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Cortical mechanisms of loss of consciousness: insight from TMS/EEG studies

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ABSTRACT

In a recent series of experiments we recorded the electroencephalogram (EEG) response to a direct cortical stimulation in humans during wakefulness, NREM sleep, REM sleep and anesthesia by means of a combination of transcranial magnetic stimulation (TMS) and high-density EEG (hd-EEG). TMS/hd-EEG measurements showed that, while during wakefulness and REM sleep the brain is able to sustain long-range specific patterns of activation, during NREM sleep and Midazolam-induced anesthesia, when consciousness fades, this ability is lost: the thalamocortical system, despite being active and reactive, either breaks down in causally independent modules (producing a local slow wave), or it bursts into an explosive and non-specific response (producing a global EEG slow wave). We hypothesize that, like spontaneous sleep slow waves, the slow waves triggered by TMS during sleep and anaesthesia are due to bistability between up- and down-states in thalamocortical circuits. In this condition, the inescapable occurrence of a silent, down state after an initial activation impairs the ability of thalamocortical circuits to sustain long-range, differentiated patterns of activation, a theoretical requisite for consciousness. According to animal experiments and computer simulations, thalamocortical bistability may result from increased K-currents, from alterations of the balance between excitation and inhibition and from partial cortical de-afferentation. We hypothesize that these factors may play an important role in determining loss, and recovery, of consciousness also in brain-injured subjects. If this is the case, some types of brain lesions may impair information transmission, above and beyond the associated anatomical disconnection, by inducing bistability in portions of the thalamocortical system that are otherwise healthy.

Key words
Bistability • Sleep • Anesthesia • Coma

Theoretical requirements for (un)consciousness

In the last decade, a growing interest in the study of the neural correlates of (un)consciousness has led to a number of experiments employing neuroimaging and electrophysiological techniques during sleep (Massimini et al., 2009a), anaesthesia (Alkire et al., 2008; Brown et al., 2010), epileptic seizures (Blumenfeld and Taylor, 2003) and coma (Laureys et al., 2004). Taken together, these studies have provided significant hints about which aspects of brain function may be critical for consciousness, and which ones may not. For example, measurements performed during seizures (Blumenfeld et al., 2003) and ketamine-induced anaesthesia (Alkire and Miller, 2005), where subjects are unconscious and unresponsive despite increased brain metabolism, suggested that the level of brain activity may not be a reliable marker of the presence of consciousness.

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Along the same lines, positron emission tomography (PET) measurements showed that brain-injured patients can recover consciousness from vegetative state, without necessarily increasing their brain metabolic rates (Laureys, et al., 2004). On the other hand, the hypothesis that the level of consciousness could be critically determined by the power/synchronization of spontaneous, fast frequency oscillations in the thalamocortical system has been questioned by recent measurements. Indeed, this hypothesis fails to explain the loss of consciousness observed during NREM sleep and generalized tonic-clonic seizures, where hyper-synchronous broad-band oscillations can be observed (Arthuis et al., 2009), or the markedly reduced level of consciousness associated with ketamine, despite preserved, or higher than wakefulness, fast frequency EEG activity recorded in the brain (Maksimow et al., 2006).

In parallel with the accumulation of empirical data, over the last decade theories of consciousness aimed at coherently account for experimental and clinical observations have been formulated. In particular, the recently developed Information Integration Theory of Consciousness (IITC) (Tononi, 2004; 2008) suggests that consciousness depends not so much on the overall level of neuronal activation, on the occurrence of specific patterns of synchronous activity, or on the ability of cortical neurons to respond to sensory inputs, but rather on the brain's capacity to sustain complex patterns of internal communication. The ITTC claims that consciousness is tied to information integration, which is contingent on the ability of multiple, specialized regions of the thalamocortical system to interact as a single entity (integration) as well as discriminate among a large repertoire of available states (information). The IITC also proposes a measure of Information Integration, Φ.

Calculating Φ of a physical system requires measuring effective information (a measure of causality) and involves perturbing directly the system in all possible ways in order to count the number of different states (information) that can be discriminated through causal interactions within the system as a whole (integration). Although the computation of Φ can be rigorously applied only to small, simulated systems (tens of elements), empirical approaches can, in principle, be developed to gauge the balance between functional differentiation and functional integration in the brain (Seth et al., 2008; Barrett and Seth, 2011). For instance, it was recently proposed that, in order to evaluate, on a very coarse grain, the brain capacity for integrated information, one may employ a perturbational approach (Massimini et al., 2009b). According to this idea, a signature of high information integration in the thalamocortical system is that it should respond to perturbations with rapidly changing activity patterns (information) that affect a large portion of the cerebral cortex (integration). On the other hand, during loss of consciousness (LOC), whether this is caused by sleep, anesthesia or coma, the brain should react to perturbations with a response that is stereotypical (loss of information) and/or local (loss of integration).

In a recent series of experiments we tested these predictions in humans. Specifically, we employed TMS in combination with hd-EEG, a technique that allows stimulating different subsets of cortical neurons and measure, with good spatial-temporal resolution, the effects produced by these perturbations on the rest of the thalamocortical system (Ilmoniemi et al., 1997). First, we studied the changes in TMS-evoked EEG responses during the transition from wakefulness to deep NREM sleep early in the night, when consciousness fades (Massimini et al., 2005; 2007). Then, we recorded the TMS-evoked responses during REM sleep, when the brain is still disconnected from the external world but consciousness comes back in the form of a dream (Massimini et al., 2010). Finally, we analyzed the cortical activations triggered by TMS during LOC induced by a pharmacological agent, midazolam, at anesthetic concentrations (Ferrarelli et al., 2010). Altogether, these TMS/hd-EEG measurements showed that, while during wakefulness and REM sleep the brain is able to sustain long-range, complex patterns of activation, during NREM sleep and anaesthesia, when consciousness fades, this ability is lost: the thalamocortical system, despite being active and reactive, either breaks down in causally independent modules (producing a local slow wave), or it bursts into an explosive and non-specific response (producing a global EEG slow wave).

In this review we suggest that the results of these studies provide some hints on the neurophysiological mechanisms that underlie reversible LOC during sleep and anesthesia. Specifically, we argue that bistability in thalamocortical networks, the key mechanism responsible for the occurrence of

sleep slow waves, may also be what prevents the brain from effectively integrating information during physiological and pharmacological LOC. Due to bistability, cortical neurons are unable to sustain balanced patterns of activation and tend to fall into a silent, hyperpolarized down-state after an initial activation. Bistability may result from increased K-currents, from alterations in the balance between excitation and inhibition and from cortical de-afferentation; thus, we discuss a possible role of these factors in pathological LOC.

Loss of consciousness during NREM sleep

Sleep is the most common condition in which consciousness is reversibly lost. It was first thought that the fading of consciousness during early, deep NREM sleep was a consequence of the brain shutting down. However, while metabolism is reduced, during NREM sleep the thalamocortical system remains active, with mean firing rates comparable to those of quiet wakefulness (Steriade et al., 2001). It was also hypothesized that sensory inputs are blocked during sleep and that they are necessary to sustain conscious experience (Coenen, 1998). Recent work, though, has shown that even during deep sleep sensory signals continue to reach the cerebral cortex, where they are processed subconsciously (Kitamura et al., 1996; Portas et al., 2000). Gamma power and synchrony have been viewed as possible correlates of consciousness, since they were found to be, on average, lower in NREM sleep compared to wakefulness (Cantero et al., 2004). However, gamma activity can be equally low in REM sleep, when conscious experience is usually vivid, while it can be high in anesthetized individuals (Vanderwolf, 2000). Moreover, intracellular recordings have shown that gamma activity is intermittently present during NREM sleep (Steriade, 2006), and other studies reported that gamma coherence is a local phenomenon that does not change between wakefulness and sleep (Bullock et al., 1995). Interestingly, similar paradoxes, where neural activity levels, access to sensory information and the degree of neural synchrony do not correlate with the level of consciousness, can be found in other conditions such as anaesthesia, epilepsy and disorder of consciousness (DOC) patients (Tononi and Laureys, 2008). In this sense, sleep represents a physiological model of unconsciousness, with commonalities to a variety of pathological conditions characterized by LOC, ideally suited to understand which aspect of brain function is key for consciousness.

Therefore, in a series of experiments we have employed TMS/EEG to measure what changes in thalamocortical circuits occur during the transition from wakefulness into NREM sleep early in the night (Massimini et al., 2005; 2007). Using a 60-channel TMS-compatible EEG amplifier, we recorded TMS-evoked brain responses while subjects, lying with their eyes closed on a reclining chair, progressed from wakefulness to deep NREM sleep. Because noise masking was played throughout the stimulation session via ear buds, subjects were unaware of TMS.

Figure 1 shows the response obtained after stimulation of rostral premotor cortex in one subject during wakefulness and NREM sleep. The black traces represent the voltage recorded from all scalp electrodes, the cortical currents associated with the main peaks of activity are depicted below. The circles on the cortical surface indicate the site of stimulation, while the cross highlights the location of maximal cortical activation. During wakefulness (Fig. 1, top), TMS triggers, a series of low-amplitude, high-frequency (25-30 Hz) waves associated with cortical activations that propagate along long-range ipsilateral and transcallosal connections. Remarkably, the exact same stimulation, applied 15 minutes later during NREM sleep stages 3-4 results in a very different picture (Fig. 1, bottom). In this case, TMS triggers a larger, low-frequency wave, associated with a strong initial cortical activation, which does not propagate to connected brain regions and dissipates rapidly. This finding can be reproduced after the stimulation of different cortical areas, as long as subjects are in slow wave sleep stages 3-4. Thus, the cortical area that is directly engaged by TMS preserves its reactivity but tends to behave as an isolated module.

TMS/EEG measurements not only indicate that during slow wave sleep the thalamocortical system tends to break down into isolated modules (loss of integration), but they also show that the ability of thalamocortical circuits to produce differentiated responses (information) is impaired. Indeed, while during wakefulness different cortical areas

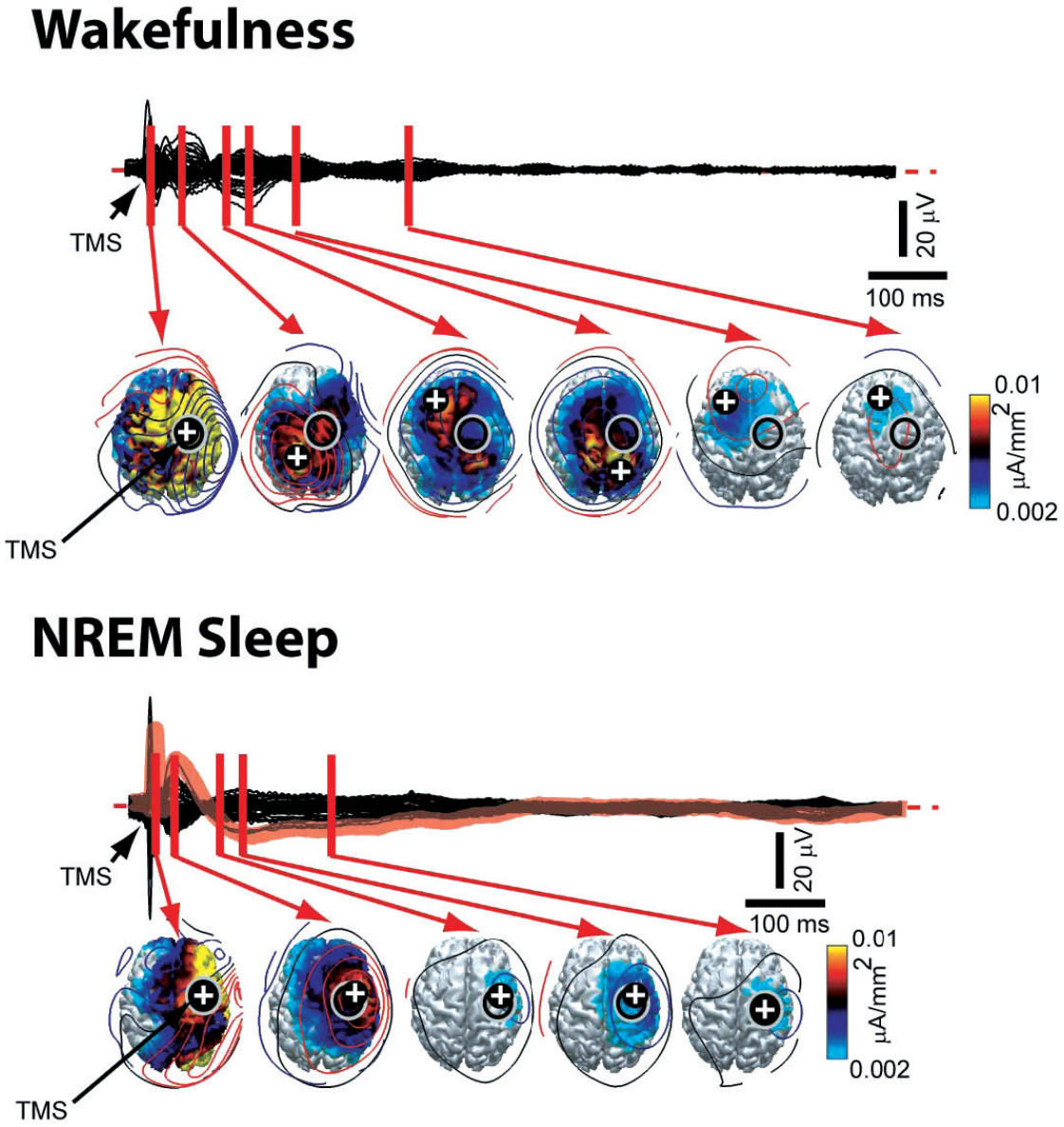

Fig. 1. - Cortical responses to TMS during wakefulness and NREM sleep. EEG traces, voltages and current densities are shown from a representative subject in which the premotor cortex was stimulated with transcranial magnetic stimulation (TMS) (black arrow). (A) During waking, stimulation evokes a complex EEG responses that initially involves the stimulation site (circle) and then, in sequence, other cortical locations (the cross indicate the site of maximum evoked current at each timepoint), producing a long-range pattern of activation. (B) During slow wave sleep, the stimulus-evoked response remains local, indicating a loss of cortical integration. At the same time, the response recorded from the electrode located under the stimulator (thick red trace) becomes a stereotypical positive-negative wave.

react to TMS with a pattern of activation which has a characteristic shape and frequency content (Rosanova et al., 2009), this distinction is clearly obliterated during sleep; the local response to TMS becomes, in all cases, a simple positive-negative wave (Massimini et al., 2007). Thus, if the reactivity of the sleeping brain is tested systematically by applying TMS at different intensities and in different cortical areas, one invariably obtains a stereotypical response: a positive wave followed by a negative rebound. Interestingly, this positive-negative component develops towards a graphoelement resembling a full-fledged sleep slow wave when TMS is delivered at increasing intensities in a scalp region

around the vertex (Fig. 2A). Indeed, the TMS-evoked potentials obtained during NREM sleep share several common features with the spontaneous slow oscillations that characterize cortical activity during sleep (Massimini et al., 2007). First, both TMS-evoked and spontaneous slow oscillations are identified based on period-amplitude criteria (negative peak amplitude of at least 80 μV and half-wave duration between 0.125 and 1 s). Second, in both cases the surface-positive portion of the wave triggers and groups thalamic spindles (a waxing–waning oscillation at ≈12-15 Hz). Third, both TMS-evoked and spontaneous sleep slow oscillations behave as traveling waves that spread smoothly over wide regions of the scalp.

In summary, TMS/hd-EEG measurements suggest that, during NREM sleep, the only way the brain can react to a direct cortical perturbation is by producing a slow wave that is either local, or global and nonspecific. What prevents the emergence of a long-range, differentiated pattern of activation during sleep? It is likely that the mechanism underlying the impaired capacity of the sleeping brain for integrated information is the same mechanism that underlies the occurrence of spontaneous sleep slow waves, which is bistability in thalamocortical circuits. Upon falling asleep, brainstem activating systems reduce their firing rates, thus increasing the influence of depolarization-dependent K+ currents in thalamic and cortical neurons (McCormick et al., 1993). These hyperpolarizing K+ conductances become stronger when neurons become depolarized and fire. Due to these currents, neurons become bistable and inevitably tend to fall into a silent, hyperpolarized state (down-state) after a period of activation (up-state). This bistability provides the mechanism for the slow oscillations of sleep, where large populations of cortical neurons spontaneously alternate between up and down-states (Steriade et al., 1993; Hill and Tononi, 2005). TMS/hd-EEG measurements, by showing that cortical stimulation triggers a stereotypical down-state, suggest that, during NREM sleep, the brain is unable to produce long-range, complex patterns of activation because of bistability. Decreased firing in activating system, increased K-conductance, and the ensuing bistability in thalamocortical networks may therefore be the reasons why information integration is impaired in early NREM sleep. As we will discuss in the last section of this paper, while sleep and the associated bistability are physiological and readily reversible processes, some brain lesions may impair consciousness through a similar mechanism.

Recovery of consciousness during REM sleep

Complete loss of consciousness during sleep is not the rule, and many awakenings yield dream reports (Casagrande et al., 1996; Stickgold et al., 2001; Fagioli, 2002). Dreams can be at times as vivid and intensely conscious as waking experiences. Dream-like consciousness occurs during various phases of sleep, including at sleep onset, during the last part of the night, and especially during rapid eye movement (REM) sleep. In a recent study we recorded TMS-evoked EEG potentials during the first REM sleep episode and compared them with the EEG responses collected during wakefulness and NREM sleep (Massimini et al., 2010). Upon entering NREM sleep early in the night TMS ceased to induce complex, widespread activations and triggered a stereotypical, local slow wave, as previously shown. However, during the transition from NREM to REM, while subjects were still behaviorally asleep, the brain's response to TMS recovered fast oscillatory components and became similar to the one obtained during wakefulness, especially during the first 100-150 ms post-stimulus. This resurgence of fast waves was associated with a partial recovery of cortical effective connectivity.

During REM sleep, while noradrenergic and serotoninergic arousing systems remain silent, brainstem cholinergic neurons come back to activity (Pace-Schott and Hobson, 2002), spontaneous slow waves disappear and the EEG becomes, at least superficially, similar to the one observed during wakefulness. However, despite this apparent resemblance with wakefulness, it is difficult to infer the degree of underlying thalamocortical bistability during REM sleep based on the presence of a low-voltage EEG alone. Indeed, it was shown that during NREM sleep TMS delivered during short stretches of low-amplitude EEG activity was still able to trigger full-fledged slow waves (Massimini et al., 2007).

The fact that during REM sleep TMS triggered a fast-frequency, wakefulness-like response suggests that

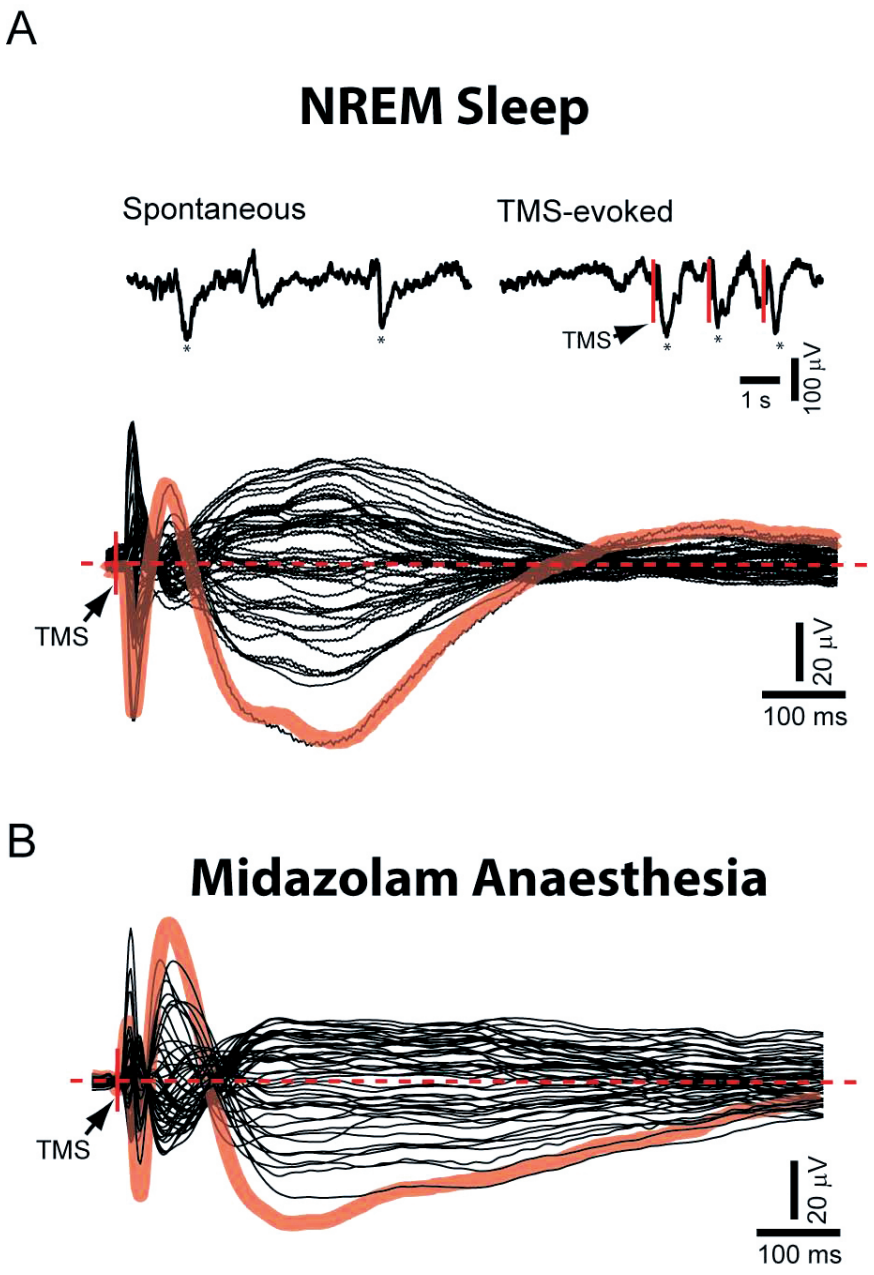

Fig. 2. - TMS triggers full-fledged sleep slow waves during NREM sleep and midazolam anaesthesia. (A, top panel) During NREM sleep, TMS delivered at high intensity (80-90% of maximal output) triggers slow waves that match the detection criteria of spontaneously occurring sleep slow waves. (A, bottom panel) The butterfly plot shows the average response to high-intensity TMS recorded during NREM sleep; TMS triggers a stereotypical, negative-positive wave that resembles the one reported in Figure 1, but that overshoots a peak-to-peak amplitude of 80 μV. (B) The same wave is triggered by high-intensity TMS during midazolam anaesthesia.

the discharge of mesopontine cholinergic neurons alone, which is restored at the transition between NREM and REM sleep, may represent a major excitatory input that can largely prevent the emergence of thalamocortical bistability during REM.

In addition, these measurements demonstrate that TMS/hd-EEG may represent an effective way to probe the internal dialogue of the thalamocortical system even in the absence of any behavioral cue (Massimini et al., 2009b). TMS triggered more

widespread and differentiated patterns of EEG activation, just as it does during wakefulness, upon entering REM sleep, a state in which subjects are conscious but almost paralyzed. Accordingly, a recent study showed that TMS/hd-EEG is effective in detecting a residual capacity for consciousness in brain injured patients who are unable to move and communicate (Rosanova et al., 2012).

Loss of consciosness during midazolam at anaesthetic doses

Besides sleep, when consciousness spontaneously fades, unconsciousness can be induced pharmacologically during general anesthesia (Alkire et al., 2008). Although several anesthetics can induce states with behavioral and electrophysiological features similar to those of deep NREM sleep, pharmacological anesthesia and sleep are not identical and may differ in terms of both neural and molecular mechanisms of action (Van Dort et al., 2008). Furthermore, whereas in sleep studies it is not feasible to evaluate consciousness reliably and repeatedly, because the depth of sleep varies unpredictably and the subjects, when awakened, may not return to sleep promptly, during pharmacologically induced LOC a subject's level of alertness may be assessed repeatedly without reversing it. For these reasons, in a recent set of experiments we asked whether cortical effective connectivity would show a breakdown, similar to the one observed in deep NREM sleep, during LOC induced by a pharmacological agent, midazolam, at anesthetic concentrations (Ferrarelli et al., 2008). We found that, while during wakefulness TMS of the premotor cortex triggered responses in multiple cortical areas distant from the site of stimulation, during midazolam-induced LOC, TMS evoked a positive-negative response that was initially larger but that remained local. As during NREM sleep, also during midazolam anesthesia we found that this stereotypical response evolved into a full-fledged slow oscillation when the cerebral cortex was stimulated at higher intensity (Fig. 2B).

The finding that both during NREM sleep and Midazolam anaesthesia TMS triggered a stereotypical, positive-negative slow wave suggests that bistability may represent a common cortical mechanism for unconsciousness. While bistability during sleep may be largely due to increased K+ currents, other mechanisms may be prevalent during anesthesia. For example, a shift in the balance between synaptic excitation and inhibition toward inhibition, determined by increased GABA activity may play a major role. At least three considerations support this mechanism. First, midazolam acts selectively on GABA receptors to induce LOC (Judge et al., 2009). Second, increased GABAergic inhibition, like depolarization-dependent K+ currents, can terminate the up-state and initiate the down-state in cortical neurons (Mann et al., 2009). Third, in a recent large-scale modeling study investigating the mechanisms underlying the breakdown of effective connectivity it was found that enhanced synaptic inhibition, due to an increase in GABA release, was effective in reducing cortico-cortical signal transmission (Esser et al., 2009). Thus, during midazolam-induced LOC, this mechanism may be engaged through an enhanced GABA-mediated inhibition via a direct, local effect within the cortex (Florian et al., 2008). In addition to cortico-cortical pathways, some mechanisms at the thalamic level may play a role in blocking the spread of spontaneous and evoked cortical activity during LOC; indeed, cortico-thalamo-cortical circuits are thought to relay driving input from one cortical area to a higher order one (Guillery and Sherman, 2002). Although the extent of these cortico-thalamo-cortical loops still needs to be fully established, it is entirely possible that an hyperpolarization of the thalamus (occurring spontaneously during NREM sleep, or pharmacologically induced by midazolam (Veselis et al., 1997)) may profoundly affect cortical effective connectivity. Interestingly, the fact that TMS evoked a large, slow, local response, largely resembling the cortical evoked responses produced by inhaled anesthetics in rats (Hudetz and Imas, 2007) suggests that these findings may be generalized to other anesthetics, and offers an opportunity to explore the molecular/neural mechanisms underlying these responses in animal models.

Possible implications for brain-injured patients

In this article we reviewed a series of TMS/hd-EEG experiments designed to test the prediction that a key requirement for consciousness is the ability of multiple, specialized cortical areas to interact rap-

idly and effectively (effective connectivity) (Tononi, 2005). The finding that a breakdown of cortical effective connectivity occurs during spontaneous (deep NREM sleep) and pharmacologically induced (i.e., midazolam at anesthetic doses) LOC, together with the finding that differentiated, widespread interactions resume during REM sleep, when conscious activity is regained in dreaming, is consistent with this theoretical prediction. More importantly, as we will discuss below, TMS/hd-EEG may offer new insight into the neurophysiogical mechanisms that underlie loss, and sometimes recovery, of consciousness after brain injuries.

The striking similarity between TMS-evoked EEG responses during sleep and midazolam-induced anaesthesia suggests common neuronal mechanisms for LOC in these two conditions. In both cases, the complex, long-range TMS-evoked activation observed during wakefulness was replaced by a stereotypical positive-negative deflection which, when TMS was delivered at high intensities, evolved into a graphoelement that matched the EEG criteria for a sleep slow wave, or a K-complex (Fig. 2). Animal (Steriade et al., 2001) and human (Cash et al., 2009) intracranial recordings have shown that both EEG sleep slow waves and K-complexes are underpinned by the occurrence of a silent, hyperpolarized down-state in cortical neurons, which is preceded and followed by a period of activation (up-state). This bimodal alternation between up- and down-states reflects an intrinsic bistability in thalamocortical circuits. This form of bistability is thought to depend on intrinsic and network properties (Sanchez-Vives and McCormick, 2000; Timofeev et al., 2000a; Hill and Tononi, 2005; Mann et al., 2009) that result, schematically, in the following sequence of events: i) due to enhanced depolarization-dependent K^+ currents and/or to an increased inhibition, cortical neurons become hyperpolarized and silent after a period of initial activation; ii) membrane hyperpolarization, in turn, facilitates the expression of hyperpolarization-activated (I_h) cationic currents that, together with spontaneous excitatory neurotransmitter release and Na^+-persistent currents, promote the resumption of neuronal firing; iii) recurrent network excitation maintain the up-state until K^+ currents and inhibition precipitate neurons into another down-state. During NREM sleep, bistability may be mainly caused by an increased activity of depolarization-dependent K^+ channels brought about by decreased brainstem cholinergic activity (McCormick et al., 1993). Inhalational anaesthetics, including nitrous oxide and isoflurane, which strongly potentiate the activity of two pore K^+ channels (Alkire et al., 2008), may act through a similar mechanism. On the other hand, increased inhibition within thalamocortical networks may play a crucial role in inducing bistability (Mann et al., 2009) in the case of other anaesthetic agents which act primarily (propofol, etomidate), or exclusively (such as midazolam at anaesthetic doses) on GABA receptors.

Can thalamocortical bistability play a role in loss of consciousness after brain injury? In this condition, as it occurs during sleep and anaesthesia, consciousness can be lost in spite of relatively preserved levels of cortical activity. Thus, it is possible that some types of brain lesions may impair information transmission, above and beyond anatomical disconnection, by inducing bistability in portions of the thalamocortical system that are otherwise healthy. We provide a few, temptative examples of how this might happen in what follows.

A direct lesion of brainstem activating systems may cause bistability through the very same mechanisms governing NREM sleep. Specifically, a reduced cholinergic, noradrenergic, histaminergic and glutammatergic ascending drive would results in enhanced leak and depolarization-dependent K^+ currents in cortical neurons (McCormick et al., 1993). As a consequence, a thalamocortical system, which is otherwise healthy, would not be able to sustain balanced patterns of activations; thus, the inescapable occurrence of a stereotypical down-state after an initial activation would prevent the emergence complex, long-range responses. A similar reduction of the activating drive from ascending arousal systems could be caused by lesions outside the brainstem. For example, an undetected cortical epileptic focus may exert a powerful inhibitory effect on brainstem activating systems (Englot et al., 2010). In principle, these causes of LOC can be overcome by promoting cortical activation with deep brain stimulation (Schiff et al., 2007) or by surgically removing the epileptic focus.

A form of bistability similar to the one observed during midazolam-induced LOC may result from cortical and subcortical lesions that alter the balance between excitation and inhibition in favour of

inhibition. 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 or may be projected by healthy areas that become hyperactive (Murase et al., 2004). Thus, cortical lesions that, by themselves, would not necessarily impair consciousness may induce LOC by causing a general unbalance between excitation and inhibition in healthy portions of the thalamocortical system. In this case, modulating with TMS, or with transcranial direct current stimulation, the output of hyperactive circuits may represent a strategy to restore the ability of thalamocortical network to sustain balanced patterns of activation. An excess of thalamocortical inhibition from a hyperactive, subcortical inhibitory area could also explain the paradoxical effects of the sedative zolpidem (Ambien), a nonbenzodiazepine hypnotic that potentiates $GABA_A$ receptors, on behavioral improvement of alertness and interactive behavior in severely brain-injured patients (Brefel-Courbon et al., 2007; Whyte and Myers, 2009). According to a recently developed model, zolpidem would act primarily by inhibiting the globus pallidus, which is hyperactive following lesions involving the dopaminergic system; in this way, zolpidem would reduce the excessive inhibitory influence of the globus pallidus on frontal thalamocortical modules (Shiff, 2009).

Another crucial event that may induce bistability following brain injury is cortical deafferentation. Indeed, a classic experimental model to induce the slow oscillation, the intracellular hallmark of bistability, involves performing partial cortical deafferentation (Timofeev et al., 2000b). Severing the white matter with a cortical undercut results in slow waves and in a continuous alternation between up- and down-states in the partially deafferented gyrus, even when the animal, and the rest of the brain, is awake (Nita et al., 2007). These experiments suggest that bistability is a default mode of cortical circuits that can be unveiled by reducing the amount of incoming activation. Hence, it is conceivable that multiple lesions in the white matter, such as the ones resulting from axonal injury, may further impair global thalamocortical function by releasing a bistable behaviour in otherwise healthy tissues. Indeed, since the introduction of EEG recordings as a clinical tool, the presence of local slow waves in the cortex of an awake subject has been linked to the presence of lesions in the white matter (Gloor et al., 1977).

Notably, bistability may be present even in the absence of clear-cut signs in the spontaneous EEG, as demonstrated by the observation that during NREM sleep TMS triggered full-fledged slow waves even on the background of low-amplitude, fast EEG activity (Fig. 2A) (Massimini et al., 2007). In all cases, evaluating the presence of bistability in the cerebral cortex of a patient with lesions of the white matter may be important. Indeed, while anatomical lesions and disconnections cannot be reversed, it may still be possible to reduce bistability and functional disconnections by acting pharmacologically on intrinsic neuronal properties. To this regard, it is worth noticing that bistability may be abolished by acetylcholine alone, as it happens during REM sleep.

All of the above considerations are highly speculative, and require specific experiments in order to give rise to a formal hypothesis. Primarily, one should confirm with intracranial recordings that the stereotypical slow wave triggered by TMS during sleep and anaesthesia reflects a clear-cut neuronal down-state; this could be done by performing magnetic (or electrical) stimulation in epileptic patients implanted with intracerebral electrodes for pre-surgical evaluation. Then, one should demonstrate that TMS triggers a similar down-state in the cortex of brain-injured, unconscious subjects and that this simple response is replaced by a long-range, complex activation when patients recover consciousness. If this is the case, bistability may be considered a common pathway through which different types of brain lesion can lead to a critical degree of functional disconnection and to LOC. To the extent that bistability proves to be an important mechanism underlying LOC it may be important to further elucidate its mechanisms in healthy and injured human brains. Indeed, while bistability can make healthy portions of the thalamocortical system completely dysfunctional, it is, in principle, reversible. Hence, it may represent a suitable target for novel therapeutic approaches in brain-injured patients in whom consciousness is impaired, in spite of preserved cortical activity.

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References

Alkire M.T. and Miller J. General anesthesia and the neural correlates of consciousness. *Progr. Brain Re.*, **150**: 229-244, 2005.

Alkire M.T., Hudetz A.G., Tononi, G. Consciousness and anesthesia. *Science*, **322**: 876-880, 2008.

Arthuis M., Valton L., Regis J., Chauvel P., Wendling F., Naccache L., et al. Impaired consciousness during temporal lobe seizures is related to increased long-distance cortical-subcortical synchronization. *Brain*, **132** (Pt 8): 2091-2101, 2009.

Blumenfeld H. and Taylor J. Why do seizures cause loss of consciousness? *Neuroscientist*, **9**, 301-310, 2003.

Barrett A.B. and Seth A. Practical Measures of Integrated Information for Time-Series Data. *PLOS Computational Biology*, **7**: e1001052, 2011.

Blumenfeld H., Westerveld M., Ostroff R.B., Vanderhill S.D., Freeman J., Necochea A., et al. Selective frontal, parietal, and temporal networks in generalized seizures. *NeuroImage*, **19**: 1556-1566, 2003.

Brefel-Courbon C., Payoux P., Ory F., Sommet A., Slaoui T., Raboyeau G., et al. Clinical and imaging evidence of zolpidem effect in hypoxic encephalopathy. *Ann. Neurol.*, **62**: 102-105, 2007.

Brown E.N., Lydic R., Schiff N.D. General anesthesia, sleep, and coma. *N. Engl. J. Med.*, **363**: 2638-2650, 2010.

Bullock T.H., McClune M.C., Achimowicz J.Z., Iragui-Madoz V.J., Duckrow R.B., Spencer S.S. Temporal fluctuations in coherence of brain waves. *Proc. Natl. Acad. Sci. U S A*, **92**: 11568-11572, 1995.

Cantero J.L., Atienza M., Madsen J.R., Stickgold R. Gamma EEG dynamics in neocortex and hippocampus during human wakefulness and sleep. *Neuroimage*, **22**: 1271-1280, 2004.

Casagrande M., Violani C., Lucidi F., Buttinelli E., Bertini M. Variations in sleep mentation as a function of time of night. *Int. J. Neurosci.*, **85**: 19-30, 1996.

Cash S.S., Halgren E., Dehghani N., Rossetti A.O., Thesen T., Wang C., et al. The human K-complex represents an isolated cortical down-state. *Science*, **324**: 1084-1087, 2009.

Classen J., Schnitzler A., Binkofski F., Werhahn K.J., Kim Y.S., Kessler K.R., et al. The motor syndrome associated with exaggerated inhibition within the primary motor cortex of patients with hemiparetic. *Brain*, **120** (Pt 4): 605-619, 1997.

Coenen A.M. Neuronal phenomena associated with vigilance and consciousness: from cellular mechanisms to electroencephalographic patterns. *Conscious. Cogn.*, **7**: 42-53, 1998.

Englot D.J., Yang L., Hamid H., Danielson N., Bai X., Marfeo A., et al. Impaired consciousness in temporal lobe seizures: role of cortical slow activity. *Brain*, **133** (Pt 12): 3764-3777, 2010.

Esser S.K., Hill S., Tononi G. Breakdown of effective connectivity during slow wave sleep: investigating the mechanism underlying a cortical gate using large-scale modeling. *J. Neurophysiol.*, **102**: 2096-2111, 2009.

Fagioli I. Mental activity during sleep. *Sleep Med. Rev.*, **6**: 307-320, 2002.

Ferrarelli F., Massimini M., Peterson M.J., Riedner B.A., Lazar M., Murphy M.J., et al. Reduced evoked gamma oscillations in the frontal cortex in schizophrenia patients: a TMS/EEG study. *Am. J. Psychiatry*, **165**: 996-1005, 2008.

Ferrarelli F., Massimini M., Sarasso S., Casali A., Riedner B.A., Angelini G., et al. Breakdown in cortical effective connectivity during midazolam-induced loss of consciousness. *Proc. Natl. Acad. Sci. U S A*, **107**: 2681-2686, 2010.

Florian J., Muller-Dahlhaus M., Liu Y., Ziemann U. Inhibitory circuits and the nature of their interactions in the human motor cortex a pharmacological TMS study. *J. Physiol.*, **586**: 495-514, 2008.

Gloor P., Ball G., Schaul N. Brain lesions that produce delta waves in the EEG. *Neurology*, **27**: 326-333, 1977.

Guillery R.W. and Sherman S.M. Thalamic relay functions and their role in corticocortical communication: generalizations from the visual system. *Neuron.*, **33**: 163-175, 2002.

Hill S. and Tononi G. Modeling sleep and wakefulness in the thalamocortical system. *J. Neurophysiol.*, **93**: 1671-1698, 2005.

Hudetz A.G. and Imas O.A. Burst activation of the cerebral cortex by flash stimuli during isoflurane anesthesia in rats. *Anesthesiology*, **107**: 983-991, 2007.

Ilmoniemi R.J., Virtanen J., Ruohonen J., Karhu J., Aronen H.J., Naatanen R., et al. Neuronal responses to magnetic stimulation reveal cortical reactivity and connectivity. *Neuroreport*, **8**: 3537-3540, 1997.

Judge O., Hill S., Antognini J.F. Modeling the effects of midazolam on cortical and thalamic neurons. *Neurosci. Lett.*, **464**: 135-139, 2009.

Kitamura Y., Kakigi R., Hoshiyama M., Koyama S., Nakamura A. Effects of sleep on somatosensory evoked responses in human: a magnetoencephalographic study. *Brain Res. Cogn. Brain Res.*, **4**: 275-279, 1996.

Laureys S., Owen A.M., Schiff ND. Brain function in coma, vegetative state, and related disorders. *Lancet Neurol.*, **3**: 537-546, 2004.

Maksimow A., Sarkela M., Langsjo J.W., Salmi E., Kaisti K.K., Yli-Hankala A., et al. Increase in high frequency EEG activity explains the poor performance of EEG spectral entropy monitor during S-ketamine anesthesia. *Clin. Neurophysiol.*, **117**: 1660-1668, 2006.

Mann E.O., Kohl M.M., Paulsen O. Distinct roles of GABA(A) and GABA(B) receptors in balancing and terminating persistent cortical activity. *J. Neurosci.*, **29**: 7513-7518, 2009.

Massimini M., Ferrarelli F., Huber R., Esser S.K., Singh,H., Tononi G. Breakdown of cortical effective connectivity during sleep. *Science*, **309**: 2228-2232, 2005.

Massimini M., Ferrarelli F., Esser S.K., Riedner B.A., Huber R., Murphy M., et al. Triggering sleep slow waves by transcranial magnetic stimulation. *Proc. Natl. Acad. Sci. U S A*, **104**: 8496-8501, 2007.

Massimini M., Tononi G., Huber R. Slow waves, synaptic plasticity and information processing: insights from transcranial magnetic stimulation and high-density EEG experiments. *Eur. J. Neurosci.*, **29**: 1761-1770, 2009a.

Massimini M., Boly M., Casali A., Rosanova M., Tononi G. A perturbational approach for evaluating the brain's capacity for consciousness. *Prog. Brain Res.*, **177**, 201-214, 2009b.

Massimini M., Ferrarelli F., Murphy M., Huber R., Riedner B., Casarotto S., et al. Cortical reactivity and effective connectivity during REM sleep in humans. *Cogn. Neurosci.*, **1**: 176-183, 2010.

McCormick D.A., Wang Z., Huguenard J. Neurotransmitter control of neocortical neuronal activity and excitability. *Cereb. Cortex*, **3**: 387-398, 1993.

Murase N., Duque J., Mazzocchio R., Cohen L.G. Influence of interhemispheric interactions on motor function in chronic stroke. *Ann. Neurol.*, **55**: 400-409, 2004.

Nita D.A., Cisse Y., Timofeev I., Steriade M. Waking-sleep modulation of paroxysmal activities induced by partial cortical deafferentation. *Cereb. Cortex*, **17**: 272-283, 2007.

Pace-Schott E.F. and Hobson J.A. The neurobiology of sleep: genetics, cellular physiology and subcortical networks. *Nat. Rev. Neurosci.*, **3**: 591-605, 2002.

Portas C.M., Krakow K., Allen P., Josephs O., Armony J.L., Frith, C.D. Auditory processing across the sleep-wake cycle: simultaneous EEG and fMRI monitoring in humans. *Neuron.*, **28**: 991-999, 2000.

Rosanova M., Casali A., Bellina V., Resta F., Mariotti M., Massimini M. Natural frequencies of human corticothalamic circuits. *J. Neurosci.*, **29**: 7579-7585, 2009

Rosanova M., Gosseries O., Casarotto S., Boly M., Casali A.G., Bruno M.A., Mariotti M., Boveroux P., Tononi G., Laureys S., Massimini M. Recovery of cortical effective connectivity and recovery of consciousness in vegetative patients. *Brain*, **135**: 1308-1320, 2012.

Sanchez-Vives M.V. and McCormick D.A. Cellular and network mechanisms of rhythmic recurrent activity in neocortex. *Nat. Neurosc.*, **3**: 1027-1034, 2000.

Schiff N.D., Giacino J.T., Kalmar K., Victor J.D., Baker K., Gerber M., et al. Behavioural improvements with thalamic stimulation after severe traumatic brain injury. *Nature*, **448**: 600-603, 2007.

Schiff N.D. Recovery of consciousness after brain injury: a mesocircuit hypothesis. *Trends Neurosci.*, **33**: 1-9, 2009.

Seth A.K., Dienes Z., Cleeremans A., Overgaard M., Pessoa L. Measuring consciousness: relating behavioural and neurophysiological approaches. *Trends Cogn. Sci.*, **12**: 314-321, 2008.

Steriade M., Nunez A., Amzica F. A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. *J. Neurosci.*, **13**: 3252-3265, 1993.

Steriade M., Timofeev I., Grenier F. Natural waking and sleep states: a view from inside neocortical neurons. *J. Neurophysiol.*, **85**: 1969-1985, 2001.

Steriade M. Grouping of brain rhythms in corticothalamic systems. *Neurosci.*, **137**: 1087-1106, 2006.

Stickgold R., Malia A., Fosse R., Propper R., Hobson J.A. Brain-mind states: I. Longitudinal field study of sleep/wake factors influencing mentation report length. *Sleep*, **24**: 171-179, 2001.

Timofeev I., Grenier F., Steriade M. Impact of intrinsic properties and synaptic factors on the activity of neocortical networks in vivo. *J. Physiol., Paris*, **94**: 343-355, 2000a.

Timofeev I., Grenier F., Bazhenov M., Sejnowski T.J., Steriade M. Origin of slow cortical oscilla-

tions in deafferented cortical slabs. *Cereb. Cortex*, **10**: 1185-1199, 2000b.

Tononi G. An information integration theory of consciousness. *BMC Neurosci.*, **5**: 42, 2004.

Tononi G. Consciousness, information integration, and the brain. *Prog. Brain Res.*, **150**: 109-126, 2005.

Tononi G. Consciousness as integrated information: a provisional manifesto. *Biol. Bull.*, *215*: 216-242, 2008.

Tononi G. and Laureys S. The neurology of Consciousness: an overview. In: *The neurology of Consciousness*. Academic Press Inc.: 375-411, 2008

Van Dort C.J., Baghdoyan H.A., Lydic R. Neurochemical modulators of sleep and anesthetic states. *Int. Anesthesiol. Clin.*, **46**: 75-104, 2008.

Vanderwolf C.H. Are neocortical gamma waves related to consciousness? *Brain Res.*, **855**: 217-224, 2000.

Veselis R.A., Reinsel R.A., Beattie B.J., Mawlawi O.R., Feshchenko V.A., DiResta G.R., et al. Midazolam changes cerebral blood flow in discrete brain regions: an H2(15)O positron emission tomography study. *Anesthesiology*, **87**: 1106-1117, 1997.

Whyte J. and Myers R. Incidence of clinically significant responses to zolpidem among patients with disorders of consciousness: a preliminary placebo controlled trial. *Am. J. Phys. Med. Rehabil.*, **88**: 410-418, 2009.