

Increasing Cortical Excitability: A Possible Explanation For The Proconvulsant Role Of Sleep Deprivation

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Study Objective: Sleep deprivation (SD) is known to facilitate both seizures and interictal epileptiform abnormalities. For this reason, it is often used in the routine diagnostic workup of epileptic patients as an activating procedure for eliciting epileptiform and/or seizure patterns in their EEGs. In order to evaluate the effects of SD on cortical excitability, we studied the effects of sleep loss on healthy subjects by transcranial magnetic stimulation (TMS).

Design and Participants: Seven normal subjects underwent TMS examination in baseline condition and after total sleep deprivation. The TMS investigation included two protocols: a) the evaluation of motor evoked potential and silent period parameters recorded in response to single-pulse magnetic stimulation; and b) the evaluation of the time course of intracortical motor activity tested with paired-pulse TMS applied at inter-stimulus intervals of 1-6 ms.

Setting: Clinical neurophysiology laboratory in a general hospital.

Interventions: None

Results: After SD, the principal finding observed using single-pulse TMS was a decrease of the silent period duration, whereas a reduction of the intracortical inhibition, in particular at inter-stimulus intervals 1 and 2 ms, was found, using the paired-pulse TMS.

Conclusion: Our findings suggest that SD may modify cortical excitability, seen as the balance between inhibitory and excitatory cortical phenomena, which could reduce the epileptic threshold.

Keywords: Transcranial magnetic stimulation, sleep deprivation, cortical excitability, epilepsy

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INTRODUCTION

SLEEP AND EPILEPSY ARE INTIMATELY RELATED AND INFLUENCE EACH OTHER.¹ FOR EXAMPLE, FLUCTUATIONS OF ALERTNESS INDUCE QUALITATIVE AND quantitative changes of EEG epileptiform abnormalities. Conversely, epileptic activity may alter sleep architecture.² In particular, sleep deprivation (SD) is capable of influencing ictal/interictal epileptic activities.³ SD may induce seizures in patients with epilepsy and may activate focal and, particularly, generalized interictal epileptiform discharges in almost 50% of epileptic patients.⁴ SD is therefore used in clinical settings as an activating procedure of EEG in the diagnosis of epilepsy.⁵ The mechanisms underlying such phenomena are controversial.⁶⁻⁹ Two main hypotheses have been suggested: a) the induced activation of epileptic phenomena is a direct consequence of SD, so that it is "per se" capable of promoting an increased cortical excitability¹⁰; b) the induced epilepsy is mediated by vigilance modification as drowsiness following SD, often documented in the EEG recordings.¹¹

A few studies concerning the mutual influences of cortical excitability, interictal epileptiform abnormalities, and sleep/wake

rhythm have been reported.¹²⁻¹⁴

Transcranial magnetic stimulation (TMS) is a safe and non-invasive diagnostic technique for the study of the motor pathways in healthy and diseased humans.^{15,16} Standard TMS measures, such as excitability threshold and amplitude of motor evoked potential, are very sensitive for detecting both physiological and pathological conditions involving central motor pathways. In addition, more complex TMS protocols, such as paired-pulse TMS and silent period, allow the documentation of motor intracortical inhibition.¹¹

The aim of our study was to use TMS in order to explore the effects of total SD upon the balanced excitatory/inhibitory properties of the brain in healthy subjects.

SUBJECTS AND METHODS

Seven healthy right-handed volunteers (4 males, 3 females; age range 26-38 years) were included in our study and submitted to TMS. All participants were required to maintain a regular sleep schedule the week before the study sessions, as verified by actigraphy and sleep diary. All the subjects expressed full informed written consent to the study.

TMS, using single or paired stimuli, was delivered via a focal butterfly-shaped coil connected to one or two Magstim 200 stimulator units through a Bi-stim module (Magstim, Whitland, Dyfed, UK). Stimulation was always applied on the nondominant hemisphere, because it has been proposed that there is an asymmetry in the excitability of cortical inhibitory mechanism between the two hemispheres.^{18,19} Motor evoked potentials were recorded from the opponens pollicis of the left hand via surface electrodes applied in a belly-tendon montage.

The experimental protocol was repeated twice for each subject, so that motor cortex excitability was separately assessed in two

Disclosure Statement

This was not an industry supported study. Drs. Scalise, Desiato, Gigli, Romigi, Tombini, Marciani, Izzi, and Placidi have indicated no financial conflicts of interest.

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different conditions: basal condition after a full night of spontaneous sleep, and SD condition after a period of at least 24 hours of active wakefulness. The interval between the two sessions was not strictly predefined, but it was longer than one week. In order to prevent the effect of circadian factors, all recording sessions were performed in the late morning. To control vigilance fluctuations, subjects were required to stay alert with eyes open and body muscles relaxed during the TMS sessions. Subject behavior was continuously checked by the technician throughout the recording. The order of the basal condition and SD condition session was counterbalanced across subjects.

Each recording session included two protocols: 1) Single pulse TMS, with evaluation of motor evoked potential, motor threshold, and silent period parameters;^{15,16} 2) Paired-pulse stimulation, with evaluation of time course of intracortical inhibition tested at 1-6 ms inter-stimulus intervals.¹⁷

Single-Pulse TMS: Motor Evoked Potential Amplitude, Motor Threshold, and Silent Period

The optimal scalp position of the coil was assessed by moving the coil in 1-cm steps over the presumed hand motor area. Coil location was determined as the site that elicited the optimal motor evoked potential amplitude during muscle relaxation with the lowest threshold. The coil handle was held backward in a lateral (45°) direction from the interhemispheric line.^{15,20} For motor threshold measurement, motor evoked potentials were recorded during relaxation of the target muscle.^{15,16} At threshold TMS value, a moderate contraction allowed the detection of both motor evoked potential and silent period parameters in the 500 ms following TMS. The mean of 3 trials was used to define the following parameters:

- Motor threshold (%), expressed as the percentage of the stimulator's maximal output, defined as the intensity required to elicit detectable motor evoked potentials with amplitudes of 0.05-0.15 mV in 50% of the stimuli.¹⁵
- Motor evoked potential amplitude (mV), defined as the peak-to-peak amplitude between the largest negative and positive deflections following stimulus onset.¹⁵
- Silent period duration (ms), measured from the motor evoked potential to the rebound of voluntary EMG activity (absolute duration of silent period).²¹⁻²³

Paired-Pulse TMS: Time Course of Intracortical Inhibition

Motor evoked potentials were recorded during complete relaxation of the target muscle. The coil was held tangential to the skull with the handle pointing backwards at 45° lateral to the midline. Usually the optimal responses were elicited when the coil was placed 5-6 centimeters along the coronal line from Cz point (10-20 International System). A conditioning-test design was used to investigate the time course of motor evoked potential inhibition. Paired stimuli were applied with conditioning pulses delivered 1, 2, 3, 4, 5, or 6 ms before test stimulation. The intensity of the conditioning pulse was maintained below the threshold necessary for evoking responses in contracted muscles (70% of the individual resting motor threshold). Test pulses were delivered suprathereshold in order to elicit "relaxed" motor evoked potentials (110%-120% of the individual resting motor threshold). In each block, test and conditioning pulses at the different inter-stimulus intervals were randomly intermixed. In order to achieve a com-

plete set of inter-stimulus intervals, several blocks of trials were performed. Each block included 16 trials, with 8 having the test stimulus alone (unconditioned motor evoked potential) and 8 having pairs of conditioning test pulses delivered at 1 of the 6 inter-stimulus intervals (conditioned motor evoked potential). The sequence began and ended with the unconditioned trials, with the conditioned motor evoked potential trials in between.^{17,24} Mean amplitudes of unconditioned and conditioned motor evoked potentials were calculated separately for each inter-stimulus interval. The amplitude of conditioned motor evoked potentials was expressed as the percentage of unconditioned motor evoked potentials amplitude. The time course was defined as the mean amplitude variation of conditioned MEPs (expressed as the percentage of "unconditioned" motor evoked potentials amplitude) at each inter-stimulus interval.

Data Analysis

Motor threshold, motor evoked potential amplitude, and silent period duration differences between conditions (basal condition vs SD condition) were tested using paired t-tests. Analysis of paired-pulse TMS used the factors of condition (basal, SD) and inter-stimulus interval (1-6 ms). Post hoc tests among the means were performed using paired t-tests adjusted for the number of comparisons (Fisher protected least significant difference). Differences at $P < 0.05$ were considered significant.

RESULTS

Single-Pulse TMS: Motor Evoked Potentials, Motor Threshold, and Silent Period

Comparison between excitability threshold in basal condition and in SD condition showed no significant difference (51.4 ± 1.8 vs 53 ± 0.6 , respectively; $P > 0.5$). Similarly, amplitude of "contracted" motor evoked potentials was comparable in the two conditions ($6.1 \text{ mV} \pm 0.7$ vs 5.9 ± 1 , respectively; $P > 0.5$). By contrast, as seen in Figure 1, silent period duration in the SD condition was significantly shorter than in the basal condition ($47.4 \text{ ms} \pm 24.1$ vs $67.6 \text{ ms} \pm 17.8$, respectively) ($t = 3.75$; $df = 6$; $P < 0.001$).

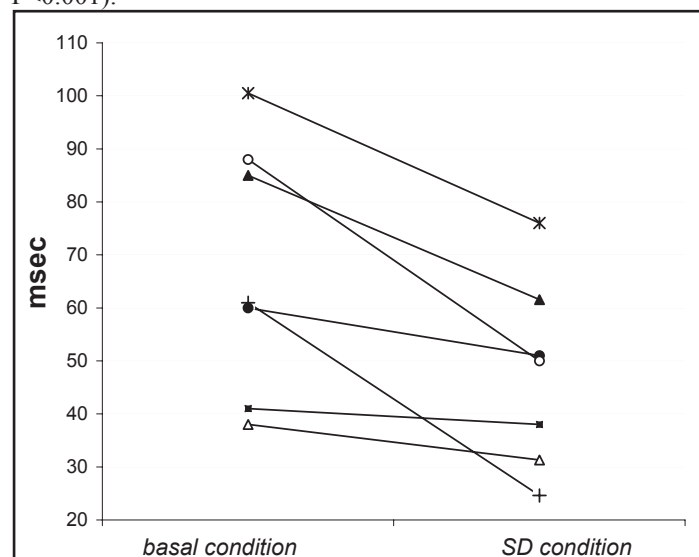


Figure 1—Single-pulse TMS. For each subject the duration of the silent period has been tested in basal condition and after SD, with a consistent reduction of the silent period duration after SD.

Paired-Pulse TMS: Time Course of Intracortical Inhibition

All the subjects exhibited a normal inhibitory profile to paired-pulse TMS in the basal condition.

There were significant effects of condition ($F_{(1,72)} = 7.51$; $P < 0.01$). In fact, the mean amplitude of motor evoked potentials after SD was higher than the mean amplitude of motor evoked potentials in basal condition, as seen in Figure 2. Post hoc t-tests comparing the groups at each inter-stimulus interval showed significant group differences at 1 ms ($t = 2.7$; $df = 6$; $P < 0.05$), and 2 ms ($t = 4.9$; $df = 6$; $P < 0.01$).

DISCUSSION

The purpose of this study was to examine the effect of SD on cortical excitability. In order to test the hypothesis that SD may induce a modification of intracortical inhibitory mechanisms in healthy subjects, we used single- and paired-pulse TMS. The most important results were that the sleep deprived subjects showed a marked decrease of the central motor inhibition and a reduction of silent period duration. Motor threshold, reflecting the excitability of the neuronal membranes, and motor evoked potential amplitude, which is related to the number of corticospinal neurons activated, were not different among conditions. Taken together, the motor threshold and motor evoked potential amplitude results indicate that pyramidal tract function is not influenced by SD. Instead, the reduction of intracortical inhibition to paired short-interval stimuli and the silent period duration, which are considered suitable markers of inhibition/excitation balance at the cortical level,^{17, 25, 26} suggest an influence of SD on the neurotransmitter system of the intracortical circuitry and brainstem nuclei. The reduction of silent period duration reported in our study in SD condition indicates a reduction of central motor inhibition.

The putative generators of silent period are located at different levels of the neuraxis (spinal, suprasegmental), although the brain seems to have a predominant role.^{22,27-31} The same multiple sites might be involved, at least in part, in the sleep/wakefulness rhythm. Pontine GABA-ergic processes in the nucleus pontis oralis play a critical role in generating and in maintaining wakefulness.³² The stimulation of the medullary reticular formation promotes inhibitory mechanisms upon both motoneurons and interneuronal transmission.³³ Thus, SD could be able to alter one of these inhibitor mechanisms, as suggested by the reduction of the silent period duration.

The inhibition tested with paired stimulation is mediated by intracortical, rather than spinal, mechanisms in healthy subjects. Weak magnetic conditioning stimuli specifically engage cortical motor inhibitory circuits.^{17,34} The administration of lorazepam enhances the early intracortical inhibition to paired-pulse TMS.^{35,36} Cortical inhibition is decreased in patients with juvenile myoclonic epilepsy; in these cases a dysfunction of the motor cortex has been supposed because of the presence of myoclonic jerks without loss of consciousness.^{14,24} The important decrease of motor inhibition after SD shown in our study may depend on modification of cortical excitability mediated by neurotransmitters, possibly the GABA-ergic system.

Sleep deprivation has been tested employing single- and paired-pulse TMS in healthy volunteers.^{12,13} Civardi and colleagues,¹² in accordance with our results, documented the lowering of inhibition to paired stimuli after SD of at least 24 hours. However, in the same study, silent period duration remained unchanged. This find-

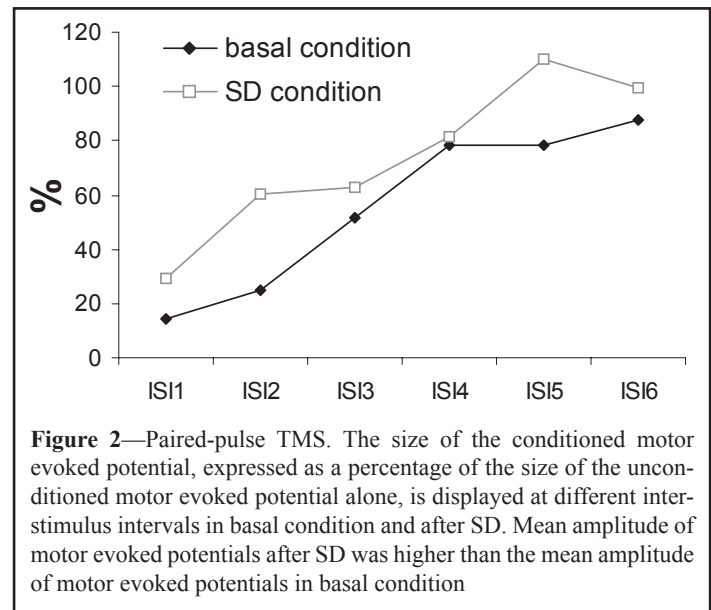


Figure 2—Paired-pulse TMS. The size of the conditioned motor evoked potential, expressed as a percentage of the size of the unconditioned motor evoked potential alone, is displayed at different inter-stimulus intervals in basal condition and after SD. Mean amplitude of motor evoked potentials after SD was higher than the mean amplitude of motor evoked potentials in basal condition

ing is in contrast with our results. Technical aspects (intensity of the stimuli, measurement of the silent period duration, etc.) might account for the differences. Another possibility is that the impact of SD on silent period may depend on which hemisphere is stimulated. In the study by Civardi and colleagues,¹² the left hemisphere (usually dominant) was stimulated, while we stimulated only the non-dominant hemisphere. Thus, an interhemispheric asymmetry in cortical excitability might explain the different results of our study, although additional studies are required to test this hypothesis.

Serial measurements of motor threshold, silent period and intracortical inhibition were performed during the night in the study by Manganotti and colleagues.¹³ Methodological differences and the different temporal windows adopted for observation may explain the discrepancies reported with respect to our results, as well as to those published by Civardi and colleagues.¹²

Manganotti and colleagues¹⁴ in a more recent paper investigated the effect of partial SD in subjects with juvenile myoclonic epilepsy compared to normal subjects. In contrast with both the previous studies^{12,13} and our results, in normal subjects the authors did not find SD related differences in TMS parameters. We cannot explain this discrepancy, and we wonder if it could be due in part to the fact that, in Manganotti and colleagues' study, the actual duration of the SD is not strictly predefined, being specified only with regards to nighttime deprivation.

The hyperexcitability of the primary motor cortex after SD may be in response to a significant stress suffered by the brain, in contrast with the restorative processes occurring during sleep that are particularly important for proper brain functioning.^{37,38}

Although the restricted number of subjects and age limitation to adulthood prevent any generalization, we suggest that the temporary loss of inhibition, induced by total sleep deprivation in healthy subjects might be important for explaining the facilitatory influence of SD upon seizures. The SD, in fact, may modify the cortical excitability, seen as the balance between inhibitory and excitatory cortical phenomena, which could reduce the epileptic threshold. Additional studies are required to confirm this hypothesis, especially in younger subjects. Another limitation of the study is that the occurrence of subtle vigilance fluctuations (e.g., microsleeps) and related cortical excitability level changes

during TMS cannot be completely ruled out; technical difficulties prevented a simultaneous recording of EEG during TMS sessions. Although we hope that future technical developments will permit a more precise control of the vigilance conditions, we are confident that in our study the verbal instruction and clinical supervision minimized possible biases due to vigilance fluctuations.

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