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Sleeping while awake:

a neurophysiological investigation on sleep during wakefulness.

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Abstract

Sleep and wakefulness are considered two mutually exclusive states. The alternation between those two states seems to be a defining characteristic of our life, a ubiquitous phenomenon demonstrated in every animal species investigated so far. However, during the last decade, advances in neurophysiology have blurred the boundaries between those states.

The mechanisms of sleep have always intrigued neurophysiologists and great advances have been made over the last century in understanding them: we now know that the defining characteristic underlying sleep activity is a specific pattern of neuronal activity, namely the slow oscillation.

The slow oscillation, which is characterized by the periodic alternation between periods of activity (ON-periods) and periods of hyperpolarization and neuronal silence (OFF-periods) is the default mode of activity of the sleeping cortex. This alternation is due to the tendency of neurons to fall into a silent period after an initial activation; such tendency is known as "bistability".

There is accumulating evidence that sleep-like bistability, and the ensuing OFF-periods, may occur locally in the awake human brain in some pathological conditions, in sleep transition, as well as after sleep deprivation. Therefore, to the extent that bistability and OFF periods represents the basic neuronal features of sleep, a paradigm shift is in place: from a neurophysiological perspective sleep can intrude into wakefulness.

In this thesis, I explore the fluid boundaries between sleep and wakefulness and investigate their possible implications on the problem of personal persistence over time. Moreover, I study the clinical implications of the intrusion of sleep into wakefulness in patients with focal brain injury due to stroke. Specifically, I aim to:

1) show how the sleep-like bistability can be responsible for the loss of function in stroke patients. This may have implications for understanding the pathophysiology of stroke and helping to foster recovery;
2) establish the basis for a model of local sleep that might be present in the everyday life, id est the sensation of sleepiness. Indeed, sleepiness could reflect islands of sleep during wakefulness;
3) advocate the biological criterion of identity, in which the continuity necessary for maintaining ourselves over time could be represented by never resting activity in the brain.

Contents

Introduction

The neurophysiology of sleep is tightly linked with the pathophysiology of several neurological disorders. In this thesis, I will show how the intrusion of sleep-like mechanisms in awake human cortex who suffered brain injury may lead to functional impairment. In support of the novel concept that, from a neurophysiological perspective, fluid boundaries are in place between sleep and wakefulness, I will propose a possible mechanism of local sleep during the sleep transition. Furthermore, I will study the implication of this paradigm shift in the problem of the personal persistence over time in the metaphysical debate.

In chapter one, I will summarise the state of the art of sleep research. Beginning with the notion of global sleep I will explore its biological mechanisms from a micro to a macro scale, from single cells to the whole brain. Here, I claim that the former rigid boundaries between sleep and wakefulness should be revised considering the new findings of local sleep.

In chapter two, I will show how the perturbational approach has unique advantages in detecting bistability. Furthermore, I will discuss the implications of this approach in the clinical field of disorders of consciousness, where it has been shown to be a promising diagnostic tool.

Despite the well-known feature of local slowing in the electroencephalogram (EEG) patterns of brain injured patients, the relationship between this slowing and sleep has never been formally characterized. In chapter three I will discuss the relationship between sleep and brain injuries, explaining how sleep could have a role in the functional impairment of brain injured patients and how confirming this finding could promote recovery in those patients.

In chapter four, I will show the main experimental findings of this thesis: the presence of local sleep in awake stroke patients with unilateral, focal brain lesions. In this group of patients, sleep-like features are present locally in the area surrounding the lesion. These findings are statistically significant and could be used to facilitate the recovery of stroke patients. Indeed, while anatomical lesions and disconnections may hardly be reversed, it might be possible to reduce local sleep-like bistability and improve overall network connectivity and complexity, thus improving the recovery of function.

In chapter five, I will focus on the notion that sleep can intrude in wakefulness and vice versa. I will imply that local sleep may underlie the sensation of sleepiness and that sleepiness may reflect the subjective experience of local sleep during wakefulness. Furthermore, in this chapter I will discuss my future plans to work with people with specific kind of epilepsy, namely generalized epilepsy with absences. These subjects present a generalised, transient episodes of loss of consciousness reflected

by a slowing in the EEG patterns. In a novel application of the perturbational approach, I will investigate possible links between those episodes and sleep mechanisms.

Finally, in chapter six I will investigate the possible implications of the fluid boundaries between sleep and wakefulness in the metaphysical debate on the personal persistence over time. In this framework I will designate my ontological commitment towards organisms. In line with it, I will opt for a diachronic identity relation. I will claim that the never-ending activity present in the brain could advocate for a biological criterion of identity that lead us to persisting over time.

In sum, in this thesis I provide further evidence for the paradigm shift in neuroscience, based on which sleep and wakefulness may no longer be two discrete states. These findings have therefore implications for both philosophy and medicine.

Chapter one. Sleep and wakefulness

The dichotomy between Sleep and Wakefulness seems to be an unavoidable feature of human life. It has important implications on both human experience and biology. Every animal species examined so far under the magnifying lens of neurophysiologists has shown a behavioural switching between two states: wakefulness and sleep. Specifically, in the animal realm of eukaryotes, within all species with a nervous system, this alternance depends on the intrinsic mechanisms of the neurons. Mircea Steriade and Sanchez Vivez among others, have characterized those mechanisms, describing how, from micro-scale to meso-scale, from molecules to cortical circuits, neurons change their firing patterns, which are the main observable behaviours in neuronal cells.

We now know that, during physiological sleep, neurons act with a bistable pattern of activation: they fire repetitively and fast and then remain silent resulting in an alternation of two periods, called ON-periods and OFF-periods respectively.

Characterizing Sleep

Two separate biological mechanisms regulate the sleep-wake cycle, interacting together and balancing each other. According to the flip-flop switch model, this regulation depends on a mutually inhibitory interaction between sleep centres and the arousal systems (Saper et al., 2001). The wake state is characterized by ascending activation of the brain cortex. Brainstem, midbrain and diencephalon constitute the evolutionary conserved network of the activating system, which contains neuromodulator and neurotransmitters such as glutamate, serotonin, catecholamines, histamine, acetylcholine and several neuropeptides able to orchestrate different states at the behavioural level. In recent years, histaminergic and orexinergic neurons have been shown to be integral for the hypothalamic wake-promoting centre (Figure 1). On the other hand, the activation of the anterior hypothalamus, along with the inhibition of the posterior nuclei and the central midbrain tegmentum seems to orchestrate the onset of sleep (Saper et al., 2001). Specifically, GABAergic neurons in the hypothalamic ventrolateral preoptic nucleus (VLPO) inhibit the waking system, thus leading to the transition towards sleep.

The cortex is active day and night. During slow wave sleep, usually at night, the electroencephalogram (EEG) voltages increase. In this state high-voltage δ-waves (0.5–3 Hz) are present, indicating a high degree of cortical synchronization. During wakefulness, instead, low voltage and fast frequency (mainly β and γ, between 20 and 60 Hz) waves are present in the EEG. It has been suggested that

behavioural awareness depends on external factors (the characters of the external world, such as light or odours) that cause cortical function to become desynchronized, active due to sensory ascending afferents (Steriade and McCarley, 2013).

By stimulating and lesioning the brain stem reticular formation, Moruzzi and Magoun (Moruzzi and Magoun, 1949) demonstrated cortical arousal in the cat and formulated the concept of the ascending reticular activating system (ARAS). The neuronal projections constituting the ARAS reach the cerebral cortex through the non-specific thalamus, the medial and intralaminar nuclei and extra-thalamic pathways. During the following decades, stimulations, lesions, and brain transections in combination with electrophysiological recordings (from EEG to single cells) have been used to determine the structures involved in the regulation of sleep and waking. Acute preparations of high brainstem transection (Bremer, 1935) or isolated forebrain display continuous, slow, synchronous, high-amplitude activity, similar to that seen during deep slow wave sleep.

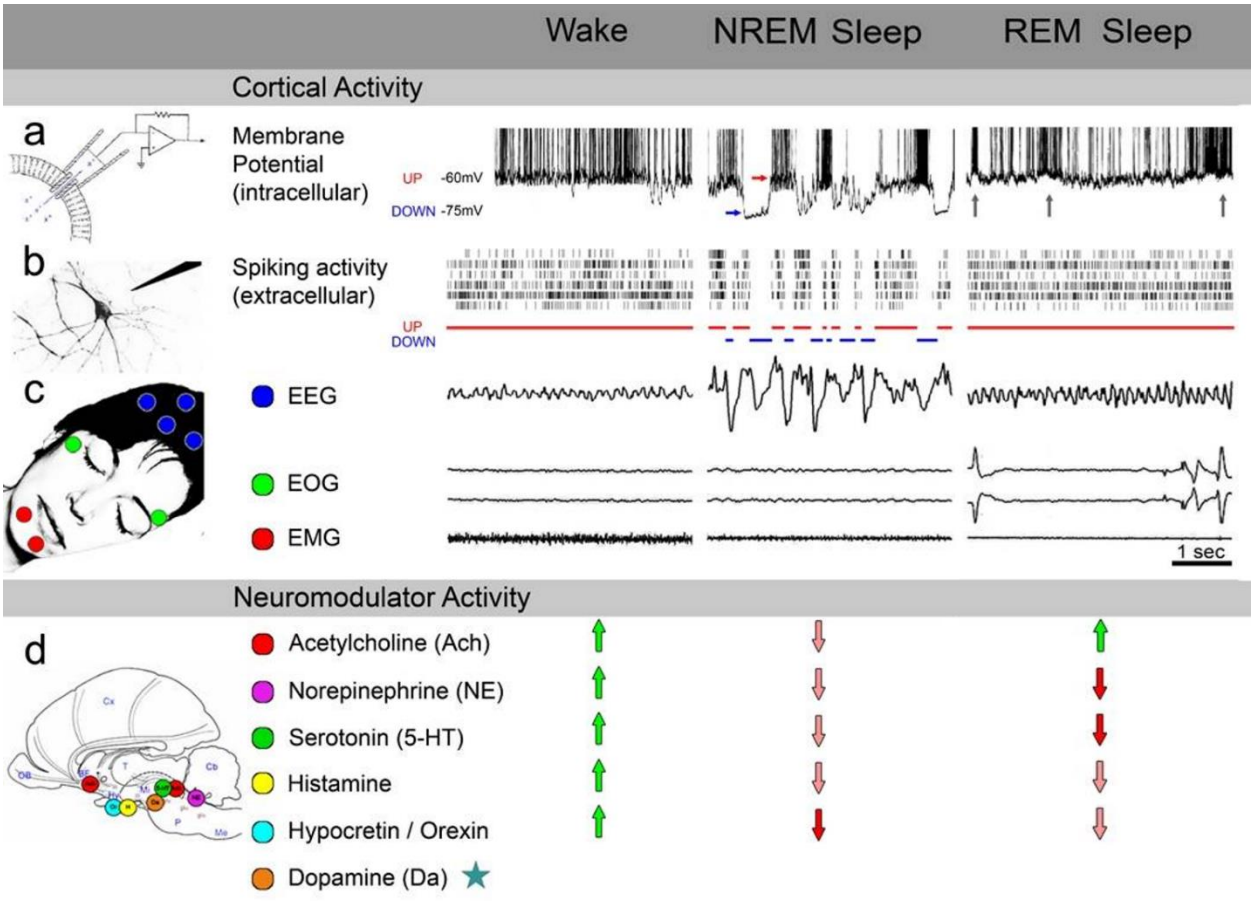

Figure 1. A comparison of cortical activity (upper panel) and neuromodulator activity (bottom panel) in wake, NREM sleep and REM sleep. Modified from Nir and Tononi, 2010.

Sleep consists of two fundamental states: rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep. REM sleep is characterized by a rapid and low-voltage EEG, irregular activity, rapid eye movement and low muscle tone. During REM sleep, neuronal activity is very similar to that during wakefulness. REM sleep is associated to an increase in the activity of limbic and amygdala networks, which coexists with an activity decrease in dorsal prefrontal regions (McNamara et al., 2010). NREM sleep is associated with deactivation of thalamic functions and emergence of synchronized wave activity throughout neocortical sites. According to the American Academy of Sleep Medicine (AASM), NREM sleep is divided in three different stages, nominally N1, N2 and N3. Stage N1 is the transition between wakefulness and sleep (Iber, 2007). During this stage, alpha waves disappear and theta (4-7 Hz) waves appear. N1 constitutes 5% of the total time of sleep. Hallucinations may occur both in the beginning and the end of this state. Stage N2 is an intermediate stage of sleep where both cardiac and respiratory rhythms slow down. It is characterized by sleep spindles (waves of 8-14 Hz that occur for at least 0.5 seconds), resulting from interactions between thalamus and cortex, and K-complexes. A K-complex is a slow biphasic wave with a brief negative high-voltage peak - usually greater than 100 μV – followed by a slower positive complex after 350 and 550 ms and a final negative peak at 900 ms. K-complexes are often followed by spindles. They are produced spontaneously or in response to auditory (Cash et al., 2009), visual, pain, tactile and respiratory stimuli (Webster and Colrain, 1998). Spindles are generated mainly over central regions, whereas K-complexes occur primarily over prefrontal and frontal regions (McCormick et al., 1997). Finally, during stage N3 (or slow-wave sleep), slow waves can be singled out inspecting EEG patterns across the scalp (Massimini et al., 2004). A similar spatio-temporal organization of such NREM rhythms can be observed under different types of anaesthesia.

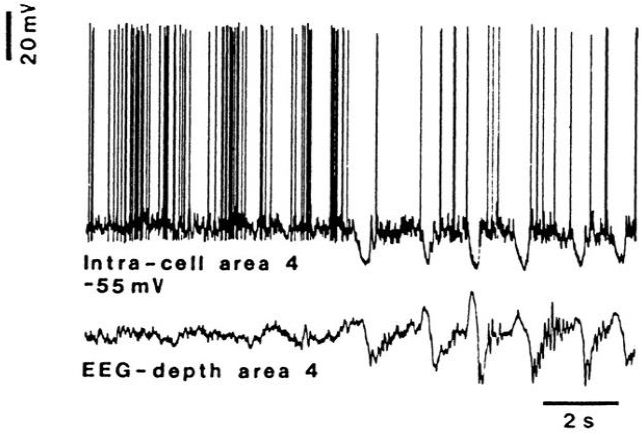

Figure 2. Intracellular recording from cortical area 4 of cat, showing the synchronization of cellular slow oscillation and EEG when neurons display prolonged hyperpolarization. Modified from Steriade et al., 1993a.

At the cellular level, sleep slow waves consist of slow oscillations (< 1Hz) of the membrane potential of cortical neurons that alternate between periods of silence (hyperpolarizing or Down) and tonic spiking activity (depolarizing or Up) (Steriade et al., 1993a), a physiological cortical phenomenon known as cortical bistability, shown in Figure 2.

In 1993, Steriade and collaborators described the existence of a slow oscillatory activity (<1 Hz) under different types of anaesthesia in cats. Neuronal intracellular recordings from different cortical areas (visual, motor and associative cortex) showed an alternating pattern of membrane potential between a depolarized level with superimposed action potentials (Up states) and a hyperpolarized level (Down states). The depolarizing phase matches with the presence of excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potential (IPSPs) simultaneously (Steriade, 2001), indicating that both excitatory and inhibitory neurons are synchronized at this frequency (<1Hz) (Steriade et al., 1993a).

The slow depolarizing envelope made by synaptic activity and superimposed spikes suggest that both intrinsic properties of neurons and synaptic activity contribute to the maintenance of this rhythm. In the cats, different anaesthetics were associated with slightly different frequencies of slow activity (urethane 0.3-0.4 Hz, ketamine combined with nitrous oxide or xylaziner 0.6-1 Hz) (Steriade et al., 1993a). This activity does not appear in an isolated region in the cerebral cortex but, especially under anaesthesia, it occurs almost synchronously in large portions of the cortical mantle, due to cortico-cortical connections (shown in Figure 3, SWS side) (Amzica and Steriade, 1995a). When these connections are interrupted the synchronization disappears (shown in Figure 3, Wake side) (Amzica and Steriade, 1995b). The slow oscillations may also be observed in thalamocortical and thalamic reticular nucleus neurons as well as the caudate nucleus, the subthalamic-pallidus network, basal forebrain, mesopontine, and medullary brain stem (Bevan et al., 2002; Magill et al., 2000; Steriade, 2006; Wilson and Kawaguchi, 1996), due to the interplay between cortical and subcortical structures (Steriade et al., 1993b). Their cortical origin is highlighted by the observation of their persistency in the cerebral cortex of anesthetized cats after injuring the thalamus by injection of kainic acid at several different thalamic sites (Steriade et al., 1993c). Furthermore, they disappeared from the thalamus in decorticated cats (Timofeev and Steriade, 1996) and were present in ferret isolated cortex preparations in vitro (Sanchez-Vives and McCormick, 2000).

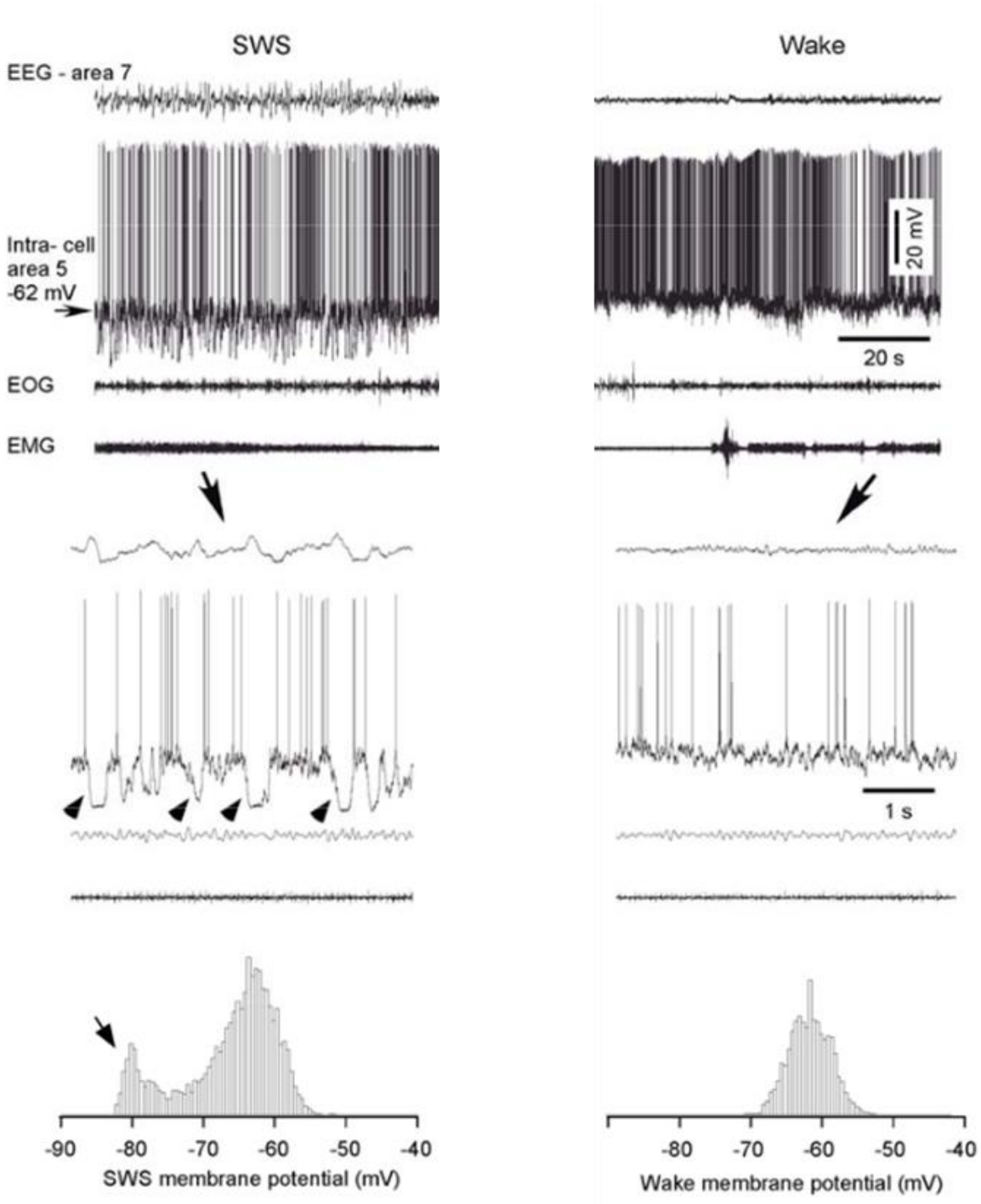

Figure 3. Cortical intracellular correlates of natural slow-wave sleep (SWS) and waking states. The four traces depict (from top to bottom): EEG from area 7, intracellular activity of area 5 RS neuron (membrane potential is indicated, -62 mV), EOG and EMG. High-amplitude and low-frequency field potentials, intracellular cyclic hyperpolarizing potentials and stable muscle tone are distinctive features of SWS. Low-amplitude and high-frequency field potential oscillations, tonic firing with little fluctuations in the membrane potential, and muscle tone with periodic contractions are characteristics of the waking state. The histograms of membrane potential in SWS and wake are illustrated below. Note the bimodal distribution of the membrane potential and the presence of hyperpolarizing mode of membrane potential during SWS (indicated by arrow). Modified from Timofeev et al., 2001.

Following the initial studies in anaesthetised cats, slow wave activity was also demonstrated during NREM sleep in cats (Steriade et al., 1996) and humans (Achermann and Borbély, 1997; Amzica and Steriade, 1997; Steriade et al., 1993b). Timofeev and colleagues compared the properties of slow oscillations during ketamine-xylazine anaesthesia and NREM sleep using extracellular and intracellular recordings in cats (Chauvette et al., 2011). During slow wave sleep, Local Field Potential (LFP) spectra showed higher power in the slow/delta (0.1-4 Hz) and spindle frequency range, while under anaesthesia the power in gamma band was higher. Moreover, slow oscillations during anaesthesia were more rhythmic and synchronous.

The intracellular recordings showed that Down state duration was longer and the amplitude of membrane potential around the transition between Up and Down state was greater under anaesthesia (Chauvette et al., 2011).

The slow oscillations have been described also in cortical slices maintained in vitro. Sanchez-Vives and McCormick showed that isolated cortical slices generate slow oscillations in the absence of neuromodulators (Sanchez-Vives and McCormick, 2000).

Oscillations of the local field potentials (LFPs) or electroencephalogram (EEG) at frequencies around 1 Hz are the hallmark of the slow-wave sleep. By means of high-density (256 channel) scalp EEG recordings, Massimini and collaborators described this activity in humans as a travelling wave which originates in a particular cortical area and travels across the cortex, following an anteroposterior gradient (Massimini et al., 2004).

However, the timing and the precise features of the underlying cellular events (i.e. the alternation of active and silent states of thalamocortical network) can be assessed from the phase of slow waves, as recorded from the cortex or the scalp. With simultaneous recordings of the LFP and intracellular activity of 2–3 neocortical cells, Mukovski and collegues showed that high–gamma-range (20–100 Hz) components in the LFP have significantly higher power when cortical cells are in active states as compared with silent-state periods (Figure 4) (Mukovski et al., 2007).

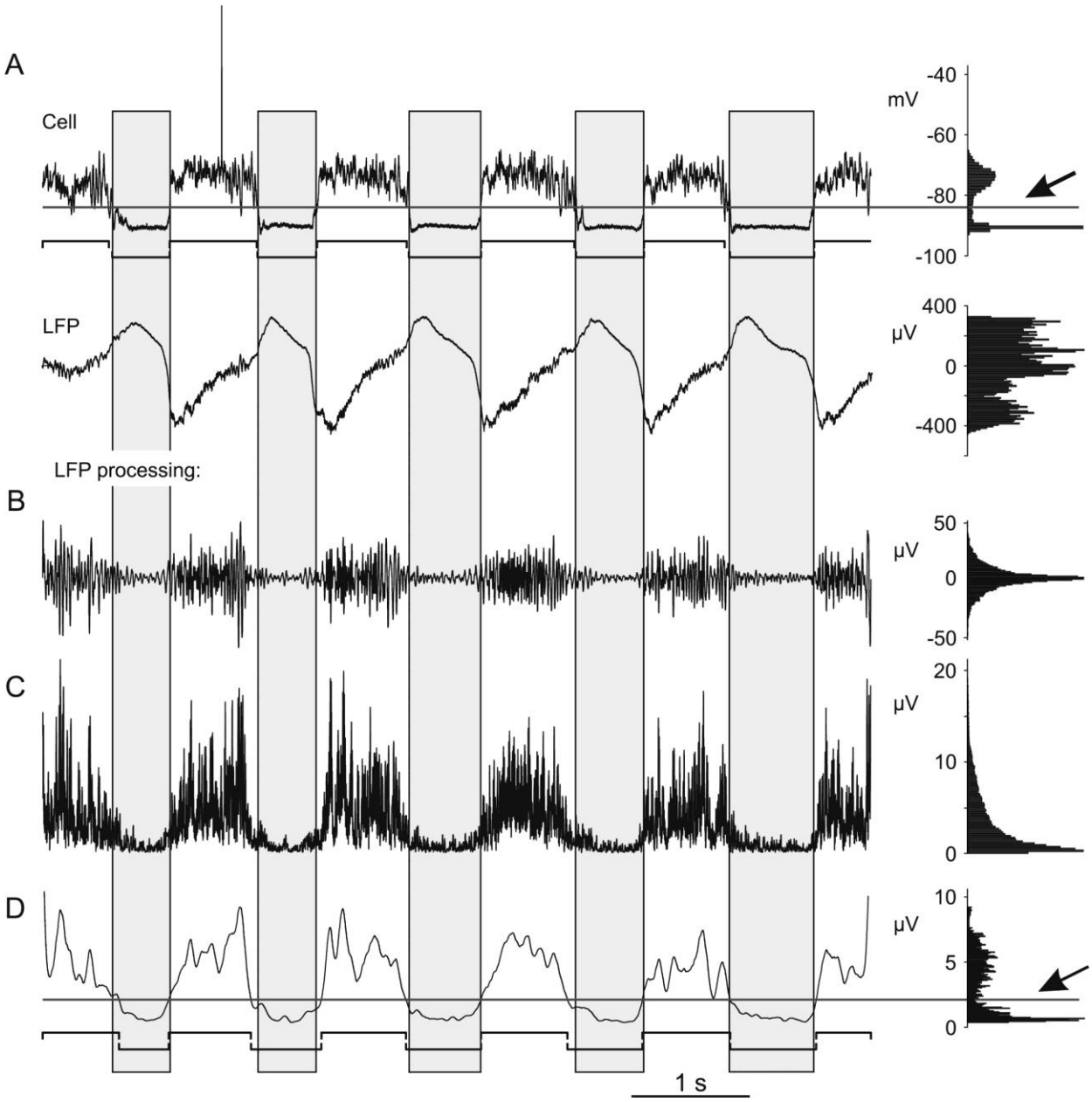

Figure 4. Processing of the LFP signal for detection of active and silent states. Simultaneously recorded membrane potential and LFP signal (A) and sequential steps of the LFP processing (B–D). (A) Simultaneously recorded membrane potential and LFP signal. The oblique arrow shows a gap in the bimodal distribution of the membrane potential. The horizontal line shows the level for separation of active and silent states in the membrane potential trace. Active and silent states in the cell are marked with horizontal bars below the membrane potential trace. The grey vertical bars mark periods of silent states in the cell. Histograms on the right-hand side show distributions of membrane potential and LFP signal (A) or processed LFP (B–D). (B) Extraction of the frequency components, which are most different between active and silent states, by band-pass filtering in the range 20–100 Hz. (C) Standard deviation of the filtered LFP calculated in a running window of 5-ms length. This operation is equal to root mean square in this case. (D) Linear filtering (50ms frame) of the signal from (C). The distribution of the processed signal is clearly bimodal; the oblique arrow shows the gap between the modes. The horizontal line shows the level, which was used to separate active and silent states. Detected states are shown with horizontal bars below the trace. Note that the states detected in the LFP correspond, with little deviations, to the states detected from the intracellular recording. From Mukovski et al., 2007.

In the last several years, slow wave activity has been investigated from a different point of view, focusing on which types of neurons are involved, whether it is an important feature of modulation of behaviours, such as memory and learning (Diekelmann and Born, 2010; Huber et al., 2004; Mander et al., 2013; Takashima et al., 2006), as well as whether different brain disorders are reflected in alterations of such slow rhythms (Diederich et al., 2009; Kyung Lee and Douglass, 2010; Landsness et al., 2011; Plante et al., 2012; Soekadar et al., 2013; Tasali et al., 2008).

In summary, several features that modulate slow oscillations have been established (Sanchez-Vives et al., 2010):

- Slow oscillations can be generated by isolated cortical networks, in which travelling waves propagate by mean of cortico-cortical connections (Sanchez-Vives and McCormick, 2000).
- Slow oscillations recruit cortical neurons, both excitatory and inhibitory, that fire during the Up state, whereas they remain in relative silence during the Down state (Steriade et al., 1993b).
- There is an excitatory-inhibitory balance, which is present during the whole Up state (Compte et al., 2008, 2009; Shu et al., 2003).
- Synaptic inhibition has a central role in the modulation. As synaptic inhibition decreased, there is an increase in firing rate in Up states, with a corresponding decrease in the frequency of Up/Down cycles and an increase in the duration of the down state.
- Computational models and experimental results demonstrate that K+ currents have a central role in the synaptic inhibition mechanisms.
- Hyperpolarizing currents are involved in the termination of the Up state.
- K+ channels are involved in the regulation of the network activity.
- Recurrent glutamatergic excitation is a key element in controlling network excitability and oscillatory properties.
- Transition between Down and Up states originate from cortical layers 5 and 6.
- The maximum activity is found in layer 5 and can persist in layer 6. Those activities reflect the nearby cortical columns activations contributing to the travelling Up-states.
- The cortical origin of Up state onset was further confirmed by studies employing thalamic inactivation. This was not associated with changes in the spatial distribution of events characterizing Up state onsets. On the other hand, the thalamic input was found to contribute to the maintenance of Up states.

- The collected evidence for both Up state initiation and termination confirms cortico-cortical connectivity as the main drive for Up/Down Slow oscillations; the thalamus has only a marginal role.

Slow oscillations derive from bistable patterns of activation that can occur locally

Altogether, those findings indicate that sleep is a neuronal property and that it can be regulated at the local level of cortical columns (Krueger et al., 2008). Furthermore, the density of intracolumnar connections is higher than the intercolumnar ones, favouring state segregation and allowing state synchrony between cells pertaining to the same column (Panzeri et al., 2003).

In 1993 Kruger and Obal (Krueger and Obál, 1993) postulated that sleep begins as a local event, involving oscillations of inhibition and excitation and is thus "quantal" in nature. Neuronal and humoral systems bring neurons in the bistable state.

Among others, the proposal by Tononi and Cirelli developed in the beginning of the new millennium, namely the Synaptic Homeostasis Hypothesis (SHY; Tononi and Cirelli 2003), might explain the function of sleep and is in line with the local nature of sleep. Tononi and Cirelli claim that during wakefulness plasticity increases or decreases in specific brain areas and, in turn, sleep intensity increases or decreases in those areas, as reflected by the amount of slow wave activity (Kattler et al., 1994; Huber et al., 2004; Vyazovskiy and Tobler, 2008; Hanlon et al., 2009; Lesku et al., 2011). Additionally, the generation of local slow waves could be caused by a change in the excitability or the amount of adaptation of each individual neuron. Thus, the need for cellular maintenance could cause individual neurons to show lower excitability and stronger adaptation (Vyazovskiy et al., 2013). Along these lines, OFF-periods might occur locally where most needed, providing a potential explanation for local sleep patterns. On the other hand, when individual neurons fall below a certain cellular stress threshold, their excitability is restored, leading to a wake-like pattern of activity.

For these reasons, a new definition of sleep which refers more to the underlying neuronal mechanism, rather than the behaviour, occurs: "synchronization of neuronal OFF-periods".

Chapter 2. Assessing cortical bistability with perturbations

At the cortical level, the key feature of NREM sleep is the occurrence of OFF-periods, reflecting a profound hyperpolarization in the membrane of cortical neurons. This phenomenon, often referred to as cortical bistability, is caused by the enhancement of adaptation (or activity-dependent) K+ currents, brought about by decreased levels of neuromodulation from brainstem activating systems and/or by increased inhibition (Rosanova et al., 2018). During sleep, the synchronous bistable dynamics of cortical neurons is reflected at the extracellular level in large slow waves underpinned by high-frequencies (>20Hz) suppression. These features are detectable by spontaneous activity at the EEG (Piantoni et al,2013; Menicucci et al, 2013). Furthermore, due to the activity-dependent nature of bistability, it is better revealed using perturbations that can probe directly the impulse-response properties of the cortex. Indeed, a direct cortical perturbation may trigger activity-dependent K+ currents and an OFF-periods even when K+ channels are de-inactivated. It could also recruit local inhibitory circuits, leading to OFF-periods when the excitation–inhibition level is more unbalanced towards the latter. Finally, it may force hyperpolarized thalamocortical neurons to fire bursts of action potentials back to the cortex and then fall into a prolonged silence when these cells are in a bursting mode.

In other words, perturbations could reveal the presence of adaptation mechanisms regardless of their background EEG pattern, of the prevalence of spontaneously occurring slow waves and of the pre-stimulus ongoing activity (Rosanova et al., 2018).

Transcranial magnetic stimulation (TMS)

A direct, non-invasive way to perturb the cortical surface of the brain involves the combination of navigated TMS and high density (hd)-EEG (Ilmoniemi et al., 1997) (Figure 5). TMS/hd-EEG was first implemented by Ilmoniemi and colleagues (Ilmoniemi et al., 1997) and further developed by other investigators (Bonato et al., 2006; Ferrarelli et al., 2012, Ferreri et al., 2011; Massimini et al., 2005; Miniussi and Thut, 2010; Rosanova et al., 2009).

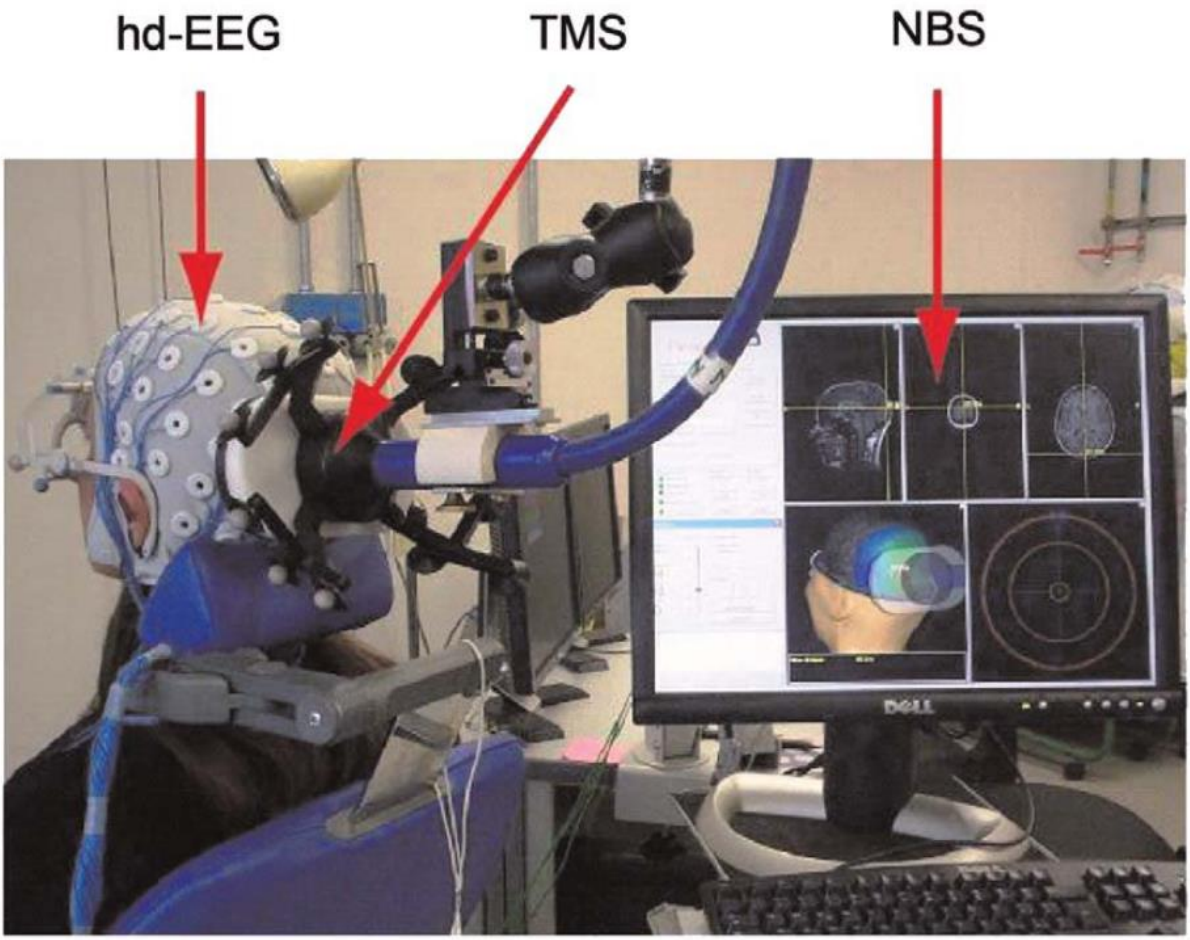

Figure 5. TMS/EEG setup. In this example, a subject is sitting on ergonomic chair while TMS is targeted to occipital cortex. The red arrows indicate, from left to right, the three fundamental elements that compose the set-up: (1) a cap for high-density (60 channels) EEG recordings (hd-EEG) that is connected to a TMS-compatible amplifier; (2) a focal figure-of-eight stimulating coil (TMS), held in place by a mechanical arm; (3) the display of the navigated brain stimulation system (NBS). This system employs an infrared camera (not visible in this picture) to navigate TMS on a 3D reconstruction of the subject's MRI. The location and the intensity of the electric field induced by TMS are estimated and displayed in real time. To prevent the subject from perceiving the click associated with the coil discharge, noise masking is played through inserted earplugs.

TMS is based on the physical principle of electromagnetic induction, discovered by Faraday in 1831. Faraday's experiments showed that currents (and voltages) were only induced by a changing, or "time varying" magnetic field, and not by a static field. If a pulse of current passing through a coil placed over a person's head has enough strength and short enough duration, rapidly changing magnetic pulses are generated that penetrate scalp and skull to reach the brain with negligible tissue attenuation. Those magnetic pulses induce secondary ionic currents in the brain (eddy currents) that penetrates the membranes of the neurons, resulting in excitatory or inhibitory postsynaptic potential.

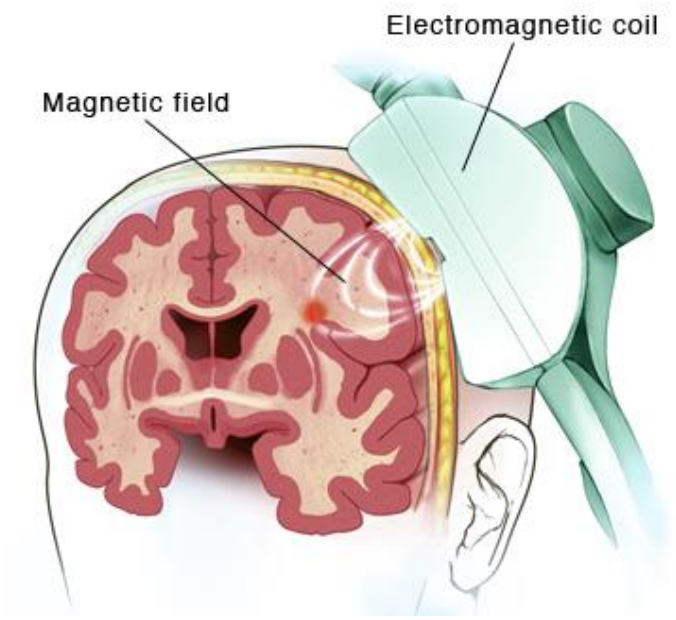

Figure 6. Schematic illustration of Transcranial Magnetic Stimulator. From the Internet (© Mayo Foundation for medical education and research. all rights reserved).

The magnetic field fades off rapidly with distance from the coil (Roth et al., 1991) (illustrated in Figure 6), so it is usually assumed, unless the stimulus intensity is very high at the surface of the brain, that the stimulus activates neural elements in the cortex or in the subcortical white matter (limiting direct stimulation to the outer parts of the cerebral cortex under the skull).

The neuronal elements that are directly excited by the TMS are still unclear. However, experimental and modelling studies strongly suggest that axons, rather than cell bodies, are the most likely targets of the stimulus as they have the lowest threshold for activation to the brief electrical currents induced by TMS. Most mathematical models of neuronal stimulation are further evolutions of the Hodgkin and Huxley model. In this class of models, the transmembrane potential, V, is mathematically represented by the following equation:

$$\lambda^2 \frac{\mathrm{d}^2 V}{\mathrm{d}^2 x^2} - V = \tau \frac{\mathrm{d}V}{\mathrm{d}t},$$

where λ and τ represent respectively the axonal membrane space and the time constant.

The capacity of TMS to depolarize neurons depends on the "activating function", which causes enough transmembrane current to flow and depolarize the membrane. In order to represent also this

external current source applied to the axon, such as the one induced by the TMS, the previous equation can be modified by adding an "activating function" as follows:

$$\lambda^2 \frac{\mathrm{d}^2 V}{\mathrm{d}^2 x^2} - V = \tau \frac{\mathrm{d}V}{\mathrm{d}t} + \lambda^2 \frac{\mathrm{d}^2 A}{\mathrm{d}x\,\mathrm{d}t}$$

According to this mathematical model (Roth et al., 1991), stimulation will take place at the point where the spatial derivative of the induced electric field is maximal. Modelling (Abdeen and Stuchly, 1994; Ruohonen and Ilmoniemi, 1999) together with experimental studies conducted on peripheral axons in vitro (Maccabee et al., 1993) confirmed this showing that axon fibres are more sensitive to electric fields induced by TMS where they bend, i.e., where the spatial derivative of the induced electric field is maximal (see Figure 7).

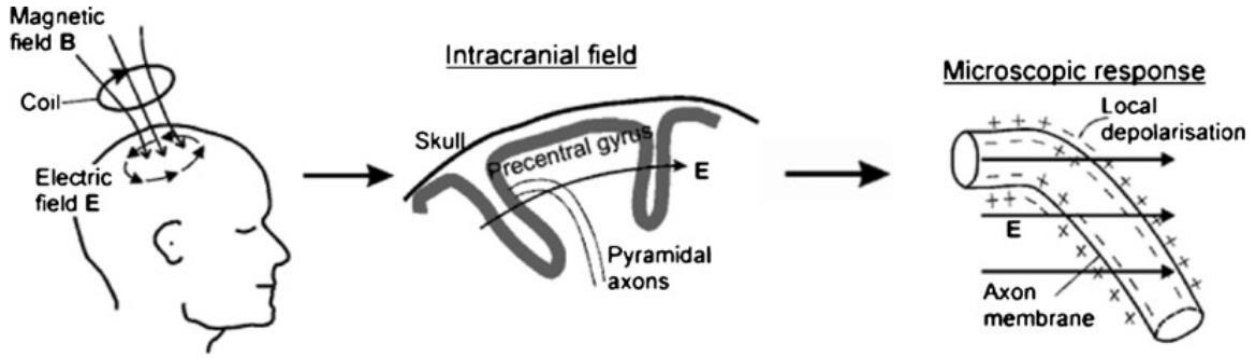

Figure 7. Neural targets of TMS. From left to right a schematic representation of the electric field (E) induced into the subject's head by the magnetic field (B) after a brief pulse of electrical current is passed through the TMS coil; the macroscopic (cortical gyri) and microscopic targets (bent axons) of the electric field E induced intracranially by the TMS coil (reproduced from Ruohonen and Ilmoniemi, 1999).

From the site of cortical stimulation, neuronal excitation propagates along the available connection pathways, via intra and interhemispheric association fibres, to other cortical areas and deeper neural structures and, via projecting fibers, to subcortical structures and the spinal cord.

TMS-compatible hd-EEG

Using multi-channel EEG amplifiers that are compatible with TMS (Virtanen et al., 1999) one can record, starting just a few milliseconds after the pulse, the impact of the perturbation on the stimulated target and in distant cortical areas.

As described above, with TMS the cerebral cortex is stimulated directly by generating a brief but strong magnetic pulse (<1ms, 1-2 T) through a coil applied to the surface of the scalp. The rapid change in magnetic field strength induces a current flow in the tissue, which results in the activation of the underlying neuronal population. The synchronous volley of action potential initiated in this neuronal population propagates along the available connection pathways and can produce significant electrical activations in different cortical regions. When applying TMS simultaneously with EEG recordings, one issue to overcome is the powerful electric field that is induced by the discharge of the TMS coil on the electrical leads. Considering a typical pulse intensity of about 1 T and a rise time of 0.1ms, the voltage induced in the electrodes underlying the stimulator can reach an amplitude of 10 V. This voltage, being several orders of magnitude larger than the signal produced by the brain (tens of microvolts) can cause large artifacts in the recordings and may put an ordinary EEG amplifier out of the operating range for a few seconds.

Today, it is possible to deal with this electric artifact induced by employing specific hardware devices or with an on-line artifact subtractions. Virtanen and colleagues (Virtanen et al., 1999) developed a 60-channel TMS-compatible EEG system (shown in Figure 8) that includes gain-control and sample-and-hold circuits to block the artifact induced by TMS in the leads. This system pins the acquired signal to a constant level for a couple of milliseconds around the pulse and records TMS-induced EEG potentials that are completely free from artifacts. Similarly, Iramina and colleagues (Iramina et al., 2003) recorded EEG responses to TMS by means of a TMS-compatible amplifier provided with a sample-and-attenuate stage that actively reduces the signal during the TMS pulse. An alternative way to deal with the TMS artifact has been implemented by Thut and his colleagues (Thut et al., 2005). They used a slew-limited amplifier that prevents the electronics from saturating during the TMS pulse resulting in a short-lasting artifact that decays within 30ms. Finally, Bonato and colleagues (Bonato et al., 2006) have used an MRI-compatible DC amplifier with a wide dynamic range to successfully record TMS-evoked potentials preceded by a short artifact lasting between 10 and 20 ms. With this method, recordings must be obtained without any filtering, as these might interact with the TMS artifact, producing ripples for up to a second.

Figure 8. TMS Compatible EEG cup. From the Internet.

Recently, Litvak and colleagues (Litvak et al., 2007) have proposed an offline method to effectively reduce the TMS-induced artifact. This method may be in principle applied also to signals recorded by a wide dynamic range, 24-bit 64 channels EEG amplifiers, even if they are not TMS-compatible. The method is based on a multiple step procedure as follows: (1) Single TMS-EEG trials are interpolated by means of a spline function within the 12 ms around the stimulus (-2 to 10 ms); (2) Poststimulus residual artifacts are removed by calculating the average TMS-EEG responses across all trials, computing their topographies based on principal component analysis (PCA) over a limited, manually defined time-window. The procedure has been used to compare, at a group level, local and spatially extended EEG responses to TMS recorded in schizophrenic patients and healthy controls, replicating results obtained by other labs by using dedicated TMS/EEG equipment (Levit-Binnun et al., 2010). Although the algorithm designed by Litvak and colleagues is not suitable for analyses at single subject level, at least in principle it can be employed by all those researchers and clinicians that do not own a TMS-compatible EEG amplifier, yet are interested in studying connectivity and excitability in neuropsychiatric disorders. Nowadays, several studies have demonstrated that it is possible to record artifact-free TMS-evoked potentials that reliably reflect the state of excitability of underlying cortical circuits (Bonato et al., 2006; Esser et al., 2006; Kähkönen et al., 2005; Massimini et al., 2005).

Navigated brain stimulation (NBS)

The precise and reproducible placement of the TMS coil over the cortical area of interest is a challenging task. In order to measure local excitability and effective connectivity at different cortical sites and across different subjects or populations, it is important to localize precisely the cortical areas to be targeted by the stimulator. Various methods may be applied to perform a precise and reproducible cortical targeting.

Up to a few years ago, only two methods were commonly employed to achieve reproducible targeting within and across individuals. The first method involves the estimation of the motor threshold (MT), i.e., the lower output of the TMS stimulator able to elicit a consistent and visible twitch of a peripheral muscle, typically of the thumb contralateral to the stimulated hemisphere. In order to stimulate other cortical areas than the motor cortex, the coil was moved referring to the location used to estimate the MT. The stimulation intensity was expressed in terms of percentage of MT. A second method is based on the 10-20 EEG electrode system and assumes that there is a consistent matching between EEG electrodes locations and underlying cortical regions across subjects. This method does not account for the interindividual variability of the skull shape and may lead to errors up to 20 mm in different directions (Herwig et al., 2003). Nevertheless, this approach is fast, economical and may be viable when studying large cortical regions.

To target the same regions in different subjects with higher accuracy and on a finer anatomical scale, one must employ a neuroimage-guided, stereotactic neuronavigation system: this strategy requires the acquisition of either structural or functional MRIs or CT scans. In addition, single-subject functional neuroimaging or average functional atlases ("probabilistic approach") can be used to guide the brain navigator (Paus et al., 1997). Regardless of the neuroimaging data employed, the stereotactic navigation systems can locate the relative positions of the subject's head and the TMS coil by means of an optical, or magnetic, tracking system. The system may also consider the individual's head shape, the coil position and scalp-to-cortex distance to calculate the electric field induced by TMS on the cortical surface. In this case, during the experiments, the TMS intensity may be adjusted according to the maximum electric field intensity (expressed in Volts/meter) estimated on the cortical surface, rather than relying on individual motor threshold, or on the percentage of maximum stimulator output.

Although these estimations are indicative, they should always be interpreted cautiously, since they may include very large errors due to coregistration/navigation mismatches. In these cases, correcting maximal stimulator output simply based on scalp-to-cortex distance (Stokes et al., 2005) is the safest option. To further standardize stimulation parameters, the maximum of the electric field should always

be on the convexity of the targeted gyrus, with the induced current perpendicular to its main axis. With most systems, the coordinates of the stimulator are usually input to a virtual aiming device of the navigation software and can be used during the experiment to ensure stability of the position, angle, direction, and intensity of the stimulation. The neuronavigation systems also allow digitizing the electrode positions by means of a pen visible to the optical tracking system as well as to store this information in the navigation computer, at the end of each experimental session. This allows performing off-line, accurate source modelling of the TMS-evoked EEG responses.

By integrating TMS with MR-guided infrared navigation systems, it is also possible, in most cortical regions, to render the perturbation controllable and reproducible.

In light of the above, the integrated use of neuro-navigation systems, TMS and multichannel TMS-compatible EEG amplifiers constitute a brain scanning method in which stimulation is navigated into any desired brain target and the concurrently recorded scalp potentials are processed into source images of the TMS-evoked neuronal activation (Komssi and Kähkönen, 2006). It is worth highlighting some of the specific advantages that TMS-EEG may offer as a tool to probe the brain:

1) TMS-evoked activations are intrinsically causal (Paus, 2005). Thus, unlike methods based on temporal correlations, TMS-EEG immediately captures the fundamental mechanism that underlies integration, that is the ability of different elements of a system to affect each other.

2) TMS-EEG bypasses sensory pathways and subcortical structures to probe directly the thalamocortical system. Therefore, unlike peripherally evoked potentials and evoked motor activations, TMS-EEG does not depend on the integrity of sensory and motor systems and can access any patient (deafferented or paralyzed). Moreover, with TMS one can stimulate most cortical areas (including associative cortices) employing several different parameters (intensity, angle, current direction), thus probing a vast repertoire of possible responses, above and beyond observable ongoing brain states.

3) TMS-evoked potentials can be recorded with millisecond resolution, a time scale that is adequate to capture effective synaptic interactions among neurons.

4) TMS-EEG does not require the subject to be involved in a task and the observed activations are not affected by the willingness of the subject to participate nor by his effort and performance. Hence, this approach is well suited to assess the objective capacity of thalamo-cortical circuits independently of behaviour.

5) TMS-EEG can be made portable in order to overcome the logistical and economic hurdles that may separate patient populations of interest from advanced imaging facilities.

Studying bistability and its impact on cortical networks

By employing TMS-EEG, it is possible to measure the strength of immediate reaction of the stimulated cortical area (an index of cortical excitability) as well as the strength of the subsequent long-range activations (an index of cortical connectivity). Moreover, by recording the dynamics of the cortical response to TMS it is possible to infer on the complexity of the underlying network (Massimini et al., 2009), also considered to be a reliable neural correlate of consciousness. Complex behaviour depends on the ability of multiple, functionally specialized (functional specialization) cortical areas to sustain balanced patterns of reciprocal interactions (functional integration).

The ability to integrate information can only be demonstrated from a causal perspective. Thus, one must employ a perturbational approach (effective connectivity) and examine to what extent subsets of neurons can interact causally as a whole (integration) to produce responses that are specific for that particular perturbation (information) (Massimini et al., 2009). Moreover, one should probe causal interactions by directly stimulating the cerebral cortex, in order to avoid possible subcortical filtering or gating. Finally, since causal interactions among thalamocortical neurons develop on a sub-second time scale, it is very important to record the neural effects of the perturbation with the appropriate temporal resolution. In short, one should find a way to stimulate different subsets of cortical neurons and measure, with good spatial-temporal resolution, the effects produced by these perturbations in the rest of the thalamocortical system.

Brain networks in which functional integration is lost will react to TMS with a response that is simple because it is local. Similarly, networks in which functional specialization is lost will react to TMS with a response that is simple, because it is redundant. Only complex networks, where functional specialization and functional integration are balanced, will react to TMS with a complex response where many integrated areas react in a distinct, differentiated way (Massimini et al., 2009).

By applying TMS-hdEEG (Casali et al., 2013; Massimini et al., 2005; Tononi and Massimini, 2008) and intracranial (Pigorini, 2014; Pigorini et al., 2015) electrical stimulation Massimini and colleagues observed that, compared to wakefulness, during (NREM) sleep the composite fast response was replaced by a large, stereotyped slow wave (altered excitability) and by a clear-cut reduction of the spatial spread of the response (reduced connectivity), as shown in Figure 9 (Massimini et al., 2005).

Overall, findings from these studies suggest that the evoked slow waves and the associated OFF periods impact local and global cortico-cortical information transmission and, in turn, the overall network connectivity and complexity. The same stereotyped, low complexity TMS-evoked response observed during NREM sleep was obtained in unconscious subjects undergoing general anaesthesia

induced with propofol, midazolam as well as xenon (Ferrarelli et al., 2010, Sarasso et al., 2015). Moreover, in these conditions, the slow wave-like initial response was associated with a dramatic reduction of the complexity of the ensuing TMS-evoked EEG activity (Casali et al., 2013). Interestingly, these slow waves evoked by direct cortical perturbations matched the EEG criteria for a sleep slow wave, or a K-complex (Massimini et al., 2007). As shown by animal (Steriade et al., 2001) and human (Cash et al., 2009) intracranial recordings, both EEG sleep slow waves and K-complexes are underpinned by the occurrence of a silent, hyperpolarized OFF period in cortical neurons after an initial activation (ON period). This bimodal alternation between ON and OFF periods reflects the aforementioned intrinsic bistability in thalamocortical circuits that is thought to depend on neuronal and network properties (Compte et al., 2003; Sanchez-Vives and McCormick, 2000; Steriade et al., 1993a) and it is mainly caused by an increased activity of leak K+ channels, brought about by decreased brainstem cholinergic activity (McCormick et al., 1993).

In light of this, the authors interpret their findings proposing that during NREM sleep and anaesthesia, due to bistability, portions of the thalamocortical system, which are otherwise healthy, are no longer able to sustain balanced patterns of activation induced by the TMS pulse because of the inescapable occurrence of a local OFF (the down state) period that prevents the emergence of complex, long-range patterns of activation. Similarly, exploiting an intracerebral perturbational approach, Pigorini and colleagues showed that the slow waves evoked during NREM by intracerebral stimulation are underpinned by a suppression of high frequencies (>20 Hz) of the local field potential (LFP) following an initial activation (Pigorini et al., 2015; Figure 10). Interestingly this behaviour strongly reflects the features of spontaneous sleep slow wave (see Figure 4), and therefore, can be used as a proxy to detect the occurrence of cortical down state (Cash et al., 2009; Valderrama et al., 2012).

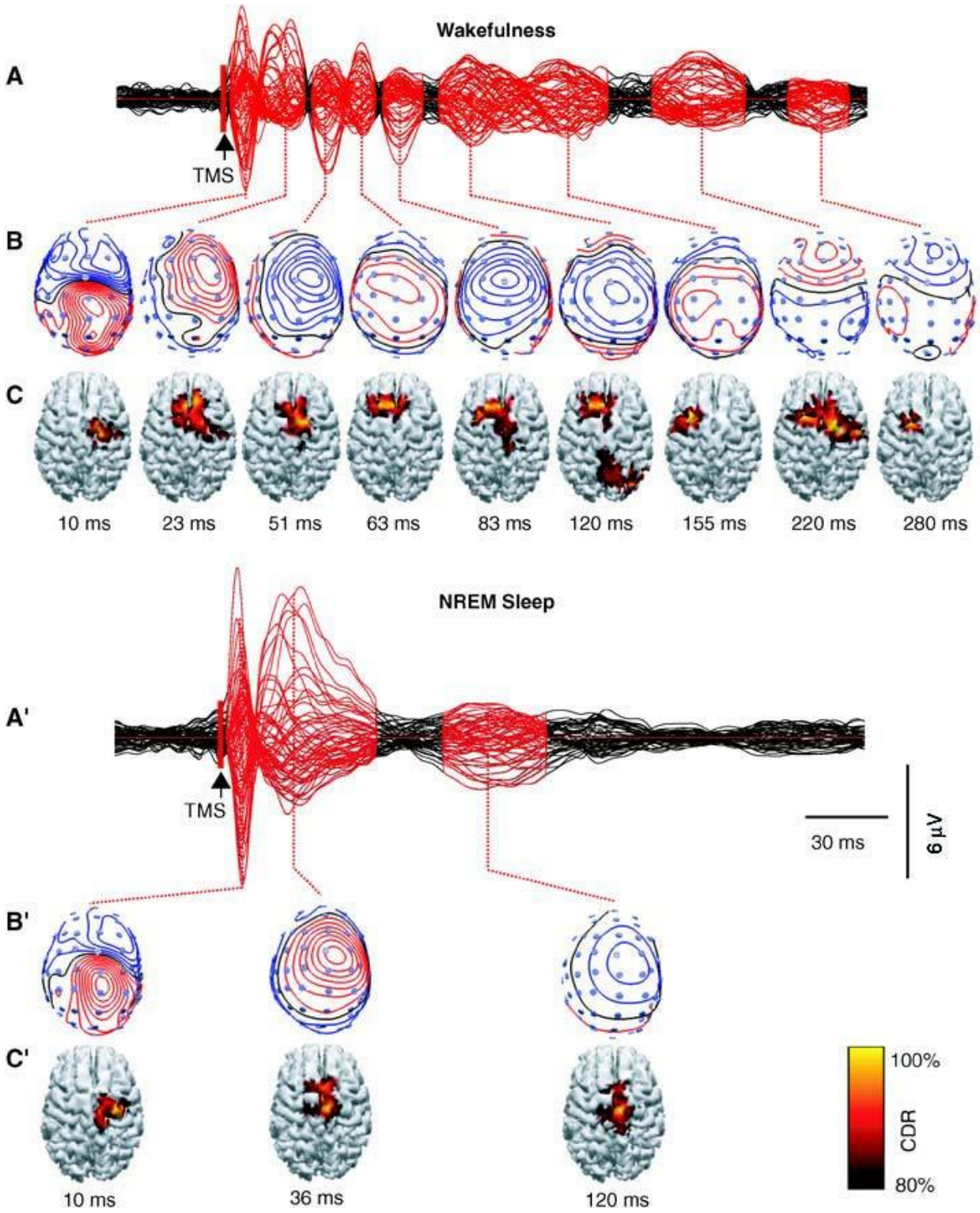

Figure 9. Spatiotemporal dynamics of scalp voltages and cortical currents evoked by TMS during wakefulness (A) and sleep (A'). Averaged TMS-evoked potentials recorded at all electrodes, superimposed in a butterfly diagram (black traces; the horizontal red line indicates the average reference), for the same subject. The time of TMS is marked by a vertical red bar. The red portions of the traces indicate the times at which TMS induced a significant response. From Massimini et al., 2005.

Furthermore, in the aforementioned work of Pigorini and colleagues, this effect has been shown to occur globally during NREM sleep and is reflected in the break-off of phase-locked responses to a perturbation (Pigorini et al., 2015) as measured by the phase locking factor (PLF) (Palva et al., 2005).

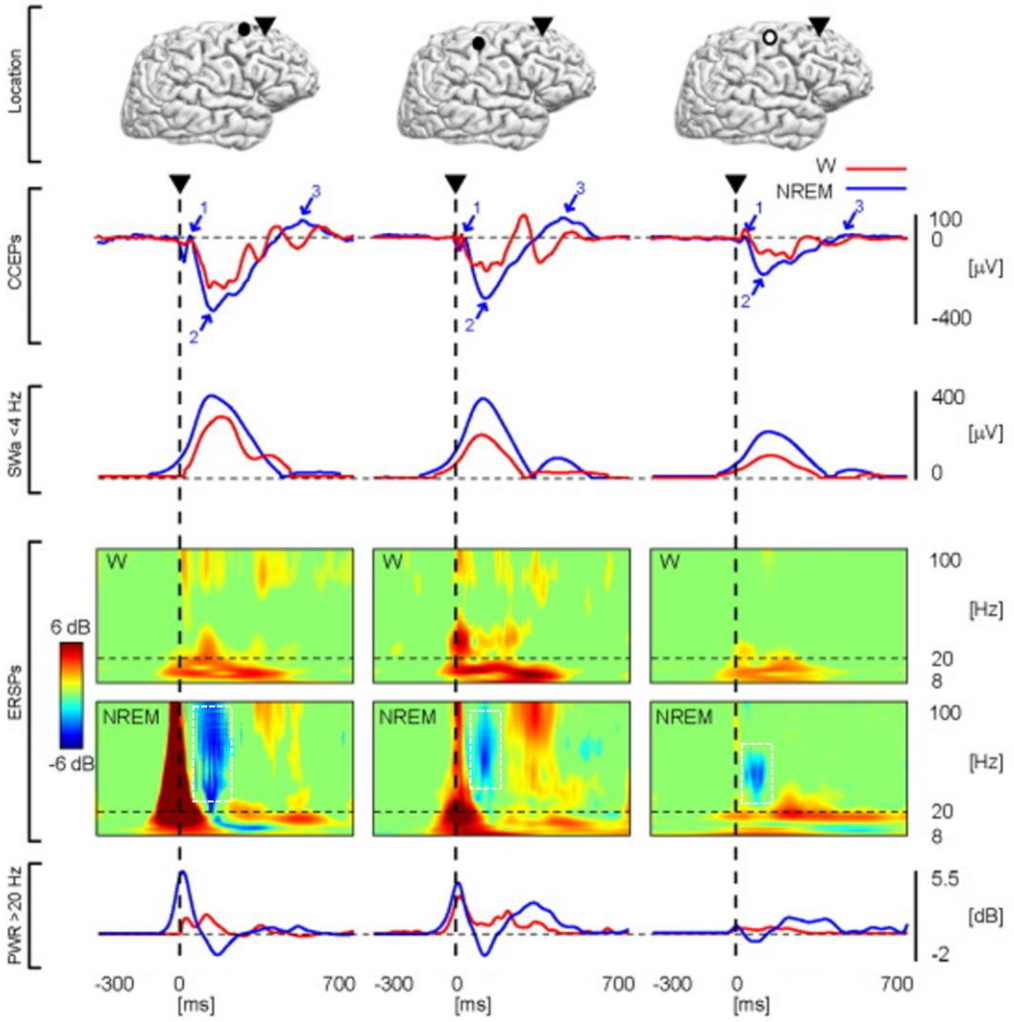

Figure 10. During NREM, Single Poulse Electrical Stimulation (SPES) triggers a slow-wave-like response that is associated with high frequency. Location: the position of the stimulating contact is depicted (black triangle) over a 3D brain reconstruction (lateral view) of the individual's brain (Subject 1). Black circles and white circles show the position of three representative recording bipolar derivations from right and left hemisphere, respectively. Cortico-Cortical Evoked Potentials (CCEPs): the corresponding average responses from these contacts during wakefulness (W-red) and NREM (NREM-blue). Blue arrows and numbers indicate the three components of CCEPs evoked during NREM. SWa < 4 Hz: amplitude of the slow (< 4 Hz) wave component calculated as squared absolute value of the CCEPs after 4 Hz low-pass third order Chebyshev filtering. ERSPs: time-frequency power spectra of CCEPs recorded in W and NREM. Time-frequency decomposition is applied at a single trial level using Wavelet Trasform (Morlet, 3 cycles) and significance for bootstrap statistics is set with $\alpha < 0.05$. Blue colour indicates a significant reduction compared to the baseline, while red indicates significant increase. The dashed horizontal line indicates 20 Hz. PWR > 20 Hz: time series of high frequency power (> 20 Hz). Statistical differences from baseline are assessed (for each contact) by assuming a Rayleigh distribution of the values of the baseline (from – 300 ms to − 50 ms). Dashed vertical lines and triangles represent stimulus onset. Dashed white boxes indicate suppression of high frequencies (>20 Hz). Modified from Pigorini et al., 2015.

Similarly, in a recent work by Rosanova and colleagues reported the occurrence of bistable dynamics after TMS associated with a break-off of PLF in vegetative state patients, suggesting that a similar phenomenon may also occur due to brain injuries. Interestingly, those patients who evolved from the vegetative state to the minimally conscious state, and then emerged from the minimally conscious state showed a progressive recovery of EEG high frequency power and a restoration of PLF duration, closely matching their clinical evolution (Figure 11).

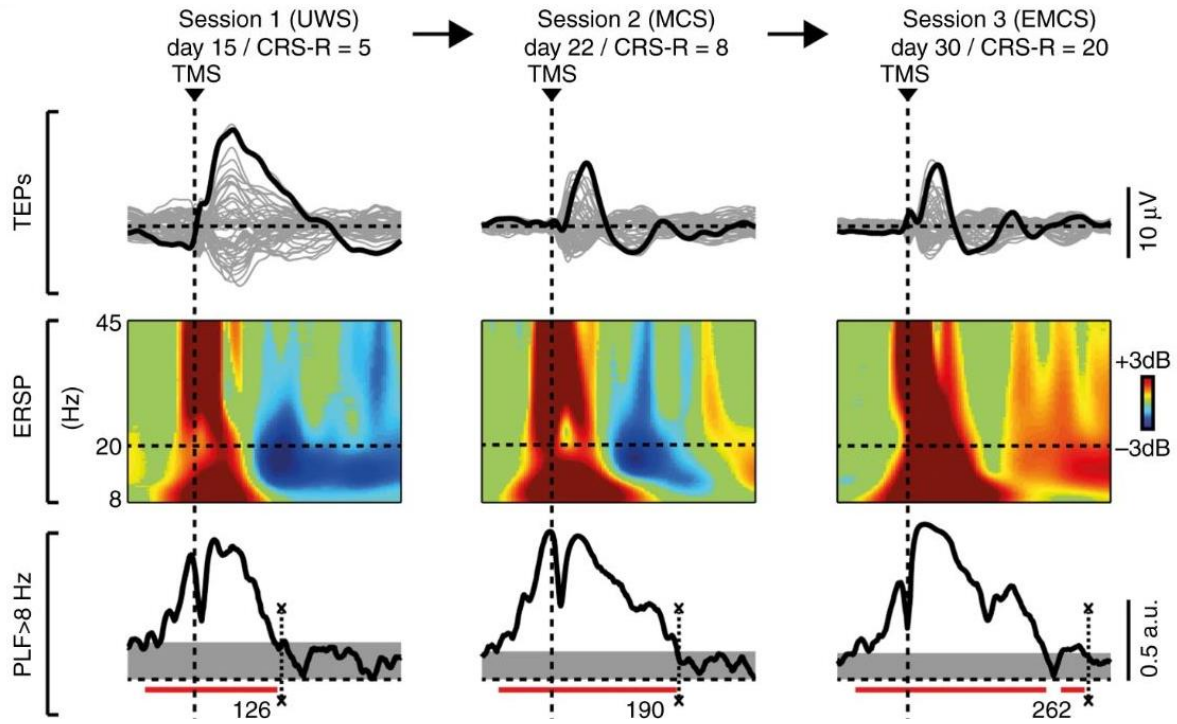

Figure 11. Longitudinal measurements in one UWS patient who evolved to EMCS, through MCS. In a representative patient the first behavioural and TMS/EEG assessments were carried out 48 h after withdrawal of sedation, as patient exited from coma. The butterfly plot of the TMS-evoked EEG potentials recorded from all 60 channels (grey traces), the corresponding ERSP and the PLF time course of the channel with the largest response are shown for each clinical diagnosis (Unresponsive Wakefulness Syndrome (UWS), Minimally Consciousness State (MCS), and Emerged from Minimally Conscious State (EMCS)). In the ERSP plot, red colour indicates a significant ($\alpha < 0.05$) power increase compared to the baseline, blue colour a significant power decrease and the green colour a non-significant activation. The dashed horizontal line marks the 20 Hz frequency bin. The last significant ($\alpha < 0.01$) time point in the PLF (above 8 Hz) is marked by a thin dashed vertical line. Time points above statistical threshold (grey shaded area) are underlined by a red horizontal line. The thick dashed vertical line indicates the occurrence of TMS. Adapted from Rosanova et al., 2018.

A key finding of the present work is the demonstration of a pathological form of sleep-like OFF-periods in the brain of UWS patients. Specifically, targeting neuronavigated TMS to intact portions of both their frontal and parietal cortices invariably elicited a stereotypical slow wave associated with a high-frequency (>20 Hz) suppression activity matching that of healthy sleeping subjects. Notably,

these sleep-like OFF-periods were never found when the same cortical areas were stimulated in awake healthy subjects (Rosanova et al., 2018, see Figure 12).

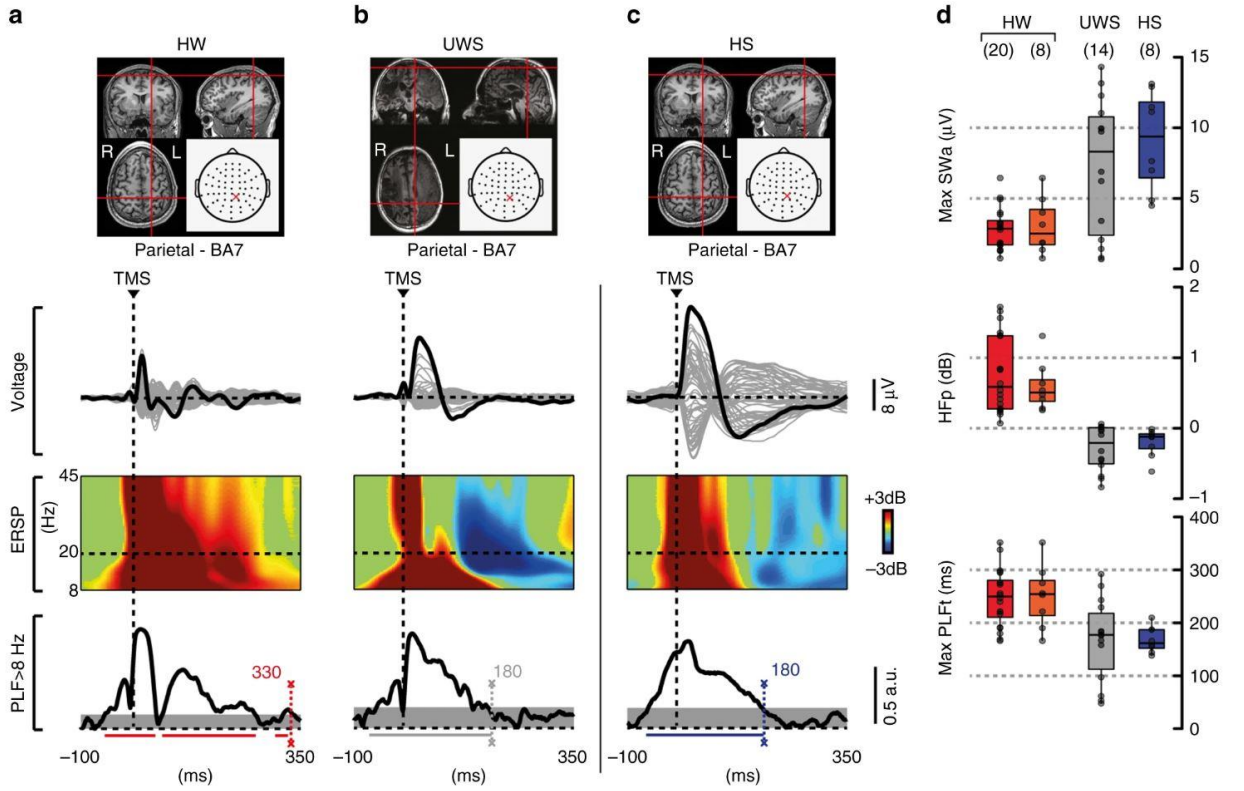

Figure 12. TMS evokes a sleep-like OFF-period and an early drop of PLF in UWS patients. Results for a representative healthy subject during wakefulness (HW) and NREM sleep (HS) and a representative UWS patient are shown for parietal stimulations (BA7). a–c MRIs and cortical targets as estimated by the Navigated Brain Stimulation system are shown (top). A dashed vertical line marks the occurrence of TMS. Butterfly plots of the TMS-evoked EEG potentials recorded at all 60 channels (grey traces) are depicted. Event-related spectral perturbation (ERSP) and PLF are presented for the electrode with the larger response (black trace). In the ERSP plot, significance for bootstrap statistics is set at $\alpha < 0.05$ (absence of any significant activation is coloured in green): statistically significant increases of power compared to baseline are coloured in red, while blue represents significant power decreases. The dashed horizontal line indicates the 20 Hz frequency bin. PLF time points above statistical threshold (grey shaded area) are indicated at the bottom by a coloured horizontal line. The coloured-dashed vertical line indicates the timing of the last significant ($\alpha < 0.01$) PLF time point. d From top to bottom, boxplots of slow wave amplitude (max SWa), high-frequency power (HFp), and duration of PLF (max PLFt) for HW (red and orange), HS (blue) and UWS (grey) are shown. Boxplot displays the median (centre line), the first and third quartiles (bounds of box). The whiskers extend from the bound of the box to the largest/smallest value no further than 1.5* inter-quartile range. Outlier datapoints are indicated by dots outside whiskers. From Rosanova et al., 2018.

The authors underpin the presence of sleep-like features because of an adaptation mechanism that cause the transient falling of intact portion of the thalamocortical system into a quiescent OFF-period. In extreme cases, the thalamocortical system might be largely intact but functionally constrained to a pathological tendency towards OFF-periods, due to a predominance of adaptation currents.

These findings show the presence of sleep-like features in awake human brains, due to brain injuries. This presence during wakefulness could reflect the presence of local sleep. Furthermore, this work showed a causal link between the functional impairment and the pathological presence of bistability, drawing a first link between neuronal events and global brain dynamics relevant for pathological loss and recovery of consciousness. This corroborate the hypothesis that bistability and OFF-periods may be in a key position to impair overall brain complexity.

In view of these results, detecting the presence of cortical sleep-like bistability and tracking its evolution over time, may offer an objective reference to titrate therapeutic strategies aimed at restoring consciousness. In this respect, it will be crucial to further elucidate the relationships between cortical bistability, neuronal OFF-periods through experiments across scales, species, and models. Studying in depth from ionic channel modelling to whole-brain simulations and macroscale measurements at the patient's bedside (Rosanova et al., 2018).

For these reasons, it would be critical to quantify the presence of differentiation and integration in cortical networks (Casali et al, 2013), a crucial requirement for consciousness according to theoretical neuroscience (Tononi et al, 2016). Considering this premises, I will describe an available measure able to respond to this need.

Cortical complexity

Complex behaviour depends on the ability of multiple, functionally specialized (functional specialization) cortical areas to sustain balanced patterns of reciprocal interactions (functional integration) (Tononi and Edelman, 1998). In theoretical neuroscience, the balance between functional specialization and functional integration within thalamocortical networks is defined as "complexity" and is considered a key feature of a healthy brain (Bassett and Bullmore, 2009; Sporns et al., 2000). Network complexity, so understood, is in turn contingent on the optimal interplay between local cortical excitability and long-range connectivity. For example, alterations of long-range connectivity may directly affect functional integration and the complexity of the network (Zalesky et al., 2012). On the other hand, excessive or insufficient excitability in one cortical node may have long-range effects that involve the rest of the network. For example, the diaschisis phenomenon (i.e. the remote depression of function in non-injured cortical areas) is another critical alteration of cortical excitability and connectivity, which may take place in brain injured patients. Though difficult to assess objectively, diaschisis may profoundly alter the functional structure of cortical networks and cognitive recovery.

Clearly, understanding whether a change in a certain direction is leading to recovery of network complexity and of function is a fundamental step for developing effective therapeutic strategies.

The perturbational complexity index

Given the above, the need of indexes able to measure the cortical complexity is clear. In order to capture brain complexity by means of a synthetic, quantitative index, Casali and colleagues recently developed a theory-driven empirical measure, the so-called Perturbational Complexity Index (PCI), which can be measured at the patient's bedside (Casali et al., 2013). PCI gauges the amount of information contained in the integrated response of the thalamocortical system to a direct perturbation. The idea is that brain complexity could be estimated empirically by perturbing the cortex ("zapping") to engage distributed causal interactions and measuring the information content of the ensuing responses by algorithmic compressibility ("zipping"). Among others, the Perturbational Complexity Index developed by Casali and colleagues (Casali, 2013), can measure the level of integration and information of the brain during a given state.

Casali and colleagues proposed PCI for measuring the amount of information contained in the integrated response of the thalamocortical system to a direct perturbation. The assumption is that the ability level in sustaining consciousness can be estimated empirically by perturbing the cortex to engage distributed interactions and measuring the information content of the ensuing responses by algorithmic compressibility. Operationally, PCI is defined as the normalized Lempel–Ziv algorithmic complexity (Lempel and Ziv, 1976) of the overall spatiotemporal pattern of significant cortical activation, measured by EEG and triggered by a direct cortical perturbation with TMS (see a schematic representation in Figure 13). The index is expected to be low when causal interactions among different cortical areas are reduced (loss of integration), since the matrix of activation determined by TMS will be spatially restricted. PCI will also be low if many interacting cortical areas react to the perturbation, but they do so in a stereotypical way (loss of differentiation) (Sarasso et al., 2014).

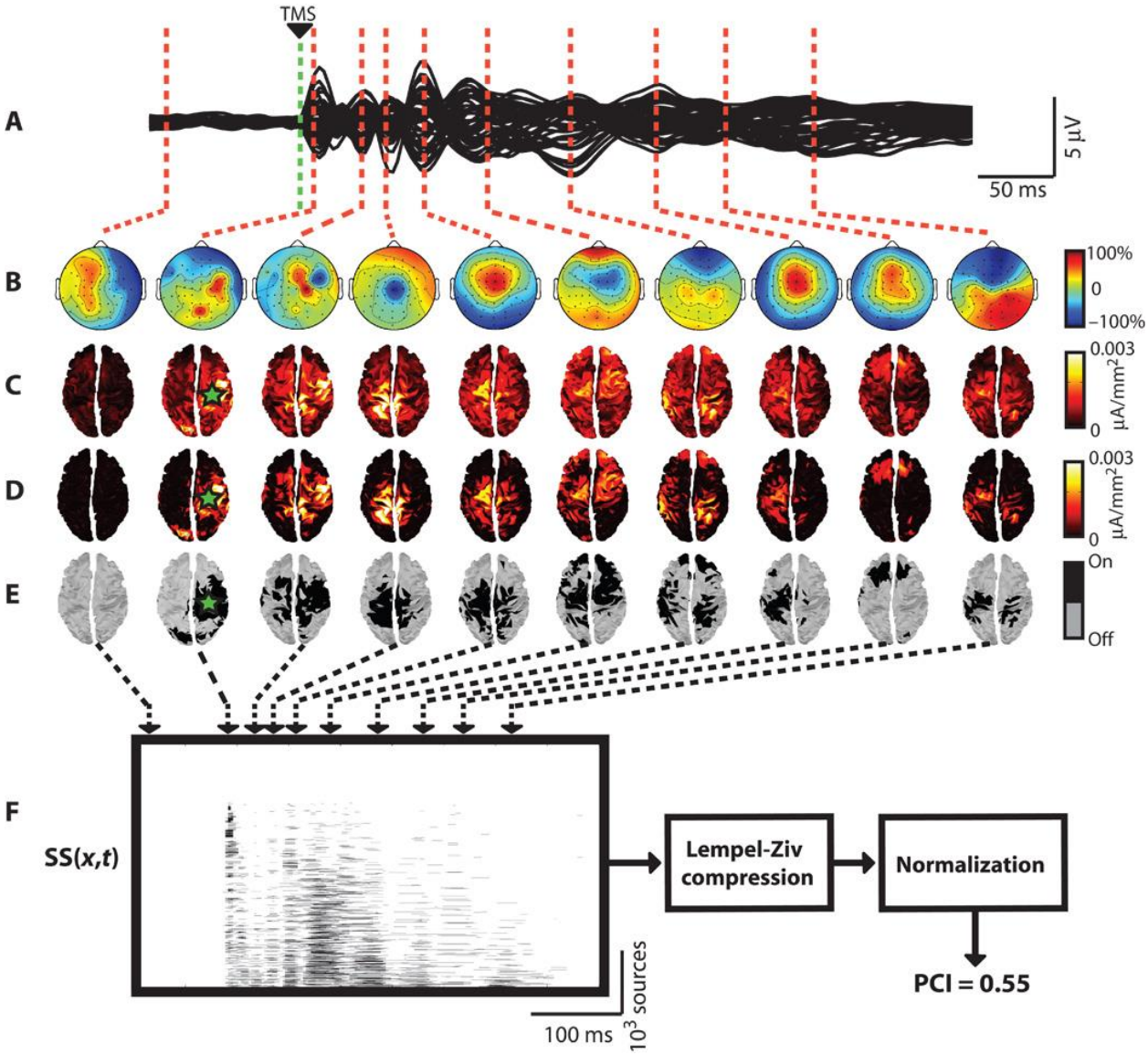

Figure 13. The PCI is calculated from TMS-evoked potentials. (A) The black traces show the superposition of the averaged TMS-evoked potentials (150 trials) recorded from all EEG channels (butterfly plot of 60 channels) in one representative subject during wakefulness. (B) The color-coded maps show the instantaneous voltage distributions at selected latencies [auto-scaled between the maximum (+100%) and the minimum (−100%) instantaneous voltages]. (C) The corresponding distributions of cortical currents are calculated by means of a weighted minimum norm inverse solution. (D) Significant TMS-evoked cortical currents are estimated by applying a nonparametric bootstrap-based statistical procedure at the source level. (E) A binary spatiotemporal distribution of significant sources (SS) is extracted: SS(x,t) = 1 for significant sources (x) and time samples (t); SS(x,t) = 0 otherwise. The sources in the matrix SS(x,t) are sorted, from bottom to top, on the basis of their total activity during the post-stimulus period. (F) The information content of SS is estimated by calculating the Lempel-Ziv complexity measure. PCI is defined as the information content of SS, normalized by the average information contained in the spatial and temporal distribution of the response to TMS that is statistically significant in respect to the pre-stimulus value, in other words the correspondent source entropy. Green star, site of TMS stimulation. From Casali et al., 2013.

In 2013 Casali and colleagues (Casali, 2013) tested PCI in healthy controls and patients with disorders of consciousness. PCI was reproducible within and across subjects and depended exclusively on the level of consciousness in all conditions. The index was always high in wakefulness, irrespective of TMS stimulation site and intensity, but dropped drastically when subjects lost consciousness in NREM sleep and after administration of propofol, midazolam and xenon. In all these conditions, PCI was invariably reduced resulting in a clear-cut distinction between the distributions of the conscious and unconscious states. Crucially, PCI was as low as in NREM sleep and anaesthesia in patients diagnosed with Unresponsive Wakefulness Syndrome (UWS), but higher in subjects who regained consciousness, including minimally conscious state, emerging from MCS (EMCS) and LIS patients, as confirmed few years later by Casarotto and colleagues (Casarotto et al., 2016, Figure 14).

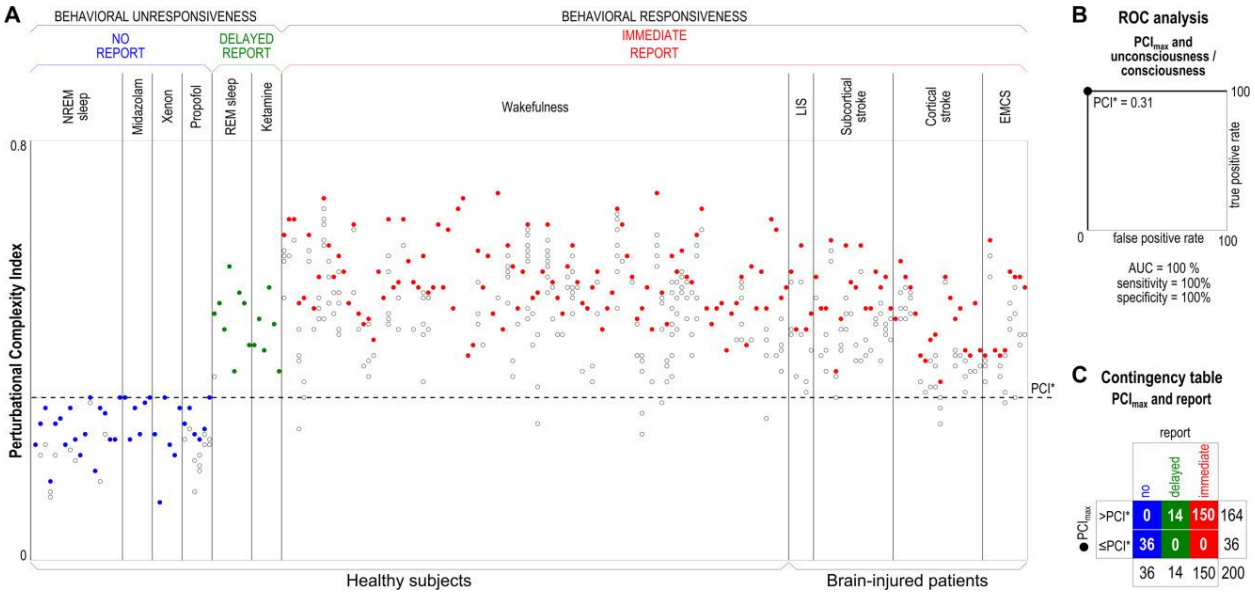

Figure 14. (A) Each circle represents the Perturbational Complexity Index (PCI) value computed from the cortical responses to transcranial magnetic stimulation (TMS) of one stimulation site. Several PCI values computed in each individual are aligned along vertical columns. PCI values are computed from TMS-evoked potentials recorded in healthy subjects and conscious brain-injured patients during different conditions. Individuals are grouped by condition, and within each condition are sorted by increasing age. For each individual, the maximum PCI value (PCImax) is represented by a solid circle, whereas lower PCI values are represented by open circles. During non–rapid eye movement (NREM) sleep and anaesthesia with midazolam, xenon, and propofol, subjects were behaviourally unresponsive and did not provide any report upon awakening. During dreaming and ketamine anaesthesia, subjects were behaviourally unresponsive but provided delayed subjective reports upon awakening. During wakefulness, both healthy subjects and conscious brain-injured patients could immediately report their subjective experience. (B) Receiver operating characteristic (ROC) curve analysis applied to PCImax values for computing the optimal cut off (PCI* = 0.31) that discriminates between unconsciousness (as assessed through the absence of any subjective report) and consciousness (as assessed through the presence of either an immediate or a delayed subjective report). Area under the curve (AUC) is 100%; using PCI* as a cut off, sensitivity and specificity both result in 100%. (C) Contingency table obtained by slicing through the PCImax values with PCI*, also highlighted by a dashed horizontal line in panel A. EMCS = emergence from minimally conscious state; LIS = locked-in syndrome; REM = rapid eye movement. From Casarotto et al., 2016.

PCI has high sensitivity (94%) in detecting minimally conscious patients and allowed identifying a significant percentage (about 20%) of vegetative state/unresponsive wakefulness syndrome (UWS) cases with high brain complexity, who had a higher chance of eventually recovering consciousness.
Despite its accuracy, PCI comes with some limitations. In fact, it can only be computed on spatiotemporal matrices of cortical activations that are obtained after an intensive processing of TMS/hd-EEG data, including forward modelling (Hallez et al., 2007), source estimation (Baillet et al., 2001) and permutation-based statistics at the single-trial level. For these reasons the possibility of estimating perturbational complexity directly at the level of EEG sensors may have critical advantages. Because PCI has a limited application to signals other than TMS/hd-EEG evoked potentials, its application on other kinds of recording is not immediate (D'Andola et al., 2018; Comolatti et al., 2019). In contrast, intracranial stimulations/recordings in humans (Lewis et al., 2012; Pigorini et al., 2015) and in animal models (Bettinardi et al., 2015; Olcese et al., 2016; Vyazovskiy et al., 2009; Vyazovskiy et al., 2013) as well as intra and extracellular responses recorded from cortical slices (D'Andola et al., 2018; Sanchez-Vives et al., 2017) offer unique opportunities to study the mechanisms of neuronal dynamics, network complexity and consciousness (Storm et al., 2017).

Perturbational complexity index – State Transition

The binary sequences of activation and deactivations compressed in the original PCI are sequences of transitions between different states, namely a "response state" and a "non-response" (or "baseline state"). Thus, high values of perturbational complexity can be found in systems that react to the initial perturbation by exhibiting multiple and irreducible patterns of transitions between response and non-response states. Following this intuition, Comolatti and colleagues developed the Perturbational Complexity Index – State Transition (Comolatti, 2019), combining dimensionality reduction and a novel metric of complexity derived from recurrence quantification analysis (RQA) to empirically quantify spatiotemporal complexity at the sensors level as the overall number of non-redundant state transitions (ST), caused by the perturbation, present in the signal's principal components (PC).
It has been validated on a dataset of 719 TMS/hd-EEG sessions recorded from 108 healthy subjects during wakefulness, NREM sleep and anaesthesia (propofol, midazolam and xenon, see a representative subject in Figure 15) and 108 brain-injured patients with disorders of consciousness (DOC). In less than a second PCI-ST approximated the performance of the original PCI in both healthy subjects and patients. In addition, it was tested on 84 stereotactic EEG recordings from nine people with epilepsy, both during wakefulness and sleep.

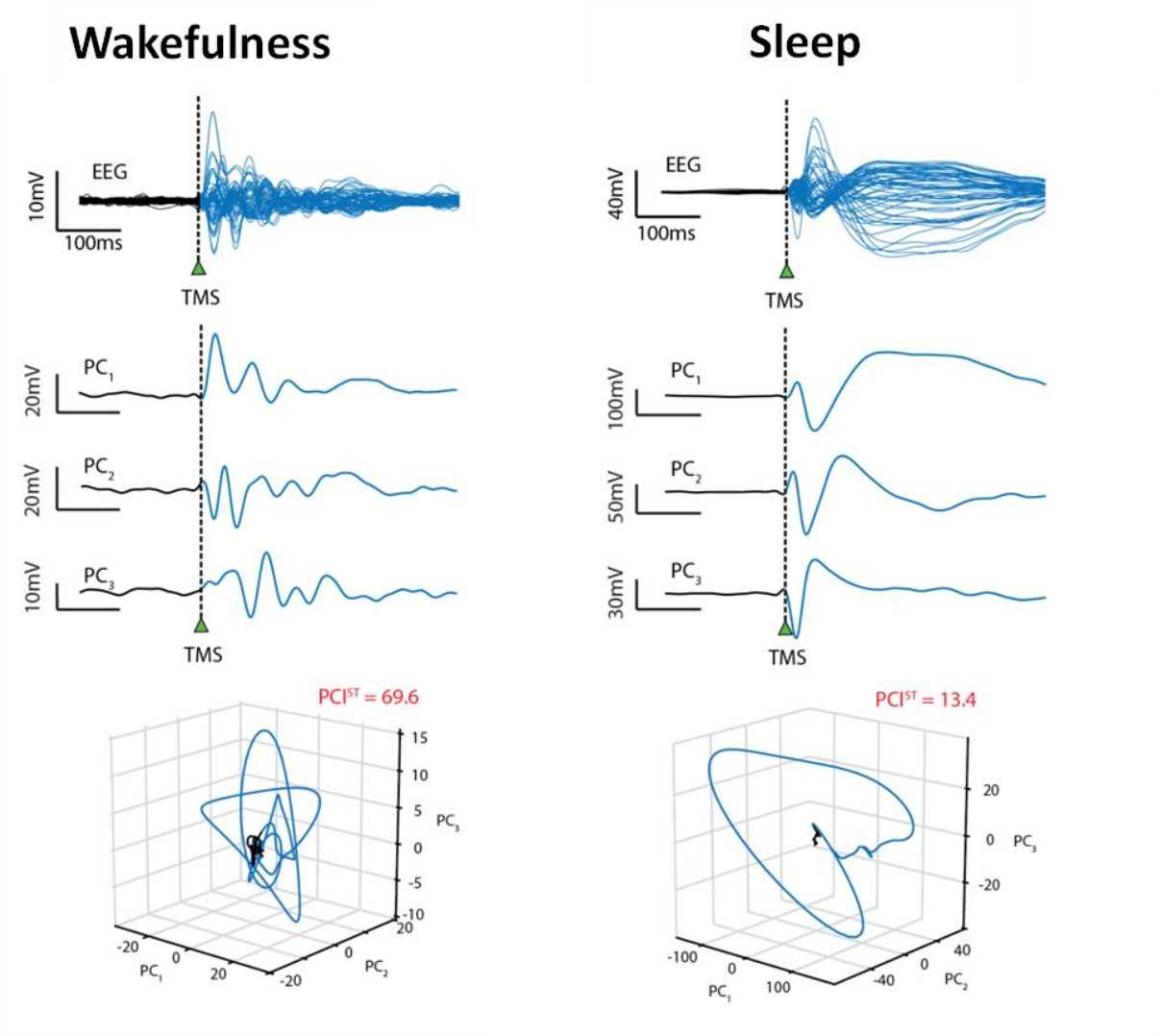

Figure 15. Representation of PCI-ST index for two different state conditions, wakefulness (left) and sleep (right). Top section: butterfly plot for averaged TMS response for all channels. Middle: a depiction of the three main principal components (PCs) involved in the response of each of the two conditions. Below: a spatial representation of PCI-ST calculation in the space of the respective three main components. Comparing the two columns, it is evident that the one referring to wakefulness is more complex not only in its averaged response, but also in the three detected principal components and in the way they react to TMS stimulation, determining higher PCI-ST value for wakefulness condition.

PCI-ST was able to detect systematic relative differences in brain complexity between wakefulness and sleep despite the substantial differences between the intracranial (SPES/SEEG) and non-invasive (TMS/EEG) recordings, showing the feasibility of PCI-ST calculation across different recording methods. PCI-ST rely on state transitions, which are computable for any system independently of its characteristics, allowing the researcher to explore the causal structure of the brain across different recording scales, from macroscopic EEG potentials, through mesoscopic local field potentials and finally, to microscopic multisite electrophysiology.

Lastly, sleep-like cortical bistability studies may constitute the endpoint of neuromodulation and pharmacological treatments aimed at restoring overall network complexity and function. In line with this, a recent microscale study employing electrical stimulation and recordings in isolated cortical slices showed that phase-locking and complex causal interactions, as assessed by PCI could be effectively restored by pharmacological interventions that reduce bistability and increase cortico-cortical excitability (D'Andola et al., 2018). This finding further suggests a causal link between cortical bistability and complexity and may have translational implications since brain slices can be considered a simplified model of the electrophysiological state of the cerebral cortex under conditions of severe deafferentation (Rosanova et al., 2018).

Chapter three. The relationship between sleep and brain injuries

Despite the physiological nature of cortical bistability, it has been shown that besides changes in K+ conductance and increased inhibition, bistability may be experimentally induced in animal models by means of cortical deafferentation (Lemieux et al., 2014; Timofeev et al., 2000). As an example, severing the white matter with a cortical undercut, results in slow waves and in a continuous alternation between ON and OFF periods in the partially deafferented gyrus, even when the animal, and the rest of the brain, is awake (Nita et al., 2007). To the extreme, cutting a slice of cortex and bathing it in the appropriate in vitro solution results in a typical pattern characterized by the alternation between ON and OFF periods (Sanchez-Vives and McCormick, 2000) suggesting that bistability is the "default mode" of the isolated cortex. Other than the abovementioned mechanism involving cortical deafferentation, bistability can be induced by alteration of the excitation/inhibition balance in intact portions of the thalamocortical system. For instance, recovery of language and motor function after brain injuries can be blocked by an excessive inhibitory activity in the peri-lesional area (Classen et al., 1997); this excessive inhibition may be generated locally by healthy areas that become hyperactive (Murase et al., 2004). In this case, bistability would be brought about by increased inhibition. Another option is that bistability can be induced by a non-physiological increase in K+ conductance. This may happen because subcortical lesions would prevent the action of ascending activating neuromodulators (McCormick et al., 1993) or following the activation of ATP-dependent K+ currents (Sun and Feng, 2013). In all cases, the engagement of bistable dynamics in portion of the cerebral cortex may have a profound impact on cognitive/motor functions. For example, local slow waves and the associated OFF periods spontaneously occurring during wakefulness have been shown to be associated with motor impairments in rats performing a pellet reaching task (Vyazovskiy et al., 2011). In a similar way, local sleep-like OFF periods occurring in peri-lesional cortical areas may account for a significant loss of function after brain injury.

Despite these electrophysiological similarities, the notion that cortical bistability may be involved in the physiopathology of brain lesions is far from being established.

Clearly, demonstrating the presence of local sleep-like bistability in brain-injured patients would be critically important. Indeed, while anatomical lesions and disconnections may hardly be reversed, it might be possible to reduce peri-lesional bistability and improve overall network connectivity and complexity and, in turn, improve the recovery of function.

Sleep-like activity may have a role in the stroke pathophysiology

Stroke is an abrupt onset of a focal neurological deficit secondary to a vascular event lasting more than 24 hours. An acute stroke refers to the first 24-hour-period of a stroke event, causing rapid loss of function. Stroke is subdivided into ischaemic and haemorrhagic, with ischaemic being more common. There are multiple aetiologies, but the common mechanism is insufficient blood supply to a cortical area, resulting in corresponding symptoms, such as hemiparesis, aphasia, visual field defect, or neglect. If recognised early, patients may be eligible for thrombolysis, which is often effective in restoring blood flow and preventing or reducing permanent ischaemic damage. Unfortunately, symptoms may go unrecognised, or occur during sleep, resulting in missed windows of opportunity for treatment.

Depending on the severity of the stroke, cellular death will occur within minutes, causing irreversible damage even after blood flow is restored. This is called the "core" of the infarct. Surrounding the core is tissue that is affected but may functionally recover if blood flow is restored. This is called the "ischaemic penumbra".

Although loss of function following stroke is the result of the direct loss of brain cells hit by the vascular event, the consequence of a stroke extends far beyond the localized structural/anatomical lesion in the brain. Increasing evidence reveals that additional symptoms result from local functional disruption in intact brain areas connected to those affected by a stroke (peri-lesional areas). These secondary events are called diaschisis (von Monakow, 1914) (i.e. the remote depression of function in non-injured cortical areas) and are hypothesized to result from a local functional alterations disrupting long-range interactions among distributed brain areas (Carrera and Tononi, 2014; Di Piero et al., 1990; Dubovik et al., 2012; Westlake et al., 2012).

Stroke electrophysiology

Organized brain activity requires the coordinated firing of a vast number of nerve cells. To maintain this coordinated firing, all neuronal cells must be adequately polarized, their axons capable of conducting action potentials and releasing transmitters to an even greater numbers of synapses. Hence, there are often serious consequences of any interruption in the normal supply of O2 and glucose.

After a stroke insult in the brain, neuronal functions are often suppressed, membrane potentials in the brain change, and many neurons are hyperpolarized. The early events, suppression of synaptic and cognitive function, sharply reduce the brain's needs of energy, enabling it to maintain the minimal metabolism required for survival. Even this minimum cannot be sustained for more than a few minutes: if ischemia is prolonged, a slowly progressive depolarization (mainly caused by glutamate

release) suddenly accelerates, owing to the activation of several inward currents. The resulting near-total depolarization and large increase in Ca++ influx – as well as Ca++ release from internal stores (including mitochondria) – leads to a rapid rise in cytoplasmic Ca++ concentration. If this does not reach the critical level that triggers the irreversible processes leading to cell death, may take place the restoring of energy supplies that reactivates the membrane pumps, which in turn re-establish normal ionic gradients and membrane potentials, thus enabling the return of synaptic and cognitive functions. Given all the above, after a stroke changes of cortical excitability and connectivity are thought to occur at various levels, depending on the duration of the event. Consequentially, post-stroke electrophysiological alterations of neurons are reflected at a macroscopic level on the pattern of the EEG. In particular, the electroencephalogram is characterized by a slowing in the channels that are closer to the lesioned area, as shown in Figure 16.

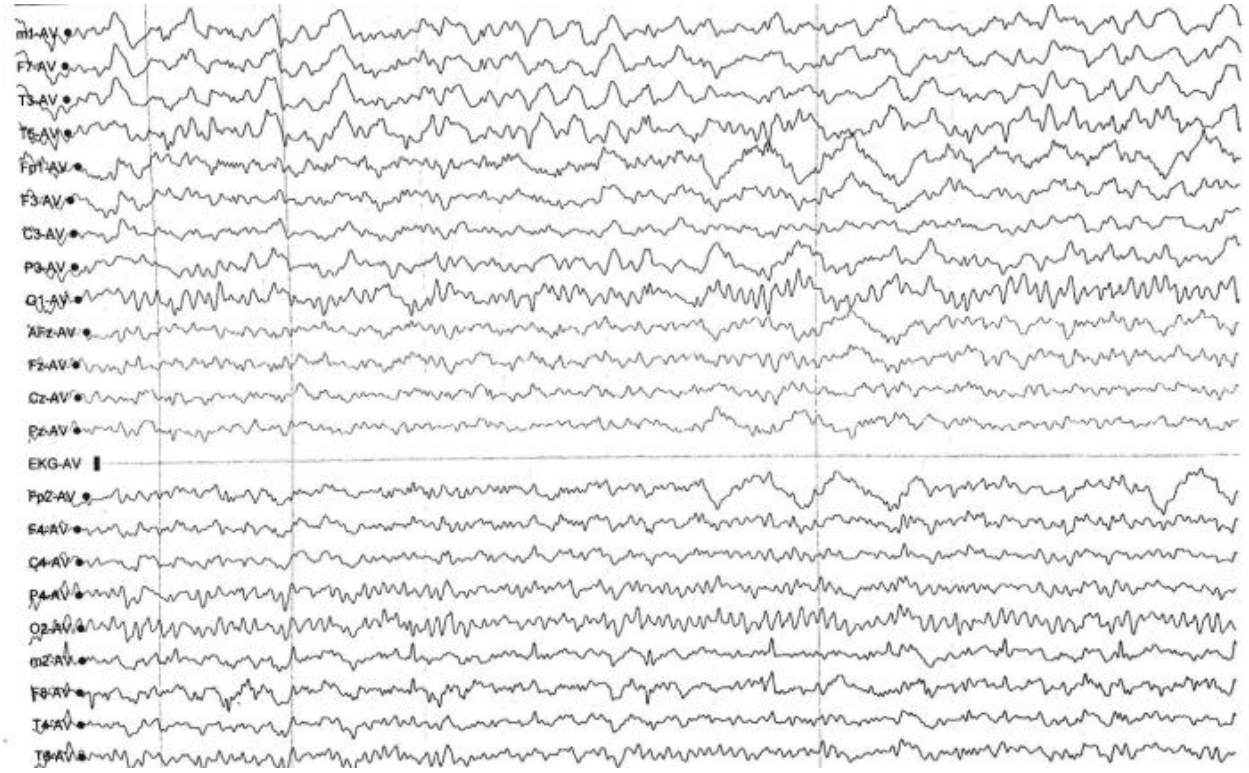

Figure 16. Lateralized slowing in a 63 years old male patient with acute ischemic stroke shows low to middle amplitude slow waves visualized in the left hemisphere and are more obvious in M1, F7, T3, T5 leads. From Bhattarai, 2014.

By the late 1930s, Sugar and Gerard (Sugar and Gerard, 1948) demonstrated, in a cat model, that ischemia selectively depresses electrical activity, first in the hippocampus and cerebellum, and then in other brain regions. As Ralph Rossen and colleagues showed in human studies (Rossen, 1943), early restoration of blood flow soon leads to apparently full recovery of function. Only after prolonged

ischemia a total or incomplete recovery of electrical signals indicates a corresponding irreversible loss of function. Thus, ischemic electrical changes are manifested by an early phase of cellular hyperpolarization, or only moderate depolarization (depending on the brain region), associated with reversible suppression of neuronal firing and no evidence of lasting damage. This is followed by a second phase, heralded by a large depolarizing shift, often leading to irreversible loss of function.

Despite these numerous observations, our understanding of the electrophysiological mechanisms behind the consequences of a stroke did not progress much over the last several decades, partially due to the absence of novel anatomical and functional brain neuroimaging findings. Consequently, the electrical properties of the stroke peri-lesional area and connected networks are still poorly defined. We are left to the notion that slow waves (the hallmarks of sleep state) can be detected in the wake EEG in the area surrounding the stroke (Niedermeyer and Silva, 2005).

For these reasons in the context of a project for the Swiss National Science Foundation, in a study that was recently published in the Journal of Neuroscience Methods, DOI: 10.1016/j.jneumeth.2018.12.011 (reported below with no changes), we have investigated the relationship between stroke and sleep (Mensen et al., 2019).

Sleep as a model to understand neuroplasticity and recovery after stroke: Observational, perturbational and interventional approaches.

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Abstract

Our own experiences with disturbances to sleep demonstrate its crucial role in the recovery of cognitive functions. This importance is likely enhanced in the recovery from stroke; both in terms of its physiology and cognitive abilities. Decades of experimental research have highlighted which aspects and mechanisms of sleep are likely to underlie these forms of recovery. Conversely, damage to certain areas of the brain, as well as the indirect effects of stroke, may disrupt sleep. However, only limited research has been conducted which seeks to directly explore this bidirectional link between both the macro and micro-architecture of sleep and stroke. Here we describe a series of semi-independent approaches that aim to establish this link through observational, perturbational, and interventional experiments. Our primary aim is to describe the methodology for future clinical and translational

research needed to delineate competing accounts of the current data. At the observational level we suggest the use of high-density EEG recording, combined analysis of macro and micro-architecture of sleep, detailed analysis of the stroke lesion, and sensitive measures of functional recovery. The perturbational approach attempts to find the causal links between sleep and stroke. We promote the use of transcranial magnetic stimulation combined with EEG to examine the cortical dynamics of the peri-infarct stroke area. Translational research should take this a step further using optogenetic techniques targeting more specific cell populations. The interventional approach focuses on how the same clinical and translational perturbational techniques can be adapted to influence long-term recovery of function.

Introduction

In adults, stroke is the first cause of permanent disability and the third cause of mortality. Major advances have been made in stroke prevention and in the management of acute stroke (e.g. thrombolysis, treatment in stroke units). Conversely, recovery from stroke can be promoted by training and exercise (neurorehabilitation) but pharmacological solutions seem to be limited (Dobkin, 2008). Currently, functional outcome still depends largely on the initial conditions of the lesion: location, severity and extension.

Stroke leads to disruption of cortical and subcortical circuits adjacent to the damaged area. Recovery is related to the reorganization and reallocation of lost functions toward spared neurons that had been mainly devoted to other activities. Our understanding of the nature of this neuroplasticity process has greatly improved over the last 2–3 decades. Animal and human data suggest that this functional remapping may be related to changes in brain excitability in the peri-infarct area and distant connected areas (Gerloff et al., 2006; Murphy and Corbett, 2009; Nudo, 2013). Animal studies suggest that these changes stem from increased GABAergic and glutamatergic transmission, the pharmacological reduction of the former and excitation of the latter can improve functional recovery (Clarkson et al., 2010; Song et al., 2017). Human studies have shown that ipsi- and contralesional cortical areas undergo changes in their activation, as assessable both at the cortical level by functional neuroimaging and at the cortico-spinal level by mean of transcranial magnetic stimulation (TMS), which parallel post-stroke functional recovery (Gerloff et al., 2006). In addition, modulation of the contralesional cortical areas by TMS has been shown to reduce post-stroke cognitive disability and improve activities of daily living (Cazzoli et al., 2012). These observations support the hypothesis that training and exercise performed

during neurorehabilitation promote recovery through a use-dependent neuroplasticity process of re-learning and functional remapping.

Every day experience reminds us of the crucial role that sufficient, quality sleep plays in the recovery of our own cognitive and physical abilities. Furthermore, local sleep, by means of an increase in slow wave activity (SWA) in delimited portions of the cortex has been found to increase following a cognitive task involving that brain region during preceding wakefulness (Huber et al., 2004; Hung et al., 2013). These local changes are also seen in structural and diffusion weighted magnetic resonance imaging (Bernardi et al., 2016); as well as positron emission tomography (Maquet, 2000). Conversely, local reduction of slow waves by closed loop acoustic stimulation leads to a reduction in the learning capacity associated with that particular cortical area (Fattinger et al., 2017). At the macrosleep-scale, optogenetic disruption of sleep continuity impairs memory consolidation in mice (Rolls et al., 2011). Therefore, the same use-dependent mechanisms which drive changes in sleep architecture may underlie the neuroplasticity found during recovery from stroke.

While sleep likely plays a key role in successful recovery, the structural and functional changes after stroke also have the potential to negatively impact sleep. In a recent meta-analysis of 15 studies, Baglioni et al., (2016), found a consistent reduction in the total amount of time acute stroke patients were asleep, further compounded with a reduction in sleep efficiency during that reduced sleep period. Interestingly, no consistent differences were reported for changes in REM sleep, furthering highlighting the specific link between stroke and NREM; in particular SWA (however see Pace et al., 2018, for translational evidence of REM changes). Further studies also suggest that beyond the direct impact on sleep, acute patients may have a higher prevalence of sleep-disordered breathing (Camilo et al., 2016; Huhtakangas et al., 2017). This would typically lead to a decrease in total sleep time, increase in sleep fragmentation, and a corresponding decrease in SWA. Sleep loss would also have the indirect effect of increasing daytime sleepiness, lowering the ability and motivation to optimally perform the tasks required for active rehabilitation of function (Gooneratne et al., 2003; Frohnhofen et al., 2013). Overall, the current state of research suggests a tight, bi-directional link between sleep and stroke. Both in terms of the effect of stroke on specific aspects of the macro and micro-architecture of sleep, as well as the potential of sleep to act as the direct and modulating driver of recovery. Understanding how brain damage, its consequences on brain activity, recovery, and sleep are interrelated is of outstanding interest for both the scientific community and society in general.

Here we describe a series of semi-independent approaches that aim to firmly establish this link through both observational and interventional experiments. Our primary aim is to describe the methodology

for future research needed to delineate competing accounts of the current data. In doing so we identify key projects needed to establish the critical role of specific aspects of sleep as the mechanism of functional impairment and as an interventional tool for its recovery. Importantly, while each of the described approaches gives us a piece of the puzzle, they each contribute to the other's explanatory and predictive roles. For each approach we describe the key missing pieces to our current understanding, the methodological rationale of future work to directly address these limitations, and the challenges each may face. In particular, we outline 3 related hypotheses: that the local damage following stroke leads to local changes in sleep architecture; that these local effects on slow waves are likely to be split into both beneficial effects of slow waves ('good waves'), and those which underlie the functional impairment ('bad waves'); and that the functional recovery following stroke can be monitored and assessed by tracking these changes in sleep architecture.

2. Observational approach

The first logical starting place for examining the relationship between sleep and stroke is its passive measurement. While most previous studies have relied on clinical polysomnography to record sleep, we recommend the use of high-density EEG (hd-EEG) to make significant progress in the field. Current setups of as high as 256-channel nets can be applied relatively quickly, and additional measurements (e.g. ECG, leg movements, respiration), can be performed synchronously. Such setups are particularly advantageous to examine the microarchitecture of sleep and track the local changes expected following focal damage after stroke. The use of the high-density array allows us to track the extent of local changes to sleep and makes the average reference a plausible solution to the referencing problem of low-density EEG montages (Dien, 1998; Lei and Liao, 2017). Macroarchitecture analysis would also benefit by having additional channels by which to score sleep stages; especially in the case where local distortions of EEG activity make scoring difficult.

One hd-EEG study reported increased slow-wave activity directly over the infarct area in both sleep and wake, yet decreased activity in the adjacent perilesional area which persisted into the chronic period of recovery (Poryazova et al., 2015). Another study demonstrated the link between highly local slow-wave activity and recovery of function in a group of chronic aphasia patients (Sarasso et al., 2014). These limited examples demonstrate the unique utility of hd-EEG in stroke patients, as well as the importance of longitudinal measures as the neural activity in sleep is likely to change along with the neuroplasticity associated with recovery. Repeated measurement from acute to chronic stages would also be useful in distinguishing the direct effect of the persistent structural damage and those

changes that relate to the functional recovery. These studies show that hd-EEG recordings are feasible in these patients and clinical settings.

Slow activity during wakefulness in the area of the stroke lesion has been consistently reported for decades (Nuwer, 1996; Yokoyama et al., 1996; Murri et al., 1998; Fernández-Bouzas et al., 2002). Decreases in these waking slow-waves predict a positive clinical outcome (Finnigan et al., 2004, 2007). Yet whether this activity and that of slow wave sleep are closely related remains unclear. This confusion is at least partly due to the fact that these phenomena have been mostly captured using spectral power analysis. With the advent of hdEEG measures and novel analytical approaches, we are now in a position to detect and analyse properties of individual slow waves (Riedner et al., 2007; Mensen et al., 2016). This approach can distinguish between several independent properties of the individual waves such as incidence, amplitude, slopes, topographic location and extension, and travelling parameters of each wave (Massimini et al., 2004). The pathological slow waves during wakefulness are likely also present during sleep, yet are masked by the appearance of normal slow waves. In depth analysis at the individual wave level of each of the wave property may be able to better characterise and distinguish the normal, good, from pathological, bad, waves. Doing so would shed new light into whether these pathological waves underlie the functional impairments in acute patients, or whether they represent similar recovery processes as those in normal sleep. Perhaps most interestingly is whether these two seemingly distinct avenues in fact represent different sides of the same coin. That is, the slow waves causing initial functional impairment are necessary for acute recovery and stabilisation, yet their persistence into chronic stages represent long-term disability. Determining the properties that separate these two processes is necessary for adequate future interventional approaches that may seek to reduce those waves that lead to impairment, while promoting only those necessary for neural recovery.

Given the diversity of functional impairments and its clear relationship to lesion location, we should expect a similar diversity in predicting distinct effects on sleep. Therefore, a key element of future research should be detailed description, segmentation and analysis of stroke lesion. While the majority of papers cited in a recent meta-analysis described the lesion location in some basic form (e.g. hemisphere, infra vs supra tentorial), relatively few of the studies segmented the lesion using the clinical MRI (whether automatic or manually; Maier et al., 2017). Fewer still included such measures as direct predictors of sleep impairment. Several parameters can be obtained that would plausibly have an impact on sleep structure such as precise location, volume, and severity (Müller et al., 2002). More secondary parameters from the segmentation process could also be explored such as the white/grey

damage ratio which may affect distinct aspects of individual waves (e.g. grey matter damage may affect wave amplitude through local synchronisation; white matter damage may block slow wave travelling). Seeing that the clinical MRI is almost always available, future work could also benefit from using specific sequences or imaging techniques to gain further insights on the impact of the lesion (e.g. diffusion tensor imaging Song et al., 2015; or functional connectivity, Hallam et al., 2018).

2.1. Measuring functional recovery

However interesting the link between sleep and stroke may be at the basic science level, at the clinical level the utility of this link is bounded by its further relationship to functional recovery. That is, the applicability of even a well-established link between the two phenomena would be limited if the variability in these factors were not then significantly related to either prediction of functional outcome or subsequent clinical treatment possibilities. Correlational analysis of the functional recovery with the parameters of slow wave activity could further differentiate the patterns of neurophysiological activity which are inherent to the neuroplasticity associated with the recovery of function (i.e. "good waves"), from those which underlie the loss of function in the first place (i.e. "bad waves"). Such an analysis could be performed on both the short-term scale comparing functional recovery measures before and after a single night of sleep. However, recovery from the acute to subacute timescales is likely to be more revealing.

Studies should therefore ensure that measures of functional recovery are integrated into the same research protocol and designed to be sufficiently sensitive to detect even small changes. Given the range of possible functional impairments, research with stroke patients must grapple with the trade-off between the personalisation of functional tasks necessary to achieve this sufficient sensitivity and broader cognitive tasks applied at the group level so that results are generalisable. Further consideration must be given to the amount of time-on-task, especially in the acute stage, such that the accuracy of the functional measures is not undermined by fatigue or lack of motivation in the patient population. In translational work, standardised behavioural tests such as the ladder walking test (Cummings et al., 2007), beam balance test (Lang et al., 2011), or single pellet reaching (Farr and Whishaw, 2002), can overcome some of these concerns. Yet, even the simple handling of the animals can be a significant stress factor, require multiple and larger groups of animals, as well as being time consuming for the researcher.

In this context, we recommend two overlapping approaches: (semi-) automatization of tasks and continuous passive measurement. In clinical work, the former can be achieved through the use of

computer-based tasks to evaluate cognitive performance, ideally in the form of simple and short, tablet-based activities using a touchscreen. On the surface this may seem to undermine the importance of the patient-researcher interaction, however, this liberates the investigator from the routine performance of the task in hand and allows them to interact more directly with the patient. Furthermore, this allows for a standardisation of tasks, and the automatic measurement of additional parameters of interest. For example, the digital adaptation of the classic test for spatial working memory, the corsi-block test, allows for precise presentation timing, multiple randomised trials, and inter-block reaction time measurements (Brunetti et al., 2014). For passive measures, actigraphy can be used to continually track the sleep/wake rhythm but also has the potential to track motor recovery (Cavalcanti et al., 2012; Bakken et al., 2014). More vision-related deficits, especially neglect, may actually be best measured through the use of passive and continuous eye-tracking (Müri et al., 2009; Delazer et al., 2018); thus, fulfilling both the need for increased sensitivity and automaticity in measuring functional recovery.

Automation of animal behavioural tasks reduces direct animal contact and experimenter time per animal, which further reduces disruption to their normal sleep rhythms (Fenrich et al., 2015; Wong et al., 2016). For example, an automated single pellet reaching task, enabled in the animal's home-cage produced improved learning curves and increased trial numbers as compared to the traditional version of the task (Fenrich et al., 2015) while allowing synchronized optogenetic interventions (Ellens et al., 2016) as well as electrophysiological monitoring. Recent advances in automatic scoring and analysis of animal movement from regular video sources could further add to the sensitivity of motor recovery while allowing for the continuous analysis over longer time periods (Mathis et al., 2018). Therefore, automation of behavioural tasks for translational stroke research will aid to study more specific outcome measures for new types of interventions, while increasing feasibility and reducing animal stress.

3. Perturbational approach

The observational approaches described above would provide profound, but ultimately correlative, insights into the relationship between sleep and stroke. To parallel observational approaches, the relationship between sleep and stroke could be observed from a causal perspective by studying effective connectivity of thalamo-cortical system employing a perturbational approach (Massimini et al., 2009). One possibility is to perturb the thalamo-cortical system through sensory stimuli, and several studies have examined auditory, somatosensory and visual evoked potentials during sleep showing a

decrease of the late components in the evoked sensory responses (Bastuji and García-Larrea, 1999; Kakigi et al., 2003). However, peripheral evoked potentials may be insensitive indicators in the context of a damaged brain after stroke because the cortical area of interest may have become disconnected or impaired. These confounds can be avoided by employing transcranial magnetic stimulation combined with EEG (TMS/EEG) This technique allows for direct stimulation of different subsets of cortical neurons and measures, with good spatial-temporal resolution, the effects on the rest of the thalamocortical system (Ilmoniemi et al., 1997). In this way, it is possible to bypass sensory inputs and to directly probe, non-invasively, the response of the cortical area of interest (Massimini et al., 2009). Recent TMS/EEG studies have shown a prominent impairment in the ability of cortical circuits to sustain complex patterns of activity following brain injury (Rosanova et al., 2012). Crucially, in the present context, this impairment extends beyond the anatomical disconnection and seems to be due to the engagement of pathological, sleep-like neuronal behavior; namely, the occurrence of cortical OFF-periods. A recent study employing intracortical single pulse electrical stimulation (SPES) and simultaneous local field potential (LFP) recordings in humans during NREM sleep (Pigorini et al., 2015), showed that the tendency of cortical neurons to fall into an OFF-period (i.e. down- state in cortical neurons - Steriade et al., 1993a; Timofeev et al., 2000) is the key mechanism that disrupts the chain of causal interactions among distant cortical areas. Although the mechanism of OFF-period following brain injury is still to be elucidated, both in vivo and in vitro studies showed that these silent periods may result from increased K-currents (Compte et al., 2003; Englot et al., 2010; Lemieux et al., 2014), from alterations of the balance between excitation and inhibition (Murase et al., 2004) and from partial cortical deafferentation (Nita et al., 2007; Timofeev et al., 2000). All these mechanisms are associated with brain lesions, impairing information transmission by inducing bistability in portions of the thalamocortical system that are otherwise healthy (Figure 17C). In line with this view, a recent TMS/EEG study showed that the residual cerebral cortex of unresponsive wakefulness syndrome patients (UWS) with multi-focal brain lesions fails to engage in complex patterns of activity because neurons tend to fall into a pathological OFF-period after an initial activation. (Rosanova et al., 2012, 2018). In both the EEG and LFP time series, these off-periods present as slow waves, visually indistinguishable from those found in NREM sleep (Massimini et al., 2007).

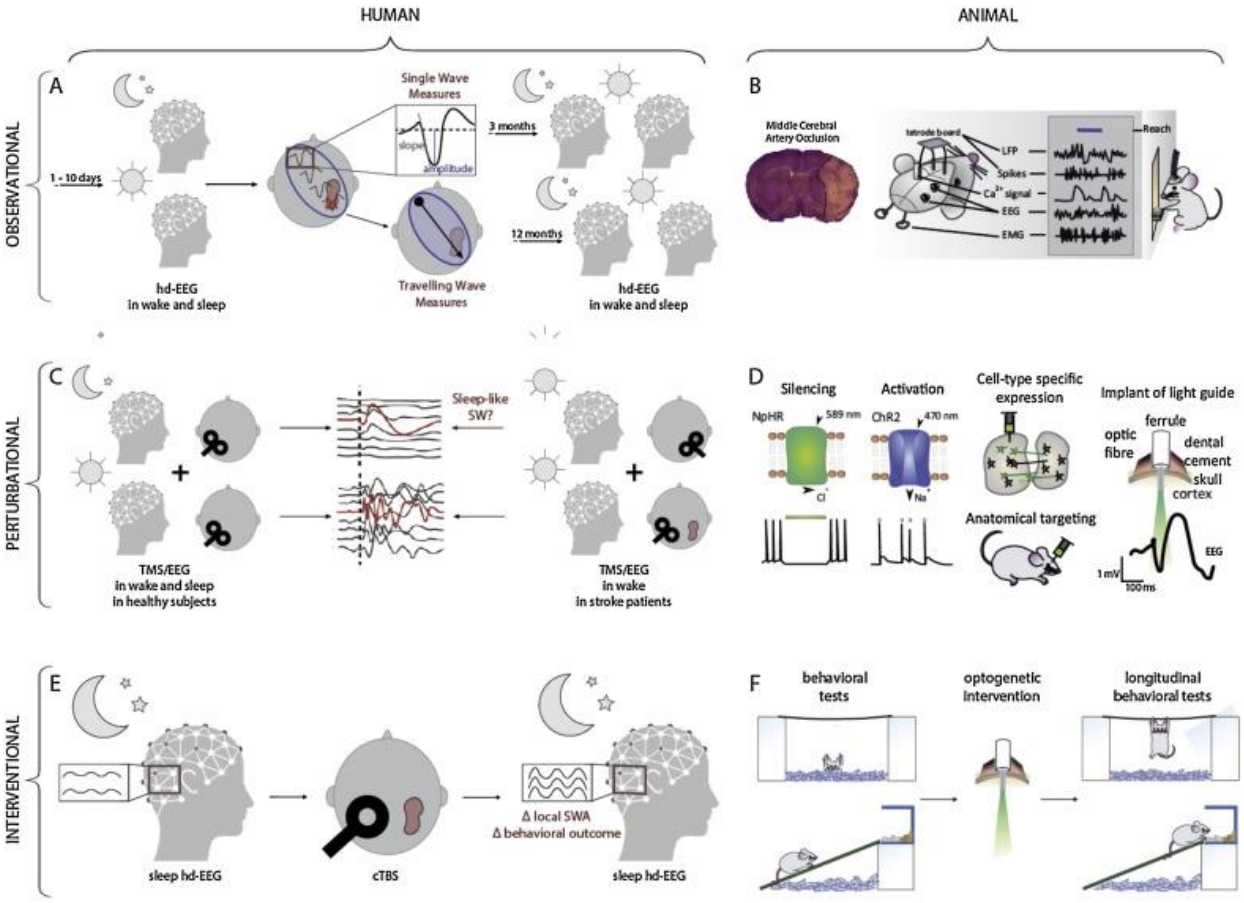

Figure 17. Three different, parallel approaches for studying the link between sleep and stroke. Red areas on topographies represent stroke lesions. Observational: A. Longitudinal hd-EEG measures in human stroke patients during wakefulness and sleep after 3 and 12 months from the baseline measures. B. Local Field Potential, Spikes, Ca++, EEG and EMG analysis in animal model of stroke, made by Middle Cerebral Artery Occlusion as in the next two approaches. Perturbational: C. Triggering, in human subjects and by means of TMS/EEG, complex responses during wakefulness and slow waves during sleep (Left) and comparing them to the response evoked by TMS in stroke patients while stimulating perilesional or contralesional areas (Right). D. Triggering slow waves in animal model of stroke by means of optogenetic stimulations. Left, NpHR and ChR2 genes chosen for the silencing or the activation of Cl− and Na+ currents respectively. Middle, Specific cell-type expression and target inclusion in the animal. Right, Light guide implant in the animal that triggers slow waves. Interventional: E. Longitudinal assessment in humans: hd-EEG measures during sleep before and after cTBS treatment in human stroke patient over contralesional cortical areas. F. Longitudinal assessment in animal model of stroke: behavioural tests before and after optogenetic intervention. From Mensen et al., 2019.

Future studies employing TMS/EEG should test whether a similar pathological, sleep-like dynamic may play a role in focal brain lesions, including the ones due to stroke. If this is the case, it will be important to deepen our understanding of the mechanistic link between sleep-like slow wave activity, OFF-periods and brain lesion. Indeed, to the extent that sleep-like dynamics represent the common functional endpoint of brain lesion, detecting its presence and tracking its evolution over time may offer a valuable read-out to devise, guide and titrate therapeutic strategies aimed at functional recovery

after stroke. To this aim, one possibility is a translational study in which intracranial stimulation and recording in humans during NREM sleep, typically those in presurgical evaluation for epileptic activity (Cossu et al., 2005), is paralleled by animal studies, both during NREM sleep and after stroke, employing similar stimulation and recording parameters.

It remains an open question, as to whether the slow-wave-like activity found in perilesional neuronal populations after stroke, especially those that seem to reflect functional impairment (i.e. bad waves), show distinct activity neurophysiological profiles, to those during NREM sleep which relate to recovery of function (e.g. good waves). Perturbational approaches in animal models offer reproducible experimental conditions to test for slow wave activity (Figure 17D). Intracranial electrical stimulation in mice has been used to evoke cortical slow waves that show comparable properties to endogenous slow waves (Vyazovskiy et al., 2009). Experimental animal models can elucidate the roles of genetically identified and anatomically defined cell populations for regulating cortical SWA. For example, chemogenetic activation of somatostatin cortical interneurons, using the Designer-Receptor-Activated-by-Designer-Drug (DREADD) approach increased SWA, while their chemogenetic silencing reduced SWA in mice (Funk et al., 2016). In contrast, activation of parvalbumin positive interneurons reduced slow-wave activity, while triggering short OFF periods (Funk et al., 2017).

Optogenetic stimulation offers the time precision of electrical intracranial stimulation and TMS, but with the additional ability to perturb only genetically specified cell populations. A recent study optogenetically activated somatostatin or parvalbumin containing cortical interneurons to induce immediate transitions from cortical up to cortical down states (Zucca et al., 2017). Comparison of optogenetically induced slow waves could teach us about the mechanisms behind naturally occurring slow waves and how they are similar to those produced through perturbation. Optogenetic stimulation can be readily applied to mice that have undergone an experimental stroke. Thus, specific targeting of neuronal cell types may aid in promoting the beneficial, recovery aspects of these "good" slow waves, while minimising their negative effects on normal brain functioning of "bad" slow waves.

4. Interventional approach

The "perturb-and-measure" approach described above can probe specific features of the thalamo-cortical system by means of single-pulse electrical or magnetic brain stimulation. Interestingly, by changing stimulation parameters we can move from a perturbational to an interventional approach. Repetitive stimulation has the potential to alter the physiology and functional organization of the brain beyond the duration of the stimulation (Esser et al., 2006; Peinemann et al., 2004; Quartarone et al.,

2005). To date, there are several demonstrations that repetitive application of TMS (rTMS) can produce longer-lasting effects, both inhibitory and excitatory depending on stimulation parameters, and thus offer potential for clinical applications post-stroke (Lefaucheur et al., 2014). Various studies have assessed the effect of rTMS on motor domains (Chang et al., 2010; Conforto et al., 2012; Emara et al., 2009, 2010; Fregni et al., 2006; Liepert et al., 2007; Mansur et al., 2005; Meehan et al., 2011; Takeuchi et al., 2008), post-stroke aphasia (Hamilton et al., 2010; Kakuda et al., 2010; Martin et al., 2009, 2004, Naeser et al., 2011, 2005b, 2005a) and neglect, significantly reducing the typical right-side bias in attention (Cazzoli et al., 2012; Koch et al., 2008; Nyffeler et al., 2009).

Importantly, in healthy individuals, high-frequency rTMS applied to motor cortex induced localized potentiation of TMS-evoked cortical EEG responses in wake, while also increasing slow wave activity during subsequent sleep (Huber et al., 2007). This study raises the critical question of the role that subsequent sleep may have in the long-term consolidation of the beneficial effects of potential rTMS intervention in stroke patients. Given the efficacy that contralesional, inhibitory rTMS has already shown in the neglect patients, we suggest using this as a model to examine the relationship between the scale of functional recovery and changes to local sleep architecture in both the ipsi and contralateral regions before and after rTMS (Figure 17E). As with most recent studies in neuromodulation, we recommend the use of theta-burst stimulation protocols, a particular form of rTMS which enables the use of lower stimulation strength, shorter total stimulation time, while actually improving the reliability and duration of the offline effects (Huang et al., 2005). Given the current literature, it is plausible that the long-term amelioration of neglect symptoms after rTMS intervention largely depend on the quality and structure of subsequent sleep.

The effects of non-invasive brain stimulation are likely to derive from modulation of long-term potentiation (LTP)-like or long-term depression (LTD)-like processes at the neuronal level (Huang et al., 2007). However, the precise mechanisms of post-stroke recovery may be difficult to understand in human research alone. A variety of stroke animal models have been developed and successfully used in stroke research to elucidate a cascade of events and mechanisms that follow the ischemic insult (Carmichael, 2005; Fluri et al., 2015). Animal models allow a controlled, homogeneous and reproducible stroke size, as well as the potential for including specific anatomical areas within the lesion (Figure 17B, left). To date, such invasive investigation of pathophysiological processes or vasculature analysis cannot be replaced by in in-vitro preparations. The interventional approach in stroke animal models through optogenetics allows the exploration of numerous stimulation paradigms and time flexibility, crucial aspects often limited within the clinical research field (Figure 17F).

Conversely, the wide range of manipulations that optogenetic provides, could render the identification of the best paradigm particularly challenging. Nevertheless, optogenetic stimulation in stroke animal models has already been shown beneficial for recovery of function (Cheng et al., 2014; Daadi et al., 2016; Shah et al., 2017; Tennant et al., 2017; Wahl et al., 2017). Thus, via direct manipulation of specific neuronal networks and investigating the effect on functional outcome we can better understand which cell types drive post-stroke recovery. With this information, we can improve stimulation patterns, as well as identify potential targets for stroke therapies. To date, however, modulation of sleep oscillations through optogenetics has never been attempted in animal models of stroke. One valid and translational approach would be to evoke sleep-like local mechanisms by mimicking spontaneous slow oscillations through either activation or silencing of specific neuronal subpopulations (see section on perturbational approaches above). Such stimulation could then be used to rescue or enhance micro and macro sleep-phenotypes in stroke models and to specifically elucidate the role of sleep-like activity for functional recovery and plasticity.

5. Conclusions

Understanding how brain damage, its consequences on brain activity and brain repair are interrelated, is still an unmet goal in clinical neuroscience. Thus, the demonstration that sleep related processes may play a role in neuroplasticity and functional outcome after stroke could represent an advancement per se. To this aim we proposed in the present work a multi-modal methodological approach that merge the potential of various standard approaches. In particular we suggest that, integrating different parallel experimental procedures, including observational, perturbational and interventional experiments, both in human and animal models, could be promising, and the integrated result is likely to exceed those obtained by the sum of the individual approaches alone.

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Chapter four. Local sleep in awake stroke patients

As described above, the perturbational approach can show the presence of cortical bistability. Despite the similarities of the two phenomena, the lateralized slow waves in the wake EEG over the area surrounding the stroke and those of sleep have never been formally connected. We hypothesize that the pathological slow waves that surround a lesion during wakefulness share the same basic electrophysiological mechanism of cortical sleep slow waves, that is bistability between ON and OFF periods. Thus, the combination of TMS and EEG over peri-lesional areas of stroke patient could shows the presence of sleep-like bistability.

Specifically, in the work that follows we have characterized the occurrence of sleep-like bistability in chronic unilateral ischemic stroke patients, by analysing the EEG responses to TMS targeted over the perilesional areas. These responses were compared to those obtained by applying TMS with the same stimulation parameters over the contralateral healthy hemisphere. More specifically, we have applied spectral analysis to the TMS evoked EEG activity with a focus on the occurrence of a slow wave component (the hallmark of the EEG sleep state) associated with the suppression of high frequencies (above 20Hz) (the EEG proxy of the underlying neuronal down state).

Sleep-like OFF periods in the perilesional areas of awake, conscious brain-injured patients

Introduction

The functional consequences of a focal brain lesion are due to direct structural damage as well as to alteration in the dynamics of intact connected areas (Carrera and Tononi, 2014; Gratton et al., 2012). Detecting the electrophysiological changes occurring in these areas and understanding their neuronal underpinnings has been so far elusive.

A lateralized slowing of the wake EEG over the area surrounding focal lesions is a classic notion derived from early EEG recordings in acute stroke patients (Gloor et al., 1977). However, since the use of EEG in stroke research was superseded by imaging techniques (both structural and metabolic) offering higher spatial resolution, our understanding of stroke electrophysiology in humans proceeded at a rather low pace. By contrast, over the last twenty years the cellular/network mechanism of physiological slow EEG oscillations has been clearly identified in in vivo, in vitro as well as in computational models (Sanchez-Vives and McCormick, 2000; Steriade et al., 1993a). For example, during sleep, slow waves in the scalp EEG reflect the occurrence of brief interruptions of the neuronal firing (OFF periods) associated with a slow oscillation of the membrane potential of cortical neurons.

OFF periods are due to the tendency of cortical neurons to fall into a silent hyperpolarized state after an initial activation and represents the most dramatic change in the functional regime of neuronal cells. The mechanisms for such cellular behaviour rely on both intrinsic as well as network properties such as increased activity of leak and adaptation K+ channels and/or by increased inhibition within thalamocortical networks. Similar alterations may also occur as a consequence of brain injury due to a disconnection of ascending activating fibers and/or a reduction of lateral excitatory connectivity (Nita et al., 2007; Sun and Feng, 2013; Timofeev et al., 2000).

Despite their common features and possible mechanistic determinants, the lateralized slow waves occurring after stroke have never been systematically connected to the electrophysiology of sleep slow waves. Such a putative connection is particularly interesting when considering that slow waves and neuronal OFF periods can occur locally not only during sleep, but also during wakefulness (some brain regions can be silent while others are active) with important consequences on behaviour such as motor impairments, as shown in awake sleep deprived rats performing a pellet reaching task as well as cognitive lapses in awake humans (Nir et al., 2017).

In light of these evidence, we here test the hypothesis that the electrophysiological alteration affecting structurally intact perilesional areas reflect a tendency of cortical neurons to fall into a silent OFF period (i.e cortical bistability (Rosanova et al., 2018), that is a pathological form of local sleep patterns in the awake injured brain.

OFF periods during non-Rapid Eye Movement (NREM) sleep can be readily detected as a suppression of high frequency power associated with a slow wave (Mukovski et al., 2007) using intracranial recordings (Cash et al., 2009; Lewis et al., 2015). By virtue of their activity-dependent nature, bistability and the associated OFF-periods can be better revealed above and beyond spontaneous activity by recording the cortical response to direct perturbations (Pigorini, 2014). Such perturbational approach has also been used to demonstrate that silent OFF periods are in a key position to disrupt cortico-cortical interactions (Pigorini et al., 2015). Furthermore, a follow up study showed that the same events can be detected non-invasively by applying transcranial magnetic stimulation combined with EEG (TMS/EEG). Specifically, multisite TMS in healthy sleeping individuals as well as in unresponsive wakefulness syndrome (UWS) patients showed the ubiquitous occurrence of OFF-periods, leading to a global impairment of causality and brain complexity (Rosanova et al., 2018).

In the present work, we apply TMS/EEG to a cohort of thirty conscious awake patients with chronic focal and multifocal brain injuries. We show that OFF periods characterize the electrophysiological state of the perilesional area surrounding a focal cortical lesion and that this alteration is associated

with a disruption of local signal complexity with respect to the contralesional hemisphere. This finding is relevant, since it connects the notion of local sleep, thoroughly described in the sleep literature, to the pathophysiology of focal brain injury and stroke. Perilesional sleep-like OFF periods may represent a valid read-out of the state of discrete cortical circuits following stroke, as well as a potential target for the development of novel therapeutic interventions and physical rehabilitation aimed at fostering functional recovery.

Methods

Experimental model and subject details

Ten patients (4 F; age [y ± SEM]: 68 ± 4) affected by cortico/subcortical lesions due to an ischemic occlusion of the MCA as well as ten patients (4 F; age y ± SEM: 54 ± 6.1) affected by severe multifocal cortico/subcortical lesions of various etiologies (ischemic, haemorrhagic or traumatic) and ten patients (6 F; age y ± SEM: 72 ± 2.6) affected by unilateral purely subcortical lesions (either lacunar ischemic or typical haemorrhagic) in a sub-acute to chronic stage (>1 months; mean duration in months ± SEM: 12.9 ± 3.9; 7.6 ± 2.5; 19.5 ± 8.2, respectively) were included in the study. For the MCA ischemia and subcortical lesion groups, clinical evaluation included the NIH Stroke Scale (NIHSS; Kwah and Diong, 2014) (average [min/max] NIHSS score: 8 [4/17] and 5.5 [2/10], respectively; Wilcoxon rank sum test $Z=2.1$, $p=0.03$) as well as the Modified Barthel Index (MBI; Shah et al., 1989). MBI scores were lumped into three categories: severe (MBI: 0-49), moderate (MBI: 50-74) and mild (MBI: 75-90). The Coma Recovery Scale-Revised (CRS-R; (Giacino et al., 2004) was applied for the clinical evaluation of the severe multifocal lesions group.

Anatomical lesions were assessed by means of T1-weighted Magnetic Resonance Imaging (MRI) or Computerized Axial Tomography (CAT) scans acquired before the experimental sessions. For all patients, exclusion criteria were: positive remote or familiar history for epilepsy, positive remote or familiar history for convulsive events, presence of metallic cranial implants, presence of TMS incompatible equipment (pace-maker, drugs dispenser, cochlear implants, intracranial stimulator), pregnancy, positive history for alcohol or drug abuse and positive history for psychiatric conditions.

The experimental protocols were approved by the local ethical committees of the following Institutions: Istituto di Ricovero e Cura a Carattere Scientifico Fondazione Don Gnocchi Onlus, Fondazione Europea per la Ricerca Biomedica Onlus in Milan, Italy, and Medical School of the University of Liege in Liege, Belgium. A written informed consent was obtained for all the patients participating in the study.

Experimental procedures

During the entire duration of the experiment, patients were awake and with their eyes open. If signs of drowsiness appeared, recordings were momentarily interrupted and subjects were stimulated using the CRS-R (Giacino et al., 2004) arousal facilitation protocols.

The presence of cortical lesions guided the selection of TMS targets (Gosseries et al., 2015; Rosanova et al., 2018) based on the individual anatomical T1-weighted MRI or CAT scans acquired for all the patients included in the study within 1 week prior to the TMS/EEG assessment. Specifically, depending on the location and spatial extent of lesions in each individual patient, we included in the present study the analysis of two TMS/EEG measurements, one over the affected hemisphere (either frontal – Brodmann Area (BA) 4/6, or parietal – BA7; perilesional stimulation site) and one over the unaffected hemisphere (contralesional stimulation site). The precision and reproducibility of the TMS pulses with respect to the selected targets was guaranteed by means of a Navigated Brain Stimulation (NBS) system (Nexstim Ltd., Finland).

For each of the three patient groups, the stimulation sites were operationalized as follows. In the case of ischemic unilateral MCA stroke patients, the perilesional stimulation site corresponded to the intact portion of the BA affected by the lesion (either BA4, BA6 or BA7) while the contralesional stimulation site to the homologue contralateral cortical area. In the case of patients affected by severe multifocal cortico/subcortical lesions we followed the same criteria applied for the cortical lesion patient group and we targeted TMS over the intact portion of the same BA affected by the lesion (either BA4, BA6 or BA7; perilesional stimulation site) as well as over the homologue contralateral cortical area (contralesional stimulation site), unless also directly affected by a lesion itself or inaccessible to TMS due to the presence of intracerebral drainage/shunt. In this case, the contralesional stimulation site was chosen as a BA spared from lesions/shunt either over the same or the contralateral hemisphere. Finally, for unilateral subcortical stroke patients, the perilesional stimulation site consisted in a frontal (either BA4 or BA6) target over the affected hemisphere, while the contralesional stimulation site to the homologue contralateral cortical area. A detailed description regarding the lesions as well as the contralesional and perilesional stimulation sites for each individual patient are shown in Appendix Table 1.

Stimulation pulses were delivered with a Focal Bipulse figure-of-eight coil (mean/outer winding diameter ~50/70 mm, biphasic pulse shape, pulse length ~280 μs, focal area of the stimulation 0.68 cm2) driven by a Mobile Stimulator Unit (eXimia TMS Stimulator, Nexstim Ltd., Finland). For all the TMS/EEG measurements, the location of the maximum electric field induced by TMS on the cortical

surface (hotspot) was always kept on the convexity of the targeted cortical gyrus, with the induced current perpendicular to its main axis. Each cortical target was stimulated with an estimated electric field, orthogonal to the gyral crown, of about 120V/m corresponding to a percentage of the maximal stimulator output (% MSO) intensity comparable between contralesional and perilesional stimulation sites for each patient group (mean ± SEM: 65.3 ± 1.97 vs 67.1 ± 2.61 for the MCA ischemia group, 62.6 ± 3.7 vs 67.8 ± 2.58 for the severe multifocal cortico/subcortical lesion group and 59.2 ± 1.73 vs 60.9 ± 2.24 for the subcortical lesion group; for all comparisons $p>0.05$, paired t-test). In each TMS/EEG measurement, at least 200 stimulation pulses were delivered with an interstimulus interval randomly jittering between 2000 and 2300 ms (0.4–0.5 Hz).

EEG recordings

TMS/EEG data recording and pre-processing

EEG data were recorded using a TMS-compatible 60-channel amplifier (Nexstim Ltd, Finland), which gates the magnetic pulse artefact and provides artifact-free data from 8 ms after stimulation (Virtanen et al., 1999). Raw recordings were referenced to a forehead electrode, online filtered between 0.1-350 Hz, and sampled at 1450 Hz. Two additional sensors were applied to record the electroculogram (EOG). As previously recommended (Ter Braack et al., 2015), during all TMS/EEG recordings a masking sound was played via earphones and a thin layer of foam was placed between coil and scalp for abolishing the auditory potentials evoked by TMS coil's discharge.

Data analysis was performed using Matlab R2012a (The MathWorks Inc.). TMS/EEG recordings were visually inspected to reject trials and channels containing noise or muscle activity, as in previous works (Casarotto et al., 2016; Gosseries et al., 2015; Rosanova et al., 2018). Then, EEG data were band-pass filtered (1-45 Hz, Butterworth, 3rd order), downsampled to 725 Hz and segmented in a time window of ± 600 ms around the stimulus. Bad channels were interpolated using the spherical function of EEGLAB (Delorme and Makeig, 2004). Recording sessions either with more than 10 bad channels or with less than 100 artifact-free trials were excluded from further analysis. Then, trials were re-referenced to the average reference and baseline corrected. Finally, Independent Component Analysis (ICA) was applied in order to remove residual eye blinks/movements and TMS-evoked and spontaneous scalp muscle activations.

Resting-state EEG recording and pre-processing

Preceding TMS/EEG measurements and using the same EEG recording apparatus, a wake resting state hd-EEG recording (up to 10 minutes with eyes open) was performed for the MCA ischemia patients (n=10) to assess the presence of the lateralized slowing in the theta/delta frequency range typical of unilateral brain injuries characterized by cortical infarction (Macdonell et al., 1988).

Spontaneous EEG data were off-line filtered (0.5–40 Hz) with a 3rd order IIR Butterworth digital filter with an attenuation of -3dB at 0.5 and 40 (using the filtfilt function in the MATLAB signal processing toolbox). Continuous data were then split into contiguous 2-second segments. Artifactual segments were excluded from the analysis based on visual inspection (max/min retained EEG segments: 284/69). Bad channels were rejected based on visual inspection (≤ 10% of channels per recording). Rejected channels were then interpolated using spherical splines. The signal for each channel was then re-referenced to the average of all channels. After reducing the number of independent components to the number of good, non-interpolated channels by performing Singular Value Decomposition, independent component analysis [ICA; [S11]] was used to remove ocular, muscle, and cardiac pulse artifacts using EEGLAB routines (Delorme and Makeig, 2004). For each EEG derivation, power spectral density (PSD) estimates were computed using the Welch's method with 2-s Hanning windows and 50% overlap (applying the pwelch function in the MATLAB signal processing toolbox). For each patient, average PSD calculated over the same four channels used for TMS/EEG analysis (contralesional, perilesional) across segments was computed for each frequency bin and then averaged across bin divided into classical frequency ranges: delta (0.5-4.5Hz), theta (4.5-8 Hz), alpha (8-12 Hz), beta (12-30 Hz) and gamma (30-40 Hz) (Figure 21). In parallel to the PSD analysis, filtered continuous EEG data were re-referenced according to a longitudinal bipolar montage based on the 10-20 system (Niedermeyer and Silva, 2005) including the following EEG derivations: 'Fp1_F5', 'F7_T3', 'T3_T5', 'T5_O1' 'Fp1_F3', 'F3_C3', 'C3_P3', 'P3_O1', 'Fz_Cz', 'Cz_Pz' ,'Fp2_F6', 'F6_T4', 'T4_T6', 'T6_O2', 'Fp2_F4', 'F4_C4', 'C4_P4', and 'P4_O2'. These data were then visually inspected by a clinical neurophysiologist to assess the presence of EEG anomalies (Apendix Table 2 and Figure 21).

Quantification and statistical analysis

In order to detect the presence of local sleep-like activity in the perilesional areas of awake, conscious brain injured patients, we followed the same methodological rationale as in Pigorini et al., Neuroimage 2015, and Rosanova et al., 2018. Specifically, for each patient group (n=10 per group), we compared

the TMS/EEG measurements performed over the contralesional and the perilesional stimulation sites and we aimed at quantifying (1) the presence of TMS-evoked slow waves over the perilesional area as well as (2) the correspondent occurrence of a cortical down state. Operationally, these variables can be quantified respectively as (1) the amplitude of low-frequency EEG components (< 4 Hz), and (2) the modulation of post-stimulus high-frequency EEG power (> 20 Hz) (Cash et al., 2009; Csercsa et al., 2010; Menicucci et al., 2013; Mukovski et al., 2007; Valderrama et al., 2012). We refer the reader to (Pigorini et al, 2015 and Rosanova et al., 2018) for detailed methodological description. In brief, to assess the presence of TMS-evoked slow waves, single trials were low-pass filtered below 4 Hz (second-order Chebyshev filter), re-referenced to the linked mastoids, averaged and eventually rectified. For each channel, the Slow Wave amplitude (SWa) was computed as the cumulated amplitude of the rectified signal within the 8-350 ms time window. On the other hand, to assess the presence of the correspondent cortical OFF-period, we applied the event related spectral perturbation (ERSP) procedure implemented in EEGLAB (Delorme and Makeig, 2004). Specifically, single trials were time-frequency decomposed between 8 and 45 Hz using Wavelet transform (Morlet, 3.5 cycles; as in Rosanova et al., 2009, Ferrarelli et al., 2012 and Premoli et al. 2017) and then normalized with the full-epoch length (here ranging from -350 to 350 ms) single-trial correction (Grandchamp et al., 2012; Rosanova et al., 2018). The resulting ERSPs were averaged across trials and baseline corrected (from -350 to -100 ms; Grandchamp et al., 2011). Furthermore, power values that were not significantly different from baseline (bootstrap statistics, $\alpha<0.05$, number of permutations = 500) were set to zero. The time course of significant high-frequency EEG power was obtained by averaging ERSP values above 20 Hz (Mukowskyi et al., 2007). The down state was detected as a significant decrease in power above 20 Hz with respect to baseline (Cash et al., 2009; Csercsa et al., 2010; Menicucci et al., 2013; Mukovski et al., 2007; Valderrama et al., 2012). For each TMS/EEG measurement, these indexes, labelled SWa and HFp, were computed at the single-channel level and then averaged over the four channels closest to the stimulation site (like to Casarotto et al., 2013 and Rosanova et al., 2018) for group analysis.

Analysis of TMS/EEG local signal complexity

For each patient, we first estimated the maximum global spatiotemporal dynamics of the TMS-evoked response, by computing the Perturbational Complexity Index based on the quantification the temporal complexity of the principal components of the EEG response to TMS (PCIst), as described in (Comolatti et al., 2019). In addition, we applied a generalization of the original method and, for each

stimulation session, we calculated PCIst restricted to the four channels closest to the stimulation site. This way, we were able to assess the impact of perilesional sleep-like OFF periods on local signal complexity.

In general, PCIst is an index that combines dimensionality reduction and a novel metric of recurrence quantification analysis (RQA) to empirically quantify perturbational complexity as the overall number of non-redundant state transitions (ST) caused by the perturbation. Briefly, principal component analysis (PCA) of the response is performed in order to obtain the spatial modes of the signal and a method derived from RQA is then applied to quantify the number of "state transitions" present in each component. For both the global and local signal complexity estimates, PCIst was computed using the code available at github.com/renzocom/PCIst using the same parameters for component selection (max_var=99%; min_snr=1.1) and state transition quantification (k=1.2), as in (Comolatti et al., 2019).

Statistical analyses

For SWa, HFp, local signal complexity, as well as for resting-state EEG PSD, within-group comparisons between stimulation sites (perilesional, contralesional) were performed by means of the non-parametric Wilcoxon signed-rank test ($p<0.05$; $n=10$). When testing between-group differences ($n=30$), mixed-model ANOVAs were performed with GROUP as categorical predictor and SITE as within-subject factor. To test contrasts, post hoc two-tailed t tests were used ($p<0.05$, Bonferroni corrected).

Results

In this work, we assessed the EEG responses to TMS in three groups of conscious awake brain-injured patients (total n=30) of various etiologies and disease severity. Specifically, we included i) a group of ten patients affected by unilateral cortico/subcortical lesions due to an ischemic occlusion of the middle cerebral artery (MCA ischemia group), ii) a group of ten patients affected by severe multifocal cortico/subcortical lesions (severe multifocal lesions group) of various aetiologies, including traumatic brain injuries and iii) a group of ten patients affected by unilateral lacunar ischemic or typical haemorrhagic purely subcortical lesions (subcortical lesions group). For a complete description of patient demographics and details, see Appendix Table 1.

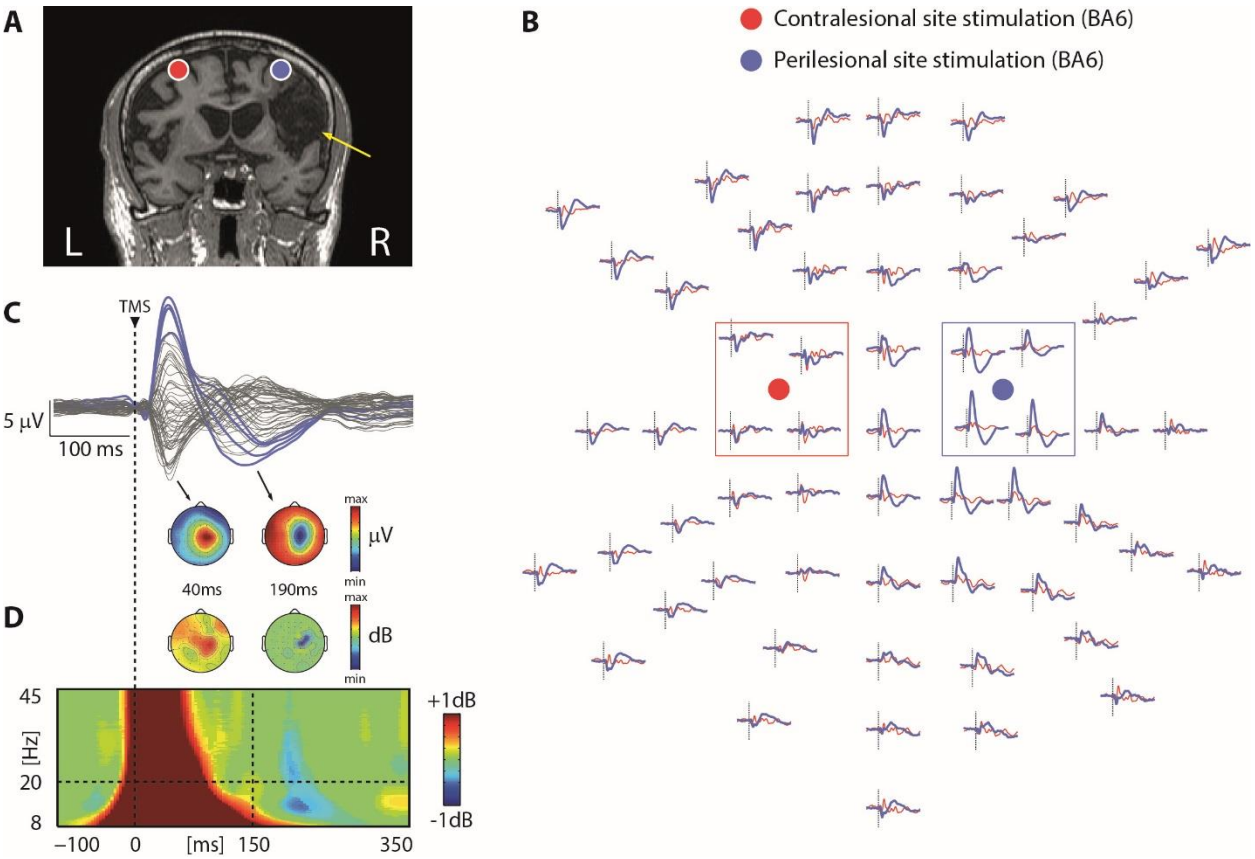

Figure 18. TMS reveals local, sleep-like slow waves associated with cortical OFF-periods over the affected hemisphere. Results from one representative patient from the MCA group (patient n.10 from Table1) are shown for both the contralesional (red) and perilesional (blue) stimulation sites. Panel A. MRI and cortical targets (BA6) as estimated by the Navigated Brain Stimulation system are shown. The yellow arrows highlight lesion location. Panel B. Superimposition of TMS-evoked potentials of the two stimulated sites for all EEG sensors (arranged based on the channel layout is displayed). For each channel, a dashed vertical line marks the occurrence of TMS. Colour-coded circles represent the position of the coil over the scalp. Colour-coded boxes highlight the four channels closest to TMS for both stimulation sites. Note the occurrence of a local slow wave over the right hemisphere for the perilesional stimulation site. Panel C. Butterfly plot of the TMS-evoked EEG potentials recorded at all 60 electrodes for the perilesional stimulation site (top). The four EEG electrode closest to TMS (indicated by the blue box in Panel A) are displayed in colour. The instantaneous voltage topography of the positive and negative deflections of the slow wave is depicted below. Panel D. Event-related spectral perturbation (ERSP) averaged across the four EEG electrodes closest to TMS (bottom). Significance for bootstrap statistics is set at $\alpha < 0.05$ (absence of any significant difference from baseline spectrum is coloured in green): statistically significant increases of power compared to baseline are coloured in red, while blue represents significant power decreases. The dashed horizontal line indicates the 20 Hz frequency bin. As in Panel B, the instantaneous voltage topography of the averaged power between 20 and 45 Hz is depicted above for the same timepoints. Note the occurrence of a local significant suppression of high-frequency power limited to the perilesional stimulation site concurrent with the negative deflection of the slow wave. For both Panels B and C, a dashed vertical line at time 0 marks the occurrence of TMS.

Each patient underwent a single experimental session which included two TMS/EEG measurements performed with TMS targeted to intact cortical portions of both the affected (perilesional stimulation site) and the unaffected (contralesional stimulation site) hemispheres (Figure 18A, see STAR Methods for details regarding the specific TMS targeting). This experimental procedure allowed for a direct intra-subject comparison, thus minimizing the variability of the dependent variables often present in case-control studies. Specifically, in each of the three groups, we assessed the occurrence of a TMS-evoked slow wave (<4 Hz) associated with the presence of a cortical OFF period, i.e. a significant high frequency (>20 Hz) suppression of EEG power compared to baseline (Mukovski et al., 2007; Cash et al., 2009)confined to the perilesional stimulated site (Figure 18B,C and D). Furthermore, we aimed at assessing the effects of local cortical OFF-periods on local signal complexity, as measured by a recently proposed index of perturbational complexity (PCIst; Comolatti et al., 2019).

TMS reveals local, sleep-like cortical OFF-periods over the affected hemisphere only in patients with cortico/subcortical lesions irrespective of the aetiology

In the MCA ischemia group, TMS-evoked EEG potentials (TEPs) obtained from the stimulation of the contralesional hemisphere were low-amplitude, fast-frequency recurrent scalp waves like those previously observed in healthy awake individuals (Figure18B and Figure 19A, red trace). When TMS was applied using the same stimulation parameters over the affected hemisphere, TEPs were characterized by a local slow EEG potential (Figure 18B, C and Figure 19A, blue traces) associated with an initial broad-band activation followed by a significant suppression of high frequency EEG power, starting roughly at 150 ms after TMS (mean±SEM: 167±14 ms) over the four channels closest to the stimulation site (Figure 18B and C). Notably, this local pattern of activation, matching the criteria for an OFF-period, was found for all ten patients only over the perilesional stimulation site and never over the contralateral unaffected stimulation site. Consistently, low-frequency (<4 Hz) EEG amplitude (SWa, see STAR Methods section) obtained over the perilesional site was significantly higher compared to the contralesional homologue site (Wilcoxon signrank test $Z=2.4$, $p=0.01$; Figure 19B). Also, the suppression of high frequency power (HFp, see STAR Methods) was significantly different between the two stimulated sites (Wilcoxon signrank test $Z=2.8$, $p=0.005$), and only present over the perilesional stimulation site (all ten patients displayed negative values (range [min/max]: -0.01/-0.6); see Figure 19D). Finally, this pattern of TMS-evoked response over the perilesional site was invariably found in all subject, irrespective of the presence of slow waves spontaneously occurring in their background EEG (Figure 21).

Similar results were obtained in the severe multifocal lesions group. Specifically, TMS-evoked EEG potentials obtained from the perilesional stimulation site were characterized by a slow wave associated with an initial broad-band activation followed by a significant suppression of high frequency EEG power, starting at 158±16 ms (mean±SEM) over the four channels closest to the stimulation site (Figure 19D). Consistently, and similar to the unilateral MCA ischemia group, both SWa and HFp (Figure 19D and E) obtained over the perilesional site were significantly different compared to the contralesional site (Wilcoxon signrank test $Z=2.3$, $p=0.01$ and $Z=2.8$, $p=0.005$, respectively). Importantly, also in this group, the suppression of HFp was present for all ten patients only over the perilesional stimulation site (range [min/max]: -0.06/-1.09, see Figure 19E).

Overall, results in these two groups of patients confirmed that, irrespective of the aetiology, TMS in the presence of cortico/subcortical lesions featured a sleep-like slow wave associated with the presence of a cortical OFF-period confined to the perilesional stimulated site. Further confirming the sleep-like nature of the observed findings, Figure 22 shows the results obtained for a representative patient (patient n.14, see Appendix Table 1) from the severe multifocal lesions group. In this patient, in addition to the two TMS/EEG measurements performed during wakefulness (Figure 22B), the same stimulations were also performed while the patient was asleep (Figure 22C) for the entire duration of the recordings, as assessed by the presence of prolonged eye closure and typical NREM sleep graphoelements -i.e. sleep spindles and slow waves- in the spontaneous EEG. Results show a striking similarity between the TMS-evoked response obtained over the perilesional stimulation site during wakefulness and those obtained ubiquitously over both stimulation site during sleep, particularly with respect to the presence of significant high-frequency power suppression concurrent with the negative deflection of the slow wave (the hallmark of cortical OFF periods during sleep).

At odds with the above, TMS-evoked EEG potentials obtained in the subcortical lesions group (Figure 19C) were never associated with the presence of a slow wave or high frequency EEG power suppression over neither the perilesional nor the contralesional stimulation site (Wilcoxon signrank test $Z= 0.7$, $p=0.44$ for SWa and $Z=0.8$, $p=0.38$ for HFp, see Figure 19F). Interestingly, for both SWa and HFp, the values obtained in this group of patients over both sites were like those obtained over the contralesional stimulation site in the MCA ischemia and the severe multifocal lesions groups (Figure 19D, E and F).

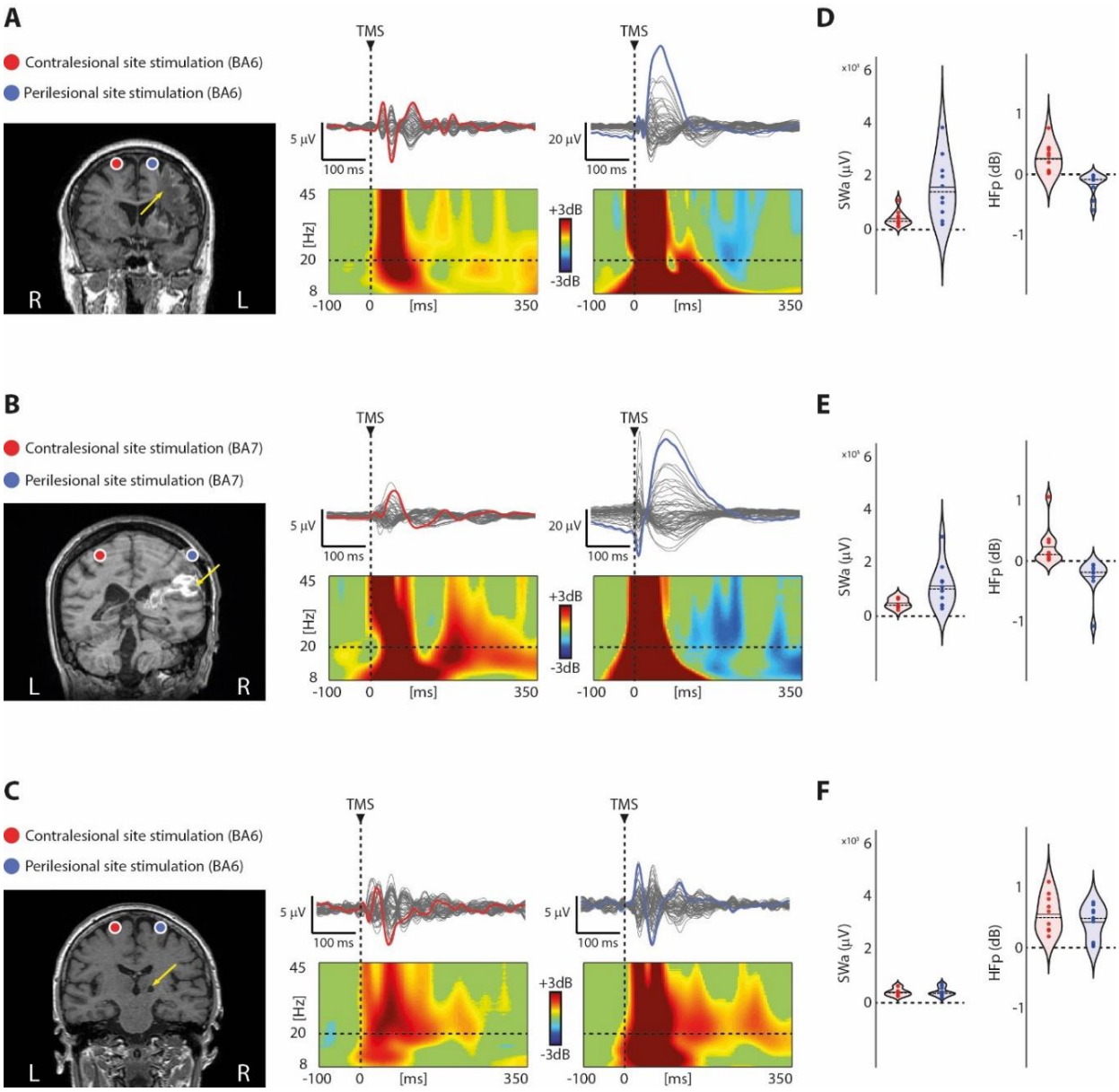

Figure 19. Perilesional cortical OFF-periods are present in all patients with cortico/subcortical lesions irrespective of the aetiology. Panel A, B and C. Results from three representative patients, one for each group (patients n.4, 12 and 23 from Appendix Table 1, respectively) are shown for both contralesional (red) and perilesional (blue) stimulation sites. For each panel, MRIs and cortical targets as estimated by the Navigated Brain Stimulation system are shown (left). The yellow arrows highlight lesion location. Butterfly plots of the TMS-evoked EEG potentials recorded at all 60 electrodes (traces) are depicted (right). A dashed vertical line marks the occurrence of TMS. Event-related spectral perturbation (ERSP) is presented for the EEG electrode (colored trace) with the largest early response, selected among the four channels closest to TMS. In the ERSP plot, significance for bootstrap statistics is set at $\alpha < 0.05$ (absence of any significant difference form baseline spectrum is colored in green): statistically significant increases of power compared to baseline are colored in red, while blue represents significant power decreases. The dashed horizontal line indicates the 20 Hz frequency bin. Panel D, E and F. Violin plots and individual values of slow wave amplitude (SWa, see STAR Methods; top) and high-frequency power (HFp, see STAR Methods; bottom) calculated for the contralesional (red) and perilesional (blue) stimulation sites for the three groups. Violin plots display the median (dashed line) and the mean (solid line) of the distribution kernel.

To test this, we performed a mixed-model ANOVA with GROUP as categorical predictor and SITE as within-subject factor. For both SWa and HFp, values obtained in the subcortical lesions group over both the perilesional and contralesional sites were statistically different from those obtained over the contralesional site of both MCA ischemia and severe multifocal lesions, as revealed by post-hoc comparisons (all p-values >0.2, Bonferroni corrected). To further explore this, in one patient affected by multiple lacunar periventricular ischemic white matter lesions (patient n.24, see Appendix Table 1) we performed a more extensive mapping including two additional stimulations over BA 7 of both the affected and the unaffected hemispheres (Figure 23). Results confirmed the absence of TMS-evoked slow waves and OFF-periods irrespective of the stimulated hemisphere and cortical area.

The presence of local, sleep-like cortical OFF-periods affects local signal complexity

We then assessed the effects of the local occurrence of TMS-evoked slow waves and OFF-periods on local signal complexity. To this aim, for each patient and stimulated site, we quantified the temporal complexity of the principal components of the signals calculated over the four channels closest to TMS (Figure 20A, B and C). In line with our hypothesis, the complexity of the TMS-evoked responses was found markedly reduced over the perilesional compared to the contralesional stimulation site for the MCA ischemia group (Wilcoxon signrank test $Z=2.4$, $p=0.01$; Figure 20A). Once again, similar results were found for the severe multifocal lesions group where differences in local signal complexity between stimulation sites were found at trend level (Wilcoxon signrank test $Z=1.5$, $p=0.1$; Figure 20E). The same analysis applied on the subcortical lesions group showed no differences in local signal complexity between stimulation sites (Wilcoxon signrank test $Z=1.2$, $p=0.2$; Figure 20F). Altogether, these findings suggest that, when present, local sleep-like cortical OFF-periods affect local signal complexity.

Of note, the somehow smaller effect observed in the group of patients affected by severe multifocal lesions may be explained by the presence of brain lesions often involving both hemispheres, partially affecting also the local signal complexity of the contralesional stimulation site. This was confirmed by a mixed-model ANOVA with GROUP as categorical predictor and SITE as within-subject factor (GROUP*SITE interaction effect: $F(2, 27)=3.3$, $p=0.04985$). Indeed, local signal complexity for the perilesional stimulation site was found similar to that observed for the MCA ischemia group ($p>0.05$, Bonferroni corrected; Figure 20D) and significantly lower than that observed in the subcortical lesions group ($p=0.000001$, Bonferroni corrected; Figure 20F).

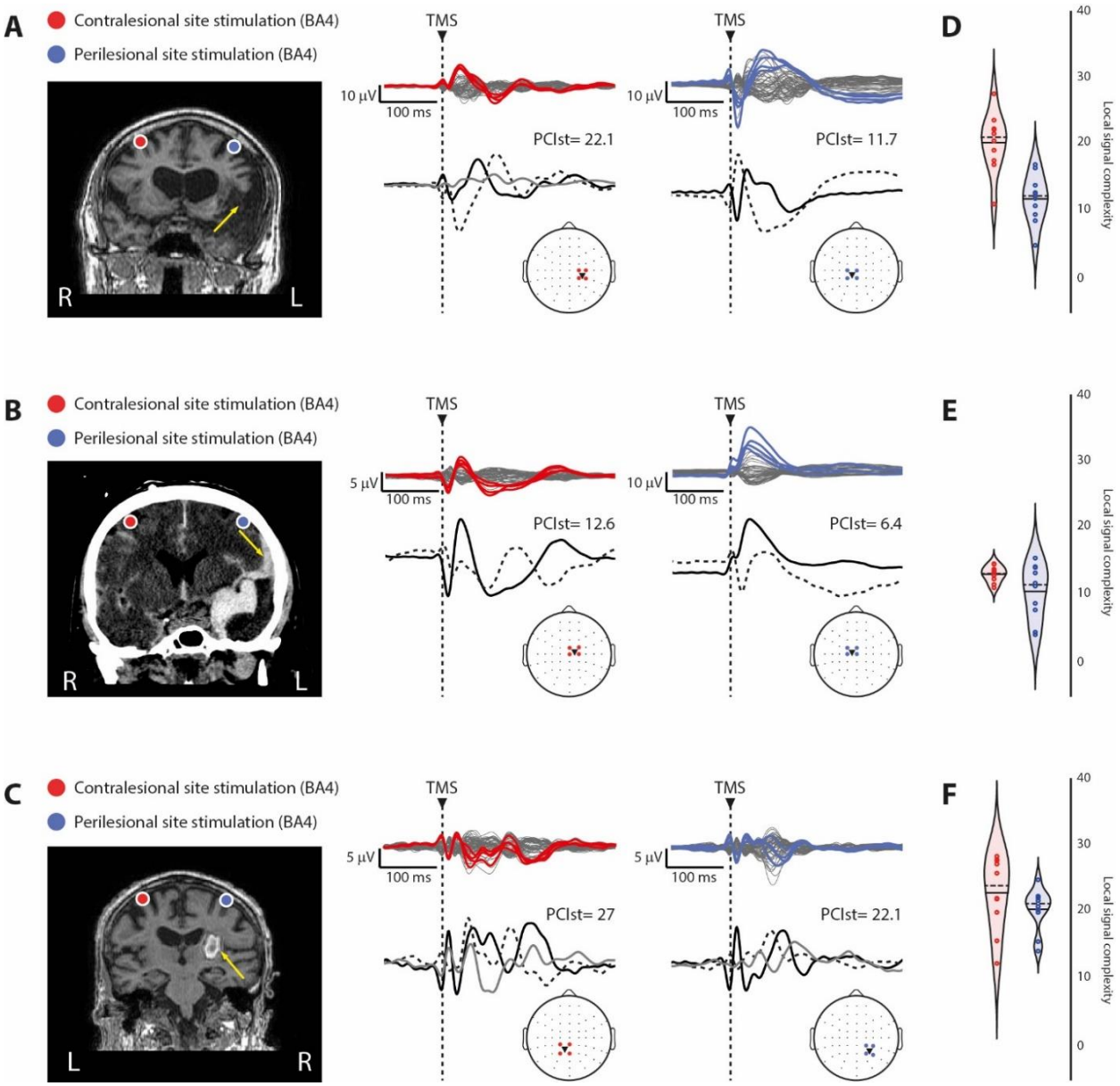

Figure 20. The presence of local, sleep-like cortical OFF-periods affects local signal complexity. Panel A, B and C. For each panel, brain imaging (MRI for Panel A and C, CAT scan for Panel B) and cortical targets as estimated by the Navigated Brain Stimulation system are shown for three representative patients, one for each group of patients (patients n.1, 13 and 22 from Appendix Table 1, respectively) are shown for both the contralesional (red) and perilesional (blue) stimulation sites. The yellow arrows highlight lesion location. Butterfly plots of the TMS-evoked EEG potentials recorded at all 60 electrodes (traces) are depicted (top). For each stimulated site, the four EEG electrode closest to TMS are displayed in colour and topographically projected on the template channel layout (bottom). The correspondent local signal complexity values (calculated by applying PCIst restricted to the four channels, see STAR Methods) and the time-course of the principal components based on singular value decomposition are depicted (bottom). A dashed vertical line marks the occurrence of TMS. Panel D, E and F. Violin plots and individual values of local signal complexity calculated for the contralesional (red) and perilesional (blue) stimulation sites for the three groups. Violin plots display the median (dashed line) and the mean (solid line) of the distribution kernel.

Local signal complexity was reduced for the contralesional stimulation site in the severe multifocal lesions group compared to the other two groups (all p-values <0.0002, Bonferroni corrected; Figure 20D, E and F).

Furthermore, we estimated the maximum global spatiotemporal dynamics of the TMS-evoked response by computing PCIst calculated on the sixty channels following the stimulation of the contralesional site (see Appendix Table 3). The aim of this analysis was to assess PCIst as an index of the capacity of thalamocortical circuits to engage in complex patterns of causal interactions, typically present in conscious awake individuals (Massimini et al., 2005, 2009). Results at the group level were in line with previous observation (Casarotto et al., 2016) on conscious brain-injured individuals and showed that, for all three groups, average maximum PCIst values (mean ± SE: 41.1 ± 2.4, 28.5 ± 3.1 and 42.5 ± 2, respectively) were above the statistical threshold (PCIst threshold value: 23.02; Comolatti et al., 2019) for consciousness obtained from a benchmark population (382 TMS/hd-EEG sessions performed on 108 healthy subjects). At the individual level, only two patients (both pertaining to the severe multifocal lesions group; patient n.17 and 18, see Appendix Table 1) out of thirty were found below this threshold further confirming the role of multifocal brain lesions in partially affecting overall signal complexity. As a result, a ONE-way ANOVA (GROUP effect $F(2, 27)=9.3$, $p=0.00082$) showed a significantly lower maximum PCIst value for the severe multifocal lesions group compared to the other two groups (all p-values <0.004, Bonferroni corrected).

Discussion

In this study, we applied TMS/EEG to a cohort of thirty conscious awake patients with chronic focal and multifocal brain injuries of various aetiologies. We showed that local sleep-like slow waves associated with OFF-periods characterize the electrophysiological state of the perilesional area surrounding cortico/subcortical lesions (Figure 18, Figure 19 and Figure 21).

More recently, in a cohort of awake unresponsive brain-injured patients we reported a similar EEG response to TMS, suggesting that a pathological form of sleep-like cortical bistability may occur also following severe brain injuries (Rosanova et al., 2018). These findings established a first attempt to link a cellular phenomenon with a well characterized physiological mechanism to the pathophysiology of brain injuries. However, in this case the structural as well as the functional (Compte et al., 2009) involvement of key regions (e.g. brainstem and cortico-striatal mesocircuits) may prevent a critical amount of diffuse neuromodulation keeping the entire thalamocortical system in a balanced, wake-like state in response to transient inputs.

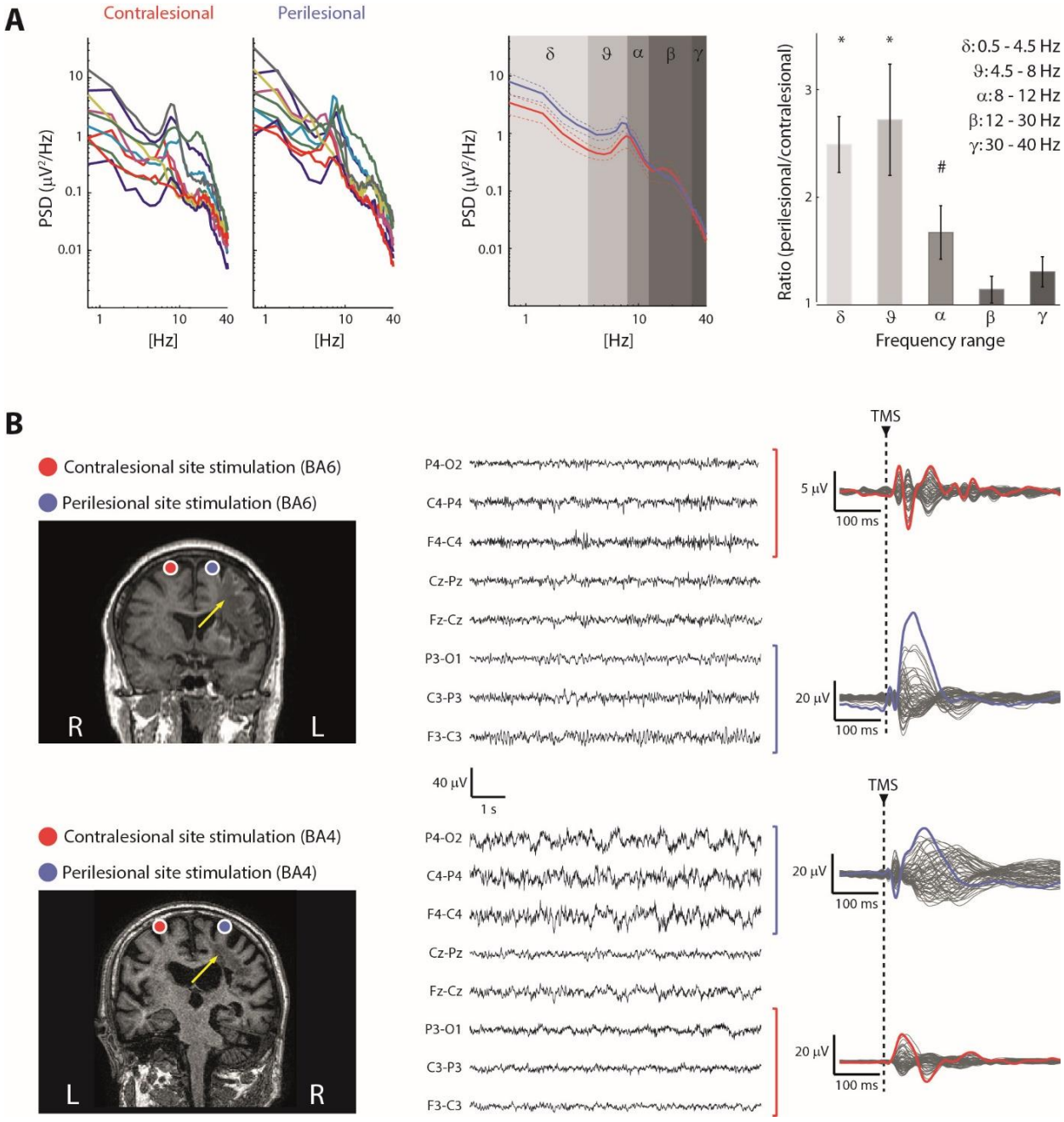

Figure 21. Resting-state EEG recordings in the MCA ischemia group. Panel A. For each patient, individual Power Spectral Density (PSD) calculated over the same four channels used for TMS/EEG analysis (contralesional, red; perilesional, blue) is shown (left). Average PSD (±SEM) across patients. Shaded grey boxes identify classical frequency ranges (middle). Ratio between perilesional and contralesional site PSD averaged across bins divided into classical frequency ranges: delta (0.5-4.5Hz), theta (4.5-8 Hz), alpha (8-12 Hz), beta (12-30 Hz) and gamma (30-40 Hz) (right). Group analysis showed a significant increase of delta and theta EEG activity over the perilesional site (*: p-values <0.008 for both delta and theta; Bonferroni corrected α: 0.01), confirming the typical EEG pattern found in unilateral brain injuries characterized by cortical infarction (Macdonnel et al., 1988). Alpha frequency was also found increased over the perilesional site (#: p=0.02 uncorrected). Panel B. MRIs and cortical targets as estimated by the Navigated Brain Stimulation system are shown for two representative patients (patient n.4 and n.5 from Appendix Table1, left). The yellow arrows highlight lesion location. The visual inspection of EEG (here displayed with a reduced longitudinal bipolar montage focused on the regions explored

by TMS, middle) confirmed the PSD findings and highlighted heterogeneous EEG patterns (see also Appendix Table 2) characterized by the lateralized presence of either theta rhythms (top) or slow waves (bottom). Notably, in both cases, applying TMS (dashed vertical line) resulted in a clear-cut evoked EEG slow wave over the perilesional site (blue traces on the right), thus showing the added value of TMS in revealing the presence of perilesional slow waves (and of the associated OFF-periods, not shown here, but see Figure 18, Figure 19 and Figure 22) irrespective of the presence of slow waves spontaneously occurring in the background EEG.

This resulted in the ubiquitous presence of electrophysiological signature of cortical bistability across different cortical areas of both hemispheres (Rosanova et al., 2018) and prevented a systematic assessment of the impact of focal lesions on cortical bistability.

Similar electrophysiological events were previously found in sleeping and anesthetized healthy controls and have been shown to reflect a profound membrane hyperpolarization of cortical neurons (Ferrarelli et al. 2010). During physiological sleep this phenomenon -often referred to as cortical bistability- is caused by the enhancement of adaptation (or activity-dependent) K+ currents, brought about by decreased levels of neuromodulation from brainstem activating systems and/or by increased inhibition. Due to these mechanisms, cortical neurons tend to plunge into a silent, hyperpolarized state, lasting few hundreds of milliseconds, after an initial activation. The occurrence of synchronous membrane hyperpolarization in cortical neurons is reflected at the extracellular level in large slow waves associated with transient suppressions of high-frequency (>20 Hz) activity that may be detectable in spontaneous activity both in the local field potential and in the EEG. However, due to its activity-dependent nature, bistability and the associated OFF-periods can be better revealed using a perturbational approach, whereby the impulse-response properties of cortical neurons is probed by means of direct activations (Pigorini et al., 2014).

In this respect, a key finding of the present work is the demonstration of a pathological form of local sleep-like cortical bistability occurring in circumscribed intact portions of the cortex adjacent to focal cortico/subcortical brain injuries of conscious awake brain-injured individuals.

Studying cortical reactivity and connectivity in patients with stroke TMS-EEG may identify salient neural mechanisms underlying motor disabilities and lead to novel biomarkers of stroke pathophysiology which can then be used to assess, monitor, and refine rehabilitation approaches for individuals with significant disability to improve outcomes and quality of life after stroke.

Furthermore, the results have shown a reduction in brain signal complexity in the brain areas surrounding the stroke. As a global reduction in brain signal complexity may be held responsible for unconsciousness, as in physiological NREM sleep and pathological UWS, the same mechanism on a local scale may partly account for behavioural deficits in stroke patients.

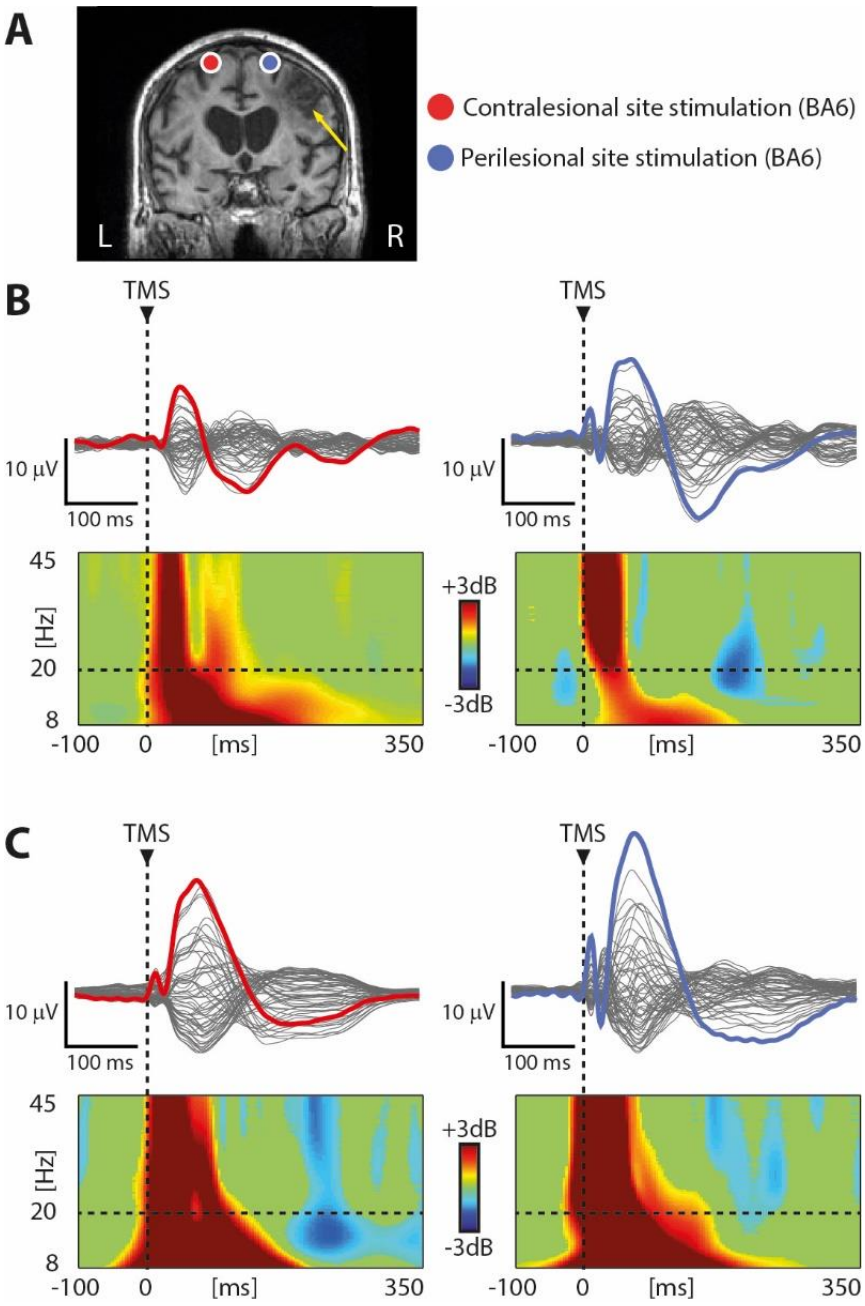

Figure 22. The TMS-evoked response over the perilesional stimulation site during wakefulness is like the typical TMS response ubiquitously observed during NREM sleep. Results from one representative patient (patient n.14 from Appendix Table 1) are shown for both the contralesional (red) and perilesional (blue) stimulation sites. Panel A. MRI and cortical targets (BA6) as estimated by the Navigated Brain Stimulation system are shown. The yellow arrows highlight lesion location. Panel B and C. Butterfly plots of the TMS-evoked EEG potentials recorded at all 60 electrodes (traces) during wakefulness (Panel B) and NREM sleep (Panel C) are depicted. A dashed vertical line marks the occurrence of TMS. Event-related spectral perturbation (ERSP) is presented for the EEG electrode (colored trace) with the largest early response, selected among the four channels closest to TMS. In the ERSP plot, significance for bootstrap statistics is set at $\alpha < 0.05$ (absence of any significant difference form baseline spectrum is colored in green): statistically significant increases of power compared to baseline are colored in red, while blue represents significant power decreases. The dashed horizontal line indicates the 20 Hz frequency bin. Note the similarity between the EEG response and ERSP features to TMS of the perilesional site stimulation during wakefulness and those observed during sleep over both stimulated sites.

Putative mechanisms underlying local cortical bistability following brain lesion

Intracortical inhibition

Other than the above-mentioned mechanism involving cortical deafferentation, bistability can be induced by alteration of the excitation/inhibition balance in intact portions of the thalamocortical system. For instance, recovery of language and motor function after stroke can be blocked by an excessive inhibitory activity in the peri-lesional area (Classen et al., 1997); this excessive inhibition may be generated locally by healthy areas that become hyperactive (Murase et al., 2004). In this case, bistability would be brought about by increased inhibition.

A possibility is that structural lesions may lead to functional changes that enhance adaptation mechanisms and hence the tendency of intact portions of the thalamocortical system to transiently fall into a quiescent OFF-period.

In an extreme case, the thalamocortical system may be largely intact but functionally constrained to a pathological tendency towards OFF-periods due a predominance of adaptation currents.

Vascular lesions may also induce bistability by engendering a state of cortico-cortical disfacilitation, that is by reducing recurrent excitation.

Chronic consequences of acute neuroprotection

Another possibility, particularly relevant for ischemic vascular events, is that the initial neuroprotective role played by the K-ATP channels present in neuronal (Heurteaux et al., 1993) as well as in astrocytes and microglial cells (Ortega et al., 2012; Simard et al., 2006), may have chronic functional consequences on the cortical tissue surviving the insult. Specifically, during the initial phase of ischemia, energy failure reduces the ATP/ADP ratio, which activates neuronal K-ATP sensitive channels (typically closed during physiological conditions), causing hyperpolarization of the neuronal cell membrane and suppression of neuronal activity. Depending on the length of the hypoxic challenge, the cell membrane potential may not follow the typical speedy recovery and stabilization thus prolonging the hyperpolarized state of cortical neurons. Although the precise mechanisms underlying the K-ATP sensitive channels activation following ischemia remain unclear, it is possible that they would initially serve a transient, positive effect that prevents cell death that turns, on the long run, into the instantiation of a chronic hyperpolarized state compatible characterized by neuronal excitability profiles similar to those of physiological sleep-like bistability (Sun and Feng, 2013).

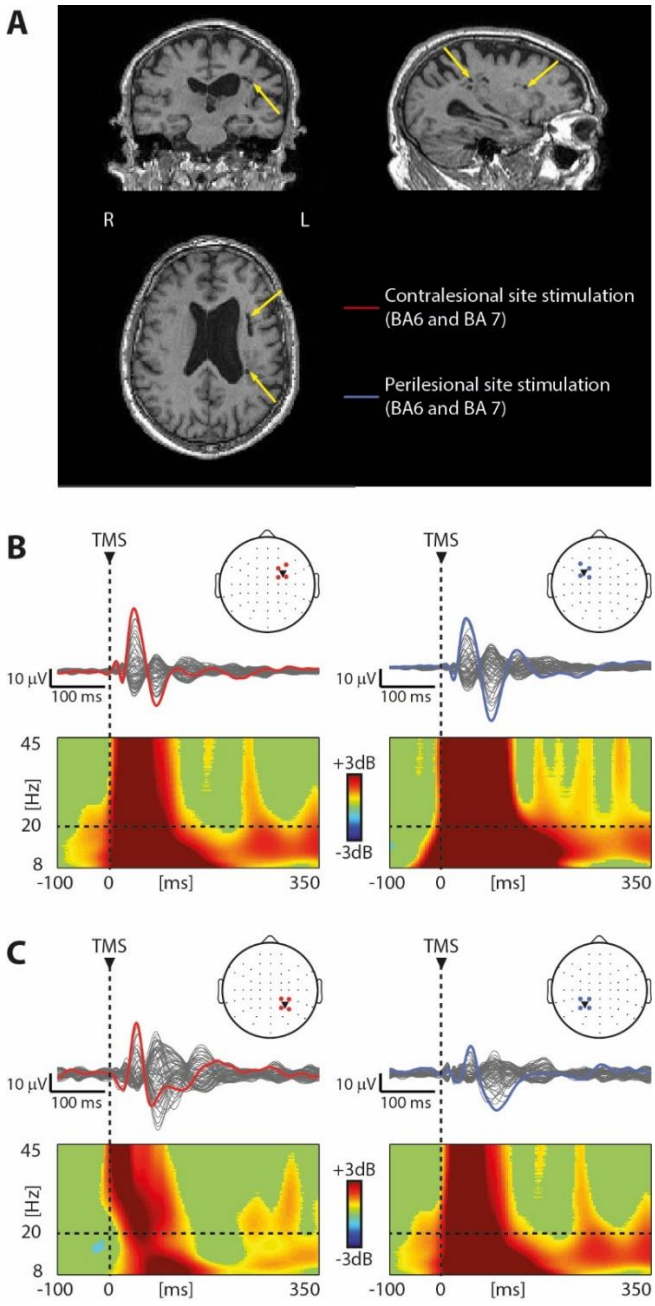

Figure 23 The absence of TMS-evoked slow waves and OFF-periods irrespective of the stimulated hemisphere and cortical area in the group of patients affected by unilateral lacunar ischemic or haemorrhagic subcortical lesions. Results from one representative patient (patient n.24 from Appendix Table1) are shown for both the contralesional (red) and perilesional (blue) stimulation sites. Panel A. MRI and cortical targets (BA6) as estimated by the Navigated Brain Stimulation system are shown. The yellow arrows highlight lesion location. Panel B and C. Butterfly plots of the TMS-evoked EEG potentials recorded at all 60 electrodes (traces) during wakefulness (Panel B) and NREM sleep (Panel C) are depicted. A dashed vertical line marks the occurrence of TMS. Event-related spectral perturbation (ERSP) is presented for the EEG electrode (coloured trace) with the largest early response, selected among the four channels closest to TMS. In the ERSP plot, significance for bootstrap statistics is set at $\alpha < 0.05$ (absence of any significant difference form baseline spectrum is coloured in green): statistically significant increases of power compared to baseline are coloured in red, while blue represents significant power decreases. The dashed horizontal line indicates the 20 Hz frequency bin. Note the absence of TMS-evoked slow waves and OFF-periods across all stimulated sites.

Deafferentation

Besides changes in K+ conductance, bistability may be experimentally induced in the animal model by means of cortical deafferentation (Lemieux et al., 2014; Timofeev et al., 2000). As an example, severing the white matter with a cortical undercut, results in slow waves and in a continuous alternation between ON and OFF periods in the partially deafferented gyrus, even when the animal, and the rest of the brain, is awake (Nita et al., 2007). To the extreme, cutting a slice of cortex and bathing it in the appropriate in vitro solution results in a typical pattern characterized by the alternation between ON and OFF periods (Sanchez-Vives and McCormick, 2000) suggesting that bistability is the "default mode" of the isolated cortex.

Synaptic scaling

In parallel, cell loss induced by stroke may induce changes in the amount of synaptic inputs impinging on the spared tissue surrounding the lesion. Recently, it has been proposed that plastic changes occurring in the central nervous system may follow a homeostatic activity-dependent mechanism observed both in vivo and in vitro where both cortical and hippocampal neurons can use their own activity as a feedback signal to modify intrinsic excitability and to maintain total synaptic strength at a roughly constant level (Desai et al., 2002; Turrigiano, 1999).

In this case, after stroke the spared tissue may turn into a hyperexcitable state induced by the prolonged reduction of synaptic inputs driven by the insult.

Whatever the mechanism involved, to the extent that sleep-like cortical bistability represents the key electrophysiological alteration of peri-lesional cortical areas, it may constitute the endpoint of neuromodulation/pharmacological treatments aimed at restoring overall network complexity and function. Indeed, while anatomical lesions and disconnections may hardly be reversed, it might be possible to reduce peri-lesional bistability and, in turn, improve the recovery of function.

Longitudinal electrophysiological assessment in one patient suggests a causal role of sleep-like bistability in stroke

In one patient (patient 4 Appendix Table 1) a retest adopting the same procedures described in the materials and methods section was performed. Specifically, after the patient underwent two weeks of physical therapy aimed at restoring the affected motor function, TMS was targeted over the same two cortical areas stimulated in the first session (Figure 24A, B, and C). By applying the same analysis procedure to the TMS-evoked responses at this follow-up assessment we observed: i) an overall reduction of the amplitude of the EEG potentials (Figure 24A and A'), ii) a reduction of the ensuing

slow wave (<4 Hz) component (Figure 24B and B'), iii) the disappearance of the suppression of high frequency components (>20 Hz) that attained at the level of the contralateral, healthy site (Figure 24C and C').

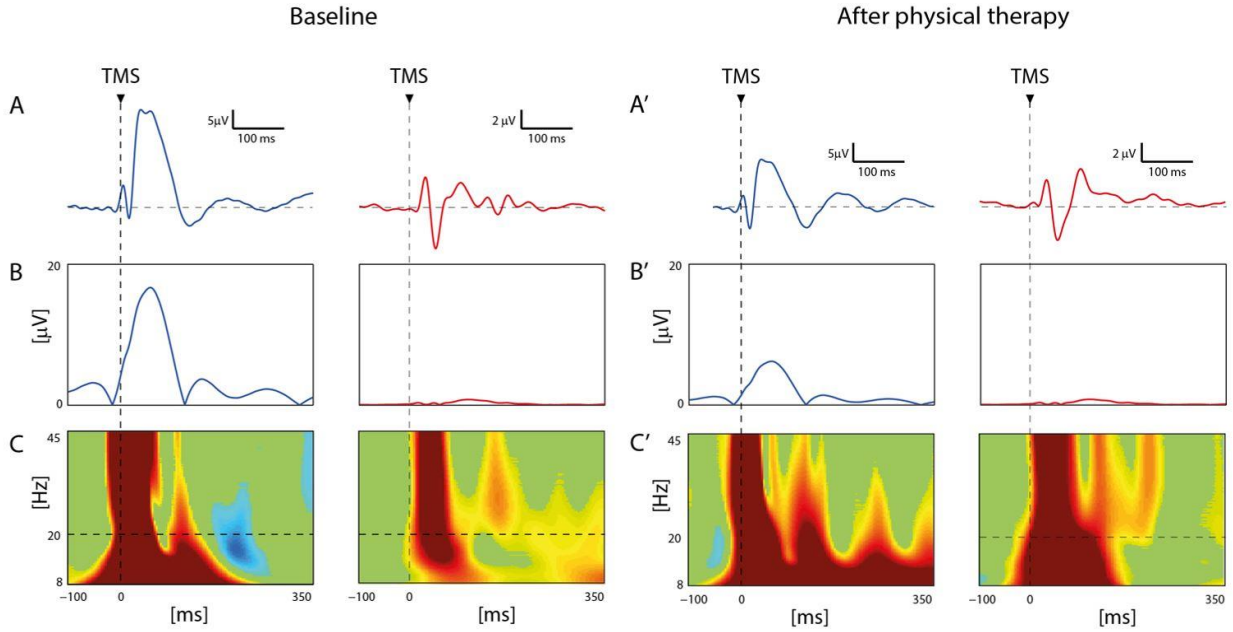

Figure 24. Longitudinal electrophysiological assessment in one patient suggests a causal role of sleep-like bistability in stroke. Both in the "baseline" and in the "after physical therapy" panels are presented stimulations on the perilesional area on the left and stimulations on the contralateral area on the right. A. Average across trial of the channel closest to the stimulation site. B. Slow wave amplitude < 4 Hz: amplitude of the slow (< 4 Hz) wave component calculated as squared absolute value of the TMS/EEG evoked potential after 4 Hz low-pass third order Chebyshev filtering. After bootstrap statistic ($\alpha < 0.05$), non-significant time points (with respect to baseline, from − 300 to − 50 ms) were set to zero. C. Event-related spectral perturbation (ERSP): time-frequency power spectra of EEG response recorded. Time-frequency decomposition is applied at a single trial level using Wavelet Transform (Morlet, 3 cycles) and significance for bootstrap statistics is set with $\alpha < 0.05$. Blue colour indicates a significant reduction compared to the baseline, while red indicates significant increase. The dashed horizontal line indicates 20 Hz. PWR > 20 Hz: time series of high frequency power (> 20 Hz). A', B', and C'. Same as in A, B, and C derived from TMS/EEG assessment performed after 2 weeks of physical therapy.

The longitudinal assessment performed in this work, showing a decrease in the evoked-slow wave components and a regain in EEG high frequency power over the perilesional area of stroke patients after two weeks of intense physical therapy, is in line with this observation and suggests a key role of local down-states in disrupting cortico-cortical information transmission also in the presence of focal brain injuries. The longitudinal behavioural assessment by means of standardized clinical scales and the correlation with the neurophysiological data presented here is also of outmost relevance and will represent a mandatory step for my future work on this topic.

Furthermore, future works should be aimed at extensively investigating this possibility including a large cohort of patients with appropriate controls. Also, it would be interesting to investigate the role of neuromodulatory interventions aimed at fostering the recovery of function by directly acting on cortical bistability such as anodal tDCS, a non-invasive, cheap and easy-to-apply modality which could be used as a stand-alone technique or as an adds-on technique to enhance corticomotor excitability and the efficacy of motor training approaches. In pre-clinical studies, tDCS applied to the affected hemisphere produced encouraging effects (Bastani and Jaberzadeh, 2012; Fusco et al., 2013; Hummel and Cohen, 2006; Hummel et al., 2005).

Conclusions

In this study, we applied TMS/EEG to a cohort of thirty conscious awake patients with chronic focal and multifocal brain injuries of various aetiologies and showed that local sleep-like slow waves associated with OFF-periods characterize the electrophysiological state of the perilesional area surrounding cortico/subcortical lesions, and that this alteration is associated with a disruption of local signal complexity.

Knowing that sleep-like cortical bistability represents the key electrophysiological alteration of peri-lesional cortical areas in the patients tested in the work, we may conclude that behaviour can be impaired due to portions of the cerebral cortex of various extent (from a few centimetres to an entire hemisphere) going offline and entering a potentially reversible sleep-like state in the context of a subject who is otherwise awake.

In addition, evaluating the presence of local sleep-like bistability in brain-injured patients is critically important. Indeed, while anatomical lesions and disconnections may hardly be reversed, it might be possible to reduce peri-lesional bistability and improve overall network connectivity and complexity and, in turn, improve the recovery of function. Moreover, connecting a fundamental sleep mechanism to brain injuries may lead to a major paradigm shift between Neuroscience and Cognitive Science: the new concept of fluid boundaries between sleep and wakefulness, where one can intrude into the other and vice versa (Sarasso et al., 2014).

Chapter five. Sleep can intrude in wakefulness and vice versa

One of the most intriguing features of the neuronal bistability is that can occur locally both during sleep (some brain regions can be ON while others are OFF) (Nir et al., 2011), as well as during wakefulness (some brain regions can be bistable while others are not) (Hung et al., 2013; Magnin et al., 2010; Sarasso et al., 2014a; Vyazovskiy et al., 2011). Interestingly, the occurrence of local intrusion of wakefulness into sleep characterizes both behavioral dissociation typical of pathological NREM sleep parasomnias (Terzaghi et al., 2009) as well as some rare example of unihemispheric sleep in marine mammals (Mukhametov, 1987). On the other hand, local slow waves and the associated OFF-periods occurring during wakefulness have been shown to be responsible for motor impairments in rats performing a pellet reaching task (Vyazovskiy et al., 2011), but never confirmed before in humans. Reporting the presence of sleep-like bistability in peri-lesional areas help us to climb one step further in the understanding of the sleep-wake cycle.

Local Sleep

Krueger and Obál postulated that sleep could be "quantal" in its nature. In fact, it begins as a local neuronal group event involving oscillations of inhibition and excitation (Krueger and Obal, 1993; Krueger et al., 2008). If this is true, it would follow that during the presence of local sleep, the other areas could be in the waking state. At the behavioural level, the number of neurons in this bistable state determine whether the brain may be asleep or awake.

Along the same line of thoughts, in the work below published in Frontiers in Neuroscience, Sleep and Circadian Rhythms DOI: 10.3389/fnins.2019.01086 (reported below with no changes), we hypothesized that the feeling of sleepiness may be a local phenomenon (D'Ambrosio et al., 2019). Moreover, it could be a brain state in which the number of neurons in sleep state is under the threshold of physiological sleep, but higher than the wakefulness one.

Sleepiness as a local phenomenon

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*These two authors contributed equally to the manuscript

Abstract

Sleep occupies a third of our life and is a primary need for all animal species studied so far. Nonetheless, chronic sleep restriction is a growing source of morbidity and mortality in both developed and developing countries. Sleep loss is associated with the subjective feeling of sleepiness and with decreased performance, as well as with detrimental effects on general health, cognition, and emotions. The ideas that small brain areas can be asleep while the rest of the brain is awake and that local sleep may account for at least some of the cognitive and behavioural manifestations of sleepiness are making their way into the scientific community. We herein clarify the different ways sleep can intrude into wakefulness, summarize recent scientific advances in the field, and offer some hypotheses that help framing sleepiness as a local phenomenon.

Introduction

Epidemiological data have shown that, over the last decades, we are seeing a concerning decrease in both the duration and the quality of sleep in developed and developing countries (Dinges, 1995; Broman et al., 1996; Dinges et al., 1997; Liu and Zhou, 2002; Krueger and Friedman, 2009; Maric et al., 2017). The progressive shift toward "24-h societies" has been accompanied by an increase in "sleepiness" and its associated detrimental effects on the individual's performance, cognition, emotions, and general health (Dinges et al., 1997; Van Dongen et al., 2003a; Chee and Choo, 2004; Banks and Dinges, 2007; Bernert and Joiner, 2007; Knutson et al., 2007; Goel et al., 2009, 2014; Couyoumdjian et al., 2010; Grandner et al., 2010; Krause et al., 2017). Thus, understanding the regulatory mechanisms of sleepiness and their implications for human health is urgent and of utmost importance (Garbarino et al., 2016).

Although the concept of sleepiness might sound intuitive, at a closer look its definition is far from trivial, and neither is the answer to fundamental issues like what sleepiness is from a neurobiological standpoint. Attempts to operationalize the subjective feeling of sleepiness for clinical and research purposes have led to the development of a number of tools, some based on subjective ratings (e.g., the Epworth Sleepiness Scale), others on objective measures like cognitive performance (e.g., reaction

time test, driving-simulators) and electroencephalography (e.g., multiple sleep latency test (MSLT) or polysomnography (PSG)). Despite the reliability of these validated measures, their agreement remains poor as they capture different aspects of sleepiness, differentially influenced by endogenous, exogenous, and situational factors. For tackling and overcoming the complex phenomenon of sleepiness, previous studies have employed and suggested a twofold approach of identifying "sleepy" patients based on combined subjective and objective sleepiness and/or physiological and biochemical biomarkers (Olson et al., 1998; Kritikou et al., 2014); this approach may be more valuable than any single measure of sleepiness (Fleming et al., 2016) but is not yet exhaustive.

In the last few years, science has produced compelling evidence supporting the idea that both sleep and wakefulness are under local regulation (Siclari and Tononi, 2017; Krueger et al., 2019). These ideas were influenced by studies performed during the transition between wake and sleep, where it was found that some brain areas may fall asleep, or awaken, before others (Pigarev et al., 1997; Magnin et al., 2010; Marzano et al., 2013; Sarasso et al., 2014a, b; Siclari et al., 2014). In further support of this view, sleep homeostasis can be modulated on a local level by active or passive tasks or via local synaptic potentiation (Kattler et al., 1994; Miyamoto et al., 2003; Huber et al., 2004, 2006, 2007; De Gennaro et al., 2008; Vyazovskiy and Tobler, 2008; Hanlon et al., 2009; Lesku et al., 2011).

The presence of local sleep was also demonstrated through the observation that slow waves and spindles, the two major spontaneous electroencephalographic oscillations of sleep that arise from complex re-entrant circuits in the thalamocortical system, often occur out-of-phase in different brain regions (Andrillon et al., 2011; Nir et al., 2011).

This case is particularly compelling as both sleep spindles and slow waves are dependent on the hyperpolarization of thalamic relay and cortical neurons, respectively. This occurs during NREM sleep due to the progressive decrease of noradrenergic, serotonergic, and cholinergic neuromodulation from brainstem activating systems. As such, being under the influence of diffuse neuromodulatory systems, their occurrence, particularly for slow waves, has been long assumed to be an ubiquitous feature of virtually every neural cell of the sleeping brain (Steriade et al., 1993b) occurring in a remarkably synchronous way (Volgushev et al., 2006).

Even more dramatically, during non-rapid eye movement (NREM) sleep, slow waves may coexist with transient local wake-like activity (Rector et al., 2005, 2009; Nobili et al., 2011; Peter-Derex et al., 2015). Similarly, local isles of sleep may intrude upon wakefulness (Rector et al., 2005, 2009; Vyazovskiy et al., 2011a; Hung et al., 2013; Quercia et al., 2018).

We herein clarify the different ways sleep can physiologically intrude into wakefulness, summarize the main findings on this topic, and offer a global framework to interpret sleepiness as a local phenomenon.

The intrusion of sleep into wakefulness

From a neuro-physiological standpoint, sleep may intrude upon wakefulness in the form of local sleep, electroencephalogram (EEG) slowing, and microsleep.

Local Sleep during wakefulness

Local sleep is a complex physiological phenomenon occurring within anatomically discrete brain locations (Krueger et al., 2019). Experiments in isolated cortical slabs (Kristiansen and Courtois, 1949), as well as in slice preparations (Steriade et al., 1993c) and cell cultures (Corner et al., 2008; Hinard et al., 2012), confirmed that slow waves—a key electrophysiological graphoelement characterizing the sleep state—is essentially an intrinsic property of cortical cells ensembles. Rector et al. (2005) provided the first indirect evidence of local sleep in living intact animals using surface evoked potentials (SEP) in rats. They showed that while, on average, wakefulness is characterized by low SEP amplitude and NREM sleep by high SEP amplitude (Hall and Borbely, 1970), SEP amplitude fluctuates over time during both states and it is frequently different between hemispheres and nearby cortical columns. Moreover, the longer a cortical column produces low-amplitude wake-like SEP the more it will begin to produce large-amplitude sleep-like SEP (Rector et al., 2005).

Further indirect evidence in favour of local sleep emerged from cortical multiunit recordings in rats during sleep deprivation (SD): firing rates increased continuously for the first 3 h of SD and showed no further significant change in the last hour. This "ceiling-effect" was interpreted as the consequence of the increase in the number of local neuronal silent periods (or OFF-periods) (Vyazovskiy et al., 2009).

Local sleep had been observed more directly in rats in 2011 (Vyazovskiy et al., 2011a, b). After a period of prolonged wakefulness, cortical neurons tended to fall silent for brief periods, as they do during NREM sleep. These OFF-periods were associated with slow oscillations in the slow/theta range in local field potential (LFP) recordings, as also confirmed by a study applying micro-stimulation during prolonged wakefulness (Vyazovskiy et al., 2013). Local OFF-periods occurred asynchronously across brain regions and increased with time spent awake. Most strikingly, they occurred in behaviorally

awake animals, and their presence over motor areas negatively affected motor performance during a sugar pellet reaching task.

More recently, use-dependent, local sleep-like EEG theta events have been found to occur during prolonged wakefulness in humans (Hung et al., 2013). As in the former studies on rodents, also in humans the occurrence of these events over frontal or posterior scalp regions was selectively associated with negative behavioural outcomes on executive functions or visuomotor control tasks, respectively (Bernardi et al., 2015; Quercia et al., 2018).

These findings lead to the intriguing hypothesis that deficits in sensory, psychomotor, and cognitive aspects of behaviour after SD may arise as a result of altered neuronal responsiveness to incoming stimuli due to these OFF-periods.

Global and local EEG slowing

Under SD conditions, which are similar to what occurs during NREM sleep, the firing rates of neurons in ON-periods, as well as the number and duration of OFF-periods, the number of neurons participating synchronously in OFF-periods and the low-frequency content (particularly in the theta range) of the EEG increase (Vyazovskiy et al., 2011b). At odds with NREM sleep, though, is the fact that during SD these events typically involve an isolated portion of the cortex possibly reflecting the occurrence of more or less widespread local cortical isles of sleep during prolonged wakefulness. In further support of this hypothesis, an fMRI connectivity analysis indicated that prolonged wakefulness is associated with a decrease in measures representing the mean strength of coupling among brain areas, resembling the breakdown in connectivity typical of slow waves sleep (Bernardi et al., 2015; Kaufmann et al., 2016).

In humans (Cajochen et al., 1995; Aeschbach et al., 1997; Finelli et al., 2000; Strijkstra et al., 2003; Fattinger et al., 2017), as in rats (Franken et al., 1991; Vyazovskiy and Tobler, 2005), EEG power in the lower frequency bands (especially theta) progressively increase with the time spent in quiet waking. This slowing can be captured by surface EEG and is paralleled by subjective sleepiness - at least in humans (Aeschbach et al., 1996; Bernardi et al., 2015) - and by a decrease in behavioral performance (Gorgoni et al., 2014; Fattinger et al., 2017). Transcranial magnetic stimulation (TMS) measures converge with EEG measures in indicating that SD has severe effects on cortical activity. SD is associated with an increased TMS resting motor threshold and cortical facilitation, at least in females, and these changes clearly predict changes in EEG theta activity (De Gennaro et al., 2007).

The increase in low frequencies in the EEG is associated with the subsequent homeostatic increase of sleep slow-wave activity (SWA) during NREM sleep in both humans (Finelli et al., 2000) and rats (Borbély et al., 1984; Tobler and Borbely, 1986), as well as with the increase of theta and delta power during REM sleep (Tinguely et al., 2006). Notably, superimposed to the homeostatic process, several studies reported a strong circadian modulation of the waking EEG (Aeschbach et al., 1996; Dumont et al., 1999; Cajochen et al., 2002) so that the intrusion of low frequencies is restrained when sustained wakefulness coincides with the biological day, while it is completely free to manifest itself during the biological night (Cajochen et al., 2002). Similarly, cortical excitability assessed using TMS coupled with scalp EEG is robustly correlated with circadian dynamics and with endocrine markers of circadian amplitude (Ly et al., 2016).

Interestingly, these regulatory mechanisms may act locally. The aforementioned increase in low frequencies has a fronto-central predominance, mainly in the theta range during wakefulness (Finelli et al., 2000; Strijkstra et al., 2003) and in the SWA range during subsequent recovery sleep (Cajochen et al., 1999; Finelli et al., 2000; Leemburg et al., 2010), and it can be observed also after repeated partial sleep restriction (Plante et al., 2016).

More recently, studies performed in humans showed that the increase in waking theta EEG activity during SD displayed regional, use-dependent changes (Hung et al., 2013; Gorgoni et al., 2014; Bernardi et al., 2015; Nir et al., 2017). A first study took advantage of high-density EEG technology to show an increase in theta power over left frontal brain regions after a language-based task and over posterior parietal regions after a visuomotor task. The same regions displayed local increases in SWA power during subsequent recovery sleep (Hung et al., 2013). A subsequent study demonstrated that the occurrence of theta waves in task-related regions coincided with specific performance errors in humans (Bernardi et al., 2015). Another study used intra-cranial electrodes in human neurosurgical patients performing a psychomotor vigilance task (PVT) at baseline and during SD. Cognitive lapses involved local state-dependent changes in neuronal activity in the medial temporal lobe (MTL). Specifically, immediately before cognitive lapses the spiking responses of individual neurons were attenuated, delayed, and lengthened while, during cognitive lapses, LFPs showed a relative local increase in slow activity (Nir et al., 2017). In line with these findings, a study using a driving simulator to evaluate the effect of sleepiness at the wheel, found that a local increase in theta EEG activity over the motor regions (as localized by EEG source modeling techniques) was associated with an increased risk of line departures (Ahlstrom et al., 2017).

Microsleep

In this review, microsleeps are defined as short episodes of sleep-like activity that satisfy criteria for stage 1 sleep (theta replacing alpha rhythm) except for their short duration of up to 15 s (Priest et al., 2001; Blaivas et al., 2007; Hertig-Godeschalk et al., 2019). Usually, blinking artifacts characteristic of full wakefulness disappear, often accompanied by the appearance of slow eye movements. However, behavioral changes, such as eye-closure and nodding-off, are not defining features of microsleeps, as they may or may not be present during microsleep episodes (Torsvall and Akerstedt, 1988; Boyle et al., 2008). Regardless, microsleeps may be associated with significant cognitive impairment -e.g., poorer performance during a continuous task under driving-simulator conditions (Boyle et al., 2008)- and are strictly associated with subjective sleepiness. Indeed, some evidence suggests that microsleeps analysis in MSLT might be a more sensitive and specific test for excessive daytime sleepiness (EDS) as compared to MSLT alone (Tirunahari et al., 2003).

Microsleep episodes are more frequent after a sleep-restricted night compared to a normally rested night (Friedman et al., 1978; Horne and Pettitt, 1985; Poudel et al., 2018) and can be followed by a brief recovery in performance (Poudel et al., 2018).

Traditionally, microsleeps have been hypothesized to be global brain phenomena that reflect the transient shutdown of activating systems, with the parallel activation of sleep promoting centres (Sechenova, 2011; Silkis, 2013). However, recent evidence describes microsleep in terms of intermediate states between sleep and wakefulness (Hertig-Godeschalk et al., 2019), possibly reflecting their local nature. Supporting this notion, a recent fMRI study during one night of SD described local decreased activation over frontal, parietal, and occipital associative cortices as well as increased activation in the default mode network (DMN) associated with slow reaction times responses at the PVT (typically reflecting the occurrence of microsleeps), showing how these different patterns of activation and deactivation could depend on circadian phases as well as homeostatic sleep pressure and the interactions between the two (Zhu et al., 2018).

Neurobiology and neurophysiology of sleepiness

In simple terms, just as hunger is the physiological need for food, sleepiness can be described as the physiological need for sleep. Very few theoretical constructs about sleepiness are available in the literature (Cluydts et al., 2002; De Valck and Cluydts, 2003). Conceptually, sleepiness is the consequence of an imbalance between the sleep drive (level of activation of sleep circuits) and the

wake drive (level of activation of arousal systems) (Cluydts et al., 2002). When wake still prevails but sleep pressure is high, we experience sleepiness.

The concept of sleepiness, therefore, closely relates to the concept of "sleep debt." While Horne originally proposed that sleep debt was uniquely the consequence of the loss of "core" or "obligatory sleep" (referred as the first 4–5 h of sleep) but not of "optional" or "facultative" sleep, which "fills the tedious hours of darkness until sunrise" (Horne, 1985), empirical research indicated that sleep debt accumulates linearly. Although clearly influenced and modulated by circadian factors (Shekleton et al., 2013), according to this line of research, sleep debt may be defined as the cumulative hours of sleep loss with respect to a subject-specific daily need for sleep (Van Dongen et al., 2003b).

Another closely related concept of sleepiness is "sleep inertia," a physiological condition of subjective drowsiness, decreased alertness, and impaired cognitive and sensory-motor performance that arises during the transition between sleep to wakefulness. It has been shown that the subjective feeling of sleep inertia lasts on average 15–30 min, although objective measure of alertness and performance do not return to waking baseline until 2–4 h after waketime (Jewett et al., 1999). Electroencephalographic, evoked potential, and neuroimaging studies suggested that sleep inertia involves the intrusion of sleep patterns during wakefulness (Trotti, 2017), bringing the concepts of sleepiness and sleep inertia even closer and further corroborating the notion that vigilance states are not necessarily discrete.

But how does this translate from a neurophysiological standpoint? According to the data presented in the previous section, sleepiness may be associated with the occurrence of local sleep during wakefulness in the presence of a positive sleep debt. We will now focus on possible regulatory mechanisms of local sleep and their interaction with global processes.

Local regulation of sleep

Great progress has been made in characterizing the brain centres responsible for the orchestration of sleep and wakefulness as global behavioural states (Jones, 2005; Saper et al., 2005; Szymusiak and McGinty, 2008; Siegel, 2009; Brown et al., 2012). Although anatomically widespread, these centres act in a coordinated fashion in modulating whole-brain activity, thus allowing for a clear behavioural distinction between wake and sleep states (for a review see Saper et al., 2001; Scammell et al., 2017; Eban-Rothschild et al., 2018). According to Saper's flip-flop switch model, sleep regulation depends on a mutually inhibitory interaction between sleep centres and the components of the arousal systems (Saper et al., 2001), located both in cortical and subcortical structures.

Although the described mechanisms may orchestrate sleep globally, sleep is fundamentally an intrinsic property of the cerebral neurons and can be regulated locally at the level of cortical areas as small as cortical columns (Krueger et al., 2008). This columnar state segregation is favoured by the fact that the functional intracolumnar connections are denser than intercolumnar connections allowing greater activity and state synchrony between cells pertaining to the same column (Panzeri et al., 2003).

The very first model-based postulation of local sleep was published in 1993 and 1995 by Krueger and Obal (1993) and Krueger et al. (2008), who postulated that sleep begins as a local neuronal group event involving oscillations of inhibition and excitation and is thus "quantal" in nature. From this perspective, these authors considered sleepiness as a statistical phenomenon, the perception of which arises when a sufficient number of neuronal groups become "bistable."

Coordination of neuronal group sleep results from both neuronal and humoral systems. As proposed by the Synaptic Homeostasis Hypothesis (SHY; Tononi and Cirelli, 2003, 2006), when neuronal plasticity during wakefulness is increased or decreased in specific brain areas, sleep intensity, as reflected by the amount of SWA, selectively increases or decreases in those areas (Kattler et al., 1994; Huber et al., 2004; Vyazovskiy and Tobler, 2008; Hanlon et al., 2009; Lesku et al., 2011). Indeed, increased synaptic connectivity means more synchronous oscillations and increased slow wave activity. Alternatively (or additionally), local slow wave generation could be due to a change in the excitability or amount of adaptation of individual neurons. Along these lines, a build-up in the need for cellular maintenance could cause individual neurons to show lower excitability and stronger adaptation (Vyazovskiy et al., 2013). OFF-periods would therefore occur locally where most needed, thus providing a potential explanation for local sleep patterns. Similarly, as soon as individual neurons fall below a certain cellular stress threshold, their excitability is restored, leading to a more wake-like pattern of activity.

Considerable evidence also suggests a role for local paracrine signaling pathways in the regulation of both global and local sleep. In this vein, it is known that sleep pressure correlates with the concentration of -among others- nitric oxide (NO) (Gautier-Sauvigné et al., 2005; Kalinchuk et al., 2011), adenosine, and various cytokines (Imeri and Opp, 2009) such as interleukin-1 (IL-1) and tumor necrosis factor (TNF). These substances are synthesized by metabolically or synaptically active cells and are released in a local fashion (Latini and Pedata, 2001; Porkka-Heiskanen and Kalinchuk, 2011). Again, Krueger et al. (1995b) played a pivotal role in delineating this humoral component (Krueger, 2008). They hypothesized that sleep at the neuronal group level is regulated by paracrine substances whose production and catabolism rates are synaptic use-dependent (Krueger et al., 1995a).

According to their model, Adenosine 5'-γ-ThiotriPhosphate (ATP) released during neurotransmission and acting on purine P2 receptors induces the release of IL1 and TNF. These cytokines in turn act on neurons to change their intrinsic properties (Krueger, 2008), directly or indirectly, altering the production of neuroendocrine substances and neurotransmitters, for example the growth hormone releasing hormone and NO, which are known to be involved in sleep-wake regulation (Krueger et al., 1995b). More recently, evidence in support of this model has been shown by Nguyen et al. (2019).

Finally, there is another mechanism able to explain regional sleep differences, especially the aforementioned frontal predominance of the low frequency effect. Different regions might be more susceptible to sleep due to intrinsic differences in some of their activating inputs. In other words, even widespread projections from centres regulating sleep globally may present with topographical differences that may affect sleep-wake regulation at the level of cortical macro-areas. For example, recent evidence suggested a region-specific dissociation between cortical noradrenaline levels during the sleep/wake cycle (Bellesi et al., 2016). Compared to the motor cortex, in the medial Prefrontal Cortex (mPFC) noradrenaline levels are higher and changes in its concentration during sleep and wake are slower. Furthermore, SD leads to a decrease in noradrenaline only at the level of mPFC, suggesting that noradrenergic neurons targeting the prefrontal cortex may undergo fatigue earlier or more markedly than other projecting cells from locus coeruleus. An increased susceptibility of noradrenergic projections to the frontal cortex might explain frontal cognitive executive function impairments associated with sleepiness (Jones and Harrison, 2001), including why the frontal lobes display more evident electrophysiological signs of deep sleep after prolonged wakefulness (Plante et al., 2016). These mechanisms may, alone or in combination, explain the occurrence of local sleep during wakefulness, leading to the subjective feeling of sleepiness.

As a last note, it is worth mentioning here that, despite the fact that we mainly focused on the cortex when we tried to explain the origins of local sleep, NREM sleep patterns in vivo emerge from the interplay between the cortex and the thalamus, more specifically the thalamic reticular nucleus (TRN). It has been reviewed recently how cellular and functional TRN heterogeneity may account for some features of local NREM sleep (Vantomme et al., 2019). By experimentally modulating the activation and firing of the TRN neurons, it was indeed possible to rapidly induce slow wave activity (Lewis et al., 2015) as well as sleep spindles (Fernandez et al., 2018) in spatially restricted regions of the cortex. However, at the current state of the art, it remains unclear how the TRN contributes in terms of physiological conditions and what the signals that activate TRN neurons locally are. Further research

will need to clarify these aspects and disentangle the causative role -if there is one- of TRN and the cortex in this loop-network.

Interplay between local and global regulation

Having highlighted local sleep regulatory mechanisms, it remains to be discussed how they interact with the different processes orchestrating sleep in a global fashion.

According to Saper's model (Saper et al., 2005), sleep regulation relies on three main streams: the "homeostatic" (Porkka-Heiskanen et al., 1997, 2000; Huang et al., 2007, 2011, 2014), the "circadian" (Chou et al., 2003; Fuller et al., 2006), and the "cognitive/emotional" (Chou et al., 2002; Sakurai et al., 2005; Yoshida et al., 2006). Each drive can potentially act globally as well as locally. Aside from the well-known homeostatic local sleep modulation discussed above, there is evidence of regional modulation of brain circadian rhythmicity. This has been demonstrated by a recent fMRI study quantifying changes in brain responses to a sustained-attention task across the circadian cycle, during baseline wakefulness, SD, and after recovery sleep (Muto et al., 2016). Subcortical areas exhibited a dominant circadian modulation that closely followed the melatonin profile but had no significant influence on sleep debt. Cortical responses also showed significant circadian rhythmicity, the phase of which varied across brain regions, as well as a widespread negative influence exerted by sleep pressure. The mechanisms of this local modulation are unknown, although the authors suggested the potential role of clock gene expression. Intriguingly, an EEG study showed that circadian rhythms modulate the incidence amplitude, frequency, and slope of slow waves (the latter being the most accurate marker of synaptic strength), with a dominant effect on central and occipital areas (Lazar et al., 2015).
Moreover, global regulatory mechanisms, particularly regarding the homeostatic and the circadian components, may influence local sleep regulation. In this respect, the extent of brain areas displaying sleep features (and thus the associated behavioural impairments and subjective feeling of sleepiness) may rest on the level of synchronization between global regulatory mechanisms. As such, asynchrony and shift of phase between the homeostatic and the circadian drive may result in local sleep without a global state transition (see Figure 25, conceived for schematizing these concepts without fitting any biological data for the sleep drives or for the number of neurons in OFF-periods). Likewise, the cognitive/emotional system may modulate the interaction between the homeostatic and the circadian drives and keep the subject awake despite strong circadian and homeostatic sleep-promoting inputs, accentuating their desynchronization (Horne, 1985).

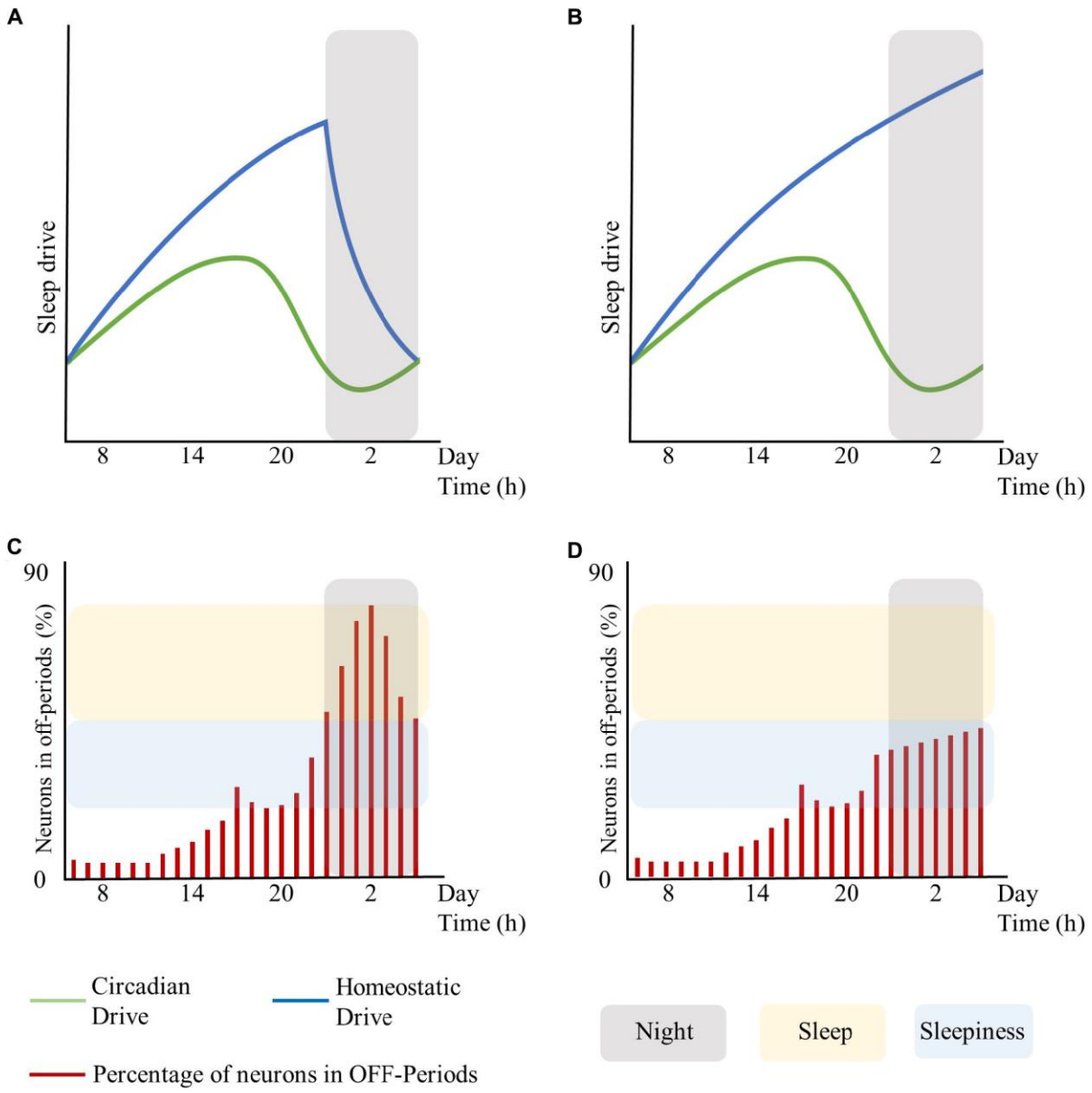


Figure 25. Interplay between global sleep drivers and cortical neuronal firing. The figure is intended to schematize the concepts described in this work without fitting any biological data for sleep drives or the number of neurons in OFF-periods. Top panels represent the time-course of the circadian and homeostatic drive over 24 h. Bottom panels represent the percentage of neurons in OFF-periods across 24 h. (A) circadian and homeostatic drives under physiological conditions. (B) circadian and homeostatic drives out of phase due to sustained wakefulness. (C) percentage of cortical neurons in OFF-periods under physiological conditions. (D) percentage of cortical neurons in OFF-periods during sustained wakefulness. Red bars: percentage of cortical neurons in OFF-periods. Blue lines: homeostatic drive. Green lines: circadian drive. Grey areas: night period. Yellow areas: sleep. Light-blue areas: sleepiness. The figure has been realized by fitting the mathematical function published in Daan et al. (1984).

In summary, local sleep may arise as an intrinsic property of each regulatory drive of the slow-wave cycle or by the desynchronization between these drives acting globally.

In turn, local sleep may affect brain centres responsible for sleep and wake as global behavioural states. As such, the occurrence of isolated local OFF-periods during wakefulness could subsequently lead to global sleep through the involvement of neuro-modulatory systems responsible for the generation of NREM sleep (Saper et al., 2010; Brown et al., 2012). Similar to focal onset seizures with impaired awareness, local changes in cortical activity may lead to profound global alteration of the vigilance state associated with loss of consciousness through the progressive involvement of other brain regions, such as midline subcortical structures including the thalamus, the hypothalamus, and the brainstem (Englot and Blumenfeld, 2009). Recordings in the ventrolateral preoptic nucleus (VLPO) neurons showed that their firing rates increase during sleep, almost doubling during recovery sleep after SD, but did not increase during prolonged wakefulness. Thus, as homeostatic sleep drive accumulates, it may influence other neurons in the brain, such as the median preoptic neurons, which provide input to the VLPO (Saper et al., 2010). Alternatively, there could be a threshold in the number of areas showing sleep signs during wakefulness, which may imply behavioural impairments and sleepiness at first, and only when passing the threshold of the transition to global sleep. Specifically, it is hypothesized that whole organism sleep is an emergent property of the collective neuronal assemblies (Rector et al., 2009), as when networks of neuronal assemblies are coupled, they will tend to synchronize (Roy et al., 2008). As such, when the number of neuronal groups entering the sleep state exceeds a significant threshold, other groups will follow (Rector et al., 2009) thus enabling the full-fledged transition from wakefulness to sleep (see Figure 25, conceived for schematizing these concepts without fitting any biological data for the sleep drives or number of neurons in OFF-periods).

Sleepiness typically arises in conditions of SD and/or prolonged wakefulness. The prevalence of the so called "insufficient sleep syndrome" is estimated to be between 1 and 4% of the population (Ohayon, 2008) and two to four times higher in individuals sleeping less than 6 h per night compared to individuals sleeping between 7 and 8 h per night (Ohayon, 2012).

The great majority of sleep disorders determine sleepiness through the curtailment of total sleep time and/or sleep fragmentation without primarily affecting the function of sleep-promoting centres. Examples are sleep breathing disorders like obstructive sleep apnoea, circadian rhythm sleep-wake disorders like shift-work disorder, sleep related movement disorders like restless leg syndrome, and objective insomnia. Central disorders of hypersomnolence like narcolepsy type 1 and 2 or idiopathic

hypersomnia involve instead a pathologic imbalance between sleep promoting and wake promoting pathways in favour of the former, determining an increased sleep need and/or the abrupt intrusion of sleep into wakefulness due to an instability of the switching mechanisms between sleep and wakefulness. Also, parasomnias like sleepwalking and sleep terrors have been associated with subjective daytime sleepiness, via as of yet unknown mechanisms (Carrillo-Solano et al., 2016).

Sleepiness is also related to mental and organic diseases that may directly or indirectly affect sleep (Ohayon, 2012). These disorders may cause a disruption of the sleep-wake schedule due to changes in behaviour, dysregulate sleep centres orchestrating sleep due to neurological lesions or more subtle abnormalities, or nociceptive, immunomodulatory, or other modulatory inputs.

Conclusions

While familiar to all on a subjective level, sleepiness is a complex matter that science is just beginning to understand. We have herein summarized how, during prolonged wakefulness, the occurrence of local neuronal OFF-periods may relate to the well-known negative consequences on performance observed in this state. As suggested by the reviewed literature, this phenomenon of local sleep during wake may account for at least some of the cognitive and behavioural manifestations of sleepiness. Under this perspective, sleepiness may reflect the transition between different vigilance states, being an epiphenomenon of these "fluid boundaries" (Sarasso et al., 2014a).

This interpretation is probably the key that will help develop new measures to quantify sleepiness in the near future. From a clinical perspective, high-density EEG, which allows an optimal spatial and temporal resolution to capture local isles of EEG slowing, may represent a valuable technological support. Moreover, a better characterization of the role of the circadian rhythm and of its interaction with other drives that modulate the sleep-wake cycle is warranted. Finally, a promising future line of research will be on the linking of the neurophysiological concepts of local sleep and sleepiness to interindividual variability in susceptibility to sleepiness (Van Dongen et al., 2004; Kuna et al., 2012; Rupp et al., 2012; Goel et al., 2013; Spaeth et al., 2015). This will open the way to a more personalized sleep medicine that will have a considerable impact on human health and promote occupational well-being, benefiting the society as a whole.

Future directions - transient sleep-like activity

Several different pathologies may cause transitory sleep-like behavioural activity during wakefulness that can last from seconds to minutes, with epilepsy being one of them. Specifically, epilepsy is a group of disorders characterised by liability to seizures. Among numerous different types of seizures, absence seizures manifest as loss of conscious perception and accompanying behavioural arrest that can last for less than a second. As a transient and recurrent phenomenon, these present a unique opportunity for investigate transitory moments of loss of consciousness. We tried to investigate this phenomenon with a perturbational approach, hypothesizing that during the absence seizure there could be the presence of sleep-like bistability. Therefore, we performed TMS/EEG sessions on a person with epilepsy characterized by absences during and not the advent of seizures (ictal and inter-ictal assessment). If the hypothesis is correct, we could confirm once more a strong relationship between the mechanisms of sleep and the ones of consciousness.

Absence seizures

Absence seizures characterize an idiopathic generalized epilepsy type. This specific type peak incidence is at age 5-7 years and 60-70% of patients are female (Alarcon et al., 2009). Typical absence seizures include clonic components, atonic components, tonic components, automatisms or autonomic components. Seizures can be very frequent, in fact, they may occur hundred times per day (pyknolepsy). Hyperventilation virtually always induces seizures, which is a useful diagnostic test in clinic and EEG. Infrequent generalized tonic clonic seizures (GTCS) can be seen in adolescence or adult life, not preceding or concomitant with the period of active absence seizures.

Memory function and cognition are not afflicted by this condition. Usually, the history of epilepsy in the family of the patients have a recurrence of the pathology. Interestingly for our study, patients have a normal inter-ictal EEG. During absences, instead, the EEG shows paroxysmal abnormalities, such as generalized spike-and-waves with a frequency of 2.5-3.5 Hz.

Neuroimaging findings are normal, and medical management is the mainstay of treatment. Patients respond to the medical treatment in the 70-90% of the cases, but 40% of them develop tonic-clonic seizures during the adolescence (Alarcon et al., 2009).

TMS/EEG assessment in one subject with absences

Within the framework of a collaboration with the Department of Clinical and Experimental Epilepsy of the University College of London Hospitals, we performed the TMS/EEG assessment on a subject affected by generalized epilepsy with absence seizures. We used the protocol described in the experiments on stroke patients above, but only in one area, specifically, medial in the premotor area. The stimulation has been assessed repetitively in one day of recording with the subject sit on a chair during the daytime. Our participant reported 10 to 30 absence seizure per hour that lasted less than a second on average.

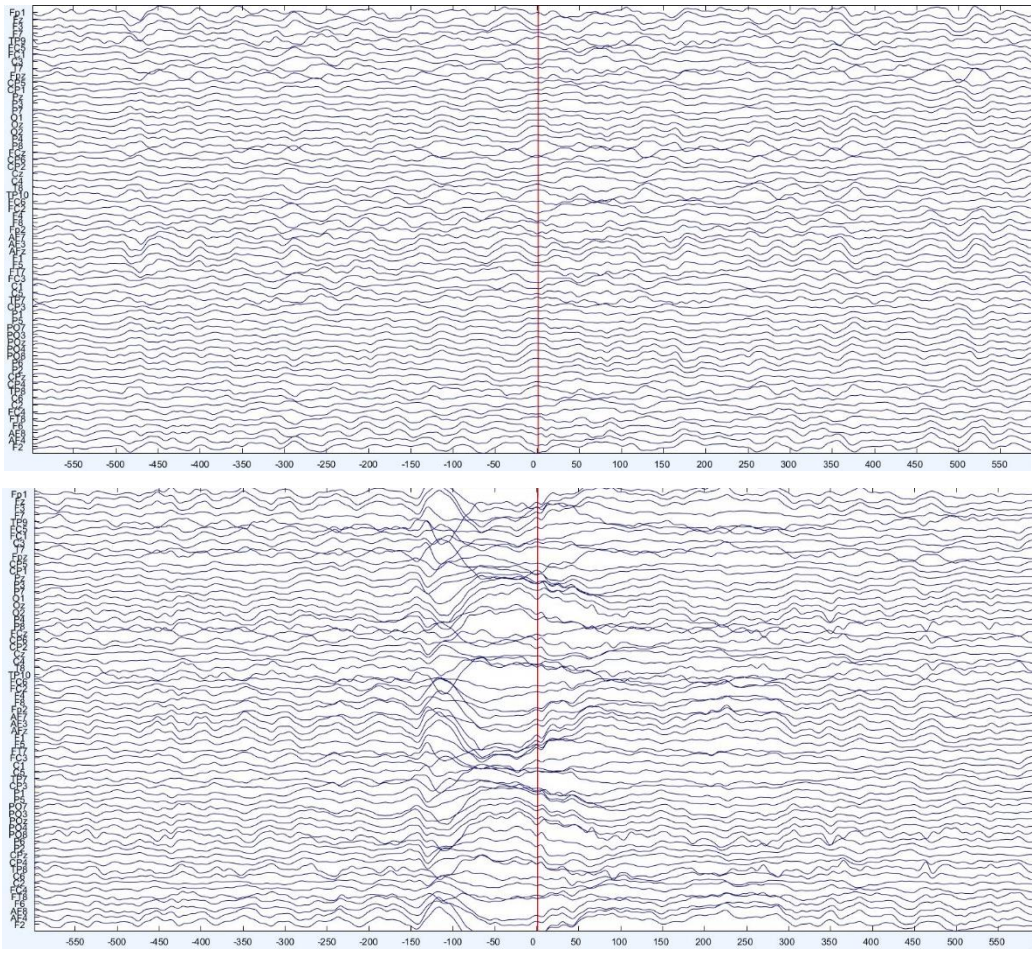


Figure 26. Upper panel. EEG traces of one representative trial with no seizure. In the figure are represented channels per time, showing the 1200ms around the TMS pulse (red vertical line). Bottom panel. EEG traces of one representative trial with an absence seizure, onset at 150ms before the TMS pulse. In the figure are represented channels per time, showing the 1200ms around the TMS pulse (red vertical line).

Clinical inspection of the data showed the presence of absence seizure within the 300ms before the TMS pulses in the 30% of the trials (see Figure 26). We computed the same type of analysis reported

in the work on stroke described in chapter four, focusing on the time-frequency decomposition of the channel under the stimulation.

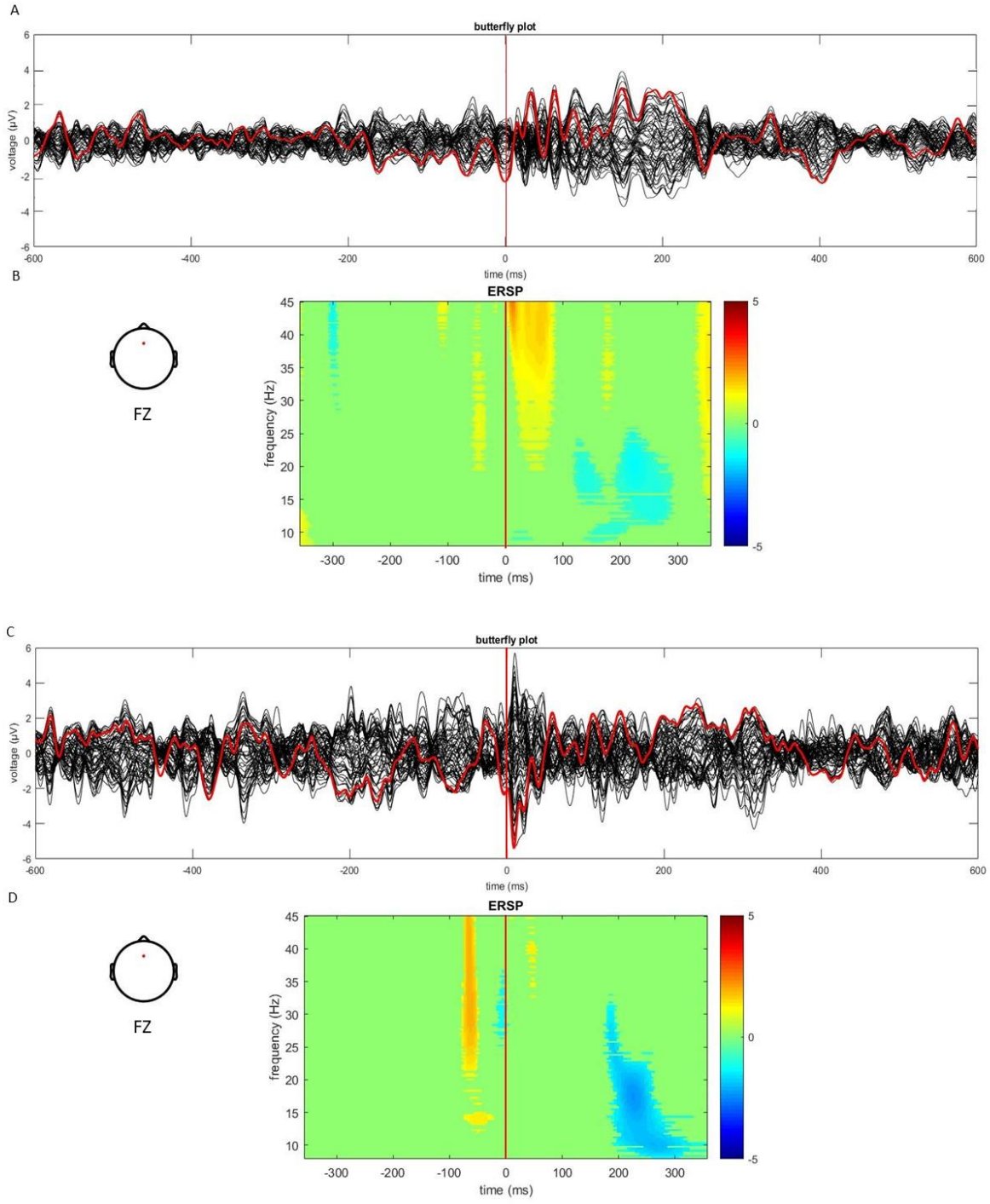


Figure 27. Butterfly plot and time-frequency decomposition of the average of the TMS/EEG trials performed on the medial premotor left cortex in the inter-ictal (panel A, B) and the ictal (panel C and D) period, in one person with generalized epilepsy characterized by absence seizures. A. Average of all the trial performed at least 2 seconds far from

any absence seizure. The black lines represent all the channels, the red line represent the closer channel to the stimulation FZ. B. Time frequency decomposition of the averaged evoked response to the TMS pulses of the channel FZ performed at least 2 seconds far from any absence seizure. C. Average of all the trial performed maximum 300ms after an absence seizure. The black lines represent all the channels, the red line represent the closer channel to the stimulation FZ. D. B. Time frequency decomposition of the averaged evoked response to the TMS pulses of the channel FZ performed maximum 300ms after an absence seizure. Red vertical line represents the onset of the TMS pulses.

The results shown above are made extrapolating only the trials in which a clinically confirmed absence seizure was within the 300ms before the TMS pulses (Figure 27, panel C,D) and confronting it with trials in which no trace of absence seizure was recorded in the 2 seconds around the TMS pulses (Figure 27, panel A,B).

TMS/EEG assessment during absence seizures may share similar features with the ones occurring during sleep

In this pilot study, we found a suppression of the high frequencies (>20Hz) underpinned by a slowing in the EEG in the trials assessed maximum 300ms after an absence seizure (see Figure 27, in which the time-frequency decomposition show a lack of power in the high frequencies and an increase in the higher when perturbing during absences). The occurrence of these two findings together, as previously discussed, is the marker of the cortical sleep-like bistability. Further corroboration of these findings by similar experiments in more subjects could demonstrate a shared mechanism between the loss of consciousness in absence patients and sleep mechanisms.

Starting from the aforementioned quantal hypothesis of sleep from Krueger and Obál (Krueger and Obál, 1993), the perturbational approach in absence patients could suggest a possible quantal hypothesis for the conscious experience; as behavioural sleep is present when the number of neurons in bistable state reach a certain threshold, loss of consciousness could occur for the same reason.

Chapter six. Beyond neuroscience

The hypothetical quantal nature of sleep and the relation between physiological sleep and consciousness could lead us far beyond the neurophysiology's domain. The mechanisms described so far in this work could trespass into that of philosophy, specifically, into the problem of personal persistence through time.

Personal persistence in philosophy

Our everyday life is surrounded by things and organisms that change over time, some of them drastically change, while other change slightly. Then how can they remain the same? And how can people change and still be the same?

We will focus on how we persist over time and what constitutes our identity over time. The problem of personal persistence grounds its roots long time ago and philosophers have been dealing with it since then. Locke, who discussed the problem of personal persistence in the Essay Concerning Human Understanding, is regarded as the first philosopher who clearly posed the persistence question. He proposed mental experiments in which all memories and consciousness are through different bodies, arguing that a person persists over time if his memories and conscious experience persists, regardless of any physical changes. Hume then claimed for a revisionist account of persistence (Treatise of Human Nature), denying any persistence of material things over time, accounting for a conception of personal persistence as nothing but a fiction, a mistake. In this view, the world is free of people, but reach of collection of senses and perceptions, that we unify under concepts and single personal names. Furthermore, in the last years analytic metaphysicians focused the problem of personal persistence on the criteria of identity over time.

A criterion of personal identity over time can be defined as the completion Φ of the following: *Necessarily, given x at time t and y at a different time t*, where at least one of those is a person, x = y if and only if Φ* where '=' stands for the relation of numerical identity, and 'Φ' stands for the constitutive condition whereby the identity of x and y is determined. A criterion of identity should be thought as the conjunction of a necessary and a sufficient condition for identity over time, for the formula above maintains that x and y are the same thing if Rxy (i.e., Rxy → x=y: sufficient condition) and only if Rxy (i.e. x=y → Rxy: necessary condition). In addition to being necessary and sufficient, any constitutive conditions of identity over time should also be informative. Informativity is a further essential feature of any criteria of personal persistence (Buonomo, 2018).

If we ask for the condition Φ that makes a person at t identical to a person at t*, we are assuming that, in order to persist over time, a person should necessarily remain a person; this assumption might be called "personal essentialism". This means that the problem of personal identity over time is combined with personal essentialism about persistence, in other words an essentialist version of the problem of personal identity over time. In contrast, the persistence question, asking for what it takes for x at t to be identical to y at t* (where at least one is a person), does not entail such a premise.

Another time we are searching answer in other questions, namely the questions about what we are, what does it take for something to be a person. If we accept the psychological Lockean account in which a person is a rational thinking being (Locke, essay con. Humans), then the condition to persist over time would be necessarily psychological. In this framework, being an embryo in the past, or to become an unresponsive wakefulness syndrome patient in the future, are irrelevant considerations, as neither embryos nor UWS patients conceived as Lockean "rational thinking beings".

We might think, for instance, that our persistence consists in our memory, or in our physical continuity, or in some other constitutive criteria. Similarly, we might think that we ought to take a person at t^1 as the same person at t^2 in virtue of their memories, or their physical features, or in virtue of other aspects.

Traditional accounts of personal identity

There are three classes of account of personal identity over time:

1. the ones that support psychological criteria of personal persistence; 2. accounts that support somatic criteria 3; accounts that deny the existence of any constitutive condition of personal persistence.

1. Psychological accounts of personal persistence argue that people are identical over time in virtue of some psychological aspects. For these accounts *necessarily, given x at time t and y at a different time t*, where at least one of those is a person, x = y if and only if x and y are connected by such and such determinate psychological relations*. This approach has traditionally most advocates, with substantial differences among each other, mainly about the kind of psychological relation that should stand between x at t and y at t* in order to be constitutive of their identity. For instance, one could account for the continuity of memories, preferences and beliefs, the continuity of the first-person perspective, or the continuity of experiences.

2. Somatic accounts rely on the physical relations as necessary and sufficient conditions for persistence. For these accounts *necessarily, given x at time t and y at a different time t*, where at least one of those is a person, x = y if and only if x and y are connected by such and such determinate physical relations*. Here, some advocate for

bodily continuity as the relevant relation, so that the continuity of the human body constitutes the criterion of personal identity over time. Others support the idea that personal persistence rests upon the continuity of the biological organism, namely the "biological" or "animalist" account of personal identity; whereas others argue that personal persistence is based on the continuity of an essential part of our body, such as the brain.

3. Anti-criterialist accounts simply reject the existence of any criteria of personal persistence. They argue that the only necessary and sufficient conditions of personal identity over time are not informative, such as *necessarily, given x at time t and y at a different time t*, where at least one of those is a person, x = y if and only if x and y are the same person over time*. Then, anti-criterialist accounts look at identity over time as something primitive, unanalysable and unexplainable through any further facts.

Persisting over time through local wakefulness

In collaboration with Valerio Buonomo, we have tried to approach the problem of personal persistence from a biological point of view. Furthermore, we tried to take into account recent findings in system biology and to choose a criterion of identity coherent with it. System biology conceives living organisms as self-organizing dynamical systems, which are able to demarcate themselves from the environment. We argue that, in order to justify our ontological commitment towards organisms as explained by system biology, we should give up the idea that strict identity is fundamental and, at the same time, to recognize the metaphysical priority of a non-standard-diachronic identity relation. As things stand, the existence of organisms as described by system biology would require a modification of the metaphysical framework and, in particular, it requires the introduction of a different fundamental notion of identity for organisms.

Organisms and their identity: metaphysical issues from system biology

In the recent years, empirical research in system biology has increased significantly, and have provided philosophy of biology with important issues that are significant for our understanding of the nature of organisms. There are systems of processes exhibiting autonomy, such as cells and organisms (Kitano, 2000). If organisms are dynamical systems that are not reducible to their processes, no informative condition of synchronic identity seems possible. Then, any reduction of the identity conditions for living organisms in terms of primitive strict identity leads us to unpleasant consequences, such as the denial of the existence of organisms or the commitment to a "mysticism of identity". Appealing to a different kind of (non-standard) identity, such as the Lewisian relation of

temporal counterpart relation among constituents at different times, on which the unity - and then the identity - of living beings rests upon; suggesting that this strategy constitutes a promising way to account for living systems as real entities. Our claim is that the individuality and the existence of living organisms, as described by system biology, might be explained properly in terms of diachronic identity and in terms of temporal counterparthood (see Lewis 1971, 1976 for the theory of temporal counterpart referred in this work). In our view, an individual as conceived by system biology, can be represented by a group of "temporal counterparts" in relation with each other.

Consider the general formula of diachronic identity: (DI) *given x at t1 and y at t2, x = y iff Φ*

and the perdurantist (for which an object extends not only in space, but also in time) reading of it:

(DI-Per) *given a stage x at t1 and a stage y at t2, x and y are unified iff Φ*

The first thing we may notice is that in (DI-Per) x and y are not related by a strict identity, but instead by a relation of unity in virtue of a certain Φ, where Φ consists in a certain continuity and connectedness among x and y.

Consider now more closely the case of living organisms, as presented by system biology. As we have seen, according to system biology, the individuality of living beings rests upon the functional integration of their components, which in turn are defined in terms of what they do. It follows that organisms are organized systems, in which a multiplicity of constituents depends on each other for their own existence and maintenance. If so, it is the continuity of the organization which constitutes a central condition for the persistence of living organisms, ensuring the identity of the organism besides several kinds of changes (material changes, structural changes, or functional changes). If we are right on that, then a possible way to spell out the identity of living organisms may consist in considering them as cross-temporal systems, whose identity is maintained through the functional relation that unifies its constituents. In particular, given the lack of constitutive conditions of identity at a time for living organisms (i.e. the lack of constitutive conditions of synchronic identity), we shall conclude that what grounds the ontological condition of identity for living organisms is a determinate kind of continuity or connectedness among constituents at different times. Let us then consider the following option *given x at t1 and y at t2, x and y compose the same living organism iff there is a determinate continuity between x and y*. We shall now specify what we take as a continuity between x and y. As we have seen above, system biology conceives organisms in terms of dynamic interactions among processes, that organize themselves within a system, which enables them to demarcate themselves from the environment. Moreover, we argue that the identity of an organism over time, although hard to chase synchronically, may be understood in terms of the continuity of its parts at different times.

Dealing with living organisms with the scientific world of system biology, we suggest considering continuity in terms of functions: "being continuous" for a living organism would be explained as "aiming to the same function". So, we have that *given x at t1 and y at t2, x and y compose the same living organism iff x and y aim to the same function.* Still, this definition is too general, and asks for a specification of what kind of function we should consider as fundamental for the constitution of identity over time of organisms. We guess a reasonable way to define this function, that should preserve the fundamental ideas of dynamicity of processes as well as a form of continuity in terms of aiming to the same function, may be the notion of dynamic equilibrium. Take a system able to demarcate itself from the environment. According to system biology, this system is made of processes, in which both endogenous and exogenous entities interact with each other. This organization produces at least two fluxes, one inward, and one outward. With a wider view, we can argue that from the moment in which the system becomes able to demarcate itself to the moment in which it ends, there is a dynamic equilibrium in those inward and outward fluxes. Extending to living organisms the well-known Michaelis Menten account on enzymatic function (Michaelis and Menten 1913), we can argue that from birth to death, living organism are in a stationary state that maintains the dynamic equilibrium needed to keep the system self-organized and able to remain demarcated from the environment. If we are correct about this, then the identity of living beings over time might be formulated as follows:

given x at t1 and y at t2, x and y compose the same living organism iff x and y are in dynamic equilibrium, i.e. they are arguments of the same function of dynamic equilibrium.

From a metaphysical perspective, the dynamic equilibrium among parts should then be understood as the standard counterpart relation among constituents at different times, on which the unity - and then the identity - of living beings rests upon.

In conclusion, we argue that the scientific world of system biology leads to accept a different relation between synchronic and diachronic identity, for the latter is the most fundamental kind of identity for organisms as living systems, given their intrinsically diachronic nature. In contrast, when we speak about the identity of an organism at a time, we refer in a 'loose and popular sense' to nothing but a part of the organism, which is individuated by abstraction in virtue of its being part of a larger time-extended system.

Local wake-like activity could be a kind of continuity

Roughly, according to the view presented above, organism x at t^1 and organism y at t^2 are the same organism if and only if x and y are connected by some kind of continuity or connectedness (without requiring that x and y are strictly identical to each other). Furthermore, we concluded that a diachronic identity account represents the most fundamental kind of identity for organisms as living systems, whose nature results intrinsically diachronic.

In line with this view we should hypothesize the presence of a kind of continuity in the electrical properties that underlies our personal identity. As showed by the works presented in these chapters we now can see at the mechanisms of the sleep-wake cycle from a local perspective. The local nature of them could suggest that even during physiological sleep, some areas could be always awake, taking turns with others in maintaining the continuity necessary for our persistence over time.

Conclusions

In sum we provided new evidence that confirms the presence of local sleep in awake human cortices. Among others, we demonstrated the presence of sleep-like bistability in the peri-lesional areas of stroke patients (see Figure 28, 29). Furthermore, connecting a fundamental sleep mechanism to brain injuries, we enlightened the relationship between local sleep and impairment.

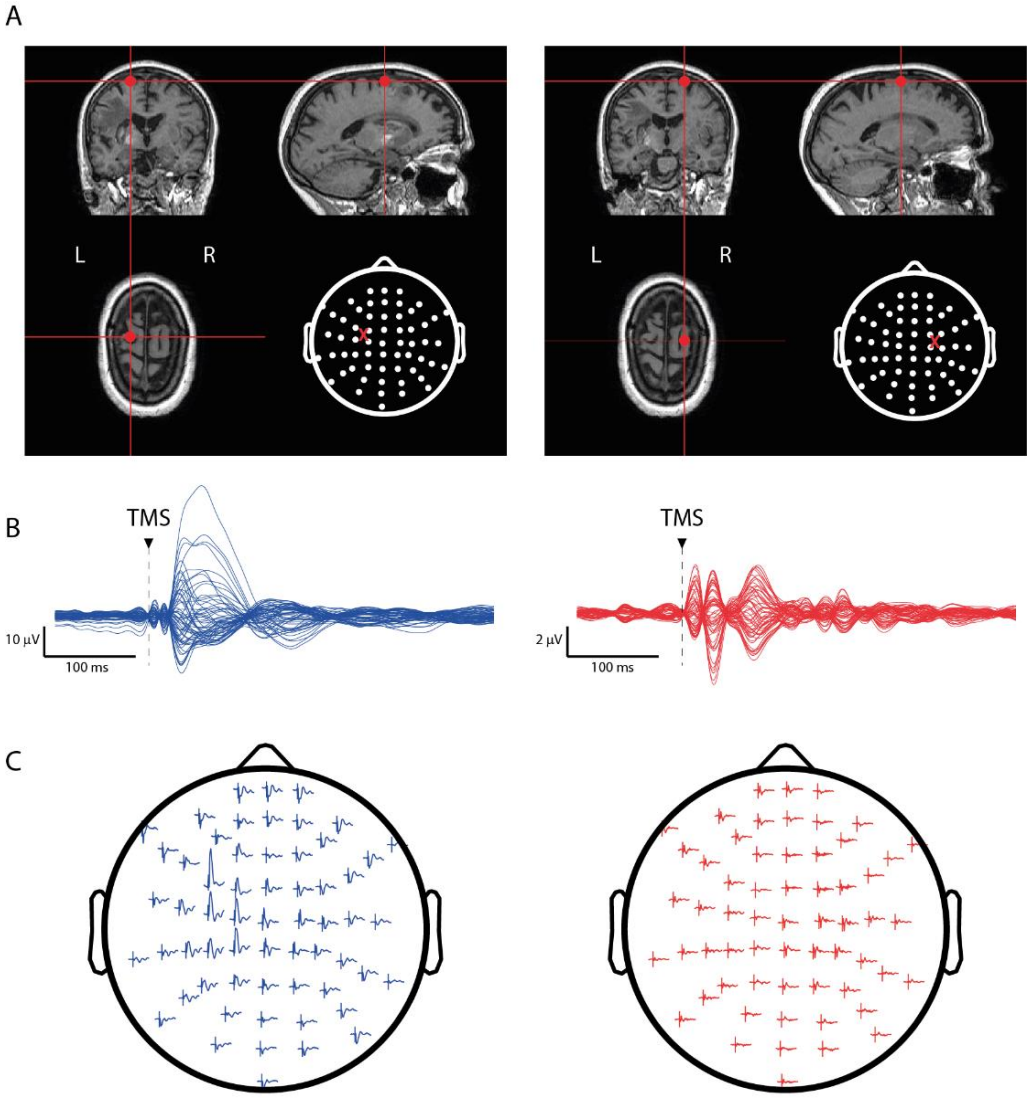


Figure 28. TMS over perilesional areas results in a local stereotypical positive negative high amplitude response. All panels refer to the affected hemisphere on the left and to the healthy hemisphere on the right for a representative patient (patient 4 Appendix table 1), blue traces show the EEG evoked response of the perilesional TMS, while red traces show the response to TMS of the contralateral healthy site A. MRI resonance of patient and a topographic EEG channel distribution, red dot on the MRI and red cross on the channel topography represent the stimulation site. B. Butterfly plot of the average response for each EEG channel between -100ms and +400ms post stimulus (note the different scale between the blue and the red traces) C. Topographical distribution average voltages for the two conditions.

Sleep and wakefulness have been considered global states regulated by subcortical circuits in a top-down fashion, as described in chapter one, from a behavioural perspective. In the last years we have discovered that both are in essence local processes. Moreover, our work may lead to a major paradigm shift between Neuroscience and Cognitive Science: the new concept of fluid boundaries between sleep and wakefulness, where one can intrude into the other and vice versa (Sarasso et al., 2014b).

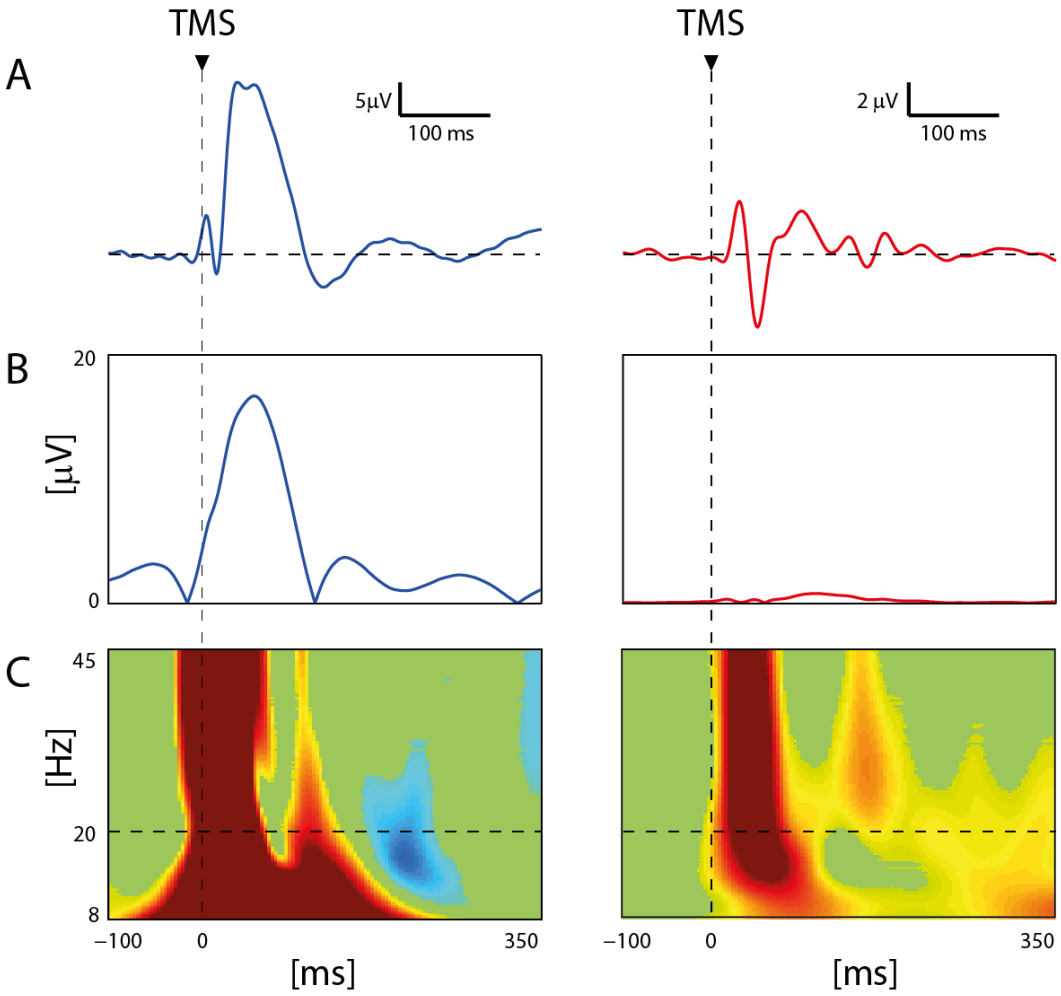


Figure 29. TMS triggers a local slow wave over perilesional areas underpinned by the occurrence of a cortical down-state. Data from a representative patient (patient 4 Appendix table 1) are presented, perilesional area assessment on the left and contralateral area assessment on the right. A. Average across trial of the channel closest to the stimulation site. B. Slow wave amplitude < 4 Hz: amplitude of the slow (< 4 Hz) wave component calculated as squared absolute value of the TMS-EEG evoked potential after 4 Hz low-pass third order Chebyshev filtering. After bootstrap statistic ($\alpha < 0.05$), non-significant time points (with respect to baseline, from − 300 to − 50 ms) were set to zero. C. Event-related spectral perturbation (ERSP): time-frequency power spectra of EEG response recorded. Time-frequency decomposition is applied at a single trial level using Wavelet Transform (Morlet, 3 cycles) and significance for bootstrap statistics is set with $\alpha < 0.05$. Blue colour indicates a significant reduction compared to the baseline, while red indicates significant increase. The dashed horizontal line indicates 20 Hz. PWR > 20 Hz: time series of high frequency power (> 20 Hz).

Here we have showed and reviewed experimental works supporting this view. In particular, we have characterized the presence of local sleep-like activity in stroke patients and in one piloting study on a subject with epilepsy. At least from an electrophysiological perspective we could claim that features of sleep and wakefulness coexist across diffuse brain areas.

The processes described in the work of chapter four, namely the circadian and the homeostatic ones, can modulate sleep intensity (Borbély,1982; Borbély and Achermann, 1999). The interaction of these processes and the modulatory effects they exert on the wake -and sleep- promoting systems allow an unambiguous separation between the state of wakefulness and sleep, from both a behavioural and a neurophysiological perspective. However, recent experimental evidence in clinical and basic research settings, including the one presented in this work, suggests that the wake and sleep-regulatory mechanisms might have different time constants for different brain areas, thus partially redefining (at least at the electrophysiological level) the classical definition of wake and sleep as separate, discrete states. If so, this could have implications beyond the neurosciences, in the field of philosophy.

Specifically, our work could shed light on the problem of the personal persistence over time; accounting for a diachronic nature of the living beings, as conceived by new findings in system biology, we may opt for a biological criterion of identity, in which the continuity necessary for maintaining our self over time could be represented by an activity in the brain that never stops.

Appendix

Table 1

Patient number	Gender	Age (years)	Etiology	Disease duration (months)	Disability	NIHSS	Lesion	Contralesional stimulation site	Perilesional stimulation site
MCA ischemia									
1	m	61	IS	5	moderate	9	right cortical MCA territory ischemia	left BA4	right BA4
2	m	80	IS	28	mild	4	right cortical MCA territory ischemia	left BA7	right BA7
3	f	39	IS	19	moderate	6	right cortical MCA territory ischemia	right BA6	left BA6
4	f	58	IS	2	moderate	16	left fronto-insular ischemia	right BA6	left BA6
5	m	78	IS	36	moderate	8	right fronto-insular ischemia	left BA4	right BA4
6	f	74	IS	2	severe	17	right temporo-parietal ischemia	left BA7	righjt BA7
7	m	65	IS	22	mild	7	right fronto-parietal ischemia	left BA7	right BA7
8	m	75	IS	3	moderate	9	right fronto-parietal ischemia	left BA4	right BA4
9	f	72	IS	3	mild	7	left cortical MCA territory ischemia	right BA4	left BA4
10	m	77	IS	9	moderate	8	right cortical MCA territory ischemia	left BA6	right BA6
Severe Multifocal (Cortico/Subcortical)									
11	m	38	TBI	1	MCS	N/A	right frontal subdural hematoma	left BA7	right BA4
12	f	60	HE/IS	2	EMCS	N/A	right fronto-temporo-parietal hemorrhage. Ischemic and hemorrhagic sequelae. Lenticular, thalamic and mesencefalic hematoma	left BA7	right BA7
13	f	62	HE	1	MCS	N/A	meningeal hemorrhage due to an aneurysm of the left sylvian artery	right BA4	left BA4
14	m	87	IS	22	EMCS	N/A	right fronto-parietal as well as bilateral cerebellar and occipital ischemic lesions	left BA6	right BA6
15	m	54	TBI	25	EMCS	N/A	left lateral and medial temporal lobe traumatic lesion	right BA6	left BA6

16	f	66	IS/HE	13	MCS+	N/A	right temporo-parietal-occipital ischemic/hemorrhagic lesion. Left occipital ischemic/hemorrhagic lesion. Bilateral frontal ischemic lesions	right BA6	right BA7
17	f	53	IS	87	MCS-	N/A	bilateral cortico-subcortical insulo-temporo-parietal ischemic lesions	right BA4	left BA4
18	m	32	TBI	29	MCS-	N/A	bilateral frontal and left parietal traumatic brain injuries. Dilation of the right lateral ventricular head	left BA6	left BA7
19	m	23	TBI	14	EMCS	N/A	traumatic diffuse axonal injury with multiple hemorrhagic lesions involving the left cerebellar hemisphere, the splenium of the corpus callosum, the left thalamus and the frontal lobes bilaterally	left BA4	right BA7
20	m	71	IS/HE	1	MCS-	N/A	moderately edematous and partially hemorrhagic ischemia of the superficial and deep left sylvan territory	right BA6	left BA6
Subcortical (lacunar infarction + intracerebral hemorrhage)									
21	m	79	HE	24	moderate	5	right thalamic hemorrhage	left BA4	right BA4
22	m	75	HE	2	severe	10	right basal ganglia hemorrhage	left BA4	right BA4
23	f	76	IS	8	mild	6	right capsular-lenticular ischemia	left BA6	right BA6
24	m	59	IS	19	mild	2	left periventricular ischemia	right BA6	left BA6
25	f	82	HE	3	severe	10	left thalamo-capsular hemorrhage	right BA6	left BA6
26	m	59	IS	3	mild	6	right corona radiata ischemia	left BA6	right BA6
27	f	66	IS	2	moderate	6	right paramedian ischemia	left BA4	right BA4
28	f	77	IS	2	mild	2	right peritrigonal ischemia	left BA4	right BA4
29	m	75	IS	11	mild	2	right nucleo-capsular ischemia	left BA6	right BA6
30	m	70	IS	2	mild	3	right corona radiata ischemia	left BA6	right BA6

Table 2

Patient number	Lateralized anomalies	Focal anomalies			PDR anomalies		Bilateral anomalies	
		Location	Frequency band	Incidence	Presence	Side prevalence	Location	Side prevalence
1	marked	right F-C-T	delta-theta	sub-continuous	yes	right suppressed PDR	N/A	N/A
2	none	N/A	N/A	N/A	yes	right PDR slowing	N/A	N/A
3	mild	right T	sharp waves	intermittent	no	N/A	N/A	N/A
4	mild	left F-C	theta	intermittent	no	N/A	N/A	N/A
5	marked	right C-P-T	delta	continuous	yes	right suppressed PDR	N/A	N/A
6	mild	right P	delta	sub-continuous	no	N/A	bifrontal	left
7	marked	right T-P	delta-theta	sub-continuous	yes	right suppressed PDR	N/A	N/A
8	none	N/A	N/A	N/A	yes	right suppressed PDR	N/A	N/A
9	mild	left F-T	delta	sporadic	no	N/A	N/A	N/A
10	mild	right P-O	theta	sub-continuous	no	N/A	N/A	N/A

posterior dominant rhythm (PDR)

Table 3

Patient number	*MCA ischemia*	Patient number	*Severe Multifocal (Cortico/ Subcortical)*	Patient number	*Subcortical (lacunar infarction + intracerebral hemorrhage)*
	Maximum PCIst		**Maximum PCIst**		**Maximum PCIst**
1	54.3	**11**	31.8	**21**	29.7
2	43.4	**12**	41.1	**22**	45.7
3	54.3	**13**	34.7	**23**	44.0
4	33.9	**14**	33.7	**24**	39.5
5	37.6	**15**	26.7	**25**	38.7
6	40.9	**16**	29.5	**26**	53.9
7	33.2	**17**	*11.7*	**27**	45.9
8	39.2	**18**	*12.7*	**28**	44.0
9	37.5	**19**	25.9	**29**	42.0
10	36.9	**20**	37.1	**30**	41.5
Mean	41.1	**Mean**	28.5	**Mean**	42.5
SE	2.4	**SE**	3.1	**SE**	2.0

Image Index

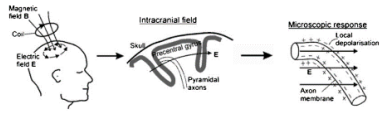
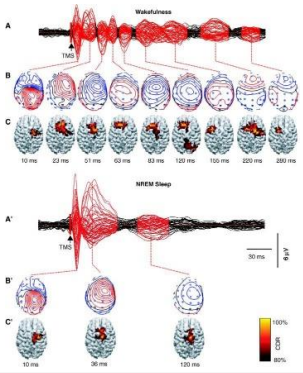
Figure 7	Figure 8	Figure 9
		
Neural targets of TMS. From left to right a schematic representation of the electric field (E) induced into the subject's head by the magnetic field (B) after a brief pulse of electrical current is passed through the TMS coil; the macroscopic (cortical gyri) and microscopic targets (bent axons) of the electric field E induced intracranially by the TMS coil. Reproduced from Ruohonen and Ilmoniemi, 1999.	TMS Compatible EEG cup. From the Internet.	Spatiotemporal dynamics of scalp voltages and cortical currents evoked by TMS during wakefulness (A) and sleep (A'). Averaged TMS-evoked potentials recorded at all electrodes, superimposed in a butterfly diagram (black traces; the horizontal red line indicates the average reference), for the same subject as in Figs. 1 and 2. The time of TMS is marked by a vertical red bar. The red portions of the traces indicate the times at which TMS induced a significant response. From Massimini et al., 2005.
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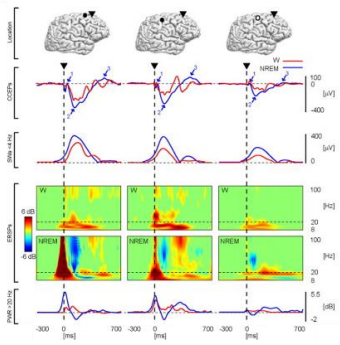
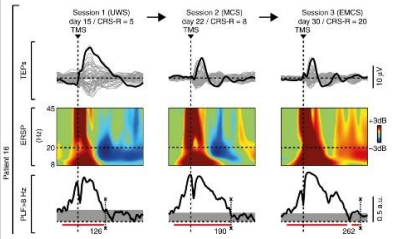
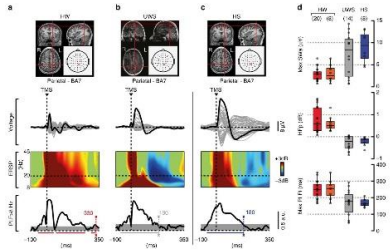
Figure 10	Figure 11	Figure 12
		
During NREM, SPES triggers a slow-wave-like response that is associated with high frequency. Location: the position of the stimulating contact is depicted (black triangle) over a 3D brain reconstruction (lateral view) of the individual's brain (Subject 1) [...]. Modified from Pigorini et al., 2015.	Longitudinal measurements in one UWS patient who evolved to EMCS, through MCS. In Patient 16 the first behavioral and TMS/EEG assessments were carried out 48 h after withdrawal of sedation, as patient exited from coma. The butterfly plot of the TMS-evoked EEG potentials recorded from all 60 channels (gray traces), the corresponding ERSP and the PLF time course of the channel with the largest response are shown [...]. Adapted from Rosanova, Fecchio, 2018.	TMS evokes a sleep-like OFF-period and an early drop of PLF in UWS patients. Results for a representative healthy subject during wakefulness (HW) and NREM sleep (HS) and a representative UWS patient are shown for parietal stimulations (BA7). a–c MRIs and cortical targets as estimated by the Navigated Brain Stimulation system are shown (top). A dashed vertical line marks the occurrence of TMS. Butterfly plots of the TMS-evoked EEG potentials recorded at all 60 channels [...]. From Rosanova, Fecchio, 2018.
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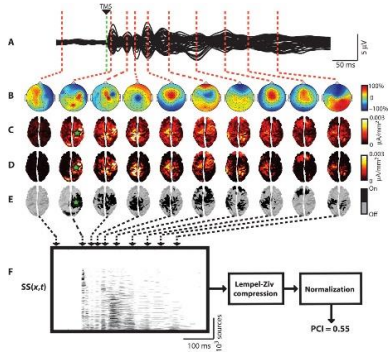
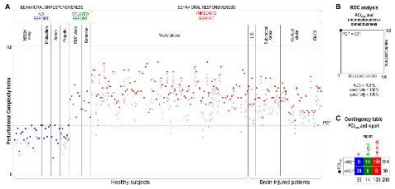
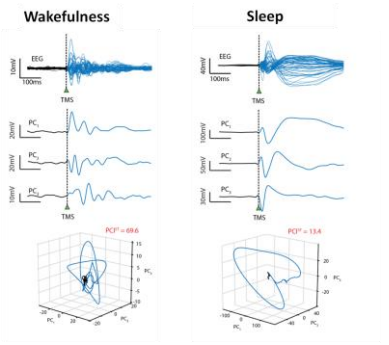
Figure 13	Figure 14	Figure 15
		
The PCI is calculated from TMS-evoked potentials. (A) The black traces show the superposition of the averaged TMS-evoked potentials (150 trials) recorded from all EEG channels (butterfly plot of 60 channels) in one representative subject during wakefulness. (B) The color-coded maps show the instantaneous voltage distributions at selected latencies [auto-scaled between the maximum (+100%) and the minimum (−100%) instantaneous voltages] [...]. From Casali, 2013.	A. Each circle represents the Perturbational Complexity Index (PCI) value computed from the cortical responses to transcranial magnetic stimulation (TMS) of one stimulation site. Several PCI values computed in each individual are aligned along vertical columns. PCI values are computed from TMS-evoked potentials recorded in healthy subjects and conscious brain-injured patients during different conditions. Individuals are grouped by condition [...]. From Casarotto et al., 2016.	Representation of PCI-ST index for two different state conditions, wakefulness (left) and sleep (right). Top section: butterfly plot for averaged TMS response for all channels. Middle: a depiction of the three main principal components (PCs) involved in the response of each of the two conditions. Below: a spatial representation of PCI-ST calculation in the space of the respective three main components [...]. From Comolatti et al., 2019.
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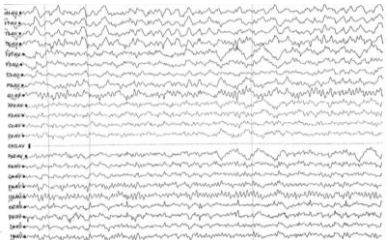
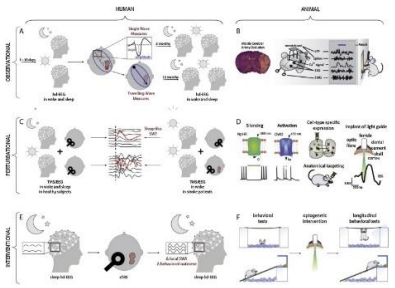
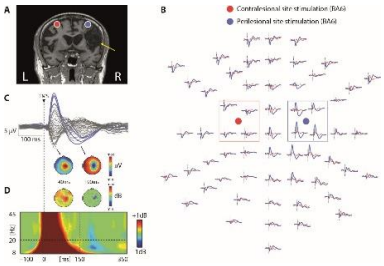
Figure 16	Figure 17	Figure 18
		
Lateralized slowing in a 63 years old male patient with acute ischemic stroke shows low to middle amplitude slow waves visualized in the left hemisphere and are more obvious in M1, F7, T3, T5 leads. From Bhattarai et al., 2014.	Three different, parallel approaches for studying the link between sleep and stroke. Red areas on topographies represent stroke lesions. Observational: A. Longitudinal hd-EEG measures in human stroke patients during wakefulness and sleep after 3 and 12 months from the baseline measures. B. Local Field Potential, Spikes, Ca++, EEG and EMG analysis in animal model of stroke, made by Middle Cerebral Artery Occlusion as in the next two approaches [...]. From Mensen et al., 2019.	TMS reveals local, sleep-like slow waves associated with cortical OFF-periods over the affected hemisphere. Results from one representative patient (patient n.10 from Table1) are shown for both the contralesional (red) and perilesional (blue) stimulation sites. Panel A. MRI and cortical targets (BA6) as estimated by the Navigated Brain Stimulation system are shown. The yellow arrows highlight lesion location [...].
Page 40	Page 49	Page 61

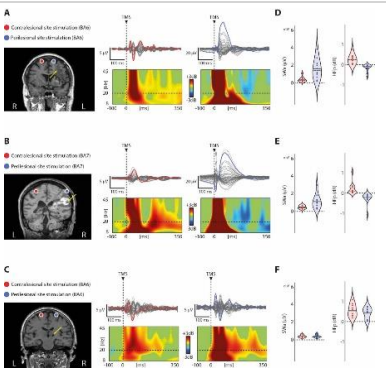
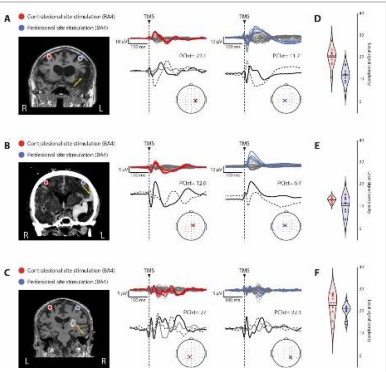
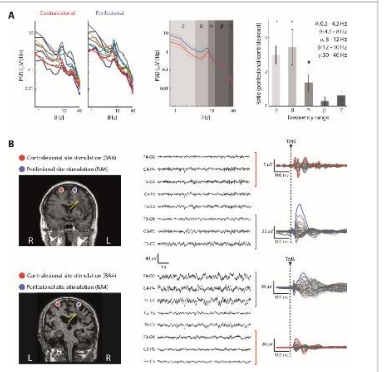
Figure 19	Figure 20	Figure 21
		
Perilesional cortical OFF-periods are present in all patients with cortico/subcortical lesions irrespective of the aetiology. Panel A, B and C. Results from three representative patients, one for each group (patients n.4, 12 and 23 from Appendix Table 1, respectively) are shown for both contralesional (red) and perilesional (blue) stimulation sites. For each panel, MRIs and cortical targets as estimated by the Navigated Brain Stimulation system are shown (left) [...].	The presence of local, sleep-like cortical OFF-periods affects local signal complexity. Panel A, B and C. For each panel, brain imaging (MRI for Panel A and C, CAT scan for Panel B) and cortical targets as estimated by the Navigated Brain Stimulation system are shown for three representative patients, one for each group of patients (patients n.1, 13 and 22 from Appendix Table 1, respectively) are shown for both the contralesional (red) and perilesional (blue) stimulation sites [...].	Resting-state EEG recordings in the MCA ischemia group. Panel A. For each patient, individual Power Spectral Density (PSD) calculated over the same four channels used for TMS/EEG analysis (contralesional, red; perilesional, blue) is shown (left). Average PSD (±SEM) across patients. Shaded gray boxes identify classical frequency ranges (middle). Ratio between perilesional and contralesional site PSD averaged across bins divided into classical frequency ranges [...].
Page 64	Page 65	Page 68

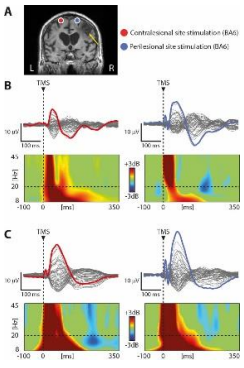
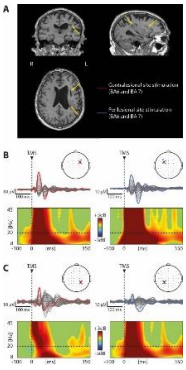
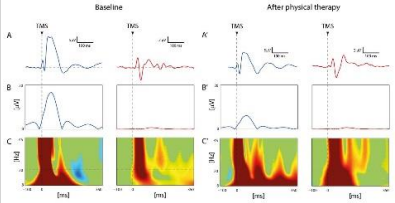
Figure 22	Figure 23	Figure 24
		
The TMS-evoked response over the perilesional stimulation site during wakefulness is similar to the typical TMS response ubiquitously observed during NREM sleep. Results from one representative patient (patient n.14 from Appendix Table 1) are shown for both the contralesional (red) and perilesional (blue) stimulation sites.[...].	The absence of TMS-evoked slow waves and OFF-periods irrespective of the stimulated hemisphere and cortical area in the group of patients affected by unilateral lacunar ischemic or haemorrhagic subcortical lesions. Results from one representative patient (patient n.24 from Appendix Table1) are shown for both the contralesional (red) and perilesional (blue) stimulation sites. Panel A. MRI and cortical targets (BA6) as estimated by the Navigated Brain Stimulation system are shown [...].	Longitudinal electrophysiological assessment in one patient suggests a causal role of sleep-like bistability in stroke A. Average across trial of the channel closest to the stimulation site. B. Slow wave amplitude < 4 Hz: amplitude of the slow (< 4 Hz) wave component calculated as squared absolute value of the TMS/EEG evoked potential after 4 Hz low-pass third order Chebyshev filtering. After bootstrap statistic ($\alpha < 0.05$), non-significant time points were set to zero [...].
Page 70	Page 72	Page 74

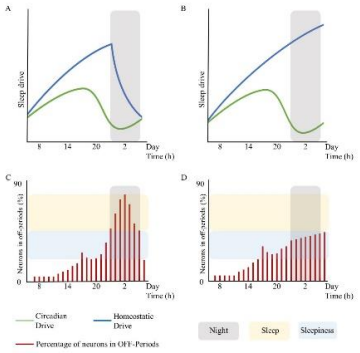
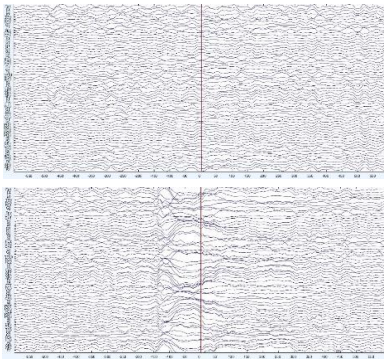
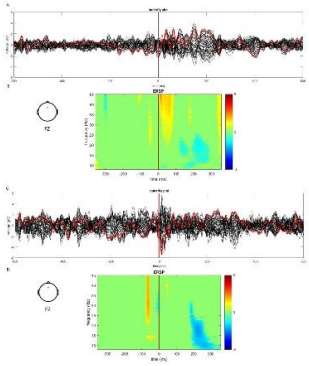
Figure 25	Figure 26	Figure 27
		
Interplay between global sleep drivers and cortical neuronal firing. The figure is intended to schematize the concepts described in this work without fitting any biological data for sleep drives or the number of neurons in OFF-periods. Top panels represent the time-course of the circadian and homeostatic drive over 24 h. Bottom panels represent the percentage of neurons in OFF-periods across 24 h. (A) circadian and homeostatic drives under physiological conditions [...]. From D'Ambrosio et al. 2019.	Upper panel. EEG traces of one representative trial with no seizure. In the figure are represented channels per time, showing the 1200ms around the TMS pulse (red vertical line). Bottom panel. EEG traces of one representative trial with an absence seizure, onset at 150ms before the TMS pulse. In the figure are represented channels per time, showing the 1200ms around the TMS pulse (red vertical line).	Butterfly plot and time-frequency decomposition of the average of the TMSEEG trials performed on the medial premotor left cortex in the inter-ictal (panel A, B) and the ictal (panel C and D) period, in one absence epilepsy patient. A. Average of all the trial performed at least 2 seconds far from any absence seizure. The black lines represent all the channels, the red line represent the closer channel to the stimulation FZ [...].
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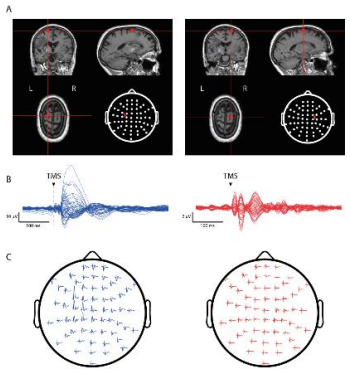
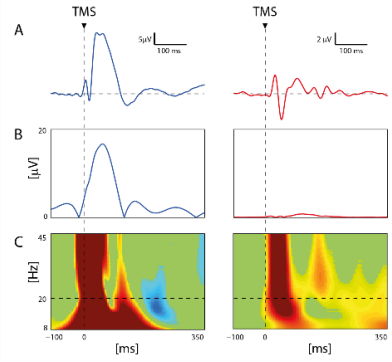
Figure 28	Figure 29	
		
TMS over perilesional areas results in a local stereotypical positive negative high amplitude response. All panels refer to the affected hemisphere on the left and to the healthy hemisphere on the right for a representative patient (patient 4 Appendix table 1), blue traces show the EEG evoked response of the perilesional TMS, while red traces show the response to TMS of the contralateral healthy site [...].	TMS triggers a local slow wave over perilesional areas underpinned by the occurrence of a cortical down-state. For a representative patient (patient 4 Appendix table 1). A. Average across trial of the channel closest to the stimulation site. B. Slow wave amplitude < 4 Hz: amplitude of the slow (< 4 Hz) wave component calculated as squared absolute value of the TMS-EEG evoked potential after 4 Hz low-pass third order Chebyshev filtering [...].	
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List of abbreviations

ATP: adenosine triphosphate

ARAS: ascending reticular activating system

AASM: American Academy of Sleep Medicine

CCEPs: cortico-cortical evoked potentials

CT: computerised tomography

CRS-R: coma recovery scale–revised

DALYs: disability-adjusted life years

DC: direct current

EEG: electroencephalography

EF: electric field

EI: effective information

EMCS: emergence from minimally conscious state

EMG: electromyogram

EOG: electrooculogram

EPSPs: excitatory postsynaptic potentials

ERSP: event-related spectral perturbation

GABA: ϒ-aminobutyric acid

GMFP: global mean field power

hd-EEG: high-density electroencephalography

IPSPs: inhibitory postsynaptic potential

ICA: independent component analysis

LFP: local field potential

LMFP: local mean field power

LOC: loss of consciousness

MAS: Modified Ashworth Spasticity

MCS: minimally conscious state

MR: magnetic resonance

MRI: magnetic resonance imaging

NBS: navigated brain stimulation system

NIHSS: The National Institutes of Health Stroke Scale

NREM: non-rapid eye movement

PCI: perturbational complexity index

PET: positron emission tomography

PLF: phase locking factor

REM: rapid eye movement

rt-PA: recombinant tissue plasminogen activator

SWa: maximum amplitude of the evoked slow wave

SWS: slow wave sleep

TEPs: TMS-evoked potentials

TIA: transient ischaemic attack

TMS: transcranial magnetic stimulation

UWS: Unresponsive Wakefulness Syndrome

VS: vegetative state

Achievements earned during the doctoral school

A.A. 2016/2017:

- March 2017 - Talk - Nordic Network for Philosophy of Science, Copenhagen, Denmark. Presentation of the paper "Organisms, processes, and the priority of diachronic identity" with Valerio Buonomo.
- September 2017 - Poster Presentation - Science Factory V, Aalto University, Helsinki, Finland. Presentation of the poster "Cortical perturbations reveal local sleep-like down states in cortical perilesional areas".

A.A. 2017/2018:

- March 2018 - Poster Presentation - Neuronest, Università degli studi di Milano, Milano, Italia. Presentation of the poster "Simultaneous hd-EEG and stereo-EEG recordings during intracerebral single pulse electrical stimulation: from macro to meso-scale".
- September 2018 - Publication - Science abstract: "Cortical perturbations reveal local sleep-like down states in cortical perilesional area"- published on the journal of sleep research, DOI: 10.1111/jsr.12751. Made with collaborators of the laboratory of Physiology. This work characterizes neurophysiological markers of local sleep like bistability, during wakefulness, in 30 cortical brain-injured patients.
- September 2018 – Poster presentation regarding the abstract mentioned above at ESRS2018.
- Best Abstract Award at ESRS 2018. 1st Prize. Success rate 1/700.

A.A. 2018/2019

- December 2018: Winning of the grant IBRO-PERC meeting support for the conference Sleep and Wakefulness: a dyadic life.
- February 2019: Publication – "Sleep as a model to understand neuroplasticity and recovery after stroke: Observational, perturbational and interventional approaches". Published in Journal of Neuroscience Methods. DOI: 10.1016/j.jneumeth.2018.12.011
- March 2019: Organization - Event: "Sleep and Wakefulness: a dyadic life". With the sponsorship of UNIMI and International Brain Research Organization and with a not-money sponsorship of Sinergia SNSF – 110 people attended from 18 countries.
- June 2019: Publication – "iEEG-BIDS, extending the Brain Imaging Data Structure specification to human intracranial electrophysiology". Published in Nature – Scientific Data. DOI: 10.1038/s41597-019-0105-7.
- September 2019: Publication – "Sleepiness as a local phenomenon". Published in Frontiers in Neuroscience - Sleep and Circadian Rhythms.

Essays written during the doctoral school

In preparation/submitted:

- Philosophy article: "Organisms, processes, and the priority of diachronic identity" - article with Valerio Buonomo - in preparation. The aim of this paper is to elaborate a theory of identity for living organisms, conceived by system biology (SB) as self-organising dynamical systems that are able to demarcate themselves from their environment, focusing on what, if anything, this theory implies about the metaphysical debate.

- Science article: "Cortical perturbations reveal local sleep-like down states in cortical perilesional areas" - in preparation. This work characterizes neurophysiological markers of local sleep like bistability, during wakefulness, in 30 cortical brain-injured patients.

- Science article: "How to maximize the cortical responses to the TMS pulses" - in preparation. This work defines the main guidelines to gain the maximal cortex responses within a Transcranial Magnetic Stimulation experiment in humans.

- Science article: "TMS/EEG during absences reveals sleep-like features" - in preparation. This work aims to show sleep-like feature during epileptic absence seizures.

- Science article: "TMS evoked responses in Dravet Syndrome Patients" - in preparation. This work aims to characterize the EEG responses evoked by TMS in Dravet epileptic patients.

- Science article: "Symmetricity in the cortex of Alternating Hemiplegia of Childhood (AHC) patients" - in preparation. This work aims to characterize the cortical neurophysiological features of the AHC patients.

- Science article: "TMS/EEG Symmetricity investigation on healthy subjects" - in preparation. This work aims to characterize the TMS evoked EEG responses in healthy subjects.

- Science article: "Reproducibility of TMS/EEG responses" – in preparation. This work is a multicentric study. It aims to standardize the assessment of the TMS/EEG technique.

- Science article: "Graphic User Interface for the TMS/EEG assessment". This work aims to show a unique tool needed for the standardization of the TMS/EEG assessments.

Published:

- Science abstract: "Cortical perturbations reveal local sleep-like down states in cortical perilesional area"- published in the journal of sleep research. This work characterizes neurophysiological markers of local sleep like bistability, during wakefulness, in 30 cortical brain-injured patients. First author.

- Science article: "Sleep as a model to understand neuroplasticity and recovery after stroke: observational, perturbational and interventional approaches" – published in Journal of Neuroscience Methods. This work presents the different approaches to indagate the motor impairment and to foster the recovery of the stroke patients.

- Science article: "iEEG-BIDS, extending the Brain Imaging Data Structure specification to human intracranial electrophysiology" - Published in Nature – Scientific Data. Intracranial electroencephalography (iEEG) data offer a unique combination of high spatial and temporal resolution measurements of the living human brain. To improve internal (re)use and external sharing of these unique data, we present a specification for storing and sharing iEEG data: iEEG-BIDS.

- Science article: "Sleepiness as a local phenomenon" - Published in Frontiers in Neuroscience – Sleep and Circadian Rhythms. The idea that small brain areas can be asleep while the rest of the brain is awake, and that local sleep may account for at least some of the cognitive and behavioural manifestations of sleepiness, are making their way into the scientific community. I herein clarify the different ways sleep can intrude into wakefulness, summarize recent scientific advances in the field, and offer some hypotheses that helps framing sleepiness as a local phenomenon. First author.

Riassunto

Il sonno e la veglia vengono comunemente considerati come due stati distinti. L'alternanza tra essi, la cui presenza è stata dimostrata in ogni specie animale studiata fino ad oggi, sembra essere una delle caratteristiche che definisce la nostra vita. Allo stesso tempo, però, le scoperte portate alla luce negli ultimi decenni hanno offuscato i confini tra questi due stati.

I meccanismi del sonno hanno sempre affascinato i neurofisiologi, che infatti, nell'ultimo secolo, li hanno caratterizzati in dettaglio: ora sappiamo che all'attività del sonno sottostà una specifica attività neuronale chiamata slow oscillation.

La slow oscillation, che è costituita da (ancora una volta) un'alternanza tra periodi di attività e periodi di iperpolarizzazione e silenzio neuronale (OFF-periods), è la modalità base di attivazione del cervello dormiente. Questa alternanza è dovuta alla tendenza dei neuroni surante lo stato di sonno, di passare ad un periodo silente dopo un'attivazione iniziale, una tendenza a cui viene dato il nome di bistabilità neuronale.

Molti studi hanno dimostrato come la bistabilità neuronale tipica del sonno ed i relativi OFF-periods, possano accadere anche durante la veglia in particolari condizioni patologiche, nelle transizioni del sonno e durante le deprivazioni di sonno. Per questo motivo, se accettassimo che la bistabilità neuronale e gli OFF-periods rappresentino una caratteristica fondamentale del sonno, allora dovremmo ammettere che stiamo assistendo ad un cambio di paradigma: da una prospettiva neurofisiologica il sonno può intrudere nella veglia.

In questa tesi ho analizzato i nuovi -fluidi- confini tra sonno e veglia e le possibili implicazioni di questi nel problema della persistenza personale attraverso il tempo. Inoltre, ho studiato le implicazioni cliniche dell'intrusione di sonno nella veglia in pazienti con lesioni cerebrali focali di natura ischemica. In particolare, i miei obiettivi sono stati:

1) Dimostrare come la bistabilità neuronale possa essere responsabile della perdita di funzione nei pazienti affetti da ischemia cerebrale e come questo potrebbe avere implicazioni nello studio della patofisiologia dell'ischemia cerebrale e nella sua terapia;
2) Stabilire le basi per un modello di sonno locale presente nella vita di tutti i giorni: la sensazione di sonnolenza. Infatti, essa potrebbe riflettere la presenza di porzioni di corteccia in stato di sonno, ma durante lo stato di veglia;
3) Difendere il criterio biologico di identità, che troverebbe nell'attività cerebrale la continuità necessaria al mantenimento della nostra identità nel tempo.