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Cortical reactivity and effective connectivity during REM sleep in humans

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Abstract

We recorded the electroencephalographic (EEG) responses evoked by transcranial magnetic stimulation (TMS) during the first rapid eye movement (REM) sleep episode of the night and we compared them with the responses obtained during previous wakefulness and NREM sleep. Confirming previous findings, upon falling into NREM sleep, cortical activations became more local and stereotypical, indicating a significant impairment of the intracortical dialogue. During REM sleep, a state in which subjects regain consciousness but are almost paralyzed, TMS triggered more widespread and differentiated patterns of cortical activation, that were similar to the ones observed in wakefulness. Similarly, TMS/hd-EEG may be used to probe the internal dialogue of the thalamocortical system in brain injured patients that are unable to move and communicate.

Introduction

According to a recent proposal (Tononi, 2004, 2008) consciousness depends critically not so much on firing rates, synchronization at specific frequency bands, or sensory input *per se*, but rather on the brain's ability to integrate information, which is contingent on the effective connectivity among functionally specialized regions of the thalamocortical system. Effective connectivity refers to the ability of a set of neuronal elements to causally affect the firing of other neuronal groups within a system (Lee, Harrison, & Mechelli, 2003). To test this proposal, our group has recently investigated whether the reduction of consciousness that occurs during non-rapid eye movement (NREM) sleep early in the night (Hobson & Pace-Schott, 2002; Stickgold, Malia, Fosse, Propper, & Hobson, 2001) may be associated with changes in cortical effective connectivity (Massimini et al., 2005). To this aim, transcranial magnetic stimulation (TMS) was combined with high-density electroencephalography (hd-EEG) to explore how the activation of one cortical area (the premotor area) is transmitted to the rest of the brain during wakefulness and NREM sleep. Result showed that, during quiet wakefulness, TMS triggered an initial response at the stimulation site that was followed by a sequence of fast waves (15–30 Hz) that moved to connected cortical areas several centimeters away. Upon entering NREM sleep stages 3/4, the brain's response to TMS became a single large positive-negative slow wave that rapidly extinguished and did not propagate beyond the stimulation site. Based on this finding, we hypothesized that a significant breakdown of cortical effective connectivity

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may actually explain why subjects awakened during NREM sleep early in the night report little, or no, conscious experience.

Of course, blank reports upon awakening from sleep are not the rule, and many awakenings yield dream reports (Casagrande, Violani, Lucidi, Buttinelli, & Bertini, 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, such as at sleep onset, during the last part of the night and, especially, during rapid eye movement (REM) sleep. Here we report the results of experiments in which we were able to record TMS-evoked potentials during the first REM sleep episode of the night and to compare them with the responses obtained during previous wakefulness and NREM sleep. During the transition from NREM to REM, while subjects were behaviorally asleep, the brain's reaction to TMS recovered fast oscillatory components and became similar to the one obtained during wakefulness, especially during the first 100–150 ms post-stimulus. The resurgence of fast waves was also associated with a partial recovery of cortical effective connectivity. These results suggest that directly measuring cortical reactivity and effective connectivity with TMS/hd-EEG may help evaluating the brain's capacity for conscious experience in the absence of overt behavior, such as in sleeping subjects or in non-communicative, brain injured patients.

Methods

Subjects

Ten subjects (age 21–34, 3 females) were involved in the study. All participants gave written informed consent and the experiment was approved by the University of Wisconsin Human Subjects Committee. Prior to the experiment a neurological screening was performed to exclude potential adverse effects of TMS. The data presented here are from the five subjects in whom at least 30 trials of TMS-evoked potentials could be recorded during REM sleep.

TMS targeting

Cortical TMS targets were identified on T1 weighted MR images (resolution 0.5 mm) of the subjects' whole heads acquired with a 3T GE Signa scanner. In order to ensure precision and reproducibility of stimulation we employed a Navigated Brain Stimulation (NBS) system (Nexstim Ltd, Helsinki FI). The NBS device located (with an error <3 mm) the relative positions of the subject's head and of the TMS coil by means of an optical tracking system. NBS also calculated the distribution and strength of the intracranial electric field induced by TMS. The coordinates of stimulation were input to a software aiming tool that ensured throughout the session the reproducibility of position, direction, and angle of the stimulator. We targeted the rostral part of the right premotor cortex (r-PM), 1 cm ahead of the anterior commissure and 2 cm lateral to the midline. This area is accessible through a central scalp position, far from any major head or facial muscle.

Stimulation parameters

Stimulation was performed by means of a Magstim figure-of-eight coil (model P/N9925), with a wing diameter of 70 mm, connected to a Magstim Rapid biphasic stimulator. Resting motor threshold (rMT) was measured prior to the experiment as the lowest stimulator output at which at least 5 out of 10 pulses, delivered on the optimal hand motor area, resulted in a motor evoked potential of 50 μV or greater in the abductor pollicis brevis (2). We stimulated r-PM at 110% of rMT, corresponding to a stimulator output between 54 and 67% and to a maximum estimated electric field on the target between 90 and 120 V/m. TMS was delivered with an interstimulus interval jittering randomly between 2 and 2.3 s.

EEG recordings

We recorded the spontaneous and TMS-evoked EEG by means of a 60 carbon electrodes cap and a specifically designed TMS-compatible amplifier (Nexstim Ltd, Helsinki, FI). The artifact induced by TMS was gated and saturation of the amplifier was avoided by means of a proprietary sample-and-hold circuit that kept the analog output of the amplifier constant from 100 μs pre- to 2 ms post-stimulus (Virtanen, Ruohonen, Naatanen, & Ilmoniemi, 1999). To further optimize TMS compatibility, the impedance at all electrodes was kept below 3 KΩ. The EEG signals, referenced to an additional electrode on the forehead, were filtered (0.1–500 Hz) and sampled at 1450 Hz with 16 bit resolution. Four extra sensors were used to record the electrooculogram (EOG) and the chin electromyogram (EMG). In most cases, no signs of TMS induced magnetic artifact were detected and in all cases the EEG signals were artifact-free from <10 ms post-stimulus. At the end of each experiment, a pen visible to the infrared camera was used to digitise the EEG electrode positions on the subject's head. During EEG recording, subject's perception of the clicks produced by TMS coil's discharge was eliminated by means of inserted earplugs continuously playing a masking noise. Volume of the masking noise (always below 90 dB) was adjusted immediately prior to the experiment until the subjects reported that the TMS click was not perceptible. A thin layer of foam was placed between coil and scalp (resulting in less than 1 mm thickness when coil was pressed against the head) in order to attenuate bone conduction. As previously demonstrated, this procedure effectively prevented any contamination of EEG signals by auditory potentials elicited by TMS-associated clicks (Massimini et al., 2005) (Massimini et al., 2007).

General experimental procedures

Subjects were invited to the lab in the evening (9:30 PM) and were requested to stay awake until 1:00 AM. After setting-up the EEG cap and selecting the TMS target, recordings started at around 2:00 AM. We chose this timing in order to extend the recordings to a circadian phase (early morning) that is more favorable to the occurrence of REM sleep (Dijk & Czeisler, 1995). During the experiment subjects were laying eyes closed on a reclining chair with a head-rest that allowed a comfortable and stable head position. Participants were requested to stay awake while at least 200 trials were recorded. Afterward, we allowed the subjects to fall asleep while the spontaneous EEG was monitored and TMS was continuously delivered. In order to avoid overheating of the coil, we interrupted and restarted the stimulation according to the EEG pattern of interest. Responses during NREM sleep were collected in 7 out of 10 subjects. Five of these subjects subsequently entered REM sleep for at least 2 minutes and were included in the analysis. Throughout the recording session the stability of stimulation coordinates were continuously monitored. If a movement of >5 mm occurred, the session was interrupted and the coil was repositioned. At the end of the experiment, the stimulation coordinates were recorded and the electrode positions were digitized.

Dream reports

In order to evaluate subjective experience we collected a dream report in all the subjects (n=5) who entered REM sleep. Two subjects awakened spontaneously during REM sleep while the remaining three were awakened as soon as the REM sleep episode was judged to be over, based on the on-line inspection of the EEG, EOG and EMG traces. Upon awakening subjects were asked to remember what was going in their mind in the time prior to waking (Stickgold et al., 2001). All reports were collected by dictation into a portable digital audio recorder (Olympus DS-50). Recordings were transcribed and subsequently edited for Total Recall Count, or TRC (Antrobus, 1983), removing extraverbal utterances, nonwords, repeated words and secondary elaborations. TRCs were obtained using the word-count function of Microsoft Word.

Data analysis

Data analysis was performed in Matlab (The Matworks, MA, USA) and Curry 5.0 (Philips GmbH, Germany). Sleep stages were scored off-line according to Rechtschaffen and Kales (1968) on two EEG channels (C3, C4) re-referenced to the mastoid, one EOG channel and one EMG channel. We classified the single trials according to the stages during which they were collected. Trials containing noise, muscle activity or eye movements were rejected. The trials collected during wakefulness, NREM sleep stage 3–4 and REM sleep were averaged separately. The averaged signals were baseline corrected and band-pass filtered between 2 and 100 Hz. In all subjects, the averaged TMS-evoked potentials was characterized, during wakefulness, by two consecutive positive peaks (peak 1 and peak 2) occurring at around 20 and 60 ms, respectively (fig. 2). We characterized changes in the shape of the early EEG response to TMS by detecting significant changes of the amplitude of peak 1 and peak 2 across states of vigilance. To this aim, we measured, on each single-trial response, the maximum voltage at around (±10 ms) the latency of peak 1 and peak 2 (as detected during wakefulness). After that, we performed a Student's T-test on the single-trial amplitude distributions obtained in the different conditions.

In order to perform source modeling, the averaged responses, the MRI sets and the electrode coordinates were input to the software package Curry 5.0. Following a semi-automatic segmentation of the individual MRI images we implemented a Boundary Element Model (BEM) of the head having 3 compartments of fixed conductivities (scalp: 0.33 S/m; skull: 0.0042 S/m; brain: 0.33 S/m). The cortical surface was also reconstructed with a 6 mm resolution and modeled with approximately 14,000 rotating dipoles. Next, the electrode positions were projected onto the skin surface and the lead field matrix was calculated. We estimated the current density on the cortical surface using the Minimum Norm Least Squares (L2 Norm) method (weighted for depth bias removal) (Hamalainen & Ilmoniemi, 1994). To identify the cortical areas involved by TMS-evoked activity we proceeded as follows: i) we calculated the global mean field power GMFP (Hamalainen & Ilmoniemi, 1994) as the mean of the absolute voltage recorded from electrodes in average reference ii) to identify the latencies were TMS evoked a significant response, we detected the time intervals where the post-stimulus amplitude of the GMFP was 3 standard deviations larger than pre-stimulus activity; iii) within these intervals, we detected the time of the local maxima of the GMFP; iv) at these time points, we estimated the location of maximum neural activity using the L2 norm. Thus, we estimated the location of the maximum activation (top 10%) at each significant time sample, using Matlab 7.0, the identified sources were then plotted on the individual cortical surface and color-coded according to the latency of their involvement.

Results

While TMS was delivered, the following percentages of sleep stages were obtained across the five subjects who entered REM sleep: wakefulness: 45.4%; stage 1: 9.1%; stage 2: 15.6%; stages 3–4: 22.6%; REM 7.3%. In all cases, at least 150 single-trial evoked responses could be retained during wakefulness and NREM sleep. In four subjects, all the trials averaged in the NREM sleep condition were collected during stages 3 and 4, while in one subject trials collected during NREM stages 2, 3 and 4 had to be averaged together in order to obtain a stable NREM response. TMS/hd-EEG measurements during REM sleep were more challenging. Indeed, this sleep stage tends to occur unpredictably and is rather unstable, especially during the first sleep episode. As a consequence, periods of clear-cut REM sleep were much shorter. Specifically, we obtained, across all subjects, the following amount of seconds (and of TMS-evoked single trials) during REM sleep: 132 s (42 trials), 163 s (53 trials), 169 s (52 trials), 230 s (92 trials), 348 s (145 trials). While TMS-evoked potentials have an excellent signal-to-noise ratio, a limited number of trials hampers the reliability of the average response, especially at late

latencies, where intertrial variability increases. Thus, to assess the reliability of the overall scalp-recorded average response at different latencies, we detected the time-samples where the GMFP was significant (post-stimulus amplitude > of 3 standard deviations of pre-stimulus; see also Methods and (Massimini et al., 2005)). In each subject, this test was repeated for each condition (including wakefulness, NREM sleep) using the maximum number of trials collected during REM sleep. In all subjects, a significant response could be detected during the first 100–150 ms, while in the subject in whom 145 single trials could be analyzed the significant response extended to 300 ms. For this reason, the analysis of TMS-evoked potentials was restricted to the first 150 ms in all cases except for this subject, in whom source modeling was also performed. All subjects, prompted upon awakening from REM sleep, provided a dream report. TWC varied largely across subjects (ranging from 45 words to 237 words) and tended to correlate ($p<0.1$) with the length of the period that subjects spent in REM sleep.

In all vigilance states, TMS elicited a time-locked response that could be appreciated on a single-trial basis. Fig. 1A displays the single-trial responses recorded from one electrode located under the stimulator (corresponding to FC2, in the 10–20 system) during a transition from wakefulness through stage 1 and NREM to REM sleep. In this representation, signals are band-passed from 15 to 100 Hz, in order to highlight the presence of fast oscillations in the single-trial responses. Fig. 1B shows the corresponding averages (filtered 2–100 Hz) calculated in the four vigilance states. During wakefulness, TMS triggered a sustained response made of a sequence of time-locked, high-frequency (20–35 Hz) oscillations in the first 100 ms. As soon as the subject transitioned into stage 1, the amplitude of the first positive component (between 0 and 40 ms) increased by 40%, while the subsequent waves were markedly dampened. During NREM sleep the response changed markedly; the first positive component became larger (120% increase), slower and was followed by a negative rebound after which the response extinguished. Upon transitioning to REM sleep the cortical response to TMS recovered wakefulness-like fast oscillations, despite the subject being behaviorally asleep.

Figure 2 shows that the resumption of fast oscillations during REM sleep was reproducible across subjects and, at the same time, it highlights significant differences between the REM sleep and the wakefulness response. In REM sleep, compared to wakefulness, the first positive component (peak1) was larger ($p<0.05$) and the second (peak 2) was smaller ($p<0.05$) in each subject (Student's T-test, comparing single-trial amplitude distributions). During NREM sleep, peak 1 was larger compared to both REM sleep ($p<0.001$) and wakefulness ($p<0.001$), while peak 2 was replaced by a negative wave.

In one subject, we were able to collect a sufficient number of trials to perform source modeling and to compare REM sleep to NREM sleep and wakefulness also at longer latencies (Fig. 3). This analysis revealed that the complex pattern of long-lasting, long-range activation triggered by TMS during wakefulness broke down during NREM sleep and that it partially recovered, within the first 150 ms post-stimulus, during REM sleep. Fig. 3 also shows that long-latency components that were present in wakefulness were obliterated during REM sleep. Unfortunately, due to the short duration of clear-cut REM sleep periods, the long latency response could not be analyzed in the other subjects.

Discussion

The level and quality of conscious experience can vary dramatically across the sleep-wake cycle. During NREM sleep early in the night, consciousness can nearly vanish (Pivik & Foulkes, 1968) (Suzuki et al., 2004) (Hobson & Pace-Schott, 2002) despite persistent neural activity in the thalamocortical system (Steriade, Timofeev, & Grenier, 2001). Why is it so? The present work confirms the results of previous measurements (Massimini et al., 2005) by showing that, during NREM sleep stages 3 and 4 early in the night, thalamocortical circuits

remain active and reactive but lose their ability to interact and to produce complex, integrated responses. Indeed, in this state, TMS failed to trigger a sustained, long-range pattern of activation and instead evoked a simple positive-negative wave that remained local. Interestingly, this stereotypical response has been shown to share fundamental characteristics with the slow waves that occur spontaneously during NREM sleep (Massimini et al., 2007). This evidence suggests that the mechanisms underlying the generation of slow waves, may also be responsible for blocking the emergence of specific long-range responses during NREM sleep (Tononi & Massimini, 2008) (Massimini, Tononi, & Huber, 2009). Upon falling asleep, due to a dampening of brainstem noradrenergic, serotoninergic and cholinergic activating systems (Steriade, 2004), cortical neurons become bistable and inevitably tend to fall into a silent, hyperpolarized state (down-state) after a period of activation (up-state) (Timofeev, Grenier, & Steriade, 2001). This mechanism provides the mechanism for the slow oscillations of sleep, where large populations of cortical neurons spontaneously alternate between up and down-states (Hill & Tononi, 2005). In addition, bistability may also be contributed for by a shift in the balance of synaptic excitation and inhibition toward inhibition due to changes in the neuromodulatory milieu (Esser, Hill, & Tononi, 2009). In any case, bistability may prevent the emergence of sustained, complex thalamocortical interactions. Thus, in this condition, any local activation, whether occurring spontaneously or induced by a stimulus (like TMS), will converge into a silent neuronal down-state and into a stereotypical EEG slow wave. While this seems to occur at least during deep NREM sleep early in the night (Tononi & Massimini, 2008), it is hard to predict what would happen during REM sleep. In this state, while noradrenergic and serotoninergic arousing systems remain silent, brainstem cholinergic neurons come back to activity (Pace-Schott & Hobson, 2002), spontaneous slow waves disappear and the EEG becomes, at least superficially, similar to the one of 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 sleep was still able to trigger full-fledged slow waves (Massimini et al., 2007). The present experiments demonstrate that, during REM sleep, TMS evokes a response that is more similar to the one observed in wakefulness, namely, a sequence of fast oscillations (Fig. 1 and 2) occurring in the first 150 ms. This evidence suggests that the discharge of mesopontine cholinergic neurons alone may represent a major excitatory input that can largely prevent the emergence of thalamocortical bistability during REM sleep.

Despite obvious similarities, the REM and the wakefulness responses also showed consistent differences; in REM sleep, the first positive component (peak 1) was always larger and the second one (peak 2) was always smaller compared to wakefulness (Fig. 2). In this sense, the REM response tended to share some features with the NREM sleep response, where peak 1 reached its maximum amplitude and peak 2 disappeared. Notably, the REM sleep response recorded in the present experiment strongly resembled the TMS-evoked potential obtained during sleep stage 1 in a previous work (Massimini et al., 2005), suggesting that stage 1 and REM sleep may be supported by a similar degree of cortical activation.

Source modeling revealed that, as in wakefulness, the resumption of fast oscillations during REM sleep was associated with a pattern of activation that was more complex and widespread compared to one of NREM sleep. This observation, although limited to a single subject, corroborates the hypothesis that cortical effective connectivity may play a role in the shifts of conscious experience that occur during sleep. Indeed, the subject in Fig. 3 spent the longest time in REM sleep (348 s) and reported a long dream recall (237 words) upon awakening. The persistence, to some degree, of long-range cortico-cortical effective connectivity has been also reported during stage 1 (see Fig S2 in (Massimini et al., 2005), another sleep stage associated with frequent and long dream reports (Foulkes, 1966). Fig. 3 also shows that long-latency components that were present in wakefulness were obliterated during REM sleep. This finding,

is consistent with the notion that late component of peripherally evoked potentials are also dampened during REM sleep(Goff, Allison, Shapiro, & Rosner, 1966;Wesensten & Badia, 1988). In future works it would be interesting to systematically collect TMS/hd-EEG measures of thalamocortical bistability and effective connectivity during the whole night and to correlate them with dream reports. This approach, might represent a valid attempt to study the neural correlates of consciousness during sleep on a finer time scale, beyond the REM/NREM sleep dichotomy and beyond traditional sleep staging. Similarly, exploring brain activity on a finer spatial scale with functional neuroimaging already shed light on the neural mechanisms underlying changes in the quality of consciousness across the sleep-wake cycle (Maquet et al., 1996) (Maquet et al., 2005).

Besides their possible relevance for sleep physiology, the present experiments demonstrate that TMS/hd-EEG may represent an effective way to probe the internal dialogue of the thalamocortical system in the absence of any behavioral cue. TMS triggered more widespread and differentiated patterns of EEG activation, just as it does during normal wakefulness, upon entering REM sleep, a state in which subjects are conscious but almost paralyzed. Hence, in the future, this technique may be employed as an aid to evaluate the brain's capacity for consciousness in brain injured patients who are unable to move and communicate (Massimini, Boly, Casali, Rosanova, & Tononi, 2009).

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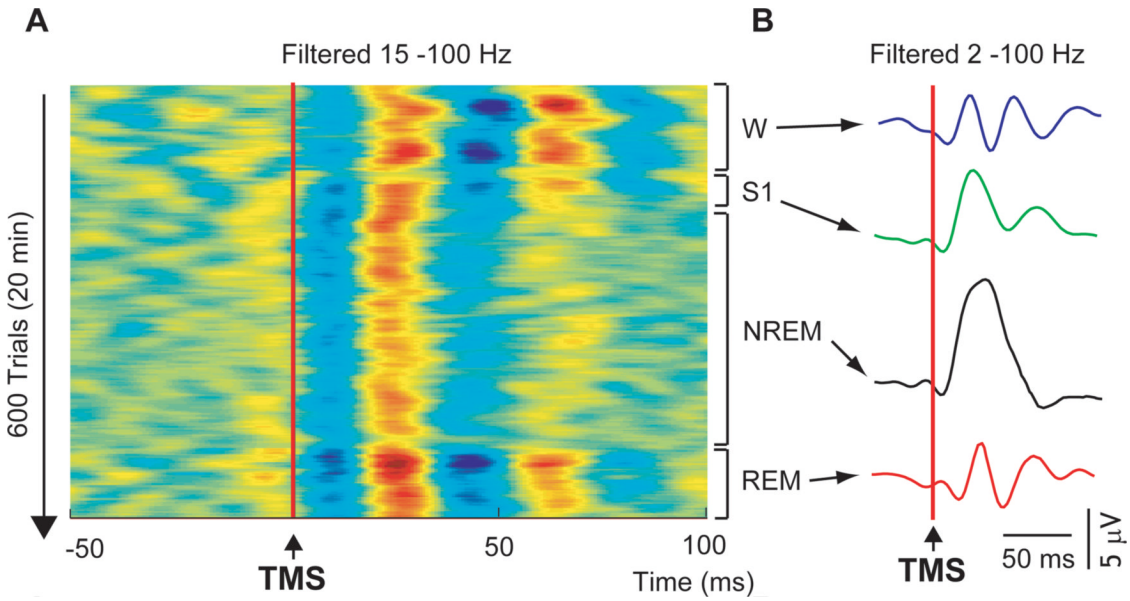

Fig.1.

(A) Single trial TMS-evoked responses are recorded from one channel located under the stimulator (FC2) while a subject transitions from wakefulness (W), through sleep stage 1 (S1) and NREM sleep (NREM) to REM sleep. The red line marks the time of TMS. Single-trial EEG data are band-pass filtered (15 to 100 Hz) and color coded for voltage (red for positive, blue for negative). **(B)** The averaged responses (filtered from 2 to 100 Hz) calculated in the four vigilance states are depicted. The onset of REM sleep is associated with a resumption of TMS-evoked fast oscillations.

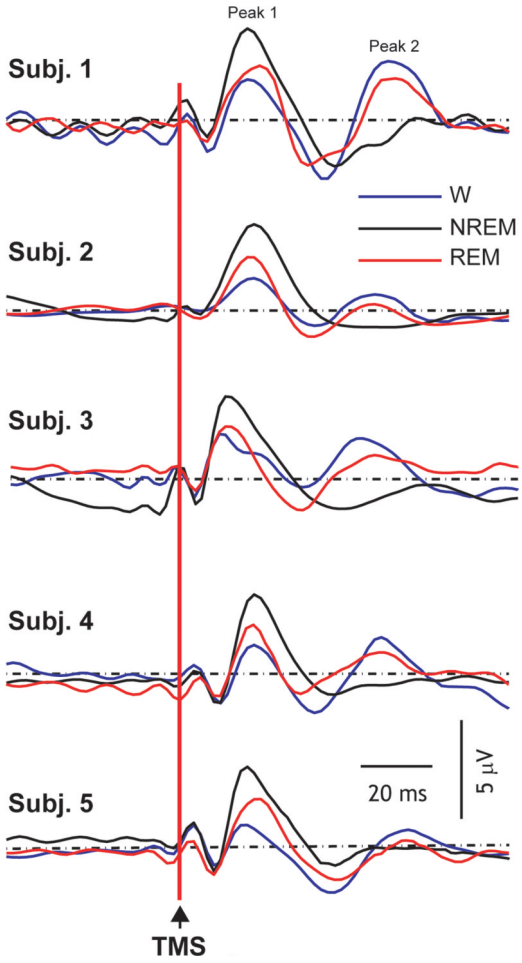

Fig.2.

The averaged responses obtained in all subjects during wakefulness (blue traces), NREM sleep (black traces) and REM sleep (red traces) are compared. The red line marks the time of TMS. TMS-evoked potentials undergo systematic changes across states of vigilances.

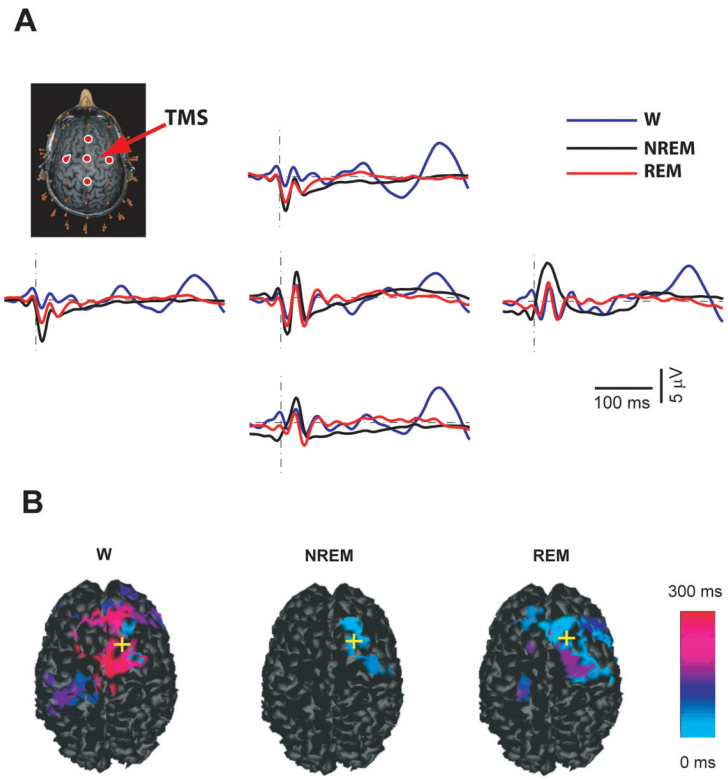

Fig. 3.

(A) The TMS-evoked potentials recorded from one subject, in whom a long stretch of REM sleep could be recorded, are displayed (blue: wakefulness, black: NREM sleep, red: REM sleep). The traces were recorded from the channels indicated by red dots in the upper left panel, where the site of stimulation on the subject's MRI is also indicated by (red arrow). **(B)** Spatiotemporal cortical maps of TMS-evoked cortical activation during wakefulness, NREM and REM sleep. For each significant time sample, maximum current sources were plotted and color-coded according to their latency of activation (light blue, 0 milliseconds; red, 300 milliseconds). The yellow cross marks the TMS target on the cortical surface. During REM sleep, the resumption of TMS-evoked fast oscillations was associated with a partial recovery of cortical effective connectivity.