest to TMS hotspot) and globally (over the scalp), in young (18-30 y) and older participants (50-70 y). The approach at the sensor level has been chosen because the source reconstruction steps might have added noise to data, masking the subtle temporal profile changes of the cortical response complexity, and also because a sensor level approach is faster to compute (Comolatti et al., 2019). The main objective is still to investigate whether brain response complexity changes during prolonged wakefulness and sleep deprivation, when vigilance level considerably varies. We hypothesise that (1) cortical response complexity will reflect the dual impact of sleep homeostasis and the circadian signal. In particular, because of the increasing intrusion of slow EEG oscillations during the biological night, we expect lower complexity value at night, around the nadir of vigilance performance. (2) Since EEG brain activity during both sleep and wakefulness undergoes a relative shift towards more high-frequency and oscillation power in ageing, we further anticipate that cortical response complexity will be higher in the older group over the entire protocol. Results disclose that global cortical complexity response profile changes with time spent awake, but –against our initial thoughts–

complexity tends to increase at the beginning of the biological night and to decrease the following biological day (state-like effect). Cortical complexity response is higher in the older compared to the young participants (trait-like effect) and is associated with worse vigilance performance. Thus, non-linear Lempel-Ziv complexity can provide additional insights to classical linear approaches, and further characterise the neurophysiological mechanisms of cortical activity.

1 **Dynamics of cortical response complexity during sleep deprivation and circadian misalignment in**

2 **young and older healthy individuals and association with vigilance performance**

3

4 **Abstract**

5 Characterization of the complexity of EEG responses has provided important insight in cognitive function as

6 well as in the brain bases of consciousness and vigilance. Whether brain response complexity changes during

7 prolonged wakefulness and sleep deprivation –when vigilance level considerably varies– is not fully elucidated

8 yet. In the present study, we repeatedly assessed EEG responses to transcranial magnetic stimulation (TMS)

9 over 34h of sleep deprivation under constant routine conditions in healthy younger (N = 13; 5 women; 18-30

10 yo) and older (N = 12; 6 women; 50-70 yo) individuals, while they were performing a vigilance task. Response

11 complexity was computed both at the global (all scalp sensors) and local (around TMS hotspot) levels using

12 the Lempel-Ziv algorithm, which measures the randomness of a given time series. Complexity response was

13 significantly higher in older compared to young volunteers over the entire protocol. Global complexity

14 response significantly changed with time spent awake, with a potential increase from the beginning to the

15 middle of the biological night, followed by a potential decrease from the middle of the biological night to the

16 following afternoon. An unexpected different link between vigilance performance and brain response

17 complexity was detected across age groups: higher response complexity was associated with lower

18 performance in the older group, particular in the morning sessions. These findings show the evolution of

19 cortical complexity between different level of vigilance, experienced during sleep deprivation and circadian

20 misalignment in two age groups. Lempel-Ziv complexity can provide additional insights to classical linear

21 approaches, and further characterise the neurophysiological mechanisms of cortical activity.

22

23

Introduction

In our 24/7 society, how the brain deals with sleep loss and circadian misalignment, and how performance is affected by it is a timely research focus. Sleep homeostasis and the circadian timing system have been established as essential to these matters. Sleep homeostasis progressively increases during prolonged wakefulness. Under well-rested circumstances during the biological day, the circadian signal opposes this progressive increase in sleep need, so that brain function and performance remain relatively stable over ~16h of wakefulness (A A Borbély, 1982; Alexander A. Borbély, Daan, Wirz-Justice, & Deboer, 2016). The circadian signal then stops opposing sleep need and promotes sleep during the biological night, so that sleep can be initiated and maintained throughout the biological night. However, if wakefulness is extended into the biological night, cognition and vigilance are jeopardized, particularly at the end of the night. If wakefulness is further prolonged, the circadian signal will re-oppose sleep need, thus triggering a partial restoration of vigilance and behavioural performance during the next biological day, even if sleep did not take place.

These changes are reflected in variations in the prevalence of different oscillations of the electroencephalogram (EEG). The EEG repertoire remains relatively stable towards higher frequencies during the day, but shows a sharply increase in lower frequencies at night if one remains awake, followed by a partial decrease the following day, reflecting the dual impact of sleep homeostasis and the circadian system (Cajochen, Wyatt, Czeisler, & Dijk, 2002). If sleep occurs at night, the initial high power in lower frequency progressively decreases, reflecting sleep need dissipation. These dynamic changes in EEG oscillation composition has been related to molecular, cellular and system level changes. These insights have however been gained through linear analyses of the EEG (e.g. Fourier transformation).

In the last decades, non-linear mathematical approaches have been applied to infer temporal structure of brain activity (Stam, 2005b). Lempel-Ziv complexity (LZC) algorithm is one of these approaches, which characterizes mostly the degree of randomness of the time series (Lempel & Ziv, 1976): it is related to the number of distinct substrings and to the their recurrence rate along a given sequence. LZC reflects the underlying activeness and information processing capacity of the underlying neurons (Hu & Zhang, 2019) and is therefore brain state-dependent. LZC has been proven to successfully differentiate between different consciousness and vigilance states (alert wakefulness, light and deep slow wave sleep, rapid eye movement (REM) sleep, disorders of consciousness, anaesthesia) (Casali et al., 2013; Mateos, Guevara Erra, Wennberg,

& Perez Velazquez, 2018; Tosun, Dijk, Winsky-Sommerer, & Abasolo, 2019). LZC and related measures of irregularity have been shown to be high during normal wakefulness and REM sleep and low during non-REM (NREM) sleep, with a progressive decrease from light to deeper sleep stages. This progressive increase in the regularity of the signal depends at least in part on changes in the balance between high-frequency and low-frequency EEG powers, resulting in a hypersynchronous EEG signal during deep sleep (Aboy, Hornero, Abásolo, & Álvarez, 2006b). Two studies investigated whether cortical complexity changes during partial sleep deprivation in rats (Abásolo, Simons, Morgado da Silva, Tononi, & Vyazovskiy, 2015; Tosun, Abásolo, Stenson, & Winsky-Sommerer, 2017) and found no significant change in complexity. Furthermore, a study in young humans also reported no significant changes in spatial complexity over 28h of sleep deprivation (Gaggioni et al., 2018). Whether LZC EEG complexity changes during prolonged wakefulness remains to be established in humans and in particular over the biological night during total sleep deprivation. Likewise, whether the dynamics of EEG complexity during prolonged wakefulness varies in ageing –when sleep homeostasis and the circadian system undergo profound modifications (Schmidt, Peigneux, & Cajochen, 2012) (Derk Jan Dijk & Duffy, 1999; Landolt, Rétey, & Adam, 2012; Münch et al., 2005)– remains to be investigated. Furthermore, how these changes may be related to vigilance performance is also not known.

To answer these questions, we computed LZC complexity of EEG brain responses to transcranial magnetic stimulation (TMS), recorded in 9 TMS-EEG sessions acquired over 34h of prolonged wakefulness, under strictly controlled constant routine conditions (Duffy & Dijk, 2002), in heathy younger and older adults of both sexes. This protocol allows to detect the combined influence of sleep homeostasis and the circadian system on TMS-induced cortical response, which mimicked normal brain stimulus processing (Burke, Fried, & Pascual-Leone, 2019). Concomitantly to TMS-EEG recordings, participants performed a vigilance task, allowing for correlations with simultaneous LZC complexity that was computed both globally (over the entire scalp) and locally (around TMS hotspot). We hypothesised that cortical response complexity would reflect the dual impact of sleep homeostasis and the circadian system on brain function. In particular, because of the increasing intrusion of slow EEG oscillations during the biological night, we expect lower complexity value at night, around the nadir of vigilance performance. Since EEG brain activity during both sleep and wakefulness undergoes a relative shift towards more high-frequency and oscillation power in ageing (Carrier, Land, Buysse, Kupfer, & Monk, 2001), we further anticipated that cortical response complexity would be

80 higher in the older group over the entire protocol. Finally, we expected significant associations between LZC

81 complexity and vigilance performance, particularly during the biological night.

82

83 **2. Material and Methods**

84 Except for Lempel-Ziv complexity analyses, all procedures are as in (Gaggioni et al., 2019).

85

86 2.1. Participants. The study was approved by the Ethics Committee of the Medicine Faculty of the University

87 of Liège. Participants gave their written informed consent and received a financial compensation. Twenty-six

88 healthy participants were enrolled, 13 older adults (62.6 y ± 3.8; 7 women) and 13 young (22.8 y ± 2.9; 5

89 women). Exclusion criteria included: Body Mass Index (BMI) < 18 and > 28; recent psychiatric history, severe

90 trauma, sleep disorders; addiction; chronic medication affecting the nervous system; smokers, excessive

91 alcohol (> 14 doses/week) or caffeine (> 3 cups/day) consumption; night shift workers during the last year;

92 transmeridian travel during the last two months; anxiety or depression; poor sleep quality; excessive self-

93 reported daytime sleepiness; early signs of dementia (in older participants). Anxiety was measured by the 21

94 item Beck Anxiety Inventory (BAI ≤ 14) (Beck, Brown, Epstein, & Steer, 1988); mood by the 21 items Beck

95 Depression Inventory II (BDI-II ≤ 14) (Steer, Ball, Ranieri, & Beck, 1997); sleep quality by the Pittsburgh

96 Sleep Quality Index Questionnaire (PSQI ≤ 7) (Buysse, Reynolds 3rd, Monk, Berman, & Kupfer, 1989);

97 daytime sleepiness by the Epworth Sleepiness Scale (ESS ≤ 11) (Johns, 1991); early signs of dementia using

98 Mattis scale (Mattis S, 1998). Chronotype was also assessed using the Horne-Östberg Questionnaire (Horne

99 & Östberg, 1976). As in (Gaggioni et al., 2019), one older participant was discarded from all analyses because

100 his performance was 3 interquartile ranges above or below the 25th and 75th percentile of the older participant

101 sample. The final sample included therefore 13 young and 12 older participants. Further sample characteristics

102 are reported in (Gaggioni et al., 2019).

103

104 2.2. Experimental protocol. At least a week before the experiment, participants completed a preparatory TMS-

105 EEG session to determine optimal TMS parameters for artefact-free recodings. Participants also completed a

106 screening night of sleep to exclude major sleep disorders (periodic leg movement with perceived leg

107 impatience and/or apnea-hypopnea index > 15/h). During the 7 days preceding the study, they kept a regular

108   sleep-wake schedule (± 15 min; verified using wrist actigraphy –actiwatch, Cambridge Neurotechnology, UK–

109   and sleep diaries). Schedule and duration were based on at least 10 days of unconstrained actimetry recordings.

110   Participants were requested to abstain from all caffeine and alcohol-containing beverages for 3 days preceding

111   the study.

112   Participants were first maintained in dim light for 5.5 h (< 5 lux) and trained to the cognitive test

113   batteries, prior to sleeping at their habitual bedtime, for their habitual duration (in complete darkness).

114   Following awakening and brief showering, the experiment consisted in a 34h constant routine sleep deprivation

115   protocol (i.e. light < 5 lux, temperature ~19°C, regular isocaloric liquid meals and water, semi-recumbent

116   position, no time-of-day information, sound proofed rooms) (Duffy & Dijk, 2002). The TMS-compatible

117   electrode cap was placed upon awaking. TMS-evoked EEG potentials (TEPs) were recorded 9 times (1000,

118   1600, 2000, 2200, 0100, 0500, 0700, 1000, 1600, for a subject sleeping from 2300 to 0700) (**Fig. 1A**).

119

120   2.3. TMS-evoked EEG response acquisitions and preprocessing. As in (Huber et al., 2013; Julien Q.M. Ly et

121   al., 2016), the left or right superior frontal gyrus was set as stimulation target for right or left-handed,

122   respectively. Stimulation target was located on individual structural MRI by means of a neuronavigation

123   system (Navigated Brain Stimulation; Nexstim). This device allows for reproducible evoked EEG responses

124   and precise target location (FDA approval for presurgery). The neuronavigation system ensured that hotspot

125   location remained constant across sessions within an individual (± 2 mm). TMS pulses were generated by a

126   Focal Bipulse 8-coil (mean/outer winding diameter ca. 50/70 mm, Nexstim, Helsinki, Finland). The intensity

127   of TMS pulses (I, %), the estimated induced electric field (EF, V/m), and the distance between the coil and the

128   cortical hotspot ($Dist$, mm) did not diverge between the two age groups (I_{young} = 54.2 ± 4.5, I_{older} = 55.2 ± 5.2;

129   EF_{young} = 108.5 ± 16, EF_{older} = 116.2 ± 16.6; $Dist_{young}$ = 17.9 ± 2.2, $Dist_{older}$ = 17.5 ± 2.2 –all comparisons P >

130   0.6). Each TMS-EEG session included 250-300 trials. Interstimulus intervals were randomly jittered between

131   1900 and 2200 ms. TMS responses were recorded with a 60-channel TMS-compatible EEG amplifier (Eximia;

132   Nexstim), equipped with a proprietary sample-and-hold circuit that provides TMS artifact free data from 5 ms

133   post-TMS (Virtanen, Ruohonen, Naatanen, & Ilmoniemi, 1999). Electrooculogram (EOG) was recorded with

134   two additional bipolar electrodes. Participants wore the EEG cap during the entire protocol, and electrodes

135   impedance was set below 5 kΩ prior to each recording session. Signal was band-pass-filtered between 0.1 and

500 Hz and sampled at 1450 Hz. Each TMS-EEG session ended with a neuronavigated digitization of the location of each electrode. Auditory EEG potentials (AEP) evoked by TMS and bone conductance were minimized by diffusing a continuous loud white masking noise through earplugs, and applying a thin foam layer between the EEG cap and the TMS coil. Each session was followed by a sham session consisting in 30-40 TMS pulses delivered parallel to the scalp while white noise was diffused at the same level. Absence of AEP was checked online on Cz between 0-500 ms post-TMS (all sessions were AEP-free). Data of sham sessions were not considered any further.

EEG data were preprocessed using SPM12 (Statistical Parametric Mapping 12, http://www.fil.ion.ucl.ac.uk/spm/) implemented in Matlab 2015 (The Mathworks Inc, Natick, MA). Processing included the following: visual rejection of artefact, re-referencing to average of good channels, low-pass filtering at 80 Hz, resampling from 1450 to 1000 Hz, high-pass filtering at 1 Hz, epoching between –100 and 300 ms around TMS pulses (at 0 ms), baseline correcting (-100 to -1 ms pre-TMS), robust averaging (Leonowicz, Karvanen, & Shishkin, 2005).

2.4. Compensatory tracking task (CTT). This visuomotor task probes vigilance (i.e. the ability to sustain attention over prolonged periods of time) and was performed during the TMS-EEG recordings (as in (Huber et al., 2013; Julien Q.M. Ly et al., 2016)). It consists in keeping a constantly randomly moving cursor on a target located in the centre of a computer screen, using a trackball device. The task was chosen because it only requires continuous smooth and limited movement of a single finger and allows for continuous vigilance monitoring during TMS-EEG recordings. Performance was computed as the average distance (in pixels) between the cursor and the target during TMS-EEG recordings, following removal of lapses. If signs of drowsiness were detected while performing the task during TMS-EEG sessions, the experimenter briefly touched the participant. Transitory lapses of vigilance resulted in temporary increases of the target-cursor distance, and could be automatically detected offline. A lapse was identified when the cursor was located outside a central 200 by 200 pixel box surrounding the target for > 500 ms from the last trackball movement. The lapse period ranged from the last trackball movement until the lapse detection. TMS evoked responses occurring during and < 1 s from a lapse period were discarded from analyses.
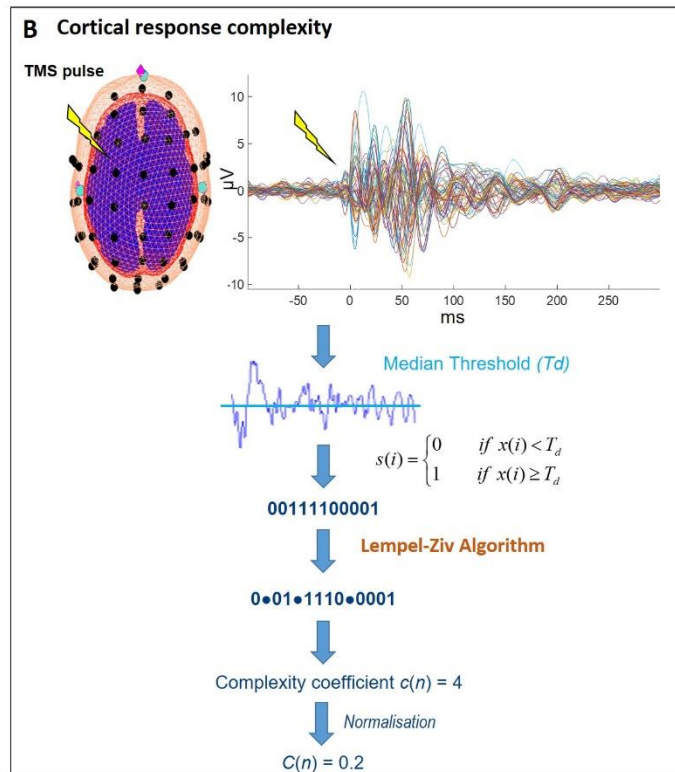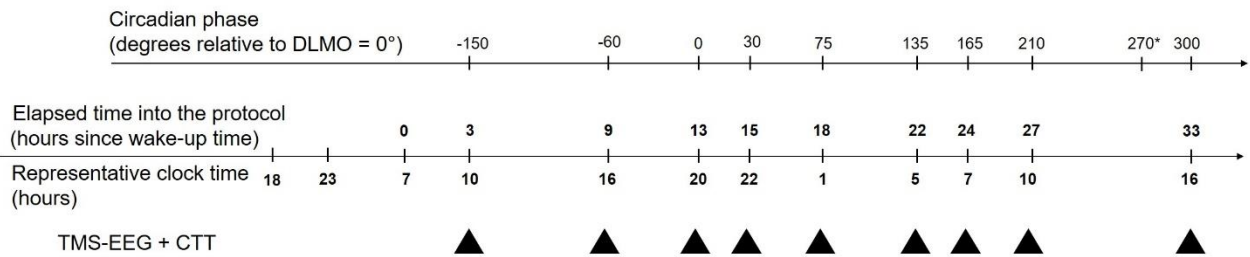
164   <u>2.5. Lempel-Ziv complexity</u>.

165 The cortical response complexity at the sensors level (global and local) was inferred by applying the non-linear

166 LZC algorithm. LZC is a scalar metric that approximates the amount of non-redundant information contained

167 in a substring of EEG signal by estimating the minimal size of the "vocabulary" necessary to describe the

168 string (Hu & Zhang, 2019). (Lempel & Ziv, 1976) The coarse-graining approach converts first the original

169 signal into 0-1 sequence through comparing the amplitude values with the threshold. The median of the

170 averaged evoked response of each channel is used as the threshold, because of its robustness to outliers. Then,

171 the binary sequence is scanned from left to right by the Lempel–Ziv algorithm. The complexity counter [c(n)]

172 is increased by one-unit every time a new subsequence of consecutive characters is encountered (**Fig. 1B**). To

173 obtain a complexity measure that is independent of the sequence length, it must be normalized [C(n)], resulting

174 in a scalar metric ranging between 0 and 1: the lower limit shows a stationary signal with no varying dynamics,

175 while the upper limit shows a very complex signal with multiple complex dynamics (Aboy et al., 2006b)

176 (Zhang, Roy, & Jensen, 2001). The LZC algorithm was applied on a time window between 8 and 300 ms post

177 stimulus (1 sample every ms), avoiding potential artefact (Virtanen et al., 1999), while participants were

178 performing a vigilance task. Thus, the temporal complexity inferred from the TEPs over the 60 EEG channels

179 is a mixture of the magnetically evoked responses with visuomotor CTT components. The *global response*

180 *complexity* was computed by averaging the response complexity value over all good channels in a given

181 session. Thus, global response complexity is dependent on the cortical activation triggered by TMS and the

182 global brain state (state-dependency). The *local response complexity* was computed by considering only the

183 electrodes within a circle of 35 mm ray and centre the maximal electric field generated by TMS (Barker, 1991;

184 Thielscher & Kammer, 2002). The angular distance between the centre and the sensors was calculated by

185 applying the sphereFit Matlab function. Thus, local response complexity is substantially more dependent on

186 the direct cortical activation evoked by TMS rather than the global brain state.

187

A   Protocol

Circadian phase
(degrees relative to DLMO = 0°)   -150   -60   0   30   75   135   165   210   270*   300

Elapsed time into the protocol
(hours since wake-up time)   0   3   9   13   15   18   22   24   27   33

Representative clock time   18   23   7   10   16   20   22   1   5   7   10   16
(hours)

TMS-EEG + CTT   ▲   ▲   ▲   ▲   ▲   ▲   ▲   ▲   ▲

B   Cortical response complexity

TMS pulse

Median Threshold *(Td)*

$$s(i) = \begin{cases} 0 & \text{if } x(i) < T_d \\ 1 & \text{if } x(i) \geq T_d \end{cases}$$

00111100001

Lempel-Ziv Algorithm

0•01•1110•0001

Complexity coefficient *c(n)* = 4

Normalisation

C(n) = 0.2

Figure 1. Experimental protocol and cortical response complexity computation. A. After a baseline night of sleep, 12 older and 13 young healthy participants underwent 34 h of sustained wakefulness under constant routine conditions. Cortical response complexity was assessed 9 times using TMS-EEG over 1.5 circadian cycle. During TMS-EEG sessions, a visuomotor compensatory tracking task (CTT) was administered. Saliva samples were collected hourly for melatonin, allowing a posteriori data realignment and interpolation based on individual endogenous circadian timing (inferred based on dim light melatonin onset – DLMO). Time is expressed in circadian phase (degrees - °; 15° = 1h), and equivalent elapsed time awake (h). Representative clock time is for a participant with a 2300–0700 sleep-wake schedule. * Data were not extrapolated > 15° from the last recording: resampling at 300° could not be carried out in most participants, and was done at 270° instead. **B.** Computation of the cortical response complexity: for each of the EEG channels of the butterfly response evoked by TMS, the coarse-graining approach first converts the original averaged signal into 0-1 sequence, through comparison of the amplitude values with a given threshold (Td). The median value of the amplitude values is chosen as Td. Then, the Lempel-Ziv algorithm counts the number of different patterns in the sequence. The final complexity measure is normalised.

2.6. Hourly salivary melatonin samples were first placed at 4°C, prior centrifugation and congelation at -20°C within 12 hrs. They were measured by radioimmunoassay (Stockgrand Ltd, Guildford, UK), as previously described (English, Middleton, Arendt, & Wirz-Justice, 1993). Most samples were analyzed in duplicate. The limit of detection of the assay for melatonin was 0.8 ± 0.2 pg/ml using 500 μL volumes, while it was 0.37 ±

205   0.05 nmol/L using 500 μL volumes (Read, Fahmy, & Walker, 1977). Estimation of individual's dim light

206   melatonin onset (DLMO = phase 0°) was determined based on raw values. The 4 first samples were disregarded

207   and maximum secretion level was set as the median of the 3 highest concentrations. Baseline level was set to

208   be the median of the values collected from "wake-up time + 5 h" to "wake-up time + 10 h". DLMO was

209   computed as the time at which melatonin level reached 20% of the baseline to maximum level (linear

210   interpolation). No group differences of the DLMO onset/offset (hh:min) were reported (*DLMO-onset$_{young}$* =

211   21.43 ± 01:11, *DLMO-onset$_{older}$* = 21:43 ± 00:38; *DLMO-offset$_{young}$* = 08:21 ± 01:01, *DLMO-offset$_{older}$* = 07:55

212   ± 01:05 –all comparisons P > 0.3).

213

214   <u>2.7. Statistical analyses</u>. The circadian phase of all data points was estimated relative to individual DLMO (i.e.

215   phase 0°, 15° = 1 h) that is a gold standard marker of endogenous circadian phase, signalling the beginning of

216   the biological night (Pevet & Challet, 2011). All data points were resampled following linear interpolation at

217   the theoretical phases of the TMS-EEG sessions in the protocol (**Fig. 1A**): -150°, -60°, 0°, 30°, 75°, 135°, 165°,

218   210° and 270°. Data were not extrapolated beyond 15° (i.e. 1 h), such that resampling at 300° could not be

219   carried out for the majority of the participants and was advanced at 270° instead. Data points situated 3

220   interquartile ranges above or below the 25th and 75th percentile were defined as extreme outliers and removed

221   (up to two data points were removed per analyses, i.e. 1-2% data points per analysis).

222   Statistical analyses were performed with SAS version 9.3 (SAS Institute, Cary, NC, USA). T-test on

223   independent samples compared group characteristics (Chi squared for proportion comparisons). Wilcoxon

224   rank-sum test compared melatonin values (non-normal distribution). Generalized linear mixed models (PROC

225   GLIMMIX) were applied to compute all statistics following determination of the dependent variable

226   distribution (using Allfitdist Matlab function). Subject (intercept) effect was included as random factor.

227   Circadian phase was included as the repeated measure together with an autoregressive estimation of

228   autocorrelation of order 1 [AR(1)], and the covariance structure specified both subject and group effect. In all

229   GLMMs, degrees of freedom were estimated using Kenward-Roger's correction (reported between brackets

230   for each test). If an interaction term was significant, simple effects were assed using post-hoc contrasts

231   (difference of least square means) adjusted for multiple testing with Tukey's procedure. Betas (i.e. regression

232   coefficient) were derived by applying the ESTIMATE statement; they were not corrected for multiple

233   comparisons. Upper and lower confidence limits were derived by applying the CL statement. Regressions were

234   used for visual display only, and not as a substitute of the full GLMM statistics.

235   When analysing the time course of a given variable (i.e. response complexity and CTT performance),

236   GLMM model included circadian phase, age group and their interaction. When seeking for associations

237   between response complexity and CTT performance, GLMM model included response complexity, circadian

238   phase, age group and all double/triple interactions. T-tests were performed on beta coefficients to analyse

239   group differences in the link between cortical response complexity and CTT performance. Semi-partial R2

240   (Rsp2) was reported for each significant effect of interest as described in (Jaeger, Edwards, Das, & Sen, 2017).

241

242   **3. Results**

243   As a first step, we focussed on global TMS-response complexity (i.e. over all scalp sensors) for which a GLMM

244   revealed a significant main effect of circadian phase ($F_{(8,145)} = 2.27$, $P = 0.026$). Post hocs indicated that this

245   effect was driven by an increase of global response complexity (statistical trend) from the beginning to the

246   middle of the biological night (i.e. 5 hrs after DLMO) (0°-75° | β = -0.052 | SE = 0.017 | lower-CL = -0.104 |

247   upper-CL = 0.001 | P = 0.055), as well as a decrease of global complexity response (statistical trend) from the

248   middle of the biological night to the following afternoon of the second circadian day (75°-270° | β = 0.058 |

249   SE = 0.019 | lower-CL = -0.001 | upper-CL = 0.117 | P = 0.056) (**Figure 2A**). A simple age-group effect was

250   also detected ($F_{(1,23.12)} = 8.23$, $P = 0.009$): during a wake extension of 34 hrs, older participant displayed an

251   overall higher value of global cortical complexity response than young (young-old | β = -0.130 | SE = 0.045 |

252   lower-CL = -0.224 | upper-CL = -0.036 | P = 0.009). No significant circadian phase x age-group interaction

253   was found.

254

255   As a second step, we focussed on local TMS-response complexity, i.e. only considering channel within

256   a 70 mm dimeter sphere surrounding the TMS hotspot, as it is less concerned with global brain state (cf.

257   method). GLMM with local LZC value as dependent variable yielded a tendency for a main effect of circadian

258   phase ($F_{(8,134.9)} = 1.83$, $P = 0.076$), and a significant main effect of age-group ($F_{(1,23.07)} = 5.74$, $P = 0.025$).

259   As for global response complexity, older people had a higher value than young participants (young-old | β = -

260   0.035 | SE = 0.014 | lower-CL = -0.064 | upper-CL = -0.005 | P = 0.025). Again, no significant circadian phase

261   x age-group interaction was found (**Figure 2B**).

262

263   We then considered performance to the CTT, which probes vigilance. GLMM with CTT performance

264   as dependent variable yielded a simple effect of circadian phase (F(8, 131.9) = 9.64, P < 0.0001), with worse

265   performance at the end of the biological night as compared to the first and second circadian day (-150° to 0°,

266   210°, 270° < 135°, 165°, P < .05; to facilitate reading, estimates and confidence limits of each comparisons of

267   the CTT are omitted) as well as a trend for a simple effect of age group (F(1, 23.92) = 3.74, P = 0.065), with

268   young performing generally better than older participant all long the protocol (young-old | β = -0.159 | SE =

269   0.082 | lower-CL = -0.328 | upper-CL = 0.011 | P = 0.065). A statistical tendency for circadian phase x age-

270   group interaction (F(8,131.9) = 1.99, P =0.052; **Fig. 2C**). Group differences were detected at all circadian

271   phases except the last three assessments (young < older; -150° to 135°, P < 0.05; 165° to 270°, P > 0.05),

272   indicating a different response to sleep loss: smaller differences in performance between age groups were

273   found towards the end of the protocol.

274

Global cortical complexity response

Local cortical complexity response

Vigilance task

275
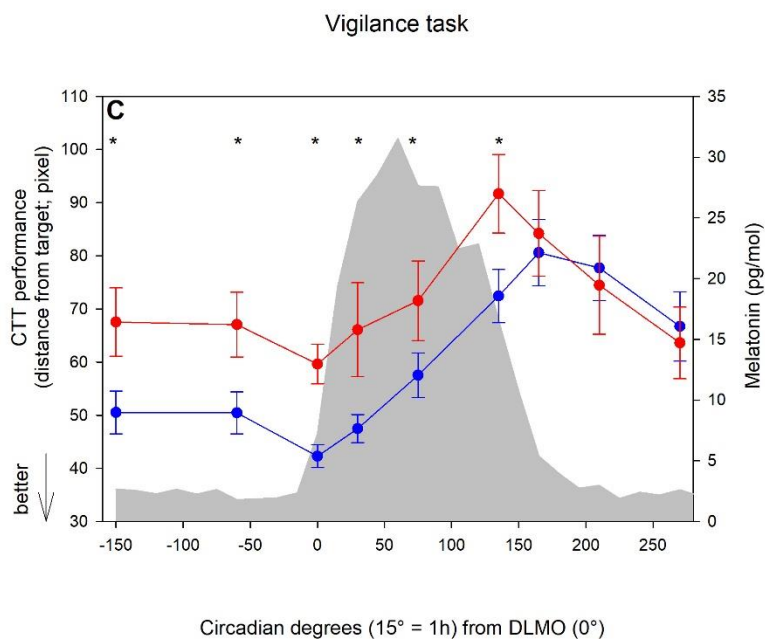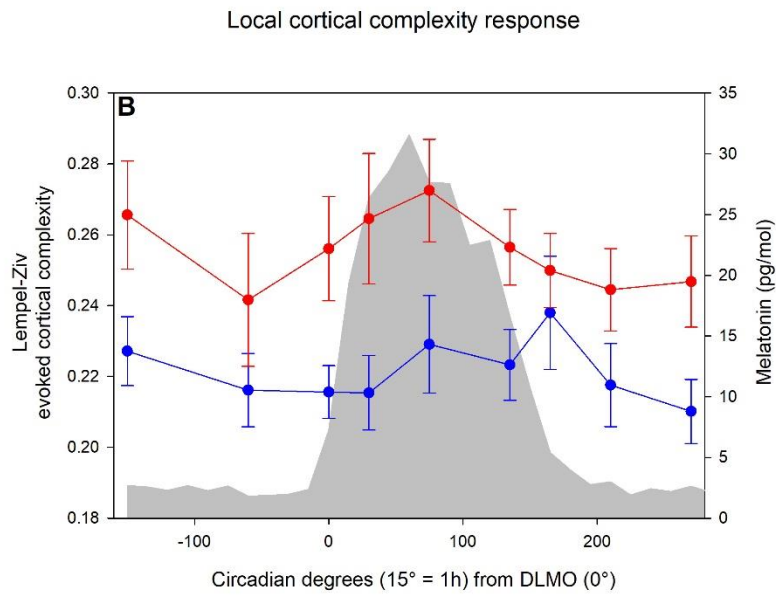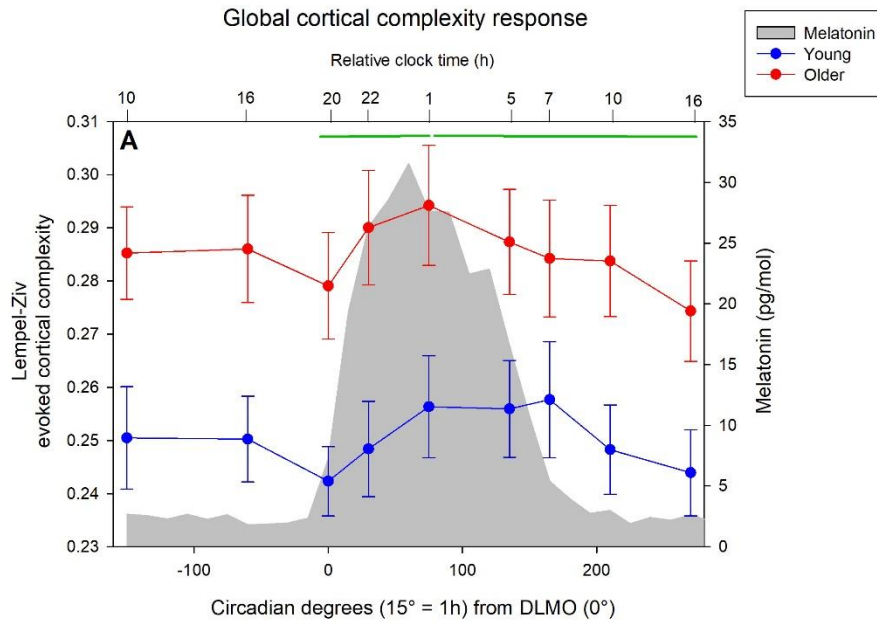
276 **Figure 2. Global and local cortical complexity response dynamics and vigilance dynamic during 34 h of prolonged wakefulness**
277 **in young and older adults (mean ± SE).** Time course of all measures is expressed relative to individual melatonin onset (DLMO =
278 phase 0°; 15° = 1h). Average melatonin profile is displayed in grey. Green bars denote a tendency between circadian phases. *
279 significant group differences by circadian phase.

280

281 We finally tested whether global and local LZC measures were associated with CTT performance. At

282 the global cortical level, A GLMM with CTT performance as dependent variable yielded a significant global

283 complexity x age-group x circadian phase triple interaction ($F_{(8, 143.6)} = 2.19$, $P = 0.031$), a significant global

284 complexity x age-group interaction ($F_{(1, 127.5)} = 5.36$, $P = 0.022$) as well as age-group x circadian phase

285 interaction ($F_{(8, 141.1)} = 2.41$, $P = 0.018$), and a simple effect of age-group ($F_{(1, 126.3)} = 3.99$, $P = 0.048$)

286 (**Table 1**). Age groups showed different association between LCZ and CTT values irrespective of circadian

287 phase (young-old | β = -3.735 | SE = 1.613 | lower-CL = -6.927 | upper-CL = -0.544 | P = 0.022), with higher

288 global complexity response significantly associated with worse CTT performance in the older group (old | β =

289 2.547 | SE = 1.095 | lower-CL = 0.373 | upper-CL = 4.722 | P = 0.022) (**Fig. 3, upper right panel**). When

290 considering each circadian phase separately, significant different associations between global response

291 complexity and CTT performance were found between the two age groups in the morning of the first and

292 second circadian day (i.e. 24h apart) (-150°, young-old | β = -6.848 | SE = 3.144 | lower-CL = -13.062 | upper-

293 CL = -0.634 | P = 0.031) and (210°, young-old | β = -8.209 | SE = 2.865 | lower-CL = -13.867 | upper-CL = -

294 2.551 | P = 0.005), again with higher global complexity response significantly associated with worse CTT

295 performance in the older group (-150°, old | β = 4.897 | SE = 2.528 | lower-CL = -0.143 | upper-CL = 9.937 |

296 P = 0.057) and (210°, old | β = 5.969 | SE = 1.922 | lower-CL = 2.148 | upper-CL = 9.789 | P = 0.003) (**Fig. 3,**

297 **left panel**). At the local level, GLMM yielded a significant local complexity x age-group interaction ($F_{(1,}$

298 $_{172.8)} = 4.12$, $P = 0.044$) (**Table 1**), with a different relationship between local complexity response and CTT

299 in the two age groups (young-old | β = -2.173 | SE = 1.071 | lower-CL = -4.286 | upper-CL = -0.060 | P = 0.044)

300 (**Fig. 3, lower right panel**).

301

302 **Table 1.** Association between global and local cortical response complexity and vigilance performance.

303 Factors including cortical response complexity are in italic. Statistically significant results are in bold.

GLOBAL cortical response complexity	CTT performance
	(distance from target)

Cortical response complexity	$F(1,127.5) = .71$ $P = .40$
Circadian period	$F(8,141.1) = 1.66$ $P = .11$
Age group	**$F(1,126.3) = 3.99$ $P = . 0.048$ $R_{sp}^2 = .03$**
Cortical response complexity x age group	**$F(1, 127.5) = 5.36$ $P = . 02$ $R_{sp}^2 = .04$**
Cortical response complexity x circadian period	$F(8, 143.6) = 1.14$ $P = .34$
Age group x circadian period	**$F(8, 141.1) = 2.41$ $P = .018$ $R_{sp}^2 = .12$**
Cortical response complexity x age group x circadian period	**$F(8, 143.6) = 2.19$ $P = .031$ $R_{sp}^2 = .11$**

304

LOCAL cortical response complexity	**CTT performance** **(distance from target)**
Cortical complexity response	$F(1, 172.8) = .02$ $P = .902$
Circadian period	$F(8, 137.4) = .66$ $P = .727$
Age group	$F(1, 152.4) = 1.84$ $P = .178$
Cortical complexity response x age group	**$F(1, 172.8) = 4.12$ $P = .044$ $R_{sp}^2 = .02$**
Cortical complexity response x circadian period	$F(8,139) = .39$ $P = .924$
Age group x circadian period	$F(8, 137.4) = 0.73$ $P = .662$
Cortical complexity response x age group x circadian period	$F(8,139) = .49$ $P = .860$

305 GLMMs including CTT as dependent variable and left column variables as predictors. Degrees of freedom are indicated between

306 brackets and were estimated using Kenward-Roger's correction.

307

308
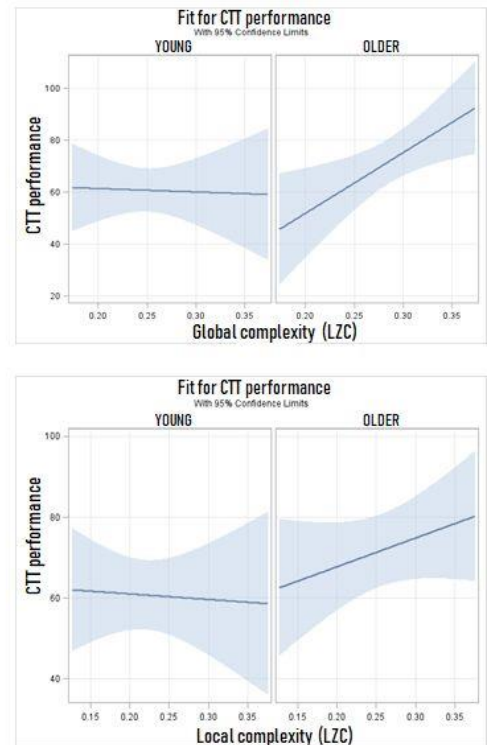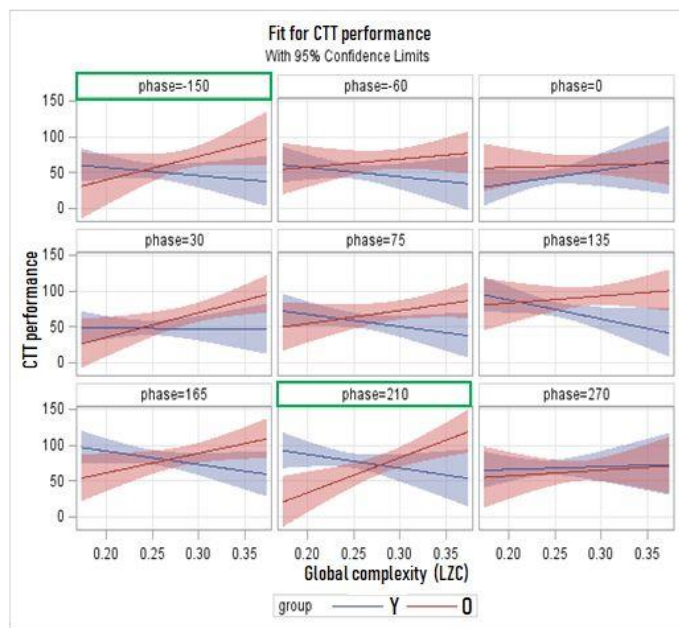
Figure 3. Associations between cortical complexity response and vigilance CTT performance. Left panel: associations between global complexity response and CTT performance in young and older individuals and across the different circadian phases (significant circadian phases are highlighted in green). **Right panel**: associations between global (**upper**) and local (**lower**) complexity response and CTT performance in young and older individuals, independently of the circadian phase.

Discussion

We investigated the dynamics of global and local cortical response complexity with time spent awake and according to the internal circadian clock in young and older participants. We further sought for correlations between response complexity and performance to a vigilance task. Despite the fact that TMS stimulation parameters remained constant over the 9 sessions, we find that whole-scalp TMS-induced response complexity significantly changed during a strictly controlled 34h sleep deprivation protocol. Results indicate that these variations are driven by an increase during the first part of the biological night followed by a progressive decline the following biological day. We observe a similar dynamic when considering local response complexity - around the TMS hotspot – but overall changes represent a statistical trend only, with no clear difference between individual circadian phases. While we do find that both global and local response complexity is higher in older individuals compared with younger ones, we have no statistical indications that response complexity dynamic over the protocol is different between age groups. Finally, we find that global

and local response complexity dynamics are associated with vigilance performance variations, but the relationship is different between younger and older individuals, the latter having higher response complexity associated with poorer vigilance performance, particularly in the morning hours while well rested and following total sleep deprivation.

Given that the experiment was conducted under constant routine conditions, the changes in global response complexity are very likely to be driven, at least to a large extend, by the dual influence of sleep homeostasis and circadian rhythmicity on brain function (D. J. Dijk & Czeisler, 1995) – in line with our hypothesis. Likewise the decrease in global response complexity from the second part of the biological night to the following biological day may be the consequence of a further increase in slow EEG oscillations as sleep deprivation progresses (Vyazovskiy et al., 2011), which would render the signal more regular and less complex, and/or of the circadian alerting signal, which counteracts the randomness of the time series during the following biological day (D. J. Dijk & Czeisler, 1995). In contrast, we had not anticipated the increase in response complexity in the first part of the biological night. It is conceivable that the initial intrusion of low-frequency EEG activity (i.e. slow waves, theta waves) during the first part of the biological night generates a disharmony between the different frequencies bands composing the EEG, resulting in a more diverse oscillation repertoire and higher response complexity (Timofeev et al., 2020; Vyazovskiy et al., 2011). Circadian phase 0° represents individual DLMO that corresponds to the end of the so-called "wake-maintenance zone" (D.-J. Dijk & Czeisler, 1994), i.e. when the circadian signal maximally promotes wakefulness to counter linear increasing sleep homeostasis. The release of the circadian wake-promoting signal could also contribute to a more mixed EEG oscillation composition, increasing signal complexity in the first part of the night.

Interestingly, LZC values were reported to become progressively more variable in the course of a partial sleep deprivation in rats (Abásolo et al., 2015). This was proposed as reflecting a progressive increase in state instability, so that LZC would constitute a metric sensitive to prior sleep-wake history that would be relatively independent from the absolute levels of slow wave activity (Abásolo et al., 2015). The dynamics in global LZC may therefore be directly related to increased EEG state instability during sleep deprivation, rather than just the slow oscillation composition of the EEG. The fact that LZC measures should be interpreted as a harmonic variability metric was also stated in (Aboy et al., 2006b). This interpretation is interesting for those

356 states that do not fit the normal continuum from deep sleep to vigilant wakefulness (e.g. sleep deprivation and

357 REM), and in which a "sleeplike" might coexist with wakefulness. One could potentially consider sleep

358 deprivation as a hybrid physiological states, in which slower waves intruded wakefulness, and consciousness

359 and vigilance are altered (Coenen, 1998).

360   Cortical excitability as indexed by the slope/amplitude of the early (0-30ms) TEP around the TMS

361 hotspot changes with time spent awake (Huber et al., 2013) and circadian cycle (J.Q.M. Ly et al., 2016) in

362 younger individuals, while variation are not as pronounced in older ones (Gaggioni et al., 2019). Here,

363 variations in local LZC values, considered only around the TMS hotspot, are not as sharp as global LZC and

364 we find no indication of age-group difference in the dynamics. This suggest that local LZC and cortical

365 excitability, but also local and global LZC measures, encompass partly distinct phenomenon. Single-pulse

366 TMS over the frontal cortex induces a long range (0-300ms) response in the (fast) beta range in the vicinity of

367 the stimulation site (Rosanova et al., 2009), which could be interpreted as a transient synchronization of

368 spontaneous activity within the beta band (Paus, Sipila, & Strafella, 2001). One could postulate that this reset

369 is constant whatever the time-awake and circadian phase so that local TMS response complexity considered

370 over the 300ms post TMS could undergo less variation over the protocol relative to global LZC, which depends

371 more on overall brain state, and is independent of local cortical excitability.

372   We stress that, although global LZC significantly varies over the protocol, posthoc comparisons only

373 yielded statistical trends in the variations detected between circadian phases. Variations of global LZC at

374 specific circadian phases need therefore to be interpreted with caution, as well as the overall trend suggesting

375 local LZC variation, and replication of the results over large samples is needed.

376   Both at the global and local level, complexity response was significantly higher in older than young

377 participants during the entire 34h sleep deprivation protocol. A curvilinear relationship between age and

378 complexity was reported, with complexity maxima reached by the sixth decade of life (Bruce, Bruce, &

379 Vennelaganti, 2009; Fernández et al., 2012), and older participants of our sample were 62 yo on average. In

380 addition, older individuals have a higher EEG frequency content and lower slow-waves activity (SWA) during

381 sleep (Carrier et al., 2001). The age group difference we detect is therefore expected and in line with the

382 literature.

383   We find however that the association between cortical response complexity and performance to a

384   vigilance task, which dramatically changed in both group across the 34h of sleep deprivation, changes over the

385   lifespan. A higher level of cortical complexity response was associated with worse vigilance performance in

386   the older group, and especially in the morning, whit the correlation estimate being stronger in the morning

387   after sleep deprivation. Interestingly, higher EEG complexity –based on the correlation dimension– was also

388   found during a decrease of alertness compared to the fully awake state (Matousek et al., 1995). A higher

389   cortical randomness might be conducive of perceptual changes and hallucination episodes (Schwartzman et

390   al., 2019) in the morning after sleep deprivation (Waters, Chiu, Atkinson, & Blom, 2018), when the level of

391   vigilance is perturbed.

392   Different non-linear measures exist that can be used to assess brain response complexity, such as

393   entropy and correlation dimension (Friston, Tononi, Sporns, & Edelman, 1995). The concept of neural

394   complexity was elaborate by combining both time and spatial component of the signal to reflect the interplay

395   between functional segregation and integration within neural systems (Tononi, Sporns, & Edelman, 1994).

396   Neuronal response complexity stand therefore as an intermediate state between randomness and order (Stam,

397   2005a). LZC computation adopted here instead only reflects differentiation (and not integration) and interpret

398   complexity as degree of randomness, or degrees of freedom in a large system of interacting elements. However,

399   the advantage of the LZC method is the simplicity (does not require any inputs selection), the robustness to

400   noise, the computational efficiency, and it can be calculated even for short data segments (milliseconds range)

401   (Zhang, Zhu, Thakor, & Wang, 1999). Previous studies further showed that the binary (i.e. 0–1) conversion of

402   the signal is adequate to estimate the LZ complexity in biomedical signals (Aboy, Hornero, Abásolo, &

403   Álvarez, 2006a). The disadvantage is that LZC is sensitive somewhat to signal amplitudes (Sarlabous et al.,

404   2009). This may explain in part the age group difference we detect as EEG signal amplitude typically decrease

405   with age, particularly during sleep (Carrier et al., 2001), but did not affect the dynamics in LZC complexity

406   nor its link with vigilance performance.

407   In conclusion, we provide novel insights in the brain response complexity dynamics during prolonged

408   wakefulness and sleep deprivation. We concluded that Lempel-Ziv complexity seems to be a valid indicator

409   of the harmonic variability within the cortical system. Despite an overall higher level of response complexity

410   in older compared to young adults, the dynamics of response complexity was not significantly difference

411 between age groups. We find a different relationship between response complexity and vigilance performance

412 in the two age groups, which warrant further investigation.

413

414 **Bibliography**

415 Abásolo, D., Simons, S., Morgado da Silva, R., Tononi, G., & Vyazovskiy, V. V. (2015). Lempel-Ziv

416   complexity of cortical activity during sleep and waking in rats. *Journal of Neurophysiology*, *113*(7),

417   2742–2752. https://doi.org/10.1152/jn.00575.2014

418 Aboy, M., Hornero, R., Abásolo, D., & Álvarez, D. (2006a). Interpretation of the Lempel-Ziv complexity

419   measure in the context of biomedical signal analysis. *IEEE Transactions on Biomedical Engineering*,

420   *53*(11), 2282–2288. https://doi.org/10.1109/TBME.2006.883696

421 Aboy, M., Hornero, R., Abásolo, D., & Álvarez, D. (2006b). Interpretation of the Lempel-Ziv Complexity

422   Measure in the Context of Biomedical Signal Analysis. *IEEE TRANSACTIONS ON BIOMEDICAL*

423   *ENGINEERING*, *53*(11). https://doi.org/10.1109/TBME.2006.883696

424 Barker, A. T. (1991). An Introduction to the Basic Principles of Magnetic Nerve Stimulation. *Journal of*

425   *Clinical Neurophysiology*, *8*(1), 26–37. https://doi.org/10.1097/00004691-199101000-00005

426 Beck, A. T., Brown, G., Epstein, N., & Steer, R. A. (1988). An Inventory for Measuring Clinical Anxiety:

427   Psychometric Properties. *Journal of Consulting and Clinical Psychology*, *56*(6), 893–897.

428   https://doi.org/10.1037/0022-006X.56.6.893

429 Borbély, A A. (1982). A two process model of sleep regulation. *Human Neurobiology*, *1*(3), 195–204.

430   Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7185792

431 Borbély, Alexander A., Daan, S., Wirz-Justice, A., & Deboer, T. (2016). The two-process model of sleep

432   regulation: a reappraisal. *Journal of Sleep Research*, *25*(2), 131–143. https://doi.org/10.1111/jsr.12371

433 Bruce, E. N., Bruce, M. C., & Vennelaganti, S. (2009). Sample Entropy Tracks Changes in

434   Electroencephalogram Power Spectrum With Sleep State and Aging. *Journal of Clinical*

435   *Neurophysiology*, *26*(4), 257–266. https://doi.org/10.1097/WNP.0b013e3181b2f1e3

436 Burke, M. J., Fried, P. J., & Pascual-Leone, A. (2019). Transcranial magnetic stimulation:

437   Neurophysiological and clinical applications. In *Handbook of Clinical Neurology* (Vol. 163, pp. 73–

438   92). https://doi.org/10.1016/B978-0-12-804281-6.00005-7

439   Buysse, D. J., Reynolds 3rd, C. F., Monk, T. H., Berman, S. R., & Kupfer, D. J. (1989). The Pittsburgh

440   Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res*, *28*(2),

441   193–213.

442   Cajochen, C., Wyatt, J. K., Czeisler, C. A., & Dijk, D. J. (2002). Separation of circadian and wake duration-

443   dependent modulation of EEG activation during wakefulness. *Neuroscience*, *114*(4), 1047–1060.

444   Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12379258

445   Carrier, J., Land, S., Buysse, D. J., Kupfer, D. J., & Monk, T. H. (2001). The effects of age and gender on

446   sleep EEG power spectral density in the middle years of life (ages 20-60 years old). *Psychophysiology*,

447   *38*(2), 232–242. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11347869

448   Casali, A. G., Gosseries, O., Rosanova, M., Boly, M., Sarasso, S., Casali, K. R., … Massimini, M. (2013,

449   August 14). A theoretically based index of consciousness independent of sensory processing and

450   behavior. *Science Translational Medicine*, Vol. 5, pp. 198ra105-198ra105.

451   https://doi.org/10.1126/scitranslmed.3006294

452   Coenen, A. M. L. (1998). Neuronal Phenomena Associated with Vigilance and Consciousness: From

453   Cellular Mechanisms to Electroencephalographic Patterns. *Consciousness and Cognition*, *7*(1), 42–53.

454   https://doi.org/10.1006/ccog.1997.0324

455   Dijk, D.-J., & Czeisler, C. A. (1994). Paradoxical timing of the circadian rhythm of sleep propensity serves

456   to consolidate sleep and wakefulness in humans. *Neuroscience Letters*, *166*(1), 63–68.

457   https://doi.org/10.1016/0304-3940(94)90841-9

458   Dijk, D. J., & Czeisler, C. A. (1995). Contribution of the circadian pacemaker and the sleep homeostat to

459   sleep propensity, sleep structure, electroencephalographic slow waves, and sleep spindle activity in

460   humans. *Journal of Neuroscience*, *15*(5 I), 3526–3538. https://doi.org/10.1523/jneurosci.15-05-

461   03526.1995

462   Dijk, Derk Jan, & Duffy, J. F. (1999). Circadian regulation of human sleep and age-related changes in its

463   timing, consolidation and EEG characteristics. *Annals of Medicine*, Vol. 31, pp. 130–140.

464   https://doi.org/10.3109/07853899908998789

465   Duffy, J. F., & Dijk, D. J. (2002). Getting through to circadian oscillators: why use constant routines? *J Biol*

466   *Rhythms*, *17*(1), 4–13.

English, J., Middleton, B. A., Arendt, J., & Wirz-Justice, A. (1993). Rapid direct measurement of melatonin in saliva using an iodinated tracer and solid phase second antibody. *Ann Clin Biochem*, *30 (Pt 4)*, 415–416.

Fernández, A., Zuluaga, P., Abásolo, D., Gómez, C., Serra, A., Méndez, M. A., & Hornero, R. (2012). Brain oscillatory complexity across the life span. *Clinical Neurophysiology*, *123*(11), 2154–2162. https://doi.org/10.1016/j.clinph.2012.04.025

Friston, K. J., Tononi, G., Sporns, O., & Edelman, G. M. (1995). Characterising the complexity of neuronal interactions. *Human Brain Mapping*, *3*(4), 302–314. https://doi.org/10.1002/hbm.460030405

Gaggioni, G., Ly, J. Q. M., Chellappa, S. L., Coppieters 't Wallant, D., Rosanova, M., Sarasso, S., … Vandewalle, G. (2018). Human fronto-parietal response scattering subserves vigilance at night. *NeuroImage*, *175*, 354–364. https://doi.org/10.1016/j.neuroimage.2018.03.055

Gaggioni, G., Ly, J. Q. M., Muto, V., Chellappa, S. L., Jaspar, M., Meyer, C., … Vandewalle, G. (2019). Age-related decrease in cortical excitability circadian variations during sleep loss and its links with cognition. *Neurobiology of Aging*, *78*, 52–63. https://doi.org/10.1016/j.neurobiolaging.2019.02.004

Horne, J. A., & Östberg, O. (1976). A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. *International Journal of Chronobiology*, *4*(2), 97–110. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1027738

Hu, L., & Zhang, Z. (2019). EEG Signal Processing and Feature Extraction. In *EEG Signal Processing and Feature Extraction*. https://doi.org/10.1007/978-981-13-9113-2

Huber, R., Maki, H., Rosanova, M., Casarotto, S., Canali, P., Casali, A. G., … Massimini, M. (2013). Human cortical excitability increases with time awake. *Cereb Cortex*, *23*(2), 332–338. https://doi.org/10.1093/cercor/bhs014

Jaeger, B. C., Edwards, L. J., Das, K., & Sen, P. K. (2017). An R 2 statistic for fixed effects in the generalized linear mixed model. *Journal of Applied Statistics*, *44*(6), 1086–1105. https://doi.org/10.1080/02664763.2016.1193725

Johns, M. W. (1991). A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep*, *14*(6), 540–545.

Landolt, H. P., Rétey, J. V, & Adam, M. (2012). Reduced neurobehavioral impairment from sleep

deprivation in older adults: Contribution of adenosinergic mechanisms. *Frontiers in Neurology*, *APR*(April), 1–11. https://doi.org/10.3389/fneur.2012.00062

Lempel, A., & Ziv, J. (1976). On the Complexity of Finite Sequences. *IEEE Transactions on Information Theory*, *22*(1), 75–81. https://doi.org/10.1109/TIT.1976.1055501

Leonowicz, Z., Karvanen, J., & Shishkin, S. L. (2005). Trimmed estimators for robust averaging of event-related potentials. *Journal of Neuroscience Methods*, *142*(1), 17–26. https://doi.org/10.1016/j.jneumeth.2004.07.008

Ly, J.Q.M., Gaggioni, G., Chellappa, S. L., Papachilleos, S., Brzozowski, A., Borsu, C., … Vandewalle, G. (2016). Circadian regulation of human cortical excitability. *Nature Communications*, *7*. https://doi.org/10.1038/ncomms11828

Ly, Julien Q.M., Gaggioni, G., Chellappa, S. L., Papachilleos, S., Brzozowski, A., Borsu, C., … Vandewalle, G. (2016). Circadian regulation of human cortical excitability. *Nature Communications*, *7*, 11828. https://doi.org/10.1038/ncomms11828

Mateos, D. M., Guevara Erra, R., Wennberg, R., & Perez Velazquez, J. L. (2018). Measures of entropy and complexity in altered states of consciousness. *Cognitive Neurodynamics*, *12*(1), 73–84. https://doi.org/10.1007/s11571-017-9459-8

Matousek, M., Wackermann, J., Palus, M., Berankova, A., Albrecht, V., & Dvorak, I. (1995). Global dimensional complexity of the eeg in healthy volunteers. *Neuropsychobiology*, *31*(1), 47–52. https://doi.org/10.1159/000119171

Mattis S. (1998). Mattis S. Dementia Rating Scale professional manual. *Psychological Assessment Resources*. Retrieved from https://www.mendeley.com/research-papers/dementia-rating-scale-professional-manual-4/?utm_source=desktop&utm_medium=1.18&utm_campaign=open_catalog&userDocumentId=%7B57 49d0f1-f538-4512-b890-61f8e3e4c787%7D

Münch, M., Knoblauch, V., Blatter, K., Schröder, C., Schnitzler, C., Kräuchi, K., … Cajochen, C. (2005). Age-related attenuation of the evening circadian arousal signal in humans. *Neurobiology of Aging*, *26*(9), 1307–1319. https://doi.org/10.1016/j.neurobiolaging.2005.03.004

Paus, T., Sipila, P. K., & Strafella, A. P. (2001). Synchronization of neuronal activity in the human primary

motor cortex by transcranial magnetic stimulation: An EEG study. *Journal of Neurophysiology*, *86*(4), 1983–1990. https://doi.org/10.1152/jn.2001.86.4.1983

Pevet, P., & Challet, E. (2011). Melatonin: Both master clock output and internal time-giver in the circadian clocks network. *Journal of Physiology-Paris*, *105*(4–6), 170–182. https://doi.org/10.1016/j.jphysparis.2011.07.001

Read, G. F., Fahmy, D. R., & Walker, R. F. (1977). Determination of cortisol in human plasma by radioimmunoassay. Use of the 125I-labelled radioligand. *Annals of Clinical Biochemistry*, *14*(6), 343–349.

Rosanova, M., Casali, A., Bellina, V., Resta, F., Mariotti, M., & Massimini, M. (2009). Natural frequencies of human corticothalamic circuits. *Journal of Neuroscience*, *29*(24), 7679–7685. https://doi.org/10.1523/JNEUROSCI.0445-09.2009

Sarlabous, L., Torres, A., Fiz, J. A., Gea, J., Galdiz, J. B., & Jané, R. (2009). Multistate Lempel-Ziv (MLZ) index interpretation as a measure of amplitude and complexity changes. *Proceedings of the 31st Annual International Conference of the IEEE Engineering in Medicine and Biology Society: Engineering the Future of Biomedicine, EMBC 2009*, 4375–4378. https://doi.org/10.1109/IEMBS.2009.5333488

Schmidt, C., Peigneux, P., & Cajochen, C. (2012). *Age-related changes in sleep and circadian rhythms : impact on cognitive performance and underlying neuroanatomical networks*. *3*(July), 1–11. https://doi.org/10.3389/fneur.2012.00118

Schwartzman, D. J., Schartner, M., Ador, B. B., Simonelli, F., Chang, A. Y.-C., & Seth, A. K. (2019). Increased spontaneous EEG signal diversity during stroboscopically-induced altered states of consciousness. *BioRxiv*, 511766. https://doi.org/10.1101/511766

Stam, C. J. (2005a, October). Nonlinear dynamical analysis of EEG and MEG: Review of an emerging field. *Clinical Neurophysiology*, Vol. 116, pp. 2266–2301. https://doi.org/10.1016/j.clinph.2005.06.011

Stam, C. J. (2005b, October 1). Nonlinear dynamical analysis of EEG and MEG: Review of an emerging field. *Clinical Neurophysiology*, Vol. 116, pp. 2266–2301. https://doi.org/10.1016/j.clinph.2005.06.011

Steer, R. A., Ball, R., Ranieri, W. F., & Beck, A. T. (1997). Further evidence for the construct validity of the Beck depression Inventory-II with psychiatric outpatients. *Psychol Rep*, *80*(2), 443–446.

Thielscher, A., & Kammer, T. (2002). Linking physics with physiology in TMS: A sphere field model to

determine the cortical stimulation site in TMS. *NeuroImage*, *17*(3), 1117–1130. https://doi.org/10.1006/nimg.2002.1282

Timofeev, I., Schoch, S. F., LeBourgeois, M. K., Huber, R., Riedner, B. A., & Kurth, S. (2020, June 1). Spatio-temporal properties of sleep slow waves and implications for development. *Current Opinion in Physiology*, Vol. 15, pp. 172–182. https://doi.org/10.1016/j.cophys.2020.01.007

Tononi, G., Sporns, O., & Edelman, G. M. (1994). A measure for brain complexity: Relating functional segregation and integration in the nervous system. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(11), 5033–5037. https://doi.org/10.1073/pnas.91.11.5033

Tosun, P. D., Abásolo, D., Stenson, G., & Winsky-Sommerer, R. (2017). Characterisation of the effects of sleep deprivation on the electroencephalogram using Permutation Lempel-Ziv complexity, a non-linear analysis tool. *Entropy*, *19*(12), 673. https://doi.org/10.3390/e19120673

Tosun, P. D., Dijk, D.-J., Winsky-Sommerer, R., & Abasolo, D. (2019). Effects of Ageing and Sex on Complexity in the Human Sleep EEG: A Comparison of Three Symbolic Dynamic Analysis Methods. *Complexity*, *2019*, 1–12. https://doi.org/10.1155/2019/9254309

Virtanen, J., Ruohonen, J., Naatanen, R., & Ilmoniemi, R. J. (1999). Instrumentation for the measurement of electric brain responses to transcranial magnetic stimulation. *Med Biol Eng Comput*, *37*(3), 322–326.

Vyazovskiy, V. V, Olcese, U., Hanlon, E. C., Nir, Y., Cirelli, C., & Tononi, G. (2011). Local sleep in awake rats. *Nature*, *472*(7344), 443–447. https://doi.org/10.1038/nature10009

Waters, F., Chiu, V., Atkinson, A., & Blom, J. D. (2018, July 10). Severe sleep deprivation causes hallucinations and a gradual progression toward psychosis with increasing time awake. *Frontiers in Psychiatry*, Vol. 9, p. 303. https://doi.org/10.3389/fpsyt.2018.00303

Zhang, X. S., Roy, R. J., & Jensen, E. W. (2001). EEG complexity as a measure of depth of anesthesia for patients. *IEEE Transactions on Biomedical Engineering*, *48*(12), 1424–1433. https://doi.org/10.1109/10.966601

Zhang, X. S., Zhu, Y. S., Thakor, N. V., & Wang, Z. Z. (1999). Detecting ventricular tachycardia and fibrillation by complexity measure. *IEEE Transactions on Biomedical Engineering*, *46*(5), 548–555. https://doi.org/10.1109/10.759055

Discussion

Nos résultats montrent que le sommeil est l'état le plus naturel du cortex cérébral. Les animaux ont besoin d'être éveillés pour manger, boire, se reproduire, etc. Une fois que cela est fait, ils dorment. C'est un peu plus compliqué avec les êtres humains parce que nous devons aussi satisfaire les besoins intellectuels, sociaux et culturels, mais une fois que c'est fait, nous dormons. La bonne question qu'il faut se poser est : pourquoi devons-nous être éveillés? – Prof. Igor Timofeev, U. Laval, Québec

I will start by briefly recapitulating and discussing the main results regarding the dynamics of cortical responsiveness (cortical excitability, scattering, and complexity response) during prolonged wakefulness and sleep deprivation, and the association with cognitive performance, as well as the relevance of these findings. I will then present some broad considerations about the importance of chronobiology in our human society and ecological life in general.

General discussion and contextualisation of the results

A simple take-home message one could get from the studies presented in this PhD dissertation is that cortical dynamics change with time spent awake.

This is particularly the case for cortical excitability, where a clear interaction between homeostatic and circadian processes was detected in the young participants, whereas in the older group the profile was dampened and void of any clear sleep homeostasis and circadian regulation. These findings contribute to further understanding the cortical mechanisms underlying cognitive performance and its deterioration, as observed in ageing. Importantly, these results outline the message that maintaining a clear sleep homeostasis and circadian regulation of the cortical function may be a protective factor against age-related cognitive decline.

A report by Muto and colleagues (2016) detected a sleep homeostasis and circadian rhythmicity in a large set of human brain responses underlying vigilant attention. The peak activation changed

accordingly to the brain area, i.e. the circadian rhythmicity was locally modulated (Muto et al., 2016). Here, we extended the investigation of sleep homeostasis and circadian rhythmicity regulation on three aspects of the basic cortical function –i.e. excitability, response scattering and complexity of the cortex. Based on the papers presented here, we can affirm that during prolonged wakefulness:

(*1*) local cortical excitability significantly decreased around the wake maintenance zone (i.e. in the early evening) and increased during the biological night (Ly et al., 2016). Even though no causality can be drawn from these data, it is noteworthy that the detected modifications of cortical excitability correlated with sleep homeostasis and circadian markers such as cortisol (hypothesized to relate to the strength of the circadian wake promoting signal (Dijk et al., 2012)), dissipation of slow waves activity during sleep, and theta power during wakefulness (both reflecting mainly sleep homeostasis (Cajochen et al., 2002)). TMS allows to gauge the neurophysiological state of the cortex. Increased cortical excitability during the biological night implies greater postsynaptic potentials, which may be interpreted as an increased neuronal synchrony and/or an increased reactivity, which depends on changing of the membrane conductance or reflects increased synaptic plasticity (Chellappa et al., 2016; Huber et al., 2013).

(*2*) In older participants, however, the profile of local cortical excitability was dampened, void of any clear circadian modification with time spent awake (Gaggioni et al., 2019). Although we do not demonstrate that physiological ageing has no impact on overall cortical excitability, our results support an age-related change in the dynamics of cortical excitability during prolonged wakefulness. We are not in the position to isolate the mechanisms underlying this progressive change in cortical excitability dynamics in ageing. Changes in threshold and amplitude of action potentials, as well as in their frequency, has been reported during ageing (Rizzo et al., 2015). Interestingly, a reduced suprachiasmatic nucleus activity in animal was reported, resulting in an overall dampening of its activity fluctuation over the circadian cycle (Farajnia et al., 2012). Thus, our findings suggest that reduced circadian variation of cortical dynamics is also measurable in the frontal cortex, i.e. outside

the master circadian clock. As previously said, cortical excitability may ultimately be related to synaptic strength (Rossini and Rossi, 2007). The age-related flattening of the cortical excitability could be due to age-related synaptic changes (Morrison and Baxter, 2012). That would lead to overall reduced experience-dependent synaptic modification, so that sleep would be less required for maintaining synaptic function in ageing. This is in line with the age-related reduction in sleep need build-up (Klerman and Dijk, 2008). Indeed, the process of ageing significantly affects circadian and sleep variables: the build-up of sleep pressure during wakefulness decreases (Landolt et al., 2012), the timing of the circadian system is advanced, and the strength of the circadian signal has been suggested to decrease (Dijk et al., 1999; Münch et al., 2005). However in this study, contrary to (Ly et al., 2016), we did not find any correlations between cortical excitability and the well-known markers of sleep homeostasis and the circadian alerting signal.

(*3*) Fronto-parietal cortical scattering induced by TMS seemed to become more local during the night in young participants (Gaggioni et al., 2018). Since our measure of effective connectivity was quantitatively instead of qualitatively (Casali et al., 2010), we can only hypothesize which brain mechanism would explain a diminution of cortical response scattering at night. Changes in effective connectivity may reflect changes in structural brain connectivity. A day of wakefulness was associated with widespread increases in white matter fractional anisotropy ((FA), reflecting changes in axonal microstructure), whereas sleep deprivation triggered widespread FA decreases (Elvsåshagen et al., 2015), reminiscent of the response scattering variations we observed.

(*4*) Finally, global cortical response complexity dynamics did not seem to differ between younger and older adults during 34 hrs of extended wakefulness, although it was continuously higher in the older age-group, with a potential increase during the first part of the biological night, and a potential decrease during the second part of the night and the following second biological day (Gaggioni et al., In prep.). The increase in response complexity in the first part of the biological night may be explained by the initial intrusion of low frequency EEG activity (i.e. slow waves, theta waves) during the first

part of the biological night, which would increase initially the "vocabulary" of the complexity response. The release of the circadian wake promoting signal around the beginning of the biological night could also contribute to a more mixed EEG oscillation composition, increasing signal complexity in the first part of the night. Likewise the decrease in global response complexity from the second part of the biological night to the following biological day may be the consequence of a further increase in slow EEG oscillations as sleep deprivation progresses (Vyazovskiy et al., 2011), which would render the signal more regular and less complex, and/or of the circadian alerting signal, which counteracts the randomness of the time series during the following biological day (Dijk and Czeisler, 1995). Based on these results, the complexity measure would reflect state instability, i.e. a marker of the disharmony between the different frequencies bands composing the EEG, instead of just the amount of regular pattern such as slow oscillations (Aboy et al., 2006). Regarding complexity response, a main effect of age appeared: it was significantly higher in older than young participants during the entire sleep deprivation protocol. A curvilinear relationship between age and complexity was reported, with complexity maxima reached by the sixth decade of life (Bruce et al., 2009) (older participants of our sample were 62 y on average). In addition, the higher EEG frequency content and lower slow-waves activity (SWA) during sleep of older individuals could also partially elucidate this age-trait (Carrier et al., 2001).

It is interesting to note that a clear sleep homeostasis and circadian regulation was only found in the profile of cortical excitability, whereas cortical response scattering and complexity only showed an effect of time spent awake. A plausible explanation may be that TMS has a very local effect (focal area of stimulation 0.68 cm2) thus, when investigating global TMS-induced effects, unrelated noise could have interfered with the measures and possibly masked the subtle circadian regulation.

Whether the present neurophysiological results are primarily contributed by neuronal bistability, synaptic plasticity, or impaired inhibition needs to be determined. Bistability may be a key mechanism underlying the different dynamics of cortical responsiveness. It has been shown that after

prolonged wake, neurons in the cerebral cortex of the rat can go briefly and locally "OFF line" as they do in sleep, accompanied by slower waves in the local EEG (Vyazovskiy et al., 2011). Depolarized up-states are interrupted by short hyperpolarized down-states when neurons remain silent, whereas during the up-states, both excitatory and inhibitory neurons fire synchronously. The strength and the synchrony of the population excitatory postsynaptic currents was reflected by the slope and amplitude of cortical evoked responses: low firing synchrony was associated with decreased slopes after sleep, whereas high firing synchrony was associated with increased slopes after wakefulness (Vyazovskiy et al., 2009). Thus, the higher occurrence of ON-OFF[3] episodes during sleep deprivation may result in a higher local cortical excitability (i.e. increased slope), because neurons react more in synchrony. How the occurrence of ON-OFF may change in ageing during extended wakefulness still needs to be fully determined. Given the reduced negative impact of wakefulness extension into the biological night on many cognitive measures in ageing, it is plausible that the occurrence of OFF periods will be reduced during the biological night in older participants.

Additionally, the slope of the cortical evoked responses could be an electrophysiological indicator of synaptic efficacy. The observation that the longer the preceding period of continuous wakefulness, the larger the increase in slope could indeed also reflect increase in synaptic strength (Vyazovskiy et al., 2008). Stronger synapses lead to tighter neural connections, resulting in a higher level of synchronization, and in turn in a larger slow wave during sleep. This view is included in the synaptic homeostasis hypothesis, which proposes synaptic potentiation during wakefulness and depression during sleep as one of the main driving signal of sleep need and sleep homeostasis (Tononi and Cirelli, 2006). It has been shown that molecular markers of long-term potentiation (LTP) and depression (LTD) (e.g. GluR1-containing AMPA receptor (AMPAR)) are strongly indicative of corresponding

---

[3]The terms "ON" and "OFF" periods were chosen instead of "up" and "down" or "depolarized" and "hyperpolarized" states, because the periods of neuronal activity and silence were defined based on the population extracellular activity and not based on changes in membrane potential of individual neurons as measured intracellularly (Vyazovskiy et al., 2009).

changes in synaptic strength in wake and sleep (De Vivo et al., 2017; Vyazovskiy et al., 2008). In this perspective, the dampening of cortical excitability found in older participants could be due to age-related synaptic changes (Morrison and Baxter, 2012), which would lead to overall reduced experience-dependent synaptic modification, so that sleep would be less required for maintaining synaptic function in ageing.

In principle, cortical responsiveness may also be the result of a shift in the balance of synaptic excitation and inhibition (Glutabatergic/GABAergic balance), due to changes in intrinsic conductance or in the neuromodulatory milieu (Chellappa et al., 2016). It is worth noting that ion channel function and neuromodulator concentrations are progressively altered over the lifespan (Rizzo et al., 2015).

Cortical bistability is a valid candidate to explain the profiles of cortical scattering and cortical complexity response as well. During sleep deprivation, any local activation –whether occurring spontaneously or induced by TMS– will eventually trigger a local down-state, preventing further propagation of activity. Thus, during the high-amplitude hyperpolarization periods, cortical neurons stop firing, resulting in reduced scattering at night (Massimini et al., 2005). Regarding cortical complexity response, a certain amount of ON-OFF episodes would increase initially the complexity of the signal due to the insertion of new pattern in the signal "vocabulary" (Abásolo et al., 2015).

Ultimately, the state of cortical responsiveness is essential for proper cognitive performance. In the papers presented here, we found that:

(*1*) higher local cortical excitability was associated with worse vigilance performance in the young group, independently of the circadian phase (Ly et al., 2016). As previously mentioned, higher cortical excitability may reflect an increased neuronal reactivity or synchrony, possibly implying a stereotype firing repertoires of neuronal populations that would be conducive to impaired performance. Vyazovskiy et al. stated that the wake behaviour of a sleep deprived subject might be better characterized as a covert form of "dormiveglia" (i.e. state between sleep and wakefulness), due

to the coexisting of ON-OFF periods in the brain. They showed that the increasing occurrence of local OFF periods during prolonged wake was associated with worsening performance in a sugar pellet reaching task (Vyazovskiy et al., 2011).

(*2*) Higher local cortical excitability was associated with worse executive performance in the younger group, whereas it was associated with better executive performance in the older group (Gaggioni et al., 2019). Since the dampening of cortical excitability dynamics during prolonged wakefulness in older participants may reflect a less adaptable brain, underlying reduced cognitive flexibility in ageing, this result shows that older participants displaying a margin ability in increasing cortical excitability (i.e. cortical resilience) perform better in task requiring a high degree of cognitive flexibility, such as executive function (Gajewski and Falkenstein, 2018). Why these subtle synaptic alterations would be related only with executive performance requires further investigation. Evidences suggest that early age-specific and subtle neural changes are nested primarily in the frontal cortex areas (Masliah et al., 1993), sustaining high order abilities (Wang et al., 2011), so that executive functions are among those most vulnerable to the aging process (Verweij et al., 2014). In this study, we could not replicate the correlation between cortical excitability and vigilance performance. Even though we claimed that the link with performance has to be considered as preliminary, a different sample size as well as a more severe statistical approach (examining the significance of an effect with all the other effects in the model) could explain the lack of correlation in *phase II*. Overall, based on these results, the main idea is that the link between cortical excitability and cognitive performance would follow two different trajectories depending on age: an inverted U-shape for the young, with an optimal level of cortical excitability beyond which performance would be negatively related to higher cortical excitability. In young individuals, cortical excitability would be close to this optimal level during the circadian day while well rested, as indicated by mostly high and stable performance, but the significant rise in cortical excitability found during the biological night would be detrimental for cognition. In contrast, in older individuals, the link between cortical

excitability and performance would be linear. Modifications of cortical excitability, through changes in the circadian system and in the build-up of the need for sleep, are reduced or compromised in older individuals: the optimal level beyond which the association becomes negative is not reached. In this vein, an attempt was done by fitting the data linking cortical excitability and executive performance of the two age-group. Preliminary data seemed to support a linear association for the older and a curvilinear for the young group. With the final aim of improving cognitive performance in ageing, and since the association between cortical excitability and executive performance was positive in older adults, it may imply that cognition could be improved in ageing by targeting on neuron excitability (e.g. neurostimulation), but this remains to be formally tested with a large sample size (see also perspectives section below).

(*3*) At night, a diminished fronto-parietal scattering was associated with worse vigilance performance in young participants (Gaggioni et al., 2018). This result recalls a study that linked higher FA within the fronto-parietal cortex while well rested with better PVT performance during sleep deprivation (Cui et al., 2015). In contrast, participants with lower FA values within multiple brain regions while well rested had worse performance to a visuomotor task after sleep deprivation (Rocklage et al., 2009). In our case, integrating a complementary covariate measure of the structural connectivity (e.g. white-matter projections between fronto-parietal areas) could have contributed to unravelling the mechanistic routes.

(*4*) Finally, higher global cortical response complexity was associated with worse vigilance performance in the older group, and especially in the morning following a night without sleep (Gaggioni et al., In prep.). In line with our result, higher EEG complexity was also found during a decrease of alertness compared to the fully awake state (Matousek et al., 1995). A higher cortical randomness may be conducive of perceptual changes and hallucination episodes (Schwartzman et al., 2019) in the morning after sleep deprivation (Waters et al., 2018), when the level of vigilance is perturbed. Interestingly, when performing the study in older people, I witnessed 2-3 older people

experiencing short episodes of visual hallucination exactly in the morning following the night of sleep. Unfortunately, the protocol did not include any measures of alertness level such as the Observer's Assessment of Alertness/Sedation scale (OAA/S) (Ferrarelli et al., 2010), except for the Karolinska Sleepiness Scale.

Finally, it is worth noting that complementary facets of the age-related cortical dynamics provide a quite complex picture regarding their relations with behavioural performance. For instance, in the older group, a higher cortical excitability is associated with better executive performance, whereas a higher global response complexity is associated with worse vigilance performance. This is important to keep in mind especially in the case of a TMS therapy approach: the chosen neurorehabilitation strategy may be helpful for a particular cognitive domain, but having negative effect on another cognitive domain. However, it is important to note that a causality between higher cortical excitability and for example higher response complexity was not investigated in the work presented here.

Perspectives

Our results show that cortical excitability profile (measured under constant conditions) is very likely under the effect of sleep homeostasis and circadian rhythmicity. This dual regulation of the cortical function is important during a normal waking day, especially during the evening around the wake maintenance zone, and become even more clear-cut during sleep deprivation and circadian misalignment (as experienced for example during jetlag, shift work or chronic sleep deprivation –see also section below). The level of cortical responsiveness is related to cognitive performance, both during normal daytime performance and performance during wake extension. Healthy ageing and to a greater extend age-associated pathologic conditions such as mild cognitive impairment, Parkinson, Alzheimer and dementia show both sleep-wake desynchronization as well as cognitive impairment during daytime (Altena et al., 2010; Stranahan, 2012; Naismith et al., 2010). Based on our results, one could tentatively postulate that the daily fluctuation of cortical responsiveness, as a possible

candidate underpinning the neurobehavioral performance, may be dysregulated as well (Blautzik et al., 2014). Alteration of cortical responsiveness has been reported in disorders of consciousness (Bodart et al., 2018; Sarasso et al., 2015), chronic insomnia (Van Der Werf et al., 2010) and stroke (Huynh et al., 2016), however the temporal profile of those pathological conditions is unknown. If we assume a simple equation like less daily modulation at the cortical level may be indicative of neurodegenerative diseases, then neurorehabilitation could constitute a possible way to restore an adequate cortical responsiveness that subtends recuperated cognitive performance (Schmidt and Bao, 2017). However, it is still unknown if neurostimulating the cortex at a given time point would effectively restore the wake-dependent profile of it. In this perspective, it seems more plausible that restoring a better sleep-wake regulation could reestablish an optimal temporal profile of cortical responsiveness, rather than the other way around.

Focusing on the results of cortical excitability, older participants showed to be void of any clear homeostatic and circadian regulation compared to younger participants. As an explorative analysis, we investigated if older people with a higher build-up of cortical excitability over the biological night would perform better during a normal waking day, when well-rested. The idea behind this explorative analysis was to demonstrate that older individuals with preserved homeostatic and circadian regulation of the cortex would perform better compared to those with low homeostatic and circadian regulation. In others words, that the age-related variability in circadian rhythmicity (and related sleep-wake regulation) could potentially explain the daytime cognitive performance. Indeed, connections between sleep-wake disturbances and cognitive impairment have been found in older adults (Yaffe et al., 2014), and abnormal circadian rhythmicity has been observed to be more severe in people with age-related neurodegenerative diseases, including Alzheimer's disease and related dementias, and Parkinson's disease (Leng et al., 2020, 2019a). Furthermore, a recent study showed that longer napping duration (a possible marker of impaired circadian sleep-wake regulation) is associated with

greater cognitive decline and higher risk to develop cognitive impairment over a decade (Leng et al., 2019b). Unfortunately, we could not detect a significant association in our limited sample (N=12).

Yet, in another study of the lab, the preserved wake-dependent cortical excitability dynamics in older people was correlated with better cognitive fitness (Van Egroo et al., 2019). Maxime, Justinas, Daphne and the rest of the team summarised cortical excitability dynamic over 20 h of extended wakefulness as the regression coefficient of a linear fit across the time points. Results disclosed that a young-like cortical excitability profile (i.e. a preserved cortical excitability dynamics) was associated with better executive performance, whereas an old-like profile with a worse cognitive performance. However, this study did not cover an entire circadian cycle, leaving the question about age-related variability in circadian rhythmicity and changes in cognition still open. It also leaves the open question on whether acting on cortical excitability or brain response propagation, through behavioural intervention which are known to restore circadian rhythmicity (e.g. proper timed exposure to zeitgeber stimuli such as light (Van Someren and Riemersma-Van Der Lek, 2007)), can positively affect cognition. From a clinical point of view, it would be interesting to determine if the older people with a young-like cortical excitability profile will have reduced risk of developing cognitive dysfunction associated with neurodegerative processes.

Nonetheless, in practical terms, it is onerous and time consuming to track the cortical responsiveness time series under constant routine conditions. For our findings to eventually translate to clinical practice, research should investigate if the cortical excitability profile could be reflected in other metrics of sleep and wakefulness regulation, ideally measured ambulatory, for instance by actigraphy or mobile application. Linking sleep-wake regulation to cortical responsiveness, in order to have a lens on the cortical dynamics, could represent an effective and inexpensive approach. It would be very interesting to investigate this association in our samples: it was indeed asked to every subjects to wear an actigraph –monitoring the rest-activity cycle– for two weeks prior to the in-lab

acquisitions. Even more promising would be to intervene on the sleep-wake cycle with the potential to improve cortical excitability dynamics and consequently cognitive performance.

Hence, sleep-wake regulation may represent an important factor in optimizing cognitive trajectories and healthspan. Whether and how the restoration of a circadian rhythmicity in older people –through for example a nap prevention intervention, light treatment, or by a cognitive behavioural therapy of insomnia– could impact on the cortical dynamics and ultimately improve the daytime cognitive performance is a promising and timely research topic with great societal repercussions (Oosterman et al., 2009). If the sleep-wake cycle is without any doubt a good candidate to be investigated, it would also be interesting to test if a combination of the screening questionnaires' scores related to sleep and chronotype (e.g. PSQI, MEQ, MCTQ), corrected for the age of the participants, could be predictive of the underlying cortical dynamics too.

Few considerations for future studies

Based on the studies included here, some considerations are necessary; the intent is not to cancel the promising results and the effort made to acquire the data, but rather to keep in mind that each protocol, even if thoughtfully conceived, sacrifices some aspects.

- It would have been interesting to further extend the protocols by a few hours, i.e. until the beginning of the following biological night, an interesting period characterized by substantial changes in the circadian modulation of cognitive performance. Ideally, it would have been interesting also to have a session after a recovery night of sleep, to reset the homeostatic sleep pressure back to an initial level and measure the impact of a night of sleep on cortical responsiveness after a long period of extended wakefulness (Huber et al., 2013). However, after already 8/9 repeated TMS sessions, the itchy scalp of our volunteers implored a break.

- Our results are somehow brain-region-specific. Some studies have indeed shown that the TMS evoked cortical response may change depending on the stimulated location (Castrillon et al., 2020). Similarly, the correlations between the three parameters of cortical responsiveness (measured on frontal and frontal-parietal region, as well as over the entire scalp) and the different cognitive tasks need to be interpreted as a first attempt to link basic cortical function and behavioural output. Indeed, each task elicits a specific reply in the brain, and the region we stimulated with TMS may or may not be representative of the cortical state of others brain region (especially in the view of a regionally specific homeostatic and circadian brain regulation (Muto et al., 2016)). Furthermore, except for the vigilance compensatory tracking task, all the other tasks of the behavioural test battery were performed 1h before and 1h after the TMS-EEG session, thus the acquisition of the cortical and behavioural aspects was not concomitant (for the correlations, parameters of the cognitive test battery were interpolated at the same circadian phases as the TMS-EEG sessions). Finally, it is worth recalling that an inherent difficulty of the constant routine protocol is the requirement for repetitive task administrations. This inherently excludes a wider range of cognitive processes that cannot be tested with such modalities (e.g. testing memory performance may be tricky due to the learning effect throughout the sessions).

- As previously mentioned in the method section of the thesis, a constant routine protocol does not allow the separation of the homeostatic from the circadian process. If that would have been the goal of our work, we should have adopted the gold standard forced desynchrony protocol (Dijk and Czeisler, 1995), which has in turn others limitations (e.g. extremely time-consuming, requires specific lab units in which participants live isolated from the rest for many weeks). A nap protocol (Cajochen et al., 2001) could have been a valid alternative to disentangle homeostatic and circadian processes, but it requires that every subject undergoes twice the experiment (high vs. low sleep pressure condition).

- In these studies, two age groups were investigated (i.e. 18-30 and 50-70 y), leaving space for further research to better understand the homeostatic and circadian regulation of cortical function across the lifespan (e.g. mapping cortical activity in the first two decades of life, 30-50 y, as well as in 70+ participants). It is possible that a healthy older cohort of 70+ participants could be conducive to stronger age-relate differences. However, to keep in mind that a prolonged semi-recumbent position is not recommended to older people due to increased risk of thrombosis. It would be interesting to know if there is a specific narrower age range that represents a key transition point in the dynamics of cortical responsiveness and on which it would be important to focus any interventions in order to delay the age-related decline of cognitive performance.

- It should be also mentioned that TMS allows to target mainly the cortex; the correlations between cortical responsiveness and behaviours, although compelling, represent just one facet of a more complex picture, which include cortical and subcortical network (Peters et al., 2020). Another study of the lab provided the first insights into a more global picture, including wake-dependent cortical excitability, cognitive, and brain structural integrity measures (grey matter volume, amyloid-beta protein, tau protein) (Van Egroo et al., 2019).

Take-home messages from the in lab studies

These results highlight that the dynamics of cortical responsiveness depend on both prior wakefulness (i.e. homeostatic drive for sleep) and circadian phase (i.e. the time of day in the human body that says "it's dusk", "it's dawn", etc). However, the combined effect of sleep homeostatic and circadian factors is somehow still neglected in many studies, which analyse brain function and behavioural output as just the result of sleep homeostatic causes, omitting the effect of the circadian phase. The hope is that this conceptual dichotomy will disappear in the near future. However, measuring the circadian effect requires a specific lab setting that might not be easily available in all research centres.

These studies reaffirm that the cognitive detrimental effects of sleep deprivation are due to the misalignment between sleep homeostatic and circadian forces, when wakefulness occurs at inappropriate biological time. Most animals are content to obey their internal circadian clock and let it orchestrate the expression of circadian rhythms (although refer to the following section for some cases of sleep-wake plasticity in animals). Humans, however, have a mind of their own and often disobey their internal biological clock –for example, with an increasing tendency toward around the clock activity. Unfortunately, human beings still confuse sleeplessness with vitality and high performance, promoting a culture of sleep machismo. They forget that, despite the evidence that links disturbed circadian rhythmicity with a variety of mental and physical disorders (e.g. impaired cognitive function, altered hormonal and immune function, and gastrointestinal complaints) and negative impact on safety, performance, and productivity (Colten and Altevogt, 2006). For example, shift work (i.e. repeated partial sleep deprivation) involves an important circadian disruption that overrides the entire biology and impacts the cognitive dynamics of the workers. The problem is that shift workers continue to live in a social environment that favours sleeping at night. Furthermore, the dimmer illumination of artificial lights is not usually sufficient to trigger the reset of the circadian clock: shift workers never really fully adapt to their unnatural sleep patterns, making practically impossible for humans to maintain a permanently inverted circadian sleep schedule (Thorne et al., 2008). In consequence, one should conclude that the homeostatic pressure towards sleep only works on a short-term schedule, whereas the circadian regulation always takes sleep-wake history into account in the long term and, if neglected, may cause serious health and safety problems. The conclusion that shift work can be carcinogenic has grown largely from epidemiological work. A nationwide study in Denmark found that women who work mainly at night for at least six months are 1.5 times more likely to develop breast cancer than those who work regular hours (Hansen, 2001). Another example of disrupted circadian biology is given by transmeridian flights. Jet lag is the result of the slow adjustment of the biological clocks to a new temporal light/darkness environment in the new time zone resulting in a temporary disruption of the entire circadian network, with physiological

rhythms not aligned to each other. Even the annual 1-hour switch to and from Daylight Saving Time is a hot debate (Roenneberg et al., 2019) and has been associated with an increase in ischaemic stroke during the first two days after transition (Sipilä et al., 2016). Even by excluding dramatic situations like the ones reported, in every day basis, it has been shown that a good proportion of the population live in constant social jetlag (i.e. a discrepancy between the individual's circadian rhythm and the social clock) (Wittmann et al., 2006): some of these are larks, some owls, and some have pretty standard rhythms that are disrupted by simply staying up at night, be it for professional of recreational reasons (i.e. chronic partial sleep deprivation). Indeed, modern lifestyles are no longer constrained by sunrise and sunset, creating a mismatch between our internal biological clock and our brave new 24/7 world. Artificial illumination from computers, televisions, cell phones, and other electronic devices can interfere with the body's ability to maintain proper circadian rhythms (Duffy and Czeisler, 2009). Finally, besides voluntary choices that disturb the normal 24-hour sleep-wake cycle, involuntary factors such as ageing exist. Since the proportion of older people in our society is constantly increasing, there is the need to promote lifestyle activities and social resources in late-life as potential strategies to mitigate age-related differences in circadian rhythmicity and to slow age-related cognitive decline. Clear Zeitgebers such as meal times, alarm times, and house lights become relatively more important (Lewis et al., 2020; Monk, 2010). Yet, provocatively maybe, abnormal sleep-wake regulation of older adults makes them more suitable to work at night compared to younger people (Zitting et al., 2018). Furthermore, if we think in term of community, older people could look after grandchildren at night with less suffering than parents (newborn grandchildren have an "under construction" circadian rhythmicity).

Overall, the aim of this digression is to show that humans' well-being and cognition are not just a matter of *how many* hours we have slept, but also *when* we slept. In this perspective, certain organisational aspects of our society shall be reconsidered (e.g. school start time (Skeldon and Dijk, 2019)), in order to allow adequate synchronisation between internal circadian rhythmicity and

external clock time. Furthermore, the interaction between sleep homeostatic and circadian processes evolve throughout lifespan, requiring different strategies according to the age-group (Bromundt et al., 2019). In order to do so, in-lab studies –like the ones included here– can contribute to better understanding and managing the discrepancy between diurnal rhythms and certain modern lifestyles, while accounting for age-dependent differences.

Chronobiology meets Nature: sleep-wake cycle outside the lab

In the studies included here, the joint effect of homeostatic and circadian processes at the cortical level has been measured by a sophisticated constant routine protocol, unmasking confounders of the circadian rhythmicity. This kind of setting is an abstraction of what happens in the real world: an intentionally simplified environment that however fails to capture the complexity of natural conditions. The direct transposition of the results found in the lab may not always find replicate in real life (Vanin et al., 2012). Yet, in-lab setting gives insight about what is hidden in ecological condition and may be of high importance for health.

It is of foremost importance to integrate chronobiology and ecology to gain a better understanding of what happens in ecologically realistic situations (Helm et al., 2017). Few interesting examples are reported hereafter, showing an incredible plasticity of the circadian rhythmicity (Schwartz et al., 2017). For example, birds have overcome the problem of sleeping in risky situations by developing the ability to sleep with one eye open and one hemisphere of the brain awake (Rattenborg et al., 1999). Migratory birds become restless at night around the time of migration (Zugunruhe = migration anxiety). Thus, seasonal changes in sleep are thought to reflect the birds' endogenous urge to migrate at certain times of the year. Sleep regulation in the starling is highly flexible and sensitive to environmental factors. Sleep time is 5 hrs less during summer than during winter, which is best explained by night length. Additionally, the birds sleep around 2 hrs less during full moon nights (van Hasselt et al., 2020). Under the constant light of the Arctic summer, male sandpipers have evolved

an unprecedented ability to forgo sleep while maintaining high performance in the tasks leading up to mating (Rattenborg, 2017). Interestingly, some Arctic animals only show evidence of circadian rhythms during the times of year with more or less regular sunrises and sunsets (spring and fall). Artic reindeer, exposed to continuous daylight in the Summer and darkness in the Winter, switch off the clock probably to help them maximize food intake (van Oort et al., 2005). However, even though some polar animals lose or weaken the expression of some external outputs of the circadian clock, they retain the clock for its role within the organism. Such circadian flexibility is of particular importance to animals exposed to conditions that are similar to constant conditions: Arctic, Antarctic, underground, and in the deep sea. Entrainable locomotor activity and molecular circadian clock rhythms are present in multiple species of cave animals (Beale et al., 2013), suggesting that the clock has not been lost even in arrhythmic environments. In this case, the entrainment happens by different ways than direct exposure to light. For example, the bacterial cryptochromes regulate the circadian rhythmicity of a host squid in the deep sea via a symbiotic relationship (Heath-Heckman et al., 2013). Competition for resources instead may push a species into an unnatural temporal niche. Normally, common spiny mice are nocturnal, whereas golden spinymice are diurnal. However, if the common spiny mice are removed from the area, the golden spiny mice become nocturnal (Shkolnik, 1971). Among insects, young honeybees take care of larvae continuously throughout the day and the night, but they later begin to forage outside the hive and then exhibit clear daily rhythmicity. In honey bees with clear daily rhythmicity, impaired communication has been reported following sleep deprivation (Klein et al., 2010). Interestingly, if foraging bees are induced to return to nursing, they revert to an arrhythmic pattern of behaviour, thus evincing intraindividual plasticity in circadian organization in a species with complex social behaviours (Bloch, 2010; Refinetti, 2012). Overall, circadian sleep-wake regulation in the wild appears to be far more ecologically flexible than commonly recognized from laboratory studies. Forces like food availability, predators, competitors, and mating opportunities may override normal sleep-wake cycle, suggesting that real-world ecological demands and associated motivational states might also play a role.

Unfortunately, also artificial illumination sustained by humans can have direct influences on daily or seasonal rhythms of other species (e.g. artificial light at night as a new threat to pollination (Knop et al., 2017)). Thus, some species might adjust or already have adjusted due to evolutionary reasons to the new light situation. Furthermore, fear of humans and anthropogenic disturbance are forcing daytime animals into a night mode (Gaynor et al., 2018).

Overall, ecological studies in animals have showed that the homeostatic-circadian interaction is flexible under vital circumstances. However, even in life-threatening situations, such as while driving a car, human's imperative for sleep can be so strong that with only modest sleep loss we will fall asleep. Thus, these ecological reflections may constitute a springboard to better understand the transposition of the results of this thesis in real life, where other ecological and motivational factors may or may not come into play in the regulation of the dynamics of the human brain. That would allow to further understand the harmony between the temporal organization of the rest-activity cycle, the rhythmicity of our internal physiology, and the world in which we live and have evolved.

Conclusion

Our results demonstrate that the interaction between homeostatic and circadian processes is represented at the cortical level, changes with age, and is implicated in the regulation of cognitive performance during the day and its deterioration during sleep deprivation and circadian misalignment. These findings contribute to further understanding the cortical mechanisms underlying the maintenance of daytime cognitive performance and its deterioration, as observed in ageing, shift work, jet lag, sleep-wake dysregulation, and neurodegenerative diseases.

<u>Acknowledge</u>

Time is both chronos and kairos.

I would like to thank the Jury, Profs. Cajochen, Duqué, Abásolo and the PhD thesis committee Prof. Collette, Phillips and Maquet for having accepted to be part of the final discussion of the thesis.

I am infinitely thankful to all the volunteers that took part –spontaneously– in the two studies. Nothing would have been achieved without your collaboration and sleep deprivation.

A special thanks to Dr. Gilles Vandewalle: you taught me everything I know about the fascinating world of neurophysiology and chronobiology. Thank you for working tirelessly for the lab: as the master SCN, you keep us all in phase :) and you let me free-run to find my *chronos* way.

I would like also to thank Dr. Christina Schmidt for the invaluable discussion about the circadian clock –done at all 360° phases of it– and Prof. Pierre Maquet that has been like Virgilio for Dante in *La Divina Commedia*.

To all the PIs of the Cyclotron, thank you for always being willing to share your feedback. To all the Cyclo members, thank you for the collaborative atmosphere, and in particular to the members of the Sleep and Chronobiology Lab for having shared the endeavour behind these studies. It was true team work and I will forever treasure many amusing memories together!

A special thanks to friends for the chat we had around a boulettes frites plate, for the smoothie at Belle-Ile while searching for your dress, and for the flavoured tea and mango-passion fruit pastry (あ りがとう). Thanks to the three best teachers of Neapolitan I could ask for, because life with a pinch of *'a cazzimma* is colourful.

I would like to thank infinitely Dr. Daniel Abásolo for welcoming me to his lab: the year I spent at the University of Surrey has been enriched by new knowledge, interesting encounters, and many rainbows. A special thanks to the Viking's culture for teaching me that whatever the mood is, there

is always a valid solution: going to the pub for a pint –for appreciating the pork scratchings I need a bit more time though.

Besides all the new people I met, I want to thank my family and friends (*bipe/quadri –pĕdis*), who have always been there.

> *I dedicate this work to Daniele, and his text message of that distant 22 January 2013.*

Bibliography

Abásolo, D., Hornero, R., Gómez, C., García, M., López, M., 2006. Analysis of EEG background activity in Alzheimer's disease patients with Lempel-Ziv complexity and central tendency measure. Med. Eng. Phys. 28, 315–322. https://doi.org/10.1016/j.medengphy.2005.07.004

Abásolo, D., Simons, S., Morgado da Silva, R., Tononi, G., Vyazovskiy, V. V, 2015. Lempel-Ziv complexity of cortical activity during sleep and waking in rats. J. Neurophysiol. 113, 2742–2752. https://doi.org/10.1152/jn.00575.2014

Aboy, M., Hornero, R., Abásolo, D., Álvarez, D., 2006. Interpretation of the Lempel-Ziv complexity measure in the context of biomedical signal analysis. IEEE Trans. Biomed. Eng. 53, 2282–2288. https://doi.org/10.1109/TBME.2006.883696

Adam, K., Oswald, I., 1977. Sleep is for tissue restoration. J. R. Coll. Physicians Lond.

Adam, M., Retey, J. V, Khatami, R., Landolt, H.P., 2006. Age-related changes in the time course of vigilant attention during 40 hours without sleep in men. Sleep 29, 55–57.

Aeschbach, D., Matthews, J.R., Postolache, T.T., Jackson, M.A., Giesen, H.A., Wehr, T.A., 1997. Dynamics of the human EEG during prolonged wakefulness: Evidence for frequency-specific circadian and homeostatic influences. Neurosci. Lett. 239, 121–124. https://doi.org/10.1016/S0304-3940(97)00904-X

Allada, R., Siegel, J.M., 2008. Unearthing the Phylogenetic Roots of Sleep. Curr. Biol. https://doi.org/10.1016/j.cub.2008.06.033

Allebrandt, K. V., Amin, N., Müller-Myhsok, B., Esko, T., Teder-Laving, M., Azevedo, R.V.D.M., Hayward, C., Van Mill, J., Vogelzangs, N., Green, E.W., Melville, S.A., Lichtner, P., Wichmann, H.E., Oostra, B.A., Janssens, A.C.J.W., Campbell, H., Wilson, J.F., Hicks, A.A., Pramstaller, P.P., Dogas, Z., Rudan, I., Merrow, M., Penninx, B., Kyriacou, C.P., Metspalu, A., Van Duijn, C.M., Meitinger, T., Roenneberg, T., 2013. A KATP channel gene effect on sleep duration: From genome-wide association studies to function in Drosophila. Mol. Psychiatry 18, 122–132. https://doi.org/10.1038/mp.2011.142

Altena, E., Ramautar, J.R., Van Der Werf, Y.D., Van Someren, E.J.W., 2010. Do sleep complaints contribute to age-related cognitive decline?, in: Progress in Brain Research. Elsevier B.V., pp. 181–205. https://doi.org/10.1016/B978-0-444-53702-7.00011-7

Arendt, J., Skene, D.J., 2005. Melatonin as a chronobiotic. Sleep Med. Rev. 9, 25–39. https://doi.org/10.1016/j.smrv.2004.05.002

ASCHOFF, J., 1965. CIRCADIAN RHYTHMS IN MAN. Science 148, 1427–32. https://doi.org/10.1126/SCIENCE.148.3676.1427

Aulsebrook, A.E., Jones, T.M., Rattenborg, N.C., Roth, T.C., Lesku, J.A., 2016. Sleep Ecophysiology: Integrating Neuroscience and Ecology. Trends Ecol. Evol. https://doi.org/10.1016/j.tree.2016.05.004

Balsalobre, A., Damiola, F., Schibler, U., 1998. A Serum Shock Induces Circadian Gene Expression in Mammalian Tissue Culture Cells. Cell 93, 929–937. https://doi.org/10.1016/S0092-8674(00)81199-X

Bargiello, T.A., Jackson, F.R., Young, M.W., 1984. Restoration of circadian behavioural rhythms by gene transfer in Drosophila. Nature 312, 752–754. https://doi.org/10.1038/312752a0

Barker, A.T., 1994. Magnetic Nerve Stimulation: Principles, Advantages and Disadvantages, in: Biomagnetic Stimulation. Springer US, pp. 9–28. https://doi.org/10.1007/978-1-4757-9507-3_2

Barker, A.T., 1991. An Introduction to the Basic Principles of Magnetic Nerve Stimulation. J. Clin. Neurophysiol. 8, 26–37. https://doi.org/10.1097/00004691-199101000-00005

Barker, A.T., Jalinous, R., Freeston, I.L., 1985. Non-invasive magnetic stimulation of human motor cortex. Lancet (London, England) 1, 1106–7. https://doi.org/10.1016/s0140-6736(85)92413-4

Barker, A.T., Shields, K., 2017. Transcranial Magnetic Stimulation: Basic Principles and Clinical Applications in Migraine. Headache 57, 517–524. https://doi.org/10.1111/head.13002

Basheer, R., Strecker, R.E., Thakkar, M.M., McCarley, R.W., 2004. Adenosine and sleep-wake regulation. Prog. Neurobiol. https://doi.org/10.1016/j.pneurobio.2004.06.004

Bass, J., Takahashi, J.S., 2011. Circadian rhythms: Redox redux. Nature. https://doi.org/10.1038/469476a

Beale, A., Guibal, C., Tamai, T.K., Klotz, L., Cowen, S., Peyric, E., Reynoso, V.H., Yamamoto, Y., Whitmore, D., 2013. Circadian rhythms in Mexican blind cavefish Astyanax mexicanus in the lab and in the field. Nat. Commun. 4, 1–10. https://doi.org/10.1038/ncomms3769

Berger, R.J., Phillips, N.H., 1995. Energy conservation and sleep. Behav. Brain Res. 69, 65–73. https://doi.org/10.1016/0166-4328(95)00002-B

Besedovsky, L., Lange, T., Born, J., 2012. Sleep and immune function. Pflugers Arch. Eur. J. Physiol. https://doi.org/10.1007/s00424-011-1044-0

Blautzik, J., Vetter, C., Schneider, A., Gutyrchik, E., Reinisch, V., Keeser, D., Paolini, M., Pöppel, E., Bao, Y., Reiser, M., Roenneberg, T., Meindl, T., 2014. Dysregulated daily rhythmicity of neuronal resting-state networks in MCI patients. Chronobiol. Int. 31, 1041–1050. https://doi.org/10.3109/07420528.2014.944618

Bloch, G., 2010. The Social Clock of the Honeybee. J. Biol. Rhythms 25, 307–317. https://doi.org/10.1177/0748730410380149

Bodart, O., Amico, E., Gómez, F., Casali, A.G., Wannez, S., Heine, L., Thibaut, A., Annen, J., Boly, M., Casarotto, S., Rosanova, M., Massimini, M., Laureys, S., Gosseries, O., 2018. Global structural integrity and effective connectivity in patients with disorders of consciousness. Brain Stimul. 11, 358–365. https://doi.org/10.1016/j.brs.2017.11.006

Bollinger, T., Schibler, U., 2014. Circadian rhythms - From genes to physiology and disease. Swiss Med. Wkly. https://doi.org/10.4414/smw.2014.13984

Borbély, A.A., 1982. A two process model of sleep regulation. Hum. Neurobiol. 1, 195–204.

Borbély, A.A., 1980. Sleep: circadian rhythm versus recovery process., Functional. ed. Elsevier, Amsterdam.

Borbély, A.A., Daan, S., Wirz-Justice, A., Deboer, T., 2016. The two-process model of sleep regulation: a reappraisal. J. Sleep Res. 25, 131–143. https://doi.org/10.1111/jsr.12371

Bromundt, V., Wirz-Justice, A., Boutellier, M., Winter, S., Haberstroh, M., Terman, M., Münch, M., 2019. Effects of a dawn-dusk simulation on circadian rest-activity cycles, sleep, mood and well-being in dementia patients. Exp. Gerontol. 124. https://doi.org/10.1016/j.exger.2019.110641

Brown, G.M., 1994. Light, melatonin and the sleep-wake cycle. J. Psychiatry Neurosci. 19, 345–53.

Bruce, E.N., Bruce, M.C., Vennelaganti, S., 2009. Sample Entropy Tracks Changes in Electroencephalogram Power Spectrum With Sleep State and Aging. J. Clin. Neurophysiol. 26, 257–266. https://doi.org/10.1097/WNP.0b013e3181b2f1e3

Burke, M.J., Fried, P.J., Pascual-Leone, A., 2019. Transcranial magnetic stimulation: Neurophysiological and clinical applications, in: Handbook of Clinical Neurology. Elsevier B.V., pp. 73–92. https://doi.org/10.1016/B978-0-12-804281-6.00005-7

Bushey, D., Tononi, G., Cirelli, C., 2011. Sleep and synaptic homeostasis: Structural evidence in Drosophila. Science (80-.). 332, 1576–1581. https://doi.org/10.1126/science.1202839

Cajochen, C., Foy, R., Dijk, D.J., 1999. Frontal predominance of a relative increase in sleep delta and theta EEG activity after sleep loss in humans. Sleep Res. Online 2, 65–69.

Cajochen, C., Knoblauch, V., Kräuchi, K., Renz, C., Wirz-Justice, A., 2001. Dynamics of frontal EEG activity, sleepiness and body temperature under high and low sleep pressure. Neuroreport 12, 2277–

2281. https://doi.org/10.1097/00001756-200107200-00046

Cajochen, C., Mu, M., Knoblauch, V., Blatter, K., Wirz-justice, A., 2006. Age-related changes in the circadian and homeostatic regulation of human sleep 23, 461–474. https://doi.org/10.1080/07420520500545813

Cajochen, C., Wyatt, J.K., Czeisler, C.A., Dijk, D.J., 2002. Separation of circadian and wake duration-dependent modulation of EEG activation during wakefulness. Neuroscience 114, 1047–60.

Campbell, S.S., Murphy, P.J., 1998. Extraocular circadian phototransduction in humans. Science (80-.). 279, 396–399. https://doi.org/10.1126/science.279.5349.396

Carrier, J., Land, S., Buysse, D.J., Kupfer, D.J., Monk, T.H., 2001. The effects of age and gender on sleep EEG power spectral density in the middle years of life (ages 20-60 years old). Psychophysiology 38, 232–42.

Casali, Adenauer G., Casarotto, S., Rosanova, M., Mariotti, M., Massimini, M., 2010. General indices to characterize the electrical response of the cerebral cortex to TMS. Neuroimage 49, 1459–1468. https://doi.org/10.1016/j.neuroimage.2009.09.026

Casali, Adenauer G, Casarotto, S., Rosanova, M., Mariotti, M., Massimini, M., 2010. General indices to characterize the electrical response of the cerebral cortex to TMS. Neuroimage 49, 1459–1468. https://doi.org/10.1016/j.neuroimage.2009.09.026

Casali, A.G., Gosseries, O., Rosanova, M., Boly, M., Sarasso, S., Casali, K.R., Casarotto, S., Bruno, M.A., Laureys, S., Tononi, G., Massimini, M., 2013. A theoretically based index of consciousness independent of sensory processing and behavior. Sci. Transl. Med. https://doi.org/10.1126/scitranslmed.3006294

Casarotto, S., Lauro, L.J.R., Bellina, V., Casali, A.G., Rosanova, M., Pigorini, A., Defendi, S., Mariotti, M., Massimini, M., 2010. EEG responses to TMS are sensitive to changes in the perturbation parameters and repeatable over time. PLoS One 5. https://doi.org/10.1371/journal.pone.0010281

Castrillon, G., Sollmann, N., Kurcyus, K., Razi, A., Krieg, S.M., Riedl, V., 2020. The physiological effects of noninvasive brain stimulation fundamentally differ across the human cortex. Sci. Adv. 6, eaay2739. https://doi.org/10.1126/sciadv.aay2739

Chellappa, S.L., Gaggioni, G., Ly, J.Q.M., Papachilleos, S., Borsu, C., Brzozowski, A., Rosanova, M., Sarasso, S., Luxen, A., Middleton, B., Archer, S.N., Dijk, D.J., Massimini, M., Maquet, P., Phillips, C., Moran, R.J., Vandewalle, G., 2016. Circadian dynamics in measures of cortical excitation and inhibition balance. Sci. Rep. 6, 33661. https://doi.org/10.1038/srep33661

Colten, H.R., Altevogt, B.M., 2006. Sleep disorders and sleep deprivation: An unmet public health problem, Sleep Disorders and Sleep Deprivation: An Unmet Public Health Problem. National Academies Press, Washington, D.C. https://doi.org/10.17226/11617

Comolatti, R., Pigorini, A., Casarotto, S., Fecchio, M., Faria, G., Sarasso, S., Rosanova, M., Gosseries, O., Boly, M., Bodart, O., Ledoux, D., Brichant, J.F., Nobili, L., Laureys, S., Tononi, G., Massimini, M., Casali, A.G., 2019. A fast and general method to empirically estimate the complexity of brain responses to transcranial and intracranial stimulations. Brain Stimul. 12, 1280–1289. https://doi.org/10.1016/j.brs.2019.05.013

Czeisler, C., Brown, E., Ronda, J., Kronauer, R., Richardson, G., Freitag, W., 1985. A clinical method to assess the endogenous circadian phase (ECP) of the deep circadian oscillator in man. Sleep Reserach 14:295.

Czeisler, C.A., 2016. Measuring the passage of brain time. Science (80-.). 353, 648–649. https://doi.org/10.1126/science.aah5402

Czeisler, C.A., Dijk, D., 2001. Human sleep-wake regulation, in: Takahashi, J.S., Turek, F.W., Moore, R.Y. (Eds.), Circadian Clocks. Springer.

Czeisler, C.A., Duffy, J.F., Shanahan, T.L., Brown, E.N., Mitchell, J.F., Rimmer, D.W., Ronda, J.M., Silva, E.J., Allan, J.S., Emens, J.S., Dijk, D.J., Kronauer, R.E., 1999. Stability, precision, and near-24-hour period of the human circadian pacemaker. Science 284, 2177–81. https://doi.org/10.1126/SCIENCE.284.5423.2177

Czeisler, C.A., Gooley, J.J., 2007. Sleep and Circadian Rhythms in Humans. Cold Spring Harb. Symp. Quant. Biol. 72, 579–597. https://doi.org/10.1101/sqb.2007.72.064

Czeisler, C.A., Johnson, M.P., Duffy, J.F., Brown, E.N., Ronda, J.M., Kronauer, R.E., 1990. Exposure to Bright Light and Darkness to Treat Physiologic Maladaptation to Night Work. N. Engl. J. Med. 322, 1253–1259. https://doi.org/10.1056/NEJM199005033221801

Daan, S., 2000. Colin Pittendrigh, Jurgen Aschoff, and the natural entrainment of circadian systems, in: Journal of Biological Rhythms. pp. 195–207. https://doi.org/10.1177/074873040001500301

Daan, S., Beersma, D.G.M., Borbely, A.A., 1984. Timing of human sleep: Recovery process gated by a circadian pacemaker. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 15. https://doi.org/10.1152/ajpregu.1984.246.2.r161

Dash, M.B., Douglas, C.L., Vyazovskiy, V. V., Cirelli, C., Tononi, G., 2009. Long-term homeostasis of extracellular glutamate in the rat cerebral cortex across sleep and waking states. J. Neurosci. 29, 620–629. https://doi.org/10.1523/JNEUROSCI.5486-08.2009

Dayan, E., Censor, N., Buch, E.R., Sandrini, M., Cohen, L.G., 2013. Noninvasive brain stimulation: From physiology to network dynamics and back. Nat. Neurosci. https://doi.org/10.1038/nn.3422

de Vivo, L., Bellesi, M., Marshall, W., Bushong, E.A., Ellisman, M.H., Tononi, G., Cirelli, C., 2017. Ultrastructural evidence for synaptic scaling across the wake/sleep cycle. Science 355, 507–510. https://doi.org/10.1126/science.aah5982

De Vivo, L., Bellesi, M., Marshall, W., Bushong, E.A., Ellisman, M.H., Tononi, G., Cirelli, C., 2017. Ultrastructural evidence for synaptic scaling across the wake/sleep cycle. Science (80-.). 355, 507–510. https://doi.org/10.1126/science.aah5982

Deboer, T., 2013. Behavioral and electrophysiological correlates of sleep and sleep homeostasis. Curr. Top. Behav. Neurosci. 25. https://doi.org/10.1007/7854_2013_248

DeCoursey, P.J., Walker, J.K., Smith, S.A., 2000. A circadian pacemaker in free-living chipmunks: Essential for survival? J. Comp. Physiol. - A Sensory, Neural, Behav. Physiol. 186, 169–180. https://doi.org/10.1007/s003590050017

Diekelmann, S., Born, J., 2010. The memory function of sleep. Nat. Rev. Neurosci. https://doi.org/10.1038/nrn2762

Dijk, D.-J., Archer, S.N., 2010. PERIOD3, circadian phenotypes, and sleep homeostasis. Sleep Med. Rev. 14, 151–60. https://doi.org/10.1016/j.smrv.2009.07.002

Dijk, D.-J., Czeisler, C.A., 1994. Paradoxical timing of the circadian rhythm of sleep propensity serves to consolidate sleep and wakefulness in humans. Neurosci. Lett. 166, 63–68. https://doi.org/10.1016/0304-3940(94)90841-9

DIJK, D. -J, DUFFY, J.F., CZEISLER, C.A., 1992. Circadian and sleep/wake dependent aspects of subjective alertness and cognitive performance. J. Sleep Res. 1, 112–117. https://doi.org/10.1111/j.1365-2869.1992.tb00021.x

Dijk, D., Duffy, J.F., Riel, E., Shanahan, T.L., Czeisler, C.A., 1999. Ageing and the circadian and homeostatic regulation of human sleep during forced desynchrony of rest , melatonin and temperature rhythms.

Dijk, D.J., 1995. EEG slow waves and sleep spindles: windows on the sleeping brain. Behav. Brain Res. 69, 109–116. https://doi.org/10.1016/0166-4328(95)00007-G

Dijk, D.J., Beersma, D.G.M., Daan, S., 1987. EEG Power Density during Nap Sleep: Reflection of an Hourglass Measuring the Duration of Prior Wakefulness. J. Biol. Rhythms 2, 207–219. https://doi.org/10.1177/074873048700200304

Dijk, D.J., Brunner, D.P., Borbely, A.A., 1990. Time course of EEG power density during long sleep in humans. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 258. https://doi.org/10.1152/ajpregu.1990.258.3.r650

Dijk, D J, Czeisler, C.A., 1995. Contribution of the circadian pacemaker and the sleep homeostat to sleep propensity, sleep structure, electroencephalographic slow waves, and sleep spindle activity in humans. J. Neurosci. 15, 3526–3538.

Dijk, D. J., Czeisler, C.A., 1995. Contribution of the circadian pacemaker and the sleep homeostat to sleep propensity, sleep structure, electroencephalographic slow waves, and sleep spindle activity in humans. J. Neurosci. 15, 3526–3538. https://doi.org/10.1523/jneurosci.15-05-03526.1995

Dijk, D.J., Czeisler, C.A., 1994. Paradoxical timing of the circadian rhythm of sleep propensity serves to consolidate sleep and wakefulness in humans. Neurosci. Lett. 166, 63–68. https://doi.org/10.1016/0304-3940(94)90841-9

Dijk, D.J., Duffy, J.F., Silva, E.J., Shanahan, T.L., Boivin, D.B., Czeisler, C.A., 2012. Amplitude reduction and phase shifts of Melatonin, cortisol and other circadian rhythms after a gradual advance of sleep and light exposure in humans. PLoS One 7. https://doi.org/10.1371/journal.pone.0030037

Duffy, J.F., Czeisler, C.A., 2009. Effect of Light on Human Circadian Physiology. Sleep Med. Clin. https://doi.org/10.1016/j.jsmc.2009.01.004

Duffy, J.F., Wright, K.P., 2005. Entrainment of the human circadian system by light. J. Biol. Rhythms. https://doi.org/10.1177/0748730405277983

Duffy, J.F., Zeitzer, J.M., Rimmer, D.W., Klerman, E.B., Dijk, D., Czeisler, C.A., Jeanne, F., Zeitzer, J.M., Rim-, D.W., Klerman, E.B., Dijk, D., Czeisler, C.A., 2002. Peak of circadian melatonin rhythm occurs later within the sleep of older subjects 02115, 297–303.

Elvsåshagen, T., Norbom, L.B., Pedersen, P.Ø., Quraishi, S.H., Bjørnerud, A., Malt, U.F., Groote, I.R., Westlye, L.T., 2015. Widespread changes in white matter microstructure after a day of waking and sleep deprivation. PLoS One 10, e0127351. https://doi.org/10.1371/journal.pone.0127351

Farajnia, S., Michel, S., Deboer, T., vanderLeest, H.T., Houben, T., Rohling, J.H.T., Ramkisoensing, A., Yasenkov, R., Meijer, J.H., 2012. Evidence for neuronal desynchrony in the aged suprachiasmatic nucleus clock. J. Neurosci. 32, 5891–9. https://doi.org/10.1523/JNEUROSCI.0469-12.2012

Ferrarelli, F., Massimini, M., Sarasso, S., Casali, A., Riedner, B.A., Angelini, G., Tononi, G., Pearce, R.A., 2010. Breakdown in cortical effective connectivity during midazolam-induced loss of consciousness. Proc. Natl. Acad. Sci. U. S. A. 107, 2681–2686. https://doi.org/10.1073/pnas.0913008107

Frank, M.G., 2013. Why I am not shy: a reply to Tononi and Cirelli. Neural Plast. https://doi.org/10.1155/2013/394946

Franken, P., Dijk, D.J., 2009. Circadian clock genes and sleep homeostasis. Eur. J. Neurosci. https://doi.org/10.1111/j.1460-9568.2009.06723.x

Franken, P., Lopez-Molina, L., Marcacci, L., Schibler, U., Tafti, M., 2000. The transcription factor DBP affects circadian sleep consolidation and rhythmic EEG activity. J. Neurosci. 20, 617–625. https://doi.org/10.1523/jneurosci.20-02-00617.2000

Friston, K., Harrison, L., Daunizeau, J., Kiebel, S., Phillips, C., Trujillo-Barreto, N., Henson, R., Flandin, G., Mattout, J., 2008. Multiple sparse priors for the M/EEG inverse problem. Neuroimage 39, 1104–1120. https://doi.org/10.1016/j.neuroimage.2007.09.048

Friston, K.J., 2011. Functional and Effective Connectivity: A Review. Brain Connect. 1, 13–36. https://doi.org/10.1089/brain.2011.0008

Gaggioni, G., Ly, J.Q.M., Chellappa, S.L., Coppieters 't Wallant, D., Rosanova, M., Sarasso, S., Luxen, A., Salmon, E., Middleton, B., Massimini, M., Schmidt, C., Casali, A., Phillips, C., Vandewalle, G., 2018. Human fronto-parietal response scattering subserves vigilance at night. Neuroimage 175, 354–364. https://doi.org/10.1016/j.neuroimage.2018.03.055

Gaggioni, G., Ly, J.Q.M., Muto, V., Chellappa, S.L., Jaspar, M., Meyer, C., Delfosse, T., Vanvinckenroye, A., Dumont, R., Coppieters 't Wallant, D., Berthomier, C., Narbutas, J., Van Egroo, M., Luxen, A., Salmon, E., Collette, F., Phillips, C., Schmidt, C., Vandewalle, G., 2019. Age-related decrease in cortical excitability circadian variations during sleep loss and its links with cognition. Neurobiol. Aging 78, 52–63. https://doi.org/10.1016/j.neurobiolaging.2019.02.004

Gaggioni, G., Maquet, P., Schmidt, C., Dijk, D., Vandewalle, G., 2014. Neuroimaging, cognition, light and circadian rhythms. Front. Syst. Neurosci. 8, 126.

Gajewski, P.D., Falkenstein, M., 2018. ERP and Behavioral Effects of Physical and Cognitive Training on Working Memory in Aging: A Randomized Controlled Study. Neural Plast. 2018, 3454835. https://doi.org/10.1155/2018/3454835

Gaynor, K.M., Hojnowski, C.E., Carter, N.H., Brashares, J.S., 2018. The influence of human disturbance on wildlife nocturnality. Science 360, 1232–1235. https://doi.org/10.1126/science.aar7121

Goel, N., Dinges, D.F., 2011. Behavioral and genetic markers of sleepiness. J. Clin. Sleep Med. https://doi.org/10.5664/JCSM.1348

Hansen, J., 2001. Increased breast cancer risk among women who work predominantly at night. Epidemiology 12, 74–77. https://doi.org/10.1097/00001648-200101000-00013

Hardin, P.E., Hall, J.C., Rosbash, M., 1990. Feedback of the Drosophila period gene product on circadian cycling of its messenger RNA levels. Nature 343, 536–540. https://doi.org/10.1038/343536a0

Hattar, S., Liao, H.W., Takao, M., Berson, D.M., Yau, K.W., 2002. Melanopsin-containing retinal ganglion cells: Architecture, projections, and intrinsic photosensitivity. Science (80-.). 295, 1065–1070. https://doi.org/10.1126/science.1069609

Heath-Heckman, E.A.C., Peyer, S.M., Whistler, C.A., Apicella, M.A., Goldman, W.E., McFall-Ngai, M.J., 2013. Bacterial bioluminescence regulates expression of a host cryptochrome gene in the squid-vibrio symbiosis. MBio 4. https://doi.org/10.1128/mBio.00167-13

Helm, B., Visser, M.E., Schwartz, W., Kronfeld-Schor, N., Gerkema, M., Piersma, T., Bloch, G., 2017. Two sides of a coin: Ecological and chronobiological perspectives of timing in the wild. Philos. Trans. R. Soc. B Biol. Sci. https://doi.org/10.1098/rstb.2016.0246

Herzog, E.D., Hermanstyne, T., Smyllie, N.J., Hastings, M.H., 2017. Regulating the suprachiasmatic nucleus (SCN) circadian clockwork: Interplay between cell- autonomous and circuit-level mechanisms. Cold Spring Harb. Perspect. Biol. 9. https://doi.org/10.1101/cshperspect.a027706

Horne, J.A., Östberg, O., 1976. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int. J. Chronobiol. 4, 97–110.

Huber, R., Ghilardi, M.F., Massimini, M., Tononi, G., 2004. Local sleep and learning. Nature 430, 78–81. https://doi.org/10.1038/nature02663

Huber, R, Maki, H., Rosanova, M., Casarotto, S., Canali, P., Casali, A.G., Tononi, G., Massimini, M., 2013. Human cortical excitability increases with time awake. Cereb Cortex 23, 332–338. https://doi.org/10.1093/cercor/bhs014

Huber, Reto, Mäki, H., Rosanova, M., Casarotto, S., Canali, P., Casali, A.G., Tononi, G., Massimini, M., 2013. Human cortical excitability increases with time awake. Cereb. Cortex 23, 332–338. https://doi.org/10.1093/cercor/bhs014

Hung, C.-S., Sarasso, S., Ferrarelli, F., Riedner, B., Ghilardi, M.F., Cirelli, C., Tononi, G., 2013. Local Experience-Dependent Changes in the Wake EEG after Prolonged Wakefulness. Sleep 36, 59–72.

https://doi.org/10.5665/sleep.2302

Hurd, M.W., Ralph, M.R., 1998. The Significance of Circadian Organization for Longevity in the Golden Hamster. J. Biol. Rhythms 13, 430–436. https://doi.org/10.1177/074873098129000255

Huynh, W., Vucic, S., Krishnan, A. V., Lin, C.S.Y., Kiernan, M.C., 2016. Exploring the evolution of cortical excitability following acute stroke. Neurorehabil. Neural Repair 30, 244–257. https://doi.org/10.1177/1545968315593804

Ilmoniemi, R.J., Virtanen, J., Ruohonen, J., Karhu, J., Aronen, H.J., Näätänen, R., Katila, T., 1997. Neuronal responses to magnetic stimulation reveal cortical reactivity and connectivity. Neuroreport 8, 3537–3540. https://doi.org/10.1097/00001756-199711100-00024

JOHNSON, M.P., DUFFY, J.F., DIJK, D.J., RONDA, J.M., DYAL, C.M., CZEISLER, C.A., 1992. Short-term memory, alertness and performance: a reappraisal of their relationship to body temperature. J. Sleep Res. https://doi.org/10.1111/j.1365-2869.1992.tb00004.x

Johnston, J.D., Frost, G., Otway, D.T., 2009. Adipose tissue, adipocytes and the circadian timing system. Obes. Rev. https://doi.org/10.1111/j.1467-789X.2009.00665.x

KATTLER, H., DIJK, D. -J, BORBÉLY, A.A., 1994. Effect of unilateral somatosensory stimulation prior to sleep on the sleep EEG in humans. J. Sleep Res. 3, 159–164. https://doi.org/10.1111/j.1365-2869.1994.tb00123.x

King, D.P., Takahashi, J.S., 2000. Molecular Genetics of Circadian Rhythms in Mammals. Annu. Rev. Neurosci. 23, 713–742. https://doi.org/10.1146/annurev.neuro.23.1.713

King, David P., Vitaterna, M.H., Chang, A.M., Dove, W.F., Pinto, L.H., Turek, F.W., Takahashi, J.S., 1997. The mouse Clock mutation behaves as an antimorph and maps within the W(19H) deletion, distal of Kit. Genetics 146, 1049–1060.

King, David P, Zhao, Y., Sangoram, A.M., Wilsbacher, L.D., Tanaka, M., Antoch, M.P., Steeves, T.D.L., Vitaterna, M.H., Kornhauser, J.M., Lowrey, P.L., Turek, F.W., Takahashi, J.S., 1997. Positional cloning of the mouse circadian Clock gene. Cell 89, 641–653. https://doi.org/10.1016/S0092-8674(00)80245-7

Klein, B.A., Klein, A., Wray, M.K., Mueller, U.G., Seeley, T.D., 2010. Sleep deprivation impairs precision of waggle dance signaling in honey bees. Proc. Natl. Acad. Sci. 107, 22705–22709. https://doi.org/10.1073/pnas.1009439108

KLEITMAN, N., 1949. Biological rhythms and cycles. Physiol. Rev. 29, 1–30. https://doi.org/10.1152/physrev.1949.29.1.1

Klerman, E.B., Dijk, D.J., 2008. Age-Related Reduction in the Maximal Capacity for Sleep-Implications for Insomnia. Curr. Biol. 18, 1118–1123. https://doi.org/10.1016/j.cub.2008.06.047

Knop, E., Zoller, L., Ryser, R., Gerpe, C., Hörler, M., Fontaine, C., 2017. Artificial light at night as a new threat to pollination. Nature. https://doi.org/10.1038/nature23288

Komssi, S., Kähkönen, S., 2006. The novelty value of the combined use of electroencephalography and transcranial magnetic stimulation for neuroscience research. Brain Res. Rev. https://doi.org/10.1016/j.brainresrev.2006.01.008

Kondratova, A. a, Kondratov, R. V, 2012. The circadian clock and pathology of the ageing brain. Nat. Rev. Neurosci. 13, 325–35. https://doi.org/10.1038/nrn3208

Konopka, R.J., Benzer, S., 1971. Clock mutants of Drosophila melanogaster. Proc. Natl. Acad. Sci. U. S. A. 68, 2112–2116. https://doi.org/10.1073/pnas.68.9.2112

Krings, T., Buchbinder, B.R., Butler, W.E., Chiappa, K.H., Jiang, H.J., Rosen, B.R., Cosgrove, G.R., 1997. Stereotactic transcranial magnetic stimulation: Correlation with direct electrical cortical stimulation. Neurosurgery 41, 1319–1326. https://doi.org/10.1097/00006123-199712000-00016

Krueger, J., 2008. The Role of Cytokines in Sleep Regulation. Curr. Pharm. Des. 14, 3408–3416. https://doi.org/10.2174/138161208786549281

Laing, E.E., Möller-Levet, C.S., Poh, N., Santhi, N., Archer, S.N., Dijk, D.-J., Feldman, B., Kofler, R., Schreiber, S., Nocentini, G., Riccardi, C., Macrea, M., Pack, A., Plante, D., Teske, J., Zee, P., Li, C., Maechler, M., Rossini, A., Sawitzki, G., Smith, C., Smyth, G., Tierney, L., Yang, J., Zhang, J., 2017. Blood transcriptome based biomarkers for human circadian phase. Elife 6, 16219–16224. https://doi.org/10.7554/eLife.20214

Landolt, H.P., 2011. Genetic determination of sleep EEG profiles in healthy humans, in: Progress in Brain Research. Elsevier B.V., pp. 51–61. https://doi.org/10.1016/B978-0-444-53839-0.00004-1

Landolt, H.P., Dijk, D.J., Achermann, P., Borbély, A.A., 1996. Effect of age on the sleep EEG: slow-wave activity and spindle frequency activity in young and middle-aged men. Brain Res. 738, 205–12.

Landolt, H.P., Rétey, J. V, Adam, M., 2012. Reduced neurobehavioral impairment from sleep deprivation in older adults: Contribution of adenosinergic mechanisms. Front. Neurol. APR, 1–11. https://doi.org/10.3389/fneur.2012.00062

Lempel, A., Ziv, J., 1976. On the Complexity of Finite Sequences. IEEE Trans. Inf. Theory 22, 75–81. https://doi.org/10.1109/TIT.1976.1055501

Leng, Y., Blackwell, T., Cawthon, P.M., Ancoli-Israel, S., Stone, K.L., Yaffe, K., Yaffe, K., Yaffe, K., 2020. Association of Circadian Abnormalities in Older Adults with an Increased Risk of Developing Parkinson Disease. JAMA Neurol. https://doi.org/10.1001/jamaneurol.2020.1623

Leng, Y., Musiek, E.S., Hu, K., Cappuccio, F.P., Yaffe, K., 2019a. Association between circadian rhythms and neurodegenerative diseases. Lancet Neurol. https://doi.org/10.1016/S1474-4422(18)30461-7

Leng, Y., Redline, S., Stone, K.L., Ancoli-Israel, S., Yaffe, K., 2019b. Objective napping, cognitive decline, and risk of cognitive impairment in older men. Alzheimer's Dement. 15, 1039–1047. https://doi.org/10.1016/j.jalz.2019.04.009

Lewis, P., Oster, H., Korf, H.W., Foster, R.G., Erren, T.C., 2020. Food as a circadian time cue — evidence from human studies. Nat. Rev. Endocrinol. https://doi.org/10.1038/s41574-020-0318-z

Litvak, V., Mattout, J., Kiebel, S., Phillips, C., Henson, R., Kilner, J., Barnes, G., Oostenveld, R., Daunizeau, J., Flandin, G., Penny, W., Friston, K., 2011. EEG and MEG data analysis in SPM8. Comput. Intell. Neurosci. 2011, 852961. https://doi.org/10.1155/2011/852961

Liu, A.C., Welsh, D.K., Ko, C.H., Tran, H.G., Zhang, E.E., Priest, A.A., Buhr, E.D., Singer, O., Meeker, K., Verma, I.M., Doyle, F.J., Takahashi, J.S., Kay, S.A., 2007. Intercellular Coupling Confers Robustness against Mutations in the SCN Circadian Clock Network. Cell 129, 605–616. https://doi.org/10.1016/j.cell.2007.02.047

Lo, J.C., Groeger, J.A., Santhi, N., Arbon, E.L., Lazar, A.S., Hasan, S., von Schantz, M., Archer, S.N., Dijk, D.-J., 2012. Effects of Partial and Acute Total Sleep Deprivation on Performance across Cognitive Domains, Individuals and Circadian Phase. PLoS One 7, e45987. https://doi.org/10.1371/journal.pone.0045987

Lowrey, P.L., Takahashi, J.S., 2000. Genetics of the Mammalian Circadian System: Photic Entrainment, Circadian Pacemaker Mechanisms, and Posttranslational Regulation. Annu. Rev. Genet. 34, 533–562. https://doi.org/10.1146/annurev.genet.34.1.533

Lucas, R.J., Freedman, M.S., Muñoz, M., Garcia-Fernández, J.M., Foster, R.G., 1999. Regulation of the mammalian pineal by non-rod, non-cone, ocular photoreceptors. Science (80-.). 284, 505–507. https://doi.org/10.1126/science.284.5413.505

Ly, J.Q.M., Gaggioni, G., Chellappa, S.L., Papachilleos, S., Brzozowski, A., Borsu, C., Rosanova, M., Sarasso, S., Middleton, B., Luxen, A., Archer, S.N., Phillips, C., Dijk, D.-J., Maquet, P., Massimini, M., Vandewalle, G., 2016. Circadian regulation of human cortical excitability. Nat. Commun. 7.

https://doi.org/10.1038/ncomms11828

Ly, Julien Q.M., Gaggioni, G., Chellappa, S.L., Papachilleos, S., Brzozowski, A., Borsu, C., Rosanova, M., Sarasso, S., Middleton, B., Luxen, A., Archer, S.N., Phillips, C., Dijk, D.J., Maquet, P., Massimini, M., Vandewalle, G., 2016. Circadian regulation of human cortical excitability. Nat. Commun. 7, 11828. https://doi.org/10.1038/ncomms11828

M. Stranahan, A., 2012. Chronobiological Approaches to Alzheimers Disease. Curr. Alzheimer Res. 9, 93–98. https://doi.org/10.2174/156720512799015028

Maire, M., Reichert, C.F., Gabel, V., Viola, A.U., Phillips, C., Berthomier, C., Borgwardt, S., Cajochen, C., Schmidt, C., 2018. Human brain patterns underlying vigilant attention: Impact of sleep debt, circadian phase and attentional engagement. Sci. Rep. 8. https://doi.org/10.1038/s41598-017-17022-9

Maire, M., Reichert, C.F., Gabel, V., Viola, A.U., Phillips, C., Krebs, J., Scheffler, K., Klarhöfer, M., Strobel, W., Cajochen, C., Schmidt, C., 2015. Fighting Sleep at Night: Brain Correlates and Vulnerability to Sleep Loss. Ann. Neurol. 78, 235–247. https://doi.org/10.1002/ana.24434

Maor, R., Dayan, T., Ferguson-Gow, H., Jones, K.E., 2017. Temporal niche expansion in mammals from a nocturnal ancestor after dinosaur extinction. Nat. Ecol. Evol. 1, 1889–1895. https://doi.org/10.1038/s41559-017-0366-5

Maquet, P., 2001. The role of sleep in learning and memory. Science (80-.). https://doi.org/10.1126/science.1062856

Masliah, E., Mallory, M., Hansen, L., DeTeresa, R., Terry, R.D., 1993. Quantitative synaptic alterations in the human neocortex during normal aging. Neurology 43, 192–7.

Massimini, M., Ferrarelli, F., Esser, S.K., Riedner, B.A., Huber, R., Murphy, M., Peterson, M.J., Tononi, G., 2007. Triggering sleep slow waves by transcranial magnetic stimulation. Proc. Natl. Acad. Sci. U. S. A. 104, 8496–8501. https://doi.org/10.1073/pnas.0702495104

Massimini, M., Ferrarelli, F., Huber, R., Esser, S.K., Singh, H., Tononi, G., 2005. Breakdown of cortical effective connectivity during sleep. Science (80-.). 309, 2228–2232. https://doi.org/10.1126/science.1117256

Massimini, M., Ferrarelli, F., Murphy, M.J., Huber, R., Riedner, B.A., Casarotto, S., Tononi, G., 2010. Cortical reactivity and effective connectivity during REM sleep in humans. Cogn. Neurosci. 1, 176–183. https://doi.org/10.1080/17588921003731578

Matousek, M., Wackermann, J., Palus, M., Berankova, A., Albrecht, V., Dvorak, I., 1995. Global dimensional complexity of the eeg in healthy volunteers. Neuropsychobiology 31, 47–52. https://doi.org/10.1159/000119171

McGinty, D., Szymusiak, R., 1990. Keeping cool: a hypothesis about the mechanisms and functions of slow-wave sleep. Trends Neurosci. 13, 480–487. https://doi.org/10.1016/0166-2236(90)90081-K

Meyer, C., Muto, V., Jaspar, M., Kussé, C., Lambot, E., Chellappa, S.L., Degueldre, C., Balteau, E., Luxen, A., Middleton, B., Archer, S.N., Collette, F., Dijk, D.J., Phillips, C., Maquet, P., Vandewalle, G., 2016. Seasonality in human cognitive brain responses. Proc. Natl. Acad. Sci. U. S. A. 113, 3066–3071. https://doi.org/10.1073/pnas.1518129113

Minegishi, S., Sagami, I., Negi, S., Kano, K., Kitagishi, H., 2018. Circadian clock disruption by selective removal of endogenous carbon monoxide. Sci. Rep. 8, 1–12. https://doi.org/10.1038/s41598-018-30425-6

Mistlberger, R.E., Bergmann, B.M., Rechtschaffen, A., 1987. Relationships Among Wake Episode Lengths, Contiguous Sleep Episode Lengths, and Electroencephalographic Delta Waves in Rats with Suprachiasmatic Nuclei Lesions.

Monk, T.H., 2010. Enhancing circadian zeitgebers. Sleep. https://doi.org/10.1093/sleep/33.4.421

Moore, R.Y., 2007. Suprachiasmatic nucleus in sleep-wake regulation. Sleep Med. 8, 27–33. https://doi.org/10.1016/j.sleep.2007.10.003

Moore, R.Y., Eichler, V.B., 1972. Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Res. 42, 201–206. https://doi.org/10.1016/0006-8993(72)90054-6

Morrison, J.H., Baxter, M.G., 2012. The ageing cortical synapse: hallmarks and implications for cognitive decline. Nat. Rev. Neurosci. 13, 240–250. https://doi.org/10.1038/nrn3200

Münch, M., Knoblauch, V., Blatter, K., Schröder, C., Schnitzler, C., Kräuchi, K., Wirz-Justice, A., Cajochen, C., 2005. Age-related attenuation of the evening circadian arousal signal in humans. Neurobiol. Aging 26, 1307–1319. https://doi.org/10.1016/J.NEUROBIOLAGING.2005.03.004

Mure, L.S., Le, H.D., Benegiamo, G., Chang, M.W., Rios, L., Jillani, N., Ngotho, M., Kariuki, T., Dkhissi-Benyahya, O., Cooper, H.M., Panda, S., 2018. Diurnal transcriptome atlas of a primate across major neural and peripheral tissues. Science (80-.). 359, eaao0318. https://doi.org/10.1126/science.aao0318

Murphy, S.C., Palmer, L.M., Nyffeler, T., Müri, R.M., Larkum, M.E., 2016. Transcranial magnetic stimulation (TMS) inhibits cortical dendrites. Elife 5. https://doi.org/10.7554/eLife.13598

Muto, V., Jaspar, M., Meyer, C., Kusse, C., Chellappa, S.L., Degueldre, C., Balteau, E., Shaffii-Le Bourdiec, A., Luxen, A., Middleton, B., Archer, S.N., Phillips, C., Collette, F., Vandewalle, G., Dijk, D.-J., Maquet, P., 2016. Local modulation of human brain responses by circadian rhythmicity and sleep debt. Science (80-.). 353, 687–690. https://doi.org/10.1126/science.aad2993

Naismith, S.L., Rogers, N.L., Hickie, I.B., MacKenzie, J., Norrie, L.M., Lewis, S.J.G., 2010. Sleep well, think well: Sleep-wake disturbance in mild cognitive impairment. J. Geriatr. Psychiatry Neurol. 23, 123–130. https://doi.org/10.1177/0891988710363710

Naylor, E., Bergmann, B.M., Krauski, K., Zee, P.C., Takahashi, J.S., Vitaterna, M.H., Turek, F.W., 2000. The circadian Clock mutation alters sleep homeostasis in the mouse. J. Neurosci. 20, 8138–8143. https://doi.org/10.1523/jneurosci.20-21-08138.2000

Nir, Y., Staba, R.J., Andrillon, T., Vyazovskiy, V. V., Cirelli, C., Fried, I., Tononi, G., 2011. Regional Slow Waves and Spindles in Human Sleep. Neuron 70, 153–169. https://doi.org/10.1016/j.neuron.2011.02.043

Nobili, L., Ferrara, M., Moroni, F., De Gennaro, L., Russo, G. Lo, Campus, C., Cardinale, F., De Carli, F., 2011. Dissociated wake-like and sleep-like electro-cortical activity during sleep. Neuroimage 58, 612–619. https://doi.org/10.1016/j.neuroimage.2011.06.032

O-Neill, J.S., Reddy, A.B., 2011. Circadian clocks in human red blood cells. Nature 469, 498–504. https://doi.org/10.1038/nature09702

Oosterman, J.M., Van Someren, E.J.W., Vogels, R.L.C., Van Harten, B., Scherder, E.J.A., 2009. Fragmentation of the rest-activity rhythm correlates with age-related cognitive deficits. J. Sleep Res. 18, 129–135. https://doi.org/10.1111/j.1365-2869.2008.00704.x

Panda, S., Antoch, M.P., Miller, B.H., Su, A.I., Schook, A.B., Straume, M., Schultz, P.G., Kay, S.A., Takahashi, J.S., Hogenesch, J.B., 2002. Coordinated transcription of key pathways in the mouse by the circadian clock. Cell 109, 307–320. https://doi.org/10.1016/S0092-8674(02)00722-5

Paus, T., Sipila, P.K., Strafella, A.P., 2001. Synchronization of neuronal activity in the human primary motor cortex by transcranial magnetic stimulation: An EEG study. J. Neurophysiol. 86, 1983–1990. https://doi.org/10.1152/jn.2001.86.4.1983

Peters, J.C., Reithler, J., Graaf, T.A. d., Schuhmann, T., Goebel, R., Sack, A.T., 2020. Concurrent human TMS-EEG-fMRI enables monitoring of oscillatory brain state-dependent gating of cortico-subcortical network activity. Commun. Biol. 3, 1–11. https://doi.org/10.1038/s42003-020-0764-0

Pittendrigh, C.S., 1993. Temporal Organization: Reflections of a Darwinian Clock-Watcher. Annu. Rev.

Physiol. 55, 17–54. https://doi.org/10.1146/annurev.ph.55.030193.000313

Pittendrigh, C.S., 1950. THE ECOTOPIC SPECIALIZATION OF *ANOPHELES HOMUNCULUS;* AND ITS RELATION TO COMPETITION WITH *A. BELLATOR*. Evolution (N. Y). 4, 64–78. https://doi.org/10.1111/j.1558-5646.1950.tb01383.x

Pittendrigh, C.S., Minis, D.H., 1972. Circadian systems: longevity as a function of circadian resonance in Drosophila melanogaster. Proc. Natl. Acad. Sci. U. S. A. 69, 1537–1539. https://doi.org/10.1073/pnas.69.6.1537

Porkka-Heiskanen, T., Strecker, R.E., Thakkar, M., Bjørkum, A.A., Greene, R.W., McCarley, R.W., 1997. Adenosine: A mediator of the sleep-inducing effects of prolonged wakefulness. Science (80-.). 276, 1265–1267. https://doi.org/10.1126/science.276.5316.1265

Ralph, M.R., Foster, R.G., Davis, F.C., Menaker, M., 1990. Transplanted suprachiasmatic nucleus determines circadian period. Science (80-.). 247, 975–978. https://doi.org/10.1126/science.2305266

Rattenborg, N.C., 2017. Sleeping on the wing. Interface Focus. https://doi.org/10.1098/rsfs.2016.0082

Rattenborg, N.C., Lesku, J.A., Martinez-Gonzalez, D., Lima, S.L., 2007. The non-trivial functions of sleep. Sleep Med. Rev. 11, 405–409. https://doi.org/10.1016/J.SMRV.2007.04.003

Rattenborg, N.C., Lima, S.L., Amlaner, C.J., 1999. Half-awake to the risk of predation. Nature 397, 397–398. https://doi.org/10.1038/17037

Reddy, A.B., Karp, N.A., Maywood, E.S., Sage, E.A., Deery, M., O'neill, J.S., Wong, G.K.Y., Chesham, J., Odell, M., Lilley, K.S., Kyriacou, C.P., Hastings, M.H., n.d. Report Circadian Orchestration of the Hepatic Proteome. Curr. Biol. 16, 1107–1115. https://doi.org/10.1016/j.cub.2006.04.026

Refinetti, R., 2012. Integration of biological clocks and rhythms. Compr. Physiol. 2, 1213–1239. https://doi.org/10.1002/cphy.c100088

Reichert, C.F., Maire, M., Gabel, V., Viola, A.U., Götz, T., Scheffler, K., Klarhöfer, M., Berthomier, C., Strobel, W., Phillips, C., Salmon, E., Cajochen, C., Schmidt, C., 2017. Cognitive brain responses during circadian wake-promotion: Evidence for sleep-pressure-dependent hypothalamic activations. Sci. Rep. 7. https://doi.org/10.1038/s41598-017-05695-1

Rensing, L., Ruoff, P., 2002. Temperature effect on entrainment, phase shifting, and amplitude of circadian clocks and its molecular bases. Chronobiol. Int. https://doi.org/10.1081/CBI-120014569

Rial, R.V., Nicolau, M.C., Gamundí, A., Akaârir, M., Aparicio, S., Garau, C., Tejada, S., Roca, C., Gené, L., Moranta, D., Esteban, S., 2007. The trivial function of sleep. Sleep Med. Rev. https://doi.org/10.1016/j.smrv.2007.03.001

Rizzo, V., Richman, J., Puthanveettil, S. V., 2015. Dissecting mechanisms of brain aging by studying the intrinsic excitability of neurons. Front. Aging Neurosci. https://doi.org/10.3389/fnagi.2014.00337

Roenneberg, T., Foster, R.G., 1997. Twilight Times: Light and the Circadian System. Photochem. Photobiol. https://doi.org/10.1111/j.1751-1097.1997.tb03188.x

Roenneberg, T., Kuehnle, T., Pramstaller, P.P., Ricken, J., Havel, M., Guth, A., Merrow, M., 2004. A marker for the end of adolescence. Curr. Biol. https://doi.org/10.1016/j.cub.2004.11.039

Roenneberg, T., Wirz-Justice, A., Merrow, M., 2003. Life between clocks: Daily temporal patterns of human chronotypes. J. Biol. Rhythms 18, 80–90. https://doi.org/10.1177/0748730402239679

Roenneberg, T., Wirz-Justice, A., Skene, D.J., Ancoli-Israel, S., Wright, K.P., Dijk, D.J., Zee, P., Gorman, M.R., Winnebeck, E.C., Klerman, E.B., 2019. Why Should We Abolish Daylight Saving Time? J. Biol. Rhythms 34, 227–230. https://doi.org/10.1177/0748730419854197

Rogasch, N.C., Fitzgerald, P.B., 2013. Assessing cortical network properties using TMS-EEG. Hum. Brain Mapp. 34, 1652–1669. https://doi.org/10.1002/hbm.22016

Rosanova, M., Casali, A., Bellina, V., Resta, F., Mariotti, M., Massimini, M., 2009. Natural frequencies of human corticothalamic circuits. J. Neurosci. 29, 7679–7685. https://doi.org/10.1523/JNEUROSCI.0445-09.2009

Rosanova, M, Casarotto, S., Pigorini, A., Canali, P., Casali, A.G., Massimini, M., 2012. Combining transcranial magnetic stimulation with electroencephalography to study human cortical excitability and effective connectivity. Neuromethods 67, 435–467.

Rosanova, Mario, Casarotto, S., Pigorini, A., Canali, P., Casali, A.G., Massimini, M., 2012. Combining transcranial magnetic stimulation with electroencephalography to study human cortical excitability and effective connectivity. Neuromethods 67, 435–457. https://doi.org/10.1007/7657_2011_15

Rossini, P.M., Barker, A.T., Berardelli, A., Caramia, M.D., Caruso, G., Cracco, R.Q., Dimitrijević, M.R., Hallett, M., Katayama, Y., Lücking, C.H., Maertens de Noordhout, A.L., Marsden, C.D., Murray, N.M.F., Rothwell, J.C., Swash, M., Tomberg, C., 1994. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroencephalogr. Clin. Neurophysiol. 91, 79–92. https://doi.org/10.1016/0013-4694(94)90029-9

Rossini, P.M., Rossi, S., 2007. Transcranial magnetic stimulation: Diagnostic, therapeutic, and research potential. Neurology 68, 484–488. https://doi.org/10.1212/01.wnl.0000250268.13789.b2

Saper, C.B., 2013. The central circadian timing system. Curr. Opin. Neurobiol. https://doi.org/10.1016/j.conb.2013.04.004

Saper, C.B., Chou, T.C., Scammell, T.E., 2001. The sleep switch: Hypothalamic control of sleep and wakefulness. Trends Neurosci. https://doi.org/10.1016/S0166-2236(00)02002-6

Saper, C.B., Fuller, P.M., Pedersen, N.P., Lu, J., Scammell, T.E., 2010. Sleep State Switching. Neuron. https://doi.org/10.1016/j.neuron.2010.11.032

Saper, C.B., Scammell, T.E., Lu, J., 2005. Hypothalamic regulation of sleep and circadian rhythms. Nature. https://doi.org/10.1038/nature04284

Sarasso, S., Boly, M., Napolitani, M., Gosseries, O., Charland-Verville, V., Casarotto, S., Rosanova, M., Casali, A.G., Brichant, J.-F., Boveroux, P., Rex, S., Tononi, G., Laureys, S., Massimini, M., 2015. Consciousness and Complexity during Unresponsiveness Induced by Propofol, Xenon, and Ketamine. Curr. Biol. 25, 3099–3105. https://doi.org/10.1016/j.cub.2015.10.014

Scammell, T.E., Arrigoni, E., Lipton, J.O., 2017. Neural Circuitry of Wakefulness and Sleep. Neuron 93, 747–765. https://doi.org/10.1016/j.neuron.2017.01.014

Schmidt, C., Bao, Y., 2017. Chronobiological research for cognitive science: A multifaceted view. PsyCh J. 6, 249–252. https://doi.org/10.1002/pchj.203

Schmidt, C, Collette, F., Cajochen, C., Peigneux, P., 2007. A time to think: circadian rhythms in human cognition. Cogn Neuropsychol 24, 755–789. https://doi.org/10.1080/02643290701754158

Schmidt, Christina, Collette, F., Cajochen, C., Peigneux, P., 2007. A time to think: Circadian rhythms in human cognition. Cogn. Neuropsychol. 24, 755–789. https://doi.org/10.1080/02643290701754158

Schmidt, C., Collette, F., Leclercq, Y., Sterpenich, V., Vandewalle, G., Berthomier, P., Berthomier, C., Phillips, C., Tinguely, G., Darsaud, A., Gais, S., Schabus, M., Desseilles, M., Dang-Vu, T.T., Salmon, E., Balteau, E., Degueldre, C., Luxen, A., Maquet, P., Cajochen, C., Peigneux, P., 2009. Homeostatic sleep pressure and responses to sustained attention in the suprachiasmatic area. Science (80-.). 324, 516–519. https://doi.org/10.1126/science.1167337

Schmidt, C., Peigneux, P., Cajochen, C., 2012a. Age-related changes in sleep and circadian rhythms: Impact on cognitive performance and underlying neuroanatomical networks. Front. Neurol. JUL, 1–11. https://doi.org/10.3389/fneur.2012.00118

Schmidt, C., Peigneux, P., Leclercq, Y., Sterpenich, V., Vandewalle, G., Phillips, C., Berthomier, P.,

Berthomier, C., Tinguely, G., Gais, S., Schabus, M., Desseilles, M., Dang-Vu, T., Salmon, E., Degueldre, C., Balteau, E., Luxen, A., Cajochen, C., Maquet, P., Collette, F., 2012b. Circadian preference modulates the neural substrate of conflict processing across the day. PLoS One 7. https://doi.org/10.1371/journal.pone.0029658

Schwartz, W.J., Helm, B., Gerkema, M.P., 2017. Wild clocks: Preface and glossary. Philos. Trans. R. Soc. B Biol. Sci. https://doi.org/10.1098/rstb.2017.0211

Schwartzman, D.J., Schartner, M., Ador, B.B., Simonelli, F., Chang, A.Y.-C., Seth, A.K., 2019. Increased spontaneous EEG signal diversity during stroboscopically-induced altered states of consciousness. bioRxiv 511766. https://doi.org/10.1101/511766

Sehgal, A., Mignot, E., 2011. Genetics of sleep and sleep disorders. Cell. https://doi.org/10.1016/j.cell.2011.07.004

Shimmura, T., Yoshimura, T., 2013. Circadian clock determines the timing of rooster crowing. Curr. Biol. https://doi.org/10.1016/j.cub.2013.02.015

Shkolnik, A., 1971. Diurnal activity in a small desert rodent. Int. J. Biometeorol. 15, 115–120. https://doi.org/10.1007/BF01803884

Siebner, H.R., Hartwigsen, G., Kassuba, T., Rothwell, J.C., 2009. How does transcranial magnetic stimulation modify neuronal activity in the brain? Implications for studies of cognition. Cortex. 45, 1035–42. https://doi.org/10.1016/j.cortex.2009.02.007

Siegel, J.M., 2009. Sleep viewed as a state of adaptive inactivity. Nat. Rev. Neurosci. https://doi.org/10.1038/nrn2697

Sipilä, J.O.T., Ruuskanen, J.O., Rautava, P., Kytö, V., 2016. Changes in ischemic stroke occurrence following daylight saving time transitions. Sleep Med. 27–28, 20–24. https://doi.org/10.1016/j.sleep.2016.10.009

Skeldon, A.C., Dijk, D.J., 2019. School start times and daylight saving time confuse California lawmakers. Curr. Biol. https://doi.org/10.1016/j.cub.2019.03.014

Spoelstra, K., Wikelski, M., Daan, S., Loudon, A.S.I., Hau, M., 2016. Natural selection against a circadian clock gene mutation in mice. Proc. Natl. Acad. Sci. U. S. A. 113, 686–91. https://doi.org/10.1073/pnas.1516442113

Stam, C.J., 2005. Nonlinear dynamical analysis of EEG and MEG: Review of an emerging field. Clin. Neurophysiol. https://doi.org/10.1016/j.clinph.2005.06.011

Stephan, F.K., Zucker, I., 1972. Circadian Rhythms in Drinking Behavior and Locomotor Activity of Rats Are Eliminated by Hypothalamic Lesions (suprachiasmatic and medial preoptic nuclei/retino-hypothalamic projection).

Stokkan, K.A., Yamazaki, S., Tei, H., Sakaki, Y., Menaker, M., 2001. Entrainment of the circadian clock in the liver by feeding. Science (80-.). 291, 490–493. https://doi.org/10.1126/science.291.5503.490

Storch, K.-F., Lipan, O., Leykin, I., Viswanathan, N., Davis, F.C., Wong, W.H., Weitz, C.J., 2002. Extensive and divergent circadian gene expression in liver and heart. Nature 417, 78–83. https://doi.org/10.1038/nature744

Thorne, H., Hampton, S., Morgan, L., Skene, D.J., Arendt, J., 2008. Differences in sleep, light, and circadian phase in offshore 18:00-06:00 h and 19:00-07:00 h shift workers, in: Chronobiology International. pp. 225–235. https://doi.org/10.1080/07420520802106850

Tobler, I., 1995. Is sleep fundamentally different between mammalian species? Behav. Brain Res. 69, 35–41. https://doi.org/10.1016/0166-4328(95)00025-O

Tobler, I., Achermann, P., 2007. Sleep homeostasis. Scholarpedia 2, 2432. https://doi.org/10.4249/scholarpedia.2432

Tononi, G., Cirelli, C., 2006. Sleep function and synaptic homeostasis. Sleep Med. Rev. 10, 49–62. https://doi.org/10.1016/j.smrv.2005.05.002

Tosun, P., Abásolo, D., Stenson, G., Winsky-Sommerer, R., Tosun, P.D., Abásolo, D., Stenson, G., Winsky-Sommerer, R., 2017. Characterisation of the Effects of Sleep Deprivation on the Electroencephalogram Using Permutation Lempel–Ziv Complexity, a Non-Linear Analysis Tool. Entropy 19, 673. https://doi.org/10.3390/e19120673

Tosun, P.D., Dijk, D.-J., Winsky-Sommerer, R., Abasolo, D., 2019. Effects of Ageing and Sex on Complexity in the Human Sleep EEG: A Comparison of Three Symbolic Dynamic Analysis Methods. Complexity 2019, 1–12. https://doi.org/10.1155/2019/9254309

Valomon, A., Holst, S.C., Bachmann, V., Viola, A.U., Schmidt, C., Zürcher, J., Berger, W., Cajochen, C., Landolt, H.P., 2014. Genetic polymorphisms of DAT1 and COMT differentially associate with actigraphy-derived sleep-wake cycles in young adults. Chronobiol. Int. 31, 705–714. https://doi.org/10.3109/07420528.2014.896376

Van Der Werf, Y.D., Altena, E., Van Dijk, K.D., Strijers, R.L.M., De Rijke, W., Stam, C.J., Van Someren, E.J.W., 2010. Is disturbed intracortical excitability a stable trait of chronic insomnia? A study using transcranial magnetic stimulation before and after multimodal sleep therapy. Biol. Psychiatry 68, 950–955. https://doi.org/10.1016/j.biopsych.2010.06.028

Van Eekelen, A.P.J., Kerkhof, G.A., 2003. No interference of task complexity with circadian rhythmicity in a constant routine protocol. Ergonomics 46, 1578–1593. https://doi.org/10.1080/0014013031000121598

Van Egroo, M., Narbutas, J., Chylinski, D., Villar González, P., Ghaemmaghami, P., Muto, V., Schmidt, C., Gaggioni, G., Besson, G., Pépin, X., Tezel, E., Marzoli, D., Le Goff, C., Cavalier, E., Luxen, A., Salmon, E., Maquet, P., Bahri, M.A., Phillips, C., Bastin, C., Collette, F., Vandewalle, G., 2019. Preserved wake-dependent cortical excitability dynamics predict cognitive fitness beyond age-related brain alterations. Commun. Biol. 2. https://doi.org/10.1038/s42003-019-0693-y

van Hasselt, S.J., Rusche, M., Vyssotski, A.L., Verhulst, S., Rattenborg, N.C., Meerlo, P., 2020. Sleep Time in the European Starling Is Strongly Affected by Night Length and Moon Phase. Curr. Biol. https://doi.org/10.1016/j.cub.2020.02.052

van Oort, B.E.H., Tyler, N.J.C., Gerkema, M.P., Folkow, L., Blix, A.S., Stokkan, K.-A., 2005. Circadian organization in reindeer. Nature 438, 1095–1096. https://doi.org/10.1038/4381095a

Van Someren, E.J.W., Riemersma-Van Der Lek, R.F., 2007. Live to the rhythm, slave to the rhythm. Sleep Med. Rev. https://doi.org/10.1016/j.smrv.2007.07.003

Vandewalle, G., Archer, S.N., Wuillaume, C., Balteau, E., Degueldre, C., Luxen, A., Dijk, D.J., Maquet, P., 2011. Effects of light on cognitive brain responses depend on circadian phase and sleep homeostasis. J. Biol. Rhythms 26, 249–259. https://doi.org/10.1177/0748730411401736

Vandewalle, G., Balteau, E., Phillips, C., Degueldre, C., Moreau, V., Sterpenich, V., Albouy, G., Darsaud, A., Desseilles, M., Dang-Vu, T.T., Peigneux, P., Luxen, A., Dijk, D.J., Maquet, P., 2006. Daytime Light Exposure Dynamically Enhances Brain Responses. Curr. Biol. 16, 1616–1621. https://doi.org/10.1016/j.cub.2006.06.031

Vanin, S., Bhutani, S., Montelli, S., Menegazzi, P., Green, E.W., Pegoraro, M., Sandrelli, F., Costa, R., Kyriacou, C.P., 2012. Unexpected features of Drosophila circadian behavioural rhythms under natural conditions. Nature 484, 371–375. https://doi.org/10.1038/nature10991

Verweij, I.M., Romeijn, N., Smit, D.J., Piantoni, G., Van Someren, E.J., van der Werf, Y.D., 2014. Sleep deprivation leads to a loss of functional connectivity in frontal brain regions. BMC Neurosci. 15, 88. https://doi.org/10.1186/1471-2202-15-88

Viola, A.U., Archer, S.N., James, L.M.M., Groeger, J.A., Lo, J.C.Y., Skene, D.J., von Schantz, M., Dijk, D.J., 2007. PER3 Polymorphism Predicts Sleep Structure and Waking Performance. Curr. Biol. 17,

613–618. https://doi.org/10.1016/j.cub.2007.01.073

Virtanen, J., Ruohonen, J., Naatanen, R., Ilmoniemi, R.J., 1999. Instrumentation for the measurement of electric brain responses to transcranial magnetic stimulation. Med Biol Eng Comput 37, 322–326.

Vitaterna, M.H., King, D.P., Chang, A.M., Kernhauser, J.M., Lowrey, P.L., McDonald, J.D., Dove, W.F., Pinto, L.H., Turek, F.W., Takahashi, J.S., 1994. Mutagenesis and mapping of a mouse gene, clock, essential for circadian behavior. Science (80-.). 264, 719–725. https://doi.org/10.1126/science.8171325

Vitaterna, M.H., Ph, D., Takahashi, J.S., Ph, D., 2017. Overview of Circadian Rhythms. Alcohol Res. Heal.

Vyazovskiy, V. V., Cirelli, C., Pfister-Genskow, M., Faraguna, U., Tononi, G., 2008. Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. Nat. Neurosci. 11, 200–208. https://doi.org/10.1038/nn2035

Vyazovskiy, V. V., Olcese, U., Lazimy, Y.M., Faraguna, U., Esser, S.K., Williams, J.C., Cirelli, C., Tononi, G., 2009. Cortical Firing and Sleep Homeostasis. Neuron 63, 865–878. https://doi.org/10.1016/j.neuron.2009.08.024

Vyazovskiy, V. V, Olcese, U., Hanlon, E.C., Nir, Y., Cirelli, C., Tononi, G., 2011. Local sleep in awake rats. Nature 472, 443–7. https://doi.org/10.1038/nature10009

Wager-Smith, K., Kay, S.A., 2000. Circadian rhythm genetics: From flies to mice to humans. Nat. Genet. https://doi.org/10.1038/79134

Wang, M., Gamo, N.J., Yang, Y., Jin, L.E., Wang, X.-J., Laubach, M., Mazer, J.A., Lee, D., Arnsten, A.F.T., 2011. Neuronal basis of age-related working memory decline. Nature 476, 210–3. https://doi.org/10.1038/nature10243

Waters, F., Chiu, V., Atkinson, A., Blom, J.D., 2018. Severe sleep deprivation causes hallucinations and a gradual progression toward psychosis with increasing time awake. Front. Psychiatry. https://doi.org/10.3389/fpsyt.2018.00303

Weber, F., Dan, Y., 2016. Circuit-based interrogation of sleep control. Nature. https://doi.org/10.1038/nature19773

Welsh, D.K., Logothetis, D.E., Meister, M., Reppert, S.M., 1995. Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. Neuron 14, 697–706. https://doi.org/10.1016/0896-6273(95)90214-7

Werth, E., Dijk, D.J., Achermann, P., Borbély, A.A., 1996. Dynamics of the sleep EEG after an early evening nap: Experimental data and simulations. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 271. https://doi.org/10.1152/ajpregu.1996.271.3.r501

Wittmann, M., Dinich, J., Merrow, M., Roenneberg, T., 2006. Social jetlag: Misalignment of biological and social time, in: Chronobiology International. Chronobiol Int, pp. 497–509. https://doi.org/10.1080/07420520500545979

Wright, K.P., Lowry, C.A., leBourgeois, M.K., 2012. Circadian and wakefulness-sleep modulation of cognition in humans. Front. Mol. Neurosci. https://doi.org/10.3389/fnmol.2012.00050

Wyatt, J.K., Ritz-De Cecco, A., Czeisler, C.A., Dijk, D.J., 1999. Circadian temperature and melatonin rhythms, sleep, and neurobehavioral function in humans living on a 20-h day. Am. J. Physiol. 277, R1152-63.

Xie, L., Kang, H., Xu, Q., Chen, M.J., Liao, Y., Thiyagarajan, M., O'Donnell, J., Christensen, D.J., Nicholson, C., Iliff, J.J., Takano, T., Deane, R., Nedergaard, M., 2013. Sleep drives metabolite clearance from the adult brain. Science (80-.). 342, 373–377. https://doi.org/10.1126/science.1241224

Yaffe, K., Falvey, C.M., Hoang, T., 2014. Connections between sleep and cognition in older adults. Lancet Neurol. https://doi.org/10.1016/S1474-4422(14)70172-3

Yamaguchi, S., Isejima, H., Matsuo, T., Okura, R., Yagita, K., Kobayashi, M., Okamura, H., 2003. Synchronization of Cellular Clocks in the Suprachiasmatic Nucleus. Science (80-.). 302, 1408–1412. https://doi.org/10.1126/science.1089287

Yamazaki, S., Numano, R., Abe, M., Hida, A., Takahashi, R.I., Ueda, M., Block, G.D., Sakaki, Y., Menaker, M., Tei, H., 2000. Resetting central and peripheral circadian oscillators in transgenic rats. Science (80-.). 288, 682–685. https://doi.org/10.1126/science.288.5466.682

Yoo, S.H., Yamazaki, S., Lowrey, P.L., Shimomura, K., Ko, C.H., Buhr, E.D., Siepka, S.M., Hong, H.K., Oh, W.J., Yoo, O.J., Menaker, M., Takahashi, J.S., 2004. PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. Proc. Natl. Acad. Sci. U. S. A. 101, 5339–5346. https://doi.org/10.1073/pnas.0308709101

Zehring, W.A., Wheeler, D.A., Reddy, P., Konopka, R.J., Kyriacou, C.P., Rosbash, M., Hall, J.C., 1984. P-element transformation with period locus DNA restores rhythmicity to mutant, arrhythmic drosophila melanogaster. Cell 39, 369–376. https://doi.org/10.1016/0092-8674(84)90015-1

Zeitzer, J.M., Daniels, J.E., Duffy, J.F., Klerman, E.B., Shanahan, T.L., Dijk, D.J., Czeisler, C.A., 1999. Do plasma melatonin concentrations decline with age? Am. J. Med. 107, 432–436. https://doi.org/10.1016/S0002-9343(99)00266-1

Zhang, X.S., Zhu, Y.S., Thakor, N. V., Wang, Z.Z., 1999. Detecting ventricular tachycardia and fibrillation by complexity measure. IEEE Trans. Biomed. Eng. 46, 548–555. https://doi.org/10.1109/10.759055

Ziemann, U., 2011. Transcranial magnetic stimulation at the interface with other techniques: A powerful tool for studying the human cortex. Neuroscientist. https://doi.org/10.1177/1073858410390225

Zitting, K.M., Münch, M.Y., Cain, S.W., Wang, W., Wong, A., Ronda, J.M., Aeschbach, D., Czeisler, C.A., Duffy, J.F., 2018. Young adults are more vulnerable to chronic sleep deficiency and recurrent circadian disruption than older adults. Sci. Rep. 8. https://doi.org/10.1038/s41598-018-29358-x