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Human cortical excitability increases with time awake

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Abstract

Prolonged wakefulness is associated with obvious changes in the way we feel and perform. Are these changes paralleled by measurable changes in the state of cortical circuits? We show that human cortical excitability, reflected in the immediate (0-20 ms) electroencephalographic reaction to transcranial magnetic stimulation (TMS), progressively increases with time awake, from morning to evening and after one night of sleep deprivation, and that it decreases after recovery sleep.

Main text

When we stay awake too long we become drowsy, we may experience lapses in vigilance and we feel a general sense of heaviness, or tiredness. Objectively, prolonged wakefulness leads to measurable performance impairments at all levels, ranging from simple reaction time tasks [1] to higher cognitive functions [2-4]. Clinically, staying awake increases the risk for seizures [5], the chance to encounter hallucinations [6] while it may relieve depressive symptoms [7]. By an electroencephysiological point of view, prolonged wakefulness is associated with higher spectral power in the theta range (5-7 Hz) and with larger slow waves (1–4.5 Hz) during subsequent sleep. Altogether, these observations suggest that wakefulness may not be a steady state and that human cortical circuits may undergo some fundamental changes with time awake. Specifically, it has been recently hypothesized that wakefulness is associated with a net increase in cortical excitability that is homeostatically rebalanced during sleep [8]. Thus, prolonged wakefulness was found to increase the frequency and amplitude of miniature excitatory post-synaptic currents in cortical slices [9] and the protein levels of key components of central synapses in Drosophila melanogaster [10]. In rats, wakefulness increased the firing rates of cortical neurons [11] and the slope of the local field potential (LFP) evoked by electrical cortical stimulation [12], a classic measure of synaptic strength in vivo.

In the present work we tested the hypothesis that the excitability of human cortical circuits may undergo significant changes as a function of time spent awake. Testing directly this hypothesis in humans requires by-passing sub-cortical sensory and motor pathways in order to probe non-invasively the immediate reaction of cortical neurons to a direct stimulation. Thus, we measured the slope and the amplitude of the early (first 20 ms) electroencephalographic (EEG) response to transcranial magnetic stimulation (TMS) of the left frontal cortex (**Fig.1a, b**) during baseline wakefulness, after one night of sleep deprivation and after one night of recovery sleep (**Supplementary Methods and Supplementary Fig.1**).

After one night of sleep deprivation, the slope and the amplitude of the early TMS-evoked potential (TEP), measured at the same circadian time (3 PM), increased significantly ($p<0.001$) in all subjects (**Fig.1c**). The response returned to the baseline level after one night of recovery sleep. A progressive increase of the cortical response was also observed during 12 hours of baseline wakefulness, from morning (9 AM) to evening (9 PM), reaching significance in 5 subjects out of 6 (**Fig.1d; see also Supplementary Fig.2a** for group results). Thus, staying awake brought about a gradual increase of cortical excitability that was measurable and significant at the single-subject level. This increase was largely independent from circadian factors and was reverted by one night of recovery sleep.

Typically, after a period of prolonged wakefulness subjects tend to fall periodically into short-lasting (tens of seconds) episodes of drowsiness that are associated with severe performance impairment and with transient increases of low theta (4-5 Hz) EEG power [13]. To test whether these transient lapses affected our results we recorded two additional TEPs, one before and one after the night of sleep deprivation, while subjects were engaged in a compensatory tracking task (CCT) [13] that continuously monitored their level of vigilance

(**Fig.2a**). As shown in **Fig. 2b** (and **Supplementary Fig.3**), the amplitude of single-trial cortical responses was steadily increased, irrespectively of the concurrent performance/vigilance level. Thus, prolonged wakefulness brought about a tonic increase of cortical excitability that was clearly measurable even when subjects managed to appear highly vigilant and did not show any sign of behavioural drowsiness.

As in animal models, the increased excitability of cortical neurons measured with TMS/EEG in humans may reflect a plasticity/use-dependent strengthening of cortical connections [8] and may underlie the increased amplitude of low (<9Hz) and high-frequency (13-25 Hz) EEG oscillations observed during wakefulness after sleep deprivation [14], as well as the increase in slow waves (0.4-5 Hz) that is normally observed during subsequent sleep [15]. Accordingly, we also found evidence that the changes in the slope of TEPs and the changes in sleep SWA may be related (**Supplementary Fig.4**). Clearly, further experiments will be needed in order to explore a possible contribution of neuromodulation and circadian factors to the observed changes of TEPs. Meanwhile, the present measurements demonstrate that the intrinsic excitability of human cortical circuits is not steady during wakefulness and that it progressively increases with time awake, irrespectively of the level of vigilance. This experimental observation supports the idea that sleep may help keeping cortical excitability under control and may provide a mechanism for the well known effects that sleep deprivation has on seizures, hallucinations and depressive symptoms.

Figure legends:

Figure 1: (**a**) TMS was targeted to the left frontal cortex by means of a neuronavigation system that ensured stimulation reproducibility across sessions (**b**) The traces show the averaged responses of all the channels to TMS. The black traces are recorded from a region of interest (ROI) around the stimulated site where TMS evoked a clear negative-to-positive deflection. After averaging the responses in the ROI (red trace), we measured cortical excitability as the slope and the amplitude of the early components of the evoked response (from 10±1 ms to 20±2 ms). Data from subject 1. (**c**) TEPs measured during baseline wakefulness, after sleep deprivation, and after recovery sleep. The measurements were carried out at the same time (3 PM) to control for circadian effects. (**d**) TEPs measured during baseline wakefulness, from morning to evening. (**c**,**d**) Bar graphs illustrate slope values, *** $P < 0.001$, ** $P < 0.001$, * $P < 0.05$, NS = not significant; two-tailed Bonferroni-corrected *t*-tests. Same significant differences were obtained for amplitude measurements. Upper row: subjects 1–3, lower row: subjects 4–6. The error limits of the responses indicate s.e.m.

Figure 2: (**a**) Trajectory of the tracker ball during a visuomotor compensatory tracking task, which the subjects performed during an additional stimulation session in the afternoons. Sleep deprivation was associated with transient lapses of vigilance and decreases in the task performance (increased distance of the tracker ball from the target). (**b**) Single-trial responses sorted according to the corresponding task error value are shown smoothed with a moving average spanning 5 trials. The black curve shows the task error. The task error did not correlate with the slope (and amplitude) of the early TMS-evoked EEG response in any of the 5 subjects (**Supplementary Fig.1**) suggesting that the changes of TEPs were not due to the occurrence of transient lapses of vigilance. Data from subject 1.

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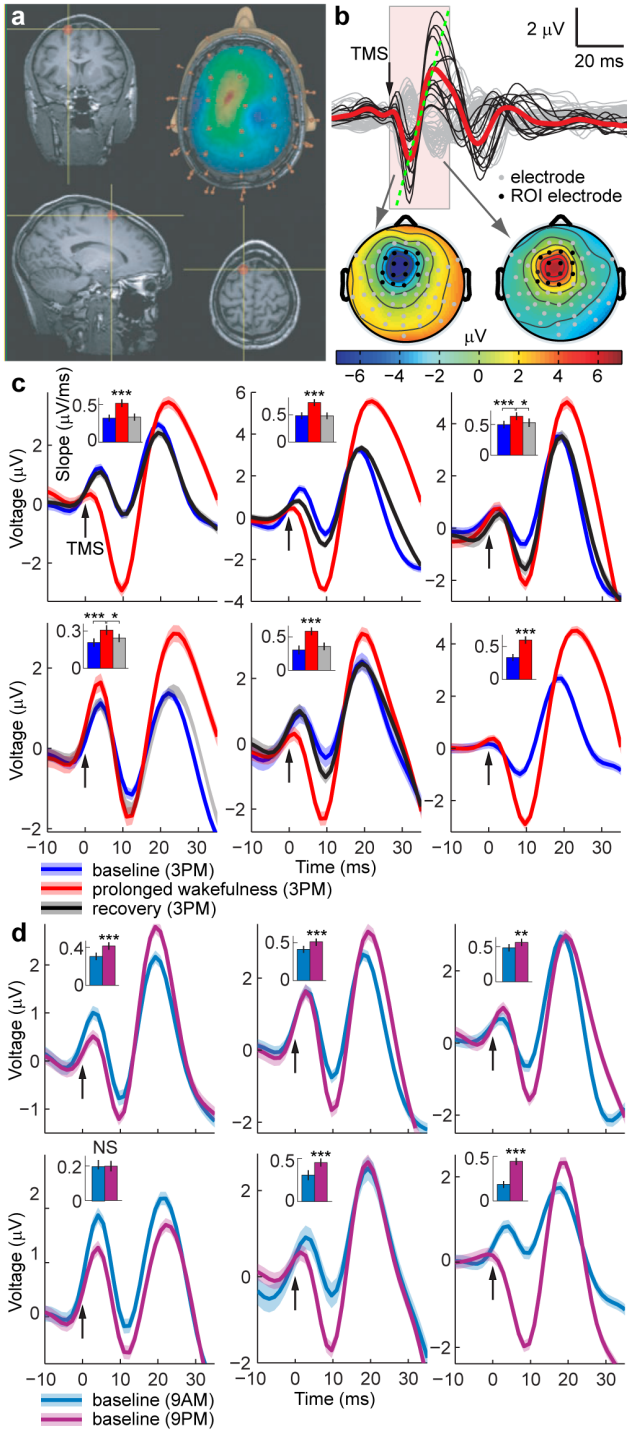

a
b
TMS
2 μV
20 ms
electrode
ROI electrode
μV
−6 −4 −2 0 2 4 6
c
Slope (μV/ms)
Voltage (μV)
TMS
Time (ms)
baseline (3PM)
prolonged wakefulness (3PM)
recovery (3PM)
d
NS
Time (ms)
baseline (9AM)
baseline (9PM)

Figure 1

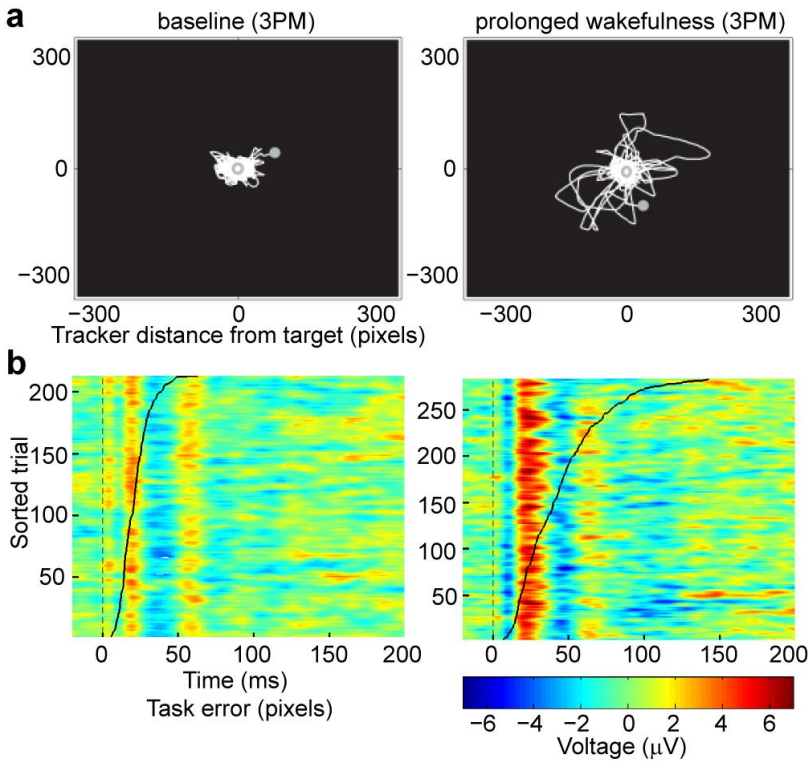

a
baseline (3PM)
prolonged wakefulness (3PM)
300
0
−300
−300
0
300
Tracker distance from target (pixels)
b
Sorted trial
200
150
100
50
250
0
50
100
150
200
Time (ms)
Task error (pixels)
−6
−4
−2
0
2
4
6
Voltage (μV)

Figure 2

Supplementary Methods

Subjects

The study was approved by the Local Ethical Committee of the Hospital L. Sacco, Milan and involved six healthy volunteers (1 female, age 25–41) who gave their written informed consent.

All subjects underwent clinical, neurological and psychiatric examinations to rule out history or presence of drug/alcohol abuse and of major medical/neurological disorders. Furthermore, subjects with medical history of seizures, convulsions, loss of consciousness and traumatic brain injury, carriers of intracranial metallic objects and/or of cardiac pace-makers were excluded to prevent potential adverse effects of TMS.

Experimental protocol

The entire experimental protocol lasted four days and is graphically depicted in **Supplementary Fig.1**. Each subject spent 4 consecutive nights and 4 consecutive days in the lab and underwent the following experimental steps:

- Adaptation night/day: adaptation to the lab environment
- Baseline night (11 pm to 7 am): subjects slept from 11 pm to 7 am and their spontaneous EEG was continuously recorded
- Baseline day (7 am to 11 pm): subjects underwent three experimental sessions (9 am; 3 pm and 9 pm). During each session we sequentially measured, (i) vigilance by means of the psychomotor vigilance task (PVT; Van Dongen, 2003; 60 trials), (ii) vigilance by means of the visuomotor compensatory tracking task (CTT - Makeig and Jolley, 1996; **Fig. 2).** (iii) TMS-evoked potentials (TEPs) and (iv) 3 minutes of spontaneous EEG (eyes open). During the afternoon sessions (3:00 pm) of the baseline day and of the day following sleep deprivation, an additional TEP measurement was carried out while subjects were engaged in the CTT.
- Sleep deprivation night: subjects were sleep deprived throughout the night under the continuous supervision of one experimenter.
- Sleep deprivation day: subjects underwent one experimental session at 3:00 pm (PVT, CTT, TEPs, TEPs simultaneous with CTT, spontaneous EEG)
- Sleep recovery night: spontaneous EEG was continuously recorded while subjects were sleeping
- Sleep recovery day: subjects underwent one experimental session at 3:00 pm (PVT, CTT, TEPs, spontaneous EEG)

During the recordings, experimenters constantly checked that subjects were awake, with their eyes opened. The subjects' eyes opening was verified off-line by means of a video camera recording synchronized with the TMS trigger.

TMS targeting

TMS was delivered on the left superior frontal cortex/supplementary motor area (Brodmann's areas 6/8) with a Focal Bipulse 8-Coil (mean/outer winding diameter ca. 50/70 mm, biphasic pulse shape, pulse length ca. 280 μs, focal area of the stimulation hot spot 0.68 cm^2; Eximia TMS Stimulator, Nexstim Ltd., Helsinki, Finland). A Navigated Brain Stimulation (NBS) system (Nexstim Ltd., Helsinki, Finland) provided with a 3D infrared Tracking Position Sensor Unit (Polaris, Northern Digital Inc., Waterloo, Canada) was used to map the positions of TMS coil and subject's head within the reference space of individual structural magnetic

resonance images (MRI) recorded from all subjects (1 T Philips scanner). This equipment allowed on-line (through a virtual aiming device) and off-line control of stimulation parameters across sessions. Stimulation intensity was adjusted to deliver an induced electric field between 120 and 130 V/m on the cortical surface, as estimated by the navigation software. Inter-stimulus interval was randomly jittered between 600–750 ms (equivalent to ca. 1.3–1.6 Hz) and about 200–300 pulses were delivered in each session.
Previous studies have shown that TEPs have good intra-individual repeatability over time, provided that the stimulation parameters are kept constant across sessions (Lioumis et al., 2009; Casarotto et al., 2010). Reproducibility of stimulation and recording was especially relevant for the present protocol. For this reason, we adopted the following criteria: (i) TMS pulses delivered with more than 2 mm error, according to the navigation system, were excluded from analysis (ii) the coil's temperature was monitored and each stimulation session started at the same level (15 °C) (iii) electrode positions were digitized on the individual MRIs and precisely replicated across session (iv) the subject's position and the environmental light were controlled and kept constant (v) subjects were requested to fixate a point on the screen (unless they were performing the CCT), while their eyes were monitored by a video camera, and wore earplugs through which a continuous noise masking was played (Massimini et al., 2005) (vi) to control for circadian factors, comparisons between baseline wakefulness, wakefulness after sleep deprivation, and wakefulness after recovery sleep were conducted at the same circadian time (3:00 PM).

EEG recording

EEG was recorded with a 60-channel TMS-compatible amplifier (Nexstim Ltd., Helsinki, Finland), equipped with a proprietary sample-and-hold circuit that prevents TMS-induced artefact by keeping the analogue output of the amplifier constant from 100 μs pre– to 2 ms post-stimulus (Virtanen et al., 1999). Vertical electrooculogram (EOG) was recorded with two additional electrodes to measure eye movements and blinks. Signals were band-pass filtered between 0.1–500 Hz and sampled at 1450 Hz. During the TMS stimulation, subjects wore inserted earplugs continuously playing a masking noise that abolished the auditory potentials elicited by TMS-associated clicks (Massimini et al., 2005, 2007). One week before being recruited in the full protocol, subjects underwent a preliminary TMS/EEG session during which optimal stimulation parameters (site, angle and intensity) were identified. As a result, TEPs that were free from artefacts (large spikes due to electrode charging/vibration) starting from 2 ms were recorded in all subjects.

Compensatory tracking task (CCT)

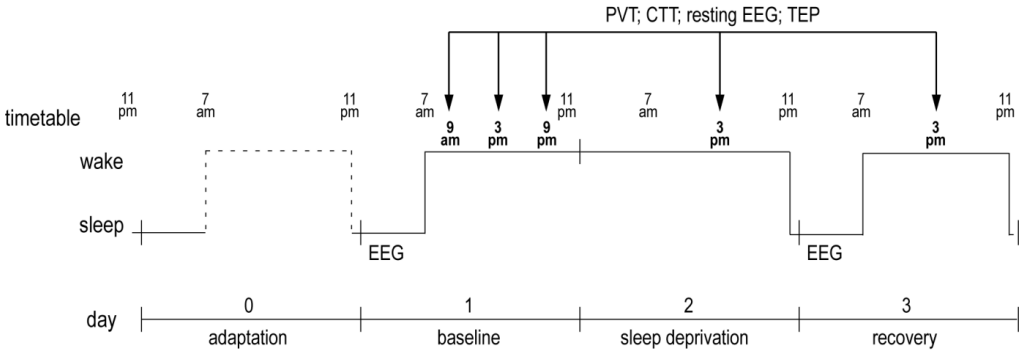
The goal of the CCT is to keep a cursor on a circular target located in the centre of a computer screen, using a trackball input device. During the test the target–cursor distance is monitored at a rate of 12 Hz. The cursor is displaced by two forces, a random buffeting force and a radial distraction force. The user must compensate these two computer-generated forces by continuously interacting with the trackball. Performance is measured as the distance, in pixels, of the cursor from the target. In this way, the CCT allows monitoring continuously the level of vigilance; transitory lapses of vigilance immediately result in temporary increases of the target–cursor distance (Makeig and Jolley, 1996). Before the actual measurements were carried out, subjects underwent a series of training sessions to avoid learning effects. The time series of the target–cursor distance were averaged to obtain the values displayed in **Supplementary Fig. 2**, while instantaneous values were used to study the correlation between short-term fluctuations in the vigilance level and the changes in TEPs, as described below.

Data analysis

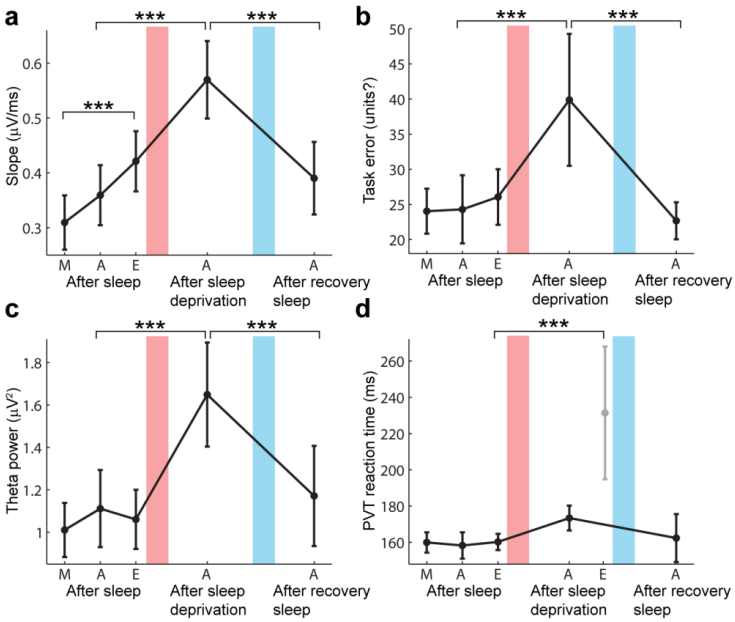
Data were analyzed using MATLAB® (The Mathworks Inc., Natick, Massachusetts, USA).
TEP analysis was focused on an early component, comprised between a negative deflection at 10 ± 1 ms and a positive deflection at 20 ± 2 ms (± standard deviation across subjects), that was highly reproducible across individuals. Continuous EEG recordings gathered during TMS stimulation were split into epochs between –80 to 300 ms around TMS pulses. Single trials and channels contaminated by artefacts or eye movements were manually rejected. Then, EEG recordings were band-pass filtered between 2–80 Hz, downsampled at 725 Hz and re-referenced to the average reference.
In order to evaluate cortical excitability, we measured the peak-to-peak amplitude and the slope of the first negative-to-positive TEP component recorded from a region of interest located immediately under the stimulator. The slope of TEPs was measured (in addition to their amplitude) since, in animal studies, the strength of population excitatory postsynaptic currents is reflected by the slope of LFPs evoked by electrical stimuli (Rall et al, 1967). Thus, in the animal model, changes in slope are thought to reflect changes in excitability related to the strengthening or weakening of cortical synapses. Accordingly, *in vivo* LTP-inducing procedures increase LFP slope (Bliss and Lomo, 1973), whereas LTD procedures reduce it (see for example, Borbely and Achermann, 2005).
The first component of the TEP (Fig. 1b) was reproducible across subjects and was characterized by a negative peak at around 10 ms, followed by a positive peak at around 20 ms, which were clearly detectable in left-frontal scalp region including electrodes AF1, AFz AF2, F5, F1, Fz, F2, FC3, FC1, FCz, FC2, C3, C1 and Cz. Peak-to-peak amplitude and slope were measured on each single trial-response obtained after averaging the TEPs recorded from these electrodes. In particular, the slope was computed (as in Vyazovskiy et al., 2008) as the mean first derivative of the up-going segment of the of the first EEG component (Fig 1b). Amplitude and slope differences between sessions were assessed by two-tailed two-sample *t*-tests applied to each subject separately (Fig. 1c,d in the main text). The obtained *p*-values were Bonferroni-corrected with factor 4 (4 comparisons of interest: 1. baseline morning vs. baseline evening, 2. baseline afternoon vs. sleep deprivation afternoon, 3. sleep deprivation afternoon vs. recovery afternoon, 4. baseline afternoon vs. recovery afternoon).
The wakefulness spontaneous EEG was recorded and analyzed in order to quantify the spectral power in the theta frequency range (4–7 Hz), a well known marker of sleep homeostatic and circadian factors. Continuous EEG recordings were re-referenced to the average reference and divided into 4-s epochs. Epochs containing artefacts due eye blinks/movements were rejected or corrected using independent component analysis (ICA). In this case, data dimensionality was reduced to 20 dimensions with principal component analysis (PCA), and afterwards the deflation approach of the fastICA algorithm (Hyvärinen and Oja, 1997) was applied to compute the independent components (ICs). For all subjects and sessions, the single IC representing the artifact was visually identified and subsequently removed from the data. Power spectra were computed (frequency resolution 0.25 Hz) and then averaged over epochs, accepted channels, and frequency bins corresponding to the theta frequency range (4–8 Hz).
When TEPs were recorded during CTT, single-trial evoked responses were matched to the concurrent level of performance. Thus, task performance was computed as the mean distance of the tracker ball from the target between –1 and +2 s around each TMS pulse. Pearson's correlation coefficient between single-trial TEP slope and task performance was computed for each subject separately to test whether short-term fluctuations in vigilance were related to cortical excitability.
At the group level, TEP slope and theta power, as well as CTT and PVT performance were compared between sessions by applying a two-tailed two-sample *t*-test with Bonferroni correction (factor 4) to the single-subject values of TEP slope, CTT performance, theta power, and 10th percentile of the longest PVT reaction time averaged across trials/epochs.

Sleep EEG recordings were band-pass filtered between 0.1–40 Hz, downsampled at 128 Hz (Matlab function ‘resample’), and re-referenced to the average reference. Sleep stages were visually scored in 20-s epochs according to standard criteria (Rechtschaffen and Kales, 1968). Non-REM and REM sleep cycles were defined as described in previous experiments (e.g. Aeschbach and Borbély 1993). Quantitative analysis of sleep EEG was carried out by estimating the spectral power of continuous 20-s epochs for all channels (Matlab FFT routine, Hanning window, averages of five 4-s epochs). Visual and semi-automatic rejection of artifact-contaminated epochs was performed by a trained experimenter as previously described by Huber et al. (2000). In one subject, (subject 6) TEPs and EEG data from the recovery night could not be analyzed due to technical problems. For the topographical display of EEG activity we used the topoplot function of the EEGLab Matlab toolbox (Delorme and Makeig, 2004).

Supplementary figure legends:

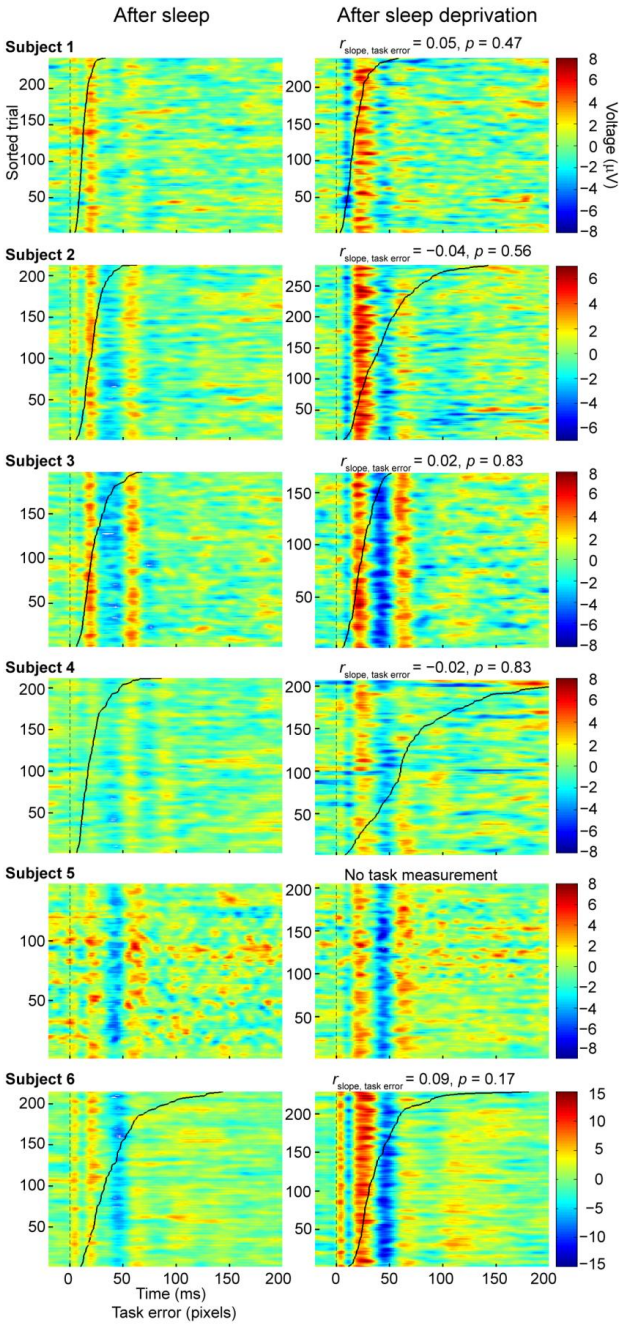


Supplementary Figure 1: Outline of the experimental protocol.
After a preliminary TMS/EEG session performed during the previous week, each subject spent one night of sleep in the lab for adaptation purposes and then was asked to come the following evening for starting the experimental procedures, that lasted three consecutive days. During the first night (baseline sleep), subjects slept normally (11:00 PM day 0 to 07:00 AM day 1) while EEG was continuously recorded. Then, subjects were sleep deprived for 40 hours (07:00 AM day 1 to 11:00 PM day 2) and were subsequently allowed to sleep again for one night (11:00 PM day 2 to 07:00 AM day 3) while EEG was continuously recorded (recovery sleep). TMS/EEG sessions were performed on days 1-2-3 at 3:00 PM (afternoon sessions) and additionally on day 1 at 09:00 AM and 09:00 PM. During each session, performance over PVT and CTT, resting EEG (2 min eyes open), TEPs while subjects were fixating a point on the screen were recorded. During afternoon sessions, additional TEPs were recorded while subjects were performing CTT.

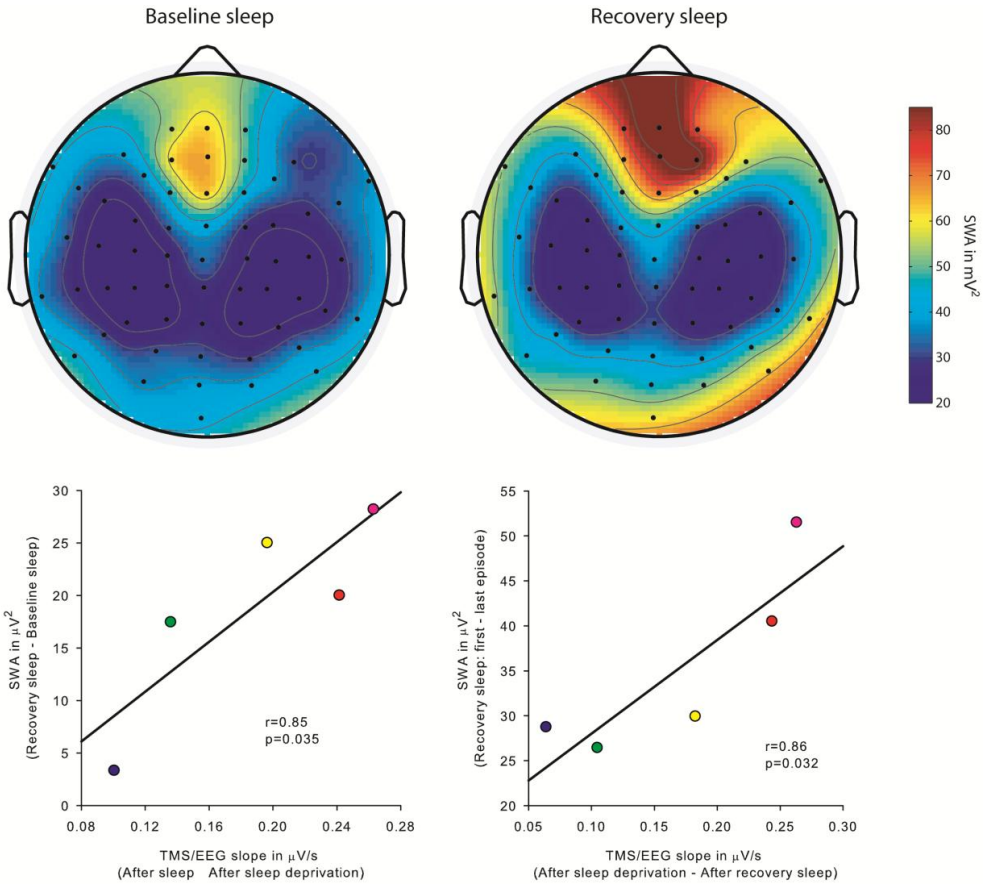


Supplementary Figure 2: Modulation of TEP slope (**a**), performance over CTT (**b**), theta power of eyes-open resting EEG (**c**), and performance over PVT (**d**) as a function of time spent awake.

After one night of sleep deprivation, the following parameters were significantly increased: slope of the early TEP component, (**b**) error in the visuomotor tracking task, (**c**) EEG power in the theta band (4–8 Hz), and (**d**) an increase in the reaction time in the psychomotor vigilance test. (**a**,**b**,**c**,**d**) *** $P < 0.001$, NS = not significant; two-tailed Bonferroni-corrected *t*-tests on the single-trial values pooled over subjects. The error bars indicate s.e.m. over subjects. M = morning, A = afternoon, E = evening. The red bar indicates a night of sleep deprivation and the blue bar a night of recovery sleep.



Supplementary Figure 3: During the 3:00 PM session on the baseline day and on the day following sleep deprivation, additional TEPs were recorded while subjects performed the CCT. Single-trial TEPs are sorted according to the corresponding task error value and smoothed with a moving average spanning 5 trials. The black trace shows the visuomotor tracking task error; the dotted line indicates the TMS pulse. The slope (and the amplitude) of TEPs was tonically increased after sleep deprivation, even during periods in which subjects showed normal performance; moreover, the slope of TEPs did not correlate with the task error in any of the 5 subjects who performed the task (Pearson's correlation coefficients *r* and corresponding *P*-values are shown above the figures of each subject). The EEG data were smoothed for visualization purposes; no smoothing was used when the correlation was calculated.



Supplementary Figure 4: (**a**) Topographic distribution of SWA during baseline and recovery sleep. Average EEG power density between 1–4.5 Hz (SWA, *n*=5 subjects) for the first two non-REM sleep episodes. SWA increases after sleep deprivation in most electrodes (**b**) Correlation between the increase in the slope of the TMS-evoked EEG response (baseline vs. prolonged wakefulness) and the increase in sleep SWA between baseline and recovery sleep. (**c**) Correlation between the decreases in the slope of the TMS evoked EEG response (prolonged wakefulness vs. recovery) and the decrease of sleep SWA during the recovery night.