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Neural correlates of attentional processes under sleep deprivation

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Summary

Most of living organisms exhibit rhythmic changes in physiological and behavioral variables concomitant with the daily light-dark cycle. In mammals, and especially in humans, this rhythmicity is regulated by an internal cellular clock, ubiquitously present in multiple organs. The overall rhythmicity observed in physiology and behavior is driven by a master clock located in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus which ticks with a period of approximately 24 hours. The light-dark cycle represents the major exogenous/environmental time cue ("Zeitgeber") synchronizing these internal rhythms to the succession of days and nights. Under entrained conditions, the endogenous clock is synchronized to the exogenous light-dark cycle ruling on earth, allowing humans to sustain vigilance during the day and to sleep at night. Entrainment has an adaptive value because it synchronizes rhythms emerging from multiple physiological systems, thereby optimizing interactions between these systems.
On a second step, it has an evolutionary value, allowing for an optimal temporal coordination of behavioural responses to the environmental requests. In human daily life, synchronization allows an individual to achieve an optimal cognitive performance during daytime, thus anticipating the behavioral needs of a diurnal species.

Modern life puts a heavy strain on our internal clock system by imposing extended working hours sometimes at unusual circadian times (i.e. shift work, jet lag). One of the most frequent consequences is the disruption of internal synchronization with detrimental effects on psychological and physiological health. Also, an abnormal night-day schedule can drive to suboptimal cognitive performance, resulting in errors and accidents. Sleep deprivation and sleep restriction induce an increase in sleep need, subjective and objective sleepiness and a concomitant decrease in neurobehavioral performance.

Nevertheless, (1) performance decrement and its cerebral correlates in response to sleep loss have been shown to depend on cognitive domains. (2) Furthermore, performance does not linearly decrease with increasing time spent awake. More specifically performance deterioration is most prominent at the end of the biological night, while this effect is attenuated during the subsequent day despite a further extension of wakefulness. Such performance rescue is most probably supported by wake-promoting activity of the circadian timing system, kicking in during day time to oppose the negative impact of the progressive rise in sleep pressure throughout wakefulness. Even though regularly experienced by shift- and night-workers, the cerebral correlates underlying both nighttime troughs and daytime gains in performance under sleep deprived conditions have not yet been investigated.

The aim of the present thesis was to address these 2 issues.

First (study 1), it might be of particular interest to find a cognitive task having the potential to underline if specific attentional components are selectively and differentially affected by sleep loss. Within this perspective, the ANT designed by Fan (2002), seemed particularly suitable to answer the question of a differential and/or selective response to sleep loss in specific attentional components and its cerebral correlates.

Second (study 2), we aimed at characterizing the cerebral correlates tracking circadian-homeostatic interaction profile commonly detected in attentional performance outputs. We thus combined a 42-h constant routine and multiple fMRI acquisitions, clustered in the morning and the evening, two periods characterized by rapid changes in the circadian modulation of cognitive performance encompassing maximal circadian sleep-wake promotion signals.

The major outputs of these 2 studies will be briefly summarized hereunder.

Study 1

An open issue is whether sleep deprivation differently and selectively affects specific attentional components, or whether the decrease in alertness is the core phenomenon responsible for the failure in the other attentional systems. Based on a cognitive model assuming that human attention can be divided into three main components (alerting, orienting and executive), allegedly supported by anatomically different brain networks, the Attentional Network Test (ANT) was designed by Fan and collaborators to probe the independency of these three brain networks. For this study, volunteers underwent an fMRI version of the ANT during rested wakefulness (RW) and sleep deprivation (SD), in a counterbalanced design. Importantly, each session occurred at the same time-of-day during SD and RW. Behavioral data show a global slowing down of reaction times (RTs), thus not supporting the hypothesis of a differential influence of sleep deprivation on the three attentional components. Analyses of functional brain imaging data revealed that the 3 attentional components can only hardly be dissociated at the cerebral level. Activity in the left precuneus and the right temporo-parietal junction was for example associated with both the alerting and orienting component of the task, while the alerting component elicited significant responses in the inferior frontal gyrus, formerly suggested to be part of the executive component. Furthermore, the data suggest that sleep loss does not selectively affect the three investigated attentional aspects. Rather, the data suggest that sleep-loss induced increased thalamic activity reflects an increase of sustained attention, contributing to the maintenance of cognitive performance when arousal is low. Such compensatory role of thalamic response argues against a selective influence of sleep deprivation on specific networks involved in orienting or executive components of attention. In conclusion these data do not support the view that the Attentional Network Test probes different and independent sets of brain areas, and that the three attentional components do not seem to be selectively affected by sleep deprivation.

Study 2

Human performance modulation over time results from the interaction between homeostatic sleep pressure and circadian rhythmicity. The cerebral correlates underlying this modulation remain virtually unexplored, just as the timing and magnitude of these influences on regional brain responses. In this work assessed the cerebral correlates underlying sustained attention in a functional magnetic resonance imaging (fMRI) environment during 42h of wakefulness under constant routine conditions. The ultimate aim was to explore whether attention-related brain responses were controlled by the combined action of circadian rhythmicity, sleep need and their interaction. Behavioural data confirm literature evidence that decrements in neurobehavioral performance, resulting from sleep loss, vary according to time of day (Schmidt et al., 2007). Performance was particularly deteriorated towards the end of the biological night, while this effect was attenuated during the subsequent day despite a further prolongation of wakefulness (Dijk et al., 1992; Cajochen et al., 1999b). Neuroimaging results showed a significant circadian periodicity in attention-related brain response profiles over almost the entire cortex, putatively reflecting the power of the circadian alerting signal in maintaining adequate vigilance levels at the cerebral level. The phase of these profiles varied significantly across brain regions, speaking in favour of a local, region-specific circadian modulation in task-related brain responses. Congruent to other literature reports, we observed an overall BOLD activity decrease in cortical areas. However, a more fine grained analysis revealed that BOLD activity (related to sustained attention performance) was maintained or even increased in the evening hours in subcortical regions, coinciding with the nocturnal melatonin rise. A significant interaction between sleep pressure and circadian rhythmicity was observed in occipital and thalamic areas. By tracking classical behavioral and electrophysiological sleep deprivation outputs at the cerebral level, our results highlight local circadian

modulation, resulting in different timing of maximal regional brain responses. Our findings have implications for the understanding of the brain mechanism involved in deterioration of cognition such as observed in shift work and ageing.

Globally the data presented in this thesis, fit into the general context of a global decrease in top-down modulation of attentional resources, by the combined action of circadian and homeostatic factors on cortical responses. Moreover, our exciting results emphasize that circadian and homeostatic factors regionally interact in the brain, probably due to a local neuronal homeostatic need, possibly linked to local use-dependent mechanisms.

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Theoretical Background

CHAPTER 1

Sleep and Circadian Rhythms

1. Introduction

Humans spend about one-third of their lives sleeping; but what is sleep? How sleep is structured and regulated? The aim of this first chapter is to provide an overview about this fascinating behavioral state, describing its organization in stages, introducing the brain structures responsible for the regulation of the sleep-wake pattern. Particular attention will then be addressed to the two mechanisms that regulate sleep and wakefulness regulation: the circadian and homeostatic processes.

2. From wakefulness to sleep

In 1912, the French psychologist Henri Piéron in his PhD thesis (entitled "Le problème physiologique du sommeil" and published in book form in 1913; Piéron, 1913) gave a definition of sleep that is still valuable. He defined sleep as a periodically necessary state, with a periodicity that is relatively independent of exterior circumstances, and which is characterized by the suspension of the sensory-motor relations that unite the individual with his environment. Prechtl defined a state as a constellation of certain patterns of physiological variables and/or certain patterns of behaviors, which seem to repeat themselves and which appear to be relatively stable (Prechtl et al., 1968). The first observation of modern age that sleep is not a homogenous process came from the work of Derbyshire and collaborators (Derbyshire et al., 1936). They noticed that in cats, when sleep was apparently less quiet (based on the twitching of their vibrissae) there were small

electroencephalographic waves as in the waking state. In mammals and birds, sleep could be divided in two main states, non-rapid eye movement (NREM) and rapid eye movement (REM) sleep, each one well characterized by specific patterns. The alternation between the two sleep states (NREM- REM) generates what is called sleep cycle, a functional and structural unit that is generally lasting between 60 and 110 minutes and that is typically repeated 4-6 times per night. Electroencephalography (EEG) is known to directly reflect the summation of synaptic (and transmembrane neuronal) currents within corticocortical and corticothalamic networks of the superficial cortical layers. EEG measurement of electrical activity along the scalp is the main instrument to detect the specific pattern of sleep and its topographical distribution. Thus changes in EEG, electromyogram (EMG) and electrooculogram (EOG) are used to classify sleep in different stages and to delineate a comprehensive frame of information about a night of sleep (polysomnography, PSG). During wakefulness (W), human EEG is characterized by low amplitude and high-frequency waves. When we close our eyes, there is a relative slowing down of cortical activity. The EEG is mostly composed by waves in the frequency range between 8-12 Hz (i.e., alpha rhythm), mainly observed over the occipital region together with normal or high chin muscle tone. Conjugate and sharply peaked eye movements could be observed during quiet wakefulness, such as eye blinks (i.e. vertical eye movements with a frequency of 0.5-2 Hz) or rapid eye movements (REMs), usually lasting <500 msec. The start of the first epoch (i.e. an epoch, based on AASM recommendations, consists of 30 seconds) scored as any stage other than W, is defined as sleep onset. The interval between lights-off and subsequent sleep onset is defined as sleep latency.

2.1. Stage N1 (NREM 1):

During the transition from wakefulness to sleep, the progressive slowing down continues but mixed frequencies are still predominant. Specifically the long sinusoidal trains of alpha activity start to be spaced out by low amplitude activity in the frequency range 4-7 Hz (i.e. theta

activity). The rapid eye movements are replaced by conjugate, ample, sinusoidal, slow eye movements (SEM) usually lasting >500 msec. Another sign of the sleep onset period is the appearance of sharp waves mainly over central derivations (i.e. vertex waves) that last <0.5 seconds, and are well distinguishable from the background activity. According to the Rechtschaffen and Kales sleep scoring manual (Rechtschaffen and Kales, 1968) and the more recent manual (Iber et al., 2007) of the American Academy of Sleep Medicine (AASM), sleep stage N1 (NREM 1) starts when alpha rhythm is attenuated and replaced by low amplitude, mixed frequency activity for more than 50% of the epoch.

2.2. Stage N2 (NREM 2):

The presence of two patterns of waves indicates the starting of sleep stage N2 (NREM2). The first consists of sleep spindles, which are described as a train of distinct waves with frequency between 11-16 Hz and consist of the waxing and waning of field potentials. They are characterized by a typical shape given by a progressive increase in amplitude followed by a gradually decrease. Sleep spindles are generated by a thalamo-cortical network, which comprises the interplay of reticular thalamic, thalamocortical and cortical pyramidal neurons. Briefly their generation is due to repetitive spike-bursts of thalamic reticular cells, which cause rhythmic inhibitory synaptic potentials in thalamocortical neurons and consequentially excitatory postsynaptic potentials in cortical cells (Steriade et al., 1985, 1987; Steriade and Timofeev, 2003). Sleep spindles are classically subdivided in two categories, with specific topographical distribution throughout the night (Dijk et al., 1993; Knoblauch et al., 2002, 2003) and specific different cerebral correlates (Schabus et al., 2007). Slow spindles (spindles in the frequency range between 11-13 Hz) are more frequent during the first part of the night over frontal regions, while fast spindles (13-16 Hz) are enhanced during the second part of the night and show a more centro-parietal distribution. A simultaneous electroencephalographic and functional MRI study assessed the hemodynamic cerebral correlates of

sleep spindles (Schabus et al., 2007). Results showed that both spindle types share a common activation pattern, involving thalami, paralimbic areas and superior temporal gyri. In the same study, slow spindles associated with increased activity in the frontal gyrus, while fast spindles were associated with activity in precentral and postcentral gyri and supplementary motor area (SMA). These results, in conjunction with those from memory studies (Schabus et al., 2004; Schmidt et al., 2006) suggest possible different functional significances and the existence of possible multiple local generators for sleep spindles (Dehghani et al., 2010).

The second pattern is called K complex (KC) (**Figure 1**, red circle). Both activities are strictly related, as observed since the first systematic description by Roth (Roth et al., 1956), which distinguished different parts of KC. He described a first di-or triphasic short sharp wave followed by a slower wave in reversed phase as compared to the preceding one, peaking at 350-550 ms, and then a final peaking near 900 ms, followed or superimposed sometimes by a 12-14 Hz rhythm (spindle).

AASM manual defines KC as a negative sharp wave immediately followed by a slower positive component standing out from the background EEG, with duration equal to 0.5 sec. or more. Numerous research groups performed simultaneous recordings of intracellular local field potential and EEG activities (Amzica and Steriade, 1998, 2002; Cash et al., 2009). This technique allowed to unravel the cellular mechanisms of KC, and of the slow oscillation.

2.3. The slow oscillation:

KC and slow waves of NREM sleep are the output of a synchronous activity in brain neuronal populations (Amzica and Steriade, 2002). This slow oscillation (<1 Hz) is the result of a cyclic fluctuation of neuronal membrane potentials between two phases, each one describing the two components of a KC. A depolarizing phase, characterized by period of intense synaptic activity and corresponding to synchronous excitations

of large neural populations, reflected at an extracellular level, as negative field potentials. This phase is corresponding, at a scalp level, with the first component of KC, the negative sharp wave, but at a more deep level (close to the cell) this phase is associated with a positive wave. The second phase is characterized by period of synaptic silence, due to a hyperpolarization of the membrane potential of those neurons as consequence of the decrease of extracellular Ca^{2+} concentrations (Massimini and Amzica, 2001). In bipolar EEG recordings, the hyperpolarization is reflected by the slower positive component of KC, while at cortical level by a negative wave (Contreras and Steriade, 1995). As aforementioned, KC and sleep spindles are strongly related. The sharp KC onset plays an important role for providing a synchronous input to thalamic neurons and in triggering sleep spindles (Amzica and Steriade, 2002).

The slow oscillation received increased interest in the last 20 years in light of several factors. As we will see later, sleep need is in part homeostatically regulated, i.e. depends on the duration of the previous wakefulness. Slow wave oscillations are considered a hallmark of homeostatic sleep pressure. However, not only the duration but also the "quality" of the wake episode has a role in the regulation of slow wave oscillations. From the 80's, researchers started to integrate sleep-wake regulatory processes with metabolic changes in the two behavioural states. A series of experiments established the hypothesis that sleep is a local and use-dependent phenomenon, suggesting restorative aspects of sleep linked with the activity occurring during wakefulness (Horne and Moore, 1985; Kattler et al., 1994; Benington and Heller, 1995). Today this idea has been integrated into what has been described as the synaptic homeostasis hypothesis or SHY (Tononi and Cirelli, 2003, 2006). Briefly the SHY relates sleep homeostasis to plastic processes linked to learning and practice phenomena occurring during wakefulness. These phenomena results in long-lasting changes in the strength and number of synaptic connections across neurons. According to the SHY, slow waves during sleep would actively promote a downscaling of synapses allowing the return to a baseline level, which is

energetically sustainable. This synaptic recalibration would also participate to memory consolidation processes.

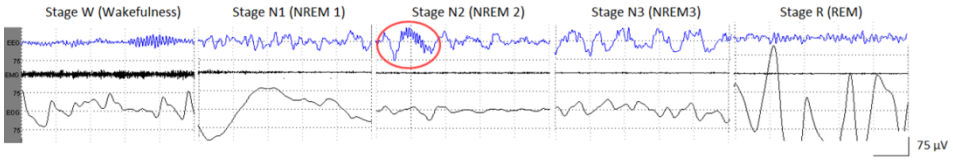

Figure 1: EEG signals during wakefulness, stage N1, stage N2, stage N3 and REM sleep in a young healthy adult. During wakefulness (eyes closed), the alpha rhythm (8-13 Hz) in the EEG gives rise to a peak around 11 Hz in the power spectrum. Sleep spindles (in the second half part of the red circle) occur preferentially during stage 2 are reflected in a peak in the spindle frequency band (11-15 Hz). High amounts of slow waves during stage N3 give rise to high power density in the slow wave range (< 4 Hz). REM sleep is dominated by activity in the theta frequency band (4-8 Hz).

2.4. Stage N3 (NREM 3):

The AASM's manual recommends scoring sleep stage N3 (NREM 3) when at least the 20% (6 sec.) of an epoch is represented by slow waves activity (SWA). SWA is defined in that manual as waves in the frequency range 0.5-2 Hz, with an amplitude >75 µV from peak-to peak (i.e. the difference between the most negative and positive points of the wave), measured over frontal regions. Stage N3 is characterized mostly by delta waves (waves in frequency range 0.5-4 Hz). It replaces the Rechtschaffen and Kales division of slow waves sleep in stage 3 and stage 4 (which was based on the proportion of slow waves in one epoch; Rechtschaffen & Kales, 1968). These slow highly synchronous oscillations reflect the firing rate of large neuronal population within the cortex. As aforementioned slow waves consist of slow oscillations between periods of intense firing (depolarizing phase, "down-states") by excitatory and inhibitory cortical neurons and period of neural silence (hyperpolarizing phase, described as "up-states" (Steriade et al., 2001). Functionally, the increase in total power, amplitude and incidence of delta waves would index enhanced sleep intensity.

2.5. Stage R (REM):

REM sleep (or stage R) is characterized by tonic and phasic components. Tonic components are present throughout REM sleep and include a

"desynchronized" EEG (i.e., high frequency and low amplitude EEG activity, paradoxically similar to the waking state) and the absence of antigravity muscular tonus. By contrast, phasic components of REM sleep are transient or periodic activities. These include rapid eye movements (REMs), sporadic muscular twitches (short irregular burst of EMG activity superimposed on low EMG tone), sawtooth waves defined as train of sharply contoured or "triangular", often serrated waves (2-6 Hz) generally with a maximal amplitude over central derivations and often, but not always, preceding a burst of REMs. Animal studies, including nonhuman primates, described another phasic component of REM sleep, represented by high amplitude spiky waves (Datta, 1997). These are mainly recorded in pontine, geniculate and occipital areas from where they take the name "ponto-geniculo-occipital (PGO) waves". PSO activity was observed to be concomitant with the bursts of REMs. Neuroimaging techniques give evidence for human PGO waves. Peigneux and colleagues, using positron emission topography (PET), found a positive correlation between REM density and activation of rostral brainstem, the lateral geniculate and the occipital cortex (Peigneux et al., 2001). More recently, using event-related fMRI, Miyauchi and collaborators showed that the pontine tegmentum, ventroposterior thalamus and primary visual cortex were activated in association with REMs (Miyauchi et al., 2009). Furthermore the time-course of blood oxygenation level-dependent responses revealed that activations in those regions started just before REMs occurrence. Therefore activation of PGO areas may reflect a switch mechanism in the brain from tonic to phasic REM sleep components.

3. Brain structures involved in wakefulness and sleep

A heterogeneous set of brain areas and neocortical structures contribute to sleep-wake regulation. Wakefulness is mediated by the ascending reticular activating system (ARAS), a theoretical construct representing ascending pathways composed of neural populations that mediate cortical desynchronization and arousal (**Figure 2A**). The ARAS begins in the medulla, ascends to the rostral pons and runs through the

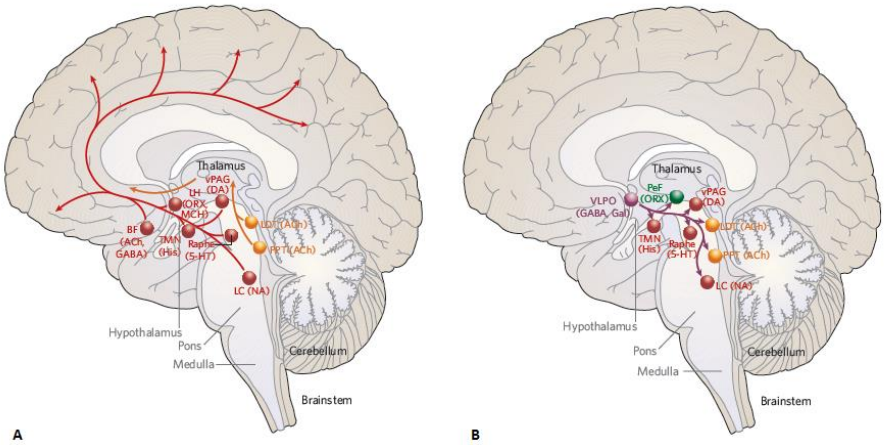

Figure 2: (A). Key components of the ascending reticular activating system (ARAS). A major input to the relay and reticular nuclei of the thalamus (yellow pathway) originates from cholinergic (Ach) cell groups in the upper pons, the peduncolopontine (PPT) and the laterodorsal tegmental nuclei (LDT). A second pathway (red) activates the cerebral cortex to facilitate input processing from the thalamus. This arises from neurons in the monoaminergic cell groups, including tuberomammilary nucleus (TMN) containing histamine, the A10 cell group containing dopamine (DA), the dorsal and median raphe nuclei containing serotonin (5-HT), and the locus coeruleus (LC) containing noradrenaline (NA). Further contribuitions to this pathway stem from peptidergic neurons in the lateral hypothalamus (LHA) containing orexin (ORX) or melanin-concentrating hormone (MCH) and from basal forebrain (BF) neurons that contain γ-aminobutyric acid (GABA) or Ach. (**B).** Key projections of the ventrolateral preoptic nucleus (VLPO) to the main components of the ARAS. It includes the monoaminergic cell groups (red) such as the tuberomammilary nucleus (TMN) containing histamine, the A10 cell group containing dopamine (DA), the raphe nuclei containing serotonin (5-HT), and the locus coeruleus (LC). It also innervates neurons in the lateral hypothalamus (LHA; green), including the perifornical (PeF) orexin (ORX) neurons, and interneurons in the cholinergic 5Ach) cell groups (yellow), the pedunculopontine (PPT) and laterodorsal tegmental nuclei (LDT) (from Saper, Scammell, & Lu, 2005).

midbrain reticular formation. It includes two major components: a first ascending pathway which activates thalamic relay neurons, and a second one that activates neurons in the lateral hypothalamus. The main origins of the first pathway are two cholinergic cell groups, the peduncolopontine (PPT) and the laterodorsal tegmental nuclei (LDT) in the mesopontine tegmentum. The PPT and the LDT project to the thalamus, specifically to the reticular nucleus, the thalamic relay and the intralaminar nuclei. These projections are thought to play a fundamental role for wakefulness, acting as a gating mechanism in

thalamocortical transmission. The neural activity of these cholinergic cells varies between different behavioral states, firing rapidly during wakefulness and REM sleep and slower during NREM sleep. The second component of the ARAS activates neurons in the lateral hypothalamic area, the basal forebrain. It originates from monoaminergic neurons in the upper brainstem and caudal hypothalamus and includes the noradrenergic locus coeruleus (LC), serotoninergic dorsal and medial raphe nuclei, the dopaminergic ventral periaqueductal grey matter and histaminergic neurons in the tuberomammilary nucleus, to finally project to the cerebral cortex. As for the first component, the firing rate of this group of cells is state-dependent, such that it is faster during wake as compared to NREM sleep, and ceases during REM sleep. Other neurons in the lateral hypothalamic area (LHA) contribute to the arousal state. One group of neurons containing orexin/hypocretin is active in the wake state and projects to different components of the arousal system, including the cholinergic basal forebrain, LC, raphe and the cerebral cortex (de Lecea and Sutcliffe, 2005). Another group of neurons in the lateral hypothalamic area contain melanin-concentrating hormone (MCH), and have similar projections as for the previous group but is active during REM sleep. The ventrolateral preoptic nucleus (VLPO) plays a key role in the inhibition of the aforementioned brain circuits (**Figure 2B**). The VLPO neurons are active during sleep and release gamma-aminobutyric acid (GABA) and galanin, two inhibitory neurotransmitters. This group of neurons inhibits the arousal systems thus promoting sleep. The interaction between the VLPO and the arousal systems is mutually inhibitory acting as a *"flip-flop"* system that avoids intermediate behavioral states and by facilitating a rapid switch between these states (McGinty and Szymusiak, 2000). It has been suggested that in elderly people, who often show sleep fragmentation and frequently complain of excessive daytime sleepiness resulting in multiple nap compensatory behaviors, a cell loss in the VLPO could be responsible for an optimal functioning of the *"flip-flop"* circuit (Saper et al., 2005). Moreover the orexin/hypocretin receptors of the LHA are considered to play a stabilizing role for the switch (Adamantidis et al.,

2007). Direct and selective stimulation of hypocretin neurons increase the probability of transition to waking states from sleep. Conversely, orexin-deficient animals or narcoleptic patients exhibit less stable sleep and wake states, and showed dysfunctional switching between these states (Saper et al., 2001).

Importantly, sleep-promoting neurotransmitters have been shown to inhibit orexin neurons, including $GABA_B$ (Xie et al., 2006) and adenosine (Liu and Gao, 2007). Further evidence, that orexin neurons are causally related to the transition from wake to sleep, builds from optical stimulation of hypocretin neurons in the hypothalamus. Adamantis and colleagues have shown that deep brain optical stimulation of these neurons increased the probability of transition from both NREM and REM sleep to wakefulness (Adamantidis et al., 2007).

4. Circadian rhythms

Circadian rhythms are a fundamental physiological process found in virtually all organisms. They could be defined as an endogenous oscillation with a periodicity of about 24 hours. In humans, this periodic rhythmicity can be observed in numerous physiological processes, such as hormone production (i.e. melatonin), body temperature regulation, blood pressure, heart rate, but also in complex behaviors as cognitive performance or sleep-wake patterns. Even if circadian rhythms are endogenous, the rhythmic changes in physiological and behavioral variables are modulated by exogenous time cues (“zeitgeber”). In other words, circadian rhythms are entrained or synchronized to the most powerful environmental cue, the 24-h light-dark cycle. In the absence of an exogenous zeitgeber, human circadian system is driven exclusively by its endogenous cycle although it no longer entrained to the natural light-dark cycle. Under these circumstances, circadian rhythms “free run”, thus oscillating in their own period, which in humans is slightly longer than 24-h. Circadian rhythms are defined by 3 basic properties: period, amplitude and phase. Period is the time interval between two identical occurrences of a reference event in successive cycle.

Amplitude corresponds to the difference between the peak and nadir of an observed parameter. Phase is the timing of a given endogenous event (e.g., melatonin midpoint).

5. Homeostatic and Circadian interaction in sleep and wakefulness regulation

The circadian component of the sleep-wake rhythm represents a clock-like process that allows the occurrence of these behavioral states at an appropriate time-of-day. Circadian processes are fundamental in sleep and wakefulness regulation. However, they are not alone in driving these complex and fascinating behaviors. Another process is involved and a mutual interaction between the two processes controls our night-day/rest-activity cycle. According to the two-process model proposed by Borbély (Borbely, 1982), sleep is regulated through the interaction of the homeostatic and the circadian processes (**Figure 3**).

The homeostatic process (process S) keeps track of prior sleep-wake history, and indexes our need for sleep. This sleep need increases exponentially when we are awake and dissipates during sleep. At the cellular level, adenosine (AD) has been proposed as a putative substrate of the homeostatic sleep process. Adenosine is a purine nucleoside involved in numerous biochemical processes. During sleep deprivation, extracellular concentration of AD increases, while during sleep recovery it decreases. The specific functions of the sleep homeostatic process in the brain have been discussed in terms of energy restoration and cellular defense (Porkka-Heiskanen, 2013), as well as synaptic plasticity (Tononi and Cirelli, 2014). The circadian process (process C) could be defined as an endogenous nearly 24 h oscillator that defines the preferred timing of the sleep-wake episodes independent from sleep-wake history (Zhu and Zee, 2012). This endogenous oscillatory process is triggered and adjusted to the external light-dark cycle by the master circadian pacemaker, the suprachiasmatic nuclei (SCN) of the anterior hypothalamus. Within the SCN, a genetic clockwork determines the

endogenous rhythm by a self-sustaining feedback loop with a duration of nearly 24 h (Jin et al., 1999; Ko and Takahashi, 2006). The two main variables considered reliable markers of the circadian process, are represented by the rhythm of melatonin and of core body temperature (CBT), when collected under special conditions.

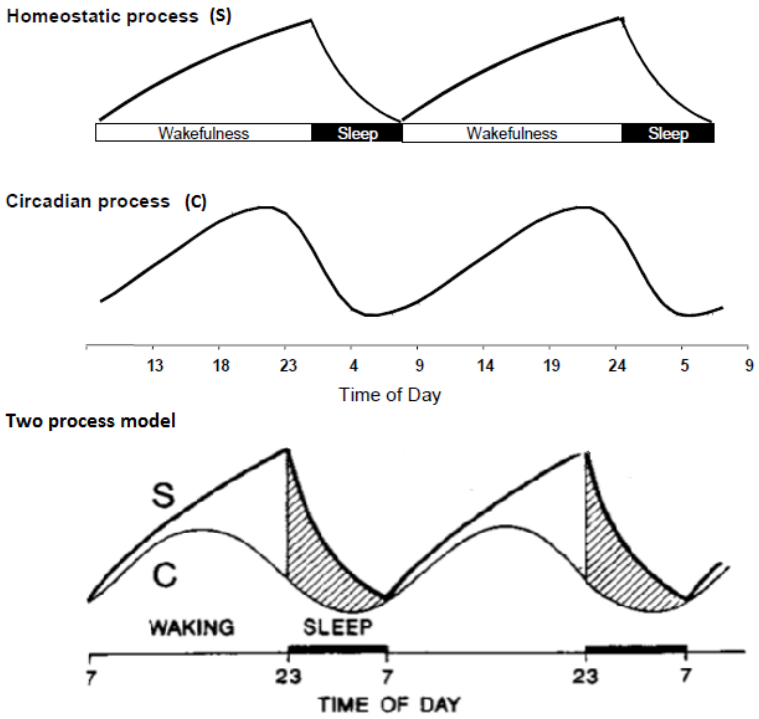

Figure 3: The two-process model of sleep-wake regulation (Borbely, 1982). S represents sleep need (homeostatic sleep process), it increases during wakefulness and decreases during sleep. Its variation is restricted to a range of values determined by the circadian process C (sinusoidal curve), which is not constant over time but varies with time-of-day (Borbely and Achermann, 1999).

Melatonin is a sleep promoting hormone secreted by the pineal gland of the epithalamus. Under entrained conditions, its secretion is very low during the day, rises few hours before habitual sleep time, peaks in the middle of the night (ca.2-h before CBT minimum), and reaches a nadir in the early morning (**Figure 4**). The circadian and homeostatic processes act in concert to ensure the consolidation and optimization of sleep-wake behavior. A paradoxical increase of the circadian signal for wakefulness during the course of the waking day counteracts the

increase in the homeostatic sleep need. While the homeostatic sleep need monotonically increases during the day (increasing sleep pressure in the early evening hours), the circadian pacemaker is counteracting this action opposing a wake promoting signal.

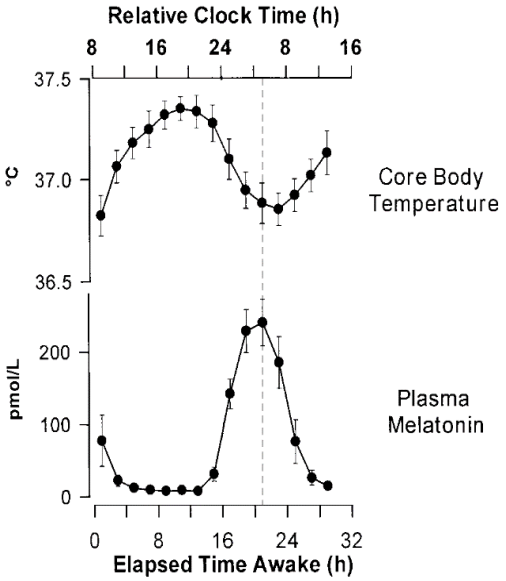

Figure 4: Time course of core body temperature and endogenous plasma melatonin. Data were binned in 2-h intervals and expressed as elapsed time from scheduled wake. Vertical reference line indicates plasma melatonin peak (modified from Cajochen, Khalsa, Wyatt, Czeisler, & Dijk, 1999)

Ironically this alerting signal reaches his peak exactly few hours before the habitual bedtime (Dijk and Czeisler, 1994). This particular moment of the evening, is called wake maintenance zone (WMZ). The WMZ could be defined as a two to three hours window of reduced sleep propensity. It occurs before the release of the sleep promoting hormone melatonin (melatonin onset) that under normal entrained condition occurs several hours prior to habitual bedtime (Shekleton et al., 2013). Similarly, there is an increase of the circadian signal to promote sleep during the biological night, when sleep pressure decreases over the sleep episode. This time-window is called the “sleep-promoting zone”. Functionally, this dual circadian and sleep homeostatic mechanism prevents us from falling asleep few hours before habitual sleep time and by waking up in the middle of the night. Ultimately, this fine-tuned coordination ensures a consolidated 16 h of wake and 8 h of sleep. This temporal coordination also guarantees optimal cognitive performance,

alertness during the day. Evidence for the WMZ was shown in forced desynchrony (FD), constant routine (CR) and multinap protocols, during which very long sleep latency were observed few hours before habitual sleep time, approximately 12 hours after the CBT minimum. The interaction of sleep homeostasis and circadian processes, however, still remains to be fully apprehended. A basic assumption of the initial model was the independency of these two processes. However, simulation of the two processes based on an additive interaction did not accurately predict subjective alertness and cognitive performance during forced desynchrony studies, suggesting a non-linear interaction instead (Dijk, Duffy, & Czeisler, 1992; Jewett & Kronauer, 1999). As an illustration, a non-linear interaction implies that the circadian amplitude depends on prior sleep duration (Dijk & Czeisler, 1995; for a review see Dijk & Franken, 2005). Evidence from animal research suggests that the circadian clock receives continuous information about the status of the homeostatic sleep process. Deboer and colleagues showed altered electric activity in the SCN after sleep deprivation, and observed a correlation between SWA during NREM sleep and electrical activity of neurons in the SCN (Deboer et al., 2003, 2007). Interestingly, data suggests that circadian gene expression in the cerebral cortex depends on previous wake history, while their expression in the SCN does not (Franken and Dijk, 2009). Neuroimaging data recently supported the idea that the interaction between the two processes is detectable at the cerebral level. Schmidt and collaborators, using fMRI, have shown that brain activity during a sustained attention task, was higher in those individuals with lower sleep pressure accumulation; moreover activity in the SCN decreases with increasing sleep pressure (Schmidt et al., 2009).

6. Forced Desynchrony and Constant Routine paradigms

A forced desynchrony (FD) is a protocol where volunteers are isolated from the usual time-cues and live in a dim light environment. Individuals are scheduled to a superimposed rest-activity cycle that enforces the normal day length. Thus FD results in shorter or longer rest-activity cycle than the habitual daily 24-hour. The resting part of the cycle is spent in the

bed in complete darkness while the activity one in dim light outside from bed. In a FD protocol the circadian phase is varied systematically, while the variation of the wake episode is simultaneously kept as constant as possible. Thus in this experimental paradigm, sleep can virtually occur at all circadian phase. Under these conditions the endogenous circadian pacemaker becomes unable to keep track of the imposed rest-activity cycles (e.g., 28 h.), as these are outside the range of its entrainment. Thus it progressively starts to desynchronize and to oscillate with a period that is slightly different from 24 h and that corresponds to its own inherent period. FD protocols are particularly indicated to assess of the interaction between circadian and homeostatic processes, as it can allows the separation and quantification of the influence of the two processes and unmask sleep-dependent changes in sleep consolidation at all circadian phases (Cajochen et al., 2004). Another paradigm typically used in chronobiology is the constant routine (CR). The CR protocol is an experimental setting allowing an accurate assessment of circadian and homeostatic influences on psychophysiological variables. The rationale is that periodic changes in external (environmental) and internal variables (e.g., sleep-wake cycle) may influence the rhythmicity of the psychophysiological variables of interest (Duffy and Dijk, 2002). Thus during a CR, participants remain under controlled constant conditions in order to minimizing the masking effects of sleep-wake cycle, physical activity, food intake, sensory stimulation and social interactions on endogenous rhythms. Constant condition implies a control for external and internal factors, including room temperature and humidity, light levels, nutritional intake, posture (semi-recumbent during wake and recumbent during sleep). Under these conditions, physiological (core body temperature, saliva melatonin or blood samples) and behavioral measurements are assessed over time. Thus CR allows for the comparison of physiological and neurocognitive performance at the same circadian phase but under different homeostatic sleep pressure levels. In other words, it is the interaction, not the separate contribution, of sleep and circadian processes that is measured.

Another protocol to unearth the relative contribution of the two processes is the multiple nap paradigm. Accordingly, the dynamics of circadian and homeostatic markers are compared between a condition of low sleep pressure (napping) and a high sleep pressure condition, under constant routine conditions. As for the CR, the separate contribution of each process (H and S) cannot be isolated.

7. Molecular correlates of circadian rhythms

Several aspects of sleep-wake regulation are highly variable between individuals as well as many of those aspects are highly stable within individuals (Tan et al., 2001; Buckelmüller et al., 2006). Studies on monozygotic (MZ) and dizygotic (DZ) twin pairs speak to a tight genetic contribution to this regulation, such that NREM sleep EEG profiles of MZ twins show higher concordance than for DZ (Ambrosius et al., 2008; De Gennaro et al., 2008) with heritability estimate of 90%. Moreover EEG patterns in MZ are remarkably stable over two different nights (Stassen et al., 1988). Genetic studies and experiments in different animal species, are contributing to a heightened understanding of sleep-wake regulatory mechanisms at the molecular level. Sleep studies in the *Drosophila* and in the mouse confirmed the existence of circadian genes (often referred as “clock genes”); moreover the “construction” of genetically altered mice starts to clarify the molecular circuitry underlying circadian rhythms. Knockout mice are one of the most used techniques to unmask sleep-wakefulness phenotype link to specific clock genes. A knockout mouse is a genetically modified mouse in which an existing gene have been inactivated, or "knocked out," by replacing it or disrupting it with an artificial piece of DNA. Knockout mice represents important animal model for studying the role of genes which have been sequenced but whose functions have not been clarified.

At a molecular level, the circadian mechanism in mammals consists of a network of interlocked transcriptional-translational feedback loops involving several clock-related genes. These genes include *CLOCK*, *BMAL1*, *PER1-3*, *CRY1-2* and so forth.

The positive feedback loop consists of two basic helix-loop-helix Period-Arnt-Singleminded (bHLH-PAS) transcriptional factors, *circadian locomotor output cycles kaput* (CLOCK) and *brain and muscle ARNT like protein 1* (BMAL1). The negative feedback loop involves the genes PERIOD and CRYPTOCHROME (Kume et al., 1999; Lee et al., 2001).

During the day, the bHLH-PAS -domain containing transcription factor CLOCK interacts with BMAL1. Thus CLOCK and BMAL1 form a heterodimer (i.e., a molecule consisting of two different subunits) that initiates the transcription by binding specific sequences, called E-Box, present in the promoter elements of *Period* (*Per1, Per2, Per3*) and *Cryptochrome* (*Cry1, Cry2*) genes and induce their expression. The resulting PER and CRY proteins are then translated in the cytoplasm. PER and CRY proteins form dimers that can translocate to the nucleus where they inhibit CLOCK-BMAL1 complex transcriptional activity. By doing so, they provide negative feedback on the promotion of their own genes by inhibiting CLOCK-BMAL1 mediated expression (Lee et al., 2001; for a review see Dijk and Archer, 2009). During the night, the PER-CRY repressor complex is degraded and thus a new cycle can be initiated. As a result, the expression of PER and CRY fall, the inhibition of BMAL1-clock complex is relieved, and the cycle starts again. This transcriptional-translational cycle takes approximately 24 hours to be completed and appears to be also regulated by post-transcriptional events, such as phosphorylation, which may affect stability, activity and even localization of clock proteins (Lee et al., 2001; Vanselow and Kramer, 2007). The aforementioned network represents a general simplified model, as additional feedback loops across clock genes, multiple interactions at both transcriptional/translational levels and posttranscriptional and posttranslational modification add complexity to the oscillatory network (Franken and Dijk, 2009). Mutations in circadian genes not only impact on rest/activity patterns or sleep-wake traits from a circadian perspective, but also influence homeostatic aspects of sleep regulation, as has been showed for *Cry1* and *Cry2*. Wiser and collaborators found that, under baseline conditions, *Cry1*, *Cry2* double knockout mice showed more time in NREM sleep, more EEG delta

power and increased NREM consolidation (Wisor et al., 2002). However, after 6 hours of SD, none of these classical hallmarks of sleep need increased. A plausible explanation is that delta power in Cry1/Cry2 knockout mice is so high after SD that cannot further increase (Wisor et al., 2002). If *Cry1* and *Cry2* are not only involved in circadian rhythms but also in the homeostatic sleep process, this may hold true for other Cry-regulated genes regulated by Cry, as for example *Per1*, *Per2* and *Per3*. *Per1* and *Per2* represent key molecular elements, and their disruption in mice leads to gradual loss of rhythmicity in constant dark conditions, thus resulting in the disruption of the circadian clock (Zheng et al., 2001). However, *Per3* appears to have redundant role in circadian clock function (Bae et al., 2001). Two studies from different and independent research groups investigated this topic in mice with non-functional *Per1*, *Per2* or *Per3* and double knockout of *Per1* and *Per2* (Kopp et al., 2002; Shiromani et al., 2004). Although deletion of the *mPer* genes does not affect sleep homeostasis, it may still be necessary. As noticed by Shiromani and colleagues, *Per* mutations affected total sleep time, sleep timing, REM sleep, and EEG delta power during sleep following SD (Shiromani et al., 2004). Thus *Per* genes are involved in the modulation of delta power during NREM sleep.

The master circadian clock, the SCN, acts as a maestro to thousands of musicians, who in this case are the individual cells of our body (Dibner et al., 2010). Indeed, through the daily entrainment to the light/dark cycle, the SCN transmits synchronizing signals to local circadian oscillators in the peripheral tissues. Clock genes have been recently found to be rhythmically expressed in most of cells, even when the cells were isolated from the SCN (Abe et al., 2002; Brown and Azzi, 2013). Individual SCN neurons can express cell-autonomous rhythmicity even in long-term organotypic cultured explants (Bos and Mirmiran, 1990). Thus, the circadian pacemaker is itself composed of potentially autonomous cellular circadian oscillators. The molecular clock mechanism oscillates in a cell autonomous fashion in peripheral tissues and also in extra-SCN regions of the brain (Nagoshi et al., 2004). The molecular organization of the peripheral clock seems to be similar to the

one observed in the SCN. As for the pacemaker, it was proposed that peripheral clocks are self-sustained and explant from several tissue exhibits robust circadian pattern in gene expression (Abe et al., 2002; for a review see Guilding and Piggins, 2007). Moreover the mechanism of circadian clock in peripheral cells tissue looks similar to the one in SCN cells: Pagani and colleagues observed a positive linear correlation between circadian period length in peripheral skin fibroblasts and physiological period length in the same subjects (Pagani et al., 2010). Collectively these data suggest that the SCN is responsible for synchronizing these peripheral oscillators, and it achieves this aim with a combination of neuronal and hormonal signals.

8. EEG correlates of circadian and homeostatic processes

Data from both FD and CR paradigms showed that both circadian and homeostatic processes contribute to EEG variation in a frequency specific manner in both sleep and wakefulness. A reliable marker of the homeostatic sleep pressure can be derived from EEG slow wave activity (SWA, spectral power density of the frequency range between 0.75-4.5 Hz). Evidence that SWA is primarily dependent on the duration of the prior wakefulness comes from two main observations. SWA progressively decreases throughout the course of the sleep episode, relatively independent from circadian phase. Second, sleep deprivation studies showed that, when wakefulness is extended during the biological night, SWA is augmented at the beginning of subsequent sleep episode. As previously mentioned, increasing sleep intensity is defined as increase in total power, amplitude and incidence of delta waves. This assumption came from sleep deprivation studies that showed that increasing sleep pressure leads to a rebound of SWA, in the subsequent recovery sleep episode. Thus the power density in the delta band was significantly higher than in the baseline (Borbely et al., 1981). During sleep, SWA decline can be approximated by an exponential decay and it rise throughout the waking period by a saturating exponential function as assumed by the two process model. Pioneering studies using a nap approach (Dijk et al., 1987b), confirmed that EEG

power density in the lower frequencies (delta and theta) increased monotonically with increasing duration of prior wakefulness. Conversely sleep latency (defined as the times between lights-off and the first epoch of stage 2) was not a monotonic function of prior wake duration. As previously mentioned SWA decreases throughout the course of the sleep. Conversely spindle frequency activity (SFA, spectral power density in the spindle frequency range) increases as sleep progress at all circadian phases (Dijk and Czeisler, 1995; Dijk et al., 1997; Wei et al., 1999). Thus there is an inverse homeostatic relationship between SWA and SFA. After sleep deprivation, slow wave and theta activity are enhanced, while high-frequency sleep spindle activity (spectral power density in the spindle frequency range 14.25-15.50 Hz) reduces and low-frequency sleep spindle activity (12.25-13 Hz) is not markedly affected (Dijk et al., 1993). Among the index of homeostatic sleep pressure, two of them have been shown to significantly vary along the antero-posterior axis (**Figure 5**). After sleep deprivation, both the global decline of SWA and the dynamics of the initial rise of SWA in recovery sleep, are larger in frontal derivations as compared to occipital ones (Cajochen et al., 1999a). The inverse homeostatic relationship between SWA and SFA is reflected also at a topographic level. Knoblauch and colleagues (2002) compared the effect of a CR versus a multinaps paradigm on these two variables. SWA increased after high sleep pressure in the frontal and central derivations as compared to the parietal and occipital ones, while central and parietal high spindle frequency activity decreased after high and increased after low sleep pressure (Knoblauch et al., 2002). These regional EEG changes could reflect a higher recovery need of frontal regions, in accordance with neuropsychological data and neuroimaging studies, showing that frontal cortical areas are particularly affected by sleep deprivation. PET studies showed selective deactivation during NREM sleep (Maquet et al., 1997) and decrease of regional cerebral metabolic rate of glucose in frontal regions after sleep deprivation (Thomas et al., 2000). Another EEG marker of the homeostatic sleep process occurs during wakefulness: the increase of theta activity (5-8 Hz) with time spent awake (Torsvall and Akerstedt, 1987; Cajochen et

al., 1995; Aeschbach et al., 1997; Finelli et al., 2000; Strijkstra et al., 2003).

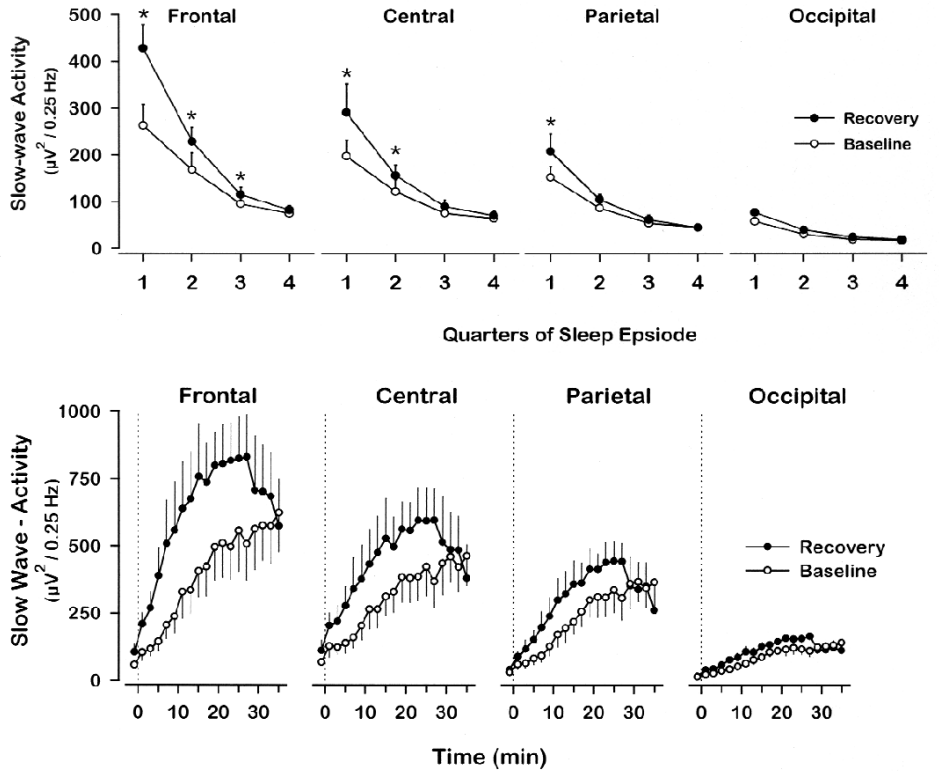

Figure 5. Top panel: Dynamics of SWA during NREM sleep across quartiles (2 h intervals) of baseline and recovery sleep, in frontal, central, parietal and occipital derivations. Asterisks indicate significant differences. **Bottom panel:** Evolution of SWA during the first 36 min after sleep onset in baseline and recovery sleep (from Cajochen et al., 1999a).

Under SD, the increase of theta power during the extended wakefulness correlated positively with the SWA increase in the first NREM sleep cycle in the subsequent recovery sleep relative to the baseline night (Finelli et al., 2000). This correlation indicated that two variables are closely related and may derive from a common process. Moreover subjective sleepiness also positively correlates with increased theta power during extended wakefulness (Akerstedt and Gillberg, 1990; Cajochen et al., 1995), particularly in frontal derivations (Strijkstra et al., 2003). Animal data show similar results for association between theta power and SWA (Vyazovskiy and Tobler, 2005). In rodents, the rise in EEG power in the 5–7 Hz band during SD predicted the subsequent enhancement of SWA

in NREM sleep (Vyazovskiy and Tobler, 2005). However data from FD experiments did not show a robust homeostatic component of theta power activity for all circadian phases (Cajochen et al., 2002). In summary, quantitative analysis of EEG data, during extended wakefulness, indicate that its frequency-specificity is influenced by both circadian and homeostatic factors. Low-frequency (<8 Hz) increase with time spent awake (Cajochen et al., 1995), thus more homeostatically-driven, while alpha activity undergoes a more clear circadian modulation (Cajochen et al., 2002).

9. Brain structures involved in circadian regulation of sleep and wakefulness

In mammals, the circadian pacemaker is located in the suprachiasmatic nuclei (SCN), a bilaterally paired nucleus located in the anterior hypothalamus, laterally to the third ventricle above the optic chiasm, where the two optic nerves cross paths. This position is relevant cause enables the SCN to receive direct projections from the retina throughout the retinohypothalamic tract (RHT). A secondary visual pathway projects to the SCN from the intergeniculate leaflet (Ferrara et al., 2006), a ventral thalamic component of the lateral geniculate nucleus. Receiving direct photic input from the retina is an extreme important property of the SCN neurons, as this input brings information about the most powerful environmental cues, light, thus allowing for the entrainment to the day/night cycle (Welsh et al., 2010). The light signal is received from a specialized set of retinal ganglion cells containing the photopigment melanopsin (Saper et al., 2005). The SCN is made up of two anatomic subdivisions, a “core” and a “shell” region. The first, islocated immediately above the optic chiasm, contains vasoactive intestinal polypeptide (VIP) neurons and receives visual input from the retina and from the intergeniculate leaflet of the lateral geniculate. The second subdivision comprises vasopressin-producing (AVP) neurons and receives nonvisual input from the brainstem, hypothalamus, basal forebrain and limbic cortex (Moore, 2007). The conjunction of these subdivisions generates the overt circadian

expression of the SCN (Chellappa and Cajochen, 2010). Despite his fundamental role, the SCN itself has only few outputs to the sleep regulatory centers and its action is thought to be mediated by multiple and divergent pathways (Deurveilher and Semba, 2005).

A principal neuronal output pathway from the SCN terminates at the adjacent subparaventricular zone (SPZ) and the dorsomedial nucleus of the hypothalamus (DMH). Lesions of ventral part of the SPZ disrupt circadian rest-activity rhythm, but have minimal effect on circadian temperature rhythm. Conversely, lesions of the dorsal part of the SPZ affect circadian temperature rhythm but not circadian rest-activity (Saper et al., 2005). Both the SCN and the SPZ project to the DMH, although the majority of the projections arise from the SPZ, thus amplifying the output of the SCN (**Figure 6**). Lesions of the DMH affect circadian rhythm of sleep-wake behavior and diminish the amount of sleep suggesting an activating role of the DMH output (Chou et al., 2003). Moreover the DMH sends excitatory glutamatergic projections to the LHA, GABAergic projections to the VLPO, to the corticotropin-releasing hormone (CRH) neurons of the paraventricular nucleus (PVH) and to the lateral hypothalamic area (LHA). Thus the SCN regulates sleep-wake behavior and coordinates this rhythmicity in other brain areas and tissues *as an orchestra leader*, through multiple and divergent pathways. Nevertheless, which of these indirect projections functionally regulate arousal still remains unclear. Animals studies suggest a role for LC-norepinephrine neurons in the circadian regulation of wakefulness and arousal (Aston-Jones et al., 2001; Aston-Jones, 2005; Gompf and Aston-Jones, 2008). DMH orexin neurons play a role in modulating the day–night differences of LC impulse activity. Since the densest projection of hypocretin/orexin fiber terminates in the LC, it has been proposed that LC controls the activity of those neurons directly by inhibiting hypocretin/orexin firing and indirectly via the DMH.

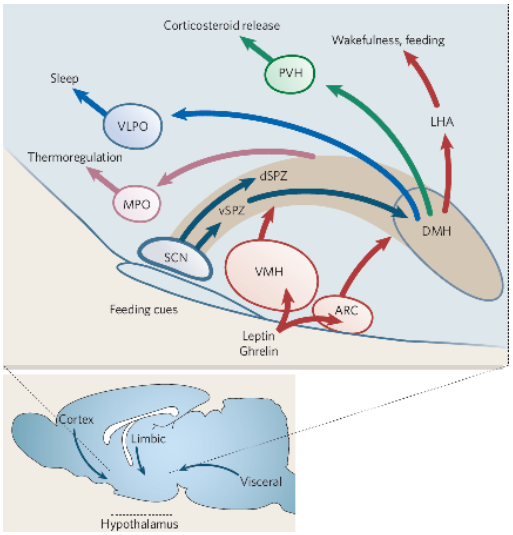

Figure 6: Outputs from the SCN to the sleep –regulatory systems.The SCN has modest projection to the VLPO neurons. The majority of its output is directed toward the ventral (vSPZ) and dorsal (dSPZ) subparaventricular zone and the dorsomedial nucleus of hypothalamus (DMH). LHA: lateral hypothalamic area; MCH: melanin-concentrating hormone; TRH thyrotropin-releasing hormone (from Saper et al., 2005).

10. Circadian and homeostatic influences on cognitive performance

As every one of us has experimented at least once in our life, long waking periods decrease our cognitive performance, while a short nap can help us to at least partially recovery those abilities. Temporal synchronization of neurobehavioral performances to environmental requests represents a fundamental issue in human daily life. A proper alignment between sleep-wakefulness and internal circadian clock is essential for neurobehavioral performance (Wright et al., 2006). Furthermore time-of-day fluctuations in performance are generated by the interaction between circadian and homeostatic processes (Carrier and Monk, 2000).

Indeed, cognitive performance does not decrease linearly with time spent awake but is modulated by the interaction of the circadian and homeostatic processes (Dijk and Archer, 2010a). The amplitude of the circadian modulation in performance depends of the level of the homeostatic sleep need (Dijk and Franken, 2005). This assumption

implies that, if sleep pressure is low, the amplitude of the circadian performance variation is small, while increasing sleep need results in a parallel amplitude increase.

Moreover, while sleep homeostasis affects subjective alertness and cognitive performance at all circadian phases, the strongest effects occur at the minimum of core body temperature (Dijk et al., 1992). Conversely, weakest effects typically occur around its maximum, as expressed by a significant interaction between circadian and homeostatic factors for both alertness and performance (Dijk et al., 1992). Surprisingly, even when homeostatic sleep pressure is very high, the circadian system is still able to counteract the detrimental effect on neurobehavioral performance. Indeed neurobehavioral performance improvement during the WMZ was more pronounced after extended wakefulness (Shekleton et al., 2013).

Importantly, when we assess the impact of circadian and sleep homeostatic processes on cognitive performance, two more sources of possible confounding effects have to be taken in account: the kind of task used as well as the inter-individual differences in task performance (Blatter and Cajochen, 2007). In fact, cognitive domain, cognitive load, task duration, task complexity, and so forth strongly impact on the measured output variable of interest as well as subject population studied (Schmidt et al., 2007). Indeed the impact of circadian and sleep homeostatic processes on cognitive performance is steadily related to task characteristics. However, deteriorating attention is usually believed to participate in performance decline consequent to sleep loss across several cognitive domains.

CHAPTER 2

Attentional Networks: Definitions and Hypothesis

1. Introduction

As mentioned in the previous chapter, temporal fluctuations in neurobehavioral performance are driven by the interaction of the circadian and homeostatic processes (Dijk et al., 1992). During a normal waking day, sleep pressure increases and its deleterious effects on cognitive performance are counteracted by an increased circadian alerting signal. When wakefulness is extended into the biological night, the circadian system no longer opposes the increasing need for sleep, and cognitive performance is jeopardized (Dijk and Archer, 2010b). A central question is whether the interaction between the circadian and homeostatic processes equally influences performance or if there is a differential effect depending on the cognitive domain. The first scenario implies an equal circadian/homeostatic variation in all cerebral functions, hypothetically related to global metabolic changes in the brain. The second hypothesis assumes a more localized, regional circadian/homeostatic variation in cerebral activity (Schmidt et al., 2007).

As we will see throughout the chapter, attentional processes play a central role within the different cognitive domains investigated under sleep deprivation. Human attention is not constant over time (Cohen, 1993).Thus it is important to investigate how attention varies over time,

as it subtends to attentional functioning. However terms like attention, vigilance, or arousal are often used interchangeably, although they do not represent the same construct. Similarly, expressions like "top-down" or "bottom-up" modulation of attentional resources are also used without specifying the corresponding conceptual framework.

Vigilance is also used with a constellation of different meanings, referring sometimes to the degree of cortical brain activity but sometimes to attentional processes.

As observed by Oken and collaborators "[…] there are activation states of cerebral cortex that impact the ability to process information where the activation itself contains no specific information. These activation states can be tonic or phasic and may be relatively global or more localized. Terms that have been used to describe these states include arousal, alertness, vigilance, and attention. Unfortunately, no terms are ideal to describe these states of cortical activation since most terms are in broad use with varied associations and there are not perfect physiological markers" (Oken et al., 2006).

Therefore, operational definitions are essential before discussing individual variability in sleep-wake regulation and its relative impact on neurobehavioral performance. Thus, in the present chapter, we will first introduce basic concepts related to the attentional processes. Then we will focus on a specific aspect of attention, regarding the temporal nature of signal detection and processing, the ability to sustain attention over time. Finally we will review a recent attentional model that emphasizes the multi-component nature of attention, and that tested the "independence" of the main components through an fMRI experimental paradigm.

2. Top-down or Bottom-up? Vigilance, sustained attention or arousal?

In daily life, global attention and especially visual attention are controlled by several factors, such as previous acquired knowledge,

expectation and current goals, but also from other factors as the properties of the object that capture our attention.

In cognitive neuroscience, the terms “top-down” and “bottom-up” do not refer purely to anatomical pathways connecting brain regions, but represent a conceptual frame to explain attentional mechanisms. As reviewed by Egeth and Yantis (1997), William James (1890) already distinguished active from passive attention, indicating with the first an attentional form that is deployed voluntary toward a stimulus, while the passive is captured automatically through stimulus characteristics. Active attention corresponds to what today is described as top-down attention. Corbetta refers to top-down signals as the internal, endogenous, knowledge-driven mechanisms aimed to guide perception, enhancing the neural processing of relevant information and allowing for the discrimination between signal and noise (Corbetta et al., 2008). Thereby top-down attention corresponds to the voluntary allocation of attentional resources to certain features, objects, or location in space.

From a top-down perspective, it is the final aim that enhances and facilitates the processing of sensory input in the visual and associative cortical regions. In this view, predictions about the stimuli of the task would be expressed by signals traveling along top-down connections from higher decision-making cortical areas to lower cortical areas (Liang and Wang, 2003).

Such internal signals interact with a “bottom-up” process (corresponding to the passive attention, in James view), mainly driven by the sensory signals produced by targets characteristics and saliency and its sensory context present in the visual scene.

Thus, a “bottom-up” perspective is assuming that the ability to correctly detect a target depends on the target’s sensory salience, which would trigger attentional processing by recruiting only, in subsequent step, higher cortical areas. Importantly these two processes do not represent a dichotomic construct but an overlapping organizational principle. In

the majority of situations, both properties of an object and goals of an individual determine the attentional phenomenon.

In real-life settings, an example of the interaction between top-down and bottom-up processes is provided by Corbetta and Shulman (2002). If we are searching for a friend wearing a red hat, in a crowd of people, our attention will be captured more often by people that are wearing red clothes, compared to people wearing clothes of different colours. Thus the target characteristic (the sensory distinctiveness or bottom-up) interacts with the goal (the top-down) of finding a red object. However, a good prerequisite to find our friend with the red hat is to have a good vigilance level, also in order to detect all red objects present in our field of view.

The term vigilance describes a state of readiness to detect and respond to certain small changes occurring at random time intervals in the environment (Mackworth, 1948).

Vigilance is also often used as synonymous of tonic alertness or tonic arousal. Tonic alertness corresponds to the level of "cortical activation" induced by the brainstem activating structures (as the ARAS, cf. first chapter). Coull defined arousal as a state of physiological reactivity of an individual, ranging from sleep at one end to excitement or panic at the other (Coull, 1998). In this thesis (and typically in sleep research), the term arousal is selectively used to indicate the level of cortical activation but also transient changes in EEG oscillations rather than to a long-lasting ('tonic') state of responsiveness.

In cognitive neuroscience vigilance indicates the ability to maintain a sufficient attentional efficiency level to a task, over extended periods of time (Parasuraman et al., 1998). Thus vigilance is used a synonymous of sustained attention due to the fact that situation requiring vigilance are typical of sustained attentional task (Leclercq, 2002). Sustained attention is studied mainly through monotonous and long lasting tasks. Accordingly, the numbers of trials are low, and require continuous

monitoring to adequately react and detect events with rare occurrence (Coull et al., 1996).

However as recapitulated by (Coull, 1998) vigilance could be thought as a longer-term process, with a time-course that ranges from minutes to hours, rather than seconds to minutes (as for sustained attention). In cognitive psychology, the number of items to process and their temporal occurrence helps to differentiate vigilance task from more sustained attentional tasks. Vigilance task would then correspond to the frequency of stimuli to which the volunteers have to react, is low and their occurrence is irregular. On the other hand, sustained attentional tasks imply that participants have to react to stimuli which occur frequently, over an extended period of time. Leclercq underlined that vigilance and sustained attention could be seen as the two extreme of a continuum. Sustained attention requires a continuous and active processing of information flow that is fast. This would contrast to vigilance tasks, where the number of information that has to be processed is low (Leclercq, 2002).

One of the first “vigilance tasks” was developed by Norman Mackworth to study the effects of long-term monitoring on signal detection by radar and sonar operators. The so-called “Clock Test” consists of a clock-like device with a black pointer that moves in short jumps like the second hand of clock. At irregular and infrequent intervals, the hand advances by a double jump. The task consist in detecting when the double just occurred by pressing a response key. The doubled jump happened twelve times per half-hour in a pseudo-random manner for each half hour period (total test duration equal to two hours). Mackworth's results indicated a decline in signal detection over time, known as a vigilance decrement. The participants' event detection declined between 10 and 15% during the first 30 minutes and then continued to decline more gradually for the remaining 90 minutes. As specified by (Parasuraman, 1986), vigilance decrement is defined as "deterioration in the ability to remain vigilant for critical signals with time, as indicated by a decline in the rate of the correct detection of

signals". In the “Clock Test”, the stimuli frequency is kept low, and stimuli occurrence is irregular; thus Mackworth's task corresponds to the aforementioned definition (used in cognitive psychology) of vigilance task. A test that corresponds to the definition of sustained attention is the Psychomotor Vigilance Task (PVT; Dinges and Powell, 1985).

In cognitive neuroscience, the PVT is probably the most used task to detect daily fluctuations in sustained attention. It comprises a simple reaction time (RT) task to stimuli that occurs at random intervals. In the classical visual PVT, participants are instructed to press a response button as fast as possible each time a digital counter starts to scroll.

Although the PVT uses a shorter inter-stimulus interval, as compared to a classical sustained attention task, such simple but sustained attention demanding tasks are reliable and sensitive to the effects of sleep loss. This suggests that attentional process is among the most susceptible to the deleterious effects of sleep deprivation. However attention is a heterogeneous concept (Oken et al., 2006), and sleep deprivation affects several aspects of attention, such as phasic alertness (Drummond et al., 2005), selective (Horowitz et al., 2003) and divided attention (Drummond et al., 2001).

In the PVT, the dependent measures are reaction times (RTs) and attentional lapses (RTs > 500 ms). Attentional lapses, which refer to a failure to respond in a timely manner to an expected stimulus, could be defined as brief moments of inattentiveness. Moreover they are one of the main factors responsible for performance decrement during sleep deprivation (Dorrian et al., 2005).

3. Insights into the effects of sleep deprivation on sustained attention: Different hypotheses

At the end of the 50’s, Williams reported that lapses in RTs task increased with time spent awake, and that, even when a performance starts worsening, participants were still able to perform at appropriate

levels between lapse periods. Moreover the majority of the lapses occurred during short and brief transition to sleep onset, as assessed by EEG recordings (Williams et al., 1959).

This observation paves the way to the so-called “lapse hypothesis”, which postulates that performance during sleep deprivation is characterized by brief moments of low arousal. In this view, lapses could be due to short periods of sleep-like states (Dinges and Kribbs, 1991), momentary (3-15 sec) intrusions of microsleep episode into the waking state (Cajochen et al., 1999a), resulting in an inability to respond properly to stimuli.

The lapse hypothesis provided a new frame to sleep deprivation studies, as it drew attention to increased performance variability (Doran et al., 2001). However, microsleep could account only for some lapses, but could not be the sole mechanism for all lapses, since volunteers under well-rested conditions occasionally lapse (Anderson et al., 2010). Thus not all lapses can be attributed to microsleep, and additional factors as “disengagement” from the task or visual inattention/distraction and fatigue may be involved.

An alternative view to the “lapse hypothesis” is the “wake-state instability” theory (Doran et al., 2001). Accordingly, the detrimental effects of sleep deprivation on performance are due to lapses in attention and considerable fluctuation in tonic aspects of functioning, as indexed by larger variability of responses.

This hypothesis refers to moment-to-moment shifts in the relationship between two neurobiological systems, the first one mediating wake maintenance and the other mediating sleep initiation (Banks and Dinges, 2007; Goel et al., 2009). Due to increasing sleep pressure, sleep deprived participants would be susceptible to uncontrolled sleep initiation. Volunteers would then try to resist using greater

compensatory[1] efforts to perform the task (Doran et al., 2001). Depending on stronger drives (sleep homeostatic pressure or compensatory efforts), performance will more or less stable. Thus the global slowing in RTs, the increase in lapses and errors of commission (i.e., responses when no stimulus is presented) would be signs of a sleep initiating mechanism that repeatedly interferes with the maintenance of wakefulness while sleep pressure increases (Doran et al., 2001). The same increase in errors of commission would reflect an increase in compensatory efforts in resisting to initiate sleep. Finally the neurobehavioral performance variability appears to reflect this state instability (Dorrian et al., 2005).

Another hypothesis is the "sleep-based neuropsychological perspective", which assumes that sleep deprivation differentially affects brain regions (Harrison et al., 2000; Babkoff et al., 2005). This hypothesis is based on two observations. First sleep deprivation strongly impacts on cognitive functions associated with prefrontal cortex (PFC), with tasks measuring higher cortical-related skills such as executive functions, working memory, verbal fluency, memory for temporal order, being more affected by sleep loss. As specified by Jones and Harrison (2001), executive functions can be defined as ''the ability to plan and coordinate a willful action in the face of alternatives, to monitor and update action as necessary and suppress distracting material by focusing attention on the task at hand". The prefrontal cortex has a strong executive attention role in maintaining access to stimulus representations and goals especially in interference contexts (Kane and Engle, 2002). However, neuroimaging studies have shown that the

[1] In this context the term "compensatory" refers to the tendency of the volunteers to adjust their responses based on the previous observed RT. Basically Doran noticed that PVT performance (under sleep deprivation conditions) was characterized, already after 18 hours of wakefulness, by short/normal RTs intermixed with both lapses and anticipation (i.e. error of commission). Errors of commission and errors of omission closely covaried across the sleep deprivation period. This, for the authors, reflected an attempt to compensate (error of commission) for having missed a target (error of omission).

mechanisms of attention are a fundamental component of higher-order cognitive tasks subserved by the frontal cortex (Dorrian et al., 2005). Moreover, neuroimaging studies on sustained attention have consistently shown frontal activation, revealing a network that includes frontal and parietal regions (Culham and Kanwisher, 2001; Lawrence et al., 2003; Fan et al., 2005).

The second observation is that SWS during sleep after sleep deprivation is markedly increased in frontal regions, suggesting a stronger restoration need in these regions. Harrison and colleagues compared performance on a battery of PFC- oriented tasks, in different age groups (Harrison et al., 2000). Poorer performance was detected in healthy older volunteers; however when young participants were evaluated after 36 hours of sleep loss, similar decrement (by comparison to older volunteers in well rested conditions) in performance was observed.

Finally the authors suggested that the effects of sleep deprivation may be similar to those due to aging, as both conditions appear to slow down cognitive “throughput”. Thus according to Harrison, sleep loss would leads to a temporary and reversible “functional lesion” of frontal areas, which are essential to adequately perform executive tasks. This assumption, as we mentioned above, leads to the conclusion that tasks assessing executive functioning are more affected by sleep deprivation. However recent data seems to point in a different direction. Balkin and colleagues, using a sleep restriction protocol, evaluated the sensitivity of several measures to the detrimental effects of sleep loss (Balkin et al., 2004). The authors have shown that sustained attention (as assessed by the PVT) was more affected than response inhibition (measured by the Stroop task). Moreover a meta-analysis of the impact of sleep deprivation on cognitive variables seems to confirm Balkin results (Lim and Dinges, 2010).

Lim and Dinges (2010) computed the effect size of sleep deprivation on speed and accuracy measures in numerous cognitive domains. Close to 145 cognitive tests, related to 6 main cognitive categories, were

analyzed. Results suggested that sustained attention is as much or more affected than executive functions. More recently Lo and colleagues investigated the interaction of repeated partial and total sleep deprivation and circadian phase on performance across seven different cognitive domains. Results showed that sleep loss differentially affected performance in the seven cognitive categories, with sustained attention being, together with subjective alertness, more affected than all other tasks, including executive function tasks (Lo et al., 2012).

4. Macro-anatomical correlates of sustained attention: Evidences from functional imaging studies

Functional imaging studies (Positron Emission Tomography, PET, and fMRI) in healthy volunteers have provided insights to the neural correlates of sustained attention.

The functional system to sustained attention has been ascribed to the frontal (particularly the dorsolateral prefrontal cortex) and parietal areas (especially in the right hemisphere) as well as the anterior cingulate cortex in PET and fMRI studies (Pardo et al., 1991; Cohen et al., 1992; Fink et al., 1997; Coull et al., 1998; Portas et al., 1998; Lawrence et al., 2003; Thakral and Slotnick, 2009). Thalamic involvement has also been found to participate in the regulation of sustained attention, even if thalamic changes seems to be more related to the influence of arousal levels on sustained attention (Coull, 1998), possibly due to its privileged anatomical relationship with the ARAS.

Ventrolateral thalamic activity changes as function of arousal. Under sleep deprivation conditions, highest levels of attention-related activity are observed in the thalamus, as compared with a control condition in which participants had caffeine administration (Portas et al., 1998). However attention is a heterogeneous concept, encompassing numerous sub-processes that may interact with each other, with potential anatomical overlapping. An example is the relationship between selective and sustained attention. Selective attention could be defined as the capacity to attend, *in selective way*, to a specific stimulus

and simultaneously ignore another. Data from patients suffering from unilateral spatial neglect suggest that the parietal cortex could represent a common anatomical basis for both sustained and spatial attention and for their interaction (Robertson et al., 1995). Based on this concept, the reciprocal interaction between sustained attention and the process of selectively monitoring for target objects was investigated, throughout two tasks (Coull et al., 1998). The first task required a selective response: to press a response button whenever a pre-designated target letter appeared. The second, a non-selective task, used exactly the same stimuli but participants were asked to respond whenever any stimulus appeared on the screen. Measuring cerebral blood flow (rCBF), the authors showed that frontal, parietal and thalamic activity decreased as time-on-task (TOT) increased. Due to the experimental paradigm, this study could functionally dissociate sustained from selective attention. Results showed that rCBF decreased in frontal and parietal areas as TOT increased during the non-selective task. However this pattern was not observed during the selective task. Sustained attention was thus associated with decreased activity in the dorsolateral prefrontal cortex, inferior parietal cortex and thalamic area. Conversely selective attention evoked activity in the anterior cingulate and prefrontal cortex. Thus, decreased cortical activity together with TOT increase (sustained attention) could be counteracted by the recruitment of selective attentional processes, via a common mode of action in the right frontal and parietal cortices. Based on the anatomical overlap between region controlling for spatial attention-shifting and region controlling for sustained attentional processes, Thakral and Slotnick (2009) investigated functional anatomic organization of the parietal cortex in an fMRI design.

Two conditions were used: a motor dot and a flickering checkerboard protocol, alternating 14 s periods of sustained attention to motion or flicker, sustained perception of motion or flicker, and sustained perception of stationary dots or stationary checkboard (Thakral and Slotnick, 2009).

By a conjunction analysis, the authors subsequently identified a set of regions associated with sustained attention, including the right intraparietal sulcus, middle frontal gyrus, superior temporal gyrus, the insula and the left cerebellum.

An interesting fMRI study, characterized different aspects of attentional processes related with lapses (Weissman et al., 2006). These included the neural correlates of momentary lapses in attentional processes, the brain activity consequences of lapses, and brain regions that help to recover from the temporal slowing outcomes. However the authors used a global/local selective attention task instead of a "pure" sustained attention task. It is worth to spend few words about this kind of task, as it introduces more than one attentional subcomponent. In this task, volunteers have to identify either the large, global letter or the small, local letters of a hierarchically organised visual stimulus (**Figure 7**).

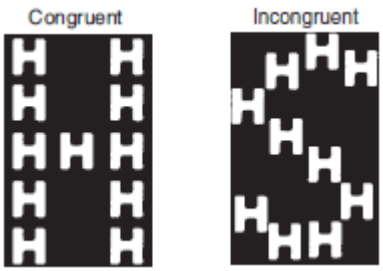

Figure 7: Stimuli used in the global/local selective attention tasks. Volunteers have to press a button as quickly and accurately as possible, to indicate the identity (H or S) of the large, global letter or the small, local letter of a hierarchically organized visual stimulus. In half the trials, the global and local letters were mapped to the same response (congruent trials; e.g., a large H made of small Hs); in the other half, they were mapped to different responses (incongruent trials; for example, a large S made of small Hs). Modified from Weissman et al., 2006.

A fixation dot, at the centre of the screen, is typically replaced by stimuli appearing at location surrounding the dot. Thus a first attentional component is introduced: a reorientation of the stimulus-driven attentional focus. Stimuli could be congruent, a global H made of local Hs or a global S made of local Ss, or incongruent, a global H made of local Ss or a global S made of local Hs. This dichotomic choice inserts an

executive component in the task, the detection and resolving processing conflict.

Results revealed that attentional lapses were associated to reduced activity in frontal regions (including the inferior frontal gyrus, IFG, middle frontal gyrus, MFG, and the anterior cingulate cortex, ACC), before behaviourally relevant stimuli are presented. Moreover reduced stimulus-triggered activity was observed in the inferior occipital cortex, IOC, bilaterally. Authors claimed that this first result suggests the failure of frontal control regions to adequately allow the perceptual processing of relevant stimuli through the sensory cortices.

Lapses were also associated to reduce task-induced deactivation in the so-called default-mode network (DMN). The DMN could be defined as a set of brain regions showing higher activity at rest, when individuals process internal or self-related information, as compared to active sustained attention tasks and goal-oriented tasks (Shulman et al., 1997; Gusnard et al., 2001). It includes part of the medial temporal lobe, part of the medial prefrontal cortex, the posterior cingulate cortex, the adjacent ventral precuneus and the medial, lateral and inferior parietal cortex (Raichle et al., 2001). This result was interpreted as a less effective suspension of task-irrelevant cognitive processes. Based on a trial-by-trial analysis, greater temporal-parietal junction (TPJ) and IFG activity in the current trial were associated with faster RT only in the subsequent trial and not in the current one. This latest result was interpreted as a recovery mechanism from lapses in attention. Basically, during a lapse, the onset of a new stimulus would result in a reorienting process of the attention to the location where the relevant stimuli are presented, thus facilitating performance at the next trial. Therefore there is a subtle line across different attentional subcomponents.

In a recent fMRI study, the Sustained Attention to Response Test (SART), developed by Robertson (Robertson et al., 1997), was used to measure sustained attention (O'Connor et al., 2011). In the SART, random series of single digits are presented at the centre of a computer screen, at the

rate of 1 per 1.15 seconds. Volunteers are instructed to press a response button to each digit with the exception of only one of them. During the control task, participants had to respond to each single digits thus eliminating to executive component of the task (response inhibition). During the SART, as compared with the control condition, greater activity was detected in the right prefrontal cortex and the thalamus, as well as in the right parietal cortex.

Grahn and Manly used two sustained attention tests in an fMRI experiment (Grahn and Manly, 2012). During the first task, participants were asked to count tones presented at long and unpredictable intervals rate, as opposed to the control condition (tones at brisk and regular rate). The second task was the SART, contrasted with a control condition in which the no-go trials (the digit to which participant have to retain to response) were removed. Using the cognitive conjunction approach suggested by Price and Friston (1997), the neuronal pathways associated with the common component of the two tasks were isolated. Activity elicited by both tasks, thus supporting the sustained attentional process, was found in the bilateral inferior frontal operculum, the anterior cingulate cortex, the inferior parietal cortex and in the bilateral premotor cortex. Interestingly, these regions have been conceptualized as part of a Multiple Demand Network, whereby flexibility adapts to current task demands (Duncan, 2010) to perform sequential goal-directed behaviours. Activity elicited by the sustained attention task, and observed in the Multiple Demand Network, could represent an endogenous activation to compensate for the low levels of exogenous task stimulation. Importantly, this study highlights that common neural systems are recruited during very different sustained attention tasks. As suggested by Sarter functional MRI studies support the idea that sustained attention could be described as a process that starts with a state of readiness to detect and discriminate stimuli, via fronto-parietal regions (Sarter et al., 2001). In turn, this supports and enhances perceptual and spatial processes through posterior areas, thus facilitating the processing of sensory input in primary, secondary and associative areas. Taken together these studies speak to an overlap of

anatomical regions recruited for a variety of attention demanding tasks. Ultimately, it emphasizes the heterogeneous nature of the attentional system and its flexibility based on current task demands.

5. Attentional Systems

Deteriorating attention is usually believed to participate in the decrease of cognitive performance observed under sleep deprivation conditions. Sustained attentional tasks are strongly affected by sleep loss. However, as pointed out by van Zomeren and Brouwer (1987), "Sustained attention can never be regarded as an independent type of attention, as it involves sustained focused attention and sustained divided attention, as well as sustained supervisory control" (p.399). This assumption bring us to a more generalized concept, as underlined by Leclercq (2002), that is the attentional components' interdependency. Accordingly, attention cannot be studied in an isolated manner and thus each attentional component cannot be isolated irrespective of the others. In his view, whatever task is developed, it will always involve, in different measures, multiple attentional competences. As a conclusion "only by cross-checking the subject's performance in different tasks, which have in common that they test some specific attentional aspect, that we will be able to give an opinion about the quality of the course of more specific processing" (Leclecq, 2002, p.49).

Recently, Posner and colleagues divided human attention system into three different subsystems, each related to specific brain circuits and related to neuromodulators (Posner and Petersen, 1990; Fan et al., 2002, 2005). Posner attentional model assumes an anatomical separation of the attention system from the data processing systems that perform operations on specific inputs.

Alerting is defined as the ability in achieving and maintaining an adequate vigilance state in order to process high priority incoming stimuli. Alerting is studied by the use of a warning signal indicating when, but not where in the visual field, a stimulus will appear. This in turn triggers the alerting attention system, which will enhance the state

of readiness to detect and respond to the stimulus. The beneficial effect of the warning signal is referred as alerting effect. This first component would involve frontal and parietal areas, as well as thalamic regions. Neuro-pharmacological studies have related the alerting effect to norepinephrine system, which arises in the locus coeruleus of the midbrain, by showing that the effect of a warning signal is reduced by norepinephrine antagonist (Posner and Petersen, 1990; Coull et al., 2001; Fan et al., 2005).

Orienting is defined by Posner in terms of the foveation of a stimulus. The act of foveating (angling the eyes to focus an object) improves the target processing efficiency. However it is possible to improve the priority of a given stimulus by attending to its location covertly without changes in the eye position (Posner, 1988). Thus orienting is experimentally manipulated by presenting a cue, which gives relevant information on the location of the event, indicating where in the space, but not when, the participant should attend to the stimulus.

When we attend to a location in the visual field, events occurring at that location are processed faster, and the phenomenon is indicated as an orienting effect. The orienting network would include the superior parietal lobe, temporal parietal junction and frontal eye field (Corbetta et al., 2000; Corbetta and Shulman, 2002). The cholinergic system, which arises in the basal forebrain, would play an important role in the orienting subcomponent, through its effects in the parietal cortex. In humans, the superior parietal lobe could be closely related to the lateral intraparietal area (LIP) in monkeys. Injections of scopolamine into the LIP area reduce the animal's ability to covertly orient (Davidson and Marrocco, 2000).

The executive control subcomponent is defined as resolving conflict among stimuli. Executive attention is supposed to monitors and resolves conflict between computations that could involve planning or decision making, error detection and so on. It is commonly measured using a task (as in the in the flanker task) in which participants confront an

incompatibility between dimension of the stimulus. In a flanker task, the target is flanked by non-target stimuli, which correspond either to the same directional response as the target (congruent flankers), to the opposite response (incongruent flankers). Responses are faster and more accurate for congruent stimuli than for incongruent ones. Conflict effect is then calculated by subtracting RTs to congruent stimuli from those to incongruent ones.

Brain imaging studies have shown that conflict monitoring recruited areas in the dorsal anterior cingulate cortex (ACC), while the lateral prefrontal cortex (LPFC) would be involved in conflict resolution (Bush et al., 2000; MacDonald 3rd et al., 2000; Botvinick et al., 2001; Fan et al., 2002, 2003, 2005). The involvement of the ACC and LPFC highlight the importance of the dopaminergic system for the executive component.

Based on this attentional subcomponent model, Posner and colleagues developed the Attentional Network Test (ANT), which evaluates the three attentional effects through the use of cues and targets within a single reaction time task.

6. The Attentional Network Test

The ANT is a combination of the cued reaction time (Posner, 1980) and the flanker task (Eriksen and Eriksen, 1974). The ANT was originally developed by Fan and collaborators (2002) to assess, within a single 30 minutes task, the validity of the three attentional networks model behaviourally, and in a later step, adapted for fMRI studies (Fan et al., 2005). We will illustrate the experimental design of the first version of the task (Fan et al., 2002).

During the task, participants are instructed to fixate a central cross for the entire experiment. Stimuli consist of a row of five aligned horizontal arrows, pointing either to the left or right. The arrow row appears above or below the fixation cross. The target is represented by the central arrow, which could point either to the same direction of the four flanker arrows (congruent condition) or to the opposite direction (incongruent

condition). In the behavioural version of the task (Fan et al., 2002), a control condition is present (neutral condition), in which the central arrow (**Figure 8**) is flanked by four horizontal lines (this condition is absent in the fMRI version of the task). Thus under this condition, there is neither an alerting nor a spatial cue. Four cued conditions are used, according to the presence of and location of the cues. In one condition, the stimulus is preceded by a visual cue (an asterisk) presented at the center of the screen (center cue). In a second condition, the cue is absent and thus the stimulus appears without any warning signal (no cue).

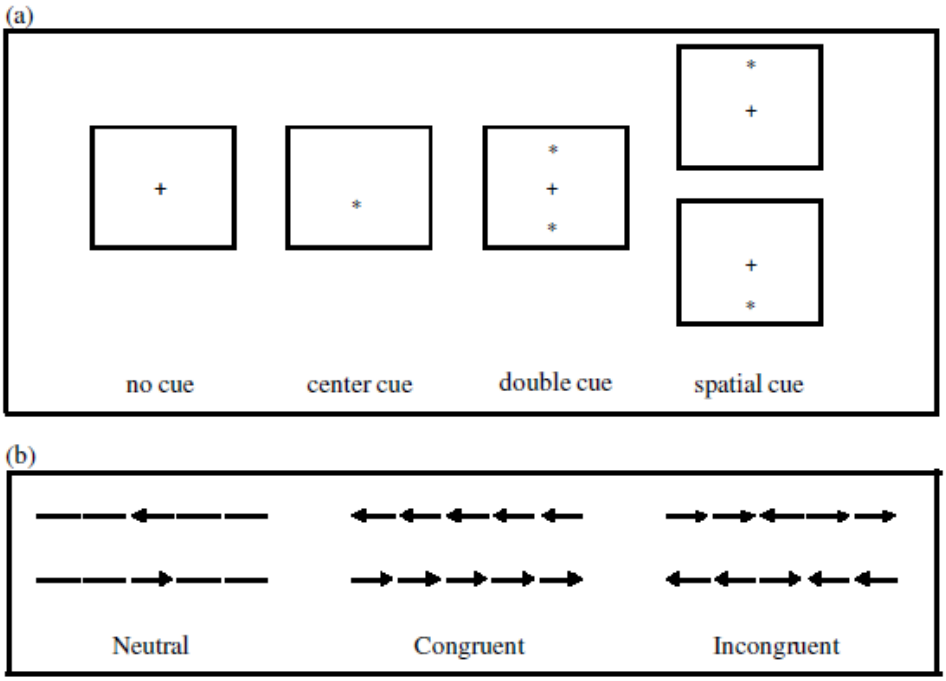

Figure 8: The ANT. As showed the task used four cue conditions (a) and three target type (b). Modified from (Fan et al., 2002).

In the spatial-cue condition, the stimulus is preceded by an asterisk presented above or below the fixation cross and is predicting the location at which the stimulus will appear. In the last cue condition, a double cue (one above and the other below the central cross) is preceding the appearance of the stimulus but without indications on where the stimulus will be displayed. Volunteers are required to

determine as quickly and accurately as possible the direction of the central arrow by pressing one key for the left direction and a different key for the right direction. The efficiency of the three attentional subcomponents is behaviourally evaluated by measuring the influence of cue and target conditions on RTs, relative to a reference condition. Thus the alerting effect is calculated by subtracting the mean RTs of the double-cue condition from the mean RTs of the no-cue condition. The logic behind the RT subtraction is based on the assumption that both the aforementioned cue conditions give indication on where the stimulus will appear. As no warning signal is presented, attention will tend to remain diffused across the two possible locations. The double cue is used because as the no-cue keep attention diffused, but at the same time also alerts the participant of the incoming appearance of the target.

The orienting effect is calculated by subtracting the mean RTs of the spatial cue condition from the mean RTs of the center cue condition. Both conditions imply a waning cue, alerting participants about the imminent appearance of the target. However, only the spatial one provides spatial information on where it will be displayed, allowing volunteers to shift the attentional focus toward the attended location. In this case, the center cue is used as control condition.

Finally the conflict effect (the executive control subcomponent) is calculated by subtracting the mean RTs of all the congruent trials (irrespective of different cue conditions) from the mean RTs of the incongruent trials. The use of neutral target condition would produce the same results due to the small differences between congruent flanker and neutral flanker conditions, and thus will be eliminated in the fMRI design.

In the original paper of Fan and colleagues (2002), correlation analyses determined that the ANT provides a reliable estimation of the efficiency of the three networks and establishes the independence of these three measures

The conflict effect was found to be the most reliable measure through test-retest correlation, whereas the alerting was less reliable. The orienting was more intermediate (Fan et al., 2002). One possible interpretation is that, while the executive control was measured directly by the task, the alerting and orienting were approached by the use of cues, which might reduce their reliability. On a next step, the independency of the three networks was investigated, first examining correlation across the measures and then through an analysis of variance (ANOVA) to determinate possible interactions between factors. If no correlations were found between the three networks (suggesting that these are functionally orthogonal), the ANOVA showed significant effects of cue and flanker type, as well as small but significant interaction between the two. Further analyses revealed that the presence of incongruent target increased RT (as expected), but both no cue and spatial cue conditions reduced the impact of incongruent flanker on RTs (relative to other cue condition). This indicated non-independence between orienting and conflict, as well as between alerting and conflict effect. These interactions suggest that the networks do not generally operate in an independent way in all situations. Moreover the authors emphasized that even if networks are independent, it would be surprising if the three did not influence each other. However in subsequent studies, the same group replicated this cue x flanker type interaction (Fan et al., 2007) and small but significant correlations were found between alerting and conflict (Fossella et al., 2002) and between alerting and conflict network scores (Lehtonen, 2008). Alerting and orienting networks are defined by cue conditions, making it difficult to separate a potential interaction between alerting and orienting from the significant interaction between cue condition and target type (Callejas et al., 2005). Thus an alternative version of the ANT was developed, in which the presence/absence of auditory signals was defining the alerting effect; the use of visual valid/invalid cues was defining the orienting, while the congruency/incongruence target type was unchanged for the conflict effect. Using uninformative cue for the orienting effect, exogenous orienting effect was measured independent

from an endogenous component. The use of auditory and visual warning signals by Callejas allows to better separate the alerting and orienting effect and thus to investigate performance as a joint function of both the effect, as well as the interaction among networks. Results showed significant interactions among all the effects: the conflict was inhibited by the alerting, but facilitated by the orienting. This last one was facilitated by the alerting, when the stimulus onset asynchrony was short (Callejas et al., 2005). Finally the three attentional networks scores, as assessed by the ANT, were found to be related to each other and thus operating in an interactive manner.

Since the first report on the ANT, the test has been widely used and an fMRI version was created to assess the brain correlates of the three attentional components (Fan et al., 2005). The fMRI adapted version of the ANT resulted in three cue conditions (no cue, center cue and spatial cue), and two target conditions (congruent and incongruent), eliminating thus the double cue condition as well as the neutral target condition. Behavioural analyses were defined similarly to the previous study with exception of the alerting effect, which was calculated by subtracting the mean RTs of the center cue condition from the mean RTs of the no cue condition. The logic was that both these conditions elicited sustained attentional resources, but the center cue provided also temporal information about the incoming target. Thus the facilitating effect of the temporal cues reduced the RTs in comparison to the no cue condition, allowing to obtain in each case positive score. To isolate brain activity associated with the alerting effect, the logic used was the opposite compared to the behavioural analysis. Thus activity elicited from the alerting effect was determined by contrasting the no cue condition to the center cue condition, in order to isolate brain regions more active in the center cue condition. Brain activity related to the orienting effect was obtained subtracting the center cue condition from the spatial one, as this indexed the location at which the target appears before its occurrence. For the conflict effect, the subtraction used to isolate the relative regions was the same used for behavioural analysis. Behavioural results were similar to the ones previously

described and no significant correlations between the network scores were found. Regarding fMRI results, the alerting network was characterized by increased activity in the inferior frontal gyrus, in the superior and inferior parietal lobe, in the fusiform gyrus, in the superior temporal gyrus and in bilateral thalamic area. The orienting effect was associated with bilateral activity in the superior parietal lobe, fusiform gyrus, superior frontal gyrus and precentral gyrus. Activity associated with the conflict effect was found in the anterior cingulate, bilateral frontal areas, fusiform gyrus and thalamus. A complete list of the regions elicited by each attentional subcomponent, as well original brain maps from the authors, is provided in annex n° 1.

Fan and colleagues capitalized that these results support the hypothesis that each network, identified by each subtraction, were rather distinct and associated with specific anatomical brain regions, providing evidences for the independency of the subcomponents. Interestingly MacLeod and colleagues, collecting data from 15 unique studies, provided an analysis of the psychometric properties of the ANT (Macleod et al., 2010). The results have shown low reliabilities (based on RTs) for the alerting and the orienting network, and moderately high for the executive control. As reported by the authors, both analysis of variance and correlational analyses suggested that the three networks measured by the task are not independent.

The independency of the attentional networks would imply a complete dissociation between relative anatomical brain regions. Areas like the fusiform gyrus were actually found to be activated in each of the three networks. The anterior intraparietal sulcus was found to be activated by both alerting and orienting effects. Common activation for the alerting and conflict were found also in the thalamus.

However the idea to integrate, within a single task, the three attentional subcomponents appears really attractive. Therefore, it would be relevant to address whether sleep deprivation has a differential and selective influence on the different components of attention.

CHAPTER 3

Interindividual variability in sleep-wake regulation: Impact on cognition

1. Introduction

Large inter-individual variability exists in the synchronization of numerous human behaviors. This is true for preferences in sleep-wake timing, optimal time-of-day to adequately perform cognitively demanding tasks, but also for sleep related variables as sleep duration or sleep architecture, as well as behavioral response to sleep loss. Experimental evidence suggests that some aspects of sleep related inter-individual variability are systematic, and could represent trait-like characteristics. However not all the variability among individuals is stable over time and is attributable to a trait: environmental factors also play a role.

Replicability and robustness are essential to define inter-individual variability as a trait-like characteristic (Van Dongen et al., 2005; Tucker et al., 2007; Van Dongen and Belenky, 2009) and to distinguish it from variability related to state-specific circumstances, as differences in sleep/wake history or related to different experimental settings. Repeated measurements, within individuals, are needed to prove that specific characteristics are stable within individuals and at the same time significantly different across subjects. Moreover these differences must be replicable over repeated experimental challenges in order to establish robustness. Another aspect that could play a fundamental role

is to establish specific inclusion/exclusion criteria, aimed to reduce systematic state-specific variability in order to boundary state-dependent variations. To assess inter-individual variability, an accurate selection of the population is essential. Indeed variability is bigger in heterogeneous samples, and this issue could be a confounding factor if specific analyses, approaches or protocols are not taking in account sample characteristics. Differences in population sample, or in experimental tasks used, could easily limit the generalization of the data or make difficult for the comparison of the results across studies.

Individual differences may be ascribed to specific phenotypes that, in turn, may be modulated by genetic factors. In this chapter, we provide an overview on key sleep-wake phenotypes, as well as try to summarize inter-individual differences in the response to sleep loss. These macroscopic system-level individual differences may be putatively linked to differences in brain function. Thus we will review studies that investigated the cerebral correlates of specific phenotypic traits and those associated to individual differences in the response to sleep deprivation.

2. Phenotypic traits in sleep-wake behavior

Individual variations in the circadian timing system impact on the daily temporal organization of human behaviors. One of the major sources of interindividual variation is the tendency to show greater morning or evening preferences to perform daily activities. Differences in timing preferences are expressed in favorite rest-activity periods, such as specific sleep habits (Taillard et al., 2003), which reflect individual's chronotype, defined here as the individual temporal preferences for the organization of activity/rest pattern. At one end of the continuum are extreme morning types ("larks"), and at the other extreme evening types ("owls"). The former wake-up very early, and have difficulties to remain awake beyond their usual bedtime.

Extreme evening types prefer to go to bed in the late night hours and experience difficulties to get up in the early morning hours. Moreover

evening types tend to have greater differences in sleep timing between work and free days (Wittmann et al., 2006). Compared to evening types, morning types go to sleep and wake-up 2-h earlier (Kerkhof, 1991; Natale and Cicogna, 2002). The morningness-eveningness chronotype can be evaluated using self-report questionnaires, which investigate timing of activities on work days and free days. The most used questionnaires are the Morningness-Eveningness Questionnaire (MEQ; Horne and Ostberg, 1976) and its variants (i.e., see Smith et al., 1989), as well as the more recently developed Munich Chronotype Questionnaire (MCTQ; Roenneberg et al., 2003). Morningness has been reported to be an important predictor of habitual sleep need, as expressed by total sleep time or time spent in bed: taking into account working and free days, morning types seem to spend less time in bed than evening types (Wittmann et al., 2006).

Importantly, it has been observed that homeostatic sleep regulation differs between chronotypes. Morning types tend to have a larger decrease of delta and theta activity across the first sleep cycles of the night (Kerkhof, 1991). Those results were confirmed by a study of Mongrain, where morning types had a faster decay rate of slow-wave activity, throughout the night, relative to evening types (Mongrain et al., 2006a). Furthermore, morning types have higher absolute SWA in anterior areas at the beginning of the sleep episode (Mongrain et al., 2006b) (**Figure 9**). Higher SWA at the beginning of the sleep episode could represent higher homeostatic sleep pressure, due to a faster build-up during wakefulness. These results go in line with data by Taillard, who showed that waking theta-alpha EEG power differs between chronotypes, with evening types showing a slower increase in sleep pressure (Taillard et al., 2003). Another source of interindividual variations is constituted by the length of the habitual sleep episode. As for “larks” and “owls”, human subjects could be classified in short and long sleepers.

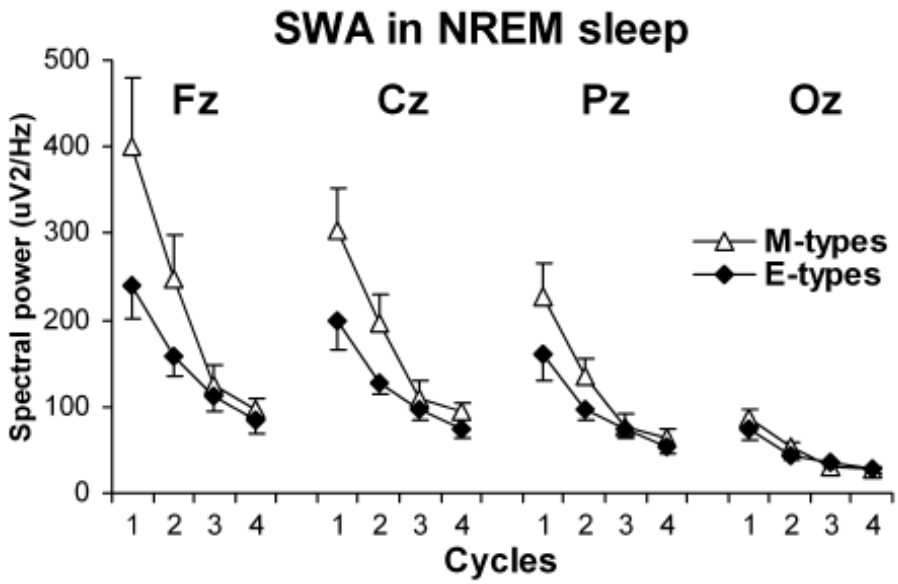

Figure 9: SWA in NREM sleep during the first four cycles, for frontal, central, parietal and occipital derivations (mean and SEM). M-types: morning types; E-types: evening types. The decrease in SWA, between cycle 1 and cycle 3 was significantly larger ($p < 0.05$) in morning types as compared to evening types, for Fz and Cz derivations (Mongrain et al., 2006b).

A short sleeper is a person who has habitual sleep duration lower than 6-h, while a long sleeper typically sleeps for more than 9 hours (Aeschbach et al., 1996). SWA decrease during sleep after sleep deprivation may not differ between short and long sleepers (Aeschbach et al., 2001). However, simulations of the homeostatic sleep process, based on the theta/low-frequency alpha activity (TLFA, power density in the 5.25-9.0 Hz band) indicate that short sleepers have higher absolute TLFA (Aeschbach et al., 2001). Moreover this difference was still present even after the recovery night, under sleep satiation conditions. Given that TLFA has a strong wake-dependent component (Aeschbach et al., 1999), short sleepers may live under higher homeostatic sleep pressure and tolerate it more than long sleepers (Aeschbach et al., 2001). However, shorter habitual bedrest duration maybe ascribed to differences in sleep debt. For instance, bedrest duration increases when individuals do not have to schedule wake time, e.g. during the weekend. Longer sleep durations during the weekend, as compared to working days, may reflect inadequate daily sleep duration and probably reflect a

society that is chronically sleep deprived. Habitual bedrest duration does not appear to be an accurate reflection of sleep need, as lifestyle may influence sleep habits, leading to self-selected sleep restriction or wake extension (Klerman and Dijk, 2005). Indeed sleep extension (16-h of sleep opportunity) for three consecutive days increases total sleep time on the first day. However this increase may decline across the days and during the third day, total sleep time can be negatively associated with habitual bedrest duration (Klerman and Dijk, 2005).

3. Genetic correlates of inter-individual differences in sleep wake regulation

Interindividual variability in morning or evening preferences may be driven by the circadian system (Landolt and Dijk, 2010). As described in the first chapter, the circadian system consists of a network of interlocked transcriptional-translational feedback loops, involving clock-related genes (Ko and Takahashi, 2006). The output of this molecular machinery might be sleep-wake phenotypes, as morningness-eveningness chronotypes (Katzenberg et al., 1998; Archer et al., 2003; Johansson et al., 2003; Pereira et al., 2005). Katzenberg and colleagues were the first to report an association of a polymorphism in the *Clock* gene with diurnal preference, as assessed by the Horne-Östberg questionnaire (Katzenberg et al., 1998). Clinical genetic studies suggested that mutations in clock genes might be involved in the susceptibility to circadian rhythm sleep disorders. In a Japanese population, the H4 haplotype for the human *Period3* gene (*hPer3*) was associated with a specific circadian sleep disorder, the Delayed Sleep Phase Syndrome (DSPS) (Ebisawa et al., 2001). DSPS is characterized by delayed sleep onset and wake-up times by three to six hours relative to conventional sleep-wake schedules. In a similar vein, Archer and colleagues focused the region of influence of the *PER3* gene, involved in DSPS and in extreme diurnal preference, to a variable number of tandem repeated (VNTR) in the exon 18 (Archer et al., 2003; **Figure 10**). The VNTR polymorphism within the coding region of *PER3* encodes either 4 or 5 tandem copies of an 18-aminoacid motif (Jenkins et al.,

2005). The VNTR four-repeat allele was associated with extreme eveningness Horne-Östberg scores and DSPS in an English population, while the frequency of the five-repeat allele was significantly higher in "morning-preference" group (Archer et al., 2003). None of the individuals homozygous for the longer allele of *PER3* (*PER*3$^{5/5}$) were found in the DSPS group and the 75% were found to be homozygous for the shorter allele (*PER*3$^{4/4}$).

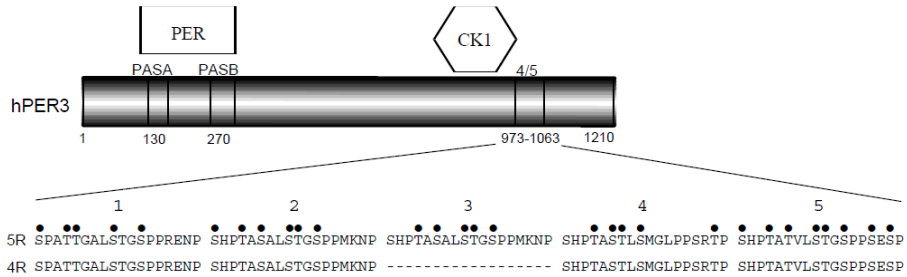

Figure 10: Schematic diagram of the human PER3 protein. Binding to other PER molecules occurs at the PAS A and B domains. The 5- and 4- repeat region amino acid sequences are shown below the sequence (5R and 4R, respectively). The repeats are numbered 1-5 and the one that is missing in the 4-repeat allele is indicated by dashes. Predicted targets for caseinkinase 1 (CK1) ε phosphorylation are indicated above by filled circles (from Archer et al., 2003).

In a later study with a larger sample, the same group reported a difference between the morning and evening types associated with *PER3* polymorphism across different age groups (Jones et al., 2007). Data from that report pointed in a direction of attenuation of the associative strength between allele frequencies and extreme preference with age; more precisely, extreme diurnal preference in young individuals, was more closely associated with the specific polymorphism than in other age groups.

Pereira and colleagues replicated the association with diurnal preference in the Brazilian population (Pereira et al., 2005). Surprisingly, the authors also found that 30% of the DSPS patients were homozygous for the 5-repeat allele. Excluded ethnic bias in the sample, these unexpected results lead the authors to hypothesize a possible latitudinal influence (Pereira et al., 2005). Day length (important for photoentrainment) at two different latitudes (London and São Paulo)

may explain that the same genotypes exposed to different photoperiods may result in different phenotypes. In a successive study, Archer and colleagues (Archer et al., 2008) assessed the association between habitual sleep timing and the rhythms of melatonin and of cortisol in individuals homozygous for the *PER3* VNTR polymorphism. A robust association between habitual sleep timing and circadian parameters was observed for both genotypes. Moreover, the association between habitual sleep timing and the variation in the circadian phase of peripheral (leukocytes) clock genes (*BMAL1*, *PER2* and *PER3*) was investigated. Only individual *PER3* rhythms correlated significantly with habitual sleep-wake timing and the circadian rhythm of melatonin and cortisol. Moreover the correlation between sleep timing and peripheral blood *PER3* expression was stronger in individuals homozygous for the longer allele of *PER3* (*PER*3$^{5/5}$), the variant of *PER3* that has been previously associated with morning preference. These data suggest a tight circadian control in *PER*3$^{5/5}$ individuals, suggesting a stronger stability of sleep-wake timing in morning types (Dijk and Archer, 2010a). Moreover these data are coherent with recent results showing a greater susceptibility in *PER*3$^{5/5}$ human subjects to the effect of light exposure on alertness and melatonin (Chellappa et al., 2012). *PER*3$^{5/5}$ volunteers exhibited a stronger alerting response[2] to blue-enriched light, reflected by a decrease of salivary melatonin in *PER*3$^{5/5}$ but not in *PER*3$^{4/4}$ participants. As showed in **Figure 11**, exposure to blue-enriched light at 6500 K, compared to light at 2500 K, induced a more pronounced decrease in the evening rise of melatonin in *PER*3$^{5/5}$ than in *PER*3$^{4/4}$. Moreover theta activity, a hallmark for sleepiness, was more reduced during short-wavelength light exposure in *PER*3$^{5/5}$ than in *PER*3$^{4/4}$ individuals. Individual sensitivity to light also impacts on cognitive brain function (Vandewalle et al., 2011). In a functional magnetic resonance imaging (fMRI) study, monochromatic blue light increased ongoing

[2] Alerting response, in this context, is conceptualized as a change in subjective (Karolinska Sleepiness Scale scores) and objective (wake theta activity) sleepiness during the light exposure period, as compared to the prelight condition.

nonvisual brain activity in thalamo-frontoparietal circuit in *PER*3$^{5/5}$ as compared to *PER*3$^{4/4}$ volunteers at specific circadian phases. These genotype differences were particularly evident in the morning after sleep loss. Collectively these data indicate that light, through its direct alerting properties, impinge differentially on a clock gene polymorphism involved in sleep-wake regulation. In the first chapter we described how the transcriptional-translational cycle, involving genes like *CLOCK*, *BMAL1*, *PER1-3*, *and CRY1-2,* takes approximately 24 hours for completion. We also described the Period gene family as a central component of the internal clock, as it provides negative auto-feedback (Archer et al., 2003).

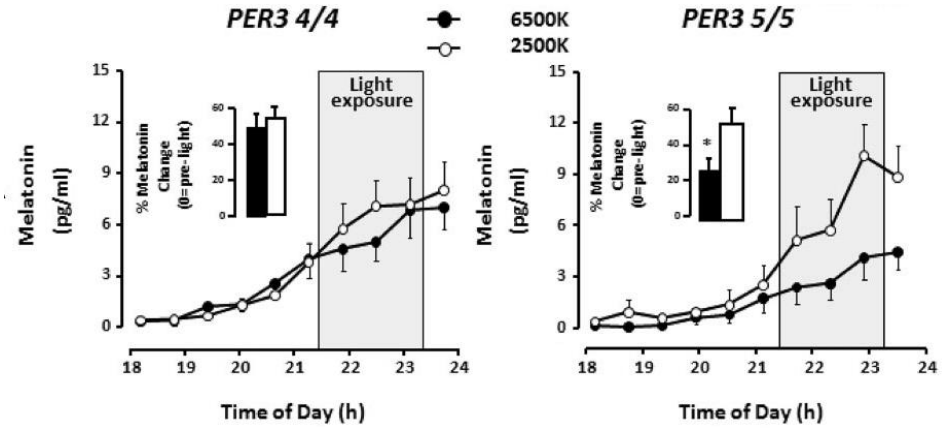

Figure 11: Light effects on melatonin in *PER3*$^{4/4}$ (left panel) and *PER3*$^{5/5}$participants (right panel). In both panels, the time course of salivary melatonin is plotted and the insets represent salivary melatonin levels during 2 h of light exposure adjusted to pre-light exposure (percentage of melatonin change). Exposure to blue-enriched light at 6500 K, compared to light at 2500 K, induced a more pronounced decrease in the evening rise of melatonin in *PER*3$^{5/5}$ than in *PER*3$^{4/4}$ (Chellappa et al., 2012).

An interesting characteristic of the *PERIOD3* polymorphism is that the repeated motifs in the protein contain numerous potential phosphorylation sites. Phosphorylation affects the stability of clock proteins, raising an interesting role for *PER3* (Dijk and Archer, 2010b). Indeed many of the genes that contribute to regulate circadian timing alter PER phosphorylation and its stability. This led to the idea that the rate of *per3* phosphorylation determines the speed of the clock (Blau, 2008). Summarizing the aforementioned data, we saw that a genetic

predisposition to the diurnal preferences is well established, with individuals homozygous for the 5-repeat allele, in the *PER3*polymorphism, associated with morning types. We also presented researches supporting the association between habitual sleep timing and phase of the rhythm of mRNA of several clock genes in leukocytes. This association was found to be particularly strong in *PER*3 compared to other clock genes. Moreover this association was more robust in individuals homozygous for the longer allele of *PER3* (*PER*$3^{5/5}$). In summary, due to its well established association with circadian and homeostatic phenotype, the *PERIOD3* polymorphism represents an interesting genetic marker to investigate the complex interaction between homeostatic and circadian processes. Thus we will focus on how the *PER3* polymorphism impacts on sleep parameters and neurobehavioral performance.

4. Inter-individual variability to sleep deprivation: Evidences from *PER3* polymorphism

Following a genotype approach, where volunteers are recruited on the basis of their *PER3* polymorphism, Viola and collaborators quantified the contribution of this clock gene to sleep-wake regulation in humans (Viola et al., 2007). After a baseline night, participants underwent a 40-hour sleep deprivation protocol under stringent constant routine conditions, followed by a 12-hour recovery sleep episode. No differences between the two allele groups were found in the amplitude and timing of cortisol or melatonin. Moreover *PER3* mRNA, extracted from peripheral blood mononuclear cells, did not differ between the two genotypes either in amplitude or in mean levels. Thus, no differences were ascribed to the circadian system. However, key variations were observed for sleep homeostasis. During baseline sleep, *PER*$3^{5/5}$ volunteers displayed a greater homeostatic sleep pressure, as indexed by shorter sleep latency, and higher percentage of time spent in slow-wave sleep. In keeping with these data, decomposition of EEG signal into its constituting frequency components revealed higher initial values of NREM sleep SWA (**Figure 12**), a steeper SWA decay, and more

REM alpha activity. During extended wakefulness, *PER*3$^{5/5}$ showed a faster build-up of homeostatic sleep pressure, as reflected by a steeper rise of waking theta EEG activity. In *PER*3$^{5/5}$, sustained wakefulness increased more rapidly the occurrence of slow eye movements (SEMs), a well-established marker of sleepiness (for a review see Cajochen et al., 2003). Furthermore neurobehavioral performance differed between the genotypes, such that, when wakefulness was extended into the biological night, a pronounced deterioration in cognitive performance was observed in *PER*3$^{5/5}$ individuals. Importantly, the genotypes differed in their response to sleep loss during a subsequent sleep. In line with the baseline night, SWA in NREM sleep and alpha activity in REM sleep were higher in *PER*3$^{5/5}$ during the recovery night.

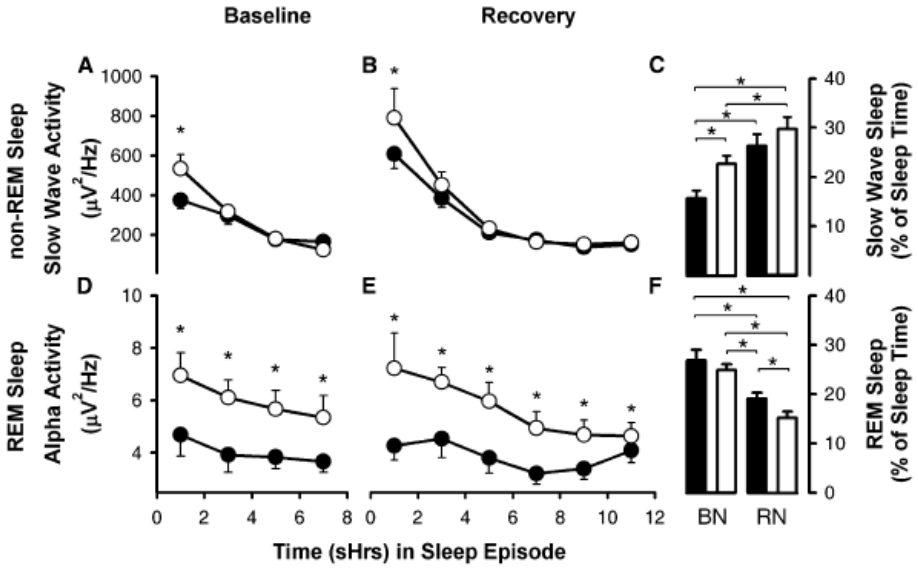

Figure 12: Dynamics of EEG Slow-Wave Activity in non-REM Sleep, EEG Alpha Activity in REM Sleep, and response to sleep deprivation differ between *PER*3$^{5/5}$ and *PER*3$^{4/4}$ volunteers (A, B, D, and E). Time courses (mean ± standard error of the mean) of slow-wave activity (0.75–4.5 Hz) in non-REM sleep (stages 1–4) and alpha activity (8–12 Hz) in REM sleep during the baseline and recovery sleep episodes for *PER3*$^{5/5}$ (open symbols) and *PER3*$^{4/4}$ (filled symbols) participants. (* indicates a significant difference between genotypes, $p < 0.05$.) (C) Slow-wave sleep time and (F) REM sleep time expressed as a percentage of total sleep time (±standard error of the mean) for baseline and recovery sleep in *PER3*$^{5/5}$ (white bar) and *PER3*$^{4/4}$ (black bar) participants (from Viola et al., 2007).

As summarized by Dijk and Archer in a recent review (Dijk and Archer, 2010a), results concerning *PER*3$^{5/5}$ individuals mimic in some way

characteristics previously observed in morning types. Shorter sleep latencies, higher percentage of SWS, higher SWA at the beginning of the night, faster decay rate of slow-wave activity throughout the night, as well as faster increase of theta activity during sleep deprivation, are some characteristics in common with morning types. Thus the hypothesis done by Mongrain, that morning types live under higher homeostatic sleep pressure due to its faster build-up during the waking period, seems to apply also to *PER*3$^{5/5}$ individuals.

In the study of Viola and co-authors, cognitive performance was evaluated based on composite scores derived from a large battery test including 12 different tasks (Viola et al., 2007). In a later work, Groeger and colleagues analyzed cognitive performance at individual tasks composing the battery test used in the previous study (Groeger et al., 2008). The authors showed that the performance decrement, in individuals homozygous for the longer repeat allele was more pronounced on executive functioning, such as more demanding versions of a working memory task. Interestingly these results go in line with several neuroimaging studies showing that executive components are particularly affected by sleep deprivation and also being related to frontal cortical areas.

PER3 polymorphism is also be involved in how we cope with partial sleep restriction. Using a partial sleep deprivation paradigm (4h of sleep) Goel al. tried to replicate the results obtained by Groeger and colleagues, by investigating the response to chronic partial sleep deprivation (Goel et al., 2009a). The rationale of using partial sleep deprivation was justified by the Authors as a condition experienced every day by millions of people in our 24 hours society. With this foreword, volunteers were selected on the basis of their *PER3* polymorphism, including *PER3*$^{4/5}$ subjects, and characterized for their cognitive performance, sleepiness and physiological response to a partial sleep deprivation that results in restricting sleep to 4 hours of time in bed for five consecutive nights.

Results showed that although during the baseline night the three genotypes did not differ significantly for any sleep measures, during the partial sleep deprivation *PER3*$^{5/5}$ volunteers showed higher slow-wave activity and slow-wave energy (delta power in NREM sleep, SWE) compared to *PER3*$^{4/4}$ and *PER3*$^{4/5}$ individuals. However no differences were observed across the genotypes for the effect of sleep loss on neurobehavioral performance. The three genotypes did not show differential responses neither at the PVT, at the Digit Symbol Substitution Task (DSST, Wechsler, 1997)or at the Digit Span task (DS, Wechsler, 1997). However when the authors compared only the two homozygote groups, using Viola approach, they found that *PER3*$^{5/5}$ participants had better cognitive throughput than *PER3*$^{4/4}$ individuals in response to sleep loss, as reflected by higher scores at DSST across days. Assessment of executive functions also failed to show significant differences across the *PER3* genotypes. There are two points that perhaps need to be further discussed, and concern the contrasting results obtained from Goel compared to the results of Groeger as well as of Viola.

As aforementioned, in the study of Viola *PER3*$^{5/5}$ participants were found to have already at baseline levels shorter sleep latency as well as higher initial values of SWA, reflecting higher homeostatic sleep pressure compared to *PER3*$^{4/4}$. This result was not replicated in the study of Goel; however, as honestly recognized also by the Authors, this difference could be related to the protocol used. The key differences between these two studies arise from a stream of reasons. First, in the study of Goel, during the first two nights (representing the baseline) was given a 10 hours opportunity of time in bed. This strategy was aimed to reduce any pre-existing difference in sleep debt between the genotypes since the results of Viola were pointing for a difference already during the baseline night. If it is true that Viola's participant had shorter habitual sleep duration, as assessed in the field study before to enter in the laboratory, it is also true that there were no significant differences between the two polymorphisms. Moreover, Viola and colleagues were the first to observe the impact of a gene polymorphism heretofore

related to clock processes onto human sleep homeostatic markers. Thus, they could not draw a definite conclusion as to why sleep baseline differences occurred. This point probably let him leaning toward a classical approach used in chronobiology, where a first step is to characterize, throughout wrist actigraphy of several weeks, individual habitual sleep-wake schedule and then adapt the protocol on those timetables. The other clarification concerns the lack of behavioral differences in executive functioning between the different polymorphisms in the study of Goel. It has been already shown that sleep deprivation leads to a decrease in cognitive performance not only depending from the cognitive domain investigated, but also in a task specific manner. Behavioral data have showed that complex tasks seem to be less sensitive to performance deterioration connected to sleep deprivation. Importantly task characteristics (as duration and complexity) are pivotal when assessing vulnerability to sleep loss (Doran et al., 2001). So even if both Goel and Viola look at possible differences in the executive functioning domain, contrasting results could emerge from difference in the kind of tests used, or in the case of the DSST could be due to task duration or to the higher number of items types. Lastly, the time-of-day when the maximal deterioration was observed in Viola et al. occurred during the biological night, and this specific time was not assessed in Goel et al., as participants were asleep. A key similarity between these studies was the absence of circadian effects, and marked differences in the homeostatic regulation of sleep. In a study designed to investigate heritability of decrement in response to sleep loss, in monozygotic and dizygotic twins, Kuna and collaborators successively assessed the effects of the polymorphisms of *PER3*. Similarly to the study of Goel, Kuna and collaborators did not find an association between the VNTR polymorphism of PER3 and neurobehavioral performance decrement in response to sleep loss(Kuna et al., 2012). The authors strongly criticize previous studies on genetic associations and performance decrements, underlying the fact that the small sample size could not only lead to false-negative but also to false-positive results. Lo and colleagues in a cross-over design of two 12-days,

where participants underwent to a partial and a total sleep deprivation, assessed neurobehavioral performance across seven different cognitive domain, in a sample stratified for PER3 polymorphism (Lo et al., 2012). As shown by the authors, great differences between the polymorphisms as well as variation across the task were observed during the morning hours after total sleep deprivation (Lo et al., 2012). Importantly subjective alertness and sustained attention were more affected than other cognitive domains, particularly in *Per3*$^{5/5}$ participants. More recently Maire and colleagues compared the effects of low and high sleep pressure conditions on circadian modulation of cognitive performance in *PER3* genotypes participants (Maire et al., 2014b). The authors compared in the same group of individuals different homeostatic sleep states, by increasing sleep pressure through a 40-h sleep deprivation or decreasing it through 10 cycles of 160 min. of wakefulness and 80 min. sleep opportunity, to assess the interaction between circadian and homeostatic processes with respect to *PER3* VNTR polymorphisms. Results has shown that *PER3*$^{5/5}$ participants produced more slow eye movements (SEMs) and had more unintentional sleep episodes (USEs) compared to *PER3*$^{4/4}$ under high sleep pressure conditions (**Figure 13**). *PER3*$^{5/5}$ volunteers produced, independently from sleep homeostatic state, more SEMs and USEs during the biological night and at the beginning of the second biological day. Moreover *PER3*$^{5/5}$ had more attentional lapses (reaction times superior to 500 milliseconds), compared to *PER3*$^{5/5}$ during the high sleep pressure condition. Finally the analysis on overall sleep episodes, in the low sleep pressure condition, revealed that *PER3*$^{5/5}$ carriers had higher total sleep time and sleep efficiency compared to the short allele group. Data from Maire seems to confirm the hypothesis of Dijk and Archer that postulates a faster build-up of the homeostatic sleep pressure in the long allele polymorphism (Dijk and Archer, 2010a). In another recent work, it has been shown not only that attentional lapses varied with test timing (as already known) but also that they were more frequent at the last portion of the attentional task used, with more numerous lapses happening during the biological night (Maire et al., 2014a). Further

analyses showed that this effect was driven by *PER3*$^{5/5}$ individuals that produced more lapses during the biological night test session compared to *PER3*$^{4/4}$ carriers. The involvement of *PER3* in sleep homeostasis and in the timing of sleep and wakefulness emerged also from animal research (Hasan et al., 2014). Hasan and colleagues recorded wheel-running activity and EEG activity in wild-type and in *PER3*-deficient mice.

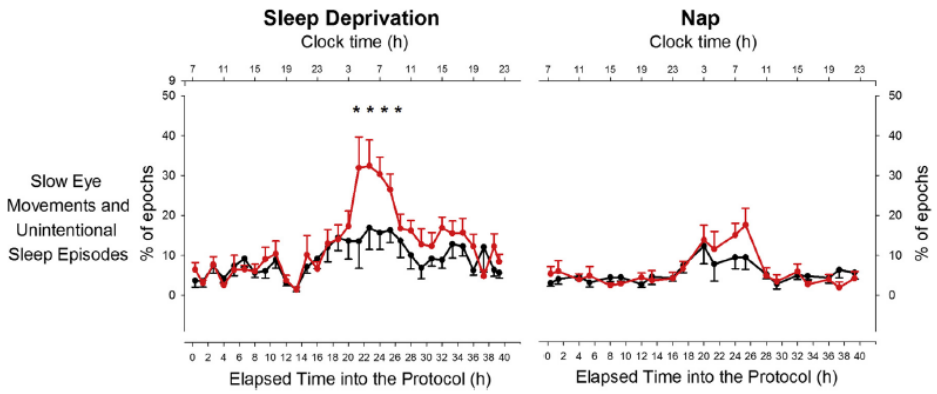

Figure 13: Time course of slow Eye Movements and unintentional sleep episodes % of epochs per bin during sleep deprivation (left panels) and nap (right panels) by genotype; *PER3*$^{5/5}$: red lines, *PER3*$^{4/4}$: black lines. Clock time indications refer to 7 a.m. wake-up time. Asterisks represent *p* values below .05 for post-hoc comparisons derived from the separate analysis computed for the SD protocol (Maire et al., 2014b).

The protocol included a 24-h baseline episode followed by a 6-h of sleep deprivation and a subsequent 18-h period of recovery sleep. Results showed that while global activity as well as vigilance states did not differ between the genotypes, the temporal distribution of these two variables and of EEG delta activity was differently modulated. Under baseline conditions no differences between the two genotypes were detected in NREM sleep, REM sleep or total time spent awake. However a 2-h resolution analysis showed that vigilance states, of the two genotypes, differ in their temporal distribution. *PER3*-deficient mice showed less wakefulness, more NREM and REM sleep at the beginning of the light period, higher NREM EEG delta activity at the beginning and the end of the baseline dark period, as well as higher delta power during the last 9-h of the recovery sleep episode, and slower REM sleep accumulation during the period of recovery sleep. A last marker of

increased homeostatic sleep pressure, the increase of theta power during REM sleep, was found to differ between the two genotypes. Thus the Authors have shown how phenotypes, emerging from functional knockout of *PER3* in mice, suggest still once a role for this gene in sleep homeostasis and waking activity. The same research group, in a more recent work, assessed circadian and homeostatic response to sleep loss in transgenic mice expressing the human 4- and 5-repeat of the *PER3* VNTR(Hasan et al., 2014). As sleep loss affect cortical clock gene expression (Mongrain et al., 2011), the Authors investigated the effect of VNTR on sleep related gene expression in the cortex and in the hypothalamus of these transgenic mice, under baseline conditions and after 12 hours of sleep deprivation. *Per3*$^{5/5}$ mice showed higher EEG theta activity during sleep deprivation as well as higher delta power during sleep. Contrary to wild-type mice and *Per3*$^{4/4}$, during the recovery sleep the 5-repeat allele mice showed a greater and more complete sleep homeostatic response, as indicated from one side by higher SWE accumulation and from the other one by returning to baseline level. No differences were found between hypothalamic and cortical expression of circadian clock genes while differences were observed in the expression of sleep homeostatic related genes (*Homer1*, *Ptgs2* and *Kcna2*were all down-regulated in the *Per3*$^{5/5}$). These results not only show coherence with human data, but further reinforce the idea of an involvement of *PERIOD3* in the homeostatic regulation of sleep and wakefulness.

5. Cerebral correlates of circadian and homeostatic interaction: Evidence from neuroimaging studies

Neuroimaging studies offer privileged insights to the comprehension of brain activity correlates. Recent neuroimaging studies capitalized on brain activity correlates of circadian misalignment or individual differences in response to sleep loss (Schmidt et al., 2009, 2012; Vandewalle et al., 2009). Schmidt and collaborators recruited healthy young individuals based on their diurnal preferences and stratified as extreme morning or evening types, as a means to investigate individual

variations in brain activity. The two groups of chronotypes underwent morning and evening fMRI sessions, according to their individual unconstrained sleep-wake schedules. This strategy was chosen to control for circadian phase differences between chronotypes, as well as equivalent amounts of time spent awake. Participants performed an fMRI version of the psychomotor vigilance task (PVT; Dinges and Powell, 1985) under low and high sleep pressure conditions (respectively 1.5 and 10.5 hours after habitual wake-up time). Functional MRI analyses focused on two main PVT outputs:

- trials associated with fastest reaction times (RTs; reaction times below 10^{th} percentile of overall RTs), which reflect optimum response capabilities (Drummond et al., 2005) that transiently recruit above and beyond the baseline alertness levels to the same extent by the two chronotypes.
- trials associated with intermediate reaction times (RTs between the 90^{th} percentile and 10^{th} percentile), which reflect an average alertness level to perform adequately and correspond to a global alertness deemed to be particularly sensitive to the effects of sleep pressure.

Under low homeostatic sleep pressure, during the morning session, only minimal differences were detected between chronotypes. However, during the evening session (higher sleep pressure), global alertness was associated with increased response in a thalamic region, compatible with the anterior part of the pulvinar, in morning as compared to evening types. The pulvinar relates to attentional domain and its activation (relative to attentional task) changes as a function of arousal (Portas et al., 1998). Results from primates point out that the pulvinar can facilitate the transmission of information regarding attentional priority across the cortex (Saalmann et al., 2012). Furthermore, in Schmidt et al. optimal attentional capability (fastest RTs) in the subjective evening was associated with larger responses, in evening compared to morning chronotypes, in a brainstem region compatible with the locus coeruleus (LC) and the suprachiasmatic area (SCA, an

anterior hypothalamic region that encompassed the SCN)(**Figure 14**). Both LC and SCA are involved in the generation of the circadian arousal promoting signal (Gaggioni et al., 2014). Moreover, the responses in the hypothalamic area decreased while homeostatic sleep pressure increased, as assessed by slow-wave activity recorded during the first sleep cycle. This suggests a direct influence of homeostatic and circadian interactions on the neural activity underpinning diurnal variations in human behavior. Finally these results provide evidence that circadian and homeostatic processes interact within hypothalamic regions to modulate human cognitive performance. In a subsequent study (Schmidt et al., 2012), the same group addressed the impact of sleep-wake regulation on brain responses in chronotypes in executive processing, using the Stroop paradigm (Stroop, 1935). From the subjective morning to evening, evening types maintained or even increased executive-related responses in a set of brain regions know to play a crucial role in successful inhibitory processes. Conversely in morning types those regions were observed to decrease under the same conditions (Schmidt et al., 2012).

BOLD activity related to the inhibitory response in the posterior part of the hypothalamus, during evening hours, was negatively related to SWA at the beginning of the night for morning types. Conversely no significant results were found for evening types. However, the hypothalamic area was more posterior compared to the one observed in the previous study, and was compatible with the lateral hypothalamus (LH). This hypothalamic portion contains orexin neurons, which have been shown playing a fundamental role in the promotion and maintenance of wakefulness and arousal signal (Saper et al., 2001; Adamantidis et al., 2007).

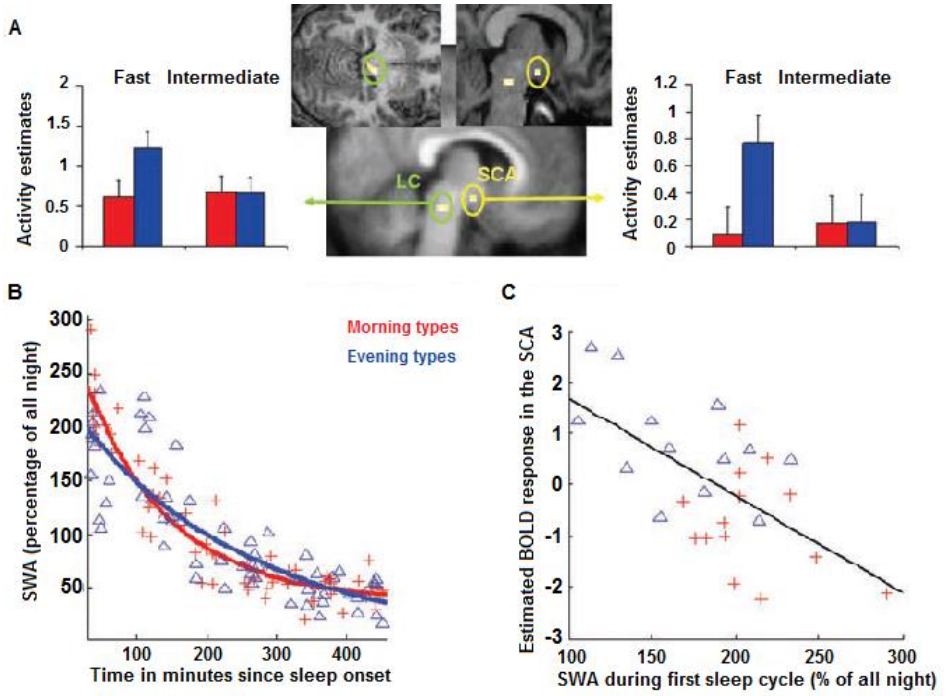

Figure 14: (A) Increased task-related response in the dorsal pontine tegmentum and the anterior hypothalamus, compatible with the LC and SCA, respectively, in evening as compared with morning chronotypes during the subjective evening. Display shows areas where BOLD activity is associated with the main effect of chronotype during the evening session for optimal alertness. Functional results are displayed at $p > 0.001$, uncorrected threshold, over the mean normalized structural MR image of the population. Corresponding parameter estimates (arbitrary units) are displayed for event indicators of fast and intermediate reaction times (red, morning types; blue, evening types). (B) Exponential decay function [$SWA_t = SWA_\infty + SWA_0 \times e^{-rt}$ (16)] adjusted on relative SWA in sleep cycles (NREM sleep) measured from the central frontal derivation for all-night EEG of the night preceding the evening scan acquisition. Red crosses, morning types; blue triangles, evening types. SWA0 and r values are significantly higher in morning relative to evening types ($p > 0.05$). (C) Regression analysis of the relation between estimated BOLD responses during optimal task performance in the SCA region and the amount of SWA during the first sleep cycle in the preceding night ($r = 0.54$, $P < 0.05$, $n = 27$). Red crosses, morning types; blue triangles, evening types (from Schmidt et al., 2009).

These findings suggest that, in evening types, when homeostatic sleep pressure is higher (evening hours), brain activity related to executive control depend on posterior hypothalamus to ensure adequate arousal signal. In morning types, the arousal signal could be less pronounced at the same circadian phase and consequently less efficient to counteract increased sleep pressure.

In an fMRI study, young healthy individuals were prospectively recruited on their *PER3* genotype, to assess the effect of sleep loss on brain activity (Vandewalle et al., 2009). Participants were involved in a working memory task (auditory 3-back task; (Cohen et al., 1997a) during morning and evening fMRI sessions. These sessions were repeated in two different experimental segments, during which fMRI sessions were separated by either sleep or total sleep deprivation. The evening session was scheduled 2-h before habitual bedtime, within the putative wake-maintenance zone, while the morning session 1.5-h after habitual wake-up time. Across a normal waking day, $PER3^{5/5}$ individuals showed reduced activation in the posterior dorsolateral prefrontal cortex (DLPFC), a region implicated in higher executive-control processes (Koechlin et al., 2003), in the evening as compared to the morning sessions. By contrast, $PER3^{4/4}$ individuals did not show changes in brain responses during the normal waking day. In a second step, comparisons were made between the two morning sessions (1.5 h vs 25 h of wakefulness, same circadian phase but under low and high sleep pressure, respectively). Briefly $PER3^{5/5}$ volunteers showed decreased activity in several brain areas (**Figure 15**), consisting of occipital, parietal and temporal cortices, as well as of DLPFC (already present as pattern across a normal waking day). However, $PER3^{4/4}$ participants did not show decreased pattern activity but instead maintained similar brain responses after sleep deprivation. Moreover in the short allele carriers, sleep deprivation led to additional increase in brain response in the ventro-lateral prefrontal cortex (VLPFC), the right middle temporal gyrus, bilateral parahippocampus, superior colliculus, in the left cerebellum, as well as a thalamic region compatible with the pulvinar. The long allele did not show any increased activity after sleep deprivation. Similarly to the previous contrast (morning after sleep vs evening before sleep deprivation) $PER3^{5/5}$volunteers did not recruit any additional brain areas to perform the task. Comparison between the morning session after sleep loss and the evening session before sleep deprivation were then computed. Results showed again increased

compensatory activations in *PER3*$^{4/4}$ individuals and decreased activation for the long allele carriers. In the morning after a night of

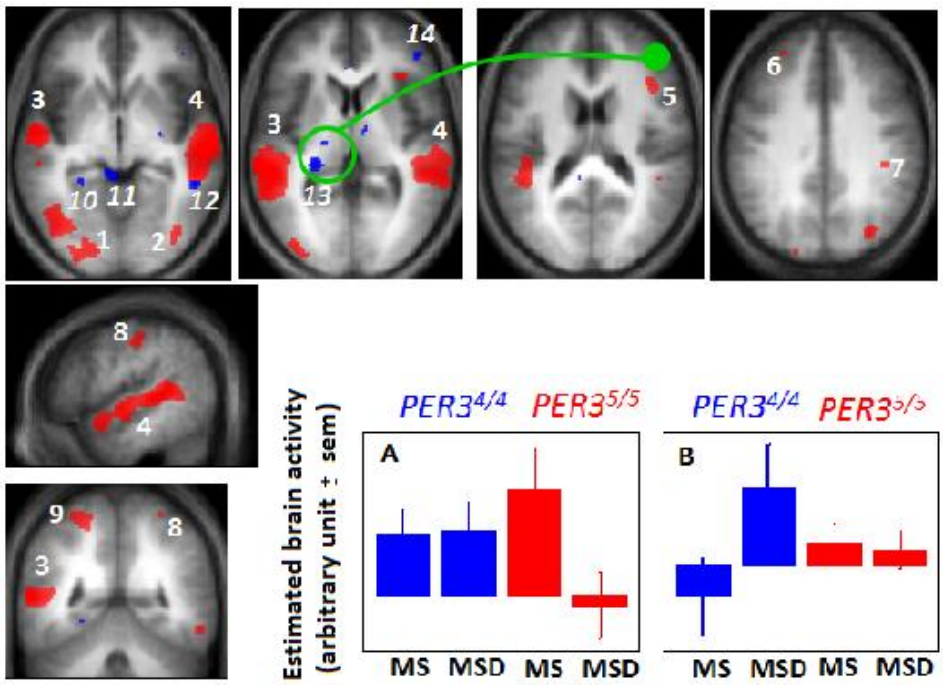

Figure 15: Difference between PER34/4 and PER35/5 individuals in the sleep loss-induced changed in brain responses to a working memory task. When comparing brain responses to an auditory 3-back task in the morning after a night of sleep (MS; 1.5 h of wakefulness) and in the morning after a night of sleep deprivation (MSD; 25 h of wakefulness), PER35/5 individuals undergo marked decreases in activation in several brain areas of the occipital (1,2) and temporal(3,4) cortices, and of the dorsolateral prefrontal (5,6) and parietal cortex (7–9), while PER34/4 individuals maintain brain responses in these areas (and do not present significant decreased activations in any brain regions). A representative profile of this brain activity change is displayed in panel A (similar profiles were observed for red areas 1–9). In contrast, when comparing the same sessions, PER34/4 individuals present increased activation (blue) in the parahippocampus (10), superior colliculus (11), temporal cortex (12), pulvinar (13), and ventrolateral prefrontal cortex (14), while no increased activation is observed in these regions in PER35/5 (and in any other brain regions). A representative profile of this brain activity change is displayed in panel B (similar profiles were observed for blue areas 10–14). A significant negative association was found between overnight change in brain response in the pulvinar (green circle) and self-reported daytime propensity to fall asleep in everyday life across all the subjects of the study (irrespective of genotype), further suggesting a central role for the pulvinar in wakefulness regulation (from Vandewalle and Schmidt, 2013).

sleep deprivation, *PER3*$^{5/5}$ showed widespread reduced activity in prefrontal, temporal, parietal and occipital areas, while *PER3*$^{4/4}$ individuals recruited supplementary brain areas, including the left

thalamus, the parahippocampus and the cerebellum. Thus the compensatory responses in *PER3*$^{4/4}$ and the decreased responses in *PER3*$^{5/5}$ were located in the same regions observed when comparing the two morning sessions, albeit the decreased activity in the long allele group was more widespread. If sleep pressure was the main mechanism underscoring differences between the two morning sessions, these differences should have been reduced between the morning after sleep deprivation and the evening after normal sleep (Vandewalle et al., 2009). Similarly if the circadian system was the primary process for the changes in brain responses, then comparing the two morning sessions should result in lack of results. Thus the interaction between the circadian and homeostatic processes is most likely to mediate cognitive brain responses.

Globally data from Vandewalle suggests that the widespread decreased activity, observed for *PER3*$^{5/5}$ individuals, in temporal and parietal cortices reflect the reduction in attentional processes consequent to sleep loss. This hypothesis is corroborate by the parallel deactivation of the occipital cortex even in absence of visual input (auditory 3-back), supporting the hypothesis of a reduced top-down control on sensory and associative areas. Moreover the additional recruitment of the VLPFC as well as of the supplemental areas, observed in *PER3*$^{4/4}$ to adequately perform the task under high sleep pressure, suggest a compensatory mechanism, as already speculate by several authors(Drummond and Brown, 2001; Chee and Choo, 2004; Drummond et al., 2004; Habeck et al., 2004). Finally Vandewalle data confirmed the differential response to sleep loss in the two genotypes, adding a new line of research to the *PER3* topic, connected with cerebral correlates as assessed by fMRI. Collectively, these two studies indicate that the daily temporal organization of cognitive brain activity depends on the interaction between circadian and homeostatic processes. However, both studies investigated the interaction of these two processes on a limited time-scale. Ultimately, this hinders a full understanding of how these processes dynamically regulate brain function. In this context, one of the aims of the present work was to investigate, changes in regional

brain responses underpinning attentional processes through more than an entire circadian cycle.

Experiments

STUDY 1

Influence of acute sleep loss on the neural correlates of alerting, orientating and executive attention components

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Abstract

The Attention Network Test (ANT) is deemed to assess the alerting, orientating and executive components of human attention. Capitalizing on the opportunity to investigate three facets of attention in a single task, we used functional magnetic resonance imaging (fMRI) to assess the effect of sleep deprivation (SD) on brain responses associated with the three attentional components elicited by the ANT. Twelve healthy volunteers were scanned in two conditions 1 week apart, after a normal night of sleep (rested wakefulness, RW) or after one night of total sleep deprivation. Sleep deprivation was associated with a global increase in reaction times, which did not affect specifically any of the three attention effects. Brain responses associated with the alerting effect did not differ between RW and SD. Higher-order attention components (orientating and conflict effects) were associated with significantly larger thalamic responses during SD than during RW. These results suggest that SD influences different components of human attention non-selectively, through mechanisms that might either affect centrencephalic structures maintaining vigilance or ubiquitously perturb neuronal function. Compensatory responses can counter these effects transiently by recruiting thalamic responses, thereby supporting thalamocortical function.

1. Introduction

A single night of sleep deprivation is detrimental to a number of cognitive abilities, ranging from phasic alertness (Doran et al., 2001) to executive functions (Harrison et al., 2000). Deteriorating attention is usually believed to participate in this decline in cognitive performance. However, attention is a heterogeneous concept (Oken et al., 2006) and sleep deprivation has been shown to affect various aspects of attention, such as phasic alertness (Drummond et al., 2005), selective (Horowitz et al., 2003) and divided attention (Drummond et al., 2001). A persistent issue is therefore whether sleep deprivation affects various components of attention selectively and differentially or whether the decrease in alertness is the core phenomenon that can explain the failure of the other attention systems (Lim and Dinges, 2010). Operational definitions of vigilance, phasic alertness and attention are detailed in Data S1.

A cognitive model assumes that human attention is supported by three main functions which are associated specifically with independent brain circuits and neuromodulators (Posner and Petersen, 1990). Following this model, the alerting component is defined as the ability to prepare and sustain alertness to process high-priority signals (Posner and Petersen, 1990). It would involve thalamic, frontal and parietal areas (Fan et al., 2005). The orientating component would allow one to attend to target items overtly or covertly, thereby improving their processing efficiency (Posner and Petersen, 1990). Orientating would involve the superior parietal lobe, temporo–parietal junctions and superior frontal cortex (Fan et al., 2005). A third, executive attention component would be involved in conflict resolution and would recruit the anterior cingulate cortex and the lateral prefrontal cortex (Fan et al., 2005).

The attention network test (ANT) was designed to probe the efficiency of these three attention networks within a single task (Fan et al., 2005). Therefore, it would be a particularly appropriate task to address whether sleep deprivation has a differential and selective influence on the various components of attention. In this study, using functional magnetic resonance imaging (fMRI) and a within-subject design, we

assessed brain responses related to the three main effects (alerting, orientating and conflict effects) during rested wakefulness and after sleep deprivation.

2. Materials and methods

2.1. Subjects:

Young, healthy subjects (n = 14, seven female; age range 19–27 years; mean age = 21) gave their written informed consent to participate in this study, which was approved by the Ethics Committee of the Faculty of Medicine of the University of Lie`ge. They received financial compensation for their participation. An interview established the absence of medical, traumatic, psychiatric or sleep disorders. All volunteers were right-handed (Oldfield, 1971), free from medication, non-smokers and moderate caffeine and alcohol consumers. None had worked on night shifts during the previous year or travelled through more than one time zone during the last 2 months. Extreme morning and evening types, as assessed by the Horne–Ostberg questionnaire (Horne and Ostberg, 1976), were not included. None complained of excessive daytime sleepiness as assessed by the Epworth Sleepiness Scale (score < 11) (Johns, 1991) or of sleep disturbances as determined by the Pittsburgh Sleep Quality Index Questionnaire (score < 7) (Buysse et al., 1989).

2.2. Protocol:

Participants completed the protocol on two separate experimental days (**Fig. 1a**), 1 week apart. Before each visit, they followed a 7-day regular and individual sleep schedule, including an 8-h sleep period, as assessed by sleep diaries and wrist actigraphy (Actiwatch, Cambridge Neuroscience, UK). Two volunteers were excluded because they did not comply with this schedule. Volunteers were requested to refrain from caffeine- and alcohol-containing beverages and intense physical activity 7 days preceding each visit. At least a week before the first visit, a short behavioural training session outside the scanner familiarized the participants to the task.

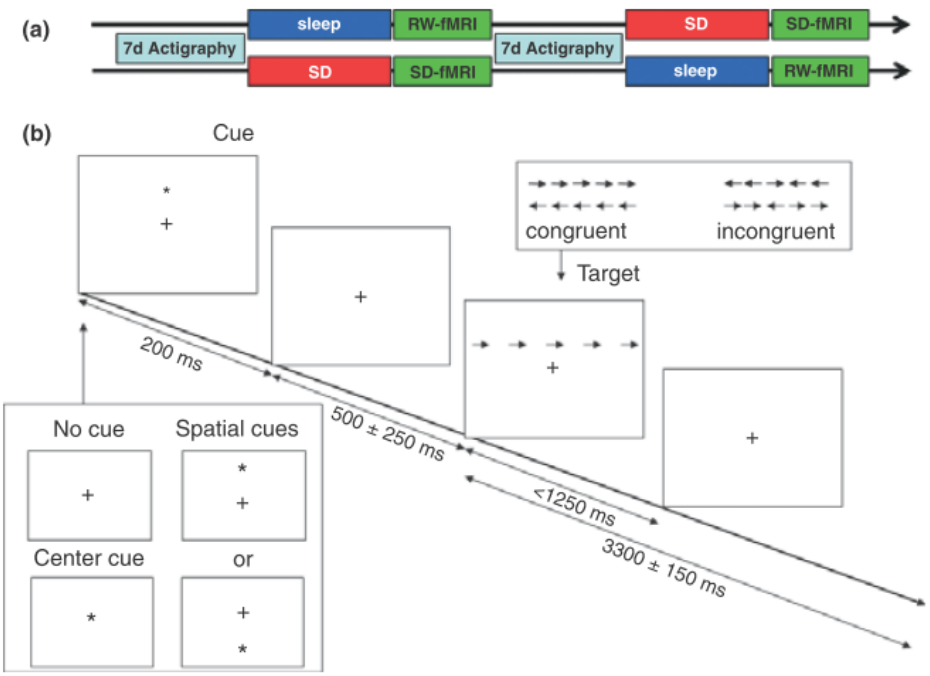

Figure 1. (**a**) Experimental design; (**b**) Description of a trial.

During one of the visits [sleep deprivation (SD)], participants came to the laboratory in the evening (19:00 h) and spent the entire night in a dim light environment (<10 lux) under constant supervision by two staff members. They were allowed one snack every 3 h. They were kept in a sitting position except to visit the bathroom. In the morning, starting from 09:00 h, volunteers underwent an fMRI session during which they carried out the ANT. The scanning sessions took place between 09:00 h and 17:00 h, corresponding to a sleep deprivation of 25–33 h, depending on the volunteer. During this time, volunteers were monitored continuously by one experimenter. For the other visit [rested wakefulness (RW)], participants followed exactly the same protocol but after a normal night of sleep at home. For each volunteer, fMRI sessions took place at the same time of day during both visits. Half the participants started with the SD session, the other half with the RW session. Subjective sleepiness was estimated immediately before scanning using the Karolinska sleepiness scale (KSS; Akerstedt and Gillberg, 1990).

2.3. Task:

The Attentional Network Task (ANT), developed originally by Fan and collaborators (Fan et al., 2005), was adapted to fMRI acquisitions (**Fig. 1b**). Stimuli were coded using cogent version 2000 (http://www.vislab.ucl.ac.uk/cogent.php) implemented in matlab version 6.1 (Mathworks Inc., Natick, MA, USA).

Participants were instructed to fixate the central cross for the entire experiment. Stimuli consisted of a row of five aligned horizontal black arrows (0.58° in size, separated by 0.06°; the five arrows row covered a total of 3.27°), pointing either to the left or right, presented against a grey background, 1.06° above or below a fixation cross. The target, i.e. the central arrow, could point either in the same direction as the other four flanker arrows (congruent condition) or in the opposite direction (incongruent condition). The participants were asked to determine as quickly and accurately as possible the direction of the central arrow of a stimulus by pressing one of two buttons on a keyboard. In one-third of the trials, the stimulus was preceded by a visual cue (an asterisk) presented at the centre of the screen for 550 ± 250 ms (central cue). In another third of the trials, the stimulus was preceded by a visual cue presented above or below the central fixation cross for 550 ± 250 ms (spatial cue), which indicated that the five-arrow-stimulus would appear in the upper or lower part of the screen, respectively. The spatial cue was always predictive of the place where the target would appear. In the remaining third of the trials, the stimulus was not preceded by any visual cue (no cue condition). The stimulus was presented for a maximum of 1250 ms, disappearing when the response was made. The intertrial interval was set to 3300 ± 150 ms. A total of 252 trials (36 for each trial type) were presented in random order and the fMRI session lasted approximately 21 min.

According to Fan et al. (2005), three attention components were evaluated behaviourally by measuring the influence of cue and target conditions on reaction times (RTs), relative to a reference condition:

• the alerting effect, by subtracting the mean RTs of the centre cue condition from the mean RT of the no cue condition;

• the orientating effect, by subtracting the mean RTs of the spatial cue condition from the mean RTs of the centre cue condition; and

• the conflict effect, by subtracting the mean RTs of all the congruent trials from the mean RTs of all the incongruent trials.

The objective of the fMRI analysis was to characterize the effects of SD on the alerting, orientating and conflict effects. Because SD was expected to affect attention processes, we considered the responses associated with optimal and global performance separately for RW and SD. Distributions of reaction times were computed separately for each of the six different trial types. We defined optimal performance as the condition corresponding to the trials associated with RTs below the 30th percentile of the RT distribution during a given state (RW or SD). Global performance corresponded to the trials for RTs which were between the 30th and 70^{th} percentiles. The slowest trials were above the 70th percentile of RTs. This strategy was adopted to eschew the problematic situation in which trials with the fastest response times are selected mainly from the sleep group, as would be observed if the RT distribution was computed across RW and SD conditions. The strategy also avoided some trials, corresponding to optimal vigilance during SD, being compared with intermediate RT trials in the RW condition.

2.4. Behavioural analysis:

Only accurate trials were included in the analyses. Trials were split into the fastest RTs (below the 30th percentile of all RTs), slowest RTs (above the 70th percentile) and intermediate RTs (between the 30th and 70th percentiles). For each trial class (percentile 30, intermediate, percentile 70), a repeated-measures analysis of variance (anova) was conducted with 'condition' (RW versus SD), 'cue' (no cue versus centre cue versus spatial cue) and 'target' (congruent versus incongruent) as within-subject factors. Degrees of freedom were adjusted using the

Greenhouse–Geisser method. Uncorrected F-values were reported together with the Greenhouse–Geisser epsilon and corrected P-values.

In a second analysis, alerting, orientating and conflict effects were derived by subtracting the mean RTs of each condition to its reference condition, as described above. A paired t-test was used to assess the session effect. Inferences were conducted at $P < 0.05$ after Bonferroni correction for multiple comparisons (n = 3).

2.5. fMRI data acquisition and analysis:

Magnetic resonance (MT) imaging was performed on a 3T MR scanner (Allegra, Siemens, Erlangen, Germany). Multislice T2*-weighted fMRI images were obtained with a gradient echo-planar sequence using axial slice orientation (32 transverse slices; voxel size = 3.4 x 3.4 x 3.0 mm^3 with a 30% of interslice gap; matrix size = 64 x 64 x 32; TR⁄TE: 2130 ⁄ 40 ms; FoV: 220 x 220 mm^2; flip angle = 90°). A high-resolution T1-weighted structural MR scan was obtained for each participant (3D MDEFT; 176 sagittal slices; TR⁄TE⁄TI: 7.92⁄2.4⁄910 ms; flip angle = 15°, field of view 256 x 224 mm^2, matrix size = 256 x 224 x 176, voxel size = 1 x 1 x 1 mm^3).

Functional volumes were preprocessed and analysed using Statistical Parametric Mapping (SPM8; http://www.fil.ion.ucl.ac.uk/spm) implemented in matlab version 7.4.0 (Mathworks Inc.). The first three volumes were discarded to account for saturation effects. Images were realigned, coregistered to the structural image, normalized spatially to a template conforming to the Montreal Neurological Institute (MNI) space, and smoothed spatially with a Gaussian kernel of 8-mm full width at half maximum.

The fMRI data analysis, based on a mixed-effects model, was conducted in two serial steps, taking into account fixed and random effects (respectively, intra- and Interindividual variance). At the first level, event types were defined according to the presence and position of the cue (no cue, central cue, ‘spatial’ cue), congruency of the target (congruent, incongruent) and the distribution of reaction times (<percentile 30,

>percentile 70, intermediate RTs). This resulted in 18 different trial types. Two further trial types of no interest (error and lapses) were also included in the design matrix.

For each subject, changes in brain regional responses were estimated using a general linear model, in which the activity evoked by each trial type was modelled as a function representing its onset, convolved with a canonical haemodynamic response function. Movement parameters estimated during realignment and a constant vector were also included in the matrix as variables of no interest. High-pass filtering was implemented in the matrix design using a cutoff period of 128 s to remove low-frequency drifts from the time–series. Serial correlations in fMRI signal were estimated using an autoregressive (order 1) plus a white noise model and a restricted maximum likelihood algorithm.

Linear contrasts estimated the three main effects of interest (alerting, orientating, executive effects) for each session (sleep versus sleep deprivation) separately as well as the differences in these effects between sessions (i.e. the sleep status · effect interaction) separately for each class of reaction times. We focused on the effects corresponding to the fastest and intermediate RTs. The slowest RTs, although modelled explicitly, were not investigated further, given that they could result from several potential factors [sleepiness, perceptual, attentional or executive deficit, task disengagement (Drummond et al., 2005)]. With regard to behavioural analyses, trials associated with intermediate RTs (percentile 30 < RTs < percentile 70) were considered to be corresponding to an average alertness level, to which we will refer as 'global' alertness. In contrast, trials associated with the fastest RTs (RTs < percentile 30) relative to the intermediate RTs were denoted as an 'optimal' alertness level (Schmidt et al., 2009). The resulting set of voxel values for each contrast constituted maps of the t-statistics [SPM(T)]. Summary statistic images were then further smoothed (6-mm FWHM Gaussian kernel) and entered into the second-level analysis, which corresponded to one-sample t-tests probing the experimental effects tested at the first level. Statistical inferences were performed after

correction for multiple comparisons on small spherical volumes (SVC; 10-mm radius) at a threshold of $P_{svc} = 0.05$, around a priori locations of activation in structures of interest, taken from the literature (see Tables 2–4).

3. Results

3.1. Behavioural results:

Subjective sleepiness was increased significantly in SD, relative to the RW condition (KSS, RW: 2.41 ± 0.79; SD: 5.33 ± 1.55, paired t-test: $t_{(11)} = 7.7$, $P < 0.001$). All volunteers maintained a response accuracy of between 97 and 98% (percentage of given responses) in both conditions.

For the sake of completeness, we first detail the effects of sleep, cue and target conditions on RTs for intermediate, fast and slow trials. In a second step, statistics on the alerting, orientating and conflict effects are summarized for the three categories of RTs. Mean values and standard deviations of each trial type appear in **Table 1**.

Table 1 Mean reaction times [and standard deviations (SD) in milliseconds] for the different trial types during the two conditions

	Sleep deprivation		*Rested wakefulness*	
Trial type	*Mean*	*SD*	*Mean*	*SD*
No cue congruent	612	77	560	63
No cue incongruent	674	66	616	58
Central cue congruent	570	61	532	60
Central cue incongruent	686	94	607	54
Spatial cue congruent	575	87	515	59
Spatial cue incongruent	632	48	601	65

For intermediate RTs (**Fig. 2a**), a repeated-measures anova with session (RW versus SD), cue (no cue, central or spatial cue) and target (congruent versus incongruent) as within-subject factors showed a main effect of session ($F_1 = 10.29$, $P = 0.008$), cue ($F_2 = 10.75$, $P = 0.001$) and target ($F_1 = 72.82$, $P < 0.001$). The cue x target interaction was significant ($F_{(2,22)}$, $F = 6.09$, $P = 0.008$, $\varepsilon = 0.82$), due to an unexpectedly smaller

conflict effect in the no cue condition ($F_{1,11}$ = 12.93, P = 0.004), independent of the sleep condition. The session x cue and session x target interactions were not significant (F_2 = 0.42, P = 0.664; F_1 = 0.73, P = 0.410, respectively), whereas the session x cue x target interaction tended to be significant (F_2 = 3.80; P = 0.06, ε = 0.67).

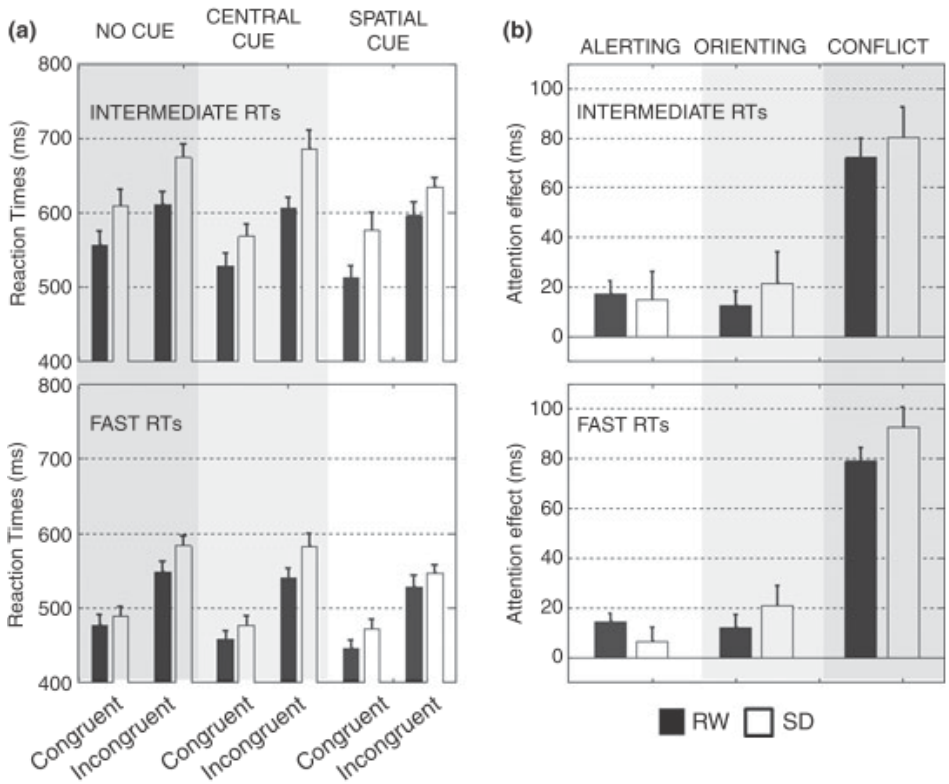

Figure 2. (**a**) Reaction times (ms) for each trial types, following cue and target conditions, during rested wakefulness (RW) and deprivation (SD). (**b**) Alerting, orientating and conflict effects (ms) during RW and SD. Black bars: RW; white bars: SD. Error bars stand for standard error of the mean. Statistical results are detailed in the text.

For fast RTs (**Fig. 2a**), a repeated-measures anova with session (RW versus SD), cue and target as within-subject factors showed a main effect of session (F_1 = 5.1, P = 0.045), cue (F_2 = 17.4, P < 0.001) and target (F_1 = 191.5, P < 0.001). The session x target interaction was significant (F_1, F = 5.0, P = 0.048), due to a larger difference in RTs between congruent and incongruent trials during SD, relative to the RW condition. The session x cue, the cue x target and the session x cue x

target interactions were not significant ($F_2 = 0.62$, $P = 0.48$; $F_1 = 1.57$, $P = 0.23$; $F_2 = 2.35$, $P = 0.13$, $\varepsilon = 0.86$).

For slow RTs (not shown), a repeated-measures anova with session, cue and target as within-subject factors showed a main effect of session ($F_1 = 19.71$, $P = 0.001$), cue ($F_2 = 9.06$, $P < 0.001$) and target ($F_1 = 20.96$, $P < 0.001$). None of the interactions were significant.

Essentially, these results showed a global slowing of RTs after SD, relative to RW, irrespective of the cue or target condition. The only exception was a larger effect of target incongruence during SD for the fastest responses. These results do not provide compelling evidence for a selective and differential effect of SD onto the different attention components.

To investigate this aspect further, we analysed the effect of SD on alerting, orientating and conflict effect (**Fig. 2b**). This analysis corresponded to the effects investigated in fMRI. There was no significant change in any of these effects from RW to SD (alerting effect, $P = 0.93$; orientating effect, $P = 0.94$; conflict effect, $P = 0.61$). Again, these results indicate that sleep deprivation slowed RTs globally in every trial category.

3.2. Functional MRI results:

First, we report the neural correlates of the alerting, orientating and conflict effects during the RW session, to allow for comparison with the literature. For this analysis, all trials (i.e. fast, intermediate and slow RTs) were considered together. In a second step, we address the effects of sleep deprivation. Because of the performance instability that characterizes sleep deprivation, these contrasts assessed fast and intermediate trials separately. Therefore, we detail separately the responses for the three attention effects, for fast and intermediate trials, during RW and SD sessions. Finally, we describe separately the session · effects interaction (i.e. between-session comparisons) for each attention effect, for fast and intermediate trials. Results are summarized in **Tables 2–4**.

3.2.1. Responses during rested wakefulness – all trials:

The alerting effect was associated with a distributed set of brain areas, including bilateral occipito–temporal regions, right anterior inferior frontal gyrus, medial prefrontal cortex and left intraparietal sulcus (**Table 2**). The orientating effect elicited a significant response in the posterior middle frontal gyrus. Finally, the conflict effect was associated with significant responses in bilateral occipito–temporal areas, left inferior and posterior frontal gyrus, left precentral gyrus and left superior parietal cortex.

Table 2 Results obtained during rested wakefulness (RW) (all trials included)

Area	x	y	z	*Z-score*	P_{SVC}	*References*
Alerting effect						
R. lateral temporo–occipital cortex	48	−68	6	3.88	0.011	(Coull *et al.*, 2001)
L. lateral temporo–occipital cortex	−42	−66	6	4.19	0.004	(Fan *et al.*, 2005)
R. superior medial frontal cortex	4	32	34	3.51	0.030	(Fan *et al.*, 2005)
R. inferior frontal gyrus	50	28	4	3.42	0.038	(Konrad *et al.*, 2005)
L. anterior intraparietal sulcus	−30	−42	34	4.21	0.004	(Thiel *et al.*, 2004)
Orientating effect						
Left MFG	−44	2	48	3.16	0.049	(Thiel *et al.*, 2004)
Conflict effect						
R. occipito–temporal cortex	44	−60	−2	3.47	0.033	(Fan *et al.*, 2005)
L. occipito–temporal cortex	−46	−68	−2	3.31	0.049	(Fan *et al.*, 2005)
L. inferior frontal sulcus	−34	32	−4	3.25	0.05	(Konrad *et al.*, 2005)
L. precentral gyrus	−36	−10	50	3.64	0.022	(Fan *et al.*, 2005)
L. superior parietal cortex	−44	−36	52	3.31	0.049	(Coull *et al.*, 2001)

Coordinates (*x*, *y*, *z*) are expressed in mm in the Montreal Neurological Institute (MNI) space. P_{svc}: probability of rejecting the null hypothesis of no activation after correction for multiple comparisons over small volumes of interest taken from the literature (reference in the last column). R., right; L., left; MFG, Middle Frontal Gyrus.

3.2.2. Responses during rested wakefulness and sleep deprivation – intermediate trials:

During RW, the alerting effect was associated with significant responses in the right inferior frontal gyrus, the right superior temporal sulcus and in bilateral temporo–occipital cortices (**Table 3**). In contrast, no significant response survived correction for multiple comparisons during the SD sessions. This is related probably to a larger signal variance: at a lenient threshold ($P_{uncorrected} < 0.001$), responses were detected in the same bilateral occipito–temporal areas as during wakefulness. In addition, no region showed a differential alerting effect between sessions (RW > SD or SD > RW).

Table 3 Results obtained during rested wakefulness (RW) and deprivation (SD) (intermediate trials)

Area	x	y	z	*Z-score*	P_{SVC}	*References*
Alerting effect RW						
R. inferior frontal gyrus	44	22	18	3.29	0.046	(Fan *et al.*, 2005)
R. superior temporal sulcus	52	−46	12	4.31	0.003	(Fan *et al.*, 2005)
R. lateral temporo–occipital cortex	50	−70	2	4.17	0.004	(Coull *et al.*, 2001)
L. lateral temporo–occipital cortex	−42	−68	6	3.88	0.010	(Fan *et al.*, 2005)
Orientating effect RW						
L. precuneus	−16	−50	34	3.47	0.050	(Thiel *et al.*, 2004)
L. temporo–parietal junction	−58	−60	35	3.45	0.050	(Coull *et al.*, 2001)
Orientating effect SD						
R. thalamus	8	−16	0	3.24	0.036	(Fan *et al.*, 2005)
Orientating effect SD > RW						
R. thalamus	8	−16	0	5.45	0.032	(Fan *et al.*, 2005)
Conflict effect RW						
L. intraparietal sulcus	−26	−48	50	3.40	0.039	(Coull *et al.*, 2001)
Conflict effect SD						
L. temporo-occipital cortex	−42	−68	−6	3.77	0.039	(Coull *et al.*, 2001)
L. thalamus	−14	−28	8	3.24	0.05	(Fan *et al.*, 2005)
Conflict effect SD > RW						
R. thalamus	18	−28	4	3.92	0.01	(Fan *et al.*, 2005)
L. thalamus	−12	−26	4	3.28	0.05	(Fan *et al.*, 2005)

Coordinates (*x*, *y*, *z*) are expressed in mm in the Montreal Neurological Institute (MNI) space. P_{svc}: probability of rejecting the null hypothesis of no activation after correction for multiple comparisons over small volumes of interest taken from the literature (reference in the last column). R., right; L., left.

During RW, the orientating effect was associated with responses in the left temporo–parietal junction and the precuneus (**Fig. 4**). In contrast, during SD, the orientating effect resulted in a significant response in the right thalamus. The only difference in response between sessions was a larger right thalamic response during SD, relative to RW.

The conflict effect was related to response in the left intraparietal sulcus during RW and in the left temporo–occipital cortex and left thalamus during SD. Similarly to the orientating effect, the only difference in responses between sessions was a larger conflict-related activity in bilateral thalamic nuclei during SD, relative to RW (**Fig. 5**).

3.2.3. Responses during rested wakefulness and sleep deprivation – fast trials:

Similarly to intermediate responses, fast responses corresponding to the alerting effect during RW were associated with increased activity in the bilateral occipito–temporal areas and right superior temporal gyrus (**Fig. 3, Table 4**). No significant response was observed during SD, except at a lenient threshold ($P_{uncorrected} < 0.001$) in the left occipito–temporal cortex. Alerting-related activity in the left precuneus and the right temporo–parietal junction was larger during RW than SD (**Fig. 3**), whereas no region was more active during SD than RW.

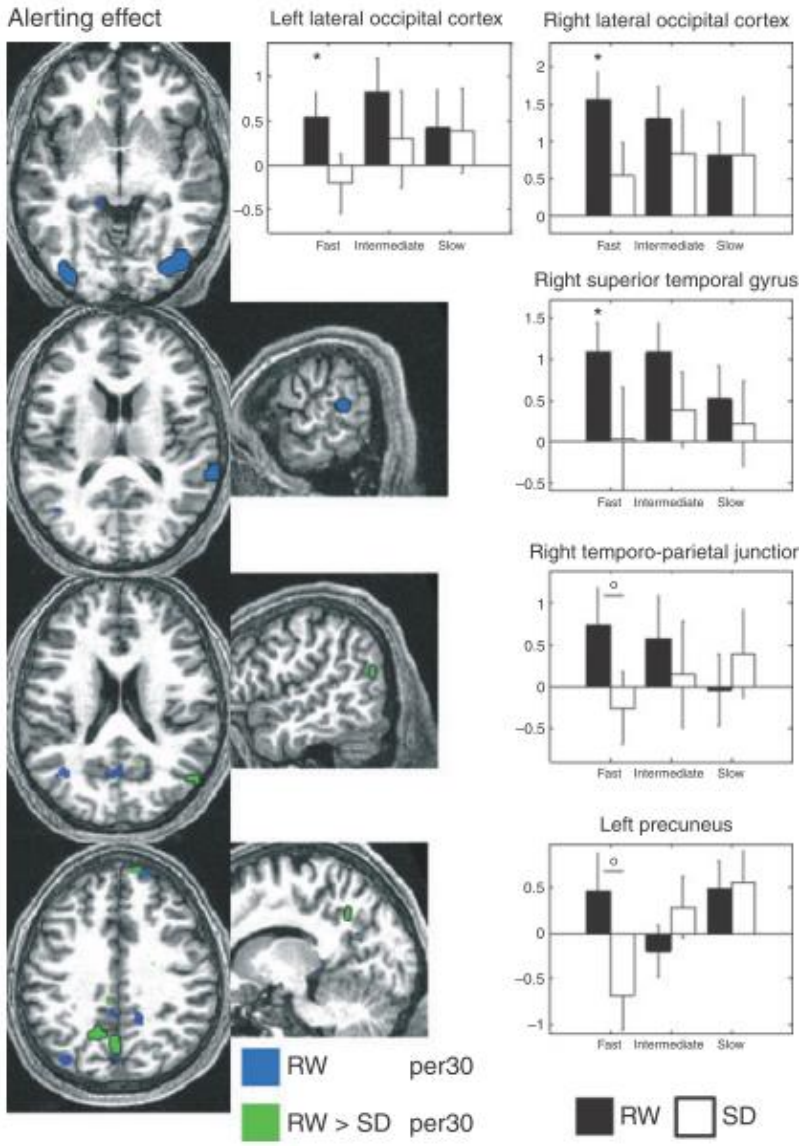

Figure 3. Brain responses associated with the alerting effect. Row 1: bilateral lateral occipital areas; row 2: right superior temporal gyrus; row 3: right temporo–parietal junction; row 4: left precuneus. Left panels: functional results are displayed at $P_{uncorrected} < 0.001$, over the normalized structural magnetic resonance (MR) scan of a typical subject. Right panels: activity estimates of each area for fast, intermediate and slow reaction times (RTs) (arbitrary units ± standard error of the mean). *Significant within-state effect (P svc < 0.05); significant state x effect interaction (P svc < 0.05).

Table 4 Results obtained during rested wakefulness (RW) and deprivation (SD) (fast trials)

Area	x	y	z	*Z-score*	P_{SVC}	*References*
Alerting effect RW						
R. lateral temporo–occipital cortex	42	−76	−4	3.53	0.025	(Coull *et al.*, 2001)
L. lateral temporo–occipital cortex	−48	−60	6	3.49	0.028	(Fan *et al.*, 2005)
R. superior temporal gyrus	68	−36	16	3.65	0.019	(Fan *et al.*, 2005)
Alerting effect RW > SD						
L. precuneus	−16	−56	36	3.72	0.014	(Thiel *et al.*, 2004)
R. temporo–parietal junction	52	−66	18	3.53	0.024	(Thiel *et al.*, 2004)
Orientating effect SD						
L. temporo-parietal junction	−48	−64	24	4.3	0.002	(Coull *et al.*, 2001)
L. precuneus	−10	−68	44	3.28	0.044	(Thiel *et al.*, 2004)
Orientating effect SD > RW						
L. temporo-parietal junction	−46	−62	20	3.75	0.015	(Coull *et al.*, 2001)
Conflict effect SD > RW						
L. thalamus	−6	−6	12	3.47	0.032	(Fan *et al.*, 2005)

Coordinates (*x*, *y*, *z*) are expressed in mm in the Montreal Neurological Institute (MNI) space. P_{svc}: probability of rejecting the null hypothesis of no activation after correction for multiple comparisons over small volumes of interest taken from the literature (reference in the last column). R., right; L., left.

In contrast, for the orientating effect, significant brain activity was detected only during SD in the left temporo–parietal junction and the precuneus (**Fig. 4**). Intriguingly, these areas correspond to those associated with intermediate responses during RW. Responses in the left temporo–parietal area were larger during SD than during RW, whereas no region had a larger orientating-related activity during RW, relative to SD.

Bold responses did not differ between congruent and incongruent trials (conflict effect), either in RW or in SD. Thalamic responses associated with the fastest trials were increased significantly during SD relative to RW (**Fig. 5**).

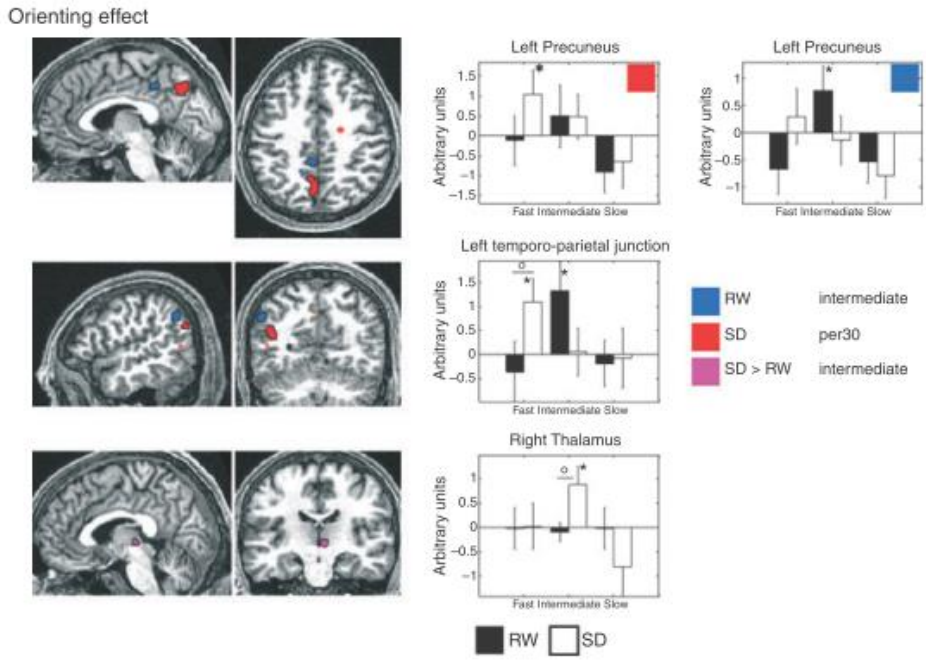

Figure 4. Brain responses associated with the orientating effect. Upper row: left precuneus; middle row: left temporo–parietal junction; lower row: right thalamus. Left panels: functional results are displayed at $P_{uncorrected} < 0.001$, over the normalized structural magnetic resonance (MR) scan of a typical subject. Right panels: activity estimates of each area for fast, intermediate and slow reaction times (RTs) (arbitrary units ± standard error of the mean). *Significant within-state effect; °Significant state x effect interaction (P svc < 0.05).

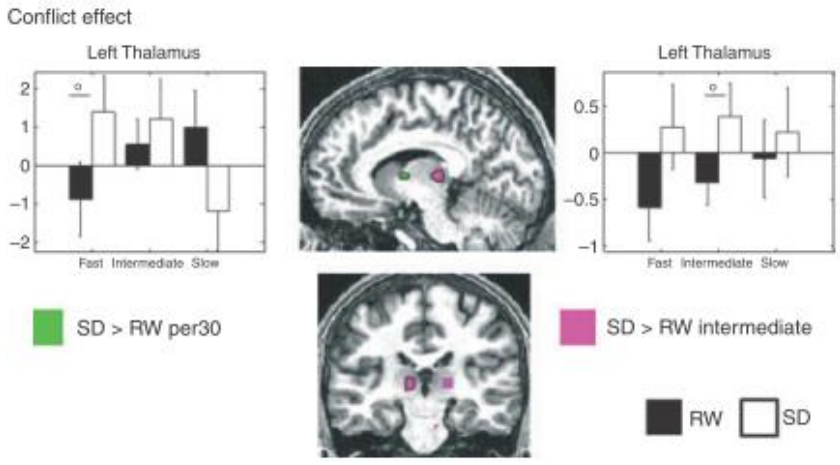

Figure 5. Differential thalamic responses associated with the conflict effect between rested wakefulness (RW) and deprivation (SD). Functional results are displayed at $P_{uncorrected} < 0.001$, over the normalized structural magnetic resonance (MR) scan of a typical subject. Lateral panels: activity estimates for fast, intermediate and slow reaction times (RTs) in each contrast (arbitrary units ± standard error of the mean). °Significant state · effect interaction ($P_{svc} < 0.05$).

4. Discussion

We used the ANT to test whether sleep deprivation would influence differentially the alerting, orientating and executive components of attention. Behavioural data did not support this hypothesis and essentially showed a global slowing of RTs for all trial types. Only the conflict effect associated with the fastest RTs was enhanced during SD, relative to RW condition. Functional MRI data did not show significant between-session changes in responses related to the alerting effect, except for those associated with the fastest RTs, which were characterized by increased activity during RW in the precuneus and right temporo–parietal junction. For the orientating effect, the precuneus and left temporo–parietal junction, which were activated during RW, could still be recruited during SD, but only when an optimal alertness level could be achieved. The orientating effect during SD was supported by an enhanced recruitment of the thalamus (intermediate RTs) and right temporo–parietal region (fastest RTs). The conflict effect was also associated with increased thalamic responses during SD relative to RW, for both intermediate and fast RTs.

4.1. Maintaining phasic alertness during sleep loss:

Sleep deprivation did not selectively modify the alerting component and slow down responses to the same extent to trials with or without a central cue. Whereas the alerting effect was associated with responses in occipital and temporal areas during RW, no statistically significant brain alerting responses were detected after SD. Moreover, the alerting effect was not associated with any significant difference in brain responses between SD and RW for intermediate RTs. These results do not support the view that the neural correlates of phasic alertness are modified specifically by sleep deprivation. On the contrary, they suggest that the main impact of sleep deprivation on brain function is related either to an impaired capacity of centrencephalic structures to maintain vigilance, or to the ubiquitous influence of local sleep pressure on neuronal activity (Krueger et al., 2008).

Intriguingly, for the fastest trials, SD resulted in decreased responses (relative to RW) in the precuneus and right temporo–parietal junction. In the absence of any behavioural correlates, this finding indicates that the activity in these areas might reflect strategies that are adopted primarily under rested conditions but are not critical to the alerting effect. These strategies would buttress a steady cognitive output during RW. In contrast, SD would reduce these cognitive resources and the corresponding brain responses.

The current findings do not confirm recent behavioural data showing, as well as global slowing in RTs, a significant effect of sleep deprivation on alerting effect (Martella et al., 2011). This discrepancy is explained potentially by their experimental protocol in which SD sessions took place at 04:00 h, a time of day associated with marked circadian decline in alertness (Dijk et al., 1992). It should be noted that this experimental design possibly underestimates the genuine behavioural and neural effects of sleep deprivation, which can be detected more easily in the early morning hours after sleep deprivation. A discussion about the contribution of increased sleep pressure and circadian rhythms on our results can be found in the supporting online information.

4.2. Thalamic and cortical compensatory responses maintain higher-order attention components during sleep loss:

For higher-order (orientating and executive) attention components, some brain responses were increased after SD relative to RW. Similar increases were reported for other cognitive tasks (Venkatraman et al., 2007) and are thought to reflect 'compensatory' responses, i.e. brain attempts to recruit neural resources transiently to maintain cognitive output despite increased sleep pressure and／or circadian misalignment (Drummond et al., 2001; Tomasi et al., 2009). Importantly, these 'compensatory' responses were detected in thalamic nuclei for the orientating and executive attention components. The thalamus constitutes a unique interface between vigilance and cognition. Increased thalamic responses were observed during sleep deprivation for working memory (Chee et al., 2008; Vandewalle et al., 2009) and

attention tasks (Chee et al., 2008; Portas et al., 1998). These 'compensatory' thalamic responses speak against a selective influence of SD on cortical circuits involved in orientating or executive attention components. In contrast, and in keeping with the conclusion drawn for alerting effects, these thalamic responses would reflect non-specific mechanisms, which transiently maintain an optimal vigilance level, thereby supporting cortical function and maintaining steady performance.

The only cortical area showing a compensatory response was the left temporo–parietal junction. These findings speak for a phasic recruitment of this area when an optimal vigilance level can be achieved during sleep loss, suggesting again the importance of vigilance level for the adjustment of cortical responses during sleep loss.

4.3. Does the ANT probe independent and segregated attention components?

The ANT was designed to probe three allegedly independent attention networks, which were segregated in specific brain areas (Fan et al., 2005). The current data acquired during RW do not support the view that the ANT probes independent sets of brain areas. For instance, the alerting effect elicited significant responses in occipito–temporal areas, in keeping with previous findings, but also in the inferior frontal gyrus, which had been implicated in conflict resolution (Fan et al., 2005). These discrepancies may have various origins, such as different versions of the tasks, the use of different statistical fMRI models or the procedures of statistical inferences (which were conservative in the current work). To maintain short scanning sessions, we transformed the ANT version for fMRI (Fan et al., 2005) in a compact form, whereas Fan and collaborators used a long version allowing them to separate responses to cues from responses to targets (Fan et al., 2005). In the same vein, MacLeod et al. (2010) demonstrated recently that the three attention networks do not operate independently at a cognitive level. In any case, the ANT does not seem adapted to our original objective, which was to

assess the effects of sleep deprivation on three independent aspects of human attention using a compact design based on a single task.

5. Conclusions

Behavioural and neural data do not support the view of a selective and differential influence of sleep deprivation on alerting, orientating or executive attention components. The recruitment of thalamus during sleep loss to support higher-order (orientating and executive) attention components suggests that sleep deprivation influences attention globally through its impact on the ability to maintain vigilance.

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7. Supporting information

Additional Supporting Information (**Appendix 1**) may be found in the online version of this article:

- **Figure S1**. Alerting, orienting and conflict effects during rested wakefulness.
- **Data S1**. Operational definitions of vigilance, alertness, arousal and attention.

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STUDY 2

Local modulation of human brain responses by circadian rhythmicity and sleep debt

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Abstract

At any point in time, human performance results from an interaction between circadian rhythmicity and sleep pressure accumulated during wakefulness. How this interaction is reflected in local brain responses is not known. Here we quantify changes in brain responses to two cognitive tasks during functional magnetic resonance imaging (fMRI) sessions scheduled across the circadian cycle during 42h of wakefulness. Results demonstrate that the temporal profile of brain responses reflects the combined influence of sleep pressure and circadian rhythmicity, and that their contribution varies across brain regions. Prefrontal responses are more strongly sleep-pressure related, while subcortical areas are under a prominent circadian modulation, apparently contributing to preserved performance during the biological day despite accumulated sleep pressure. Results reveal the local modulation of circadian rhythmicity and of its interaction with homeostatic sleep pressure. These findings have implications for the understanding of the brain mechanism involved in deterioration of cognition during sleep deprivation, such as observed in shift work and ageing.

1. Main text

Foregoing sleep and staying up at night, be it for professional or recreational reasons, is highly prevalent in modern societies (Bixler, 2009). Acute sleep loss leads to deterioration of multiple aspects of cognition, from alertness and emotional processing to attention and executive functions (Killgore et al., 2008; Lo et al., 2012), and is associated with increased risk of human errors and health hazards (Rajaratnam and Arendt, 2001). Human brain responses to various cognitive tasks decrease during acute sleep loss, consistent with its detrimental influence on brain information processing (Chee and Chuah, 2008; Ma et al., 2015a). These effects are usually attributed to the mere lack of sleep. However, despite the progressive buildup of sleep pressure during wakefulness, human performance remains remarkably well preserved until wakefulness is extended into the biological night. To account for this phenomenon, it is assumed that a putative circadian alerting signal increases during the day reaching its peak in the early evening, close to the rise of melatonin concentrations, to counter the mounting homeostatic sleep pressure (Edgar et al., 1993; Dijk and Czeisler, 1994; Dijk et al., 1997; Wyatt et al., 1999). Cognition deteriorates rapidly and substantially, when wakefulness is extended into the night and early morning hours. This is attributed to the accumulated sleep pressure and the dissipation of the circadian alerting signal (Dijk et al., 1992; Dijk and Czeisler, 1994). The neural correlates of this alerting circadian process are not known, although circumstantial evidence points to the involvement of subcortical areas (Schmidt et al., 2009) and thalamo-cortical loops (Vandewalle et al., 2009). Here, we used fMRI to assess whether during sustained wakefulness, brain responses are modulated by circadian rhythmicity and how the latter interacts with sleep pressure accumulated during elapsed time awake. Young healthy volunteers (17 men, 16 women, age: 21.12 ± 1.7) stayed awake under constant environmental and behavioural conditions for a 42-h period starting in the morning, and covering two biological days, a full biological night and the beginning of a second biological night. During this 42-h period brain responses were assessed in twelve fMRI

sessions. These were not evenly distributed and were clustered in the morning and the evening, two periods characterized by rapid changes in the circadian modulation of cognitive performance. A thirteenth fMRI session took place after recovery sleep (**Fig. 1A**).

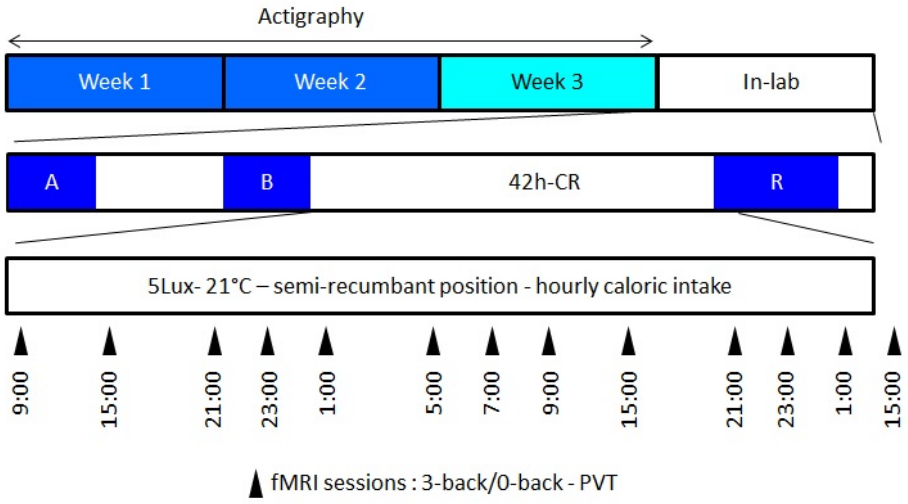

Figure 1A. Schematic representation of the experimental protocol. Actigraphy was recorded during 3 weeks prior to the laboratory study which comprised an 8h adaptation night (A), an 8h baseline night (B) and a 12h recovery night (R). From the morning of day 3, participants maintained wakefulness for 42 h under constant environmental and behavioural conditions in dim light and in a semi-supine position (45 degrees). During transfers to the fMRI room, volunteers lied down on a MR-compatible trolley, wearing ear plugs and light-proof goggles. Each of the 13 fMRI session included two runs. During the first run, participants performed randomly alternating blocks of auditory 3-back and 0-back tasks. During the second run, they performed the Psychomotor Vigilance Task (PVT). Half an hour before each fMRI sessions, KSS (Akerstedt and Gillberg, 1990), visual analog scales (VAS) and waking EEG were recorded.

Circadian phase was determined from the central circadian pacemaker driven melatonin rhythm (Pevet and Challet, 2011) which showed a typical profile with low levels during the day and a sudden increase in the late evening hours that occurred on average at 22:33 ± 00:09 (mean ± SEM). Sleep during the 12-h recovery night following the sleep deprivation was characterized by shorter sleep latency, increase sleep efficiency, total sleep time, NREM and REM sleep confirming the increase in sleep pressure relative to baseline (Supplementary **Table S1 and S2**). As expected, subjective sleepiness, negative affect, delta and

theta EEG power increased with elapsed time awake and returned to baseline after recovery sleep. These variables also showed a circadian modulation with poorest ratings observed at the end of the biological night (at approximately 8 am) and at the end of the sleep deprivation (at approximately 01 am), i.e. after melatonin had risen again (**Fig. 1B-E**).

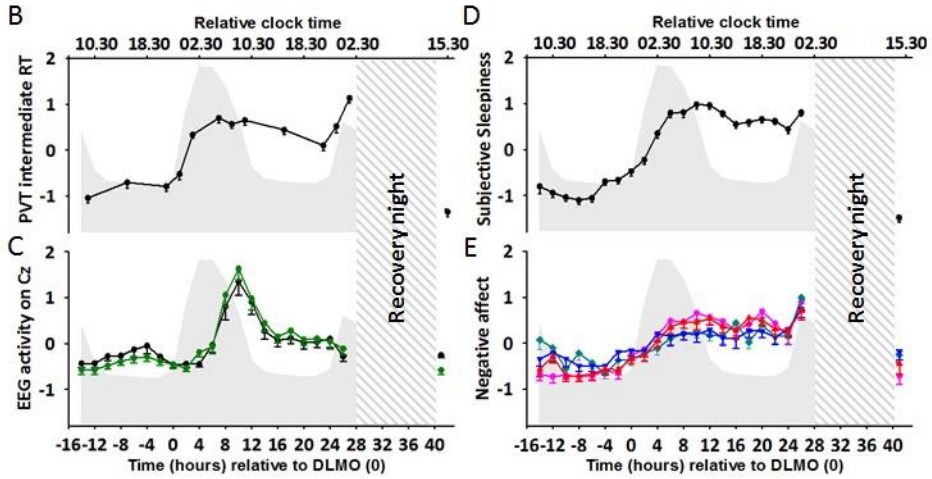

Figure 1B-E. Physiological and behavioral data, realigned to Dim Light Melatonin Onset (DLMO). The grey area illustrates the mean melatonin profile; the grey hatched area represents the recovery sleep episode. All data are normalized z-scores, mean values ± SEM. (**B)** PVT Intermediate reaction times varied significantly across the 13 fMRI sessions ($F_{12, 366}$ = 55.52; p<0.0001). **(C)** Waking EEG power in delta (0.75-4.5 Hz, black line) and theta (4.75-7.75 Hz, green line) frequency bands. A main effect of time relative to the onset of melatonin was detected for delta ($F_{21, 577}$ = 8.44; p<0.0001), theta power ($F_{21, 576}$ = 18.86; p<0.0001) and alpha activity ($F_{21, 572}$ = 3.32; p<0.0001; data not shown). **(D)** Subjective sleepiness varied significantly with time relative to melatonin onset ($F_{21, 629}$= 58.51; p<0.0001). **(E)** Subjective status: stress (cyan; main effect of time relative DLMO: $F_{21, 628}$ = 5.06; p<0.0001), anxiety (blue; $F_{21, 629}$ = 3.34; p<0.0001), happiness (red; $F_{21, 629}$= 9.86; p<0.0001), and motivation (pink; $F_{21, 630}$ = 13.59; p<0.0001). Higher scores indicate higher levels of stress, anxiety, unhappiness and demotivation.

During fMRI sessions, participants performed the psychomotor vigilance task (PVT) (Dinges and Powell, 1985) (**Fig. 1B**), a simple low stimulus frequency reaction time task. Performance on this task showed effects of elapsed time awake and circadian phase such that it remained relatively stable during the first day, significantly declined after the first onset of nocturnal melatonin secretion, with some recovery during the

second day, a further deterioration after the second melatonin onset and return to baseline after recovery sleep.

A first fMRI analysis identified voxelwise any significant 24h periodicity in brain response profiles, using two regressors of interest: 24h-period sine and cosine waves adjusted to individual circadian phase as determined from melatonin onset and computed for each individual scan time. A significant circadian modulation was observed in a large set of cortical areas, involving nearly the whole cortical mantle ($p_{FDR\ whole\ brain} < 0.05$; **Fig. 2A**, inset, **Table S3**), with the exception of the dorsolateral prefrontal cortex (DLPFC).

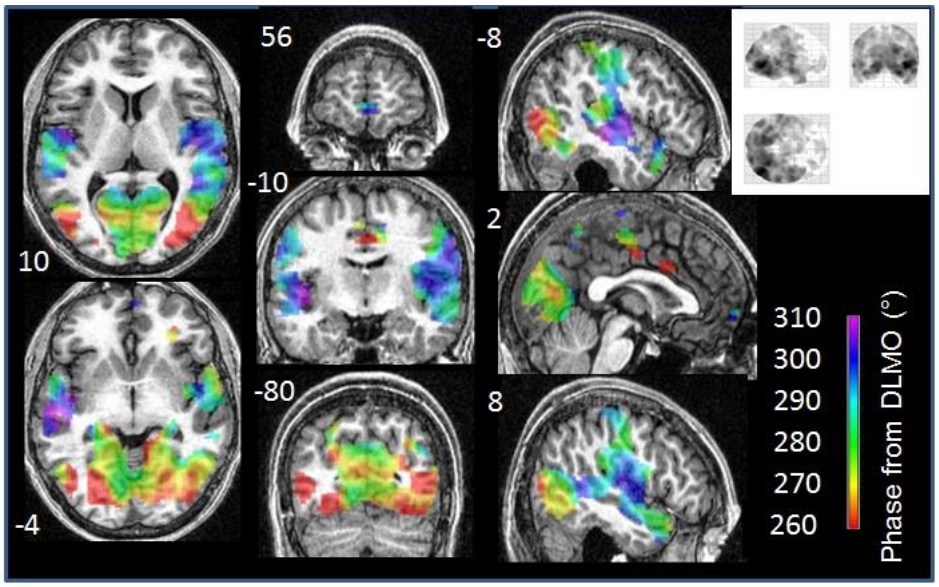

Figure 2A. PVT fMRI analysis I. (A) Circadian phase map of brain responses to PVT estimated with the Sandwich Estimator method (Guillaume et al., 2014) ($p_{FDR} < 0.05$ over the whole brain; the False Discovery Rate is currently recommended with this method). The phase of the estimated maximum of brain responses to PVT is displayed according to the color scale (melatonin onset is = 0°) and overlaid over an individual normalized T1 MR scan. Coordinates in mm along x, y and z axes. Inset: Glass brain with areas showing significant 24-h periodicity in responses.

This result indicates that circadian modulation is pervasive throughout most of the cortex. From sine and cosine parameter estimates, we computed voxelwise a complex number ($z = \beta_c + i*\beta_s$, with β_c and β_s, the

beta estimates for the cosine and sine functions) from which the phase of the circadian rhythm in responses [atan2(z)] were derived. The phase of response profiles varied significantly across brain areas (Kruskall-Wallis, χ^2 = 28.36; 4 d.f., *p*=0.0002) and spanned a phase range from 250 to 320° (**Fig.2 A**) with occipital and allocortical areas (amygdala, cingulate cortex) areas showing earlier timing of maximum responses than multimodal association areas (precuneus, temporal cortex, prefrontal areas, **Fig.2 A**). The predicted response profile peaked during the subjective afternoon and reached their nadir in the morning hours close to the offset of melatonin levels (**Fig.2C**).

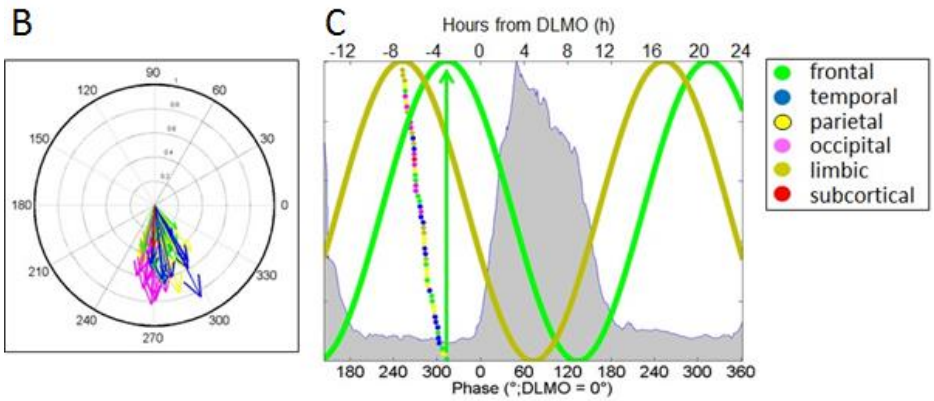

Figure 2B-C. PVT fMRI analysis I. (B) Polar representation of response phases. 0° corresponds to melatonin onset. Arrow colors correspond to the inset of panel C. **(C)** Predicted and observed time courses of significant 24-h period responses expressed as phase and approximate hours from melatonin onset. Mean melatonin profile appears in grey. Dashed sine waves illustrate the earliest (amygdala) and latest (inferior frontal gyrus) phase responses. Staggered dots show peak response times for significant 24h period responses, colored according to the legend (inset).Limbic phases ranged from 253° to 282°; occipital: 256°-302°; frontal: 256°-314°; parietal: 256°-309°; temporal: 267°-301°. Solid lines illustrate the response profile of the amygdala and inferior frontal gyrus, as determined by analysis 2. Brain responses synchronized to mean melatonin level show a trough at the offset of the melatonin rhythm, as predicted by analysis 1.

Although this analysis established a circadian modulation of regional brain responses, it assumed that the latter fluctuated as a sine wave, an assumption which does not correspond to actual time courses of most circadian biomarkers (Czeisler and Buxton, 2011). Therefore, in a second analysis, we evaluated the effective circadian modulation of PVT brain responses through an empirical marker of circadian process, the mean

melatonin levels across volunteers (**Fig. 3** inset, red line). We also assessed whether brain responses to the PVT were modulated by accumulating sleep pressure and how this homeostatic (H) sleep pressure and circadian factors (C) interact (**Fig. 3A**). This is a commonly used approach in mathematical models for human performance (Achermann, 2004; Mallis et al., 2004). Because no pure marker of homeostatic sleep pressure can be derived from empirical data obtained during sleep deprivation, it was modeled as monotonically increasing with elapsed time awake and decreasing during sleep (Fig. 3, blue). The interaction contrast ('HxC') was computed as the element by element product of the linear homeostatic contrast and the mean melatonin level (**Fig. 3**, green). A negative main effect of sleep pressure was observed in a large set of cortical areas that spanned high-order association cortices of the frontal, parietal and insular lobes and cingulate cortex as well as primary visual and sensori-motor cortices (**Fig. 3B**, blue areas; Supplementary **Table S4**). A significant main effect of circadian rhythm indicated that the time course of responses was significantly correlated with mean melatonin levels in a number of subcortical areas (midbrain, cerebellum, basal ganglia, and thalamus), in primary sensori-motor cortices, occipital areas, ventral temporal lobe, and in the association cortex of the intraparietal sulcus (Fig. 3, red areas; Supplementary **Table S5**). A significant interaction between sleep pressure and circadian rhythmicity was observed in occipital and thalamic areas (**Fig.3**, green areas; Supplementary Table S6). For all cortical areas, response profile across sessions revealed a strong decrease in response to elapsed time awake and a return to baseline levels after recovery sleep (Figure 3, left-hand activity estimate plots). In the same areas, a substantial circadian modulation was also observed, again characterized by a rapid decrease in responses during the late subjective night or early subjective morning, around the melatonin offset. However, peak responses were no longer observed during the subjective afternoon (as seen in analysis 1) but during the sessions immediately preceding the onset of melatonin secretion (solid lines, **Fig.**

2), a circadian time associated with low sleep propensity known as the wake maintenance zone (Strogatz et al., 1987; Dijk and Czeisler, 1994).

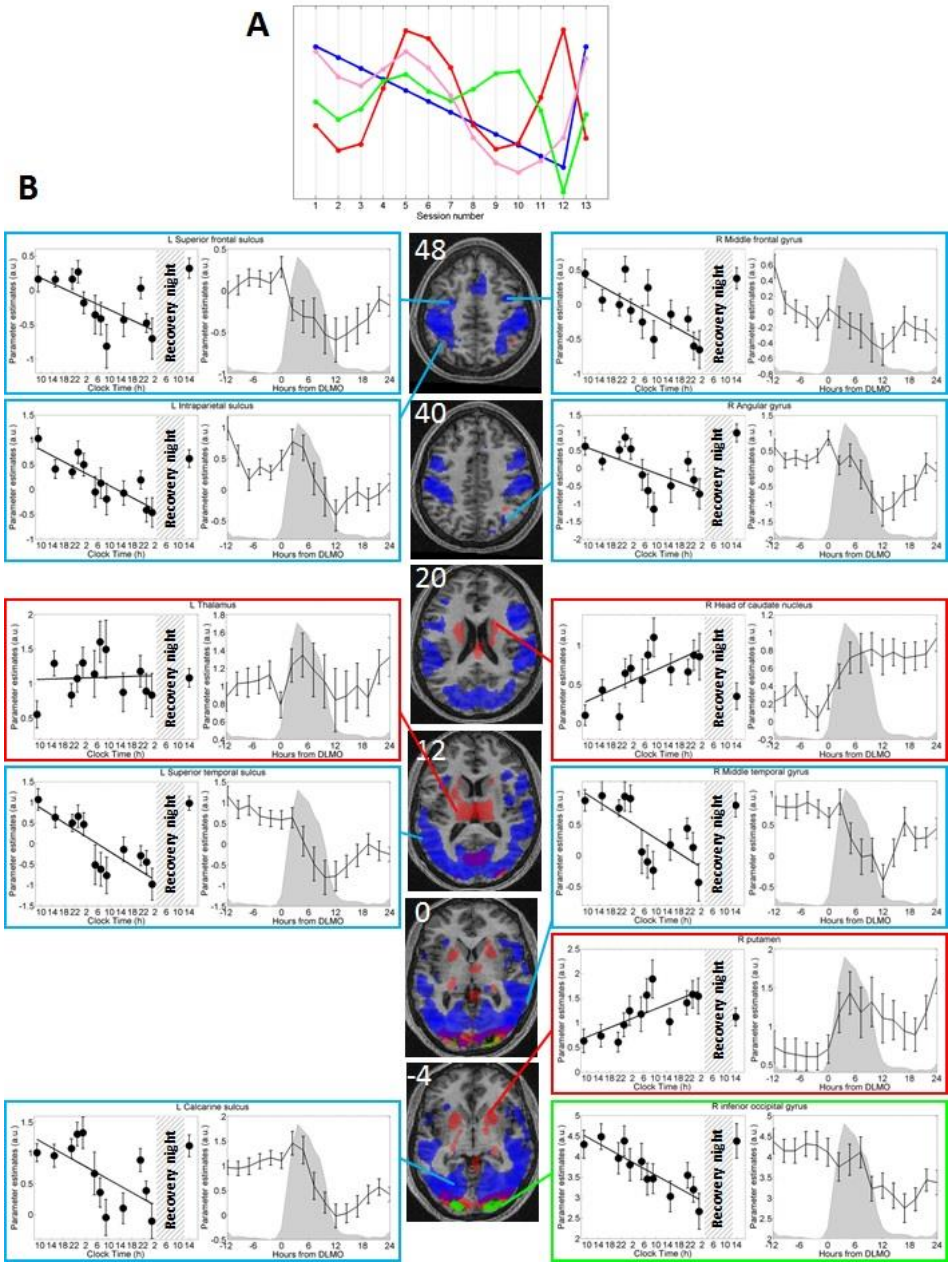

Figure 3. PVT fMRI analysis 2. (A) Response time course expected due to increasing sleep pressure during wakefulness and its reduction during recovery sleep (blue), mean melatonin level (red) and their interaction (green), used as contrasts at the random effects level. Note that the interaction is characterized by a steady level of response up to the evening sessions of day 3, in contrast to a simple 'additive' interaction (magenta). **(B)** Middle column: significant effects of

homeostatic sleep pressure (blue), circadian (red) and their interaction (green), displayed at $p_{FWE\ whole\ brain}<0.05$ over an individual normalized T1-weighted MR scan. Left- and right-hand side columns: for the main peak voxels, beta estimates are plotted against clock time (left-hand panels) and time relative to DLMO (right-hand panels; mean melatonin levels in grey). Activity estimates have been interpolated to hourly bins. Coordinates in mm along z axis. NS: non-significant.

The circadian modulation appears more tightly in phase with melatonin levels in posterior areas than in more anterior areas, accounting for the significant results in the former areas. By contrast, subcortical areas did not show significant sleep pressure associated modulation whereas their response profile shows a significant circadian modulation synchronized to the melatonin rhythm. We reasoned that the early morning circadian trough in PVT brain responses, because they lack an explicit baseline condition, might reflect a nonspecific circadian effect due, for instance, to a decrease in core body temperature or a global hormonal influence. Also, the absence of circadian modulation to DLPFC responses might reflects task dependency, as anterior prefrontal areas are not expected to participate in a simple reaction time task. To rule out this interpretation, we analyzed brain responses to the n-back task (Cohen et al., 1997b), which were also recorded during fMRI sessions. In this case, executive responses were derived from contrasting the execution of a 3-back task to a control 0-back task (3 - 0 back). Executive response profiles were not significantly modulated by elapsed time awake, because responses to both 3-back and 0-back decreased to the same extent during sleep deprivation (**Fig.4**).

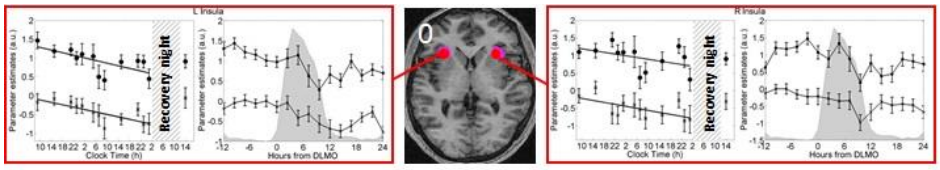

Figure 4. N-back fMRI analysis. Middle column: significant effects of circadian rhythmicity (red) displayed at $p_{FWE\ whole\ brain}<0.05$ over an individual normalized T1-weighted MR scan. Left- and right-hand side columns: brain activity estimates are plotted against clock time (left-hand panels) and time relative to melatonin onset (right-hand panels; mean melatonin levels in grey).

By contrast, responses in the bilateral anterior insula were significantly modulated by a circadian oscillation, synchronous to the melatonin rhythm ($p_{FWE\ whole\ brain} < 0.05$). Activity estimates for these brain areas showed that responses to 3-back task follow a strong circadian modulation, in contrast to responses to 0-back task in which this modulation remains at the noise level. This finding rules out a global non-specific circadian influence and speaks for the influence of a local region-specific task-dependent circadian signal.

Taken together, these findings indicate (1) a pervasive effect of local sleep pressure and circadian rhythmicity on cortical responses, (2) a prominent circadian modulation of subcortical responses, which prevails over sleep homeostatic influence; (3) an interaction of elapsed time awake and circadian factors in few task-dependent cortical areas; (4) a region-specific task-dependent modulation of circadian rhythmicity and of its interaction with local sleep need. These results suggest that the circadian rhythmicity imposed by the master clock, located in the suprachiasmatic nucleus of the hypothalamus, can be to some extent locally altered, possibly to respond to local task-related requirements. Although the molecular signals of this local modulation are unknown, it is tantalizing to suggest that clock gene expression or post-translational circadian influences are involved. Likewise, the mechanisms by which this putative local clock modulation interacts with the molecular consequences of sleep debt are not yet known, although some suggest that clock machinery would actually participate in molecular signals involved in sleep debt homeostasis (Franken and Dijk, 2009). These data have important implications for the understanding of the brain mechanisms underlying deterioration of cognition as observed in shift work, jet lag, sleep disorders and ageing.

2. Supplementary materials and methods

2.1. Participants

The study was approved by the Ethics Committee of the Faculty of Medicine of the University of Liège. Participants gave their written informed consent prior to their inclusion in the study and received a financial compensation for their

participation. They were recruited through flyers, emails, and newspaper and radio advertisements. All volunteers were right-handed (Oldfield, 1971), free from medication or psychoactive drugs, non-smokers and moderate caffeine (< 3 cups/day) and alcohol (< 7 units/week) consumers. Semi-structured interviews established the absence of medical, traumatic, psychiatric or sleep disorders. Exclusion criteria were a poor sleep quality as assessed by the Pittsburgh Sleep Quality Index (Buysse et al., 1989) (> 5 excluded); excessive daytime sleepiness (Epworth Sleepiness Scale) (> 10 excluded) (Johns, 1991); extreme chronotype (Horne and Ostberg, 1976) (scores < 31 and > 69 excluded) ; night shifts during the preceding year; travels through more than one time zone during the last three months; or a body mass index >27kg/m².

2.2. Protocol:

The experimental design consisted of a 3-week assessment of sleep timing at home followed by a 4 day laboratory protocol.

2.2.1. Ambulatory recordings:

The individual sleep history was comprehensively assessed. Rest/activity periods were measured by actigraphy (Actiwatch, Cambridge Neurotechnology, UK) and sleep diary during 3 weeks preceding the laboratory measures. The first two weeks, participants were instructed to follow a regular sleep schedule according to their habitual sleep timing. During the third week, each participant was asked to follow one of two possible sleep schedules (00:00-08:00 or 01:00-09:00). These schedules differed on average by only 18 minutes from habitual sleep time and by 8 minutes from habitual wake time and correspond to the timing imposed by subsequent fMRI sessions during the in-lab protocol. The two sleep/wake schedules were balanced across participants. Actigraphy data showed that volunteers faithfully followed the assigned schedules (mean deviation from sleep schedule: 3 $\pm$1.8 min; mean deviation from wake schedule: 9 ± 1.8 min (SD or SE).).

2.2.2. Laboratory study:

On day 0, a urine drug test was performed (10-multipanel drug test, SureScreen Diagnostics Ltd Derby, UK). Participants were trained in the MR scanner to the cognitive tasks involved in the protocol. Structural MR scans were also acquired.

Night 1 was an adaptation night during which a full polysomnography was recorded in order to rule out undetected sleep disorders (e.g., sleep breathing disorders or restless limb syndrome).

During day 1, five multiple sleep latency tests (MSLT) were performed (Carskadon and Dement, 1987). Each of them was preceded by a waking EEG recording, a measure of subjective alertness (Karolinska Sleepiness Scale, KSS) (Akerstedt and Gillberg, 1990) and measures of subjective mood (stress, anxiety, happiness, motivation) using visual analogic scales (VAS). The MSLT was conducted in accordance with standard procedures (Carskadon and Dement, 1987).

Night 2 was the baseline night, during which polysomnography was recorded. During the first 2 nights (habituation and baseline nights), the volunteers slept according to their assigned sleep/wake schedule (00:00-08:00 or 01:00-09:00).

From the morning of day 2, participants were individually isolated in soundproof, light-, temperature- and humidity- controlled rooms, under constant CCTV and EEG monitoring. They were instructed to remain awake for 42 hours under constant routine (CR) conditions. Thus volunteers remained in bed in a semi-recumbent position (45°), under dim light (<5 lux at eye level), with no information on clock time and in constant environmental conditions (temperature: 19°C ± 1; 60% humidity). Salivary samples were hourly collected for melatonin measurement. Every 2 hours, volunteers received isocaloric liquid meal substitutes. These where calculated at individual level using the Harris-Benedict formula with an activity factor of 1.3 (Harris and Benedict, 1918). During the CR protocol, twelve fMRI sessions were scheduled. They were not evenly distributed and were clustered in the morning and the evening, two periods characterized by swift modifications in circadian modulation of cognitive performance (e.g., for the 00:00-08:00 sleep schedule, fMRI sessions were scheduled at 9:00, 15:00, 21:00, 23:00, 01:00, 5:00, 7:00, 9:00, 15:00, 21:00, 23:00, 01:00). Each fMRI session included two runs. During the first run, participants performed randomly alternating blocks of auditory 3-back and 0-back tasks (Cohen et al., 1997b). During the second run, they were submitted to a psychomotor vigilance task (PVT) (Dinges and Powell, 1985). Half an hour before each fMRI sessions, KSS, VAS and a waking EEG were recorded.

Between scan sessions, a test battery consisting of auditory 3-back task, PVT and an inhibitory motor task (Sustained Attention to Response Task, SART) was performed at 2 hour intervals, starting two hours and twenty minutes after lights on, unless an fMRI scan session was planned. The order of these tests was randomized across subjects. Each session was preceded by a waking EEG recording, a KSS and a VAS. The CR-sleep deprivation was followed by a 12-hour recovery sleep episode during which participants were not allow to leave the bed or ask for lights on before the end of the scheduled sleep period.

Finally, in the afternoon of day 4, a thirteenth (last) fMRI session was conducted one hour after wake up from the recovery sleep episode, and was preceded by a waking EEG recording. Participants finished the experiments by performing the battery tests usually administered between scan sessions.

2.3. Physiological data analysis:

Melatonin: During the 42-hour CR, 43 saliva samples were collected. The first sample was obtained immediately after lights on, then at hourly intervals. Saliva samples were placed in a fridge and then centrifuged at 4°C for 10 minutes at 3000 rounds per minute. The supernatant liquid was sampled and frozen at -28°C. Salivary melatonin was measured by radioimmunoassay (Stockgrand Ltd, University of Surrey, Guildford, United Kingdom), as previously described (English et al., 1993). For each sample, 500 µL volumes were analyzed for melatonin concentration. The limit of detection of the assay was 0.8 ± 0.2 pg/ml.

Using PROC MIXED (SAS, Institute Inc., Cary, NC, Version 9.3), linear mixed model tested for the effect of clock time on melatonin secretion level. Clock time was considered as a repeated variable. All *p*-values derived from r-ANOVAs were based on Kenward-Roger (KR) corrected degrees of freedom ($p<0.05$). Before these analyses, all melatonin data have been transformed in z-score in order to avoid deviance from normality and homoscedasticity in the distributions. Melatonin data were fitted with PROC NLIN (SAS, Institute Inc., Cary, NC, Version 9.3) using the function: Value (Sample ti) = Mesor + Amplitude *Sin((Sample ti - phase)/24.2), in which the mesor = 0, amplitude = 1, and phase = 12.1, ti represents the clock time i at which a sample was collected, and value represents the value of the circadian marker variable. The circadian period was set at 24. 2 because this is the approximate average period of the human circadian melatonin rhythm (Czeisler et al., 1999) Dim

Light Melatonin Onset (DLMO) was defined as the time at which predicted melatonin level exceed 20% of the peak of the fitted curve, and Dim Light Melatonin Offset as the time at which melatonin level is below 20% of the peak of the fitted curve (Klerman et al., 2002).

2.4. EEG recordings and analyses

EEG data (sleep and waking EEG) have been recorded using a V-Amp 16 amplifier (Brain Products GmbH, Gilching Germany). EEG data were digitized at a sampling rate of 500 Hz with a bandpass filter from DC to Nyquist frequency and, for display, a 50 Hz notch filter. During the adaptation night, sleep was recorded using Ag-AgCl electrodes on 6 EEG (Fz, C3, Cz, Pz, Oz, A1, reference: right mastoid), 2 EOG, 2 chin EMG, 2 leg EMG and 2 ECG channels. Thoracic movements, nasal flow and oximetry were also recorded. During baseline and recovery nights, as well as waking EEG during CR, recordings included 10 EEG (F3, Fz, F4, C3, Cz, C4, Pz, O1, O2, A1, reference: right mastoid), 2 EOG (horizontal and vertical EOG), 2 chin EMG and 2 ECG. Before the baseline night all the electrodes were replaced by MR-compatible electrodes.

For the waking EEG recording, a modified version of the Karolinska Drowsiness Test (KDT) (Akerstedt and Gillberg, 1990) was performed. Participants were instructed to relax, avoid movement and fix a dot displayed on a screen at 75cm. Each EEG session consisted of a two-minute eyes-open period, followed by 15 seconds with eyes-closed and one minute during which volunteers were required to suppress blinks. A sound forewarned the volunteers of the beginning of each period. Throughout the recordings an experimenter monitored the participants to ensure that they remained awake. Impedances were checked and kept below 5 kΩ.

Data were analyzed by 2 independent observers. Discrepancies were resolved by consensus. EEG data were re-referenced to average mastoids. Artifacts (eye blinks, slow eye movements, body movements) were visually identified and excluded from subsequent analyses.
EEG data were scored on a 20-s epoch basis, according to the Rechtschaffen and Kales criteria (Rechtschaffen and Kales, 1968), using FASST (an SPM compatible open-source toolbox, http://www.monteforiere.ulg.ac.be/phillips/FASST.html) (Leclercq et al., 2009). NREM–REM sleep cycles were determined according to the criteria of Feinberg & Floyd (Feinberg and Floyd, 1979). Spectral analysis was computed

using the pwelch function and a Hanning window in MATLAB (7.5.0; Mathworks Inc., MA). Successive 4s epochs, overlapping by 2s were used. For data reduction, power density of artifact-free 4-s epochs was averaged over 20-s epochs.

For waking EEG, the absolute EEG power density was calculated from artifact-free 2-s epochs in the delta (0.75-4.5 Hz), theta (4.75-7.75 Hz) and alpha (8-12 Hz) frequency range, overlapping by 1 second using the pwelch function in MATLAB (7.5.0). For data reduction, power density of artifact-free 2-s epochs was averaged over 20-s epochs. Statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, North Carolina, USA). Based on the melatonin values, we obtained individual melatonin midpoints and dim light melatonin onsets (DLMO). Individuals results of power density have been realign to DLMO using 2-hours bins, for each electrode. Linear mixed-model analyses of variance for repeated measures (PROC Mixed) tested for the effect of "time relative to DLMO" on delta, theta and alpha frequency range. All raw data have been normalized using a z-score transformation before starting statistical analyses. All *p*-values were based on Kenward-Roger's corrected degree of freedom.

2.5. Tasks descriptions

PVT: The PVT is a simple reaction time task, developed to measure sustained attention (Dinges and Powell, 1985). During the task, volunteers were instructed to fixate a central cross presented on a black screen. At random interval (2-10 seconds) the fixation cross was replaced by a millisecond counter that started to scroll. In order to stop the counter, participants were instructed to press a response button as soon as possible. Participants were informed of their reaction time at each trial. The maximum trial duration was set to 10 seconds. Task duration was 10 minutes.

Auditory 3-back task: fMRI sessions included 6 blocks of 3-back and 4 blocks of 0-back task, separated by 10 to 20 second rest periods. The block order was randomly generated with the only constraint that no more than two consecutive blocks could use the same instruction. At the beginning of each block, a panel informed the participant which task was to be performed. During each block, 30 consonants were orally presented every 2 seconds. In the 3-back blocks, participants were instructed to state for each trial whether or not the current letter was identical to the consonant presented three stimuli earlier, by pressing one of two possible keys of the MR compatible

keypad. In the 0-back task, participants had to decide if the letter they just heard was a K or not. During each block, 30 consonants were aurally presented every 2 seconds. In the 3-back task, participants were instructed to state for each trial whether or not the current letter was identical to the consonant presented three stimuli earlier, by pressing one of two possible keys of the MR compatible keypad. In the 0-back task, participants had to decide if the letter they just heard was a K or not. Task duration was about 15 minutes.

2.6. Functional MRI data acquisition and analyses

Functional and structural MRI images were acquired on *3T head-only scanner (Magnetom Allegra, Siemens Medical Solutions, Erlangen) operated with the standard transmit-receive quadrature head coil.* Structural images were obtained using high-resolution T1-weighted sequence (3D MDEFT; *TR = 7.92 ms, TE = 2.4 ms, TI = 910 ms, FA = 15°, FoV = 256 x 224 x 176 mm³, 1 mm isotropic spatial resolution)* (Deichmann, 2006). *Multislice T2*-weighted functional images were acquired with a gradient-echo echo-planar imaging (EPI) sequence using axial slice orientation and covering the whole brain/most of the brain (34 slices, FoV = 192x192 mm², voxel size 3x3x3 mm³, 25% interslice gap, matrix size 64x64x34, TR = 2040 ms, TE = 30 ms, FA = 90°). In each 3-back session, between 360 and 390 functional volumes were obtained. Between 300 and 315 volumes were acquired for each PVT session. For both, 3-back* and *PVT sessions,* the first three volumes were discarded to account for T1 saturation effect. Stimuli were displayed on a screen positioned at the rear of the scanner, which the participant could see through a mirror mounted on the standard head coil. Preprocessing and data analysis were performed using Statistical Parametric Mapping (SPM8, Wellcome Trust Centre for Neuroimaging, University College London, http://www.fil.ion.ucl.ac.uk/spm) implemented in MATLAB 7.5.0 (Mathworks Inc., Sherborn, MA). *EPI time series were corrected for motion and distortion using Realign and Unwarp* (Andersson et al., 2001). Images of each participant were first realigned (motion corrected). After realignment, we spatially coregistered the mean EPI image to the anatomical structural MRI image and coregistration parameters were applied to the realigned BOLD time series. Individual anatomical MRIs were spatially normalized into the MNI space (Montreal Neurological Institute, http://www.bic.mni.mcgill.ca) with a 'unified segmentation approach (*19*). The functional MRIs were then normalized with the estimated warping and

smoothed spatially with a Gaussian kernel of 8-mm full width at half maximum (FWHM).

Functional MRI analyses were based on a mixed-effects model, and conducted in two serial steps, taking into account fixed and random effects. For each subject, changes in brain responses were estimated at each voxel, using a general linear model. According to the existing literature about PVT (Graw et al., 2004; Drummond et al., 2005; Schmidt et al., 2009), 4 regressors were entered into the model: events associated with reaction times faster than the 20^{th} percentile (fastest RTs), events associated to reaction times slower than the 80^{th} percentile (slowest RTs), events linked to intermediate reaction times (between the 20^{th} and 80^{th}percentile), events associated with lapses (trials with reaction times >500ms and <3000ms). The distribution of reaction times was estimated after exclusion of lapses throughout the 13 fMRI sessions. Trials related to fastest RT (<20^{th} percentile) were considered reflecting an optimal alertness level, while intermediate RT were considered to be corresponding to an average alertness level. The slowest RTs could result from several potential factors [sleepiness, perceptual, attentional or executive deficit, task disengagement (Drummond et al., 2005)]. For each trial type, each event was modeled as a Dirac delta function representing its onset. The ensuing vector was convolved with the canonical hemodynamic response function and used as a regressor in the individual design matrix. Six movement parameters estimated during realignment and a constant vector were also included in the matrix as a variable of no interest. High-pass filtering was implemented in the matrix design using a cutoff period of 128 s to remove low-frequency drifts from the time–series. Serial correlations were estimated with a restricted maximum likelihood algorithm using an autoregressive model.

A first analysis looked voxelwise for a modulation of PVT response (intermediate RTs) by circadian rhythms of any phase, using a sine and a cosine function of 24 h period, computed at each scan time with reference to individual melatonin onset. We used a novel statistical method (Sandwich Estimator method) which first estimates the parameters of interest with a simple Ordinary Least Square model and second estimates variances/covariances with the so-called Sandwich Estimator (SwE) which accounts for the within-subject correlation existing in longitudinal data (Guillaume et al., 2014). Summary statistic images, corresponding to the simple main effect of the task at each session for intermediate reaction times,

were fed into this analysis which accounted for between-subjects and within-subjects repetition (session) effects. Two regressors of interest were included in the design matrix: a sine wave and a cosine wave. The value of these 24h-period oscillations was adjusted to individual melatonin onset and computed for each individual scan time. The critical F contrast looked for any effect of sine and cosine functions. The resulting set of voxel values constituted a map of F-statistics [SPM(F)]. Statistical inferences were conducted at a threshold of $p_{FDR}< 0.05$ over the whole brain. The False Discovery Rate control to deal with the multiple comparison problem is currently recommended, as Random Field Theory is not yet validated for the SwE method (Guillaume et al., 2014). From the parameter estimates of the sine and cosine functions, we computed voxelwise complex number ($z = \beta_c + i*\beta_s$, with β_c and β_s, the beta estimates for the cosine and sine functions) from which the amplitude [a= abs(z)] and the phase of response [atan2(z)] were derived.

Peak voxels were grouped in 6 regions: subcortical, limbic, frontal, parietal, temporal and occipital regions. A Kruskal-Wallis test assessed the effect of regions (frontal, parietal, temporal, occipital and limbic) on phase.

The second analysis looked for circadian responses synchronized to melatonin salivary levels, for sleep homeostatic responses and the 'non additive' interaction between these 2 factors. It corresponded to a standard mixed effects model. At the fixed effects level, contrasts assessed how PVT responses were modulated by circadian, sleep pressure factors or their interactions across sessions. Because no pure marker of sleep pressure can be derived from empirical data obtained during CR (behavioral or EEG), sleep homeostat was modelled as monotonically (linearly) increasing (resp. decreasing) with time spent awake. For circadian process we used the mean melatonin levels computed across volunteers and adjusted to individual dim light melatonin onset. The interaction contrast ('HxC') was computed as the element by element product of the linear homeostatic sleep pressure contrast and the mean melatonin level, adjusted to individual dim light melatonin onset and interpolated to the scan time. The individual summary statistical images were further spatially smoothed with a Gaussian kernel of 6 mm FWHM and used in second-level analyses. The resulting set of voxel values constituted a map of *t*-statistics [SPM(T)]. Statistical inferences were performed after correction for multiple comparisons at a threshold of $p_{FWE}< 0.05$ over the whole brain.

The same analysis was conducted for the Nback task. At the fixed effects level, the contrasts consisted of the task effect (3back-0back) modulated by the homeostatic sleep debt, the mean melatonin profile and their interaction.

3. Supplementary results

3.1. Population:

Buccal DNA samples were collected and analyzed in 329 right-handed individuals, aged between 18 and 30 years old during a pre-laboratory field study. Among these individuals, 149 were homozygous for the *PER3* $^{4/4}$ allele and 33 for the *PER3* $^{5/5}$ allele. Thirty-six volunteers participated in the experiment, but three of them have been discarded from all the analyses because of incomplete melatonin data. The sample comprised twenty-two participants homozygous for the 4 allele (mean age 21,318 +/- 1,701; 10 females) and eleven for the 5 allele (mean age 20,727 +/- 1,737; 6 females). Genotyping was carried out as previously described (Viola et al., 2007), with some modifications. Briefly, buccal swab samples (Rayon swab tip, Copan 155C; Copan Italia, Brescia, Italy) were collected during the initial interview and genomic DNA was extracted using the QuickExtract system (Epicentre Biotechnologies, Madison, Wisconsin). Volunteers had been screened for their *PERIOD3* variable-number-of-tandem-repeat genotype. Genotyping was performed by PCR DNA amplification followed by fragment length analysis with agarose gel electrophoresis. PCR was carried out using GoTaq Green Master Mix (Promega, Madison, Wisconsin) with the following cycling conditions: 95°C for 3 min, followed by 40 cycles of 95°C for 1 min, 58°C for 1 min, and 72°C for 1 min. All subjects were pooled together for the current analyses such that genotype was not considered any further.

3.2. KSS and VAS

The linear mixed-model analyses of variance for repeated measures (PROC Mixed) yielded a significant main effect of “time relative to melatonin onset” on subjective sleepiness, as measured by KSS ($F_{21, 629}$= 58.51; P <0.0001), with highest levels of sleepiness at the end of the biological night as well as at the end of the CR-sleep deprivation when melatonin had risen again. Analyses of

variance revealed similar results for motivation, as indexed by VAS ($F_{21, 630}$= 13.59; P <0.0001), with lower motivation values at the end of the CR. Similar results were obtained for all the other variables measured by the VAS: happiness ($F_{21, 629}$= 9.86; P <0.0001), anxiety ($F_{21, 629}$ = 3.34; P <0.0001) and stress ($F_{21, 628}$ = 5.06; P <0.0001).

3.3. Waking EEG:

There was a significant effect of "time relative to melatonin onset" on delta, theta and alpha frequency range for each derivation tested. On Cz, for delta activity (0.75-4.5 Hz), a main effect of time relative to DLMO was detected ($F_{21, 577}$ = 8.44; P <0.0001), with highest values at the end of the biological night. A significant effect of time relative to melatonin onset was detected on Cz for theta activity (4.75-7.75 Hz; Cz; $F_{21, 576}$ = 18.86; P <0.0001). Similar results were obtained for alpha activity, in the frequency range of 8-12 Hz (Cz; $F_{21, 572}$ = 3.32; P <0.0001).

3.4. Sleep EEG :

Baseline and recovery sleep structure

The sleep stages, visually scored for both baseline and recovery nights, were expressed in minutes or as percentages of total sleep time (Table S1).Polysomnographic sleep variables were calculated using the following procedure:

- Time allowed to sleep (TAS) = time from lights-off to lights-on
- Sleeping period (SP) = time from the first epoch of sleep stage 2 to the last epoch of sleep.
- Total sleep time (TST) = time spent sleeping during the TAS
- Sleep efficiency = TST/TAS*100

Comparison between baseline and recovery nights was carried out through a PROC TTEST (SAS, Institute Inc., Cary, NC, Version 9.3). Results of the PROC TTEST are illustrated in Table S1 and in Table S2 for measures expressed as percentages. The differences observed between the two nights reflected the rebound of sleep typically observed following sleep deprivation (Daan et al., 1984; Dijk et al., 1987a). Slow wave activity (0.75-4 Hz EEG power), a reliable quantitative estimate of homeostatic sleep need (Dijk et al., 1987a; Dijk and Czeisler, 1995) was also significantly increased during recovery, as compared

to baseline sleep (baseline: 844.4 µV2 + 71.96 over frontal leads; recovery: 1810.8 µV2 + 193.7; Wilcoxon test: $p < 0.0001$).

4. Supplementary tables

Table S1. PSG Sleep variables during baseline (BSN) and recovery

	BSN			RN			BSNxRN	
Wake (min)	36.29	±	3.45	72.84	±	8.51	t = 3.98;	*P* = 0.0002
Stage 1 (min)	27.68	±	2.09	27.46	±	2.51	t = 0.07;	*P* = 0.94
Stage 2 (min)	170.78	±	4.16	206.57	±	9.88	t = 3.34;	*P* = 0.0014
Stage 3 (min)	55.88	±	2.43	84.50	±	5.80	t = 4.55;	*P* = < 0.0001
Stage 4 (min)	81.53	±	3.29	150.08	±	6.09	t = 9.90;	*P* = < 0.0001
SWS (min)	137.42	±	4.37	234.58	±	9.94	t = 8.94;	*P* = < 0.0001
REM sleep (min)	111.77	±	3.35	134.30	±	7.03	t = 2.89;	*P* = 0.0053
WASO (min)	11.87	±	1.46	38.37	±	6.63	t =3.90;	*P* = 0.0002
MT (min)	3.88	±	0.53	7.23	±	0.83	t = 3.38;	*P* = 0.0012
Latency to S1 (min)	12.91	±	1.89	8.31	±	4.40	t = 0.96;	*P* = 0.34
Latency to S2 (min)	17.83	±	1.90	6.90	±	1.23	t = 4.81;	*P* = < 0.0001
Latency to REM sleep (min)	88.04	±	7.39	73.15	±	6.08	t = 1.55;	*P* = 0.12
TAS (min)	488.03	±	2.08	684.69	±	22.33	t = 8.77;	*P* = < 0.0001
SP (min)	458.86	±	2.45	646.93	±	21.76	t = 8.59;	*P* = < 0.0001
TST (min)	419.98	±	3.68	575.46	±	18.84	t = 8.10;	*P* = < 0.0001
Sleep Efficiency	86.09	±	0.79	84.52	±	1.11	t = 1.15;	*P* = 0.25
Sleep Efficiency S1	5.67	±	0.42	3.93	±	0.32	t = 3.25;	*P* = 0.0019
Sleep Efficiency S2	34.99	±	0.86	29.81	±	0.97	t = 3.98;	*P* = 0.0002
Sleep Efficiency S3	11.45	±	0.49	12.14	±	0.70	t = 0.8;	*P* = 0.42
Sleep Efficiency S4	16.71	±	0.67	23.26	±	1.52	t = 0.39;	*P* = 0.0002
Sleep Efficiency REM sleep	22.92	±	0.70	19.30	±	0.77	t = 3.47;	*P* = 0.0009

Mean ± SEM is shown; *p* values are accounting for comparison between the two nights.

PSG, polysomnographic; WASO, wake after sleep onset; MT movement time; REM, rapid eye movement; SWS, slow wave sleep; TAS, time allowed to sleep; SP, sleeping period; TST, total sleep time.

Relative to baseline, recovery sleep was characterized by shorter sleep latency, increase sleep efficiency, total sleep time, NREM and REM sleep. Slow wave activity (0.75-4 Hz EEG power), a reliable quantitative estimate of homeostatic sleep need (Dijk et al., 1987; Dijk & Czeisler, 1995), was also significantly increased during recovery, as compared to baseline sleep (baseline: 844.4 μV^2 ± 71.96 over frontal leads; recovery: 1810.8 μV^2 ± 193.7; Wilcoxon test: $p < 0.0001$).

Table S2. PSG Sleep variables expressed as percentages of total sleep

	BSN			RN			BSNxRN	
Wake (%)	8.82	±	0.92	12.64	±	1.56	t = 2.10;	*P* = 0.03
Stage 1 (%)	6.70	±	0.55	4.68	±	0.39	t = 2.94;	*P* = 0.004
Stage 2 (%)	40.72	±	1.02	35.44	±	1.22	t = 3.31;	*P* = 0.0015
Stage 3 (%)	13.33	±	0.58	14.35	±	0.77	t = 1.06;	*P* = 0.29
Stage 4 (%)	19.39	±	0.76	27.32	±	1.57	t = 4.53;	*P* = < 0.0001
SWS (%)	32.72	±	1.01	41.68	±	1.60	t = 4.72;	*P* = < 0.0001
REM sleep (%)	26.55	±	0.72	22.87	±	0.89	t = 3.20;	*P* = < 0.0022
WASO (%)	2.90	±	0.39	6.62	±	1.21	t = 2.91;	*P* = 0.005
MT (%)	0.92	±	0.12	1.24	±	0.13	t = 1.78;	*P* = 0.07

Table S3. Statistical results: brain areas showing a significant 24h periodicity in PVT response profile.

Lateralization	Area	p(FDR-corr)	X	x	y	z
Right	**Anterior cingulate cortex**	0.023	8.217	6	46	-4
Left	**Anterior cingulate cortex**	0.027	7.690	-10	52	2
Right	**Mid-cingulate cortex**	0.008	11.683	6	8	32
Right	**Orbito-frontal cortex**	0.033	7.070	4	44	-14
Left	**Orbito-frontal cortex**	0.017	9.081	-8	46	-14
Right	**Frontopolar cortex**	0.014	9.662	2	62	-4
Right	**Inferior frontal gyrus**	0.025	7.884	58	24	12
Left	**Inferior frontal gyrus**	0.024	8.024	-60	18	14
Left	**Middle frontal gyrus**	0.048	5.962	-32	34	20
Left	**Superior frontal gyrus**	0.046	6.091	-18	56	22
Left	**Superior frontal sulcus**	0.012	10.255	-20	4	56
Left	**Frontal operculum**	0.014	9.668	-64	-10	26
Right	**Insula**	0.003	18.559	44	-6	18
Left	**Insula**	0.007	12.147	-36	-26	16
Right	**Precentral gyrus**	0.004	14.485	64	-8	30
Left	**Precentral gyrus**	0.028	7.530	-40	-18	62
Left	**Post central gyrus**	0.004	15.442	-56	-14	42
Left	**Inferiorparietal lobule**	0.024	8.006	-42	-66	28
Right	**Intraparietal sulcus**	0.010	10.806	26	-34	54
Left	**Intraparietal sulcus**	0.008	11.834	-42	-40	56
Right	**Superior parietal lobule**	0.005	14.157	26	-24	72
Left	**Superior parietal lobule**	0.007	12.377	-28	-22	70
Right	**Angular gyrus**	0.002	20.587	24	-88	28
Left	**Angular gyrus**	0.006	12.774	-24	-60	56
Right	**Precuneus**	0.013	10.014	10	-48	48
Left	**Retrosplenial cortex**	0.029	7.494	12	-38	12
Right	**Temporal pole**	0.004	14.826	44	14	-32
Left	**Temporal pole**	0.008	11.810	-44	16	-18
Right	**Superior temporal gyrus**	0.003	16.543	48	-6	2
Left	**Superior temporal gyrus**	0.008	11.817	-48	-22	12
Right	**Superior temporal sulcus**	0.002	19.485	58	-6	-12
Left	**Superior temporal sulcus**	0.004	15.101	-52	-32	6
Left	**Middle temporal gyrus**	0.003	16.579	-62	-28	-4
Right	**Amygdala**	0.028	7.608	18	-2	-28
Left	**Amygdala**	0.014	9.661	-24	0	-22
Right	**Hippocampus**	0.007	12.245	30	-30	-10
Right	**Parahippocampal gyrus**	0.002	19.937	22	-58	-4
Left	**Parahippocampal gyrus**	0.002	20.742	-14	-50	-12
Right	**Calcarine sulcus**	0.003	16.213	20	-56	12
Right	**Cuneus**	0.003	15.948	20	-84	38
Left	**Cuneus**	0.006	12.867	-8	-80	32
Right	**Fusiform gyrus**	0.002	23.052	30	-54	-16
Left	**Fusiform gyrus**	0.002	21.599	-20	-62	-4
Right	**Lingual gyrus**	0.003	17.668	20	-74	-4
Left	**Lingual gyrus**	0.003	17.720	-16	-78	-10
Right	**Occipito-temporal cortex**	0.002	26.672	44	-64	-2
Left	**Occipito-temporal cortex**	0.003	17.169	-46	-72	0
Right	**Occipital lateral cortex**	0.015	9.581	44	-80	20
	Thalamus	0.036	6.758	0	-8	6

Table S4 . Brain areas negatively influenced by homeostatic sleep pressure (i.e. elapsed time awake)
Only one representative peak voxel is included per area.

Homeostatic sleep pressure					
Lateralization Area	**Z score**	**P_{corr}**	**x**	**y**	**z**
Right Superior frontal gyrus	5,03	4,67 10-03	14	-2	68
Right Superior frontal sulcus	5,43	7,33 10-04	36	-2	54
Left Superior frontal sulcus	4,74	1,5610-02	-28	-4	46
Right Middle frontal gyrus	4,98	5,77 10-03	40	-2	42
Left Middle frontal gyrus	4,98	5,60 10-03	-38	34	20
Right Inferior frontal gyrus	5,83	1,02 10-04	56	16	0
Right Inferior frontal sulcus	6,67	9,44 10-07	40	16	24
Left Inferior frontal sulcus	5,97	4,77 10-05	-42	-2	28
Left Anterior cingulate cortex	4,53	3,53 10-02	-8	6	34
Left Mid-cingulate cortex	4,55	3,28 10-02	-14	-24	46
Right Pre-supplementary motor area	5,95	5,41 10-05	10	10	56
Left Supplementary motor area	5,11	3,26 10-03	-4	-8	56
Left Precentral gyrus	5,84	9,42 10-05	-52	0	36
Left Central sulcus	6,43	3,67 10-06	-42	-36	50
Right Postcentral gyrus	6,56	1,80 10-06	54	-30	48
Left Inferior parietal lobule	5,86	8,69 10-05	-44	-40	20
Left Intraparietal sulcus	4,94	6,89 10-03	-28	-50	46
Right Angular gyrus	4,65	2,28 10-02	20	-78	38
Right Anterior insula	5,31	1,32 10-03	34	24	8
Right Insula	4,56	3,12 10-02	40	0	0
Right Posterior insula	5,34	1,11 10-03	40	-4	14
Right Superior temporal gyrus	5,88	7,55 10-05	64	-28	22
Left Superior temporal gyrus	5,43	7,35 10-04	-62	-22	0
Right Superior temporal sulcus	6	4,11 10-05	50	-22	-8
Left Superior temporal sulcus	5,73	1,64 10-04	-52	-50	10
Right Middle temporal gyrus	6,49	2,59 10-06	54	-48	-2
Right Temporal pole	5,54	4,28 10-04	48	16	-20
Left Temporal pole	4,64	2,30 10-02	-54	8	-14
Left Calcarine sulcus	6,64	1,09 10-06	-24	-68	-2
Right Calcarine sulcus	5,26	1,61 10-03	10	-78	16
Right Cuneus	5,29	1,41 10-03	6	-84	28
Left Cuneus	5,94	5,56 10-05	-10	-84	16
Left Fusiform gyrus	6,04	3,27 10-05	-36	-48	-20
Right Fusiform gyrus	5,99	4,32 10-05	42	-52	-16
Right Lingual gyrus	5,38	9,33 10-04	30	-84	-12
Left Lateral occipital	6,59	1,46 10-06	-46	-68	6
Right Lateral occipital	6,7	7,58 10-07	44	-68	2
Left Occipital pole	6,14	1,92 10-05	-20	-94	12
Right Occipital pole	5,81	1,14 10-04	20	-92	2
Right Thalamus	4,85	1,01 10-02	18	-30	0
Left Thalamus	4,67	2,04 10-02	-20	-30	-2

Table S5 . Brain areas positively correlated with average melatonin levels (adjusted to individual DLMO)

Circadian rhythmicity					
Lateralization Area	**Z score**	**P_{corr}**	**x**	**y**	**z**
Right Occipital pole	7,62	8,75 10-10	22	-92	-2
Left Occipital pole	6,59	6,44 10-07	-10	-94	-6
Right Calcarine sulcus	5,07	1,68 10-03	8	-72	10
Right Lingual gyrus	5,91	2,87 10-05	34	-76	-18
Left Lingual gyrus	5,99	1,91 10-05	-36	-68	-22
Right Fusiform gyrus	5,89	3,27 10-05	26	-56	-24
Right Intraparietal sulcus	4,49	1,88 10-02	32	-52	40
Right Thalamus	4,81	5,20 10-03	26	-28	-2
Left Thalamus	5,22	8,52 10-04	-16	-18	12
Right Head caudate	5,01	2,21 10-03	20	8	18
Left Head caudate	4,47	2,05 10-02	-20	20	12
Right Putamen	5,21	9,12 10-04	20	10	-6
Left Putamen	5,36	4,63 10-04	-22	8	-6
Right Globus Pallidus	4,78	6,00 10-03	14	-4	-6
Left Globus Pallidus	4,34	3,33 10-02	-18	-10	-4
Right Vermis	5,33	5,16 10-04	6	-64	-18
Left Vermis	5	2,31 10-03	-4	-76	-20
Right Ventral mesencephalon	4,79	5,80 10-03	0	-20	-14

Table S6 . Brain areas positively correlated with the interaction between sleep homeostasis and circadian rhythmicity (average melatonin levels, adjusted to individual DLMO), all during PVT responses

Interaction between sleep homeostasis and circadian rhythmicity					
Lateralization Area	**Z score**	**P_{corr}**	**x**	**y**	**z**
Right Occipital pole	5,44	4,08 10-04	20	-94	-2
Left Occipital pole	4,92	4,17 10-03	-20	-92	-4
Right Lingual gyrus	4,54	1,98 10-02	32	-72	-22
Right Thalamus	4,4	3,39 10-02	16	-22	16

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General Discussion

General Discussion

1. Main results

Sleep loss substantially alters cognitive, cardiovascular, immune and metabolic regulation (Hanlon and Van Cauter, 2011). In particular, acute sleep deprivation affects multiple aspects of cognition, ranging from alertness and emotional processing to attention and executive functions (Killgore et al., 2008; Lo et al., 2012). However, understanding how cognition, and the underlying brain activity, is affected by sleep loss is difficult because at any point in time, cognitive performance results from the combined influence of two main factors: sleep pressure and circadian rhythmicity. The aim of the present work was to characterize the neural correlates of attention, under sleep loss conditions. Firstly we assessed the robustness of the Attentional Network Test (ANT; Fan et al., 2005). According with its developers, the ANT measures the efficiency of three attentional components related to different and independent cortical networks. Thus we decided to investigate the effect of sleep loss on these attentional subunits. In a subsequent study we were interested to assess whether during sustained wakefulness, brain responses associated with attentional resources were modulated by circadian rhythmicity and how the latter interacts with sleep pressure accumulated during elapsed time awake.

2. Study I

The first study aimed to investigate brain correlates of alerting, orientating and executive attentional processes using the Attentional Network Test (Fan et al., 2005), and more interestingly to assess

whether sleep deprivation *differently and/or selectively* affects specific attentional components. We first investigated the brain responses related to each attentional subunit, under rested wakefulness (RW) to compare their congruency with the literature (for this analysis all RTs were considered together). Our fMRI data acquired during RW do not support the independency of the alerting, orientating and executive attentional networks. As underlined in the corresponding publication, occipito-temporal as well as frontal regions were partially overlapping for alerting and executive components. The alerting effect was associated with activity in occipito-temporal regions, inferior frontal gyrus, medial prefrontal cortex and intraparietal sulcus. The orientating effect was related to posterior middle frontal gyrus, while the conflict effect (executive component) elicited responses in occipito-temporal regions, inferior and posterior frontal gyrus, precentral gyrus and superior parietal cortex. Globally RW activity pattern related with each attentional component is relatively congruent with literature reports (Coull et al., 2001; Thiel et al., 2004; Fan et al., 2005; Konrad et al., 2005).

2.1. The impact of sleep deprivation on attentional components:

Regarding the impact of sleep deprivation (SD), our data do not support the view of a selective effect on these attentional subunits. Behavioural results showed that, during sleep loss, reaction times (RTs) slowed down to the same extent irrespective of the cue or target condition. This result could suggest a widespread effect of sleep loss on brain activity, a non-specific influence of sleep need on neural correlates of attentional components.

In a later step we compared each effect between conditions (RW vs SD) for fMRI responses. For this analysis trials were split into intermediate and faster RTs. For the alerting effect, surprisingly, average alertness level (corresponding to intermediate RTs) did not show any significant difference between SD and RW. For optimal alertness (fastest RTs), brain activity in the precuneus and in the right temporo-parietal junction (rTPJ) decreased during SD compared to RW. Let us note that

under RW, for intermediate RTs, increased activity in the left TPJ was associated with the orientating effect. Neuroimaging evidences suggest that the TPJ is linked to stimulus-driven attentional responses to task-relevant information (Arrington et al., 2000; Coull et al., 2001; Corbetta and Shulman, 2002). Corbetta and Shulman (2002) observed activity of TPJ in response to target detection. However, when the target appeared at an unexpected location, the recruitment of this region was even enhanced. These results assign to the TPJ a role in mediating, in a bottom-up manner, automatic attention toward relevant stimuli. Now, in the context of the ANT, Fan and colleagues (2005) found significant activations in right TPJ related with alerting, while left TPJ was related to orientating effect. Our results of a decreased activity in this region during SD (relative to RW), in absence of behavioural correlates, could reflect the use of strategies that reinforce optimal cognitive response under rested conditions but that at the same time are not critical to the alerting effect. Higher sleep pressure could finally reduce these mechanisms resulting in brain activity decreases without impacts on the behavioural output.

2.2. Higher-order attentional components:

For the orientating effect, comparison between RW and SD revealed enhanced left TPJ activity under sleep deprivation conditions, when optimal vigilance (faster RTs) level can be achieved. However this pattern was not observed for intermediate RTs. As aforementioned, the TPJ mediates the automatic capture of attention by salient stimuli, particularly when the target appear at an unexpected location (Corbetta and Shulman, 2002). Thus, under SD, to achieve in orienting attention to salient stimuli at optimal vigilance level (fast RTs) and in an efficient way (the session x cue interaction was not significant), more important left TPJ recruitment is needed. Again this result supports the idea of strict link between vigilance and attention, relative to cortical brain responses. For both executive and orientating components, brain responses were found to be enhanced in thalamic areas after SD, relative to RW. Importantly, increase in thalamic responses, following

sleep deprivation, albeit not always detected (Choo et al., 2005), was often reported in fMRI studies and with very different cognitive tasks (Portas et al., 1998; Chee and Choo, 2004; Habeck et al., 2004; Schmidt et al., 2009; Vandewalle et al., 2009). A recent meta-analysis of neuroimaging studies, on the effects of sleep loss, emphasized that the only region that consistently showed increased activation is the thalamus (Ma et al., 2015). Increased thalamic activity, observed under sleep loss conditions, was often interpreted as reflecting increased cognitive efforts to compensate for the detrimental effects of sleep deprivation (Portas et al., 1998; Chee and Choo, 2004; Habeck et al., 2004; Chee et al., 2008; Schmidt et al., 2009; Tomasi et al., 2009; Vandewalle et al., 2009). Therefore, in our data, a compensatory role of thalamic response argues against a selective influence of sleep deprivation on specific networks involved in orientating or executive components of attention. Furthermore, thalamic activity would reflect non-specific mechanisms, which transiently maintain an optimal vigilance level, thereby supporting cortical function. In broad terms, following the same conclusion drawn for the alerting component, increased thalamic responses underlies the importance of vigilance to cortical function in maintaining cognitive performance under sleep loss. Thalamic changes, moreover, seem to be related to the influence of arousal levels on attention (Coull, 1998), possibly due to its privileged anatomical relationship with the ascending reticular activating system (ARAS). Therefore, the thalamus can regulate cortical arousal level through thalamocortical connections, as well as through intra-thalamic interactions (Paus et al., 1997). As change in thalamic activity was not observed here for alertness, further studies are needed to confirm the specificity/generality of thalamic activity in low versus high attentional processes. A possible scenario is that phasic alertness is a too low basic cognitive process, compared to higher-order cognitive processes, to require compensatory processes under SD. However these results could have been influenced by circadian confounding effects. This issue has been further investigated in the second study.

In summary, our behavioural and fMRI results do not completely support the hypothesis of a selective and differential influence of sleep deprivation on the three attentional components. Thalamic recruitment, during sleep loss, supported orientating and executive components suggesting that sleep deprivation influences attention through its impact on the ability to maintain vigilance[3].

3. Study II

In the second study, we investigated cognitive brain responses associated with attentional resources, during extended wakefulness over more than an entire circadian cycle. The ultimate aim was to explore whether attention-related brain responses were controlled by the combined action of circadian rhythmicity, sleep need and their interaction. As in study I, the picture emerged that sleep loss affects attention through global vigilance, we decided to opt for one of 'the most widely used measures of behavioural alertness' (Basner and Dinges, 2011), the Psychomotor Vigilance Test (PVT; Dinges and Powell, 1985). To achieve our aim we set up a sleep deprivation protocol combining a constant routine (CR) paradigm with fMRI acquisitions. The constant routine is considered as the gold standard to minimize masking effects of exogenous factors and thus distinguish external from internal drives of circadian rhythmicity (Van Dongen et al., 2004). Moreover there is an extended literature assessing the time-course of behavioural and electrophysiological variables under CR conditions. However, to our knowledge, there are no studies measuring the specific temporal profile of attentional brain responses by performing multiple acquisitions, particularly focusing on time windows encompassing circadian arousal peaks and troughs.

Our behavioural results clearly confirmed a progressive increase of subjective (KSS; Akerstedt and Gillberg, 1990) and objective sleepiness (delta and theta EEG power), as well as of negative affect (as indexed by

[3] In this context the term vigilance is used as synonymous of tonic alertness, corresponding to the level of 'cortical activation' induced by brainstem activating structures.

VAS) throughout the CR. These changes paralleled the progressive increase in RTs at the PVT, during the fMRI sessions. Again, in agreement with other literature reports, all these variables also showed a strong circadian modulation, such that sleep loss affected neurobehavioral output strongest at the end of the first biological night (approximately around 8 am) as well as at the end of the protocol (approximately around 01 pm), before to return to baseline levels after the recovery sleep episode.

In sum, our behavioural data confirm literature evidences that decrements in neurobehavioral performance, resulting from sleep loss, vary according to time of day (see Carrier and Monk, 2000; Schmidt et al., 2007 for reviews). Performance deterioration during sleep deprivation is most prominent towards the end of the biological night, while this effect is attenuated during the subsequent day despite a further prolongation of wakefulness (Dijk et al., 1992; Cajochen et al., 1999b). Such daytime performance stabilization is most probably supported by wake-promoting activity of the circadian timing system (Cajochen et al., 2004), which counteracts the detrimental impact of the progressive rise in sleep pressure throughout wakefulness (Dijk and Edgar, 1999).

3.1. Neuroimaging results:

Intriguingly, our neuroimaging results regularly acquired throughout the 42-hour protocol revealed a significant circadian periodicity in attention-related brain response profiles over almost the entire cortex, putatively reflecting the power of the circadian alerting signal in maintaining adequate vigilance levels at the cerebral level. Moreover the phase of these profiles varied significantly across brain regions, speaking in favour of a local, region-specific circadian modulation in task-related brain responses. Intriguingly occipital and limbic regions showed an earlier timing of maximal responses relative to associative areas such as temporal and prefrontal regions.

As abovementioned, the way SD affects task-related activation, differed across studies and this could be linked to several potential factors. Between these factors we saw that cognitive domain investigated, task duration and task difficulty are crucial. For instance, as reported by Chee and Chuah (2008), task difficulty increases were related to specific activation patterns ("compensatory") in some studies (Chee and Choo, 2004; Drummond et al., 2004) but not in others (Bell-McGinty et al., 2004; Chee et al., 2006). Thus, the presence of a region-specific circadian modulation in cognition-related brain responses speaks in favour of the existence of multiple local clocks regulating neurobehavioral output, at least under challenging sleep deprivation conditions. It might also be able to explain the complex pattern of BOLD activity increases and decreases in several brain regions varying according to task complexity and investigated cognitive domain (Chee and Chuah, 2008). In the context of the PVT, task requirements, more specifically the perception of visual stimuli putatively leads to a constant recruitment and thereby disproportional use of occipital regions, presenting earlier circadian trough. We thus interpreted this finding as resulting from a combined influence of task-dependent specific local demands, circadian factors and elapsed time awake.

3.2. Brain responses associated with circadian melatonin profile:

In a next step, in order to more concretely investigate circadian influences on sleep-loss-related modulation in the cerebral correlates underlying attentional performance, we related acquired brain imaging data throughout the protocol to a classical physiological marker of circadian phase (DLMO, dim light melatonin onset). For this analysis, brain imaging data were differentially weighted according to their distance to individual DLMO at acquisition. This analysis allowed the detection of brain regions in which BOLD activity followed a circadian modulation adjusted to the individual DLMO. Note that the DLMO coincides or at least surrounds the occurrence of the so called wake maintenance zone (WMZ) or 'forbidden zone for sleep'. The WMZ can be defined as a two to three hours window of reduced sleep propensity,

where a paradoxical increase in the circadian alerting signal counteracts the rise in sleep propensity (Lavie, 1986; Strogatz et al., 1987; Edgar et al., 1993; Dijk and Czeisler, 1994; Shekleton et al., 2013; Sletten et al., 2015), occurring approximately three hours before habitual bedtime. Anchoring the data to DLMO might thus be particularly appropriate if it is aimed to trace circadian wake promotion at the cerebral level.

Results highlighted a more specific set of cortical regions. BOLD activity in occipital areas, in the intraparietal sulcus, but also in subcortical areas (thalamus, basal ganglia and midbrain) showed a circadian profile coinciding with the circadian melatonin profile, strengthening the suggestion of functional relevance of the circadian timing system for neurobehavioral (or attentional) performance modulation across time. Note however that our data were collected during sleep deprivation. Under such conditions, sleep need or pressure monotonically increases throughout the protocol and thus inevitably accompanies circadian modulation of wake promotion. Thus, in order to adequately interpret our data, the temporal profile of wake promotion resulting from the combined action of both circadian and sleep homeostatic processes has to be applied to our neuroimaging data.

At the end of the biological day, the circadian drive for wakefulness is counteracting or opposing increasing homeostatic sleep pressure by maximally promoting wakefulness few hours before the habitual bedtime (Dijk and Czeisler, 1994). The combined action of both processes thus allows a consolidated period of wakefulness under entrained day-night conditions. However, as soon as wakefulness is extended into the biological night, circadian wake promotion declines and no more opposes increasing sleep pressure levels. As no pure marker of homeostatic sleep pressure can be derived from empirical data, we modeled brain responses as monotonically decreasing across fMRI sessions and then increasing again after the recovery sleep episode. This monotonic decrease was used as a proxy to assess the negative impact of increasing sleep debt onto brain responses. Ultimately the interaction between time spent awake (reflecting sleep

homeostasis influences) and the circadian process was modeled as the element by element product of the linear homeostatic contrast and the mean melatonin level; this is an approach commonly used in mathematical model for human performance (Achermann, 2004; Mallis et al., 2004).

3.3. Brain responses associated with homeostatic sleep need:

Brain responses associated with homeostatic sleep process were observed in frontal, cingulate, parietal and insular cortex as well as primary visual and sensori-motor cortices. BOLD activity in occipital and thalamic regions traced the time course of the interaction between sleep pressure and circadian rhythmicity. To further inspect the temporal response profile of these regions, we extracted brain activity estimates across sessions. Impressively, we consistently observed strong BOLD decreases in response to time spent awake, for all cortical areas, and a return to baseline levels following the 12 hour recovery sleep episode. Peak responses were observed during the sessions immediately preceding the onset of melatonin secretion, while a rapid activity decrease was detected during the late subjective night or early subjective morning, around the melatonin offset. Thus, at cortical level we observed a profile clearly recognizable as the combined effect of both circadian and sleep pressure factors. These results are in agreement with the expected negative influence of sleep debt on neuronal response. Sleep loss is associated with a number of molecular processes that are thought to dampen neural responsiveness. In particular, release of cytokines, nitric oxide (NO), prostaglandins and adenosine are believed to result in an increase in K+ conductance and, consequently, a reduced neural activity (Porkka-Heiskanen et al., 1997; Cirelli et al., 2005; Rétey et al., 2005; Krueger, 2008). It is usually considered that these processes result from the synaptic activity accrued during wakefulness, especially when it involves synaptic potentiation (Tononi and Cirelli, 2014), and are thus expected to be particularly prominent in frontal areas (Werth et al., 1997; Cajochen et

al., 1999a; Borbély, 2001; Finelli et al., 2001; Riedner et al., 2007; Dang-Vu et al., 2008).

In keeping with this perspective, a different profile was observed for subcortical regions. These showed a significant circadian modulation, steady synchronized to the melatonin rhythm but not influenced by homeostatic sleep pressure. More precisely, thalamic neural responses were quite stable until the onset of melatonin secretion; however immediately after the DLMO, during the biological night, responses increased, following the melatonin profile. This result suggests that little local sleep debt is accumulated in these subcortical areas, unmasking the underlying, probably pervasive, circadian rhythmicity of brain responses.

3.4. Global non-specific circadian influence?

Note however that with these data, a nonspecific circadian effect due to a decrease in core body temperature or a global hormonal influence (e.g. cortisol or melatonin), for example, cannot be ruled out. Thus, the early morning circadian trough in PVT brain responses might be a byproduct of such general physiological fluctuations. The main issue here is that the PVT task lacks an explicit baseline condition. To test this hypothesis, we analyzed brain responses to the n-back task (Cohen et al., 1997), which were also recorded in parallel during fMRI sessions. In this case, executive responses were derived from contrasting the execution of a 3-back task to a control 0-back task, used here as a baseline measure (3 - 0 back). We observed that the cerebral correlates underlying executive responses throughout the protocol were not significantly modulated by elapsed time awake. Indeed, brain responses to both 3-back and 0-back decreased to the same extent during sleep deprivation. By contrast, responses in the bilateral anterior insula were significantly modulated by a circadian oscillation, synchronous to the melatonin rhythm. Extracted BOLD activity estimates in these brain regions followed a strong circadian modulation during the 3-back task, in contrast to responses to 0-back. This finding rules out a global non-specific circadian influence and again speaks in favor of a region-specific

and task-dependent circadian signal underlying neurobehavioral performance under sleep loss conditions.

3.5. Regional brain modulation of circadian and homeostatic factors:

At first glance, our data seem compatible with a local use-dependent perspective on sleep deprivation and neurobehavioral performance. Our data indeed bring first exciting evidence that at the cerebral level, task performance is degraded by local, use-dependent neuronal groups subserving cognitive processes associated with the task at hand (Van Dongen et al., 2011). Importantly, our data speak in favor of local use of brain regions majorly triggered by the challenged cognitive process. Critically our data reveal the important role of the circadian timing system in this putative local use of task-related brain regions under conditions of extended wakefulness. This finding implicates, that, depending on task-demands and underlying cerebral correlates, the circadian system differentially rescues or impedes neurobehavioral function in response to sleep loss. Accordingly, specific behaviors might be endangered by sleep loss, critically depending on when it occurs over the 24-hour cycle. Importantly, our data thereby also put into perspective the simplistic, but intuitive, assumption that local use-dependent phenomena are synonym to sleep homeostasis. Even though, in current literature reports, the circadian timing system is often acknowledged as a team-mate of the sleep homeostatic process, for which designs should control, our data indicate that it might just appear impossible to speak about local-sleep-homeostatic use at the cerebral level, without considering the circadian clock, even handedly implicated in this putative local/region-specific and use-dependent phenomenon occurring during wakefulness. Attenuated responses have been repeatedly observed in both fronto-parietal attentional regions, as well as in occipito-temporal regions (Chee et al., 2008). The intraparietal sulcus (IPS) appears interesting within this context since it mediates the allocation of top-down attention to specific aspects of the environment, according to subject's goal (Ciaramelli et al., 2008). Attenuated stimulus-related activation was suggested to rise from an interaction between

compromised fronto-parietal top-down control of attention and reduced sensitivity of primary sensory cortices to top-down or bottom-up inputs (Chee et al., 2011). Indeed, even though our task is definitively not designed to dissociate top-down form bottom-up attentional processes, it is tempting to suggest that reduced activity in more integrative regions such as the IPS reflects sleep-loss-induced impairment of top-down processes. Concomitantly, occipital cortex activation in response to sleep loss might come from reduced sensitivity of the visual cortex to sensory stimuli, consequent to the continuous use of the visual cortex during sustained wakefulness (Chee et al., 2008). However, another plausible explanation is that it arises from reduced generation of top-down attention modulation from the fronto-parietal cortex (Chee et al., 2011). Within this context, it was observed that even in the absence of an explicit visual stimulation ("endogenous attention"), sleep loss led to attenuated responses within the occipital cortex as well as in the IPS. Chee suggests that the mediation of such endogenous attention by sleep deprivation results from a reduced number of functional circuits mediating top-down control of attention. Globally, Chee assumes that sleep-deprivation-induced changes in stimulus-related BOLD activation arise from an interaction between compromised fronto-parietal top-down control of attention and reduced sensitivity of visual cortex to top-down or bottom-up inputs (Chee et al., 2011).

In the context of our study, we observed circadian and homeostatic influences in several cerebral brain regions, including occipital and IPS. BOLD activity in these regions declined as soon as passing over the circadian arousal peak, delimited here by the DLMO. Maximal sleep-loss-related decrement was detected in the hours surrounding the offset of melatonin secretion, close to minimal circadian arousal promotion. Intriguingly, thalamic responses showed the reverse pattern and presented a strong circadian modulation, with maximal activity during the end of the biological night. Thalamic increases under challenging sleep deprived conditions have been repeatedly reported in the context of attentional tasks, including the first study reported in this

thesis. Portas et al. (1998) showed thalamic activity increases in a selective attention task after SD. Another study by Tomasi et al. (2009) reported compensatory thalamic activity after SD during a visual tracking task. Accordingly, it was also observed that high sleep pressure levels during night-time are associated with increased activation in thalamic structures, whereas under low sleep pressure, several cortical regions were more active (Maire et al., 2015). Our data indicate that thalamic activation boost maximally at a time when cortical regions show maximal activity decreases. Thus, it might be speculated that, while this enhanced thalamic recruitment allows the maintenance of a reactive state, the latter can only be suboptimal since cortical resources (allowing optimization of global alertness) are mostly compromised.

The main questions raised by our results are the following. Our results suggest a local brain modulation of circadian rhythmicity.

Is there a modulation of the clock machinery in the local neural populations? This would mean that the rhythmicity imposed by the SCN can be to some extent regionally altered, possibly to respond to local neuronal needs.

How does this putative clock modulation interact with the molecular consequences resulting from sleep debt (increased synaptic potentiation, genomic and proteomic consequences)? An intriguing possibility would be that changes in clock machinery would actually participate in molecular signals involved in sleep debt homeostasis.

These last two points are strictly interconnected and highlight questions about the relative independency of the sleep homeostatic and circadian processes. An interesting prospective is offered by (Franken and Dijk, 2009a). Indeed the authors argued that, at molecular level, clock genes setting internal time-of-day within the SCN (the circadian masterclock), in the forebrain, might be equally utilized to track sleep homeostatic factors (Franken and Dijk, 2009a). As observed by Franken, “cross talk” between the two processes is underlined by the observation that in Syrian hamster, sleep loss can rapidly induces a phase shift of the

circadian clock and alters gene expression within the circadian system (Antle and Mistlberger, 2000). Moreover, our exciting results emphasize that circadian and homeostatic factors regionally interact in the brain, probably due to a local neuronal homeostatic need, possibly linked to local use-dependent mechanisms (Tononi and Cirelli, 2003b).

4. Limitations

Cognitive performance critically depends on phase relationship and amplitude of the circadian and sleep homeostatic processes (Dijk and von Schantz, 2005), implying that the time-course of neurobehavioral performance cannot be predicted without an accurate assessment of both factors. Attentional processes are essential to support cognitive abilities. The present work aimed to characterize the neural brain correlates of human attentional processes under sleep loss conditions. However, attention is a heterogeneous concept. The two studies, presented here, strongly differ in relationship to the experimental design used to assess the effects of sleep loss. In the first study we controlled for time-of-day at which performance was assessed in the two conditions (rested wakefulness and sleep deprivation). This implies that intra-individual performance was assessed at the same time-of-day, reducing circadian confounding factors at individual level. At interindividual level, however, a circadian confounding factor was introduced by this approach. Performance was evaluated during a time window ranging from 9:00 AM to 5:00 PM. This issue could have minimized possible selective effects of sleep deprivation. Literature clearly showed that behavioral consequences of sleep loss are more easily detected during the early morning (close to the habitual wake up time) and late evening hours (preceding the habitual sleep time). At the same time, the specific time-of-day (temporal window) at which performance was assessed, as not characterized by important circadian changes, should not have increased too much interindividual variability. A more adequate experimental design would consists into adapt the protocol to individual sleep-wake schedules. This assumption implies to assess performance at the ANT with a different experimental design and

to scan each participant on a different day. However the main objective of the first study was to investigate the three main components of attentional system as conceptualized in the Posner's model (Posner and Petersen, 1990). Thus the rational underpinned was to assess if those attentional components were effectively independent from each other and explore the possibility of a selective and differential effect of sleep loss. In other words we desired to assess the robustness of the ANT as fast as possible, in order to eventually apply the above-mentioned task to the second study.

In the second study a rigid control of sleep homeostatic and circadian processes was used, adapting a constant routine protocol to an fMRI setting. Again, the ideal experimental design would have been to adapt the protocol to individual sleep-wake schedules; however this would have implied daily individual acquisitions. Another limit regards the *PER*3 stratification of our sample, together with high control of participants' inclusion criteria. We selected a very homogenous group of individuals, regarding the age (our mean age was 21.12 ± 1.7), the individual sleep-wake history previous to the in-lab study (3 actimetry weeks, characterized by small variability across days), as well as accurate selection of their sleep phenotype (intermediate chronotypes with habitual sleep-wake schedules as close as possible to timing imposed by fMRI sessions). At a first glance, the adoption of a homogenous sample is essential to avoid confounding effects; the latters could be linked to the between-subjects variance. However a genetic stratification together with high homogeneity of the sample raises questions about the generalizability of the results. For instance a genetic association observed in a high homogeneous population could not be generalizable to other populations. Genome-wide association studies, screening the full genome, can establish in a more robust way the association between specific phenotypes and genes, especially in light of possible multiple interactions between genetic variants and environmental factors.

5. General conclusion

The aim of the present work was to characterize neural correlates of attentional processes, under sleep deprivation conditions. We first investigated the effects of sleep loss on brain activity correlates of attentional resources, based on a cognitive model proposed by Posner and Petersen (1990). This model assumes that attention is supported by three main components associated with independent brain circuits; the attentional network test was developed to probe the independency of these attentional components. Our results do not support the view of a selective and differential effect of sleep loss on those three attentional subunits. A global increase of RTs was observed after SD for all attentional components. Activity pattern related with each attentional component was relatively congruent with literature reports under conditions of RW. For optimal responses (fast RTs) SD resulted in decreases responses in several cortical regions. Higher-order attentional components elicited brain responses increase in thalamic areas, after SD relative to RW. In accordance with the literature, thalamic responses increases were interpreted as reflecting compensatory mechanisms, which transiently maintained an optimal vigilance level, thereby supporting cortical functions.

In a second step we investigated cognitive brain responses associated with attentional resources, under sleep loss conditions, covering with our protocol more than one circadian cycle. Here the aim was to explore whether attention-related brain responses were controlled by the combined action of circadian rhythmicity, sleep need and their interaction. Behavioural data showed that the decrements in neurobehavioral performance, consequent to SD, vary according to time of day. Neuroimaging results showed that the temporal profile of brain responses reflected the combined influence of sleep pressure and circadian rhythmicity, and that their contribution varied across brain regions. Moreover, using an empirical marker of circadian process, we assessed whether brain responses to the PVT were modulated by the increase in time spent awake. Response profile of cortical areas

revealed a strong decrease in response to elapsed time awake, also congruently with results observed in the first study. Subcortical areas did not show any significant change modulated by sleep homeostatic pressure. By contrast their response profile shows a significant circadian modulation synchronized to the melatonin rhythm. A significant interaction between sleep pressure and circadian rhythmicity was observed in occipital and thalamic areas.

Altogether, our data fit into the general context of a global decrease in top-down modulation of attentional resources, by the combined action of circadian and homeostatic factors on cortical responses. Importantly the results of the second study, showed regional brain differences in the timing of maximal responses associated with sustained attention, under sleep loss condition. These data rise important questions about reciprocal influences between circadian and sleep homeostatic factors. Studies on animals with targeted clock gene disruptions showed that altered circadian factors impact on sleep homeostatic features, highlighting a non-circadian role for clock genes (Wisor et al., 2002; Franken et al., 2007; Franken and Dijk, 2009b; Mongrain et al., 2010). Furthermore human studies suggested that interindividual differences in circadian phenotypes (e.g. the association between morning preferences and *PER3*$^{5/5}$) may be linked to changes in homeostatic sleep process (Viola et al., 2007; Groeger et al., 2008) despite the lack of any differences in circadian parameters (e.g., with respect to *PER3* genotypes, no differences in melatonin, cortisol or peripheral *PER3* mRNA expression; Viola et al., 2007). Congruent with these results, our data indicate brain region-specific task-dependent modulation of circadian rhythmicity and of its interaction with local sleep need.

We investigated the cerebral correlates of attentional processes tracking circadian-homeostatic interaction profile. In a next step, it would be of great interest to assess possible differences between the two polymorphisms (*PER3*$^{4/4}$ and *PER3*$^{5/5}$). Particularly, we would explore higher-order cognitive functions, which have been shown to be particularly sensible to the effects of sleep loss in *PER3*$^{5/5}$ (Groeger et

al., 2008). Future studies need to further investigate the impact of the interplay between sleep homeostasis and circadian processes on neuronal function. Recently, amplitude and slope of cortical responses evoked by transcranial magnetic stimulation on EEG (TMS-EEG) have been shown to increase with time spent awake (Huber et al., 2013). TMS-EEG presents two main advantages. First it gauges local synaptic efficacy (Esser et al., 2006), considered to mainly depends on sleep homeostasis (Vyazovskiy et al., 2008). Secondly, compared to fMRI, TMS-EEG offers much higher temporal resolution. Using TMS-EEG recordings in constant routine or forced desynchrony protocols will open new horizons in view of our results. Thus we would be able to investigate, at a different time scale, the possibility that brain activity is regionally modulated by circadian rhythmicity and its interaction with a local neuronal sleep pressure.

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Appendix

Appendix 1

Supplemental Data of Study 1

1. Introduction

1.1. Operational definitions of vigilance, alertness, arousal and attention:

Oken and collaborators rightly observed that '[…] there are activation states of cerebral cortex that impact the ability to process information where the activation itself contains no specific information. These activation states can be tonic or phasic and may be relatively global or more localized. Terms that have been used to describe these states include arousal, alertness, vigilance, and attention. Unfortunately, no terms are ideal to describe these states of cortical activation since most terms are in broad use with varied associations and there are not perfect physiological markers ' (Oken et al., 2006).

In the present manuscript, we used the following operational definitions, largely inspired from (Leclercq, 2002; Oken et al., 2006):

- **Focal or selective attention**: the ability of the subject to process selectively some events to the detriment of others. In the ANT, the orientation effect assesses spatial orientation of attention within the visual field, i.e. the internal displacement of mechanisms of attention to the target positions.
- **Sustained attention**: the ability to maintain attention over extended periods of time, typically in attention-demanding tasks in which stimuli are infrequently delivered. The ANT is *not* a prototypical example of such tasks.
- **Phasic alertness**: the instantaneous generalized facilitation of performance induced by warning signals. In the ANT, the alerting effect is taken as an indicator of the subject's enhanced receptivity and reactivity when the information that he has to process is preceded by a warning signal (cue).
- **Tonic alertness**: takes into account daily fluctuation in performance. It corresponds to the level of 'cortical activation'

induced by brainstem activating structures. It is synonymous to **vigilance**.

Tonic alertness is also sometimes referred to as **tonic arousal**. We do not use this denomination because in sleep medicine, 'arousal' selectively refers to transient changes in EEG oscillations rather than to a long-lasting ('tonic') states of responisveness.

2. Discussion

2.1. Sleep deprivation, sleep homeostasis and circadian signals:

The changes in behavior and regional brain responses induced by sleep deprivation are usually interpreted as the consequence of an increased sleep pressure (Chee and Chuah, 2008). In fact, they result from the interaction between sleep homeostasis and circadian signals (Dijk et al., 1992; Dijk et al., 2001; Schmidt et al., 2009; Vandewalle et al., 2009). In the current study, volunteers were scanned between 9:00 AM and 5:00 PM, a period of the day at which circadian signals are likely to be sufficiently strong to counter the sleep pressure accrued during the preceding waking hours (Dijk et al., 1992). It should be noticed that this experimental design possibly underestimates the genuine behavioral and neural effects of sleep deprivation, which can be more easily detected in the early morning hours after sleep deprivation (Dijk et al., 1992; Groeger et al., 2008; Viola et al., 2007).

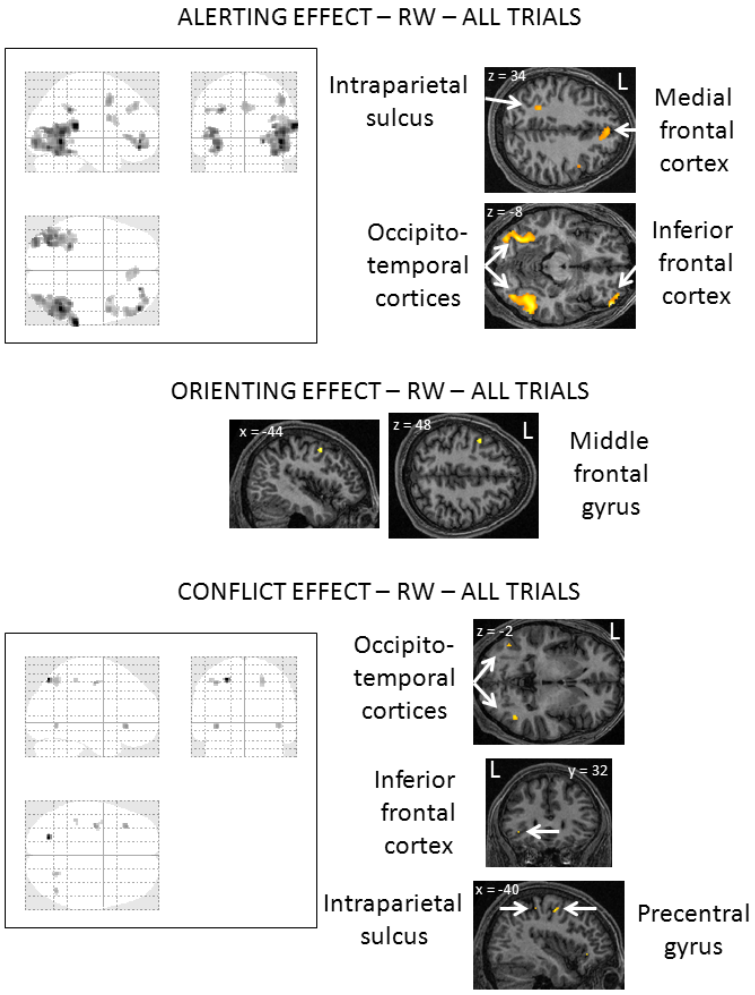

Figure S1

Figure S1. Alerting, orienting and conflict effects during rested wakefulness. For alerting (top row) and conflict (bottom row) effects, the left-hand panels show voxels significant at $P_{uncorrected} < 0.001$ on 'glass brains' displayed along cartesian axes. The right-hand panels focus on brain areas surviving correction for multiple comparisons on small volumes of interest at $p_{SVC} < 0.05$. These areas are displayed at $P_{uncorrected} < 0.001$, overlaid on an individual MR image normalized to the MNI space. For orienting effect (middle row), the left middle frontal gyrus is the only area surviving correction for multiple comparisons on a small volume of interest at $p_{SVC} < 0.05$. It is displayed at $P_{uncorrected} < 0.001$, overlaid on an individual MR image normalized to the MNI space.