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Laboratory Investigation

LABORATORY INVESTIGATION

Depth of sedation with dexmedetomidine increases transcranial magnetic stimulation-evoked potential amplitude non-linearly

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Abstract

Background: Cortical excitability is higher in unconsciousness than in wakefulness, but it is unclear how this relates to anaesthesia. We investigated cortical excitability in response to dexmedetomidine, the effects of which are not fully known.
Methods: We recorded transcranial magnetic stimulation (TMS) and EEG in frontal and parietal cortex of 20 healthy subjects undergoing dexmedetomidine sedation in four conditions (baseline, light sedation, deep sedation, recovery). We used the first component (0–30 ms) of the TMS-evoked potential (TEP) to measure cortical excitability (amplitude), slope, and positive and negative peak latencies (collectively, TEP indices). We used generalised linear mixed models to test the effect of condition, brain region, and responsiveness on TEP indices.
Results: Compared with baseline, amplitude in the frontal cortex increased by 6.52 μV ($P<0.001$) in light sedation, 4.55 μV ($P=0.003$) in deep sedation, and 5.03 μV ($P<0.001$) in recovery. Amplitude did not change in the parietal cortex. Compared with baseline, slope increased in all conditions ($P<0.02$) in the frontal but not parietal cortex. The frontal cortex showed 5.73 μV higher amplitude ($P<0.001$), 0.63 μV ms^{-1} higher slope ($P<0.001$), and 2.2 ms shorter negative peak latency ($P=0.001$) than parietal areas. Interactions between dexmedetomidine and region had effects over amplitude ($P=0.004$) and slope ($P=0.009$), with both being higher in light sedation, deep sedation, and recovery compared with baseline.
Conclusions: Transcranial magnetic stimulation-evoked potential amplitude changes non-linearly as a function of depth of sedation by dexmedetomidine, with a region-specific paradoxical increase. Future research should investigate other anaesthetics to elucidate the link between cortical excitability and depth of sedation.

Keywords: anaesthesia; consciousness; dexmedetomidine; responsiveness; sedation; transcranial magnetic stimulation-evoked potential

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Editor's key points

- Cortical excitability is an electroencephalographic parameter that increases in states of reduced consciousness.
- In this study of 20 volunteers, the effects of targeted dexmedetomidine infusion on cortical excitability was analysed using transcranial magnetic stimulation of frontal and parietal cerebral cortex.
- Evoked potential amplitude and slope, measures of excitability, increased with light and deep sedation in frontal but not parietal cortex in a nonlinear manner. Thus cortical excitability increases with sedation by dexmedetomidine in a brain region-selective manner.
- Further studies are required to determine the mechanisms of these effects, and their potential for monitoring and understanding various states of consciousness and sedation.

Anaesthesia offers a unique tool to investigate consciousness mechanisms, reversibly modulating different aspects of consciousness states depending on the nature of the drug and its dosage.[1] When an anaesthetic agent leads to an alteration of consciousness, it impacts brain functioning in its complexity,[2] connectivity,[3] and frequency range.[4] After regaining consciousness, people can experience emergence agitation; postoperative delirium, a cognitive disorder characterised by anxiety; cognitive alterations; and/or hypoactivity or hyperactivity.[5]

Dexmedetomidine (DEX) is an α_2-adrenoceptor agonist that has the potential to reduce the incidence of emergence agitation[6] and postoperative delirium compared with other anaesthetic agents.[7] The reasons for these phenomena are still unclear. The anxiolytic, analgesic, and opioid-sparing properties of DEX, as well as its absence of anticholinergic effects, improvement in sleep quality, and attenuation of postoperative inflammation have been advocated to explain the reduced incidence of postoperative delirium.[8] Moreover, a possibly more rapid transition between brain states and restoration of cortical communication might explain the positive effect on emergence agitation.

Through its inhibitory effect on the locus coeruleus, DEX reduces inhibition of the ventrolateral preoptic nucleus of the hypothalamus, which in turn exerts GABAergic inhibition of cortical arousal nuclei. This effect on subcortical sleep systems promotes a state similar to stage 2/3 non-rapid eye movement (NREM) sleep.[9–11] With DEX, cortical and subcortical region glucose consumption decreases, which correlates with the functional connectivity impairment in intrinsic consciousness networks, as well as between the thalamus and cortical regions within those networks.[11,12] Interestingly, cortico-cortical connectivity remains partially preserved during deep sedation.[12] This asymmetry between cortical and subcortical regions might account for the partially preserved semantic processing of incoming stimuli after loss of responsiveness as indicated by EEG.[13] Functional connectivity between the thalamus and key arousal structures and saliency detection networks is relatively preserved during DEX-induced deep sedation, which might explain the ability to restore responsiveness rapidly by vigorous external stimulation. Finally, DEX drives a shift towards slow-wave oscillation,[14,15] while high-frequency oscillations (e.g. beta) can accurately predict responsiveness upon behavioural assessment.[16] These findings led us to investigate the link between responsiveness, depth of sedation, and relative cortical modulation.

Transcranial magnetic stimulation coupled with high-density EEG (TMS-hdEEG) assesses brain response with a no-task paradigm, bypassing sensory cortices. TMS-hdEEG is a noninvasive neurostimulation technique that perturbs the brain through fast local changes in magnetic field, which induce electrical currents that mimic endogenous physiological activity. TMS-evoked potentials (TEP), the averaged EEG responses to the TMS pulse, capture the neural response.[2,3] TEP at the nearest electrode to the stimulation side provides information on the local modulation of TMS. We operationally define cortical excitability as the amplitude (primary outcome) of the first component (0–30 ms) of the TEP. Cortical excitability measured this way is modulated by conscious states,[17] circadian rhythms, and sleep deprivation.[18–20] It also increases during unresponsive states such as NREM sleep[21] and attentional lapses,[22] representing a promising method to investigate cortical reactivity in time and space as a function of conscious states.

We determined DEX effects on cortical excitability during different levels of sedation (baseline without sedation, light sedation, deep sedation, and recovery). Following the effects described in sleep, we expected cortical excitability (amplitude; primary outcome) to increase proportionally with depth of sedation. We hypothesised that cortical excitability would be highest during deep sedation, whereas there would be virtually no difference between baseline and the recovery after DEX. Other indices such as slope, which we expected to have a similar pattern as amplitude, and positive and negative latencies of the first TEP component (secondary outcomes) were also analysed to characterise the brain response.

Methods

Participants

A priori power analysis and sample size estimation were difficult to perform given the scarcity of research on TEP, anaesthesia, and cortical excitability. Based on study sample sizes and covariate numbers, assuming a statistical power of 0.8 and using a false-positive rate of α=0.05, we were in a position to detect a large effect size (r>0.6; r^2>0.36). We aimed to include at least 20 subjects consistent with most TMS-hdEEG studies.[2,22] Considering drop-out and possible technical problems, we recruited 30 healthy adult subjects on the University of Liège campus between February 2015 and May 2016. Participants were screened by a senior anaesthesiologist (VB) to determine any contraindications to DEX sedation, TMS, or MRI. We recruited volunteers with the following inclusion criteria: aged 18–35 yr old, absence of prior neurological, neurosurgical, or psychiatric history, no history of drug addiction, no history of adverse events during anaesthesia or previous exposure to DEX, no active chronic illness or medication, no contraindication to MRI or TMS-EEG, and no ongoing pregnancy for female participants (efficient contraception or negative pregnancy test required for inclusion). Five participants were dismissed because artifact-free TEPs could not be obtained reliably during normal wakefulness, two lost interest in the study, and two were dropped for technical or logistical reasons. One subject had a minor adverse reaction to DEX infusion (pruritus without a rash or any other symptoms

or signs), for which the experiment was aborted. In total, 20 subjects completed the entire experiment (Table 1 and Supplementary Table S1 for additional information). All subjects gave written informed consent. The study was approved by the Ethics Committee of the University and University Hospital of Liège, Belgium (number B707201422895, professor V. Seutin).

Experimental protocol

A visual summary of the protocol is shown in Figure 1a. After screening, eligible participants underwent an MRI and a TMS-hdEEG pretest during normal wakefulness to find the most suitable brain target for stimulation of the superior parietal (precuneus - Brodmann area 7) and premotor region (Brodmann area 6) in the midline. These brain targets were set for the experimental phase using neuronavigation (Nexstim, Helsinki, Finland). During the experiment, subjects were supine while venous access was secured to infuse DEX administered using a target-controlled infusion (TCI) device (height-adjusted model of Dyck[23]), providing a constant estimation of DEX plasma concentration. DEX target concentration was changed by steps of 0.5 ng ml^{-1} to achieve the desired behavioural state. Once attained, a 5-min equilibration period without change in target concentration allowed equilibration between pharmacokinetic compartments, and a blood sample was drawn immediately before and after data acquisition for offline DEX plasma concentration measurement by high-performance liquid chromatography–mass spectrometry (see Supplementary Material). Behavioural assessment of depth of sedation was performed at the same times using the University of Michigan Sedation Scale (UMSS)[24] and Ramsay scale.[25] There were four conditions for each subject: baseline, before DEX; light sedation, marked by drowsiness; deep sedation, characterised by no behavioural response or maximal allowed concentration of DEX; and recovery, upon regaining responsiveness. Physiological parameters were monitored (ECG, peripheral blood oxygen saturation by pulse oximetry, and end-tidal CO_2 levels). After a baseline TMS-hdEEG recording, DEX was increased to reach drowsiness. A 5-min break allowed concentration to stabilise, reaching light sedation, during which subjects were still able to follow a command. The level of DEX was then increased by 0.5 ng ml^{-1} increments to induce deep sedation (unresponsiveness or maximal concentration). For safety reasons, we set the maximal concentration to 2.5 ng ml^{-1}. Lastly, DEX concentration was reduced by 0.5 ng ml^{-1} steps to regain responsiveness to command, which was referred as recovery. Once responsiveness had returned, the attained DEX concentration was maintained for the duration of recordings.

Table 1 Participant characteristics and descriptive statistics of variables at the group level. Continuous variables are mean (standard deviation), and categorical variables are the count for each level.

Variables	Descriptive statistics
Number of participants (female/male)	20 (9/11)
Age, yr	23.85 (range: 19–28)
Height, cm	173.65 (8.42)
Weight, kg	70 (13.71)
Body mass index, kg m^{-2}	23.10 (3.40)
Distance electrode, mm	Frontal: 31.20 (11.21) Parietal: 47.40 (11.80)
Dexmedetomidine concentration (measured; predicted by the model), ng ml^{-1}	Baseline: 0 (0); 0 (0) Light: 1.37 (0.47); 1.3 (0.30) Deep: 3.41 (0.78); 2.35 (0.24) Recovery: 2.71 (0.47); 1.74 (0.31)

Data acquisition

Magnetic resonance imaging

High-resolution structural MRI was performed on a 3-Tesla MR scanner (Allegra Prisma, Siemens, Erlangen, Germany; 3D isometric 1×1×1 mm T1) during wakefulness on the pretesting day just before the TMS-hdEEG session. Diffusion-weighted imaging data were also acquired (not used in the study). T1 was used to perform TMS neuronavigation on the individual cortex.

TMS-hdEEG

A focal bipulse 8-coil (Nexstim) with a 3D infrared tracking position sensor was used to perform TMS. Neuronavigation was implemented using a glasses head tracker and the Navigated Brain Stimulation system (Nexstim). Neuronavigation is a system of precision targeting that uses an infrared camera to locate the TMS coil and the head in space, while using a reconstructed brain image based on the individual T1 scan. It allows online localisation of the stimulated brain area based on real-time positions of the coil and head, assuring on-target stimulation. A 64-channel TMS-compatible EEG amplifier (Eximia, Helsinki, Finland), equipped with a sample-and-hold circuit to provide TMS-artifact-free data from 5 ms post-stimulation, was used to record concurrent EEG data during TMS stimulation. The electro-oculogram was recorded with two bipolar electrodes. EEG signals were bandpass filtered between 0.1 and 500 Hz and sampled at 1450 Hz. Prior to each recording session, electrode impedance was set below 5 kΩ. Stimulation targets and intensity were set during the pretest and kept constant across all conditions. Left premotor and left parietal cortices were targeted, and the stimulation target was chosen if there was a good TEP with no muscular or magnetic artifact. We specifically targeted the left premotor cortex and parietal cortex for several reasons: 1) these regions have been associated with the consciousness network—the fronto-parietal network[26,27]; 2) premotor cortex has been stimulated in other experiments to determine cortical excitability[18,19,22]; and 3) both regions allow artifact-free EEG recording of responses to TMS (arising from muscle contraction, eye blinks). We performed acquisition on the left hemisphere to decrease interindividual variability. Intensity was adjusted individually to get a good signal-to-noise ratio, with an evoked electric field intensity at the cortical surface between 100 and 150 V m^{-1}. Each condition had 200 to 250 trials, with a frequency of 0.5 Hz and a jitter of ±200 ms. A thin foam layer under the TMS coil and white noise mask were used to minimise somatosensory stimulation and auditory evoked potentials caused by the TMS click, respectively. For more details, see the Supplementary Material.

Behavioural assessment

Behavioural assessment of depth of sedation was performed using the UMSS[24] and Ramsay scale.[25] The four conditions had different behavioural profiles: baseline, before DEX administration, was marked by a clear command-following to the

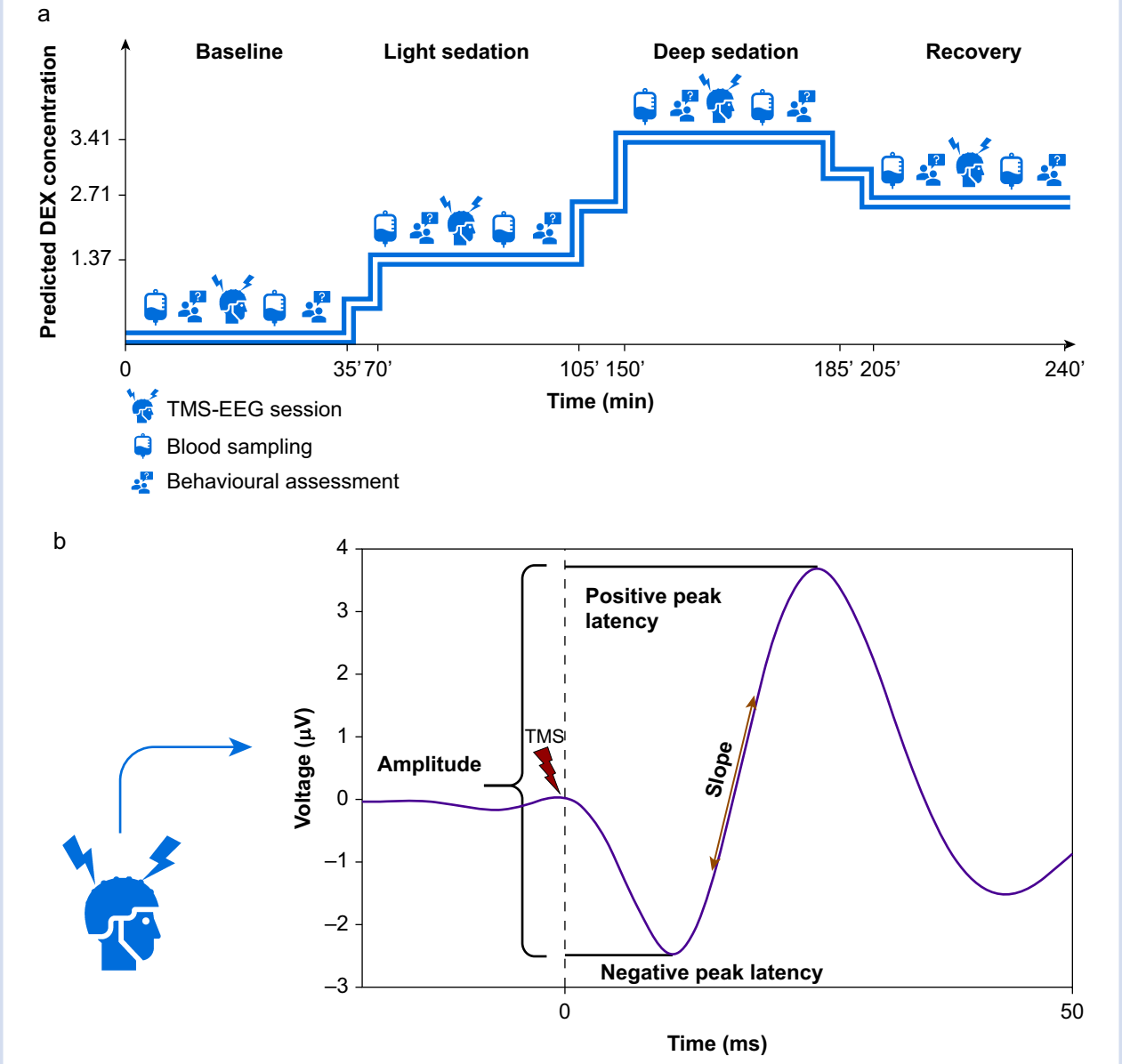

Fig 1. (a) Diagram of the protocol plotted over predicted time (x-axis, in min) and predicted dexmedetomidine (DEX) concentration (y-axis, in ng ml^{-1}). The axes are not to scale. Four conditions were investigated: baseline, light sedation, deep sedation, and recovery. Transcranial magnetic stimulation coupled with high-density EEG (TMS-hdEEG) sessions over the parietal and frontal regions were performed in each stable condition; a total of eight TMS-hdEEG sessions per subject. The additional TMS-EEG 'pretest' session to find suitable hotspot not shown. (b) Measures of cortical excitability in the TMS-evoked potential (TEP) (average TMS-hdEEG responses over trials). The red flash indicates the TMS pulse. We measured the peak-to-peak amplitude of the TEP in μV (here, around 6 μV), the latency in milliseconds of the negative peak (here, around 10 ms) and of the positive peak (here, around 20 ms), and the maximal slope of the curve in voltage over time (μV ms^{-1}). Note that here the slope is represented with the tangent line at the inflection point.

verbal request 'squeeze my hand' (Ramsay score 2, UMSS 0); light sedation was marked by drowsiness (Ramsay score 3–4, UMSS 1–2); deep sedation was characterised by no behavioural response to any verbal command (Ramsay score 6, UMSS 4) or deeper drowsiness (if maximal DEX concentration was reached but participants were still responsive); recovery was distinguished by regaining response after deep sedation (Ramsay score 3–4, UMSS 1–2). To exclude possible automatic responses to command, participants were asked to reply to one of the following questions: 'what is your date of birth?', 'what is today's date?', 'how much is 13 minus 9?', 'what is your home address?', 'what is your name?'. In case of a wrong answer, another question from the list was asked. Questions were selected randomly. No participant was asked more than two

questions. The questions were asked only in the transition between baseline and light sedation, and in recovery, when the participant showed a motor response to 'squeeze my hand'.

Blood sampling

Before and after each session, we obtained a blood sample to determine actual plasma DEX concentration. The sample was anonymised and stored at −20°C before being analysed by Orion Pharma (Orion Corporation, Orionintie , Espoo, Finland). For more information, see the Supplementary Material. The values of DEX concentration used in our analysis were the average between samples taken before and after each TMS-EEG session.

Data analysis

Preprocessing of TMS-hdEEG data

Data were analysed using MATLAB (The Mathworks Inc., Natick, MA, USA). Trial rejection was performed manually with SSP (Sisyphus Project, University of Milan) to eliminate trials with magnetic artifacts or ocular/muscular movements. A 1 Hz high-pass filter was applied to continuous data to eliminate slow oscillating noise. Afterwards, data were downsampled to 1000 Hz, then low-pass filtered to 80 Hz. Data were subsequently epoched from −100 to 300 ms post-TMS. Baseline correction between −100 and −1.5 ms was applied. Trials were then averaged using a robust averaging method[28] to minimise noise. Robust averaging eliminates strong deviants (>5 standard deviations) for each time bin.[28] An iterative channel rejection was performed until no channel showed signal with any artifact.[18,19,22]

Cortical excitability computation

We operationalised cortical excitability as the amplitude of the first component of the TEP, between 0 and 30 ms post-TMS. Amplitude, our primary outcome, is the simplest and most direct way to describe the TEP response, as a proxy of how excitable the stimulated cortex is. As TEP is a complex phenomenon, we analysed the early TEP in a multifaceted manner calculating slope and positive and negative latencies (secondary outcomes). Slope incorporates a temporal quality that is absent from amplitude, while latencies provide temporal information of the response. These indices are collectively referred to as TEP indices. The TEP was extracted from the closest electrode to the stimulation point that did not produce an artifact (distance of the electrode from the hotspot: mean [SD], 39.3 [14.0] mm). The electrode chosen differed between subjects, but was the same within subjects (same electrodes across the four conditions). For more information about which electrode was chosen for frontal and parietal cortex, see Supplementary Table S3. The latency of the negative peak is the time delay between the stimulation (t_N) and the moment at which the TEP is minimum, and ranged between 9 and 15 ms, while the latency of the positive peak (t_P) is the time delay between the stimulation and the moment at which the TEP is maximum, and ranged between 10 and 30 ms. Amplitude refers to the peak-to-peak amplitude ($A_{t_P} - A_{t_N}$), which is the microvolt change between peak (A_{t_P}) and trough (A_{t_N}), while the slope is the maximum change of the first component between t_P and t_N. The same parameters can be found in previous experiments with TMS-EEG,[18,19,22] see Figure 1b.

Statistical analysis

We ran four generalized linear mixed models (GLMMs) on SPSS (IBM© SPSS© Statistics 27, IBM Corporation, Armonk, NY, USA) to test the effect of condition (factor variable: baseline, light sedation, deep sedation, and recovery) and stimulated brain region (factor variable: frontal, posterior) over TEP indices (amplitude; slope, positive and negative latencies). A variance component matrix was used as covariance structure. We included the interaction between condition and region, condition and responsiveness, and responsiveness and region. The model also took into consideration DEX concentration as a fixed effect, and the characteristics of the TMS pulse such as mean induced electric field (V m^{-1}) and distance of the electrode from the stimulation point (in mm) as random effects. Given that seven participants were still behaviourally responsive in the deep sedation condition, responsiveness at any condition was considered in the model as a covariate (responsive *vs* unresponsive). Pairwise comparisons between conditions were performed with Bonferroni-adjusted two-tailed *t*-tests. We considered amplitude as the primary endpoint [$P_{critical}$=0.05/(2 locations×4 conditions)=0.006, corrected for multiple comparison], and slope, positive and negative latencies as secondary endpoints [$P_{critical}$=0.05]. The model in Wilkinson's notation was:

$$TEP\ indices = Condition * Region + Responsiveness * Condition + Responsiveness * Region + DEX_{blood} + Responsiveness + EF_{induced} + EDist + (1|Subject)$$

where TEP indices stand for amplitude, slope, negative latency, and positive latency, collectively.

Results

We modulated DEX concentration to induce different depth of sedation, which led to different behavioural responses. The attained concentrations (mean [SD], in ng ml^{-1}), measured in plasma for each condition were: baseline:0 (0); light sedation: 1.37 (0.47); deep sedation: 3.41 (0.78); and recovery: 2.71 (0.47). Median UMSS scores (range) were: baseline: 0 (0–0); light sedation: 2 (1–3); deep sedation: 4 (2–6); and recovery: 2 (1–4). Median Ramsay scores (range) were: baseline: 2 (2–2); light sedation: 3 (3–4); deep sedation: 6 (3–6); and recovery: 3 (2–5). Interestingly, 7 of 20 subjects (35%) were still responsive in the deep sedation condition as we did not want to exceed a safety threshold of 2.5 ng ml^{-1} theoretical target concentration. For more information about the participants, see Table 1 and Supplementary Table S1.

The condition (depth of sedation) modulated amplitude and the other TEP indices (see Fig. 2 for the grand-average TEPs, and individual values and means). As shown in Table 2, and according to the GLMMs, there was a significant interaction between depth of sedation condition and stimulation location for amplitude ($F_{[3, 149]}$=4.59, *P*=0.004) and slope

Table 2 Results of the generalised linear mixed model (GLMM) for the modulation of transcranial magnetic stimulation-evoked potential indices. We took into consideration the condition (baseline, light sedation, deep sedation, and recovery), stimulated region (frontal, parietal), their interaction (represented as an asterisk), dexmedetomidine concentration in the blood, responsiveness to command, and interaction between responsiveness, region, and condition. Significant effects are illustrated in bold. Mean values (standard deviation) of each measurement for condition are reported in Supplementary Table S3. Coefficients with 95% confidential intervals are reported in Supplementary Table S5. The corrected model represents the sum of the effects of all between-subjects effects besides the intercept, and shows if the model accounts for any variance in the dependent variable (if significant, the model is able to describe variance). The interaction between responsiveness and condition did not converge, meaning that SPSS could not estimate a stable result and it should be ignored.

Dependent variables	Models								
	Corrected model	Condition* region	Condition	Region	Dexmedetomidine concentration	Responsiveness	Responsiveness* condition	Responsiveness* region
Amplitude	**$F_{(10, 149)}=7.83$ $P<0.001$**	**$F_{(3, 149)}=4.60$ $P=0.004$**	**$F_{(3, 149)}=9.09$ $P<0.001$**	**$F_{(1, 149)}=34.57$ $P<0.001$**	$F_{(1, 149)}=3.01$ $P=0.09$	$F_{(1, 149)}=0.86$ $P=0.36$	Not converged	$F_{(1, 149)}=2.028$ $P=0.16$
Slope	**$F_{(10, 149)}=12.09$ $P<0.001$**	**$F_{(3, 149)}=4.01$ $P=0.009$**	**$F_{(3, 149)}=5.66$ $P=0.001$**	**$F_{(1, 149)}=50.28$ $P<0.001$**	$F_{(1, 149)}=3.35$ $P=0.07$	$F_{(1, 149)}=0.003$ $P=0.95$	Not converged	$F_{(1, 149)}=0.02$ $P=0.90$
Latency of negative peak	**$F_{(10, 149)}=3.195$ $P=0.001$**	$F_{(3, 149)}=0.23$ $P=0.88$	$F_{(3, 149)}=0.13$ $P=0.94$	**$F_{(1, 149)}=10.50$ $P=0.001$**	$F_{(1, 149)}=0.83$ $P=0.36$	$F_{(1, 149)}=0.07$ $P=0.79$	Not converged	$F_{(1, 149)}=0.20$ $P=0.66$
Latency of positive peak	**$F_{(10, 149)}=4.11$ $P<0.001$**	$F_{(3, 149)}=2.15$ $P=0.96$	**$F_{(3, 149)}=2.81$ $P=0.04$**	$F_{(1, 149)}=1.23$ $P=0.27$	$F_{(1, 149)}=1.11$ $P=0.30$	$F_{(1, 149)}=0.002$ $P=0.96$	Not converged	$F_{(1, 149)}=2.70$ $P=0.10$

($F_{[3, 149]}=4.01$, $P=0.009$), but not for negative or positive peak latency. In the frontal cortex, *post hoc* analysis (Table 3) showed that amplitude was higher in light sedation (Baseline-Light: −6.52 μV; adjusted $P<0.001$), deep sedation (Baseline-Deep: −4.55 μV; adjusted $P=0.003$), and recovery (Baseline-Recovery: −5.03 μV; adjusted $P<0.001$) than in baseline. In the frontal cortex, slope was different in all pairwise comparisons (adjusted $P<0.02$), such that it was higher in light sedation, deep sedation, and recovery than in baseline, and it was higher in light sedation than in deep sedation and recovery. The only contrast that did not reach statistical significance was between deep sedation and recovery (adjusted $P=0.26$). Slope and amplitude had the highest mean values in light sedation. These differences were not seen in the parietal region, where both amplitude and slope did not change between baseline and light sedation, deep sedation, and recovery. Depth of sedation (Table 2) had an effect on positive peak latency across regions ($F_{[3, 149]}=2.81$, $P=0.04$), but not on latency of the negative peak ($F_{[3, 149]}=0.13$, $P=0.22$). Irrespective of the region, positive peak latency was longer at light sedation than at baseline (adjusted $P=0.03$; Baseline-Light: −2.28 ms) (Table 3). The stimulated region (frontal *vs* parietal) had an effect on negative peak latency that was longer in the parietal region than in the frontal one (Frontal-Parietal: −2.24 ms; $F_{[1, 149]}=10.50$, $P=0.001$), but no effect was seen for positive peak latency ($F_{[1, 149]}=1.23$, $P=0.27$). Frontal cortex had higher amplitude (Frontal-Parietal: 5.73 μV, t=5.81, $P<0.001$) and slope (Frontal-Parietal: 0.63 μV ms^{-1}, t=7.51, $P<0.001$) than parietal cortex. Responsiveness to command had no effect on the studied parameters, or on its interaction with condition or region stimulated (Table 3).

Figure 2 shows the effect of conditions and brain regions. For more detailed information about the values of amplitude and the other TEP indices, see Supplementary Tables S2 and S3. We also removed the two subjects who showed the strongest effects (z-score>3) to test robustness of our findings, which gave virtually the same results (see Supplementary Material). Finally, the general shape of the TEP changed in the late components (>30 ms) as shown in previous studies.[2,29]

Covariates of mean induced electric field, which summarise TMS pulse characteristics, and distance of the electrode from where TEP was taken, had a significant effect over slope. These effects are negligible and not informative for our purposes, as they were constant across conditions and had a smaller effect size than the effects of condition or brain region. They are reported in Supplementary Table S4. The coefficients of our main model are reported in Supplementary Table S5.

Discussion

We analysed changes in cortical excitability reflected in amplitude as a function of the depth of DEX sedation in 20 healthy subjects during four conditions (baseline, light sedation, deep sedation, and recovery). Cortical excitability at the sensor level has been reported to increase during unconscious states, such as deep NREM sleep or disorders of consciousness like the unresponsive wakefulness syndrome.[21,30] Thus, we expected amplitude to increase proportionally with the depth of sedation, being maximal during the deep sedation. Depth of sedation had a strong effect on amplitude, but the effect was only present in the frontal cortex, and was not higher in deep sedation compared with light sedation, when subjects were drowsy but still able to respond to a command. This is the first time that a non-linear evolution of cortical excitability has

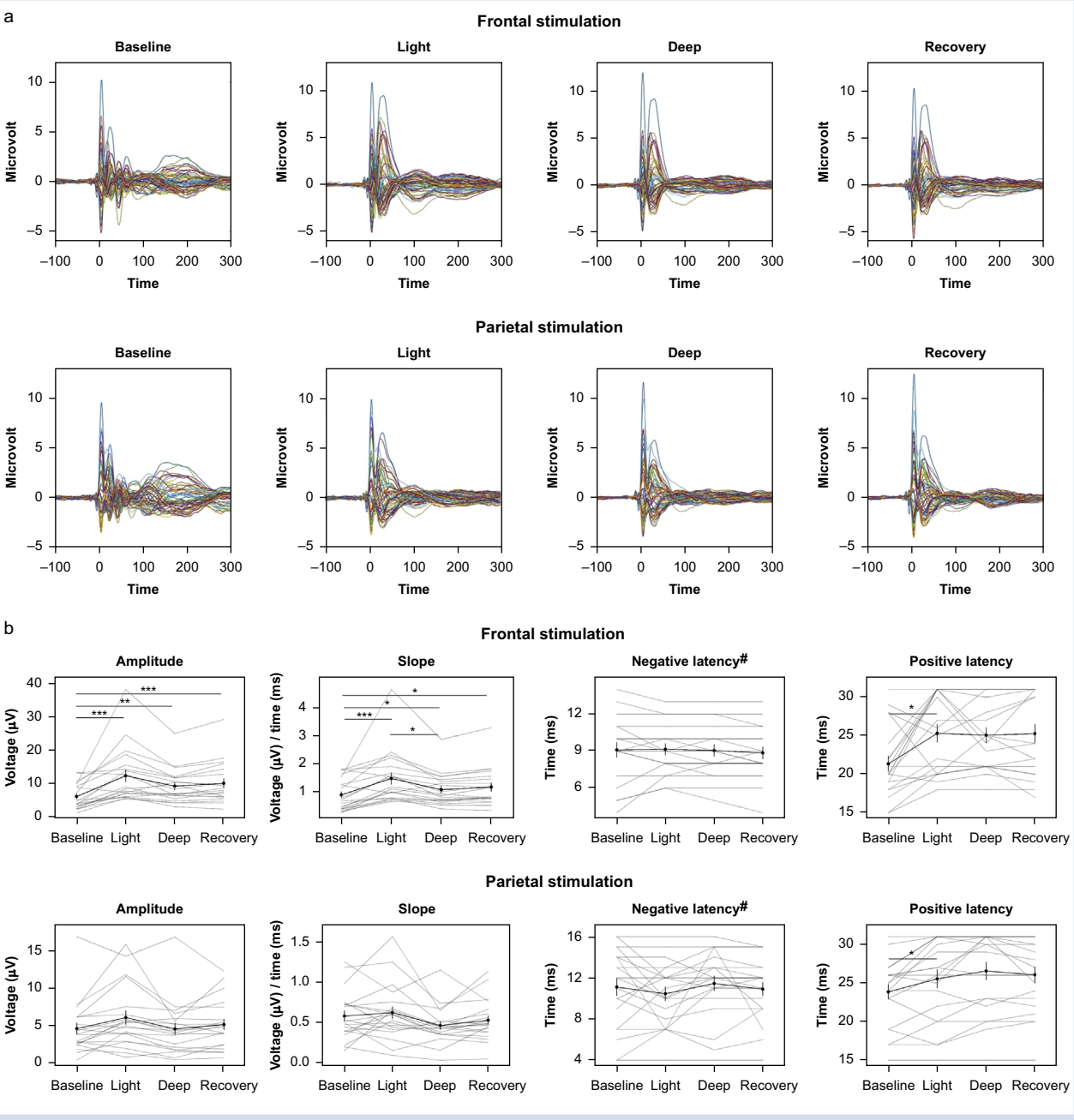

Fig 2. (a) Grand average of the transcranial magnetic stimulation-evoked potentials (TEPs) for all the subjects, divided by region (frontal and parietal) and the depth of sedation (baseline, light sedation, deep sedation, recovery). (b) Average (black line) and individual results (grey line) of amplitude (primary outcome), slope, latency of negative peak and latency of positive peak (secondary outcomes) for the four conditions (baseline, light sedation, deep sedation, recovery). Each condition is divided according to the region (frontal *vs* parietal). Error bars correspond to the standard error of the mean (SEM). The highest mean values of amplitude and slope are in the frontal cortex during light sedation. For the image without the subjects who displayed the strongest effect, see Supplementary Figure S1 and Supplementary Tables S6–S8. The asterisk represents the significant difference of negative latency between frontal and parietal cortex. *P<0.05, **P<0.01, ***P<0.001. The hashtag represents the main effect of the region over the negative latency.

been described under the action of an anaesthetic agent in a region-specific manner as a function of depth of sedation. Similar effects were seen for slope. It is important to note that our definition of cortical excitability is purely operational in this context, in that it refers to the amplitude of early TEPs, rather than to the nature of the underlying neuronal events. Various mechanisms could account for enhancement of early TEPs by DEX, including a stronger driving force in hyperpolarised postsynaptic neurones,[31] increased discharge synchrony of cortical populations[32] within cortical columns, reduction in synaptic depression,[33,34] and thalamic bursting triggered by the TMS-induced corticothalamic volley.

Table 3 *Post hoc* comparisons for amplitude and slope in the frontal cortex, and for positive latency over the depth of sedation conditions. Significant comparisons are represented in bold. Since amplitude is the primary endpoint, its $P_{critical}$ is set to 0.006, while for slope and positive latency $P_{critical}$ is 0.05.

Pairwise comparisons in the frontal region					
Measure	**Contrast**	**Estimate of the mean difference**	**Adjusted *P***	**95% Confidence interval**	
				Lower limit	**Upper limit**
Amplitude	**Baseline – Light**	**−6.52**	**<0.001**	**−9.85**	**−3.19**
	Baseline – Deep	**−4.55**	**0.003**	**−7.88**	**−1.23**
	Baseline – Recovery	**−5.03**	**<0.001**	**−8.19**	**−1.86**
	Light – Deep	1.97	0.08	−0.18	4.11
	Light – Recovery	1.49	0.13	−0.33	3.31
	Deep – Recovery	−0.47	0.23	−1.25	0.30
Slope	**Baseline – Light**	**−0.60**	**<0.001**	**−0.96**	**−0.25**
	Baseline – Deep	**−0.33**	**0.02**	**−0.63**	**−0.04**
	Baseline – Recovery	**−0.37**	**0.007**	**−0.69**	**−0.07**
	Light – Deep	**0.27**	**0.02**	**0.03**	**0.51**
	Light – Recovery	**0.24**	**0.02**	**0.03**	**0.44**
	Deep – Recovery	−0.04	0.26	−0.10	0.03
Pairwise comparisons across regions					
Measure	Contrast	Estimate of the mean difference	Adjusted *P*	95% Confidence interval	
				Lower limit	Upper limit
Positive latency	**Baseline – Light**	**−2.28**	**0.03**	**−4.42**	**−0.14**
	Baseline – Deep	−2.02	0.35	−5.00	0.95
	Baseline – Recovery	−2.06	0.23	−4.73	0.61
	Light – Deep	0.25	1.00	−1.33	1.84
	Light – Recovery	0.22	1.00	−1.26	1.70
	Deep – Recovery	−0.03	1.00	−0.90	0.83

The increase in cortical excitability recorded in the frontal cortex is in line with two recent studies of spontaneous conscious transition and TEPs.[22,35] In the first, amplitude of the TEP in the motor cortex increased during drowsiness, but did not change in unresponsiveness when participants were allowed to drift towards sleep during a detection task.[35] In the second study, amplitude of the TEP in the premotor cortex transiently increased during lapses of attention in a continuous attentive task after usual bedtime, compared with no-lapse periods.[22] These findings support the idea that reactivity of the frontal cortex is specifically altered in drowsy conditions where subjects might have impaired (but evident) behavioural responsiveness, as described here during light sedation with DEX. In support of this view, we observed higher amplitude in recovery (after regaining response to command) than in baseline. Arguably, during recovery, participants were in a state that was closer to light sedation than to baseline, and were still drowsy. In other words, our results extend with a pharmacological manipulation what has been reported in natural settings.[22,35] It is possible that these effects are specific to the sleep-like modulation by DEX and might not extend to other anaesthetics that are not α_2-adrenergic agonists. If it is an effect of the sedation *per se*, cortical excitability might be a novel index of drowsiness and sedation, the neural mechanisms of which should be investigated. However, the absence of an effect on the parietal cortex is peculiar, as there are several reports that highlight the role of parietal regions for the emergence of consciousness.[27] Future research should address this phenomenon in more detail, and regional differences in excitability[36–38] under DEX will require further mechanistic investigation.

Drug modulation of TMS-evoked responses are of paramount importance to the underlying neural dynamics of DEX, and to bridging neurochemical pathways, brain mechanisms, and behaviour. Of a number of studies that inferred cortical excitability with TMS looking at changes in resting state motor thresholds,[39,40] just a few observed TEPs.[35,41] A notable example is the study by Darmani and collegues,[41] who investigated the effect of different antiepileptic drugs on both TEP and motor-evoked potential. One issue is that TEPs (and in general evoked-responses) change from region to region,[36–38] as shown by the effect of the region over the negative latency (Fig. 2b). This is relevant, as TEPs might be modulated not only by the depth of sedation *per se* but also by changes of oscillatory activity (in a power-[38] or a phase-dependent[42] manner). In fact, depth of sedation affects spectral modification, in particular within the beta frequency band, which predicts responsiveness under anaesthesia[16] and wakefulness,[43] and in the alpha and delta bands which are modulated by DEX and state of consciousness.[15] Alpha and beta activity in *Rhesus* macaques after DEX anaesthesia differs between loss of consciousness, recovery of consciousness, and recovery of task performance to pre-anaesthesia levels.[44] Future investigations should focus on the relationship between responsiveness, natural oscillation, and TEPs.

The clinical relevance of cortical excitability is not yet clear. On one hand, it increases in NREM sleep (where there is no conscious experience) and in attentional lapses which correlates with error-making. On the other hand, protocols using

noninvasive brain stimulation directly rely on increasing cortical excitability to favour plastic phenomena.[45,46] Thus it is unclear whether it is optimal to increase or decrease cortical excitability to produce beneficial therapeutic effects. However, excitation/inhibition balance is of pivotal importance for normal functioning of the cortex.[47]

Our study shows cortical excitability changes in the frontal cortex with the same features present in almost all subjects, and a similar trend was seen for slope. However, there are still some relevant limitations. First, we recognise that there is high variability in the TMS response even if we observe a significant increase of amplitude overall (see Supplementary Figure S1 and Supplementary Tables S6–S8 for the same analysis without the participants with the strongest increase in cortical excitability). Second, we used a behavioural assessment for inferring consciousness. The absence of behavioural responses does not always coincide with unconsciousness,[48] and subjects can have relatively preserved higher-order cognitive processes (i.e. semantics) during loss of responsiveness due to DEX.[13] One could argue that consciousness assessment should be refined with bedside neurophysiological measurements. In the deep sedation condition, even if some patients were responsive, the DEX concentration in the blood was higher than expected. We considered responsiveness in our GLMM to account for different behavioural features, but the reason why some subjects were responsive in deep sedation while others were not is unclear. This could be affected by the accuracy of the model we used for TCI, and/or interindividual variability in sensitivity to DEX. The mechanisms that affect responsiveness to a drug should be approached in a systematic way. Given that brain dynamics[49] and spectral power[15] change after DEX administration in dose-dependent fashion, different patterns and biomarkers could be used to predict responsiveness. We observed that responsiveness had no effect on cortical excitability, so it cannot be used to predict responsiveness.

Another possible limitation is that we did not randomise the order of light and deep sedation. We cannot exclude that the greater excitability of light sedation is driven by an order effect. It is also possible that the stabilisation time (5 min) was not sufficient given the slow dynamics of the effect-site equilibrium. Another limitation is variability in electrode locations used to calculate cortical excitability between subjects and brain regions. However, we included the distance of the electrode in the statistical models. It is challenging to have longitudinal recording of TMS-EEG in subjects under sedation, as in our current protocol. Future studies should ideally use the same electrode for all the subjects. Additionally, we did not have any free recall after the sessions. This could have helped to understand the status of subjects, even when they did not show response to verbal command. Comparison with other drugs might elucidate possible underlying dynamics, which is relevant to understand which neuronal pathways are important in changing cortical excitability and what its links are to sedation in a drug (in)dependent manner. Finally, as cortical excitability is a complex property, future investigations should explore in more detail its neurophysiology. Increased excitability typically indicates that neurones respond more to a given input. We analysed EEG components, which can be ambiguous. As discussed by Huber and colleagues,[18] increased slope and amplitude can occur due to increased synaptic strength, neuronal bursts, or membrane hyperpolarisation, leading to stronger driving force. While the first two could be interpreted as increased excitability, the third option reflects a larger deflection of the membrane potential starting from more hyperpolarised levels but reaching the same depolarisation and firing level (reflected in a larger EEG response). The latter is likely to happen, together with bursting after DEX administration.

In conclusion, we provide evidence of non-linear evolution of cortical excitability after DEX administration. Sedation by DEX increases local cortical excitability in a region-specific manner, but cortical excitability does not differentiate between sedation levels. In particular, we found no statistically significant difference between light sedation, deep sedation, and recovery in amplitude. This is in line with recent findings that describe abnormally high cortical excitability during drowsiness in natural settings. Interestingly, the effect was present only in the frontal cortex. These results foster new questions for investigations into the nature of sedation and drowsiness that will result in a deeper understanding of cortical dynamics during anaesthesia.

Authors' contributions

Study design: OB, VB, SL, RS
Data collection: OB, SW, MK, JS, AV, VB, OG.
Data analysis: PC, VB.
Data interpretation: all authors
Manuscript draft: PC, with the help of OG and VB.
All authors revised the manuscript critically for important intellectual content and gave final approval of the revised manuscript.

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Declarations of interest

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Doctoral School for Healthy Sciences (University of Liège). OG is a research associate, GV is a senior research associate, and SL is research director at the F.R.S-FNRS.

Appendix A. Supplementary data

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