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The local regulation of human sleep: anatomo-functional bases and implications for behavior

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Publications

1. **Avvenuti G**, Bertelloni D, Lettieri G, Ricciardi E, Cecchetti L, Pietrini P, Bernardi G. (2020). Emotion suppression failures are associated with local increases in sleep-like activity. *BioRxiv*, 2020.08.04.235978.

2. **Avvenuti G**, Handjaras G, Betta M, Cataldi J, Imperatori LS, Lattanzi S, Riedner BA, Pietrini P, Ricciardi E, Tononi G, Siclari F, Polonara G, Fabri M, Silvestrini M, Bellesi M*, Bernardi G* (2020). Integrity of corpus callosum is essential for the cross-hemispheric propagation of sleep slow waves: a high-density EEG study in split-brain patients. *J Neurosci*, 40(29), 55895603. *(* denotes equal contribution).*

3. **Avvenuti G**, Leo A, Cecchetti L, Franco FM, Travis F, Caramella D, Bernardi G, Ricciardi E, Pietrini P. (2020). Reductions in perceived stress following Transcendental Meditation practice are associated with increased brain regional connectivity at rest. *Brain Cogn* 139:105517.

4. **Avvenuti G** (2017). Personal Identity and Head Transplant: A Psychological Analysis. *AJOB Neuroscience*, 8:4, 232-234.

5. Steca P, Monzani D, Pierobon A, **Avvenuti G**, Greco A and Giardini A (2017). Measuring Dispositional Optimism in Patients with Chronic Heart Failure and Their Healthcare Providers: The Validity of the Life Orientation Test – Revised. *Patient Preference and Adherence*, 11:1497-1503.

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7. **Avvenuti G**, Baiardini I and Giardini A (2016). Optimism's explicative role for chronic diseases. *Front. Psychol*. 7:295.

Oral presentations

1. *"Emotion regulation failures are associated with increases in frontal sleep-like activity during wakefulness."* **Avvenuti G,** Bertelloni D, Lettieri G, Ricciardi E, Cecchetti L, Pietrini P, Bernardi G. AIMS 2020, Web Conference (Italy), October 25-27, 2020.

2. *"The corpus callosum is essential for the cross-hemispheric propagation of sleep slow waves: a high-density EEG study in split-brain patients."* **Avvenuti G,** Handjaras G, Betta M, Cataldi J, Imperatori LS, Lattanzi S, Riedner BA, Pietrini P, Ricciardi E, Tononi G, Siclari F, Polonara G, Fabri M, Silvestrini M, Bellesi M *, Bernardi G *. World Sleep 2019, Vancouver (Canada), September 20-25, 2019. *(*denotes equal contribution).*

3. *"Il ruolo del corpo calloso nella propagazione crossemisferica delle onde lente del sonno: uno studio in pazienti con callosotomia totale."* **Avvenuti G,** Handjaras G, Betta M, Cataldi J, Imperatori LS, Lattanzi S, Riedner BA, Pietrini P, Ricciardi E, Tononi G, Siclari F, Polonara G, Fabri M, Silvestrini M, Bellesi M *, Bernardi G *. AIMS 2019, Genova (Italy), September 13-15, 2019. *(*denotes equal contribution).*

Abstracts at (inter-)national conferences

1. **Avvenuti G,** Bertelloni D, Lettieri G, Ricciardi E, Cecchetti L, Pietrini P, Bernardi G. *Emotion regulation failures are associated with increases in frontal sleep-like activity during wakefulness.* AIMS 2020, Web Conference (Italy), October 25-27, 2020.

2. **Avvenuti G,** Bertelloni D, Lettieri G, Ricciardi E, Cecchetti L, Pietrini P, Bernardi G. *Reduced sleep time is associated with increases in frontal sleep-like activity and emotion regulation failures.* ESRS 2020, Virtual Conference, September 22-24, 2020.

3. **Avvenuti G**, Handjaras G, Setti F, Leo A, Cecchetti L, Betta M, Ricciardi E, Pietrini P, Bernardi G. *Self-control performance, but not subjective vigilance level, decreases after brief periods of task practice and increases following caffeine intake.* ESRS 2020, Virtual Conference, September 22-24, 2020.

4. **Avvenuti G**, Cataldi J, Leo A, Cecchetti L, Lutti A, Draganski B, Siclari F*, Bernardi G*. *Greater cortical thickness in the occipital cortex is associated with larger slow wave amplitude and stronger cortical involvement in centro-frontal brain areas.* ESRS 2020, Virtual Conference, September 22-24, 2020.

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6. **Avvenuti G,** Handjaras G, Betta M, Cataldi J, Imperatori LS, Lattanzi S, Riedner BA, Pietrini P, Ricciardi E, Tononi G, Siclari F, Polonara G, Fabri M, Silvestrini M, Bellesi M *, Bernardi G *. *Integrity of corpus callosum is essential for the cross-hemispheric propagation of sleep slow waves: a high-density EEG study in split-brain patients.* XXVII Congresso SIPF, Ferrara (Italy), November 14-16, 2019. *(*denotes equal contribution).*

7. **Avvenuti G,** Handjaras G, Betta M, Cataldi J, Imperatori LS, Lattanzi S, Riedner BA, Pietrini P, Ricciardi E, Tononi G, Siclari F, Polonara G, Fabri M, Silvestrini M, Bellesi M *, Bernardi G *. *The corpus callosum is essential for the cross-hemispheric propagation of sleep slow waves: a high-density EEG study in split-brain patients.* World Sleep 2019, Vancouver (Canada), September 20-25, 2019. *(*denotes equal contribution).*

8. **Avvenuti G,** Leo A, Cecchetti L, Bernardi G, Franco FM, Travis F, Caramella D, Ricciardi E, Pietrini P (2018). *Transcendental Meditation reduces perceived stress and increases brain regional connectivity at rest.* OHBM 2018 Annual Meeting. Singapore, June 17-21, 2018.

9. **Avvenuti G,** Leo A, Cecchetti L, Bernardi G, Franco FM, Travis F, Caramella D, Ricciardi E, Pietrini P (2018). *Three-months Transcendental Meditation reductions in perceived stress are associated with DMN increased connectivity at rest.* 19th World Congress of Psychophysiology. Lucca (Italy), September 4-8, 2018.

Abstract

The traditional view of sleep and wakefulness as two distinct and mutually exclusive states has been recently challenged by the discovery that they actually are locally regulated and that islands of sleep- and wake-like activity may often coexist in a same individual at a given time. Importantly, it has been suggested that the local regulation of sleep may be involved in many of the essential functions of sleep in physiological conditions. Particular attention has been given to the study of the so-called 'slow waves' of sleep, which represent in particular the main hallmark of non-rapid eye movement (NREM) sleep. In fact, local changes in slow wave activity have been shown to occur in brain regions that are more actively used during wakefulness, ultimately reflecting wake- and experience-dependent plastic processes. In addition, electrophysiological events similar to sleep slow waves have been found to occur also during wakefulness and have been suggested to reflect neuronal functional fatigue and the accumulation of sleep need. Of note, while experimental research has started to shed light on the mechanisms involved in the local regulation of sleep-like activity, and on how they affect cognition and behavior, many aspects still remain to be fully clarified.

Given these premises, in the present Thesis, my aim was to advance the current knowledge on the local regulation of sleep in humans, with a specific focus on slow-wave-like activity. To this aim, I performed three different experiments. In the first study, I investigated the role of cortico-cortical white matter connections in the generation and propagation of sleep slow waves. To this aim I analyzed overnight high density (hd)EEG data collected in an extremely rare population of 'split-brain' patients and in two additional groups including neurologic patients and healthy control subjects. Obtained results demonstrated that the traveling of sleep slow waves is significantly affected by the resection of the corpus callosum, which leads to a reduced proportion of cross-hemispheric slow waves. This result demonstrates that the way sleep slow waves propagate can inform us regarding the status of brain connections and may thus offer a valuable marker for functional or structural alterations caused by traumatic or neurodegenerative disorders. On the other hand, our analyses

showed that the lack of inter-hemispheric connections is not associated with dissociations characterized by sleep rhythms in one hemisphere and wake-like activity in the other half of the brain. In addition, while we found that sleep slow waves tend to originate more often in the right than in the left hemisphere, such an asymmetry was found not to differ between split-brain patients and subjects with an intact corpus callosum. Overall, these results indicate that global state changes are coherently modulated across the cortical mantle by non-cortical (bottom-up) mechanisms.

In two additional studies, I investigated the local regulation of sleep-like activity during wakefulness and its possible effects on cognition and behavior. In particular, in one experiment I applied a single-subject multi-session design to explore whether the regional distribution of morning-to-evening increases in local sleep-like activity is dependent on the degree of experience-dependent activation or rather it mainly reflects inter-regional differences in vulnerability to neuronal fatigue. In fact, it has been shown that low-frequency power increases during wakefulness and decreases after a night of sleep, and such changes are on average more pronounced over frontal areas. Our results showed that changes in low-frequency activity may peak in different brain regions. In particular, we observed at least two main morning-to-evening variation patterns: one, more common and stronger, involving centro-frontal cortical areas, and one, less common, mainly involving sensory cortices. This observation does not support an inherent vulnerability of frontal areas and is instead potentially compatible with a use/experience-dependent regulation of electrophysiological indices reflecting functional fatigue and sleep need.

Finally, I investigated whether the occurrence of local sleep-like episodes may influence behaviors with a social relevance, such as the ability to regulate one's own emotional reactions. In particular, my aim was to test whether the occurrence local sleep-like activity within brain areas involved in emotional regulation could account for failures in the suppression of emotional expressions. Obtained results demonstrated, for the first time, that sleep-like activity in frontal and parietal areas precede emotion regulation failures. Moreover, I found that the incidence of behavioral failures is negatively correlated with a shorter sleep

duration the night preceding the experiment, in line with previous evidence linking local sleep-like episodes and sleep loss. Taken together, these results indicate that transient, local 'neuronal sleep' may represent a direct functional cause of impairment in complex and socially relevant human behaviors.

Abbreviations

CC Corpus callosum.

CP Callosotomized patients.

CSD Current-source density.

dSPM dynamical Statistical Parametric Mapping.

EEG Electroencephalogram.

EFs Executive Functions.

EMG Electromyogram.

EOG Electrooculogram.

fMRI functional Magnetic Resonance Imaging.

HCA Hierarchical Clustering Analysis.

HS Healthy subjects.

ICA Independent Component Analysis.

mPFC medial Prefrontal cortex.

NP Non-callosotomized patients.

NREM Non-Rapid Eye Movement.

PCA Principal Component Analysis.

PCs Principal components.

PSD Power spectral density.

PVT Psychomotor Vigilance Task.

REM Rapid Eye Movement.

RMS Root mean square.

RT Reaction time.

SD Sleep deprivation.

sLORETA standardized low-resolution brain electromagnetic tomography.

SMA Supplementary motor area.

SWA Slow Wave Activity.

TBI Traumatic brain injury.

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Chapter 1

Introduction

1.1 Motivation for investigating the mechanisms of local regulation of sleep and wakefulness

Sleep and wakefulness have traditionally been regarded as two separate and mutually exclusive states, each characterized by its own specific behavioral and physiological characteristics. Unquestionably, these two states are identified by well-distinct behavioral correlates, levels of mental activity and degrees of responsiveness to the external environment. Moreover, they are associated with distinct and specific neuromodulatory milieus as well as with distinct electroencephalographic (EEG) signatures reflecting changes in the patterns of brain activity and connectivity. However, the last decades of research have re-defined the boundaries of both sleep and wakefulness, demonstrating that changes in brain activity are locally regulated and that islands of sleep- and wake-like activity may even often coexist in the same individual at a given time.

Importantly, this mosaic activity has been directly related to the occurrence and content of cognitive processes during both sleep (Siclari et al., 2013, 2017; Boly et al., 2017) and wakefulness (Andrillon et al., 2019). Although the mechanisms underlying the generation of "local awakenings" during sleep and of "local sleep" during wakefulness are yet to be fully clarified, these events are clearly physiological and may subserve specific and essential functions (e.g., Wamsley and Stickgold, 2011). However, significant local alterations in the balance between wake- and sleep-like activity are also associated with a broad range of sleep

disorders, including paradoxical insomnia (Manconi et al., 2010; Lecci et al., 2019; Stålesen Ramfjord et al., 2020) and arousal disorders (Terzaghi et al., 2012; Castelnovo et al., 2018b), as well as with several psychopathological and neurological conditions (Pisarenco et al., 2014), such as major depressive disorder, ADHD, schizophrenia and traumatic brain injury.

In fact, the field of research concerning the local regulation of sleep and wakefulness has rapidly grown in recent years due to its many implications for our understanding of both the physiological functions of sleep and their alterations under pathological conditions. In particular, one of the largest and most relevant fields of investigations is certainly represented by the one focusing on the regulation of sleep slow waves. Indeed, the slow waves have been linked to many of the physiological sleep functions, including learning, memory consolidation and sensory disconnection, and represent a valuable marker of individual (and neuronal) sleep need. Importantly, events similar to sleep slow waves have been observed during periods of global wakefulness and have been demonstrated to affect cognitive and behavioural processes. In the next sections, after a general introduction about sleep and its different stages, I will describe the current state of the art of this particular field of research, which also represent the main focus of the present Thesis work.

1.2 The stages of sleep

From a behavioral perspective, sleep is typically characterized by relative immobility and reduced responsiveness to external stimuli (increased arousal threshold) (Vyazovskiy, 2015). Mental activity is also assumed to be greatly reduced for most of the night. Nonetheless, sleep is not a uniform state. Instead, all sleepers spontaneously encounter different stages of sleep, each associated with specific and distinctive features that can be identified through the evaluation of changes in brain activity (EEG), eye movements (electrooculogram, EOG) and muscle tone (electromyogram, EMG). The human sleep is classically divided into Non-Rapid Eye Movement (NREM) sleep and Rapid Eye Movement (REM) sleep; NREM sleep is then categorized into N1, N2 and N3 stages.

Wakefulness

During wakefulness the EEG pattern is characterized by fast, low-amplitude oscillations. The waking EOG shows fast, voluntary eye movements and eye blinks, while the EMG indicates tonic muscle activity together with phasic muscular activations corresponding to voluntary movements. In preparation for sleep, the eyes close and the EEG alpha activity (8-13 Hz) becomes prominent especially over the occipital areas. The falling asleep process is then associated with clear changes in EEG activity, from a high-frequency, low-amplitude signal typical of wakefulness, to a low-frequency, high-amplitude activity of deep NREM sleep. At the same time, the EMG and EOG signals show coordinated modifications, with a relaxation of muscle tone and the disappearance of voluntary eye-movements.

NREM Stage 1

During the process of falling asleep, the individual gradually disconnect from the external environment. NREM sleep stage 1 (N1) is regarded as a transitional stage between wakefulness and sleep, and is characterized by the disappearance of alpha activity and the appearance of a mixed-frequency, low-amplitude EEG

pattern dominated by theta (3-8 Hz) oscillations. The EOG reveals slow, rolling eye movements and the muscle tone relaxes. However, some motor activity may persist and individuals in N1 may experience sudden muscle contractions, called *hypnic jerks*, frequently associated with dream-like imagery (*hypnagogic hallucinations*) and a sense of falling.

NREM Stage 2

NREM sleep stage 2 (N2) usually begins after a few minutes in stage N1, and its EEG pattern is characterized by the appearance of K-complexes and sleep spindles, which are especially evident over centro-frontal brain areas. K-complexes are waveforms that consist of a high-voltage negative peak followed by a positive wave. They are often triggered by sensory stimuli coming from the external environment (i.e., sounds, lights, touches), although they may also occur without a clear triggering-event (so-called "spontaneous" K-complexes). Sleep spindles represent sudden bursts of oscillatory brain activity in the 12-16 Hz frequency range, generated by the reticular nucleus of the thalamus and projected at cortical level. They last about 1 second and occur 5-10 times per minute. Of note, K-complexes may often be followed by bursts of spindling activity. Both the EOG and the EMG show a reduced activity.

NREM Stage 3

During NREM stage 3 (N3) the EEG activity is characterized by the frequent occurrence of slow waves in the delta range (0.5-4 Hz). This sleep stage is usually prominent in the first part of the night and it is often referred to as '*slow-wave sleep*', due to the presence of slow waves, or '*deep sleep*', due to the clear increase in arousal threshold with respect to the other NREM stages. In fact, subjects in N3 are difficult to awaken and they may remain confused for some time after waking up. Indeed, the so-called 'sleep inertia', which represents a physiological state of impaired cognitive and sensory-motor performance that is present

immediately after awakening, is most evident in individuals who are awakened from this particular sleep stage.

REM sleep

Usually the sleeper enters REM sleep after having passed through the various stages of NREM sleep. REM sleep is also called '*paradoxical sleep*' because brain activity of this stage is reminiscent of that observed during relaxed wakefulness or sleep stage N1 but the arousal threshold is similar to the one of N2 or N3 sleep, and the sleeper is thus strongly "disconnected" from the environment. In fact, the EEG of REM sleep is characterized by low-voltage, fast activity, especially in the theta range. Although REM sleep is not further divided in distinct stages, two different sub-components may be distinguished. Tonic REM sleep shows the typical activated EEG and includes a generalized loss of muscle tone. Instead, phasic REM sleep is characterized by bursts of rapid eye movements and muscular twitches.

The sleep cycle

A sleep cycle is defined by the succession of NREM sleep stages followed by an episode of REM sleep. In humans, a sleep cycle lasts around 90-120 minutes, and a total of 4-5 cycles usually occur per night (Figure 1). While N3 sleep is prominent at the beginning of the night, it is less represented as the night progresses. In parallel with the decrease of deep N3 sleep, REM sleep episodes lengthen. In healthy adults the proportion of time spent in each sleep stage and the pattern of sleep stages across the night are relatively stable and consistent: typically the 5% of sleep is spent in stage N1, about the 50% in stage N2, the 20-25% in stage N3 and around the 20-25% is spent in REM sleep.

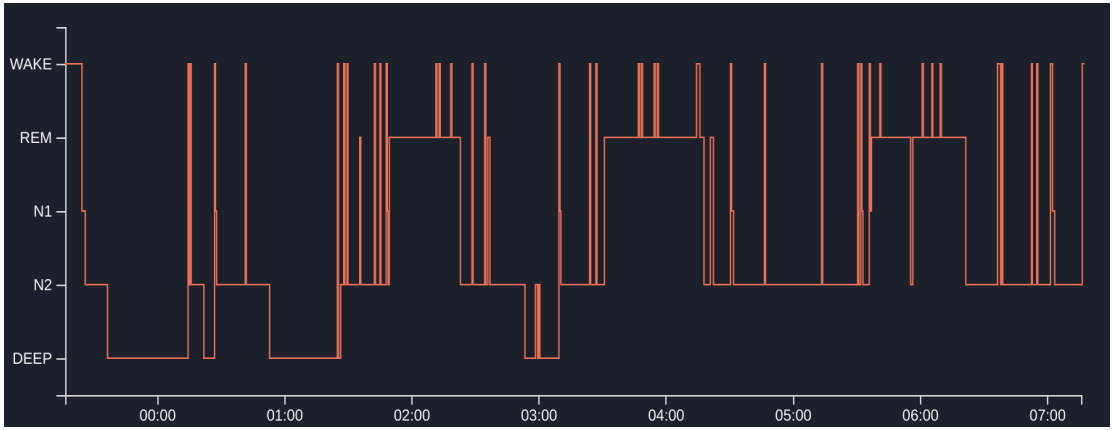

Figure 1. Hypnogram for an all-night recording in a young woman. Note the occurrence of three sleep cycles, in which slow-wave sleep (the "DEEP" row) was predominant early in the night, while the length of REM-sleep episodes increased later in the night.

1.3 Global and local regulation of sleep slow waves

While sleep slow waves are especially dominant during N3 sleep, they can be observed throughout all phases of NREM sleep. In this perspective, they certainly represent the most salient signature of NREM sleep and have been suggested to subserve many important physiological functions of this particular stage.

The sleep slow wave appears in the EEG signal when cortical neurons become *bistable* and their membrane potential undergo a *slow oscillation* (<1 Hz), with an alternation between a depolarized *up-state*, corresponding to neuronal firing (*ON-period*), and a hyperpolarized *down-state*, characterized by neuronal silence (*OFF-period*). Thus, the *slow oscillation* represents a periodic cellular process that can be directly measured only using intracellular, depth electrodes or intracranial EEG (Timofeev et al., 2020).

Given that neuronal *bistability* may be observed in isolated cortical slabs, the sleep slow wave is thought to have a cortical origin and to represent a predominantly cortical phenomenon (Sanchez-Vives and McCormick, 2000; Lemieux et al., 2014; Capone et al., 2019). However, a growing body of evidence indicates that subcortical structures such as the thalamus may provide a significant contribution to the modulation and synchronization of slow waves in physiological conditions (Neske, 2016; Gent et al., 2018; Vantomme et al., 2019; Betta et al., 2020).

The level of slow wave activity (SWA) during NREM sleep is commonly defined by computing the EEG signal power in the 0.5-4.0 Hz (*delta*) frequency range, which reflects both the amplitude and the number of sleep slow waves. Importantly, SWA has been demonstrated to reflect and track the homeostatic regulation of sleep need (Achermann and Borbély, 2003). Indeed, the individual drive to sleep is commonly assumed to depend on two factors: the circadian process C, controlled by the circadian pacemaker aligned to the 24-h day-night cycle, and a homeostatic process S, establishing that longer periods of wakefulness have to be balanced by longer periods of sleep. In particular, SWA appears to be tightly linked to this latter regulation mechanism (Cajochen et al., 1999). In fact, SWA is maximal at sleep onset, reflecting the

high level of sleep pressure, and it progressively declines during the night. Moreover, the longer an individual stays awake the higher SWA will be, and these higher levels will take longer to dissipate throughout the night (Borbely and Achermann, 1999; Achermann and Borbély, 2003; Borbély et al., 2016).

Interestingly, though, evidence indicates that the slow waves of sleep are not global, spatially uniform events, but are instead locally regulated, showing hemispheric and regional differences (Ferrara and De Gennaro, 2011; Krueger and Tononi, 2011; Nir et al., 2011; Nobili et al., 2011; Siclari and Tononi, 2017). In fact, changes in SWA after sleep deprivation or restriction are typically more evident over medial frontal areas with respect to more posterior and lateral brain regions, suggesting that different brain regions may accumulate sleep pressure at a different rate (Werth et al., 1996, 1997; Cajochen et al., 1999; Finelli et al., 2000, 2001b; Achermann et al., 2001; Bersagliere et al., 2018). In line with this, clear regional differences in SWA are typically observed also after a waking period of normal duration (Achermann et al., 2001; Bersagliere et al., 2018; Borbely and Achermann, 1999; Guillaumin et al., 2018). Moreover, several studies also showed that sleep slow waves tend to originate in relatively circumscribed cortical areas, commonly within the frontal cortex, and then propagate from their origin to other brain areas (Massimini et al., 2004; Nir et al., 2011; Muller et al., 2018). Such a propagation is assumed to depend on white matter cortico-cortical connections (Murphy et al., 2009; Buchmann et al., 2011b; Piantoni et al., 2013).

The particular cortical distribution of sleep slow waves may reflect stable anatomo-functional properties of different brain areas (Kilduff et al., 2011), but may also depend on an experience- and task-dependent regulation (Borbely and Achermann, 1999; Cajochen et al., 1999; Schwierin et al., 1999). Thus, in line with the homeostatic principle, SWA could increase more in areas that are used more intensely or for a longer time during wakefulness. However, other factors may also affect the local levels of SWA. For instance, Tamaki and colleagues recently described hemispheric asymmetries in sleep depth (based on distinct behavioral, fMRI and EEG measures) when an individual sleeps for the first time in a novel environment (such as the sleep laboratory), in line with the so-called "first-night effect" (Tamaki et al., 2016). Specifically,

they showed that the left hemisphere tends to show less SWA, relative to the right hemisphere, during the first night in the sleep laboratory, while this asymmetry ceases to be detectable during the second night spent in the same environment. This relative asymmetry in sleep depth across the two brain hemispheres may allow one half of the human brain to act as a "night-watch", remaining more vigilant and potentially easier to awaken in case of unfamiliar or dangerous stimuli detected in the surrounding external environment (Tamaki et al., 2016). Yet, the physiological mechanisms underlying this particular asymmetry are unknown.

1.3.1 Dissociated EEG activity in wake-sleep state transitions

Changes in brain activity during physiological state transitions, such as the processes of falling asleep and the awakening, do not simultaneously affect all brain regions at the same time. For example, while thalamic and cortical activity were classically considered as coupled and co-modulated across different vigilance states, this view has been recently challenged. Indeed, results from studies using intracranial recordings in humans demonstrated that thalamic and cortical regions may alternate periods of coupled activity to periods of decoupling both during N2 and REM sleep (Magnin et al., 2004; Rey et al., 2007). Furthermore, it has been demonstrated that a thalamic deactivation often precedes the onset of neocortical sleep by several minutes (Magnin et al., 2010), thus indicating an asynchronous and regionally dissociated process of falling asleep affecting cortical and subcortical structures. Similarly, a more recent study in neurosurgical patients reported that, during the transition to sleep, sleep spindles in the hippocampus preceded cortical spindles (Sarasso et al., 2014). Overall, these observations demonstrated that the process of falling asleep is characterized by a sequence of events which progressively involve subcortical (Magnin et al., 2010) and cortical regions (De Gennaro et al., 2001b, 2001a; Bódizs et al., 2005). Moreover, at cortical level, the typical sleep-related SWA increase shows an antero-posterior gradient at sleep onset (Marzano et al., 2013; Siclari et al., 2014; Bernardi and Siclari, 2019). Even during the awakening process, albeit in a more variable manner than during the process of falling asleep, regionally dissociated brain activity patterns can be typically

observed. At the cortical level, during the first minutes after awakening, posterior brain regions display sleep-like brain activity while more frontal areas show EEG activity typical of wakefulness (Ferrara et al., 2006; Marzano et al., 2011). In addition, changes of activity in subcortical regions precede those occurring in cortical areas, mirroring the pattern observed at the transition from wakefulness to sleep (Balkin et al., 2002).

Intriguingly, the occurrence of such dissociated activity patterns across different brain structures may account for phenomena observed both in physiological and pathological conditions, such as the hypnagogic experiences typical of the wake-sleep transition or the misperception of sleep latency and duration (Stickgold et al., 2000; Majer et al., 2007; Lecci et al., 2019, 2020).

These observations support the notion that sleep and wakefulness are not mutually exclusive states, and that the transition from wake to sleep is characterized by the progressive and topographically heterogeneous involvement of subcortical and cortical regions. As for what has been discussed in previous paragraphs concerning the diversification of the cortical regulation of sleep slow waves, the progressive and asynchronous engagement of subcortical structures can be due to several factors. One possibility is that cortical and subcortical structures may have a different sensitivity to brainstem and hypothalamic wake and sleep promoting activity. Another possibility is that the homeostatic process may differently influence subcortical regions relative to the neocortical ones. In this light, subcortical regions involved in learning and experience-dependent activity during prior wakefulness may accumulate grater sleep pressure and thus fall asleep faster (Ferrara et al., 2008; Sarasso et al., 2014).

1.3.2 Local, experience-dependent regulation of sleep

A local, experience-dependent modulation of human sleep slow waves has been reported in several studies (Kattler et al., 1994; Huber et al., 2004, 2006; Ferrara and De Gennaro, 2011; Hung et al., 2013; Mascetti et al., 2013; Wilhelm et al., 2014; Fattinger et al., 2017a; Bernardi et al., 2019a). These works revealed that brain areas that are intensely activated during practice and learning of

particular tasks in wakefulness show increased SWA during subsequent sleep. Thus, for instance practicing a motor learning task is associated with a subsequent increase in SWA in electrodes located over the corresponding sensorimotor cortex (Huber et al., 2004). Moreover, the local increase in SWA correlates with post-sleep, learning-related, gains in performance (Huber et al., 2004), so that a stronger increase of SWA is directly related to larger post-sleep performance improvements in the trained task. Similar, consistent findings have been obtained in animal models (Vyazovskiy et al., 2000; Hanlon et al., 2009; Lesku et al., 2011). By contrast, a relative decrease in SWA can be observed when the recruitment of particular brain areas is prevented prior to sleep. Indeed, for instance, Huber and colleagues (2006) showed that 12-hours of arm immobilization lead to lower levels of SWA over sensorimotor areas with respect to baseline sleep conditions. Also in this case, the local, homeostatic regulation of SWA was found to be correlated with post-sleep motor performance, which was worse for subjects showing lower levels of SWA. These observations led researchers to hypothesize a direct role of sleep slow waves in modulating and refining experience-dependent plastic brain changes (Tononi and Cirelli, 2014). Consistent with this view, a recent study demonstrated that a selective perturbation of sleep slow waves in the sensorimotor cortex through a closed-loop stimulation protocol, leads to detrimental effects on motor performance after sleep (Fattinger et al., 2017a).

Of note, while most evidence regarding the relationship between experience and slow waves has been obtained through studies of the sensorimotor domain, recent work revealed that a similar relationship may exist also in other domains. For instance, studies in animal models and in humans suggest that recent visual experiences during wakefulness may affect slow waves in the occipital cortex. Indeed, extended monocular visual deprivation in pigeons has been shown to result in a SWA homeostatic increase only in the non-deprived visual areas (Lesku et al., 2011). On the same line, a study in humans showed that the number of local slow waves in the occipital cortex is lower after 8-hours of binocular visual deprivation relative to sleep following normal visual stimulation (Bernardi et al., 2019a). Moreover, Mascetti and colleagues (2013) demonstrated that the number of sleep slow waves that originate in parieto-occipital areas during sleep

following a period of practice with a visual perceptual learning task is directly correlated with post-sleep performance improvement. Consistent with these observations, another study showed that extended audiobook listening leads to an increase in low-frequency EEG activity during subsequent sleep in left frontal and temporal brain areas, whereas subjects who perform a driving simulation task, which require visuo-spatial and coordination skills, show low-frequency EEG activity increases over right-parietal areas (Hung et al., 2013). While in this latter study changes in brain activity were found for frequencies beyond the classical delta range (0.5-11 Hz), taken together, these findings suggest that sleep slow waves may be tightly related to experience-dependent microstructural and functional brain changes.

The above evidence provides strong support for a strong relationship between sleep slow waves, plasticity-related processes and learning. Indeed, according to studies in animal models and based on computer simulations, experience- and learning-dependent changes in synaptic strength and density may mediate the increase in SWA within task-related brain areas (Tononi and Cirelli, 2014). As a matter of fact, all the experiences and the learning-related processes that occur during wakefulness lead to an increase in the strength of synaptic connections among cortical neurons (Tononi and Cirelli, 2003, 2006), which in turn leads to an increase in cellular energetic needs and to a decrease in signal-to-noise ratio (Sousouri and Huber, 2019). These modifications, which may eventually affect the correct brain functioning, must be (in part) reverted in order to restore an optimal brain activity. The synaptic homeostasis hypothesis, proposed by Cirelli and Tononi (2003, 2006) postulates that homeostatic changes in SWA ultimately reflect the wake-dependent potentiation of synapses and their "down-scaling" that specifically and selectively occurs during sleep. Indeed, simulation and animal studies suggest that slow wave characteristics indicating synchronization efficiency, such as amplitude and slope, may reflect the number and strength of synaptic connections among local neural population (Esser et al., 2007). Thus, the potentiation of synaptic connections that occur during wakefulness may favor a more efficient spreading of slow waves across neighboring and distant neurons, with the

emergence of large and highly synchronized slow waves (Vyazovskiy and Harris, 2013). Then, during sleep a process of synaptic depotentiation and renormalization seems to take place in most neural circuits, preferentially affecting smaller and less strong connections that may especially represent less relevant and more "noisy" connections (Vyazovskiy et al., 2008; Gilestro et al., 2009; Bushey et al., 2011; Maret et al., 2011; de Vivo et al., 2017; Diering et al., 2017; Norimoto et al., 2018). Such changes could ultimately explain the reduction in SWA that is observed throughout each night of sleep (Vyazovskiy et al., 2007a, 2007b) and may help to clarify the physiological mechanism underlying the restorative effects of sleep and its benefits for our ability to learn and form new memories each day (Tononi and Cirelli, 2014).

1.4 Local sleep-like activity during wakefulness

Sleep curtailment due to acute and/or chronic sleep deprivation (SD), as well as extended wakefulness (sleep restriction), is known to determine both objective and subjective decreases in vigilance, alertness, processing speed and attention, and an increase in sleepiness (Van Dongen et al., 2003; Killgore, 2010; Lo et al., 2012; Ma et al., 2015). However, many other distinct cognitive domains such as working memory, learning, emotional processing and self-control are also directly or indirectly affected, with consequent changes in behavior (Lim and Dinges, 2010; Pilcher et al., 2015; Lowe et al., 2017a; Honn et al., 2018). Studies combining behavioral testing and neuroimaging demonstrated that the prefrontal cortex, subserving a wide variety of higher cognitive functions (the so-called executive functions, EFs) ranging from the allocation of attentional resources to the exertion of inhibitory control over someone's own action and behavior, is particularly vulnerable to sleep loss (Goel et al., 2009). Specifically, the detrimental effects of sleep deprivation gradually arise with a breakdown of the EFs, which depend on top-down mechanisms and are responsible of all the cognitive functions that require the capacity of behavioral and cognitive control (Chua et al., 2017; Kusztor et al., 2019). In fact, there is a linear correlation between the time spent awake and the gradual decline of cognitive performance, meaning that the duration of the waking period determines the magnitude of cognitive impairment (Van Dongen

et al., 2003). On the other hand, automatic, bottom-up processes appear to be more resilient to the negative effect of sleep deprivation (Gevers et al., 2015).

Cognitive and behavioral alterations observed after extended wakefulness and sleep loss may be in part explained by the occurrence of *microsleep* episodes, that are temporary intrusions (0.5 to 15 seconds of duration) of global sleep-like activity into periods of wakefulness (Poudel et al., 2014). Yet, many behavioral alterations are not associated with the objective signs of fatigue and sleepiness - such as frequent yawning, eyes-closure, head nodding and brief lapses in consciousness - that are typically associated with *microsleep* periods (D'Ambrosio et al., 2019). Instead, a growing body of evidence suggests that typical alterations observed during sleep deprivation/restriction may depend on local sleep-like episodes affecting limited portions of the cortical mantle. Indeed, a seminal study performed in sleep deprived rats demonstrated that populations of cortical neurons may display brief episodes of neuronal silence (*OFF-periods*) associated with the appearance of delta-theta (2-6 Hz) waves, similar to those seen in the EEG during behavioral sleep and thus called "*local sleep*" episodes (Vyazovskiy et al., 2011). These local sleep-like episodes were observed while rats were apparently awake and behaviorally active and tended to involve each time only a small portion of the cortical mantle, while the overall scalp EEG continued to show the typical low-amplitude, high-frequency activity of wakefulness. Importantly, the same study also showed that the rats' behavior was directly affected by the occurrence of local sleep-like episodes, depending on their spatial localization and relative timing of occurrence. Indeed, local *OFF-periods* involving the motor cortex, but not the parietal cortex, led to performance errors during a sugar-pellet reaching task. Thus, although both the global EEG and behavior indicated that the rats were awake, local populations of cortical neurons were falling asleep with negative consequences for performance (Vyazovskiy et al., 2011).

Interestingly, it has been known for a long time that during extended wakefulness humans show an increase in low-frequency activity, especially in the delta/theta range (1-8 Hz) and over frontal brain regions (Cajochen et al., 1999; Finelli et al., 2000;

Landolt et al., 2004; Vyazovskiy and Tobler, 2005; Leemburg et al., 2010). These low-frequency waves may represent the signature of local sleep-like episodes occurring more and more frequently with time spent awake (Hung et al., 2013), and may potentially account for at least some of the cognitive and behavioral manifestations of sleep restriction or deprivation. In line with this view, overall changes in low-frequency activity are associated with changes in behavioral performance, as suggested by the correlation found between increases in theta power and increases in reaction time and lapses observed during the Psychomotor Vigilance Test (PVT), which measures sustained attention and alertness (Hung et al., 2013; Gorgoni et al., 2014). Moreover, several investigations demonstrated that, during extended wakefulness, low-frequency EEG oscillations below 10 Hz may present a specific association with behavioral errors when occurring in brain areas involved in the execution of specific tasks (Hung et al., 2013; Bernardi et al., 2015; Nir et al., 2017; Slater et al., 2017).

Importantly, changes in low-frequency activity during wakefulness seem to reflect variations in sleep need in humans (Finelli et al., 2000; Strijkstra et al., 2003). In line with this, Finelli and colleagues (2000) demonstrated that the increase in theta power over frontal regions during extended wakefulness is positively correlated with the level of SWA in the same area during the first NREM cycle of recovery sleep. Thus, low-frequency waves of wakefulness may represent, at least in part, a functional equivalent of sleep slow waves. This parallel is further supported by the observation that, as for sleep slow waves, also the low-frequency EEG activity observed during wakefulness shows regional, experience-dependent modifications (Hung et al., 2013; Bernardi et al., 2015; Quercia et al., 2018). For instance, low-frequency activity increases more in left frontal regions after extended audiobook listening, while it increases more over parietal electrodes after extended practice with a driving simulator (Hung et al., 2013). It has been suggested that local sleep-like episodes during wakefulness may ultimately reflect an increased sleep need of brain areas that are more intensely activated during wakefulness, with consequent cellular stress and accumulation of metabolic wastes (Vyazovskiy and Harris, 2013).

1.4.1 Local sleep-like activity as a neural signature of brain functional fatigue

The fact that low-frequency activity builds-up with time spent awake implies that local sleep-like episodes may not be exclusively observed when wakefulness is extended over its normal length. Indeed, a growing body of experimental evidence indicates that local sleep-like episodes and their behavioral consequences can be observed within a waking period of normal duration, with no sleep deprivation or restriction (e.g., Bernardi et al., 2015; Slater et al., 2017). For instance, even few hours of practice with specific tasks may lead to a "slowing down" of EEG activity in task-related regions, with a negative impact on behavioral performance. In fact, the coincidence of a low-frequency wave within task-related areas and the presentation of the stimulus or the preparation/execution of the behavioral response has been shown to determine commission errors or lapses (Bernardi et al., 2015; Nir et al., 2017). Thus, the incidence of local sleep-like episodes may affect the probability for an episode to occur in coincidence with a stimulus, explaining why behavioral performance becomes more and more negatively affected with time spent awake.

Several studies showed that local sleep-like episodes may significantly affect performance in a variety of cognitive tasks, including sustained attention (Fattinger et al., 2017b), impulse control (Bernardi et al., 2015), spatial navigation (Quercia et al., 2018) and complex visuo-motor coordination (Bernardi et al., 2015; Ahlstrom et al., 2017). For example, Bernardi and colleagues demonstrated that participants' performance in an impulse control task was impaired after extended practice on tasks requiring the exertion of executive functions, while the impairment was stronger in a visuo-motor control task after extended practice with a driving simulator, and that behavioral alterations were correlated with changes in low-frequency activity within task-related brain areas (Bernardi et al., 2015). Similarly, Quercia and colleagues (2018) showed that the time spent practicing with a spatial navigation task is associated with a progressive increase in delta and theta power, and that the number of delta waves occurring in task-related regions is significantly associated with performance errors. By contrast, the

intensive use of the same task-related regions during a control task that did not require learning, did not lead to similar behavioral and electrophysiological alterations. Using intracranial recordings in humans, Nir and colleagues (2017) further showed that attentional lapses (i.e., delayed behavioral response) during a psychomotor vigilance task (PVT) after a night of sleep deprivation are associated with delayed neuronal spiking in the medial temporal lobe and with a local increase in slow/theta activity. A recent work showed that a similar relationship between low-frequency activity and behavioral impairment also holds true in young children. Indeed, Fattinger and colleagues showed in children who practiced with an auditory attention task that the reaction times (RTs) associated with widespread local sleep-like episodes are significantly slower than the RTs of trials that are not associated with widespread low-frequency waves (Fattinger et al., 2017b). Moreover, the authors showed that the brain regions presenting the strongest correlation between low-frequency activity and slower RTs were those engaged during the task, located in parietal and central areas. Finally, another study contributed to clarify the possible consequences of local sleep-like episodes for real-life situations. Specifically, Ahlstrom and colleagues showed in non-sleep-deprived individuals that a high local – but not global – low-frequency activity in motor brain areas is associated with increased risk of lane departures during monotonous driving in a simulator (Ahlstrom et al., 2017).

In summary, it has been repeatedly confirmed that extensive task practice during wakefulness leads to a progressive, task-specific power increase in the low frequency range within brain regions involved in the task, and that behavioral performance worsens in relation to the slowing down of EEG activity (Bernardi et al., 2015; Nir et al., 2017; Slater et al., 2017; Petit et al., 2019; Ricci et al., 2019). Of note, however, the literature contains some discrepancies with respect to the specific frequency band that more directly reflects brain functional fatigue and the occurrence of local sleep episodes. Indeed, while waking and experience-dependent variations in EEG activity predominantly affect the theta (4-9 Hz) range (Hung et al., 2013; Bernardi et al., 2015), associations with behavioral impairment have been reported for increases in both theta (Hung et al., 2013; Ahlstrom et al., 2017; Fattinger et al., 2017b; Nir et al., 2017; Petit et al., 2019) and delta

activity (Slater et al., 2017; Quercia et al., 2018; Andrillon et al., 2020). Such a discrepancy may have different explanations. On one hand, different brain areas have been shown to be characterized by partially a different dominant frequency of EEG activity (Groppe et al., 2013). Thus, the manifestation of sleep-like activity in the theta or delta range may merely reflect inter-regional differences and may relate to the specific brain areas involved in task execution. On the other hand, delta waves may reflect more widespread and synchronized neuronal *OFF-periods* relative to theta waves. In this light, depending on the task at hand, behavioral errors may more commonly occur for more widespread relative to more local sleep-like episodes. This possibility is consistent with studies indicating that among theta waves, more widespread ones are more commonly associated with behavioral impairment (Fattinger et al., 2017b; Petit et al., 2019).

Altogether, these findings suggest that local sleep-like episodes occurring during wakefulness may represent the physiological consequence of "neuronal tiredness", and may offer an explicative model for fatigue- and sleepiness-related behavioral failures (Andrillon et al., 2019). Indeed, depending on which brain regions are affected by a local sleep-like episode, cognitive and behavioral correlates may be altered in partially distinct ways, leading for instance to commission or omission errors, and to different subjective experiences related to behavioral failures, such as mind wandering or mind-blanking (Andrillon et al., 2019). In this light, the local sleep phenomenon may provide a parsimonious explanation for a wide range of observations, including variations in behavioral performance and cognition related to time-on-task, cognitive fatigue, or sleep loss.

1.5 Aims

The principal aim of this Thesis was to advance current knowledge regarding the local regulation of sleep and wakefulness in humans, with a particular focus on slow waves.

In Chapter 2, I used overnight high-density (hd-)EEG (256 channels) to investigate the role of cortico-cortical anatomical connections in slow wave origin and propagation. In fact, the slow waves of NREM-sleep behave as traveling waves and their propagation has been previously suggested to reflect the integrity of white matter cortico-cortical connections (Massimini et al., 2004; Murphy et al., 2009; Buchmann et al., 2011a; Piantoni et al., 2013). In order to directly test this hypothesis, I investigated the role of the corpus callosum, the main bundle of fibers that connect the two brain hemispheres, in the cortical spreading of NREM slow waves. In particular, I collected overnight hd-EEG recordings in an extremely rare population of split-brain patients who underwent total callosotomy as a treatment for drug-resistant epilepsy and compared this data with that of two control groups, respectively including non-callosotomized neurological patients (n=3) and healthy adults (n=24).

In Chapter 3, I used a single subject multi-session design in order to investigate whether the topographic distribution of the build-up of local, sleep-like activity during normal wakefulness is relatively stable or rather varies across days. This particular approach allowed me to investigate whether the typical stronger frontal increase in low-frequency activity more likely reflects an experience-dependent accumulation of sleep need (Ferrara and De Gennaro, 2011; Bernardi et al., 2015), or rather a specific constitutional vulnerability (Kilduff et al., 2011). Hd-EEG (256 channels) recordings acquired during the evening were compared to those collected during the morning on the same day, and behavioral assessments of vigilance and sleepiness were used to correlate variations in EEG signal power with changes in behavioral performance.

In Chapter 4, I investigated the possible impact of sleep-like activity during wakefulness on emotion regulation. To this aim, I analyzed a subset of data that I acquired in the context of a larger experimental protocol aimed at exploring inter-subject differences

in the experience-dependent build-up of low-frequency activity. In this study, I used a combination of hd-EEG (64 channels) recordings, actigraphic monitoring, standardized behavioral tasks and psychometric assessments. For the particular study described in this chapter, I examined data collected during an emotion suppression task, in which subjects were asked to maintain a neutral facial expression while amusing video-clips were presented to them. These data were compared with those collected during a similar task in which, however, the same participants were free to express their emotional states.

Chapter 2

Integrity of corpus callosum is essential for the cross-hemispheric propagation of sleep slow waves: a high-density EEG study in split-brain patients[1]

2.1 Introduction

The transition from wakefulness to sleep is marked by profound changes in brain EEG activity, with a shift from the low-amplitude, high-frequency signals recorded in wakefulness to the high-amplitude, low-frequency slow waves (0.5-4 Hz) of NREM-sleep. In particular, sleep slow wave represents the EEG signature of a slow oscillation in membrane potential at neuronal level, characterized by an alternation between a hyperpolarized "silent" phase (*down-state*) and a depolarized phase of intense firing activity (*up-state*) (Steriade et al., 2001). Crucially, the amount of slow wave activity (SWA, expressed as the 0.5-4 Hz EEG-signal power in NREM-sleep) represents a reliable marker of homeostatically regulated sleep need (Achermann and Borbély, 2003) and has been shown to be locally modulated in a use-dependent manner, thus implying a possible relationship with plasticity-related processes (Tononi and Cirelli, 2014). Indeed, experimental studies and computer simulations have

[1] Avvenuti G, Handjaras G, Betta M, Cataldi J, Imperatori LS, Lattanzi S, Riedner BA, Pietrini P, Ricciardi E, Tononi G, Siclari F, Polonara G, Fabri M, Silvestrini M, Bellesi M*, Bernardi G* (2020) Integrity of corpus callosum is essential for the cross-hemispheric propagation of sleep slow waves: a high-density EEG study in split-brain patients. J Neurosci, 40(29), 55895603. * *denotes equal contribution.*

demonstrated that not only SWA reflects experience-dependent changes in regional synaptic density/strength, but also have indicated that slow waves may play a direct role in cellular and systems restoration and in the consolidation of newly acquired memories (Tononi and Cirelli, 2014). Recent evidence also suggested a possible implication of sleep slow waves in the clearance of neurotoxic metabolic products that accumulate during wakefulness (Xie et al., 2013; Hablitz et al., 2019).

The sleep slow waves are not stationary events. Instead, they typically behave as traveling waves at the macro-scale level of the scalp EEG, with variable cortical origin and propagation pattern (Massimini et al., 2004; Murphy et al., 2009). Such a propagation is commonly assumed to reflect the structural properties of cortico-cortical white matter connections. In line with this, structural white matter properties have been found to correlate with parameters reflecting slow waves synchronization (Murphy et al., 2009; Buchmann et al., 2011a; Piantoni et al., 2013). In this perspective, the corpus callosum (CC) would be expected to represent the main route responsible for cross-hemispheric slow wave propagation. However, correlational studies and research in human models of inter-hemispheric disconnection produced contradictory findings. For instance, two studies found a positive significant correlation between macro (volume) and micro (axial diffusivity) structural properties of the CC and parameters reflecting slow wave synchronization (i.e., amplitude and slope) in healthy adult individuals (Buchmann et al., 2011a; Piantoni et al., 2013). In contrast, a more recent work failed to replicate the correlation between slow-wave slope and axial diffusivity in healthy adult subjects, and rather described a positive correlation between indices reflecting white matter damage and slow wave synchronization in patients with traumatic brain injury (TBI; Sanchez *et al.*, 2019). In addition, while studies performed in patients with agenesis of the CC (Kuks et al., 1985; Nielsen et al., 1993) or in epileptic patients who underwent partial or total callosotomy (Montplaisir et al., 1990) showed a decreased inter-hemispheric coherence within the delta range (<4 Hz) during NREM-sleep, callosotomized patients continue to present a clear increase in inter-hemispheric coherence from wakefulness to sleep (Corsi-Cabrera *et al.*, 2006).

Given the above considerations, it is still unclear how the agenesis or the complete resection of the corpus callosum may affect sleep slow wave propagation in humans. Crucially, this matter has more general implications for the hypothesized relationship between brain structural connectivity and slow wave propagation (Murphy et al., 2009), as well as for the understanding of the mechanisms that regulate slow wave synchronization in relation to plastic and developmental processes (e.g., Mascetti *et al.*, 2013; Kurth *et al.*, 2017). Moreover, the contradictory findings reported in the literature likely result mostly from methodological limitations and discrepancies. Therefore, to determine the role of inter-hemispheric white matter connections in slow wave generation and propagation, here we analyzed for the first time overnight high-density (hd-)EEG recordings (256 electrodes) collected in a sample of five epileptic patients who underwent total callosotomy (CP; Figure 2) and in control subjects with an intact CC, including three neurological patients (NP, one epileptic male) and 24 healthy adult subjects (HS). In order to overcome limitations of previous studies related to the use of indirect indices of slow wave synchronization and propagation, we used validated algorithms to detect individual slow waves and to determine their specific origin and traveling pattern.

2.2 Materials and methods

2.2.1 Participants

Overnight hd-EEG recordings (256 electrodes; EGI-Philips) were performed at the Neurological Unit of the Marche Polytechnic University (Ancona, Italy) in five epileptic in-patients who underwent a total resection of the CC (CP, callosotomized patients; age range 40-53, two females; Figure 2). Three non-callosotomized neurological in-patients (NP, non-callosotomized patients; age range 44-66, two females) were also studied under the same experimental conditions. One of these patients was diagnosed with symptomatic generalized epilepsy due to viral meningoencephalitis occurred in infancy (this subject, indicated as NP03, was marked using a distinctive color in figures). All the non-callosotomized patients have no diagnoses of any other comorbidities affecting brain function at the time of the study. Table 1 and Table 2 report demographic and clinical characteristics for all patients. An additional control group of 24 healthy adult volunteers (HS, healthy subjects; age range 20-47, 13 females) was studied with the same hd-EEG recording system at the Lausanne University Hospital, Switzerland (analyses of NREM-sleep data from these subjects, not involving the study of inter-hemispheric slow wave propagation, have been reported in previous work; Siclari *et al.*, 2018, Bernardi *et al.*, 2019*a,b*). Prior to their inclusion into the study, HS group individuals underwent a clinical interview to exclude a history of sleep, medical and psychiatric disorders. None of the HS subjects was taking any medication at the time of the study. The study procedures were conducted under clinical research protocols approved by the local ethical committees and in accordance with the guidelines of the Declaration of Helsinki. Written informed consent was obtained from all participants.

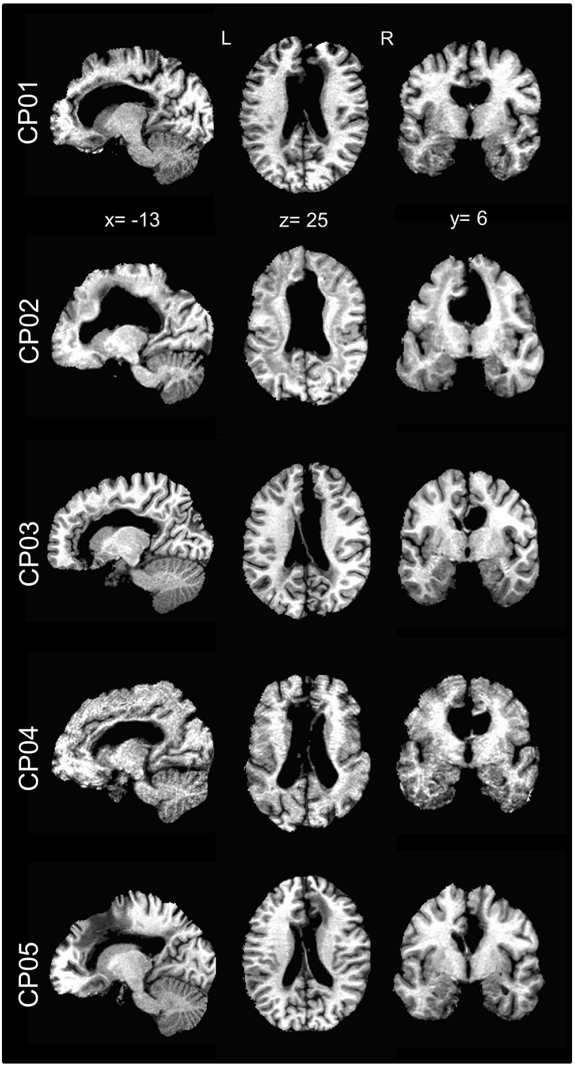

Figure 2. Anatomical MRI images of callosotomized patients. For each patient (CP01-CP05) sagittal, axial and coronal MRI images are shown in MNI space. It is possible to appreciate the complete absence of the corpus callosum in all cases.

2.2.2 Data Acquisition

One overnight hd-EEG recording (500 Hz sampling rate) was obtained for each subject. All recordings were initiated at the usual bedtime of each participant and interrupted at ~7AM. Given that callosotomized patients presented relatively low sleep quality with frequent awakenings, especially in the second part of the night, we extracted and analyzed only the first 5h of each recording, starting from the time of 'lights-off'. In order to ensure across-group comparability, analyses similarly focused only on the first 5h of data also in the healthy control subjects. Three 2-min 'resting-state' hd-EEG recordings (6-min in total) were also collected during relaxed wakefulness with the eyes closed before sleep and in the morning, about 40 min after awakening.

2.2.3 Data Preprocessing

For all patients, recordings were band-pass filtered between 0.1 and 45 Hz. Then, overnight recordings were divided into 30-sec epochs, while wake resting-state recordings were divided into 4-sec epochs. Bad channels and epochs were identified and rejected through visual inspection in NetStation 5.3 (EGI-Philips). An Independent Component Analysis (ICA) procedure was used to reduce residual ocular, muscular and electrocardiograph artifacts (EEGLAB toolbox; Delorme and Makeig, 2004). Finally, rejected bad channels were interpolated using spherical splines. A similar procedure was used to preprocess the sleep data of healthy control subjects, as described in previous work (Bernardi et al., 2019b). Data were filtered between 0.5 and 40 Hz prior to the analyses of signal power and slow wave parameters.

2.2.4 Sleep Scoring

For scoring purposes, four electrodes were used to monitor horizontal and vertical eye movements (electrooculography, EOG), while electrodes located in the chin-cheek region were used to evaluate muscular activity (electromyography, EMG). Sleep scoring was performed over 30-sec epochs according to the criteria from the American Academy of Sleep Medicine scoring manual (Iber et al., 2007). Two operators took care in marking periods containing large artifacts, arousals and non-physiological

activity (examples of included and excluded data for epileptic patients are shown in Figure 2). Only slow waves detected within artifact-free NREM-sleep (N2/N3) data-segments were analyzed.

2.2.5 Power Computation

A current-source-density (CSD) transform was applied to all EEG recordings using the CSD-toolbox (Kayser and Tenke, 2006). This method provides a reference-independent signal and improves spatial resolution by acting as a spatial filter. Of note the CSD transform was applied only for power computation and not for the detection and analysis of slow waves (see below). For each EEG derivation, power spectral density (PSD) estimates were computed using the Welch's method in 4-s data segments (Hamming windows, 8 sections, 50% overlap) and integrated within the delta/SWA (0.5-4 Hz) and the beta (18-35 Hz) frequency bands. In all sleep epochs, the power computation was performed over seven 4-s segments (28-s) after excluding the first and the last second of data.

2.2.6 Slow wave detection

Slow waves were detected automatically in a composite EEG-signal generated from linked-mastoid referenced channels, as previously described (Siclari et al., 2014; Mensen et al., 2016; Bernardi et al., 2018). This method provides a unique time reference (across electrodes) for each slow wave and facilitates the detection of both local and widespread events (Mensen et al., 2016). Specifically, a negative-going signal envelope was calculated by selecting the fifth most negative sample across a subset of 191 electrodes obtained by excluding channels located on the neck and face regions. This approach minimizes the risk of including in the envelope potential residual high amplitude oscillations of artifactual origin. Finally, the obtained signal-envelope was broadband filtered (0.5-40 Hz) prior to the application of a slow wave detection procedure based on half-wave zero-crossings (Vyazovskiy et al., 2007b; Siclari et al., 2014). Only half-waves with a duration comprised between 0.25 and 1.0 s were retained for further analyses. Of note, no amplitude thresholds were applied based on previous evidence indicating

that: i) slow waves with peak-to-peak amplitude < 75μV show the clear homeostatic changes commonly attributed to the slow waves of NREM-sleep (Vyazovskiy et al., 2007b; Bernardi et al., 2018); ii) the application of an amplitude threshold may actually preferentially select a minority of very large slow waves that have been shown to display different regulation and synchronization mechanisms as compared to the majority of slow waves (Siclari et al., 2014, 2018; Mensen et al., 2016; Bernardi et al., 2018, 2019a; Spiess et al., 2018). For all the detected slow waves, various parameters of interest were calculated and stored for a subsequent evaluation, including negative amplitude (μV), descending slope (between the first zero-crossing and the maximum negative peak; μV/ms) and involvement (mean EEG-signal calculated for all electrodes in an 80-ms window centered on the wave peak; μV). Moreover, the slow wave density (number of waves per minute) was computed in each sleep epoch (epochs in which artifactual or non-physiological activity occupied more than the 75% of time were excluded).

2.2.7 Scalp involvement distribution

For each subject, the involvement distribution (across channels) of all slow waves was analyzed using principal component analysis (PCA) as described in previous work (Bernardi et al., 2018). We recently showed that in healthy adult subjects the 95% of the variance related to slow wave involvement is explained by three principal components (PCs), with maxima located in the centro-frontal area (~70% of total variance), anterior or posterior areas (~20%) and left or right hemispheres (~5%). Here we hypothesized that callosotomized patients would present an increased variance explained by the last, uni-hemispheric PC at the expenses of the other two, symmetrical components. To test this hypothesis, we first verified through visual inspection that similar PCs explaining a similar amount of total variance were present in HS (95.0 ± 1.5%, range 92.3-97.0), NP (96.6 ± 0.6%, range 96.2-97.3; relative to HS all $P_{unc.} > 0.099$, $|z| < 1.652$) and CP (94.6 ± 1.3%, range 93.1-95.9; all $P_{unc.} > 0.193$, $|z| < 1.302$) subjects. Then, the PCs-space of each subject was rotated into a common, reference-PCs-space using the Procrustes transformation (Schönemann, 1966; Haxby et al., 2011). The

Procrustes transformation is an orthogonal transformation that minimizes the Euclidean distance between two sets of paired vectors. The reference-space was selected by iteratively applying the transformation over pairs of subjects of the HS group and then identifying the coordinate system (i.e., the subject) presenting the smallest distance with respect to the coordinate systems of all tested subjects (Haxby et al., 2011). Finally, the Procrustes transformation was applied to remap the original PCs-space of each subject (including the patients), into the new reference-PCs-space. This procedure allowed us to compare directly the first three PCs (and their explained variances) across individuals.

2.2.8 Slow wave propagation

For each detected slow wave, the pattern of propagation was calculated by determining the topographic distribution of relative delays in the local maximum negative peak, representing the moment of maximal regional recruitment (Massimini et al., 2004). In order to minimize the impact of potential localized artefacts, a *'likeness constraint'* method (Menicucci et al., 2009) was used to discard channels in which the negative wave was excessively dissimilar from a 'prototype' slow wave, defined as the wave with the largest negative peak at the reference peak timing across all channels. Specifically, we calculated the cross-correlation between the instantaneous phases (estimated using the Hilbert transform) of the prototype wave and of all other EEG signals in a symmetrical 300ms time-window centered on the reference peak. The 25th percentile of the distribution of the maximal cross-correlation values (C) was then used as a threshold to exclude events dissimilar from the prototype wave. The latency of all remaining local peaks was subsequently used to create a preliminary scalp 'delay-map'. A spatio-temporal clusterization procedure was applied to exclude potential propagation gaps: local peaks of two spatial neighbor electrodes had to be separated by less than 10 ms in order to be considered as part of the same propagation cluster. This approach ensures that all the electrodes are connected to one another through some neighboring electrodes, thus eliminating islands of channels which are likely to reflect artefacts in the local EEG signal. Then, the propagation cluster including the prototype wave was identified and the final

delay-map was extracted. The minimum delay, corresponding to the slow wave origin, was set to zero. Finally, a three-dimensional gradient (two for direction, one for timing) was computed from the delay-map to identify the main streamlines of propagation for each slow wave. Up to three streamlines were extracted: the longest displacement (the distance between the start and end points of the wave); the longest distance traveled (the cumulative sum of all coordinates of the line); and the stream of the most angular deviation from the longest displacement (minimum trajectory difference was set to 45°). The streamline corresponding to the longest distance traveled was used to compute the slow wave speed (distance divided by maximum delay; m/s).

2.2.9 Cross-hemispheric propagation

Information obtained from the propagation analysis was used to compute parameters reflecting the degree of cross-hemispheric propagation of sleep slow waves. First, for each slow wave we determined whether at least one of the propagation streamlines passed the midline (nasion-inion axis). This information was used to compute the proportion of cross-hemispheric slow waves in each subject (% of all the detected slow waves). Second, we determined the relative distribution of electrodes involved in the same (propagating) slow wave across the two hemispheres. This information was used to compute an index of channel recruitment asymmetry, defined as the number of channels in the hemisphere with less involved electrodes, divided by the total number of involved channels (%). In this index, a value of 50% indicates a symmetric distribution, while a value of 0% indicates a unilateral wave. This second parameter was also computed for slow waves subdivided into five amplitude percentile classes: 0-20, 20-40, 40-60, 60-80, 80-100.

2.2.10 Inter-hemispheric differences in slow wave latency

A resection of the CC may not completely abolish the cross-hemispheric spreading of sleep slow waves. Theoretically, an apparent hemispheric synchronization may result from volume conduction of electrophysiological signals, while a real spreading could occur through alternative pathways involving subcortical structures. In order to test these hypotheses, we analyzed the relative time-lag between homologous symmetrical electrodes in the two brain hemispheres. Unlike the analyses described above, this investigation was performed using a more conventional slow wave detection approach, in which negative half-waves were automatically identified in a subset of EEG electrodes as described in previous work (Mölle et al., 2002; Riedner et al., 2007). This analysis gave us the opportunity to verify the consistency of results obtained from the propagation analysis described above with analyses based on a more conventional detection and definition of sleep slow waves. Specifically, linked-mastoid-referenced EEG-signals of electrodes corresponding to F3 (frontal-left) and F4 (frontal-right) were filtered between 0.5 and 4 Hz and negative half-waves with duration comprised between 0.25 and 1.0 s were detected and retained for further analyses. For each slow wave with a peak-to-peak amplitude of 75μV detected in F3, we evaluated whether another negative wave of any amplitude was present in F4 within a 140ms time-window centered on the negative peak of the F3 slow wave. Of note, this window-length has been selected as it roughly corresponds to the time-interval necessary for slow waves to travel from one electrode to the other assuming a minimum propagation speed of 1m/s (Massimini et al., 2004). In addition, the amplitude threshold of 75μV was selected as it represents a criterion commonly adopted in the clinical practice for the definition of sleep slow waves. In order to verify the consistency of this analysis with evaluations of cross-hemispheric slow wave propagation described above we computed in each subject the proportion of bilateral slow waves with respect to all slow waves detected in F3. Moreover, for all bilateral slow waves we determined the absolute time-lag between their negative and positive peaks (respectively, the maximum negative peak between the positive-to-negative and the negative-to-positive zero-crossings, and the maximum positive peak after the negative-to-positive zero-crossing and before the

following zero-crossing). Finally, we also determined the proportion of bilateral slow waves showing a zero-lag time difference, which could result from volume conduction rather than a true inter-hemispheric spreading. Of note, similar results were obtained using F4 as a reference channel, or using central (C3, C4) instead of frontal electrodes (data not shown).

2.2.11 Inter-hemispheric differences in slow wave density

The CC could be involved not only in the propagation of individual slow waves but also in homogenizing sleep depth across the two hemispheres. In other words, the lack of inter-hemispheric connections could lead to the emergence of hemispheric asymmetries in the relative density of large slow waves, potentially even to a marked uni-hemispheric sleep. To test this hypothesis, slow waves were automatically detected using the conventional approach described above in three left (F3, C3, P3) and three right (F4, C4, P4) homologous electrodes (Vyazovskiy et al., 2007b). A peak-to-peak amplitude threshold of 75 μV was applied; similar results were also obtained using a 40 μV negative amplitude threshold. The density of slow waves in each epoch and channel was computed as described above. Finally, the average absolute inter-hemispheric difference in slow wave density was computed across pairs of homologous electrodes.

2.2.12 Probabilistic origin and recruitment

Next, we evaluated whether slow waves originate with a different incidence across the two hemispheres. Thus, individual slow waves were classified as having a left-hemisphere (right-hemisphere) origin if > 75% of the origin-channels (delay = 0 ms) were located in the left (right) hemisphere. An origin asymmetry index was computed as the difference in the density (waves/min) of slow waves originating in the left vs. the right hemisphere. In addition, we defined the slow wave "*probabilistic origin*" as the probability for each channel to represent the origin of a slow wave, computed with respect to all the detected slow waves. Similarly, the "*probabilistic recruitment*" was defined as the

probability for each electrode to be part of the propagation path of a slow wave.

2.2.13 Statistical Analyses

For each parameter of interest, the 5 CP and the 3 NP (Ancona dataset) were compared with the control group of healthy adult individuals (HS; Lausanne dataset). Specifically, for each patient, the relative z-score and corresponding p-value were computed with respect to the distribution represented by the healthy control group. A Bonferroni correction was applied to account for multiple comparisons across tested subjects and related hypotheses. Effects were regarded as significant only when a corrected $P < 0.05$ was observed in each of the 5 CP and in none of the 3 NP (Table 3 summarizes group- and subject-level statistics for each performed comparison). Analyses were repeated after regression-based adjustment of values to account for inter-subjects age differences. For analyses performed in individual groups (HS, CP) against the null-hypothesis of no inter-hemispheric asymmetry, a bootstrapping procedure (1,000 iterations) was applied to compute confidence intervals (bCI, $\alpha = 0.05$).

2.2.14 Data availability

Relevant data that support the findings of this study are available from the corresponding authors upon motivated request.

2.3 Results

2.3.1 Sleep structure

Table 1 and Table 2 report demographic and clinical characteristics for all patients. Table 1 also displays the sleep macro-structure in each patient and in the healthy control group. Of note, all epileptic patients (CP01-CP05 and NP03) presented altered patterns of brain activity, with bursts of spike-wave discharges, during both wakefulness and sleep (Figure 3). Such non-physiological activity was particularly evident in four patients (CP01-CP04) and limited the possibility to accurately recognize changes in sleep depth based on standard criteria (the remaining patient, CP05, is marked using a distinctive color in figures). For this reason, a distinction between N2- and N3-sleep was not made. Examined NREM epochs in CP were characterized by an increase in SWA (0.5-4Hz) and a decrease in high-frequency activity (18-35Hz) relative to wake epochs (Figure 4), thus confirming the reliability of performed sleep scoring.

Table 1. Demographic characteristics. For patients in CP and NP group demographic characteristics, questionnaires and sleep macro-architecture are presented separately for each subject. For CP patients the resection of the corpus callosum occurred in two distinct surgical interventions, for each of which the age of the patients is reported. For the HS group the values are reported as group mean ± standard deviation. Sleep stage percentages are expressed with respect to total sleep time (TST). PSQI = Pittsburg Sleep Quality Index; ESS = Epworth Sleepiness Scale; HOQ = Horne-Östberg Questionnaire; EHI = Edinburgh Handedness Inventory; PSG = polysomnography; WASO = wake after sleep onset; CP = callosotomized patients; NP = non-callosotomized patients, HS = healthy subjects.

	CP01	**CP02**	**CP03**	**CP04**	**CP05**	**NP01**	**NP02**	**NP03**	**HS (n=24)**
Age, years	53	40	47	45	42	45	66	44	27 ± 6
Gender	M	F	F	M	M	F	F	M	13F
Age at Surgery 1, years	30	16	25	14	18	-	-	-	-
Age at Surgery 2, years	45	17	26	22	19	-	-	-	-
Questionnaires									
PSQI	12	2	4	5	9	11	4	12	3.1 ± 1.5
ESS	23	4	2	0	13	7	18	1	5.9 ± 2.1
HOQ	59	44	43	61	54	52	34	45	51.8 ± 6.7
EHI	RH	RH	RH	RH	RH	RH	RH	RH	20RH
Sleep structure									
Sleep latency, min	20.5	29.5	23.5	38.5	34.0	6.0	6.5	4.0	15.4 ± 16.9
Total sleep time, min	244.5	243.5	185.5	254.0	120.5	278.0	251.5	118.5	256.6 ± 30.6
Sleep efficiency, %	81.4	81.0	61.7	84.5	40.1	92.5	83.7	39.4	85.5 ± 10.2
WASO, min	36	15.5	66.0	4.5	129.5	11.0	24.5	157.5	20.0 ± 15.3
N1 sleep, %	0.8	7.0	14.8	9.6	24.5	3.1	8.5	19.4	5.1 ± 5.0
N2 sleep, %	-	-	-	-	80.9	63.5	66.0	59.1	57.7 ± 10.4
N3 sleep, %	-	-	-	-	19.1	9.2	15.7	19.4	27.1 ± 7.5
NREM (N2/N3) sleep, %	100.0	88.7	100.0	100.0	100.0	72.7	81.7	78.5	84.8 ± 6.0
REM sleep, %	0.0	11.3	0.0	0.0	0.0	27.3	18.3	21.5	15.2 ± 6.0

Table 2. Clinical diagnosis and medications of studied patients. CP = callosotomized patients; NP = non-callosotomized patients.

	DIAGNOSED PATHOLOGY	CURRENT MEDICATIONS
CP01	Lennox-Gastaut Syndrome	Carbamazepine, Phenytoin sodium, Phenobarbital
CP02	Drug-resistant epilepsy	Carbamazepine, Levetiracetam, Sodium valproate
CP03	Early Infantile Epileptic Encephalopathy	Levosulpiride, Oxcarbazepine, Phenobarbital, Risperidone
CP04	Lennox-Gastaut Syndrome	Clobazam, Lacosamide, Phenobarbital, Rosuvastatin, Vigabatrin
CP05	Drug-resistant epilepsy	Carbamazepine, Clonazepam, Diazepam, Omeprazole, Phenobarbital
NP01	Generalized Anxiety Disorder	None
NP02	Lumbar spinal stenosis	Cholecalciferol, Esomeprazole, Lisinopril, Mometasone
NP03	Epilepsy (viral meningoencephalitis in infancy)	Enalapril+Lercanidipine, Lacosamide, Oxcarbazepine, Sodium valproate, Ursodeoxycholic acid

*Table 3. Summary of statistics related to comparisons between patients and healthy subjects. The first two columns indicate the analyses of interest. Columns three to seven include descriptive statistics for the healthy subjects (HS) group: p-value of the Kolmogorov–Smirnov test for data normality (KS Test), group level mean (Mean), standard deviation of the mean (SD), 2.5 (Prc. 2.5) and 97.5 (Prc. 97.5) percentiles of the distribution. Columns from eight to ten show the values of the parameter of interest observed in each of the three non-callosotomized patients (NP01-NP03). Columns from ten to thirteen show the values of the parameter of interest observed in each of the five callosotomized patients (CP01-CP03). Bold text indicates values that fall off the 2.5-97.5 percentiles range ($\alpha < 0.05$). Shaded green cells mark values that are significantly different from those of the HS group after Bonferroni correction. The correction was applied based on the number of tested subjects (N=8). Are exceptions the analyses marked with a *, for which the correction also took into account the number of related parameters that were tested.*

		HEALTHY SUBJECTS (N=24)					NON-CALL. PATIENTS			CALLOSOTOMIZED PATIENTS				
ANALYSIS	**PARAMETER**	**KS TEST**	**MEAN**	**SD**	**PRC. 2.5**	**PRC. 97.5**	**NP01**	**NP02**	**NP03**	**CP01**	**CP02**	**CP03**	**CP04**	**CP05**
Slow wave properties	Density	0.350	18.7	4.4	10.1	25.4	20.3	22.6	12.4	13.8	14.4	17.5	9.8	15.9
	Amplitude	0.410	50.3	15.6	32.3	96.0	62.9	35.6	42.8	55.6	78.6	**102.8**	69.4	50.3
	Slope	0.350	1.1	0.3	0.8	1.7	1.6	0.9	1.1	1.4	**1.8**	**2.5**	**2.2**	1.6
	Speed	0.991	2.3	0.3	1.8	2.9	2.1	2.2	2.1	1.9	2.5	1.9	**1.8**	**1.5**
* Involv. PCA	FrontoCentral	0.993	73.1	7.0	57.8	85.1	81.8	72.1	67.9	**32.6**	61.7	**50.4**	**47.0**	**26.8**
	Anter./Poster.	0.744	19.7	5.7	9.7	34.0	11.2	17.2	16.3	13.9	**8.5**	10.5	21.4	32.0
	Left/Right	0.119	7.2	3.1	2.7	15.3	7.0	10.8	**15.8**	**53.6**	**29.8**	**39.1**	**31.5**	**41.2**
Tra.v. Asy.	Crosshem. Pr.	0.830	63.2	3.5	54.9	69.0	65.8	57.3	57.8	**35.9**	**43.6**	**22.4**	**41.8**	**41.2**
	Asymmetry	0.652	36.8	2.4	43.3	33.4	35.8	**31.9**	33.4	**19.7**	**24.8**	**15.8**	**24.2**	**24.4**

* Asymmetry amplitude percentiles	Prc. 0-20	0.414	34.6	2.4	31.1	41.2	31.3	**28.6**	**29.5**	**21.2**	**20.7**	**14.6**	**18.9**	**20.9**
	Prc, 20-40	0.560	35.7	2.7	31.5	42.5	34.4	32.6	33.4	**20.7**	**23.8**	**15.0**	**24.0**	**23.6**
	Prc, 40-60	0.622	36.5	2.7	32.3	43.7	36.5	**30.6**	33.1	**20.7**	**25.3**	**14.5**	**24.2**	**25.2**
	Prc, 60-80	0.865	37.7	2.6	32.8	44.1	37.6	**32.2**	35.5	**19.7**	**27.4**	**16.6**	**27.5**	**25.4**
	Prc, 80-100	0.696	39.8	2.1	35.6	45.5	39.0	35.7	36.0	**16.2**	**26.7**	**18.2**	**26.5**	**26.8**
Relat. Hem. Diff.	Density 75μV	0.869	-0.3	0.7	-2.5	1.0	0.2	0.2	-0.5	**-5.4**	**-3.4**	-1.8	**-4.6**	-1.5
	Density 40μV	0.839	-0.3	0.8	-2.6	1.1	0.1	0.2	-1.0	**-5.7**	**-4.1**	-1.5	**-5.7**	-1.6
	Origins	0.908	-0.5	0.7	-2.1	0.9	**1.0**	-0.2	**2.5**	**-3.4**	**-3.1**	-1.4	-0.3	**-3.8**
Absol. Hem. Diff.	Density 75μV	0.382	1.7	0.5	1.1	2.9	1.9	**0.8**	2.0	**8.3**	**8.4**	**10.7**	**8.2**	**4.3**
	Density 40μV	0.192	1.9	0.5	1.3	3.1	2.3	**1.1**	2.6	**8.2**	**9.5**	**11.3**	**9.7**	**4.9**
	Origins	0.967	4.4	0.6	3.0	5.5	4.1	5.0	**6.3**	**7.4**	**6.8**	**7.1**	3.7	5.5

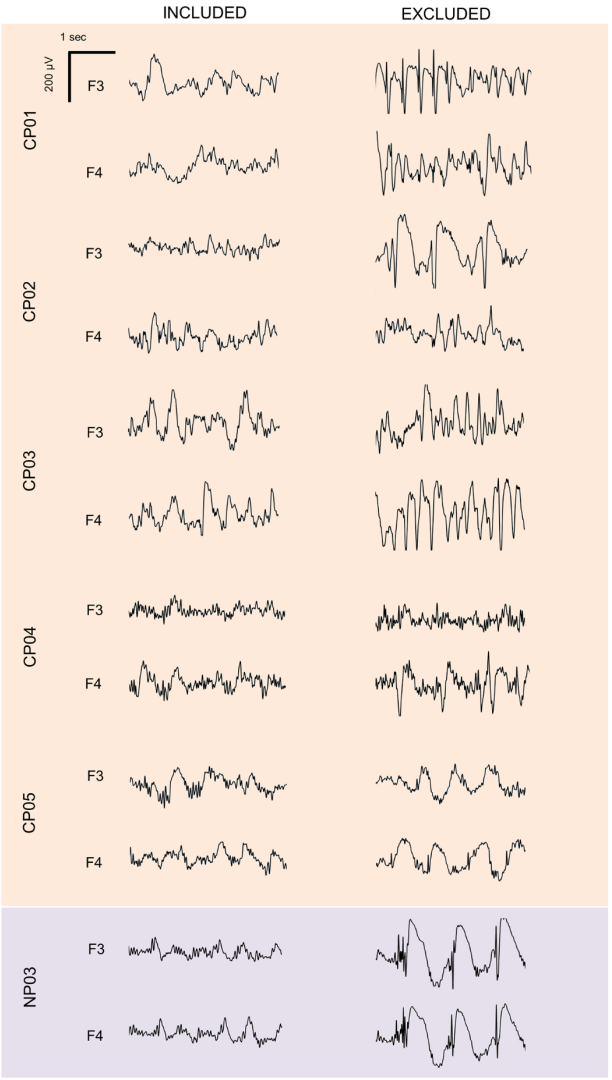

Figure 3. Examples of included and excluded data segments in epileptic patients. For each subject representative EEG-traces (0.5-25 Hz) corresponding to included (left) and excluded (right) data segments are shown for one frontal left (F3) and one frontal right (F4) electrode.

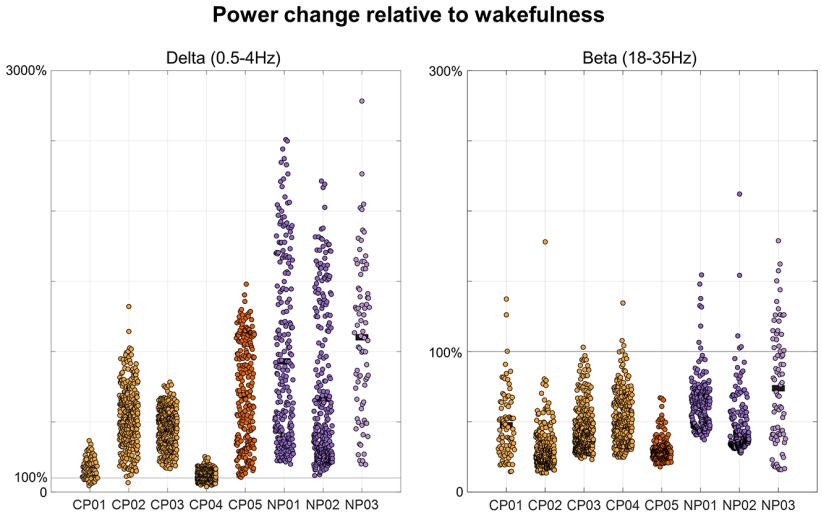

Figure 4. EEG power changes relative to wakefulness. The sleep stage classification in four of the five callosotomized patients (CP01-CP04) and of the epileptic non-callosotomized control patient (NP; NP03) was made difficult by the presence of altered patterns of brain activity. However, the reliability of the sleep scoring procedure is supported by the direct comparison of EEG power between epochs scored as NREM-sleep and eyes-closed wake recordings collected prior to sleep, which showed an increase in SWA (0.5-4 Hz) and a decrease in high-frequency activity (beta, 18-35 Hz) in all CP (CP01-CP05) and NP (NP01-NP03) subjects. Here 100% corresponds to the signal power in wakefulness. The SWA increase was overall smaller in CP relative to NP. CP = callosotomized patients; NP = non-callosotomized patients. CP are represented with orange dots (CP05 = dark orange), NP with purple dots (NP03 = light purple).

2.3.2 Slow wave characteristics

Slow wave density (CP = 20.2 ± 5.6 waves/min, range 11.7-26.3; HS = 18.7 ± 4.4 waves/min, range 9.8-25.5), amplitude (CP = 62.3 ± 21.5 μV, range 50.3-102.8; HS = 50.3 ± 15.6 μV, range 32.1-97.9), slope (CP = 1.6 ± 0.5 μV/ms, range 1.4-2.5; HS = 1.1 ± 0.3 μV/ms, range 0.8-1.8) and propagation speed (CP = 2.0 ± 0.3 m/s, range 1.5-2.5; HS = 2.3 ± 0.3 m/s, range 1.8-2.9) did not differ between CP and healthy controls as a group (Figure 5). Specifically, we found no significant differences ($P_{cor.} < 0.05$) in slow wave density (all $P_{unc.} > 0.08$, $|z| < 1.7344$), while significant effects were observed only in CP03 for amplitude ($P_{unc.} = 0.008$, $|z| = 3.3688$), in CP03 ($P_{unc.} < 0.001$, $|z| = 5.1634$) and CP04 ($P_{unc.} < 0.001$, $|z| = 4.1840$) for slope and in CP05 ($P_{unc.} = 0.002$, $|z| = 3.1509$) for speed. Results did not change after controlling for between-subjects age differences, except for speed, in which no significant differences were found in any patients with respect to HS (all $P_{unc.} > 0.01$, $|z| < 2.5532$).

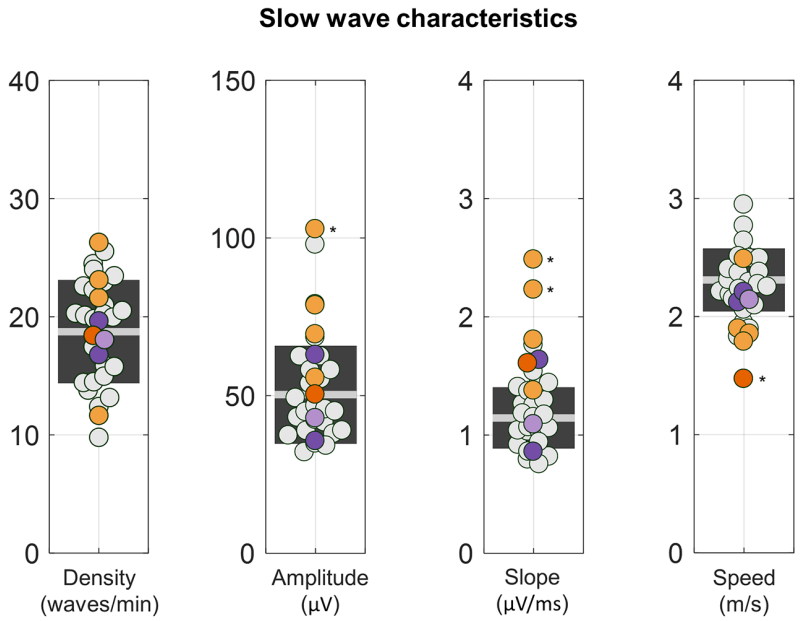

*Figure 5. Properties of NREM slow waves. For each subject we computed slow wave density (number of waves per minute), negative amplitude (μV), descending slope (μV/ms), and propagation speed (m/s). CP are represented with orange dots (CP05 = dark orange), NP with purple dots (NP03 = light purple), and HS with light-gray dots. The light-gray horizontal line represents the mean for the HS, while the dark-gray box represents 1 SD around the mean. Values observed in the 8 patients (CP and NP) were compared with the 24 HS. * $P_{cor.} < 0.05$.*

2.3.3 Slow wave involvement

Visual inspection of EEG-traces suggested that most sleep slow waves of CP may present an asymmetric scalp distribution (Figure 6).

This observation was quantitatively confirmed through a PCA-based comparison of slow wave involvement across groups (Figure 7). In fact, in HS the 95% of the variance related to scalp slow wave involvement was explained by three PCs, with maxima located in the centro-frontal area (73.1 ± 7.0%, range 57.4-85.3, of the total variance explained by the first three components), anterior or posterior areas (19.7 ± 5.7%, range 9.4-34.6) and left or right hemispheres (7.2 ± 3.1%, range 2.6-15.4), respectively. Similar values were obtained in the NP-group, with percentages corresponding to 73.9 ± 7.1% (range 67.9-81.8), 14.9 ± 3.3% (range 11.2-17.2), 11.2 ± 4.4% (range 7.0-15.8), respectively. On the other hand, in the CP-group we observed a significant increase in the variance explained by the third (left/right) component (39.0 ± 9.5%, range 29.8-53.6; $P_{cor.} < 0.05$, $|z| > 7.3503$; Bonferroni correction based on the number of tested subjects and PCs), at the expenses of the other two symmetrical components (43.7 ± 14.1%, range 26.8-61.7, for the centro-frontal component and 17.2 ± 9.6%, range 8.5-32.0, for the anterior/posterior component). In particular, the variance explained by the first component was significantly decreased in four (CP01, CP03, CP04, CP05) out of five CP.

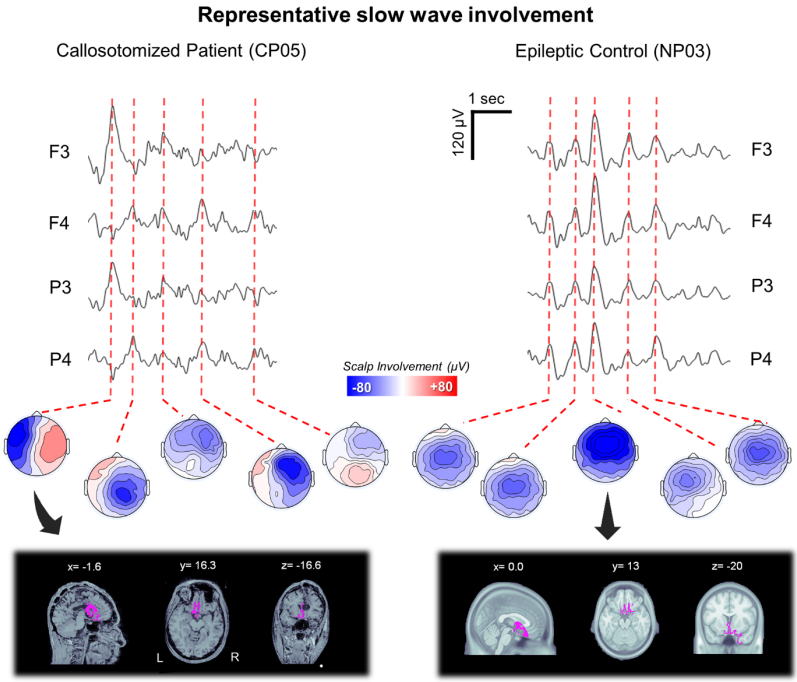

Figure 6. Representative slow wave involvement patterns in a callosotomized (CP05) and a non-callosotomized (NP03) epileptic patient. The top panel shows representative NREM-sleep EEG-traces (5-s) for two left (F3, P3) and two right (F4, P4) channels and the relative scalp involvement associated with exemplar slow waves. The bottom panel shows the source-reconstructed signal distributions for two representative slow waves. The source modeling has been performed using BrainStorm. A symmetric Boundary Element Method (BEM) was used to define the forward model, while the inverse matrix was computed using the standardized low-resolution brain electromagnetic tomography (sLORETA) constraint. The cortical maps are thresholded at 80% of the maximum signal amplitude.

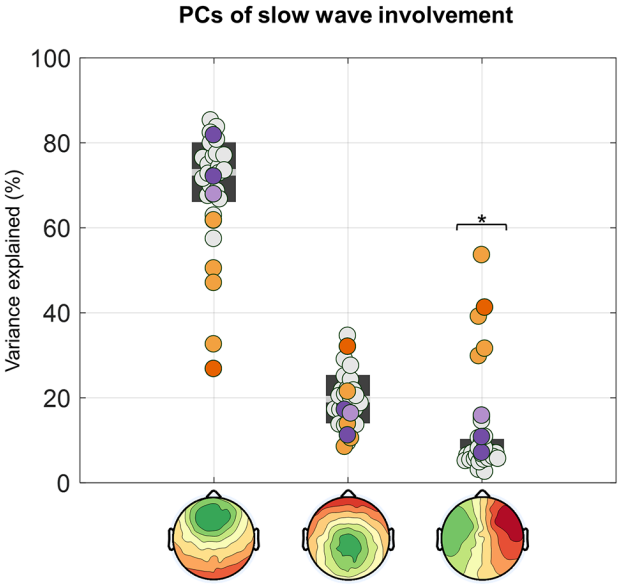

*Figure 7. PCA-based analysis of slow wave involvement. The involvement distribution (mean EEG-signal calculated across all electrodes in a 40-ms window centered on the wave peak; µV) of all slow waves was entered in a principal component analysis (PCA). The plot shows the variance explained by each of the three PCs in all subjects. CP are represented with orange dots (CP05 = dark orange), NP with purple dots (NP03 = light purple), and HS with light-gray dots. * $P_{cor.} < 0.05$.*

2.3.4 Cross-hemispheric propagation of slow waves

Next, we investigated whether alterations in the scalp distribution of slow waves in callosotomized patients could be explained by a lack of cross-hemispheric propagation of individual slow waves (Figure 8A).

The percentage of slow waves presenting a cross-hemispheric propagation was significantly reduced in CP (37.0 ± 8.6%, range 22.4-43.6) relative to HS (63.2 ± 3.5%, range 54.6-69.0; $P_{cor.} < 0.05$, $|z| > 5.5345$; Figure 8B). Consistent, slow waves in CP showed a stronger lateralization in terms of number of channels recruited along the propagation pattern in each of the two hemispheres (CP = 21.8 ± 4.0%, range 19.7-24.8; HS = 36.8 ± 2.4%, range 33.4-43.3; $P_{cor.} < 0.05$, $|z| > 4.9546$; Figure 8C). Of note, such lateralization appeared to similarly affect all the slow waves regardless of their amplitude ($P_{cor.} < 0.05$, Bonferroni correction based on the number of tested subjects and amplitude percentile classes; Figure 8D). Given that slow wave traveling was computed by applying a spatio-temporal clusterization procedure that could have concealed potential propagation discontinuities caused by cortico-subcortico-cortical loops, the same analyses were repeated without this procedure. Obtained results confirmed the above observations, by showing that all CP had a lower proportion of cross-hemispheric slow waves (all $P_{unc.} < 0.0001$, $|z| > 5.7083$) and a stronger inter-hemispheric asymmetry in slow wave spreading with respect to HS (all $P_{unc.} < 0.0001$, $|z| > 4.1063$). All results remained significant after controlling for between-subjects age difference.

Figure 9 shows the probabilistic channel recruitment of slow waves originating in the left or right hemisphere in each of the CP individuals and in the HS group. This qualitative representation further shows that slow waves tended to remain confined to the origin hemisphere in callosotomized but not in control subjects

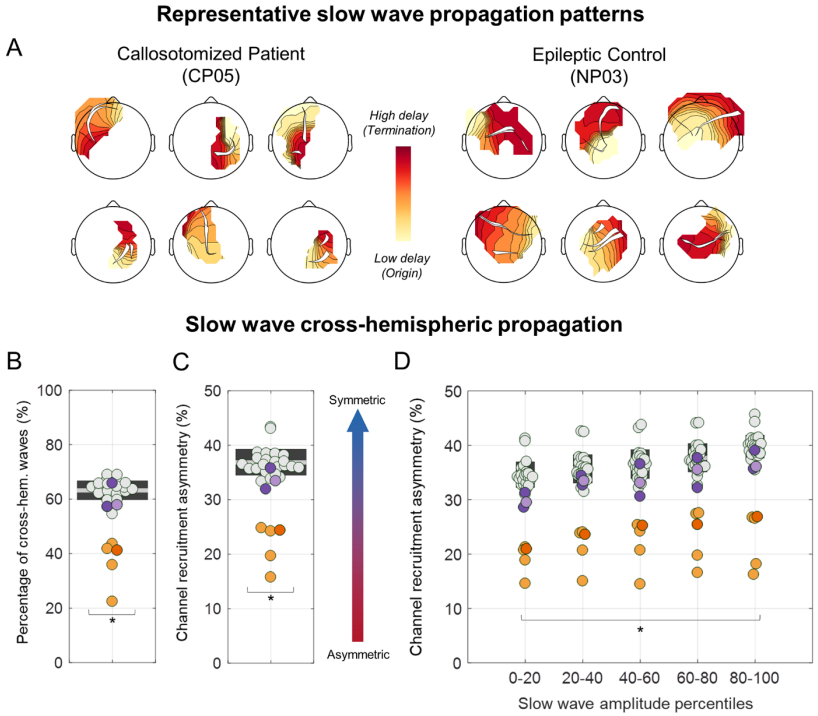

*Figure 8. Quantitative analysis of slow wave cross-hemispheric propagation. A) Traveling delay-maps and relative propagation streamlines of six representative slow waves of CP05 and NP03. In CP slow waves tended to remain confined to the origin hemisphere, while cross-hemispheric propagation was common in NP and HS. B) The percentage of cross-hemispheric slow waves was computed as the number of slow waves for which at least one of the propagation streamlines passed the nasion-inion, midline axis relative to the total number of detected slow waves. C) The recruitment asymmetry was determined by computing the number of channels in the hemisphere with less recruited electrodes divided by the total number of recruited channels across the two hemispheres. Values close to 50% indicate a symmetric distribution, while values close to 0% indicate a unilateral wave. D) This second parameter was also computed for slow waves grouped into five amplitude percentile classes (0-20, 20-40, 40-60, 60-80, 80-100). CP present a significantly reduced percentage of cross-hemispheric slow waves and an increased channel recruitment asymmetry (uni-hemispheric distribution). CP are represented with orange dots (CP05 = dark orange), NP with purple dots (NP03 = light purple), and HS with light-gray dots. * $P_{cor.} < 0.05$.*

Figure 9. Scalp probabilistic recruitment as function of hemispheric origin. The top panel shows, for each of the CP, the probabilistic recruitment (probability of channel recruitment in a slow wave) for slow waves originating in the left (left-column) or right (right-column) hemisphere. The probabilistic recruitment is expressed as a percentage with respect to the total number of detected slow waves, regardless of their origin site. The bottom panel shows the average probabilistic recruitment for CP and HS. It is evident that the involvement tended to be more symmetrical in HS with respect to CP. Moreover, this analysis suggested a relatively stronger recruitment of the right (vs. left) hemisphere in both CP and HS.

2.3.5 Inter-hemispheric differences in slow wave latency

Given that cross-hemispheric propagation was reduced but not abolished in CP, we investigated whether this depends on an apparent synchronization caused by volume conduction of EEG signals, or on a real spreading of slow waves through alternative pathways. To this aim, we analyzed the co-occurrence and degree of synchronization of slow waves detected in homologous frontal electrodes (Figure 10). In line with results described above, we found that the percentage of bilateral slow waves was significantly reduced in CP with respect to HS ($P_{cor.} < 0.05$; all $P_{unc.} < 0.0001$, $|z| > 5.2439$, Fig. 10A). Moreover, the time-lag between negative peaks of bilateral detections was significantly higher in CP relative to HS ($P_{unc.} < 0.0055$, $|z| > 2.7790$, Fig. 10A) and these results remained significant after adjustment for inter-subject age differences. A similar trend toward an increased time-lag in CP was found for positive peaks although the difference with respect to HS reached significance only in CP01 ($P_{unc.} = 0.0030$, $|z| = 2.9679$) and CP03 ($P_{unc.} < 0.0001$, $|z| = 4.6526$, Fig. 10A). After adjustment for age differences a significant effect was found also in CP02 ($P_{unc.} = 0.0013$, $|z| = 3.2269$). Finally, we found no significant differences between CP and HS with respect to the proportion of slow waves showing perfectly synchronous (zero-lag) negative peaks across the two hemispheres ($P_{unc.} > 0.1322$, $|z| < 1.5055$, Fig. 10B). Of note, for this latter analysis the distribution of HS included a clear outlier (HS10; value greater than 3 SD from the group mean) but results did not change after exclusion of this subject.

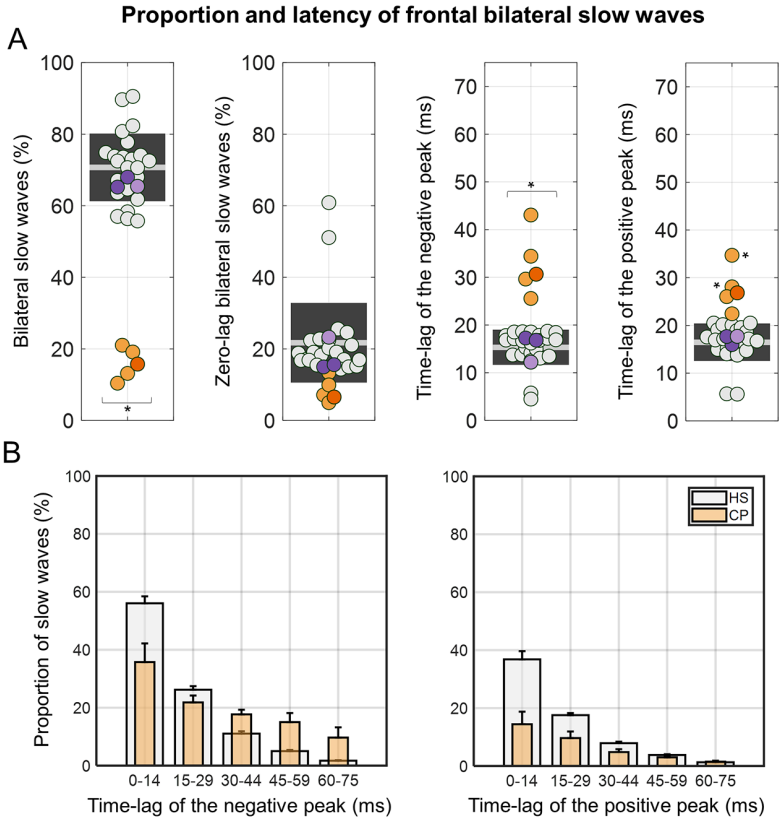

Figure 10. Slow wave synchronization across symmetrical frontal electrodes. ***A)*** *The four plots respectively show (from left to right) the percentage of bilaterally detected slow waves, the proportion of perfectly synchronous (zero-lag) slow waves that may reflect volume conduction, the absolute time-lag between the negative peaks of bilateral slow waves, the absolute time-lag between the positive peaks of bilateral slow waves.* ***B)*** *Distribution of time-lags for negative (left) and positive (right) peaks in HS and CP. Note that percentages were calculated with respect to the total number of bilateral negative peaks and that bilateral positive peaks were not found in the explored time window for some of these slow waves. * $P_{cor.} < 0.05$.*

2.3.6 Inter-hemispheric asymmetry in sleep depth

Last, we investigated whether the lack of strong inter-hemispheric connections could be responsible for an unbalanced sleep depth - as reflected by the generation and synchronization of sleep slow waves - across the two hemispheres. To this aim, we first tested whether CP and control HS presented an asymmetric incidence of large amplitude slow waves, characterized by peak-to-peak (negative-to-positive) amplitude greater than 75 μV. Specifically, we computed for each sleep epoch the relative difference in slow wave incidence across homologous electrodes of the two hemispheres. We found that, in both groups, many of the NREM-sleep epochs were characterized by an inter-hemispheric difference in slow wave density (Figure 11A and Figure 11C), with a relative hemispheric dominance that varied from epoch to epoch. Overall, however, slow wave density tended to be higher in the right, relative to the left, hemisphere in both CP (-3.38 ± 0.69 waves/min, difference left-right) and HS (-0.26 ± 0.15 waves/min), although the effect reached statistical significance only in the first group (one sample t-tests against the null hypothesis of no asymmetry; HS, $P = 0.099$, $|t_{23}| = 1.7197$, bCIs = [-0.55, 0.01]; CP, $P = 0.012$, $|t_4| = 4.3676$, bCIs = [-4.84, -2.08]). Similar results were obtained using a slow wave amplitude threshold corresponding to a negative amplitude of 40μV (HS, $P < 0.074$, $|t_{23}| = 1.8735$, bCIs = [-0.65, -0.01]; CP, $P = 0.017$, $|t_4| = 3.9427$, bCIs = [-5.39, -2.04]). Importantly, the relative inter-hemispheric asymmetry was not systematically different across CP and HS (Table 3). However, when the absolute ($|\text{left} - \text{right}|$), rather than the relative (left – right) inter-hemispheric difference in slow wave incidence was considered, this parameter was significantly greater in CP (5.6 ± 3.8 waves/min) relative to HS (1.7 ± 0.5 waves/min; $P_{cor.} < 0.05$, $|z| > 4.6552$; Figure 11A). Similar results were obtained for the 40μV amplitude threshold (HS = 1.9 ± 0.5 waves/min; CP = 6.2 ± 3.9 waves/min; $P_{cor.} < 0.05$, $|z| > 5.8069$). All results remained significant after controlling for between-subjects age differences.

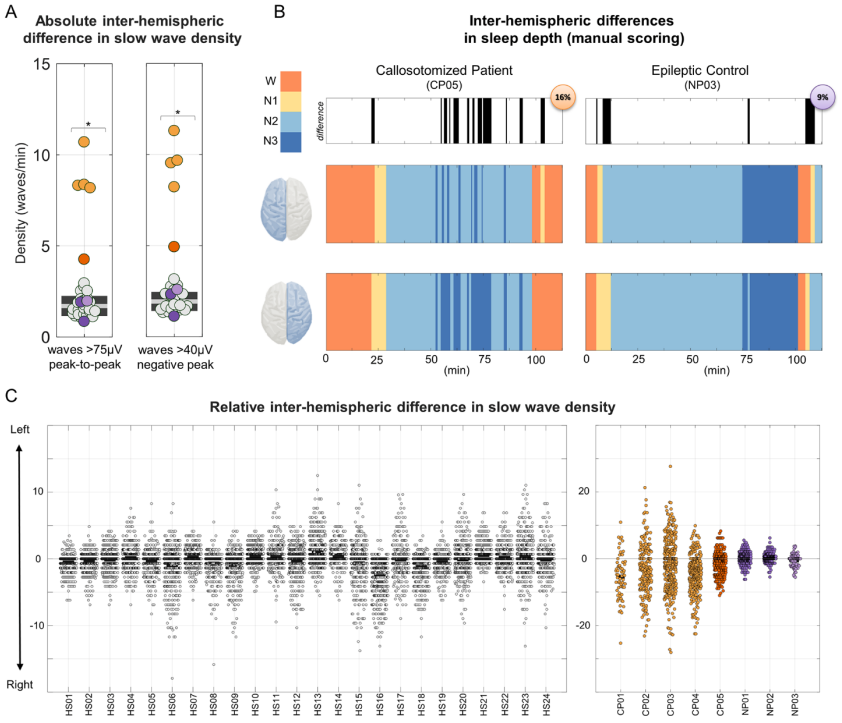

Figure 11. Inter-hemispheric difference in slow wave density. ***A)*** *Absolute inter-hemispheric difference in slow wave density. The plot on the left shows the absolute inter-hemispheric (| left-right |) difference in slow wave density. CP are represented with orange dots (CP05 = dark orange), NP with purple dots (NP03 = light purple), and HS with light-gray dots. * $P_{cor.} < 0.05$.* ***B)*** *Sleep scoring (120 minutes; 0 = time of lights off) in a callosotomized patient (CP05) and in the non-callosotomized epileptic patient (NP03). Sleep scoring was performed separately for each hemisphere (i.e., using only electrodes of the left or right side) by an operator blind to both the identity of the subjects and the evaluated brain hemisphere. In the top panel, black sections indicate epochs for which different stages were scored across the two hemispheres. Bottom panels represent the sleep scoring for the first sleep cycle in the two patients and for each hemisphere (left top, right bottom).* ***C)*** *Difference in the mean slow wave density (waves/min) across three left (F3, C3, P3) and three right (F4, C4, P4) channels. A peak-to-peak amplitude threshold corresponding to 75 µV was applied to minimize spurious cross-hemispheric detection caused by simple volume conduction. Each dot represents a different NREM-sleep epoch. The left plot represents each of the HS (HS01-HS24), while the right plot shows the CP (CP01-CP05) and the NP (NP01-NP03). Lower (negative) values indicate a higher number of slow waves detected in the right hemisphere. In both CP and HS groups there was a tendency toward a higher slow wave density in the right relative to the left hemisphere.*

In light of the above observations, we then asked whether the greater inter-hemispheric differences in slow wave incidence found in CP could be better explained by a more disproportionate slow wave generation across brain hemispheres, or simply by the lack of cross-hemispheric propagation. To this aim, we first determined the overall proportion of slow waves with a clear origin in the left or in the right hemisphere with respect to the total number of detected slow waves (Figure 12). Consistent with the above-reported results, we found that a greater percentage of waves originated in the right hemisphere in both HS (paired t-test, $P = 0.009$, $|t_{23}| = 2.8471$, bCIs = [-4.11, -0.85]; left = $39.95 \pm 2.99\%$, right = $42.45 \pm 2.47\%$) and CP (paired t-test, $P = 0.03$, $|t_4| = 3.4595$, bCIs = [-17.64, -5.58]; left = $37.33 \pm 4.04\%$, right = $49.03 \pm 3.59\%$). However, the relative and the absolute inter-hemispheric asymmetry in origin density did not show systematic differences across the two groups (Table 3). In fact, for both parameters, only three of the five CP displayed a statistically significant difference with respect to HS. Moreover, a significant difference was also found in one NP, thus indicating that differences found in the three CP were not specific.

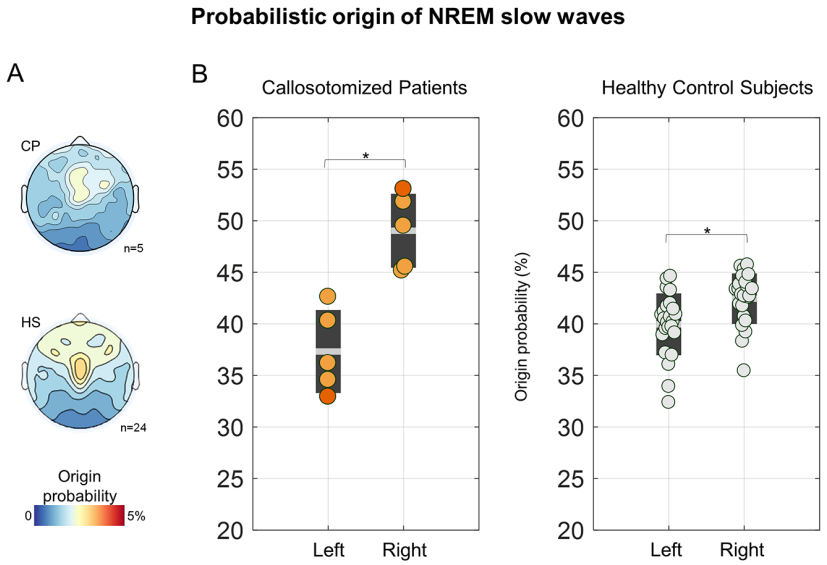

Figure 12. Differences in probabilistic origin across the left and right hemisphere. ***A)*** *Topographic map of probabilistic slow wave origin in CP (top) and HS (bottom). Similar distributions, with maxima in central-lateral and anterior areas were found in both CP and HS.* ***B)*** *A higher proportion of slow waves originated in the right vs. left hemisphere in both CP and HS. CP are represented with orange dots (CP05 = dark orange), NP with purple dots (NP03 = light purple) and HS with light-gray dots.*

2.4 Discussion

The slow waves of NREM-sleep have been shown to spread across brain areas in scalp hd-EEG recordings of healthy human individuals (Massimini et al., 2004; Murphy et al., 2009). While this macro-scale cortical traveling has been thought to be mediated by cortico-cortical white matter connections, to date only indirect, correlational evidence has supported this assumption (Buchmann et al., 2011a; Piantoni et al., 2013). Furthermore, findings reported in the literature are contradictory, likely because of methodological discrepancies and limitations. In the present study we show that a complete resection of the corpus callosum (CC), which contains the main bundle of inter-hemispheric white matter fibers, is associated with an increased incidence of uni-hemispheric slow waves, reflecting a decrease in cross-hemispheric propagation. Interestingly, our results also demonstrate that slow waves originate more often in the right relative to the left hemisphere and that this asymmetry is not significantly affected by the resection of the CC.

2.4.1 The corpus callosum is essential for the cross-hemispheric propagation of sleep slow waves

Here we demonstrate that the cross-hemispheric propagation of NREM slow waves largely depends on the integrity of callosal white matter tracts. Indeed, while in healthy adult subjects more than the 60% of all slow waves showed a clear cross-hemispheric propagation, in callosotomized patients more than the 60% of them remained confined within the cerebral hemisphere in which they originated. These results are in line with previous correlational evidence indicating a direct relationship between parameters reflecting slow wave synchronization and the (micro)structure of the anterior CC (Buchmann et al., 2011a; Piantoni et al., 2013); but see also Sanchez *et al.*, 2019). More in general, they provide support to the hypothesized relationship between patterns of slow wave propagation and structural cortico-cortical connectivity (Murphy et al., 2009; Kurth et al., 2017; Schoch et al., 2018). In this respect, our findings may appear in contrast with recent work showing a positive correlation

between indices reflecting white matter damage and slow wave synchronization efficiency in TBI patients (Sanchez et al., 2019). These discrepancies may in part be explained by the different methodological approach, as the indices investigated in the previous work (slow wave amplitude/slope) only indirectly reflect slow wave synchronization/propagation across brain areas. Another possibility is that the impact of white matter integrity may change as a function of lesion extension and involved pathways. Indeed, a damage-related "disconnection" in TBI patients may enhance the cortical propensity to locally generate and synchronize slow-wave-like events, as shown in animal models of cortical deafferentation (Timofeev et al., 2000, 2013; Topolnik et al., 2003). In fact, the neuronal bistable state typically observed during sleep has been suggested to represent a 'default' state of isolated neocortical modules (Sanchez-Vives and McCormick, 2000; Lemieux et al., 2014; Capone et al., 2019), though the thalamus and other subcortical structures seem to provide a significant contribution to shaping and synchronizing slow cortical oscillations in physiological sleep (Neske, 2016; Gent et al., 2018; Vantomme et al., 2019). In this light, extensive white matter lesions, such as those observed in TBI patients, may favor the transition of many cortical neurons into a bistable state, thus determining a paradoxical increase in slow wave generation and local synchronization. The slow-wave cortical traveling has been suggested to have a direct role in organizing information processing and plasticity in cortical networks through the local modulation of spindles and high-frequency activity (Cox et al., 2014). Thus, the alteration of cross-hemispheric propagation may lead to an alteration of plasticity-related processes requiring the interaction and coordination of activity across the two brain hemispheres. It should be noted, however, that the resection of the CC did not completely abolish cross-hemispheric slow wave propagation. While this residual bilateral cortical involvement may in part represent a spurious consequence of volume conduction, our results suggest that this issue alone is not sufficient to explain the full magnitude of the phenomenon. In fact, the proportion of perfectly synchronous (zero-lag) bilateral slow waves was not significantly affected by callosotomy. Instead, we observed a longer inter-hemispheric delay between slow-wave negative peaks in callosotomized patients, which is consistent

with the involvement of polysynaptic propagation pathways possibly including cortico-subcortico-cortical loops (e.g., (Timofeev and Steriade, 1996)). Additional mechanisms underlying residual bilateral slow waves in callosotomized patients may include direct subcortico-cortical recruitment processes (Siclari et al., 2014; Bernardi et al., 2018) and/or the involvement of anterior and posterior commissures (Mancuso et al., 2019), which were relatively spared in all the CP individuals. Interestingly, a similar change in inter-hemispheric delay was not observed for the positive peak occurring at the transition into the up-state. This observation is consistent with a role of subcortical structures in synchronizing up-state transitions across cortical neuronal populations (Lemieux et al., 2014; Neske, 2016).

Of note, while our analyses focused on the slow waves during NREM sleep, previous evidence showed that slow-wave-like activity may also occur during REM sleep (Baird et al., 2018; Bernardi et al., 2019b). Taking this observation into account, it could be interesting to study the propagation and synchronization pattern of sleep slow waves also during other sleep stages. In fact, a seminal TMS study in healthy individuals found that while during NREM sleep the functional inter-hemispheric connectivity was similar to what observed during wakefulness, transcallosal inhibition was reduced after awakening from REM sleep in the last part of the night (Bertini et al., 2004), thus suggesting that inter-hemispheric functional connectivity may undergo important changes across distinct sleep stages.

2.4.2 The resection of the corpus callosum is not sufficient for the manifestation of uni-hemispheric sleep

Our study showed that, during NREM-sleep, large slow waves are often asymmetrically distributed across the two hemispheres in healthy adult individuals. Interestingly, the absolute degree of inter-hemispheric asymmetry is significantly increased in callosotomized patients. Based on the standard definition of sleep stages, this particular condition could lead to apparent differences in sleep depth across the two brain hemispheres. Such an asymmetry could be explained either by a change in the number of slow waves originated in the two hemispheres, or simply by the loss of cross-hemispheric propagation after callosotomy.

However, since the cortical distribution of slow wave origins was not systematically and significantly affected by the resection of the CC, the observed asymmetry could be entirely explained by the reduced cross-hemispheric slow wave propagation. This observation is in line with previous evidence suggesting that the lack or resection of inter-hemispheric connections is not sufficient for the manifestation of uni-hemispheric sleep (Berlucchi, 1966; Montplaisir et al., 1990; Nielsen et al., 1993), as naturally seen in some animal species, such as birds and cetaceans (Rattenborg et al., 2000; Mascetti, 2016). On the other hand, the absence (as in birds) or small size (as in cetaceans) (Tarpley and Ridgway, 1994) of the CC may prevent the cross-hemispheric spreading of sleep slow waves, and thus represent one fundamental prerequisite for uni-hemispheric sleep.

2.4.3 Slow waves originate more often in the right than in the left hemisphere

Present results revealed that during NREM-sleep, slow waves tend to originate more often in the right than in the left hemisphere in healthy adult subjects as well as in callosotomized patients, although the relative hemispheric predominance also varies from epoch to epoch. A similar inter-hemispheric difference in SWA during NREM-sleep has been reported in some previous investigations (e.g., Goldstein *et al.*, 1972; Sekimoto *et al.*, 2000, 2007). In addition, an EEG study investigating the inter-hemispheric flow of information found that, in healthy individuals, delta activity during NREM sleep of the first sleep cycle shows a preferential right to left inter-hemispheric direction (Bertini et al., 2007). Interestingly, our observation of a similar lateralization in patients who underwent callosal resection implies that such slow wave lateralization does not depend on competitive regulatory mechanisms acting across the two hemispheres. Why then does the right hemisphere generate more slow waves than the left one during NREM-sleep? In light of the homeostatic mechanisms that regulate SWA (Borbely and Achermann, 1999) and of the known differences in hemispheric functional specialization (Karolis et al., 2019), the right hemisphere may develop a stronger function- and use-dependent "sleep need" during wakefulness that translates into higher slow

wave activity during subsequent sleep. However, this possibility is at odds with previous findings indicating a stronger rebound in SWA within the left hemisphere following extended wakefulness, relative to baseline sleep conditions (Achermann et al., 2001; Ferrara et al., 2002; Vyazovskiy et al., 2002). Of note, a recent work showed that the first night of sleep in a new environment may be associated with an increased sleep-depth asymmetry, with the left hemisphere operating as a "night watch" (Tamaki et al., 2016). This observation raises the interesting possibility of a constitutional difference in the arousal-related, bottom-up control of sleep in the two hemispheres. One could speculate that a "deeper sleep" of the right hemisphere, highly involved in attentional control, may enable a relative disengagement from environmental stimuli (Bareham et al., 2014), while a "more awake" left hemisphere could facilitate the recognition of potentially relevant communicative stimuli that are especially important in social animals (Legendre et al., 2019). More specific studies will be required to directly put these hypotheses into test.

2.4.4 Limitations

The main limitation of this study is the relatively small sample size. However, it should be emphasized that patients who underwent complete callosotomy represent an exceptionally rare population (Fabri et al., 2017). Furthermore, to overcome potential limitations related to the sample size, we performed evaluations at single subject level and applied strict criteria for the definition of "significant" group differences. Another potential limitation is that all the epileptic patients (CP01-CP05 and NP03) presented alterations in the background EEG activity caused by the underlying pathological condition. All the signals have been carefully inspected to discard segments containing non-physiological activity. Though, it is still possible for some slow-wave-like epileptic events to have been included in our analyses. In addition, according to the sleep scoring (Table 1), some of the patients presented a quite perturbed sleep (mainly CP05 and NP03), indicative of a poor sleep quality. While quality of sleep could represent a confounding effect for the interpretation of our results this is unlikely given that non-callosotomized patients, including the epileptic subject (NP03), who also had a relatively

disturbed sleep, exhibited patterns of slow-wave propagation/synchronization similar to those of the healthy control group. The use of medications, including anti-epileptic and hypnotic drugs (Table 2), may also represent a possible confounding factor. However, the medications used among callosotomized patients and the epileptic non-callosotomized patient belong to similar classes of drugs (i.e., benzodiazepines, anticonvulsants, atypical antipsychotics) and their effects on sleep architecture are mainly known to involve sleep latency and REM sleep, rather than slow wave activity and NREM sleep. Although we cannot completely exclude an influence of medications and of other mentioned confounding factors on our analyses, we note that all results were consistent across a heterogeneous sample of callosotomized patients with distinct underlying conditions, comorbidities and pharmacological therapies. Moreover, the non-callosotomized patients, including an epileptic subject, who were studied under similar conditions did not show the same pattern of slow wave differences observed in callosotomized patients as compared to the healthy adult control group. Finally, given that all patients in our sample and most of the healthy control subjects were right-handed, an investigation of the role of handedness in modulating slow wave propagation or lateralization was not possible. Future studies will be necessary to shed light on this issue.

2.5 Conclusions

This study systematically investigated the origin, distribution and traveling of sleep slow waves in complete split-brain patients. To the best of our knowledge, our results are the first demonstration that the resection of inter-hemispheric connections significantly limits the cross-hemispheric propagation of sleep slow waves without affecting the relative distribution of slow wave origins across the two hemispheres. These findings also provide further support to previous assumptions regarding the dependence of slow waves on cortico-cortical connections for their macro-scale spreading. In light of previous evidence indicating that slow waves may modulate, throughout their propagation, spindle and high-frequency activity potentially related to plastic processes, our results indicate that callosotomy may significantly affect these sleep-dependent mechanisms. On a different perspective, our findings also demonstrate that the loss of inter-hemispheric connections in adult life is not sufficient, *per-se*, to allow the appearance of uni-hemispheric sleep in humans, thus implying that in animals showing this particular behavioral state additional functional and/or anatomical mechanisms may play a pivotal role.

Chapter 3

Two main topographic patterns characterize morning-to-evening increases in low-frequency brain activity[2]

3.1 Introduction

Sleep and wakefulness are two interdependent physiological states that correspond to well-distinct global modes of brain activity. Their alternation is regulated by the circadian (C) and the homeostatic (S) processes (Borbély, 1982). While the circadian rhythm is synchronized with the 24h light/dark cycle, which is relatively constant under normal conditions, the homeostatic process reflects the continuous build-up of sleep pressure with time spent awake. Such an increase is counteracted by sleep, which is associated with a gradual decrease of sleep pressure throughout the night (Borbély, 1982). Interestingly, specific electroencephalographic (EEG) indices have been suggested to reliably reflect the homeostatic build-up of sleep pressure and its overnight dissipation. Indeed, EEG low-frequency activity (delta-theta, ≤8 Hz) increases progressively during extended wakefulness, in parallel with changes in subjective and objective markers of sleepiness (Cajochen et al., 1995; Finelli et al., 2000; Hung et al., 2013). In turn, the variation in low-frequency activity has been found to correlate with the overall level of slow wave activity (SWA, 1-4 Hz) during the first cycle of NREM sleep

[2] Avvenuti G*, Grollero D*, Betta M, Riedner BA, Tononi G, Ricciardi E, Pietrini P, Bernardi G. Two main topographic patterns characterize morning-to-evening increases in low-frequency brain activity (*Unpublished*). * *denotes equal contribution.*

(Cajochen et al., 1995, 1999; Achermann and Borbély, 2003; Borbély et al., 2016). The subsequent decrease in sleep pressure throughout the night is paralleled by a decrease in SWA, and also leads to reduced levels of low-frequency activity during wakefulness the following morning.

Evidence obtained in animal models indicates that the relative increase in low-frequency activity observed during extended wakefulness reflects an increasing occurrence of periods of neuronal silence (*off-periods*) similar to those underlying the generation of the typical slow waves of NREM sleep (Vyazovskiy et al., 2011). Indeed, in their seminal study, Vyazovskiy and colleagues demonstrated in rats that, during prolonged wakefulness, small populations of cortical neurons may often show brief, locally synchronized *off-periods* that are associated with the appearance of spatially circumscribed delta-theta waves in the surface EEG even though the global EEG features remain those of wakefulness. Importantly, these slow-wave-like episodes have been found to become more frequent and to involve larger portions of the cortex with time spent awake. In addition, the same authors showed that, when occurring in brain areas relevant for behavior, local *off-periods* may lead to performance errors in specific tasks. Based on this evidence the local sleep-like events observed in wakefulness may represent a signature of neuronal fatigue and sleep need (Andrillon et al., 2019; D'Ambrosio et al., 2019). Indeed, they have been suggested to directly reflect neuronal stress caused by extended activation during wakefulness, which may lead to changes in synaptic strength and to an accumulation of metabolic wastes (Vyazovskiy and Harris, 2013).

Increases in low-frequency activity observed in humans during extended wakefulness have been suggested to represent an equivalent of local sleep-like episodes described in rats (Hung et al., 2013; Bernardi et al., 2015). Indeed, changes in low-frequency activity and the occurrence of delta-theta waves involving task-related brain areas have been linked to behavioral impairments and may thus explain the typical performance alterations observed after sleep deprivation or restriction (Gorgoni et al., 2014; Bernardi et al., 2015; Nir et al., 2017; Quercia et al., 2018; Petit et al., 2019). Importantly, all human investigations also invariably

indicated that the wake-dependent rate of increase in low-frequency activity is not uniform across the human cortical mantle. In fact, such an increase has a greater magnitude in frontal regions relative to other brain areas (Finelli et al., 2000; Strijkstra et al., 2003). At least two distinct explanations have been suggested for this observation. On the one hand, functional and structural properties of frontal areas may be associated with higher release rates of factors reflecting cellular stress and sleep need (Kilduff et al., 2011; Vyazovskiy and Harris, 2013). In this perspective, the frontal areas would be constitutionally more vulnerable to functional fatigue. On the other hand, in line with the homeostatic principle of sleep regulation, regional differences may reflect a different degree of 'use' (activation), and thus a different rate of accumulation of sleep pressure. This view is in line with evidence indicating that extended practice with specific tasks leads to a local slowing-down of brain activity within task-related areas and to a similar increase in SWA during subsequent sleep (Ferrara and De Gennaro, 2011; Hung et al., 2013; Bernardi et al., 2015; Quercia et al., 2018; Petit et al., 2019). Therefore, the frontal cortex could show a faster build-up of functional fatigue simply because it is more often and/or more intensively activated during wakefulness due to its involvement in a wide range of cognitive functions (Jurado and Rosselli, 2007; Banich, 2009; Diamond, 2013; Fedorenko et al., 2013).

Based on the first (constitutional) view, the topographic distribution of increases in low-frequency activity should be relatively constant from day to day, with a stable peak in frontal areas. Vice-versa, the second (experience-dependent) view, suggests that low-frequency activity changes may vary greatly from one day to the other, as a function of performed activities. In light of these considerations, here we used a single-subject repeated-sampling design to investigate whether daily variations (i.e., morning-to-evening) in low-frequency activity remain topographically stable or rather display relevant inter-session variability.

3.2 Materials and methods

3.2.1 Participant

A single adult individual (age = 37 years, male, right-handed, Italian) participated in 19 standardized electroencephalographic and behavioral assessments between May 2019 and February 2020. The subject presented no clinical, neurological or psychiatric conditions potentially affecting brain function and behavior. Questionnaires regarding sleep habits and quality (e.g., Epworth Sleepiness Scale; Pittsburgh Sleep Quality Index; Horne-Östberg Morningness-Eveningness Questionnaire) were administered at the beginning of the experimental procedure and showed values within the physiological range. For the whole duration of the experimental assessment, sleep-wake cycles were continuously recorded using a wrist-worn actimeter (MotionWatch 8, CamTech). The study was conducted under a protocol approved by the local ethical committee. The subject was provided with a detailed description of the experimental procedures and was required to sign a written informed consent.

3.2.2 Experimental procedure

The subject completed on average two experimental visits per month on weekdays selected based on his work schedules and on the availability of the laboratory spaces. During each visit the subject underwent two hd-EEG recordings, one in the morning (from 9:00 AM to 11:00 AM) and one in the afternoon (from 4:00 PM to 6:00 PM), for a total of 38 EEG sessions. During each session, the hd-EEG activity was recorded both at rest (eyes-open) and during a computerized Psychomotor Vigilance Task (PVT), adapted from previous studies (Hung et al., 2013; Bernardi et al., 2015). During the 10-minutes PVT, which was used to obtain measures of alertness and vigilance (Lim and Dinges, 2010), the subject was instructed to respond as fast as possible when the "GO" word appeared on the screen, by pressing a button of the keyboard. In this task, a total of 81 "GO" stimuli were presented at randomized intervals of variable duration, ranging from 2000 ms to 10000 ms. A feedback about the reaction time (RT) was provided. After the exclusion of RTs longer than 500ms,

considered as attentional lapses, the mean RT was computed for each session. Of note, the mean RT was preferred over other measures such as the number of lapses or the standard deviation of the reaction time for two main reasons. First the present study was not performed during extended wakefulness, and we thus expected very few or no attentional lapses. Second, the mean reaction time has a more direct interpretation with respect to the standard deviation of reaction time, which is used as an index of non-well-defined "system instability". During the experimental sessions, the subject was seated on a comfortable chair in a soundproofed room.

3.2.3 Resting-state EEG data acquisition and preprocessing

High-density (hd)-EEG data were recorded with a 256-channel EEG (EGI, Eugene, OR, USA) using a sampling frequency of 500 Hz. For each experimental session (morning/evening), three 2-minutes recordings of spontaneous eyes-open EEG were acquired (6-min in total). The subject was instructed to sit quietly in front of a screen and fix a white cross on a gray background in order to minimize eye movements. Recordings were off-line band-pass filtered between 0.5 and 45 Hz (FIR filter), and a 50 Hz notch filter was applied. Each resting-state recording was divided into non-overlapping 4-sec epochs and visually inspected to identify and reject channels and epochs containing artifacts using NetStation 5.3 (EGI). Then, and Independent Component Analysis (ICA) procedure was performed in EEGLAB in order to reduce residual ocular, muscular and electrocardiograph artifacts (Delorme and Makeig, 2004). Finally, rejected bad channels were interpolated using spherical splines.

3.2.4 Power computation and hierarchical clustering analysis

After preprocessing, all EEG traces were re-referenced to average reference. For each experimental session, power spectral density (PSD) estimates were computed using the Welch's method for each 4-s data epoch (Hamming windows, 8 sections, 50% overlap; 0.25 Hz resolution). The signal power was first computed in 1 Hz-bins between 1 and 45 Hz, averaged across the 191 most "internal" electrodes, and compared across morning and

evening sessions. Of note, internal electrodes were specifically selected in order to minimize the impact of potential residual artifactual activity in channels close to temporal and neck muscles or to the eyes. This analysis was applied to confirm the occurrence of systematic morning-to-evening variations in low-frequency activity. As detailed in the Results section, based on this analysis the theta (4-8 Hz), sigma (12-15Hz) and beta (19-25 Hz) frequency bands were computed and further evaluated. However, given our specific focus on low-frequency activity only the theta band was investigated in subsequent analyses. In particular, relative topographic morning-to-evening percent variations in theta activity (for internal electrodes only) were entered in a hierarchical clustering analysis (HCA) in order to determine whether patterns of increase were stable or instead varied across experimental sessions. Specifically, the HCA was performed using the correlation distance metric and the ward method (inner squared distance) for computing the relative distance between clusters. The number of clusters was determined by thresholding the similarity trees at the level of the highest increase of intracluster distance, as described in previous work (Boly et al., 2012).

3.2.5 Source modeling of EEG data

The signals of pre-processed resting-state EEG epochs were source modeled using *Brainstorm* (Tadel et al., 2011). Specifically, the conductive head volume was modeled using a three layers symmetric boundary element method (OpenMEEG BEM; Kybic et al., 2005; Gramfort et al., 2010) and the default ICBM152 anatomical template. A standard set of electrode positions (GSN HydroCel 256) was used to construct the forward model. The source space was constrained to the cerebral cortex, which was modeled as a three-dimensional grid of 15,002 vertices. The inverse matrix was computed using the standardized low-resolution brain electromagnetic tomography (sLORETA) constraint with a regularization parameter equal to 10^{-2} λ. Finally, the signal power was calculated for each vertex in source space using the Welch's method (2-s Hamming windows, 50% overlap). The signal power was computed in the theta (4-8 Hz) frequency-band. Power maps in source space were then exported to

MATLAB for planned statistical comparisons and obtained results were re-imported in *Brainstorm* for visualization.

3.2.6 Statistics

Comparisons of EEG signal power across experimental sessions (morning *versus* evening) were performed using paired t-tests and a supra-threshold cluster correction for multiple comparisons, as described in previous work (Nichols and Holmes, 2002; Huber et al., 2006). In brief, the same statistical contrast was repeated (nPerm = 5,000) after shuffling the labels of experimental conditions (or the values of the covariate, for correlation analyses) and the maximum size of significant electrode-clusters was saved in a frequency table. Unless differently specified, the cluster-forming threshold was set to $p < 0.05$. A minimum cluster-size threshold corresponding to the 95th percentile of the resulting distribution was eventually applied to correct for multiple comparisons. Correlations between behavioral performance and EEG data were computed using the Spearman's correlation coefficient. All statistical analyses were performed in MATLAB.

3.3 Results

3.3.1 Morning-to-evening changes in global signal power

Firstly, we evaluated global, overall changes in signal power from morning to evening and across experimental sessions (Figure 13). Significant morning-to-evening increases were observed in the EEG spectral power density in the theta (4-8 Hz), sigma (12-15 Hz) and beta (19-25 Hz) frequency-bands ($p < 0.05$, Bonferroni correction).

The topographic analysis revealed diffused increases in theta, sigma and beta activity, which especially involved frontal, temporal, occipital and right-parietal electrodes ($p < 0.05$, cluster corrected; $\alpha < 0.005$ cluster-forming threshold; Figure 14). Of note, theta and beta changes showed two peaks of increase, over frontal and right-parietal channels, while sigma changes were less strong and peaked over right-parietal electrodes. These findings are in line with previous evidence indicating that variations in low-

frequency activity during extended wakefulness are accompanied by parallel variations in beta activity (Strijkstra et al., 2003; Hung et al., 2013).

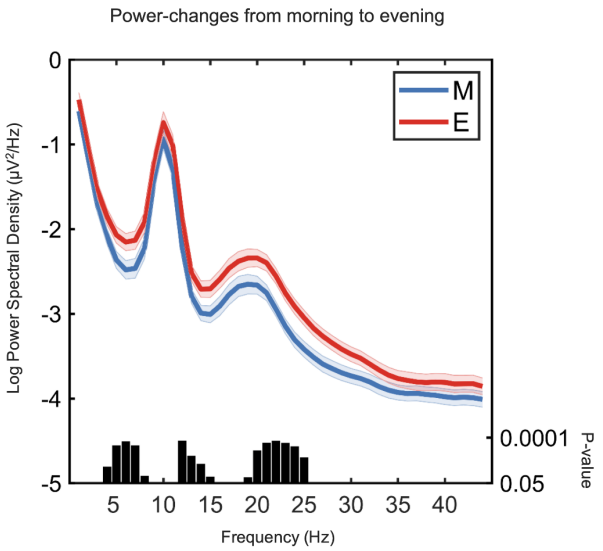

Figure 13. Mean change in EEG power spectral density (1 Hz bins) from morning (blue) to evening (red) across all (inner) electrodes. Significant changes were found in theta (4-8Hz), sigma (12-15Hz) and beta (19-25Hz) frequency ranges. Black bars mark P<0.05, Bonferroni correction. M = morning; E = evening.

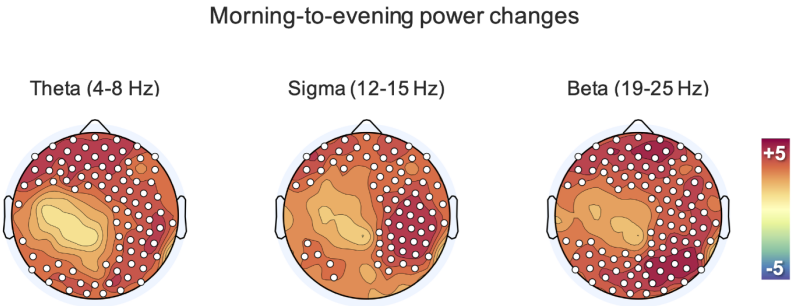

Figure 14. Significant differences in theta, sigma and beta power between morning and evening. White dots mark electrodes presenting a significant difference at P<0.05, cluster corrected (cluster-forming threshold $\alpha < 0.005$).

3.3.2 Clustering of topographic theta activity increases

In order to investigate whether theta-power changes across sessions showed a consistent topographic pattern, we performed a hierarchical clustering analysis (HCA). Two main morning-to-evening spatial patterns of theta power changes were observed (Figure 15A and 15B). The first pattern (n = 11 sessions; pattern#1) showed a predominant increase in centro-frontal areas (Figure 16A, blue color). The second topographic pattern (n = 8 sessions; pattern#2) was characterized by a predominant theta-power variation within occipital, parietal and temporal areas (Figure 16A, red color).

Of note, we found that relative to pattern#2, pattern#1 presented a significantly stronger increase in theta power over frontal electrodes ($p < 0.05$, cluster correction; Figure 16B). By contrast, pattern#2 showed relatively stronger increases in theta power over parietal electrodes, although this effect did not survive after correction for multiple comparisons ($p < 0.05$, uncorrected). Importantly, in order to exclude potential differences at baseline (i.e., in the morning), we compared the morning theta activity of the two patterns. No significant differences were found between pattern#1 and pattern#2 ($p < 0.05$, corrected, Figure 17).

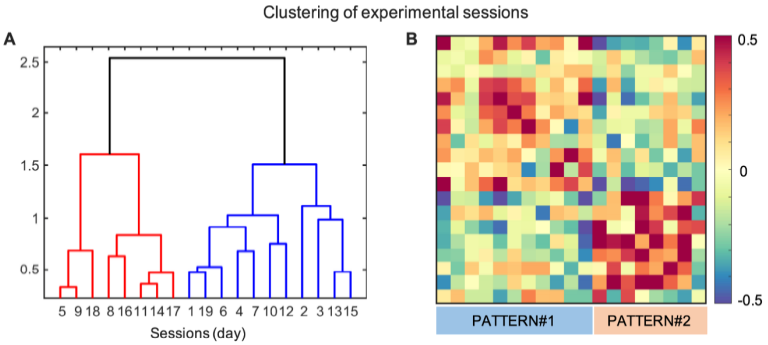

Figure 15. Clustering of experimental sessions based on morning-to-evening variations in theta power (percent variation with respect to morning activity). (A) We found two clusters, one including 11 sessions (pattern#1; blue color) and the other including 8 sessions (pattern#2; red color). (B) Across-sessions topographic-consistency matrix obtained by computing, for each pair of sessions, the Pearson's correlation coefficient across electrodes. The diagonal shows the topographic correlation of each session with the mean topographic distribution across all sessions.

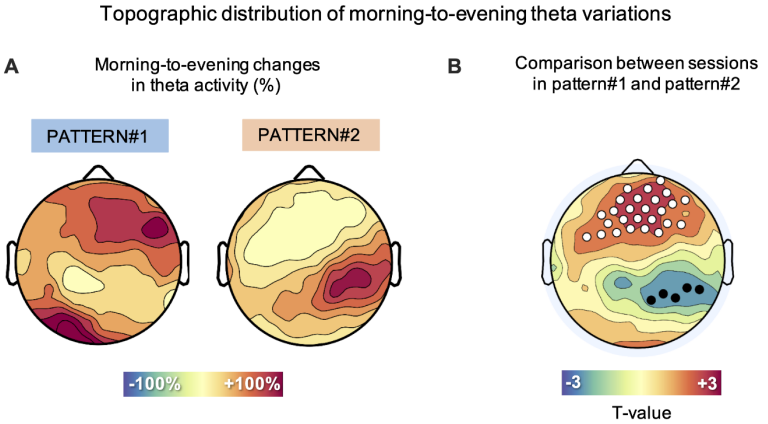

Figure 16. Topographic distribution of morning-to-evening theta variations. (A) Mean percent morning-to-evening change in theta activity for sessions in pattern#1 (N=11) and sessions in pattern#2 (N=8). (B) Comparison between sessions of pattern#1 (red) and sessions of pattern#2 (blue). Pattern#1 was associated with a stronger increase in frontal theta activity relative to pattern#2 ($p < 0.05$, cluster correction), which instead tended to present a stronger increase in parietal theta activity ($p < 0.05$, uncorrected). White dots mark $p < 0.05$, cluster-based correction; black dots mark $p < 0.05$ uncorrected.

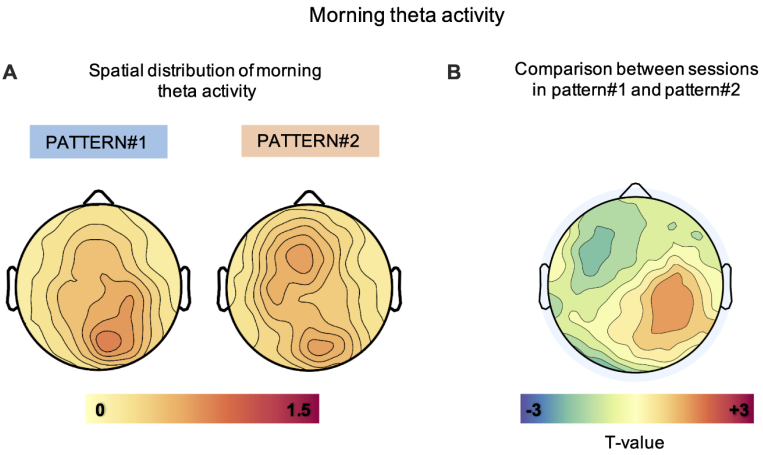

Figure 17. Between-sessions comparison of morning theta activity (baseline). (A) Spatial distribution of morning theta activity in sessions of pattern#1 and sessions of pattern#2. (B) No differences were found between pattern#1 and pattern#2 ($p < 0.05$, corrected). Of note, at $P < 0.05$ uncorrected only two electrodes located in the left temporo-occipital region showed a significant effect (data not shown).

3.3.3 Source modeling analysis of theta power variations

In order to identify the actual sources of morning-to-evening variations in theta activity, the same analysis shown in Figure 16 were repeated after source reconstruction of the EEG signals (sLORETA). Pattern#1 was characterized by theta power increases that peaked in centro-frontal areas, including somatomotor and premotor cortices (Figure 18A). Instead, pattern#2 presented theta-power variations that peaked in occipital, somatomotor and temporal areas (Figure 18B). Mean morning-to-evening variations in theta activity computed across all experimental sessions are represented in Figure 18C. This image clearly shows that, on average, the centro-frontal variation was the strongest and most evident, in line with previous investigations and with results shown in Figure 14.

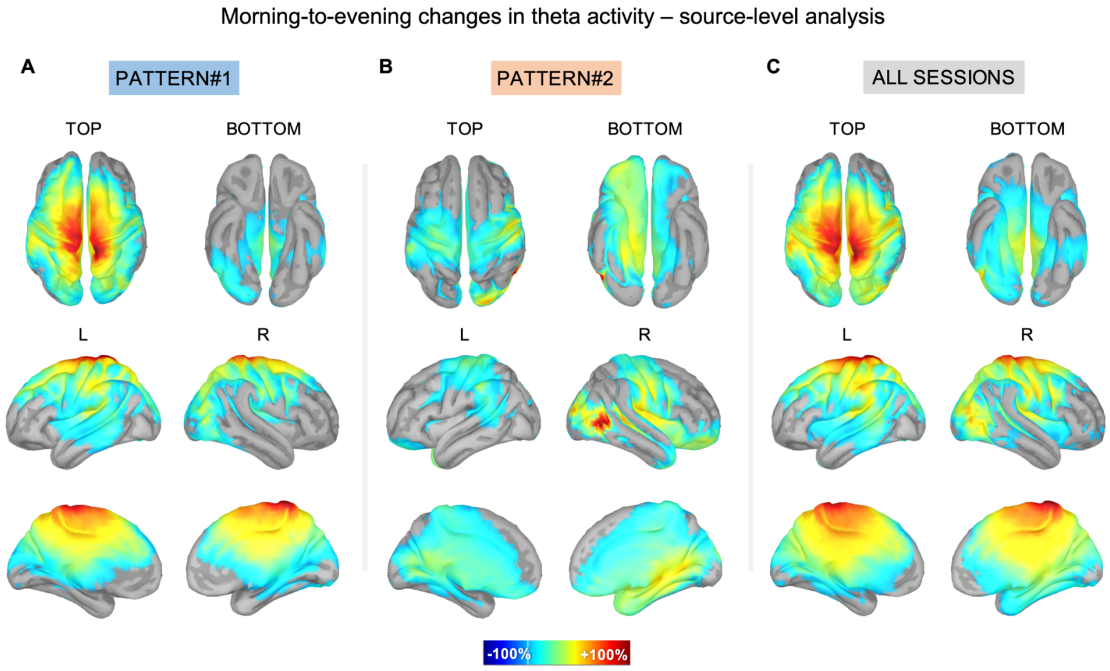

Figure 18. Source localization of morning-to-evening variations in theta activity. Pattern#1 was characterized by a theta increase in central-frontal areas, including somatomotor and premotor cortices ***(A)****. Pattern#2 was characterized by a theta increase in sensory (occipital, somatomotor, temporal) and in orbitofrontal cortical areas* ***(B)****. Average regional theta-activity variations across all sessions are represented in panel* ***(C)****.*

3.3.4 Correlation between PVT reaction time and theta power increases

Next, we evaluated whether changes in theta activity were correlated with variations in objective vigilance, as measured based on reaction time in the PVT task. Parallel to theta power changes, an increase in PVT mean reaction time was observed from morning to evening ($t18 = 7.598$, $p < 0.001$; paired t-test). However, only a weak correlation was found between morning-to-evening variations in PVT mean reaction time and changes in EEG theta power ($r>0.47$; $p<0.05$, uncorrected). As show in Figure 19, the correlation was found in parietal electrodes, while frontal theta changes showed a non-significant negative correlation with variations in PVT reaction time. Then, we investigated whether the two previously identified theta-change topographic patterns presented different correlations with variations in PVT reaction time. In line with above observations, pattern#2 was associated with a greater morning-to-evening slowing down in PVT reaction time with respect to pattern#1 ($+11.2 \pm 5.4\%$ vs. $+7.2 \pm 4.3\%$; $p < 0.1$, unpaired t-test; Figure 20A). On the other hand, pattern#1 tended to be associated with longer sleep time in the night following the experimental session, relative to pattern#2 (337.3 ± 16.7 min vs. 321.6 ± 20.0 min; $p < 0.1$, unpaired t-test; Figure 20B).

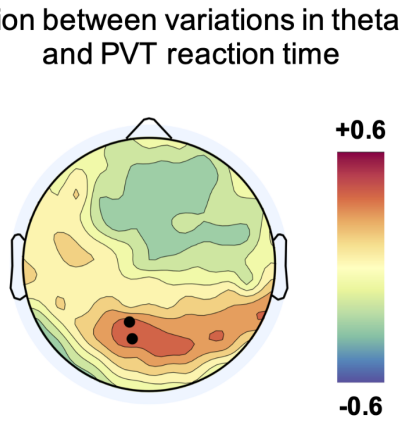

Figure 19. Spearman correlation between morning-to-evening variations in theta activity and morning to evening variations in PVT reaction time. Black dots mark significant effects at p < 0.05 uncorrected.

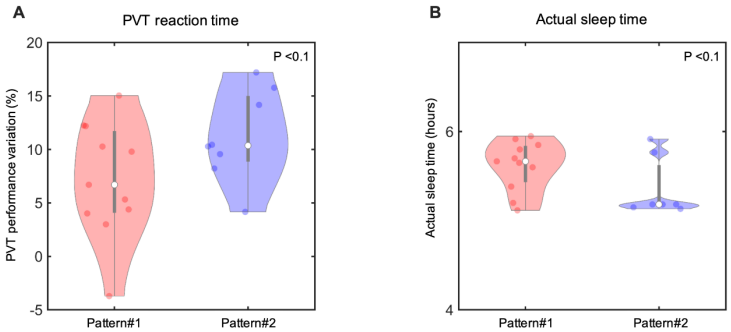

Figure 20.Spearman correlations between theta variations and behavioral measures. ***(A)*** *Variations in PVT reaction time in sessions of pattern#1 (red) and sessions of pattern#2 (blue).* ***(B)*** *Total hours of sleep in the night following the experimental session. Of note, sessions in pattern#2 tended to be associated with a greater slowing down in PVT reaction time with respect to sessions in pattern#1 (+11% vs. +7%; P < 0.1; unpaired t-test), while pattern#1 tended to be associated with longer sleep duration relative to pattern#2 (337.3 ± 16.7 min vs. 321.6 ± 20.0 min; p < 0.1, unpaired t-test).*

3.4 Discussion

Low-frequency EEG activity progressively increases during wakefulness and this increase has a greater magnitude in frontal brain areas relative to other brain regions (Finelli et al., 2000; Tinguely et al., 2006; Ferrara and De Gennaro, 2011; Hung et al., 2013; Fattinger et al., 2017b). The specific cause of this inter-regional variability is however unclear. Indeed, the greater slowing-down of frontal brain activity could reflect region-specific intrinsic characteristics, and thus, a greater "constitutional" vulnerability to functional fatigue, or may instead indicate a higher degree of "use" (activation), which in turn determines a correspondingly faster build-up in homeostatic sleep pressure. Through repeated measures in a single individual, here we showed that morning-to-evening increases in low frequency activity actually display two main topographic patterns: one, more common, peaking in centro-frontal areas, and another, less common, peaking in posterior brain regions, including occipital, parietal and temporal cortices. This observation does not support the hypothesis of a constitutionally greater vulnerability of frontal areas to functional fatigue.

3.4.1 Regional vulnerability to local, sleep-like episodes

Previous investigations regarding the effects of extended wakefulness reported a progressive slowing-down of brain activity that is typically more prominent over frontal brain areas (Finelli et al., 2000; Strijkstra et al., 2003; Hung et al., 2013; Bernardi et al., 2015). While most investigations focused on the sleep deprivation period, evidence indicates that clear changes in low-frequency activity already occur within waking periods of normal length, as indicated by analyses of morning-to-evening brain activity variations (Hung et al., 2013; Bernardi et al., 2015; Fattinger et al., 2017b). Consistent with these previous observations, here we found that low-frequency activity strongly increases from morning to evening in most cortical areas, with peak mean variations in centro-frontal regions including motor and premotor areas.

Studies in animal models (Vyazovskiy et al., 2011) and in humans (Nir et al., 2017) suggested that increases in low-frequency activity could ultimately reflect the occurrence of local, slow-wave-like events, which seem in turn to represent a signature of functional fatigue and sleep pressure. In line with this view, the occurrence of sleep-like episodes in task-related brain areas has been shown to negatively affect behavioral performance in a variety of cognitive tasks (Bernardi et al., 2015; Ahlstrom et al., 2017; Fattinger et al., 2017b; Quercia et al., 2018; Petit et al., 2019). In this perspective, the greater increase in low-frequency activity within frontal areas may contribute to explain the common observation of a particular vulnerability to sleep loss of cognitive functions that rely on these particular regions, the so-called executive functions (Goel et al., 2009; Lim and Dinges, 2010; Pilcher et al., 2015; Lowe et al., 2017b; Honn et al., 2018). On the other hand, why frontal areas and related cognitive functions are more vulnerable to functional fatigue and sleep need was largely unclear. Two main hypotheses have been proposed. Based on a first hypothesis, the frontal cortex may present an intrinsically greater vulnerability due to its (micro)structural and neurophysiological characteristics (Kilduff et al., 2011; Vyazovskiy and Harris, 2013). Alternatively, the higher increase of low-frequency activity over frontal regions could reflect a direct consequence of their intensive activity during the day, which determines a faster homeostatic increase in sleep need. This latter alternative is in line with evidence indicating that extended practice with specific tasks leads to a slowing-down of brain activity in task-related areas and to related behavioral errors (Ferrara and De Gennaro, 2011; Hung et al., 2013; Bernardi et al., 2015; Quercia et al., 2018; Petit et al., 2019). Importantly, these two hypotheses lead to different predictions with regard to expected morning-to-evening brain activity changes measured across multiple sessions. Indeed, based on the "constitutional" hypothesis, low-frequency activity should systematically increase more strongly in frontal brain areas relative to other regions, while based on the "use-dependent" hypothesis, patterns of brain activity changes may be expected to vary significantly from day to day.

Through the analysis of data collected across multiple experimental sessions in a single volunteer, here we showed that

morning-to-evening increases in low-frequency activity actually exhibit two main distinct topographic patterns, with peaks over fronto-central and posterior (sensory) brain regions, respectively. Importantly, this result was not explained by different degrees of functional fatigue already present in the first hours after awakening, since morning theta activity was not significantly different across experimental sessions. These findings do not support the assumption of a 'pure' constitutional vulnerability of frontal areas, and rather favor the hypothesis of a use- and experience-dependent regulation of frontal functional fatigue. However, we note that our results do not allow to fully disentangle the possible relative contribution of the two hypothesized mechanisms which may be, at least in part, intertwined. Indeed, the extent and way we 'use' specific cortical regions may also depend on how they are constitutionally wired. In order to definitively clarify the differential contribution of 'constitutional' and 'use-dependent' mechanisms, future studies should apply specific behavioral procedures to experimentally manipulate the use of frontal brain regions.

Interestingly, instead of multiple distinct patterns of low-frequency increase potentially related to different types of wake-dependent activities, we only identified two main patterns. Unfortunately, the subject's activities were not monitored during the days in which the experimental sessions were performed and we have thus been unable to link the two low-frequency increase patterns to specific experiences or tasks. Based on the observed regional distributions, however, it is tempting to speculate that the more common pattern (pattern#1) might reflect a more "active" state, in which the subject directly and actively engaged in one or more 'high-level' cognitive activities, whereas the less common pattern (pattern#2) could indicate a more "passive", receptive state.

Two additional considerations should be made regarding observed morning-to-evening variations in brain activity. First, changes in low-frequency activity were accompanied, with similar topographic distributions, by significant changes in higher frequencies within the sigma-beta ranges. Interestingly, while similar results have been previously reported (e.g.,Finelli et al., 2000; Hung et al., 2013), they received scarce attention, probably

due to their smaller magnitude and statistical significance. One intriguing possibility is that high-frequency increases represent an attempt of the brain to compensate for the increasing occurrence of sleep-like episodes (Strijkstra et al., 2003). However, given the relationship between slow waves and neuronal *bistability*, an alternative, and somewhat opposite explanation may be also proposed: a disruption of the excitation/inhibition balance could lead to more high-frequency activity which could, in turn, increase the number of *OFF-periods*, because of the increase in neuronal *bistability*. In this light, future investigations should evaluate more in detail the temporal and spatial relationship between low- and high-frequency activity changes during wakefulness. Second, we note that the posterior morning-to-evening increase in low-frequency activity (pattern#2) was more evident in the right, relative to the left hemisphere. This asymmetry could reflect specific anatomo-functional characteristics of the study participant. Interestingly, however recent evidence suggested that the right hemisphere may present a greater sleep "intensity", expressed in terms of slow wave activity, relative to the left one (Sekimoto et al., 2000; Tamaki et al., 2016; Avvenuti et al., 2020). In this perspective, the asymmetry observed in the present work could indicate an asymmetry in the build-up of sleep pressure already during the waking period. Along the same line, previous studies showed that EEG rhythms are strongly heritable and stable within a same individual both during sleep (Finelli et al., 2001a; De Gennaro et al., 2005a, 2008; Ambrosius et al., 2008; Gorgoni et al., 2019) and wakefulness (van Beijsterveldt et al., 1996; Smit et al., 2005). In this light, without additional data acquired on different subjects, it is not possible to draw definitive conclusions regarding the generalizability of the two patterns of low-frequency activity increase to other individuals.

3.4.2 Relationship between morning-to-evening theta increase and behavior

Specific analyses were performed to investigate whether the two distinct topographic patterns of theta power increase were associated with different behavioral correlates. As mentioned above, previous work showed that local, sleep-like episodes are

associated with performance errors when occurring in task-relevant brain regions (Vyazovskiy et al., 2011; Hung et al., 2013; Bernardi et al., 2015; Nir et al., 2017; Quercia et al., 2018). Moreover, Andrillon and colleagues (2019) recently proposed that local sleep-like intrusions during wakefulness may also determine different behavioral outcomes in response to a same task (e.g., commission *versus* omission errors), depending on their topographic involvement and timing of occurrence. They also suggested that where and when local sleep-like events occur may predict the different phenomenology of subjective experiences, such as mind-blanking and mind-wandering (Andrillon et al., 2019). In line with this view, here we found that the two topographically distinct morning-to-evening low-frequency power variations are differently associated with behavioral measures. Specifically, pattern#2 (peaking over posterior brain regions), tends to be associated with a greater reduction in vigilance and alertness relative to pattern#1 (peaking over centro-frontal areas), as reflected by the slowing-down in PVT reaction times. This finding is in line with previous work indicating that wake-dependent increases in low-frequency activity within posterior areas lead to performance impairments in tasks requiring vigilance and attention, as is the case for the PVT (Gorgoni et al., 2014; Nir et al., 2017). In fact, such alterations may depend on a failure in deploying attentional resources due to a "bottom-up" impairment caused by local, sleep-like episodes in sensory and parietal areas, rather than to a "top-down" impairment of executive (frontal) functions. On the other hand, pattern#1 tend to be associated with longer sleep duration the night following the experimental session relative to pattern#2. This result is consistent with previous evidence relating frontal low-frequency power to subjective sleepiness and increased sleep need (Finelli et al., 2000; Strijkstra et al., 2003; De Gennaro et al., 2007), as well as with studies indicating a prominent role of frontal areas in the initiation and maintenance of sleep-related oscillations (Werth et al., 1997; De Gennaro et al., 2001b, 2004; Massimini et al., 2004). Indeed, converging lines of evidence indicate that frontal areas begin to show slow waves and spindles well before more posterior regions, suggesting that they may "fall asleep" faster (De Gennaro et al., 2001b, 2005b; Marzano et al., 2013). Moreover, most slow waves seem to have a well-defined

origin in frontal and insular brain regions (Murphy et al., 2009; Nir et al., 2011; Botella-Soler et al., 2012; Timofeev, 2013; Siclari et al., 2014). In this light, a higher sleep pressure of frontal areas could favor a faster transition to sleep and its longer duration.

Taken together, although preliminary, these results suggest that, at least during waking periods of normal length, a dissociation may be observed between objective indices of alertness derived from the PVT and the overall levels of sleep pressure, since these two aspects correspond to distinct topographic patterns of low-frequency activity. Our observations also support the idea that the spatial distribution of local sleep-like events during wakefulness may lead to different behavioral outcomes and/or subjective experiences (Andrillon et al., 2019).

3.4.3 Limitations

The present work has some limitations that should be taken into account. First, empirical evidence provided here rests on a multi-session single-subject design. While this experimental design provides useful data for the investigation of intra-individual variability in ultradian changes in brain activity, it also limits the possibility to generalize findings to other individuals. To overcome this issue, future studies should perform multi-session assessments in more than one subject. Second, the lack of data relative to the daily activities carried out by the participant during the days of the experimental sessions limits any direct inference regarding the relationship between the two observed topographical patterns of local theta variations and the different tasks performed during the day. Future research should address this issue by monitoring daily activities in order to better understand the link between the spatial distribution of low-frequency increases during wakefulness and experience-dependent brain functional changes. Third, analyses investigating the relationship between the different theta-activity increase patterns and behavioral correlates were based on subsamples of experimental sessions and thus suffered from low statistical power. In order to confirm present results, future experiments should include a larger number of experimental sessions.

3.5 Conclusions

Present results integrate into the current research framework on the local regulation of sleep and wakefulness and provide novel insights on the relationship between behavioral and the electrophysiological markers of functional fatigue and sleep need. Specifically, our study provides evidence that the build-up of sleep need during waking periods of normal length is not always necessarily stronger in frontal brain areas, but may rather peak in different brain regions, likely depending on waking activities and experiences. Moreover, our data suggest that two main patterns of low-frequency activity increase could be observed, each associated with distinct behavioral correlates. In fact, the anterior (pattern#1) pattern seems associated with relatively higher sleep

pressure, while the posterior one (pattern#2) is associated with a relative impairment of vigilance and sustained attention. In this respect, our results suggest that previous indications of a direct association between sleep need and alterations of sustained attention could derive from a loss of information due to averaging across subjects or sessions. Indeed, while further studies will be necessary to clarify this issue, the present observations suggest that important information regarding variability in the development of functional fatigue and sleep need may be lost in group-level analyses.

Chapter 4

Local increases in sleep-like activity predict the occurrence of emotion suppression failures[3]

4.1 Introduction

Emotions are an essential aspect of the psychological life of human beings. In fact, they greatly affect our physiological, cognitive and behavioral responses to internal and external stimuli (Keltner and Kring, 1998; Cacioppo et al., 2000; Sapolsky, 2007; Koole, 2009). Importantly, emotions may occasionally lead to inappropriate or exaggerated reactions that could have negative consequences for our social life. Therefore, in dealing with emotions, people frequently engage in covert or overt forms of self-regulation in order to preserve a flexible and goal-oriented behavior (Koole, 2009; Kelley et al., 2015).

In general, self-regulation involves a balance between the strength of an impulse, its related reward, and the individuals' ability to resist to the impulse and to modify their behavior in accordance to relevant personal goals (Carver and Scheier, 1998; Gross and Thompson, 2007). When applied to emotions, self-regulation typically implies adjusting their type, intensity, duration and expression (Gross, 1998). Various schemata, ranging from attention allocation to cognitive reappraisal and expressive suppression (Gross, 1998; Webb et al., 2012) can be used to modify an individual's reaction to emotional states. For instance, when

[3] Avvenuti G, Bertelloni D, Lettieri G, Ricciardi E, Cecchetti L, Pietrini P, Bernardi G. (2020). Emotion suppression failures are associated with local increases in sleep-like activity. *BioRxiv*, 2020.08.04.235978.

applying expressive suppression, a response-based modulation, individuals voluntarily refrain from overtly showing their emotional state, which is kept hidden to an external observer.

Previous work investigated the neural correlates of voluntary emotion suppression by characterizing changes in brain activity during the presentation of emotion-inducing stimuli. The obtained results revealed that successful emotion suppression is associated with the activation of a broad fronto-parieto-insular network, including bilateral supplementary motor area (SMA), preSMA, anterior midcingulate cortex, anterior insula, inferior frontal gyrus, lateral orbitofrontal cortex, posterior middle frontal gyrus, dorsal temporo-parietal junction and the left posterior middle temporal gyrus (Frank et al., 2014; Kohn et al., 2014; Langner et al., 2018). Of note, the expressive suppression strategy has been shown to rely on similar brain substrates for both negative and positive emotional stimuli (e.g., Hajcak and Nieuwenhuis, 2006; Dennis and Hajcak, 2009; Korb et al., 2012; Paul et al., 2013; Morawetz et al., 2017). Yet, what may cause a failure of this emotion-regulation system and the consequent generation of undesired behavioral responses still remains largely unclear.

Interestingly, sleep loss due to restriction or deprivation is known to significantly impair the ability to regulate emotional responses and affective states (Krause et al., 2017; Ben Simon et al., 2020a, 2020b), and these changes have been suggested to depend on an altered top-down control of the medial frontal cortex on limbic structures (Yoo et al., 2007). However, the actual functional cause of this frontal impairment and whether it may also explain emotion regulation failures observed in (apparently) rested wakefulness is unknown.

Importantly, recent evidence indicates that local, temporary intrusions of sleep-like brain activity, may represent a common cause of behavioral errors when involving task-related brain regions (Hung et al., 2013; Bernardi et al., 2015; Nir et al., 2017; Slater et al., 2017; Quercia et al., 2018; Petit et al., 2019). Such local sleep-like episodes reflect spatially and temporally circumscribed neuronal *off-periods* and are associated with the appearance, in the electroencephalographic (EEG) signal, of delta-theta waves (≤8 Hz) similar to those of actual sleep (Vyazovskiy et al., 2011). Given

that they increase in number and extension as a function of time spent awake and that such changes are reverted by a night of sleep, the local sleep-like episodes have been suggested to represent a signature of brain functional 'tiredness', and a direct cause of behavioral impairment following sleep loss (Andrillon et al., 2019; D'Ambrosio et al., 2019). Of note, during wakefulness, the number of sleep-like episodes does not increase homogeneously over the cortical mantle. Instead, frontal areas appear particularly vulnerable to the development of functional fatigue relative to other brain regions (Finelli et al., 2000; Strijkstra et al., 2003).

In light of the above considerations, here we hypothesized that local sleep-like episodes occurring in frontal brain areas could be associated with emotion suppression failures. In particular, we predicted that a shorter sleep time or a reduced sleep quality could lead to a higher incidence of frontal sleep-like episodes the following morning, which would in turn result in higher probability of emotion suppression failures.

4.2 Materials and methods

4.2.1 Subjects

Nineteen healthy adults (age range = 21-31 years, mean ± SD = 26.3 ± 2.9 years, 10 females, all right-handed) were included in the study. All participants underwent a preliminary interview to exclude any clinical, neurological or psychiatric conditions potentially affecting brain function and behavior. Additional exclusion criteria comprised excessive daytime sleepiness (Epworth Sleepiness Scale score >10; Johns, 1991) and extreme chronotypes (Morningness-Eveningness Questionnaire score >70 or <30; Horne and Ostberg, 1976). Participants were asked to maintain a regular sleep-wake schedule for at least one week before each experiment. Compliance was verified by wrist-worn actigraphy (MotionWatch 8, CamTech). The study was conducted under a protocol defined in accordance with the ethical standards of the 2013 Declaration of Helsinki and approved by the Local Ethical Committee. Written informed consent was obtained from all participants.

4.2.2 Experimental procedures

Data analyzed in the present work was collected as part of a larger study aimed at investigating the behavioral consequences of extended task practice (*unpublished*). A general overview of the whole experimental protocol is provided herein.

All participants completed a practice session and two experimental visits in which EEG (EGI, Eugene, OR, USA; 64 electrodes, 500 Hz sampling rate) activity and behavioral data were recorded. In order to minimize inter-individual differences in wake-sleep rhythms and work-related fatigue, all sessions were performed with a pre-defined, fixed schedule. In particular, the practice session was performed on Friday morning from 9:30 AM to 11:30 AM, while the two experimental visits took place on the next Monday and Tuesday, from 8:30 AM to 1:00 PM.

During the practice session, participants completed five 5-min trials of a motor-response inhibition task (Garavan et al., 1999, 2002; Roche et al., 2005; Chuah et al., 2006; Bernardi et al., 2015; 300 stimuli, 10% lures). Data obtained from this procedure was used to calibrate the difficulty of the same task presented during the two subsequent experimental sessions, as described in previous work (Chuah et al., 2006).

The two experimental visits required the participants to complete partially different versions of the same tasks. Each experiment began with a ~15-min long test block (baseline; BL) including two 2-min resting-state EEG recordings with eyes open (4 min in total) and two trials of the response inhibition task. Moreover, subjective vigilance, sleepiness, mood, perceived effort and motivation were assessed using 10-point Likert scales. This initial assessment was followed by three ~45-min long task sessions (TS1-3), each one followed by a ~15-min long test block identical to the first one (Figure 21). In one of the two experimental visits the task sessions included three computerized tasks requiring high levels of impulse control, decision making and conflict resolution, i.e., an emotion suppression task (Baumeister et al., 1998; Dang, 2018), a false response task (Bernardi et al., 2015), and a classical Stroop task (Stroop, 1935). A detailed description of the emotion suppression task, which was the focus of the present study, is provided below. In the other experimental

visit, a modified version of the same tasks was presented, which required no exertion of self-control (e.g., Stroop task with consistent word-ink color). Finally, before the last task block a caffeinated or decaffeinated beverage was provided to participants. The order of the two experimental visits and the administration of caffeinated vs. decaffeinated beverages were randomized across participants. All computerized tasks were implemented using E-Prime 2 (Psychology Software Tools, Pittsburgh, PA).

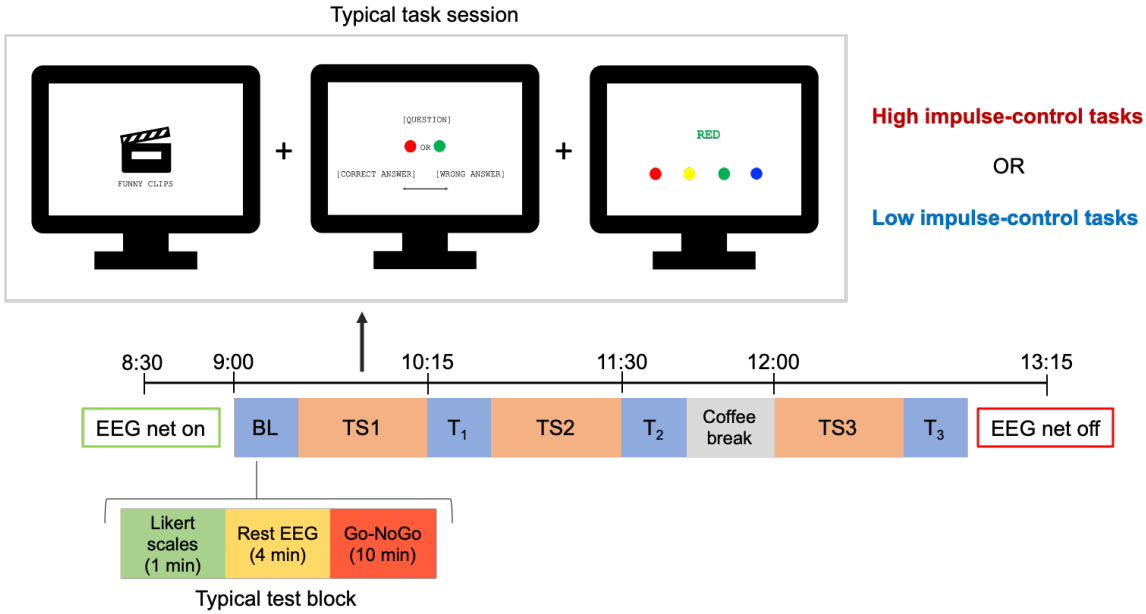

Figure 21. Experimental paradigm. The baseline assessment (BL) and each test block (T_1-T_3) included Likert scales, resting-state EEG recordings and the completion of two trials of a response inhibition task (Go-NoGo). In one of the two experimental visits (high impulse-control condition), the subjects completed an emotion suppression task, a false response task and a Stroop task. During the other experimental visit (low impulse-control condition), the participants completed a simplified version of the same tasks requiring no exertion of self-control.

4.2.3 Emotion suppression/expression task

In one experimental visit, participants completed three instances (one for each task session) of an emotion suppression task in which they were explicitly requested to suppress their facial reactions while watching amusing video-clips ('emotion suppression' condition, ES). In the task sessions of the other experimental visit, volunteers watched similar video-clips, but they were left free to express their emotional responses ('free expression' condition, FE). In both conditions, the participants' faces were video-recorded using a camera positioned above the PC-monitor and synchronized with the software used to record the EEG signals (NetStation 5.3, EGI). Of note, for each task session, the video-clips were presented in two trials in which they were alternated with 4 s of black frames, for an average total duration of ~6 min (5.9 ± 0.3 min).

In order to present different contents to participants during each task session, a total of 261 clips depicting people and/or animals in amusing situations were downloaded from the internet. An initial validation procedure was performed in an independent sample of 12 subjects (age range = 23-36 years, mean ± SD = 29.6 ± 4 years, 8 females) to ensure a balanced distribution of the emotional contents across sessions. Specifically, the volunteers were asked to rate the set of amusing clips and an additional set of 20 clips showing simple actions/activities with no obvious emotional content. The clips were presented in random order. After each video, the subjects answered the question *"How difficult would it be for you to keep a neutral expression while watching this clip?"* using a rating scale ranging from 1 (*"Not difficult at all"*) to 5 (*"Very difficult"*). As expected, the 261 amusing clips were associated in each subject with significantly higher ratings with respect to the 20 neutral clips ($p < 0.001$). For each subject, the ratings given to the amusing video-clips were re-scaled by computing the ratio with respect to the average rating of the 20 neutral clips, which were considered as an individual baseline. Then, the group-average ratios were computed for each clip, and a randomization procedure was used to assign the clips to each task trial, thus ensuring a similar distribution of ratings and a similar total duration of the video stimuli (Table 4).

Table 4. Video ratings. Mean and standard deviation (SD) of video ratings (ratio with respect to mean score of neutral videos) for each task trial, presented during the 'emotion suppression' condition (ES) and the 'free expression' condition (FE). No statistically significant differences were found across experimental conditions.

	ES	**FE**		
	mean ± SD	**mean ± SD**	**z-score**	**p-value**
TS1-1	1.85 ± 0.23	1.90 ± 0.20	-0.393	0.705
TS1-2	1.94 ± 0.29	1.87 ± 0.25	0.608	0.551
TS2-1	1.88 ± 0.27	1.97 ± 0.17	-0.718	0.488
TS2-2	1.88 ± 0.25	1.82 ± 0.28	0.464	0.661
TS3-1	1.92 ± 0.16	1.91 ± 0.24	0.083	0.946
TS3-2	1.88 ± 0.18	1.87 ± 0.26	0.103	0.924

4.2.4 Scoring of video recordings

In order to identify and quantify the occurrence of facial expression changes in each task trial, video recordings of each participant were visually inspected and scored by one of the authors (D.B.). The scoring procedure was performed in two steps. First, the operator watched each video using a custom-made program written in Psychtoolbox v3.0.1 (Kleiner et al., 2007) for MATLAB (v9.7; Natick, Massachusetts: The MathWorks Inc.). Each time a change in facial expression was identified, the scorer pressed a button and the corresponding time-point (in milliseconds) was stored. In the second step, another custom-made MATLAB program was used to re-inspect, frame-by-frame, the time-period around the tagged time-point (± 2 s) and to select the video-frame that immediately preceded the change in facial expression. In this step, each event was also accurately re-evaluated and all cases in which a clear change in facial expression was not confirmed were marked for rejection. For this whole procedure, the scorer remained blind to the experimental condition of each video. Finally, tagged events that occurred after 1 second from the end of a clip and before the beginning of the subsequent clip (i.e., during inter-stimulus black frames) were also automatically discarded.

4.2.5 EMG data analysis

A facial electromyographic (EMG) signal was derived from two EEG electrodes located below the two eyes, on the cheeks, approximately above the zygomatic muscles. The continuous signal of these two channels was referenced on homolateral, pre-auricular electrodes, and band-pass filtered between 30 and 200 Hz. A notch filter at 50 Hz was also applied.

Variations in facial EMG activity were evaluated to confirm the expected association between tagged events and changes in facial expression. Moreover, given that the estimation of expressive changes based on visual inspection of the video-clips may be inaccurate with respect to the actual beginning of muscular activity, the EMG signal was also inspected to determine the specific onset of each tagged event (Fiacconi and Owen, 2015). In particular, for the EMG inspection procedure, the root mean square (RMS) of the filtered signals was computed using a moving-window approach (1-s length; 1 time-point steps). Then, 8-s long data segments, including 4 s before and 4 s after the manually tagged onset of changes in facial expression were extracted. Finally, each individual event was visually inspected using a custom-made MATLAB function and the onset of the increase in EMG activity was marked. Cases for which a clear increase in EMG activity were not identified were discarded from further analyses. Finally, for all the retained episodes, 8-s long data segments centered on the EMG-activation onset were extracted and the total signal power (30-200 Hz) was computed in 2-s epochs using the Welch's method (*pwelch* function, MATLAB signal processing toolbox; Hamming windows, 8 sections, 50% overlap). The mean power in the 4 s after onset-time were compared to the mean signal power in the 4-s epoch preceding the same time-point at group level (i.e., after within-subject averaging across episodes). The ratios between the two data segments (post/pre) were also compared across experimental conditions (ES, FE) to investigate potential differences.

4.2.6 EEG data analysis

Continuous EEG recordings performed during the emotion suppression/expression task were band-pass filtered between 0.5 and 45 Hz. All EEG traces were visually inspected to identify and mark bad channels containing clear artifactual activity using NetStation 5.3 (EGI). Then, an Independent Component Analysis (ICA) was performed in EEGLAB (Delorme and Makeig, 2004) to remove signal components reflecting ocular, muscular and electrocardiograph artifacts. Rejected bad channels were subsequently interpolated using spherical splines. After preprocessing, all EEG traces were re-referenced to average reference and 4-s-long data epochs immediately preceding the EMG-activation onset (t = 0 s) of changes in facial expression were extracted. Finally, for each subject, the signal power in delta (1-4 Hz) and theta (4-8 Hz) frequency-bands was computed for each epoch as described for the EMG signal and averaged across episodes of interest. Paired comparisons between experimental conditions were then performed at group level.

4.2.7 Source modeling of EEG data

The signal of pre-processed EEG-epochs corresponding to the 4 s immediately preceding the onset of changes in facial expression were source modeled using *Brainstorm* (Tadel et al., 2011). Specifically, the conductive head volume was modeled using a three layers symmetric boundary element method (OpenMEEG BEM, Kybic et al., 2005; Gramfort et al., 2010) and the default ICBM152 anatomical template. A standard set of electrode positions (GSN HydroCel 64) was used to construct the forward model. The source space was constrained to the cerebral cortex, which was modeled as a three-dimensional grid of 15,002 vertices. The inverse matrix was computed using the standardized low-resolution brain electromagnetic tomography (sLORETA) constraint with a regularization parameter equal to 10^{-2} λ. Finally, the signal power was computed for each vertex in source space using the Welch's method (1-s Hamming windows, 50% overlap). Power maps in source space were then exported to MATLAB for planned statistical comparisons and obtained results were re-imported in *Brainstorm* for visualization.

4.2.8 Data selection

In order to avoid potential confounding effects related to extended practice with partially different tasks in the two experimental conditions (ES, FE), as well as to the administration of caffeinated or decaffeinated beverages in the last hour of the two experiments, our analyses of behavioral and EEG/EMG data collected during the emotion suppression/expression task specifically focused on the first task session (i.e., TS1-1 and TS1-2). However, confirmatory analyses of relevant EEG-based comparisons were performed using all available task sessions, with and without application of a within-trial normalization. In particular, the normalization of EEG-signal power was performed by subtracting the median band-specific power computed across the whole corresponding EEG recording (2-s data segments; *pwelch* method with Hamming windows, 8 sections, 50% overlap). This procedure was performed in order to exclude potential confounding effects of task-related and session-specific changes in brain activity.

4.2.9 Statistics

Comparisons of EEG-signal power across experimental conditions were performed using paired t-tests and a permutation-based supra-threshold cluster correction, as described in previous work (Nichols and Holmes, 2002; Huber et al., 2006). In brief, the same statistical contrast was repeated after shuffling the labels of the two experimental conditions and the maximum size of significant electrode-clusters ($p < 0.05$) was saved in a frequency table. A minimum cluster-size threshold corresponding to the 95th percentile of the resulting distribution was applied to correct for multiple comparisons. For comparisons performed at scalp-level, the analysis was restricted to 51 'internal' electrodes as described in previous work (Hung et al., 2013). In fact, more 'external' channels, located near the eyes, or on the temporal or neck muscles, are more likely to be affected by potential residual artifactual activity. Finally, permutation tests were used to assess the statistical significance of comparisons regarding behavioral and subjective variables and of correlations computed using the Spearman's correlation coefficient. Of note, for all cases in which the number of possible data recombinations

was greater than 10,000 this value was used to approximate the null distributions. In all other cases, the exact number of possible data recombinations was used. All statistical analyses were performed in MATLAB.

4.3 Results

4.3.1 Behavioral results

During the first task session (TS1), all subjects showed a lower number of facial expression changes in ES (2.6 ± 3.2) relative to FE (20.2 ± 10.7) condition ($p < 0.001$, $z = 3.767$; Figure 22A). Importantly, we found a negative correlation between actual sleep time in the night preceding the ES experiment and the absolute number of emotion suppression failures ($p = 0.0126$, $r = -0.57$; Figure 22B). A consistent but non-significant correlation was also found with subjective sleepiness ($p = 0.119$, $r = -0.37$), so that higher sleepiness was positively related to the number of suppression failures. Of note, self-reported mood showed instead no relationship with self-control failures ($p = 0.306$, $r = -0.25$).

Twelve participants (mean age $= 26.7 \pm 3.1$, 8 males) had at least one emotion suppression failure in the ES condition and were thus included in further analyses. Of note, in this subsample the order of experimental conditions was relatively balanced, as 7 participants completed the ES condition during the first experimental visit, while 5 subjects competed first the FE condition. This observation allows to exclude a major confounding effect of the order of experimental sessions on our analyses.

Specifically, these subjects had on average 4.1 ± 3.2 facial expression changes in the ES condition; a value still significantly lower than the one observed in the FE condition (22.9 ± 11.4; $p < 0.001$, $z = 3.0158$). In the same subjects, the analysis of variations in EMG activity (30-200Hz) of the zygomatic muscle confirmed that marked facial expression changes were associated with significant activity increases in both experimental conditions (ES: $p < 0.001$, $z = 1.682$; FE: $p < 0.001$, $z = 2.581$; Figure 23). Relative EMG changes tended to be stronger in the ES condition ($p = 0.029$, $z = 1.842$), although values also showed more variability across

subjects in this particular condition. In fact, a visual re-inspection of video-recordings in ES confirmed a broad variability in the manifestation of suppression failures, with some subjects showing "explosive" losses of control, and others only presenting minimal changes in facial expression. Instead, reactions tended to be more similar across subjects during FE.

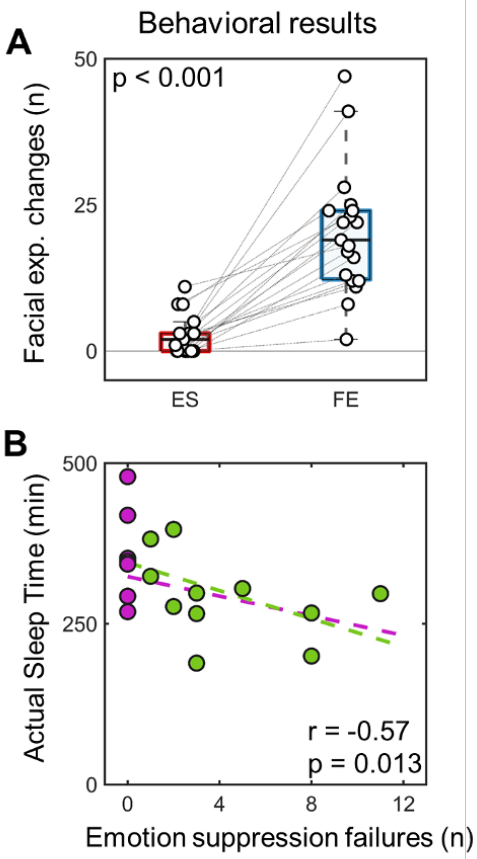

Figure 22. Behavioral results. (A) Changes of number of facial expressions in the two experimental conditions (ES = emotion suppression; FE = free expression). (B) Relationship between number of emotion suppression failures and actual sleep time the night preceding the experimental session. This analysis was performed on 18 subjects due to missing actigraphic data in one participant (Spearman's rho). The subjects who showed no changes in facial expression during TS1 are marked with red color. The remaining subjects are displayed using green color. Dashed lines represent the least-square fit for the distributions including all subjects (red) or only subjects who had at least one facial expression change (green).

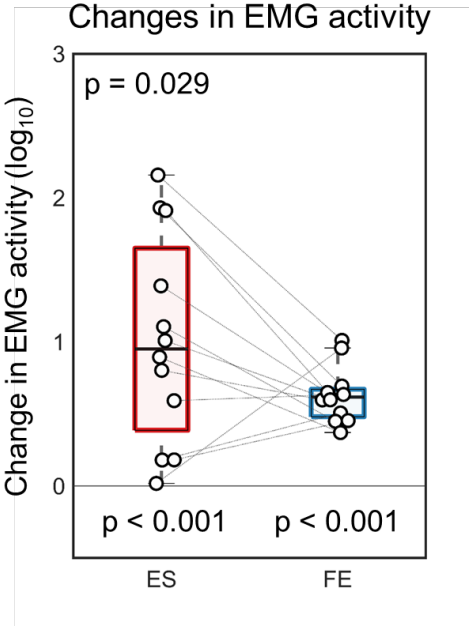

Figure 23. Variations of EMG activity associated with changes of facial expression. Signal power changes (30-200Hz) were computed as the ratio between EMG activity in the 4 seconds after the onset of facial expression changes and brain activity in the 4 seconds that preceded this event. Values were log-transformed for display purposes. Here, values greater than 0 indicate an increase.

4.3.2 Sleep quality, vigilance and mood

Several control analyses were performed to exclude potential systematic differences between experimental conditions in the twelve examined participants. First, we investigated possible differences in time spent in bed and in actual sleep time between the nights that respectively preceded ES and FE experiments (of note, this analysis was performed on 11 subjects due to missing actigraphic data from one volunteer; age 28 yrs, male). We found no evidence of systematic differences (time spent in bed: $p = 0.341$, $z = 1.004$; actual sleep time: $p = 0.492$, $z = -0.720$; Figure 24A). Similarly, we found no systematic differences in sleepiness ($p = 0.563$, $z = 0.904$; Figure 24B), mood ($p = 0.766$, $z = 0.632$; Figure 24C) and motivation ($p = 0.500$, $z = 1.134$; Figure 24D). Of note, similar results were obtained when considering 18 subjects for actual sleep time (missing data in one subject) and all 19 subjects for the Likert scales (*data not shown*).

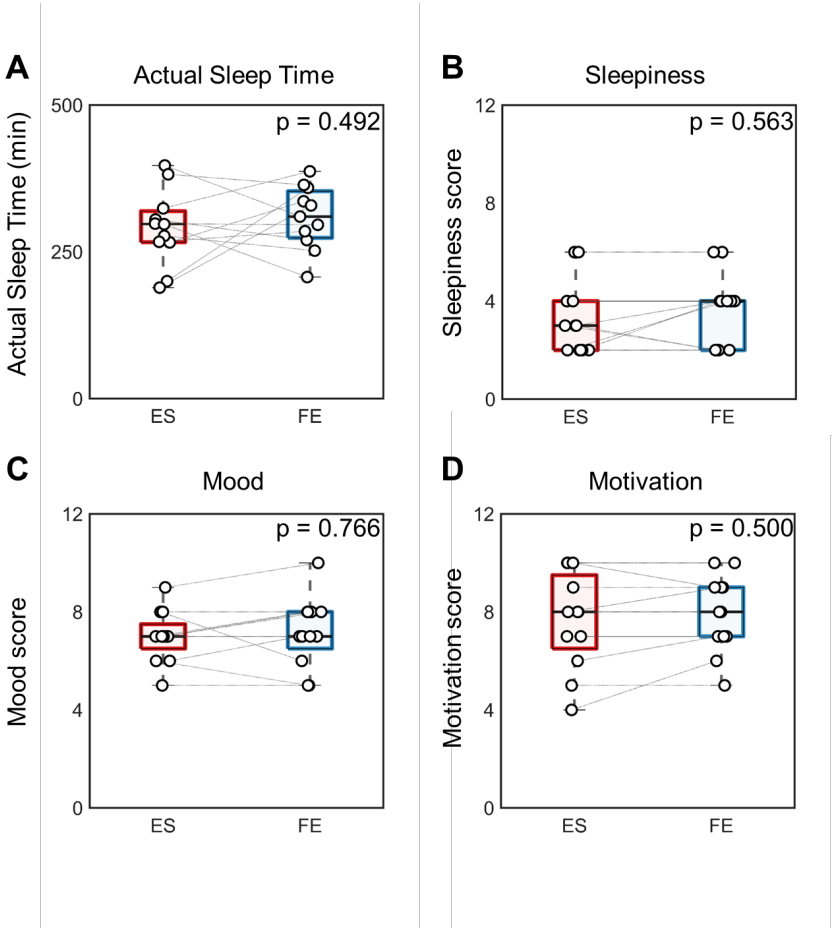

Figure 24.Variations of objective and subjective parameters. (A) Total minutes of sleep in the night that preceded each experimental session (N=11). (B-D) Subjective scores (0-10) for sleepiness, mood and motivation in the two experimental conditions (N=12). No systematic differences were found between any of the tested parameters.

4.3.3 Delta and theta EEG activity

We next evaluated whether increases in low-frequency (delta/theta) activity preceded the occurrence of emotion suppression failures (Figure 25). To this aim we compared the signal power computed in the four seconds preceding a change in facial expression across ES and FE conditions. We found that changes in facial expression were preceded by an increase in delta activity (1-4Hz) in frontal and left temporo-parietal electrodes ($p < 0.05$, corrected). These results were confirmed in additional analyses that included 15 subjects (mean age = 26.6 ± 2.8, 9 males) who had at least one emotion suppression failure across the three task sessions (TS1-TS3) of ES ($p < 0.05$, corrected; Figure 26). No significant differences in theta activity (4-8Hz) were found.

Given that changes in low-frequency activity may represent a signature of brain functional fatigue caused by insufficient sleep, we then explored the possible relationship between delta activity and actual sleep time the night preceding the ES experiment. We found that maximum delta power in frontal electrodes tended to be correlated with sleep time ($p = 0.054$, $r = -0.60$). Thus, a lower amount of sleep tended to be associated with higher levels of failure-related delta activity. Of note, a similar relationship was not found for temporo-parietal electrodes ($p = 0.664$, $r = 0.15$). Consistent but relatively weaker results were found using subjective sleepiness instead of actual sleep time (frontal: $p = 0.066$, $r = 0.55$; parietal: $p = 0.198$, $r = 0.39$).

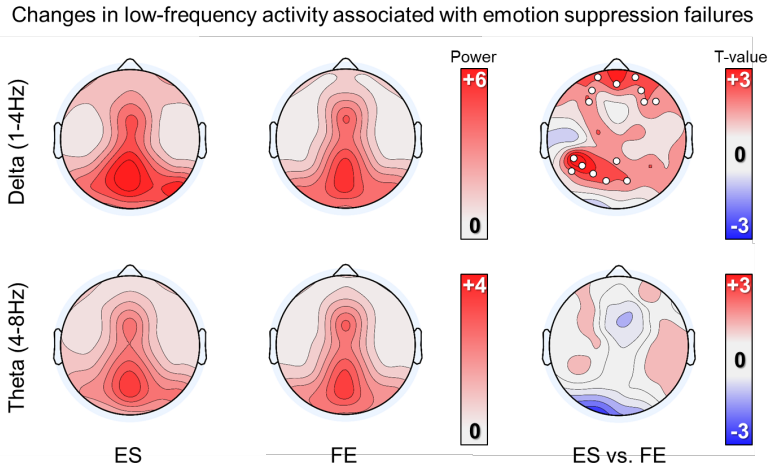

Figure 25. Low-frequency EEG activity associated with emotion suppression failures. Topographic plots on the left show absolute values of delta (top) and theta (bottom) power for the two experimental conditions (ES, FE) in the 4 s that preceded changes in facial expression. Topographic plots on the right show the statistical comparison between experimental conditions for the two frequency bands. White dots mark $p < 0.05$, cluster-based correction.

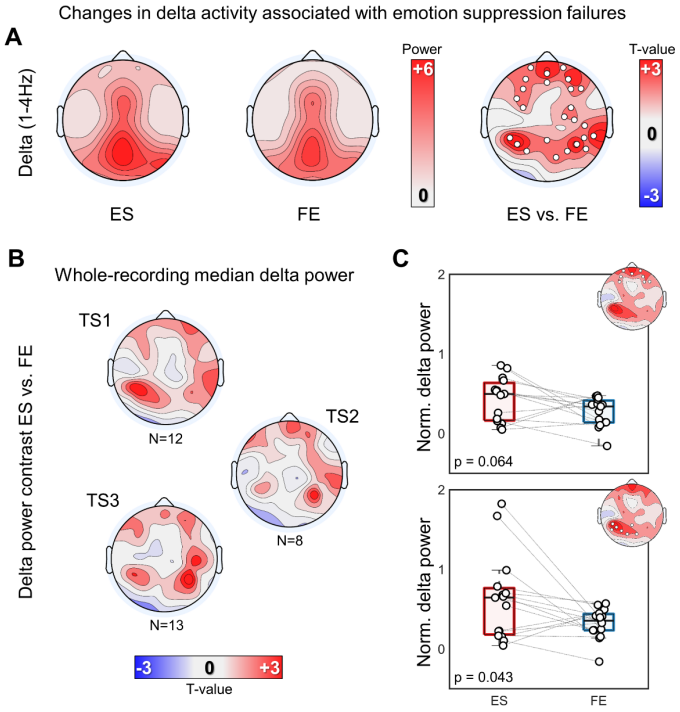

Figure 26. (A) Delta EEG activity associated with emotion suppression failures. Topographic plots on the left show values of delta activity computed for the two experimental conditions (ES, FE) in the 4 seconds that preceded a change in facial expression. Here 15 subjects that presented at least one emotion suppression failure across the three task sessions of ES were included. (B-C) In order to take into account potential inter-session variability, additional analyses were performed after a normalization of the values of each electrode achieved by subtracting the median of delta activity computed in the whole corresponding EEG recording. As shown in (B), median delta activity was not significantly different across experimental conditions for TS1 (12 subjects), TS2 (8 subjects) and TS3 (13 subjects). Boxplots in (C) represent differences in mean delta activity computed for the frontal and the temporo-parietal clusters obtained from the comparison of delta activity in ES and FE for the twelve subjects who presented at least one emotion suppression failure in TS1 (see main text). This analysis confirmed that higher delta power was present in ES relative to FE in the 4 s preceding facial-expression changes, although the effect remained significant in the parietal electrode-cluster, while only a trend was found in frontal electrodes. In topographic analyses, white dots mark $p < 0.05$, cluster-based correction.

4.3.4 Temporal and frequency-band specificity

Additional analyses were performed to verify whether the observed association between changes in delta activity and emotion suppression failures were temporally and frequency-band specific. First, we compared the median delta power of the entire recordings of ES and FE to identify potential differences in overall task-related brain activity. The median was preferred over the mean as this measure is less likely to be affected by potential outliers. This analysis revealed no significant differences between ES and FE (Figure 27A; also see Figure 26). Similarly, we found no significant differences between the two experimental conditions when comparing delta activity in the 4 s after the onset of changes in facial expression (Figure 27B; $p < 0.05$, corrected). Moreover, we compared the mean differences between ES and FE in the frontal and in the parietal electrode-clusters with null distributions ($N = 10,000$) obtained by computing the differences using 4 s epochs randomly selected within task-related recordings in place of epochs time-locked to facial-expression changes. This analysis confirmed the temporal specificity of the time-locked differences in delta power for both the frontal ($p = 0.049$, $|z| = 2.335$) and the parietal cluster ($p = 0.046$, $|z| = 2.323$).

Next, we evaluated whether additional differences between ES and FE were present in other frequency bands (Figure 28). In line with previous results, we found no significant differences for alpha (8-12Hz), sigma (12-16Hz) and beta (18-30Hz) activity.

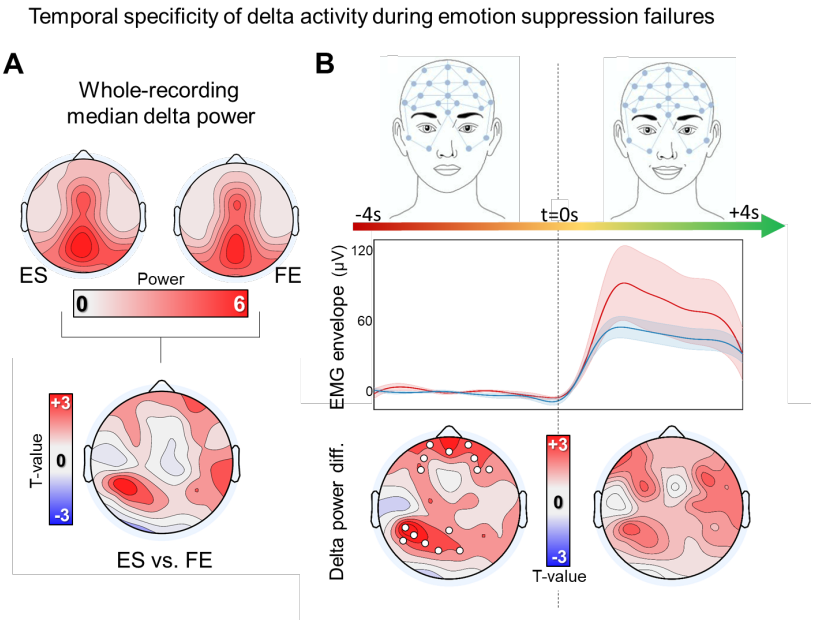

Figure 27. Temporal specificity of delta activity changes. (A) Median task-related delta power computed for the entire EEG recordings. The topographic plot at the bottom shows the contrast between delta activity of ES and the of FE. (B) Contrast between experimental conditions in the 4 seconds before and in the 4 seconds after the change in facial expression (t = 0s). The central graph shows the variation of EMG activity in the same time window (envelope of rectified filtered EMG-signal). Values were normalized before averaging by subtracting the mean EMG activity computed in the 4 seconds before the onset of changes in facial expression (t = 0s). Shaded areas correspond to the standard error of the mean. For topographic plots, white dots mark $p < 0.05$, cluster-based correction.

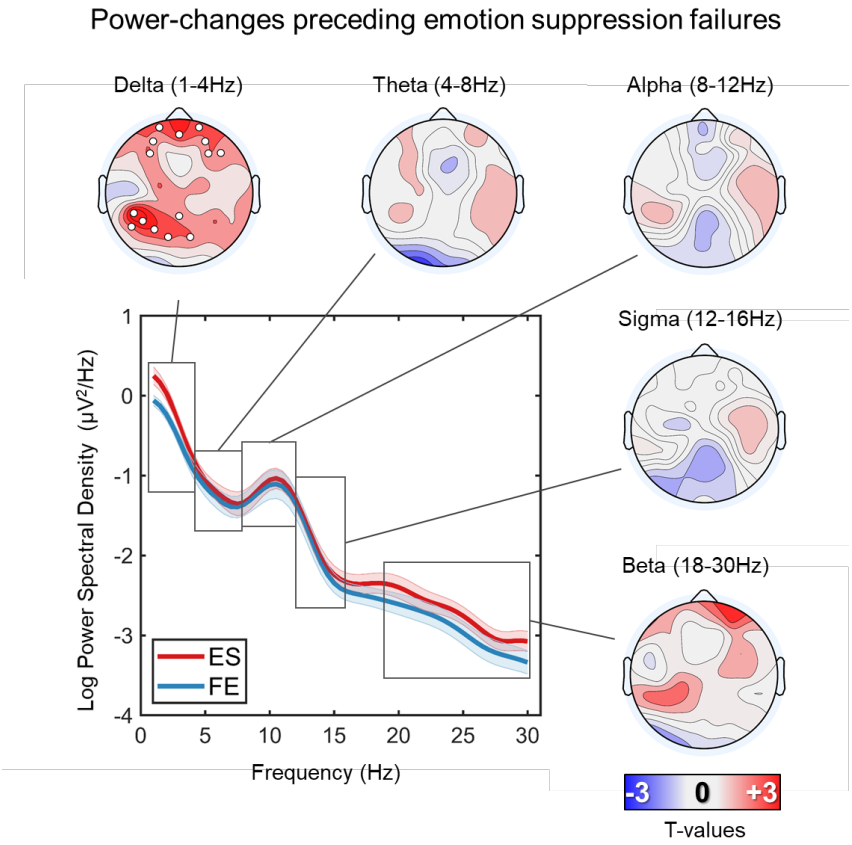

Figure 28. Differences in brain activity preceding the onset of changes of facial expression in the two experimental conditions (ES, FE). The central plot shows the power spectral density (PSD) computed across the electrodes presenting a significant difference in delta activity between ES and FE (white dots). Additional comparisons were performed for other typical frequency bands, including theta, alpha, sigma and beta. White dots mark $p < 0.05$, cluster-based correction.

4.3.5 Source modeling analysis of delta activity

In order to identify the actual sources of changes in delta activity observed in the ES condition, the same analysis shown in Figure 3 was repeated after source reconstruction of the EEG signals (sLORETA; but very similar results were obtained using dynamical Statistical Parametric Mapping, dSPM). The obtained results are shown in Figure 29 ($p < 0.05$, corrected; cluster-forming threshold set to uncorrected $p < 0.001$; also see Figure 30). In particular, we found significant clusters in the bilateral anterior cingulate and medial frontal cortex, the anterior insula, the right precuneus and the right motor/premotor cortex, including the middle and inferior frontal gyri. Of note, most of these regions overlap with key areas of the previously described as part of the emotion regulation network (Langner et al., 2018).

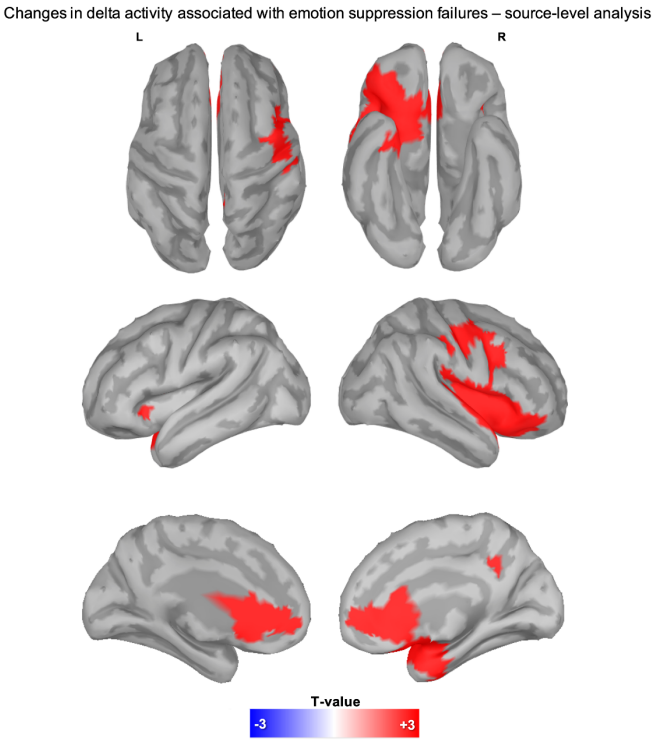

Figure 29. Delta EEG activity associated with emotion suppression failures. Significant differences ($p < 0.001$, cluster-based correction) in delta activity between ES and FE in the 4 seconds that preceded the onset of changes in facial expression.

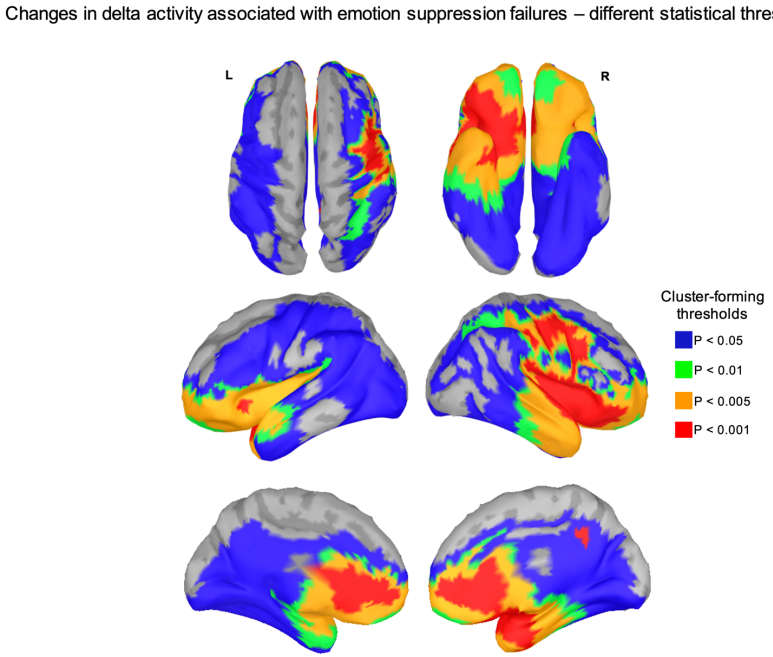

Figure 30. Changes in delta activity associated with emotion suppression failures are reported for four different cluster-forming thresholds, corresponding to $p < 0.05$ (blue), $p < 0.01$ (green), $p < 0.005$ (orange), $p < 0.001$ (red). The cluster-level threshold was $p < 0.05$ in all cases.

4.4 Discussion

Previous evidence indicates that frontal, parietal and limbic brain regions are activated during successful emotion suppression (Dörfel et al., 2014; Frank et al., 2014; Kohn et al., 2014; Langner et al., 2018). However, the neural correlates of emotion suppression failures were unknown. Here we demonstrated that self-control failures in an emotion suppression task are preceded by increases in low-frequency (delta) activity over frontal, insular and parietal regions. In addition, a positive correlation was found between sleep time the night before practice with the emotion suppression task and the absolute number of self-control failures, so that shorter sleep duration was associated with a poorer behavioral performance. These results indicate that intrusions of sleep-like activity in the brain network responsible for emotion regulation affect the efficacy of emotion suppression in healthy individuals. In this light, the occurrence of local, sleep-like episodes may offer a possible neurophysiological mechanism for previous observations regarding the effects of sleep loss on emotion regulation.

4.4.1 Neural correlates of emotion suppression failures

Previous work showed that a neural network encompassing frontal, cingulate and parietal regions is recruited during emotion suppression tasks (Langner et al., 2018). However, to the best of our knowledge, no study investigated the neurophysiological events that could underlie failures in emotion suppression. Here, we showed that increases in delta (1-4 Hz) activity over frontal and parietal areas encompassing the brain network involved in impulse control and emotion regulation precede the occurrence of emotion suppression failures. Of note, while delta activity is considered as a typical hallmark of sleep, a growing body of evidence indicates that temporary regional increases in low-frequency, delta-theta activity may often occur also during wakefulness. Indeed, seminal work in rats demonstrated that locally synchronized neuronal *off-periods* similar to those underlying the generation of sleep slow waves (0.5-4 Hz) occur more frequently as a function of time spent awake (Vyazovskiy et

al., 2011). Moreover, rather than being uniform across the cortical mantle, this increase appears to be of greater magnitude in brain areas that are more intensively 'used' during wakefulness (Hung et al., 2013; Quercia et al., 2018; Bernardi et al., 2019a; Petit et al., 2019). Interestingly, the occurrence of sleep-like events in brain areas related to the execution of ongoing activities has been shown to potentially determine temporary behavioral impairments in a variety of different tasks, including impulse control, visuo-motor coordination and stimulus categorization (Bernardi et al., 2015; Nir et al., 2017). Based on these observations, it has been suggested that local sleep episodes may reflect the neurophysiological correlate of *'functional fatigue'* and sleep need (Bernardi et al., 2015; Andrillon et al., 2019; D'Ambrosio et al., 2019). Our present results extend the previous literature by demonstrating that local, sleep-like episodes occurring in brain areas involved in emotion regulation predict – and thus, represent a potential cause of – failures in emotion suppression. Interestingly, our results also showed, for the first time, that sleep-like episodes related to behavioral errors do not occur only after sleep deprivation or extended task practice but may be observed also in apparently well-rested individuals, during the first hours of the morning. This finding has important implications for our understanding of the actual influence of local sleep regulation on human behavior.

4.4.2 Poor sleep quality and emotion suppression failures

We found that emotion regulation failures - measured as the inability to voluntary suppress changes in facial expression -, are more common in individuals who had shorter sleep duration. Moreover, shorter sleep tended to be associated with higher delta activity over frontal regions in those who fail at suppressing their emotional expressions. These results are consistent with evidence indicating the existence of a tight interplay between sleep and emotion regulation. Indeed, both acute and chronic sleep loss determine alterations of mood and emotional reactivity, as well as increased stress, anxiety and depression (Zohar et al., 2005; Anderson and Platten, 2011; Minkel et al., 2012; Mauss et al., 2013; Beattie et al., 2015; for a recent review, also see Krause et al., 2017). The sleep-deficient individual may often display greater emotional reactivity to stimulus salience independently from valence, biased cognitive evaluation and flawed behavioral

expression (Gujar et al., 2011a; Ben Simon et al., 2015). In addition, affective/mood disorders and sleep disturbances are often found associated, thus implying a potential role of inadequate sleep in the development or worsening of these clinical conditions (Benca et al., 1992; Goldstein and Walker, 2014). In line with this, good sleep quality has been associated with enhanced emotional well-being and is commonly considered as a protective factor for humans' emotional functioning (van der Helm et al., 2011; Gruber and Cassoff, 2014; Palmer and Alfano, 2017; Watling et al., 2017).

Neuroimaging studies demonstrated that one night of sleep deprivation impairs the medial prefrontal cortex (mPFC) top-down regulation on limbic brain areas, resulting in an *'executive dysfunction'* (Yoo et al., 2007; Gujar et al., 2011b; Gruber and Cassoff, 2014; Ben Simon et al., 2020a). Notably, even a single night of slight sleep curtailment, from 1 to 2 hours, has been shown to robustly impair mPFC activity and its related limbic functional connectivity and to determine an increased emotional distress in healthy adult individuals (Killgore, 2013). In light of these considerations, our results indicate that alterations in the top-down control exerted by the mPFC on limbic areas may be ultimately determined by the occurrence of local sleep-like episodes in this brain region.

4.4.3 Limitations

It is important to acknowledge that this study has some limitations. First, while the original sample included 19 subjects, some of the analyses have been performed on a reduced sample of 12 subjects. This depended on the fact that emotion suppression failures are relatively uncommon in healthy young and rested individuals. Importantly, however, the same analyses repeated after including additional experimental sessions and subjects (N=15) confirmed the results obtained in the reduced sample (Figure 26). Another important limitation is related to the fact that estimates of brain activity in ES and FE relied on a different number of events, since changes in facial expression were always more common in FE relative to ES. However, we found that increases in delta activity prior to emotion suppression failures relative to facial-expression changes in FE remained significant when compared with a null distribution obtained using the same

number of data epochs non-time-locked with respect to facial-expression onsets. This observation indicates that the observed effects are not explained by relative differences in the amount of data available in each condition. In future investigations, sleep restriction or deprivation paradigms could be used to increase the incidence of emotion suppression failures and thus obtain a greater statistical power.

Moreover, here we only used emotional stimuli with a positive valence, thus limiting the possibility to generalize our results to other emotional stimuli, such as negative and/or aversive stimuli. However, it should be noted that the expressive suppression strategy has been shown to rely on similar brain substrates for both negative and positive emotional stimuli (e.g., Hajcak and Nieuwenhuis, 2006; Dennis and Hajcak, 2009; Korb et al., 2012; Paul et al., 2013; Morawetz et al., 2017).

Finally, while the video scorer was blind to the experimental condition, we cannot exclude that he could have possibly guessed whether the videos were obtained in the ES of FE condition. To avoid this issue, future studies should rely on automatic procedures for the detection of facial-expression changes.

4.5 Conclusions

Our study shows that emotion suppression failures are associated with temporary, sleep-like episodes occurring in key brain areas involved in emotion regulation. Importantly, given previous observations indicating that the incidence of local sleep-like episodes increases with time spent awake, the same functional mechanism may contribute to emotional dysfunctions commonly observed following sleep restriction or deprivation. Of note, while objective indices of sleep duration appeared to significantly correlate with behavioral impairment, the relationship was less clear for subjective reports of sleepiness. In line with previous findings, this observation suggests that individuals may not correctly perceive their actual level of brain functional fatigue (Benoit et al., 2018).

An impaired emotional regulation may substantially affect - and, potentially, disrupt - social interactions. In this light, understanding the mechanisms that underlie the loss of control over emotional responses may have broad important implications. In particular, future studies should investigate whether specific individual factors may favor a faster/greater build-up of or a greater vulnerability to local sleep-like episodes in specific brain areas or networks. Moreover, it will be important to clarify whether alterations in the local regulation of sleep need may be responsible for alterations of emotional regulation observed in psychopathological conditions.

Chapter 5

Conclusions

The studies included in this Thesis were aimed at expanding the current knowledge of the local regulation of sleep and wakefulness in humans. In particular, one study (Chapter 2) examined the role of cortico-cortical anatomical connections in determining the origin and propagation of slow waves during NREM sleep. Furthermore, two studies investigated how local, sleep-like activity is regulated during wakefulness (Chapter 3), and how it may influence behaviors with a potential social relevance (Chapter 4).

In the experiment described in Chapter 2, we demonstrated that the brain cortical propagation of human sleep slow waves directly depends on cortico-cortical, anatomical constraints. In particular, by studying a rare population of "split-brain" patients, we showed that the inter-hemispheric spreading of sleep slow waves is tightly related to the integrity of inter-hemispheric white matter tracts. While previous correlational studies already provided indirect support for such a relationship (Murphy et al., 2009; Buchmann et al., 2011b; Piantoni et al., 2013), our study is the first to demonstrate a direct, causal dependence of slow-wave spreading on white matter connections. Interestingly, however, we also found that the absence of the corpus callosum does not affect the overall coordination of state-shifts across the two hemispheres. Indeed, we did not observe clear state dissociations characterized by sleep rhythms in one hemisphere and wake-like activity in the other half of the brain. In addition, while we found that sleep slow waves tend to originate more often in the right than in the left hemisphere, such an asymmetry is not different between split-brain patients and subjects with an intact corpus callosum. Overall, these results indicate that global state changes are coherently modulated across the cortical mantle by non-

cortical (bottom-up) mechanisms. At the same time, the state of cortical connections profoundly affects the spreading of sleep oscillations. Given the growing body of evidence indicating that slow waves are involved in many important physiological processes including learning and brain plasticity, our observations highlight a possible direct role of cortical connections in coordinating such functions at the network level. In this light, we hypothesize that pathological alterations in brain connectivity could give rise to a vicious circle in which alterations in the coordination of sleep rhythms may ultimately worsen the symptomatology of, or slow-down the recovery from, the underlying clinical state. It is important to note that our results also point at slow waves as potential valuable readout of the state of cortical connections in both healthy individuals and pathological populations. In this perspective, our findings represent an important foundation for future basic and translational investigations regarding the fundamental functions of sleep slow waves in humans. Finally, it would be interesting to investigate whether other sleep rhythms, such as sleep spindles, may be similarly affected by callosal resection. In fact, sleep spindles are thought to propagate and be synchronized at cortical level through thalamo-cortical connections rather than cortico-cortical pathways (Lüthi, 2013; Fernandez and Lüthi, 2019) and thus their activity may be expected to remain unaltered after total callosotomy.

In the following Chapters, we focused on the investigation of the regulation and implications of local, sleep-like activity during wakefulness. These local increases in low-frequency, delta-theta activity, have been shown to reflect the global and local levels of sleep need, as they are linearly correlated with the time spent awake and with time-on-task, and of functional fatigue, as they have often been observed in association with behavioral performance impairments. The EEG manifestation of the sleep-like episodes is a negative wave similar to slow waves observed during actual sleep, and previous work in rats has shown that the underlying generation mechanism is also the same, namely a temporary hyperpolarization (*off-period*) of cortical neurons.

Interestingly, both sleep slow waves and sleep-like events of wakefulness have been shown to increase more strongly in frontal

areas relative to other brain regions after extended wakefulness (Finelli et al., 2000; Strijkstra et al., 2003). However, the physiological mechanism underlying these regional differences was still to be clarified. In particular, it was unclear whether the frontal cortex may present a constitutional vulnerability related to its microstructural and functional organization, or rather its faster/stronger increase in sleep pressure may simply reflect its frequent recruitment in most cognitive tasks. Preliminary results presented in Chapter 3 indicate that morning-to-evening increases in low-frequency activity reflecting the occurrence of sleep-like episodes are not topographically stable across days/sessions, but rather show relevant intra-individual variability. Specifically, by employing a single-subject multi-session design, we found that at least two different topographic patterns of morning-to-evening variations could be observed. These distinct patterns also tended to be associated with different behavioral correlates. Taken together, our findings indicate that variations in sleep-like activity do not necessarily peak in frontal areas, and that a constitutional vulnerability of the frontal cortex to functional fatigue is not sufficient to explain previously observed inter-regional differences. Of note, however, our results do not allow to entirely exclude a possible predisposition of the frontal regions to local sleep-like episodes, and a combination of both constitutional and use-dependent factors may certainly be involved. It is also worth noting that our results revealed only two main topographic configurations of morning-to-evening variations in sleep-like activity, one more frontal, and one more posterior, involving sensory regions. Further investigations will be necessary to determine whether such patterns resulted from different performed activities and experiences associated with a different engagement of anterior (frontal) and posterior (sensory) brain areas during the day.

A growing body of evidence links local, sleep-like events during wakefulness and alterations in cognitive processes and behavioral performance. In particular, it has been shown that local sleep-like episodes may affect performance in a wide variety of task depending on their timing of occurrence and cortical distribution. Yet most of previous research focused on relatively simple tasks requiring basic cognitive control (e.g., sustained attention, visuo-motor coordination, response inhibition), and it

was thus unclear whether the local sleep phenomenon could have a relevant impact in more naturalistic conditions. In Chapter 4, we thus investigated whether a complex behavior such as emotional regulation, which relies on the integration of different cognitive functions such as attention, perception, and impulse control, may be affected by local, sleep-like events. Here, we demonstrated, for the first time, that sleep-like activity in brain regions relevant for emotion regulation precede emotion regulation failures in a task that required emotion suppression during movie-watching in apparently rested individuals. In addition, we found that the incidence of behavioral failures was negatively correlated with the amount of sleep achieved the night before the experiment, in line with previous evidence linking local sleep-like episodes and sleep loss. Overall, our findings confirm that local, sleep-like events affect behavior when occurring in task-relevant regions, and suggest that similar, transient cortical deactivations may represent at least one of the functional causes that contribute to emotional dysfunctions frequently exacerbated following sleep restriction or deprivation. More in general, they also indicate that the local sleep phenomenon may have a relevant impact for complex, real-life conditions.

In conclusion, the results of the works included in the present Thesis shed new light on the mechanisms that underlie the regulation of sleep and wakefulness in physiological conditions. Taken together, they clearly indicate that the boundaries between sleep and wakefulness are extremely fluid, and that the co-existence of these two states across different regions of the brain is the rule rather than the exception. Most importantly, the resulting ever-changing mosaic of sleep- and wake-like activity continuously shape our subjective experience and our behavior by affecting both regional and network-level brain functional processing. Of note, it is becoming increasingly clear that alterations in the balance between sleep- and wake-like activity could be associated with, and potentially be the cause of, important pathological conditions. In fact, alterations of the local regulation of sleep and wakefulness may account for symptoms and manifestations of several sleep disorders such as disorders of arousal (Castelnovo et al., 2018b) and paradoxical insomnia (Stålesen Ramfjord et al., 2020). Moreover, a rapidly growing body of evidence indicates that similar local alterations may be

implicated also in other pathological conditions, including psychiatric and neurological disorders such as major depressive disorder (Nutt et al., 2008; Plante et al., 2013), ADHD (Andrillon et al., 2019; Furrer et al., 2019; Miano et al., 2019), schizophrenia (Monti and Monti, 2005; Castelnovo et al., 2018a; Manoach and Stickgold, 2019), traumatic brain injury and stroke (Modarres et al., 2016; Sarasso et al., 2019). Indeed, alterations of the regional balance between sleep and wakefulness observed in these conditions could represent manifestations of underlying brain plasticity impairments or could themselves represent a direct cause of impairment for plastic, experience-dependent adaptations. Although in most cases the causes of the local dysregulations are still unclear, these alterations may offer new important targets for therapeutic approaches.

It is our hope that the findings of the studies presented in this Thesis work will represent a foundation for future investigations aimed not only at improving our understanding of the physiological bases of local sleep regulation, but also at furthering our knowledge regarding how and why these may become altered in pathological states.

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