Brain oscillations and frequency-dependent modulation of cortical excitability

Dennis J. L. G. Schutter, Ruud Hortensius

Experimental Psychology, Utrecht University, Utrecht, The Netherlands

Background
Noninvasive brain stimulation is a powerful way to modify excitability of the cerebral cortex in humans and is increasingly used to treat psychiatric disorders. The observed clinical effects are in the moderate range and it has been suggested that the efficiency of brain stimulation depends on the underlying cortical state.

Objective
To isolate and manipulate brain rhythms associated with cortical excitability.

Methods
In the first experiment electroencephalography (EEG) and transcranial magnetic stimulation (TMS) were interleaved to study associations between brain oscillations and the amplitude of the motor evoked potential (MEP) during isometric contraction. Results of the first experiment were used in a second experiment to selectively modulate cortical excitability levels by applying transcranial alternating current stimulation (tACS).

Results
A linear regression showed that MEP amplitude could be modeled by $\theta$ (4-7 Hz) and $\beta$ (13-30 Hz) oscillations recorded over the left and right M1. Significant increases in cortical excitability were found after $\theta$ (5 Hz)-$\beta$ (20 Hz) tACS as compared with baseline and $\alpha$ (10 Hz) tACS.

Conclusions
Scalp-recorded brain oscillations can serve as a proxy for the effective modulation of cortical excitability by mimicking natural brain rhythms using weak electric currents.

© 2011 Elsevier Inc. All rights reserved.

Keywords brain oscillations; cortical excitability; electroencephalogram; motor cortex; motor evoked potential; transcranial alternating current stimulation; transcranial magnetic stimulation

This work was supported by an Innovational Research Grant (VIDI 452-07-012) from the Netherlands Organization for Scientific Research (NWO). Correspondence: Dr. Dennis J. L. G. Schutter, Experimental Psychology, Utrecht University, Heidelberglaan 2, Utrecht 3584CS, The Netherlands. E-mail address: d.schutter@uu.nl
Submitted December 23, 2009; revised July 9, 2010. Accepted for publication July 9, 2010.

1935-861X/S - see front matter © 2011 Elsevier Inc. All rights reserved.
The discovery that noninvasive brain stimulation technique such as transcranial magnetic stimulation (TMS) and electric current stimulation can excite nerve cells and modify excitability levels in the cerebral cortex led to new clinical applications in the treatment of psychiatric disorders.\(^1\)\(^2\) Particularly, TMS has been systematically explored as a relative painless neuromodulation technique in the treatment of major depressive disorder. Despite its moderate therapeutic efficacy, the effects of TMS are nonetheless similar to the effects found with antidepressant medication.\(^3\)\(^4\) Part of the explanation as to why TMS is moderately effective may have its root in the varying degree to which noninvasive brain stimulation techniques are able to produce stable and clinically relevant therapeutic effects. Although the effects of TMS are likely to be influenced by the underlying electrophysiology of the target regions relative few studies have investigated the role of brain oscillations in the effects of TMS on cortical excitability levels.\(^5\)\(^6\) Rhythmic activity reflects the synchronization of oscillations of large neuronal populations in a particular frequency range and constitutes an important organizing principle of brain function.\(^6\) Repeating the initial findings of Zarkowski and colleagues,\(^7\) Sasseng and colleagues\(^8\) observed an inverse relationship between the presence of spontaneous alpha (8-12 Hz) oscillations and cortical excitability in the motor cortex during rest. More recently, associations between rolandic midrange beta (12-18 Hz) oscillations and motor cortex excitability were taken as further evidence for a connection between spontaneous fluctuations in electric brain activity and cortical excitability.\(^9\) It is therefore reasonable to assume that the TMS related modulation of neural excitability critically depends on the presence of specific brain oscillations generated by the target tissue and associated regions.\(^8\)\(^10\) In support of this notion, recent evidence shows that the application of weak alternating currents is able to modulate visual cortex excitability\(^11\) and influence motor behavior in a frequency dependent manner.\(^12\) In contrast, single sessions of transcranial alternating current stimulation (tACS) at 1, 10, 15, 30, and 45 Hz to the primary motor cortex did not result in strong changes in motor-evoked potential (MEP) amplitude.\(^13\) Apart from the low stimulation intensity and the relative short stimulation duration (2-5 minutes) applied in this study, tACS at a single frequency may be less effective in altering motor cortex excitability. However, recent findings suggest that when stimulating at higher intensities, a significant reduction in cortical excitability can be observed after 20 minutes of bilateral 15 Hz tACS over the primary motor cortex.\(^14\) Another possible way of increasing the efficacy of tACS is to isolate the brain rhythms associated with cortical excitability to determine the optimal stimulation frequency.\(^6\)\(^15\)

The aim of the first experiment was therefore to study the interrelations between brain oscillations and cortical excitability. To that end, we simultaneously recorded brain oscillations from the scalp using the electroencephalogram (EEG) and MEP amplitude to single-pulse TMS over the primary motor cortex (M1) during isometric contractions of the contralateral hand. In the second experiment, we used the results from the first experiment to apply tACS in a frequency dependent manner to augment cortical excitability.

### Materials and methods

#### Experiment 1

**Participants**

Eight healthy volunteers aged from 21 to 31 years (mean age ± SD, 24 ± 3.39 years) participated in this experiment. Volunteers had no psychiatric or neurologic history and no contraindications for TMS as confirmed by safety screening.\(^10\) Except for the three women using oral contraceptives, all subjects were medication free. Written informed consent was obtained. The experiment was approved by the local ethics committee.

**Electroencephalogram**

A nine channel bilateral mastoid referenced EEG recording was obtained by using TMS-compatible Ag/AgCl-tipped active electrodes filled with Parker Spectra 360 electrode gel (Parker Laboratories, Fairfield, NJ). Electrodes (F3, F4, Fz, C3, Cz, C4, P3, Pz, and P4) were positioned according to the International 10/20 EEG system. Recordings were made relative to common mode sense (CMS) with the ActiveTwo system (BioSemi, Amsterdam, The Netherlands). A sampling rate of 256 Hz (bandwidth [3 dB]: 52 Hz) was used and data were stored for offline analysis. The ActiveTwo system allows extremely low-noise recordings free of interference. The ground consists of a feedback loop between the active CMS and passive driven right leg (DRL) electrode driving the subject’s average potential as close as possible to the analog-to-digital converter reference voltage in the A/D-box. As a result no impedance measurements or gain adjustments are needed with the ActiveTwo system (www.biosemi.com).

**Electromyogram**

Electromyogram (EMG) signals were recorded with active Ag-AgCl electrodes using a second ActiveTwo system (BioSemi) from the right abductor pollicis brevis (APB). Parker Spectra 360 electrode gel (Parker Laboratories) was used as a conducting medium. A belly-tendon montage was used with the active electrode placed at the muscle belly of the right APB, the reference electrode located at the proximal phalanx of the thumb, and the CMS-DRL electrodes attached to the wrist. A sampling rate of 2048 Hz (bandwidth [3 dB]: 417 Hz) was used.

**Single-pulse TMS**

Single suprathreshold pulses were delivered to the left primary motor cortex with a biphasic Neopulse magnetic brain stimulator with a maximum output of 4160 A peak/1750 VAC peak using a modified eight-shaped iron core coil (Neotonus, Atlanta, GA). The interstimulation interval
Brain oscillations and cortical excitability

(ISI) was between 0.1 and 0.2 Hz and stimulus intensity was set at 120% MT. The angle of the Neotonus iron-core coil to the mid-sagittal line was 90° and the induced electric current in the brain flowed in an anterior-to-posterior direction.

Procedure

Before the experiment participants were screened for contraindications to TMS,16 and handedness was assessed using the Edinburgh Handedness Inventory (mean ± SD group: 39.25 ± 16.11).17 On arrival at the laboratory the participants were explained to the subjects and informed consent was obtained. Volunteers were seated in a chair with their arms placed on a table with the palm of the hand facing upward and their head placed in a chin-rest. For effective stimulation during testing, a custom-made plastic tube of 2 cm in length was attached underneath the coil to correct for the coil-electrode-scalp distance. The distance corrected resting motor threshold (MT) of the left hemisphere (mean ± SD, 79.63 ± 6.32) was determined in accordance with the standardized visual thumb movement procedure.18 First, the coil was placed halfway the vertex and ear with a starting intensity of 40% and an ISI of 0.2 Hz. The coil was moved in different directions and TMS intensity was gradually increased to find the site that most reliably elicited thumb twitches. Next, the intensity was decreased until 5 of 10 consecutive pulses (100% MT) resulted in a MEP. After this initial step, the coil was again moved over the scalp to search for other sites (100% MT) that most reliably elicited thumb twitches. For effective stimulation during testing, a custom-made plastic tube of 2 cm in length was attached underneath the coil to correct for the coil-electrode-scalp distance. The distance corrected resting motor threshold (MT) of the left hemisphere (mean ± SD, 79.63 ± 6.32) was determined in accordance with the standardized visual thumb movement procedure.18 First, the coil was placed halfway the vertex and ear with a starting intensity of 40% and an ISI of 0.2 Hz. The coil was moved in different directions and TMS intensity was gradually increased to find the site that most reliably elicited thumb twitches. Next, the intensity was decreased until 5 of 10 consecutive pulses (100% MT) resulted in a MEP. After this initial step, the coil was again moved over the scalp to search for other sites that exceeded the 50% thumb movement criterion and TMS intensity was decreased so that 5 of 10 consecutive pulses resulted in a MEP.18,19 The site in which the lowest intensity was needed for the 50% thumb movement criterion was used for single pulse TMS. The site (“hot spot”) was marked on the scalp using a felt pen. Next, EMG and EEG electrodes were attached according to the International 10-20 System. The “hot spot” corresponded to within a 1 cm radius of the C3 electrode. The iron core coil was fixated using a mechanical arm and the coil-scalp distance of 2 cm was kept constant throughout the experiment. Subjects were instructed to relax, close their eyes, and make no head movements and were monitored during the experiment. To obtain a relative constant state of alertness of the subjects and to lower the threshold for obtaining a MEP to single-pulse TMS participants were instructed to make an isometric contraction between the thumb and index finger at 30% ± 5% of maximum force before the TMS pulse. Participants received oral feedback from the experimenter to adjust their contraction before the pulse if necessary. Distance corrected TMS intensity was set at 120% MT (mean maximum machine output ± SD, 94% ± 7.17). Sixty single pulses of TMS whereas simultaneously recording EEG and EMG signals were given. Total duration of single pulse TMS was 5 minutes. Figure 1 shows a schematic illustration of the combined TMS-EEG setup.

Participants were naïve to the aim of the study. Stimulation parameters were in accordance with the International Federation of Clinical Neurophysiology safety guidelines.20

Data reduction and analysis

EEG signals were segmented offline into 1 second bins from 1010 until 10 milliseconds prior the pulse. Epochs were band-pass filtered (1-30 Hz, roll-off: 24 dB/octave) and trials with amplitudes exceeding ± 50 μV were removed. Spectral amplitudes (μV) in the δ (1-3 Hz), θ (4-7 Hz), α (8-12 Hz), and β (13-30 Hz) frequency bandwidths were estimated by using a fast Fourier transformation (Hanning window: length 10%). Mean amplitude for the δ, θ, α, and β frequency range was calculated for each single trial over the eight participants.7 EMG signal was high-pass filtered (3 dB cutoff frequency: 20 Hz, roll-off: 24 dB/octave filter). Isometric prepulse EMG was epoched from 1010 milliseconds until 10 milliseconds before the TMS pulse as the recording signal around pulse onset often contained noise. The rectified MEP amplitude was quantified as the peak-to-peak amplitude of the maximal EMG response after single-pulse TMS. Mean isometric prepulse EMG activity and MEP were calculated for each single trial over the eight participants.7 The designation of an artifact in one of the recordings resulted in removal of that epoch for all records to ensure the remaining data were identical for all sites in time. Mean ± SD number of included trials across the participants was 53.25 ± 7.81.

To examine the relations between brain oscillations and cortical excitability, a stepwise linear regression analysis (method: probability of F to enter < .05, probability of F to enter > .1) was used with MEP as the independent variable.

Figure 1 Single-pulse TMS over the left primary motor cortex to record the MEP from the right APD and scalp locations from which EEG was recorded 1 second before TMS pulse onset.
and mean spectral amplitude in the δ, θ, α, β, and frequency bands recorded at the C3 and C4 electrode and isometric prepulse EMG entered as predictor variables. The α level of significance was set at .05 (two-tailed).

Results

Step-wise linear regression analyses showed that, in addition to electromyographic activity resulting from the isometric right-hand contraction, MEP amplitude of the right APB muscle in response to single pulse TMS over left M1 could be modeled by θ (4-7 Hz) and β (13-30 Hz) oscillations recorded over the left and right M1, $F(5, 4) = 3.70; P = .006, R^2 = 0.26$. Table 1 shows the standardized beta coefficients for each predictor.

Experiment 2

Participants

Six healthy subjects (three females) aged between 21 and 28 years (mean ± SD, 23.33 ± 2.94 years) participated in the experiment. Three subjects had also participated in the prior experiment. Volunteers had no psychiatric or neurologic history and no contraindications for TMS as confirmed by safety screening. None of the volunteers had damaged skin tissue or a skin disease. Except for the three women using oral contraceptives, all subjects were medication free. Written informed consent was obtained. The experiment was approved by the local ethics committee.

Electromyogram and single-pulse TMS

Settings and procedure for EMG and single-pulse TMS were identical to experiment 1.

tACS

Stimulation was given with a battery-driven Eldith DC-stimulator Plus (NeuroConn GmbH, Ilmenau, Germany) through conductive-rubber electrodes placed in sponges saturated with tap water and Parker Spectra 360 electrode gel (Parker Laboratories). Both electrodes were 5 × 7 cm and were fixed with two rubber bands on the scalp. A bipolar electrode montage was used. One electrode was placed over left M1 (C3) and the second electrode was placed over right M1 (C4) in accordance with the International 10-20 EEG system (Figure 2). Stimulation was sinusoidal and current intensity was set at 1000 μA with a maximum current density of 28.57 μA/cm² and a total charge of 0.017 C/cm². Intensity was ramped up and down for 1 second and impedance was < 10 kΩ.

Procedure

In this single-blind within-subjects counterbalanced design θ-β and α-α tACS were applied in separate sessions. The two sessions were separated by at least 24 hours to exclude carryover effects and took place at the same time of day. In the θ-β tACS, condition stimulation frequency was set at 5 Hz (5 minutes), followed by 20 Hz (5 minutes). In the α-α tACS, condition stimulation frequency was set at 10 Hz (10 minutes). Half-way during θ-β tACS, the frequency parameter was changed from 5 to 20 Hz. Stimulation was briefly stopped to change the frequency setting. To minimize procedural differences between sessions, the procedure was identical across sessions, except for adjustments in stimulation frequency in the α-α tACS condition.

Before the start of the first session, participants were screened for contraindications and their handedness was assessed using the Edinburgh Handedness Inventory (mean ± SD, 45.4 ± 2.61). After explaining the procedure, informed consent was obtained and volunteers were seated in a comfortable chair. MT was assessed by means of the standardized visual thumb movement (see Experiment 1). The MT did not differ between the θ-β (mean ± SEM, 46.67 ± 1.12) and α-α tACS session (mean ± SEM, 45.83 ± 1.33), $t(5) = 0.75, P = .49$. The position was marked with a felt pen on the scalp to ensure identical placement of the coil throughout the experiment. Next, EMG electrodes were attached. Preceding tACS baseline cortical excitability was determined by the averaged MEP amplitude (MEPbaseline) to 20 single-pulses at 120% MT to left M1. During tACS, the room was dimly lit and participants were

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Standardized beta coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predictor</td>
<td>Standardized beta coefficient</td>
</tr>
<tr>
<td>EMG</td>
<td>0.244</td>
</tr>
<tr>
<td>Left M1 θ</td>
<td>0.302</td>
</tr>
<tr>
<td>Left M1 β</td>
<td>−0.313</td>
</tr>
<tr>
<td>Right M1 θ</td>
<td>−0.261</td>
</tr>
<tr>
<td>Right M1 β</td>
<td>0.429</td>
</tr>
</tbody>
</table>

Figure 2 Experimental tACS setup targeting the left (C3) and right (C4) primary motor cortex (M1).
seated in a comfortable chair, instructed to relax, and keep eyes open. Twenty MEPs to single-pulse TMS at 120% MT were collected 5 minutes (MEP<sub>post5</sub>) and 15 minutes after tACS (MEP<sub>post15</sub>). The stimulating coil was held in place by the experimenter over the left M1. Quality of the EMG signal during tACS was monitored by the experimenter in an adjacent control room throughout the experiment. Interactions between experimenter and participant were kept to a minimum throughout the experiment. Participants were naive to the aim of the study. Stimulation parameters were set according to the International Federation of Clinical Neurophysiology safety guidelines.  

Data reduction and analysis

EMG signal was offline high-pass filtered (3 dB cutoff frequency: 20 Hz, roll-off: 24 dB/octave). The rectified MEP amplitude was quantified as averaged peak-to-peak amplitude of the maximal EMG response after single-pulse TMS. Mean MEP<sub>baseline</sub> amplitude was set at 100%. MEP<sub>post5</sub> and MEP<sub>post15</sub> values were determined by calculating the percentage change from baseline MEP amplitude.

A general linear model (GLM) for repeated measurements with tACS (2) and time (2) as within subject factors was applied to investigate the anticipated increase in MEP amplitude after 0-β tACS as compared with α-α tACS. One and paired samples t tests were performed to examine changes to baseline and changes in cortical excitability after 0-β tACS as compared with α-α tACS. The α level of significance was set at .05 (two-tailed).

Results

Experiment 2

The GLM for repeated measurements demonstrated a significant main effect of tACS on MEP amplitude, F (1,5) = 8.11; P = .04. Even though no main effect of time on MEP amplitude was observed, F(1,5) = 0.18; P = .69, the tACS x time interaction reached significance, F(1,5) = 7.31; P = .04. Post hoc tests demonstrated a significant increase to baseline in MEP amplitude 5 minutes after 0-β tACS, t(5) = 3.11; P = .03. Furthermore, a significant difference was found between the 0-β and α-α tACS 5 minutes after stimulation, t(5) = 3.44; P = .02. The increase in MEP amplitude was no longer significantly different from baseline 15 minutes after 0-β tACS t(5) = 2.24; P = .08, neither was the difference between 0-β and α-α tACS t(5) = 0.94; P = .39. Finally, no baseline or time-related changes in MEP amplitude were found in the α-α tACS condition (both P values >.17). Figure 3 illustrates the baseline and time course of the relative change in MEP amplitude after 10 minutes of 0-β and α-α tACS.

Discussion

Results from the first experiment show that cortical excitability of the left primary motor cortex (M1) as indexed by the MEP from the right APB to magnetically induced activation of the left M1 depends on peripheral muscle activity tonus and electric activity recorded from the ipsi- (C3) and contralateral (C4) M1 in the θ (4-7 Hz) and β (13-30 Hz) frequency range. These findings suggest that in addition to general activation of the corticospinal tract, cortical networks comprised of the left and right M1 oscillating in the θ and β frequency range seem to contribute to local levels of cortical excitability.

The current findings seem at variance with an earlier TMS study that found an inverse relationship between α oscillations and motor cortex excitability.  

Prior TMS research by Sauseng and colleagues has found additional evidence to suggest that the 10 Hz rhythm originate in the postcentral cortex is more related to sensory processes, whereas the 20 Hz rhythm is localized in the anterior part of the central sulcus and is more closely tied to motor functions. In our study, participants kept their eyes closed and were instructed to make isometric contractions, whereas in the former study participants kept their eyes open and were instructed to relax. In other words, the relation between brain oscillations and cortical excitability in the previous study may have been more sensory driven, whereas the relations observed in the current study may have been more motor driven. In agreement with this notion, earlier work on this topic has found that beta-oscillations are associated with the sensorimotor system during isometric contraction. Another study has found a relation between the theta frequency band and voluntary tremor. These studies may point toward theta and beta band driven networks dedicated to motor control.

In sum, even though our results are in good agreement with several other studies, our findings and the findings by Sauseng and colleagues are necessarily mutually exclusive.

Figure 3 Frequency-dependent increase in cortical excitability after (5 Hz)-β(20 Hz) tACS applied to the ipsi- (C3) and contralateral M1 (C4). Post5: 5 minutes after tACS, post15: 15 minutes after tACS.
To determine whether the oscillations as found with the linear regression model represent a genuine electrophysiologic correlate of cortical excitability, we performed a second pilot experiment in which we applied 5 Hz (θ) and 20 Hz (β) tACS at 1 mA intensity over left and right M1 and successfully increased cortical excitability.

The ability of weak electric currents to alter cortical excitability is suggested to involve modification of the balance between inhibitory and excitatory processes in the cerebral cortex. Interestingly, brain oscillations in the θ frequency range have been associated with long-term potentiation that is considered an important synaptic phenomenon underlying increased excitability of nerve cells.

Analogous to our findings, a newly developed TMS paradigm called θ burst stimulation has proven highly effective in augmenting neuronal excitability by applying trains of 50 Hz TMS pulses to the cortex in a 5 Hz repetitive fashion. It should, however, be mentioned that depending on duration of the trains one finds either increases (i.e., intermittent TBS) or decreases in cortical excitability (i.e., continuous TBS). Indeed, the current tACS stimulation would fall in the cTBS category and our effects are opposite of what is found for cTBS. However, an important difference between cTBS and the current tACS lies in the fact that in the cTBS condition trains of three pulses are given at 5 Hz rate, whereas in our tACS paradigm one sinusoidal pulse at 5 Hz rate was given.

Furthermore, pretreatment with stimulation in the 5-6 Hz (θ) range has shown to significantly increase the effects of subsequent stimulation. In agreement, a previous study demonstrated increased inhibitory effects of 1 Hz repetitive TMS after priming stimulation in the θ frequency range (i.e., 4-8 Hz). Similar to the TMS study by Ivry and colleagues tACS in the θ frequency range may have acted as a primer for subsequent TACS in the β frequency range. Together with the notion that TMS studies have shown that stimulation frequencies around 20 Hz augment cortical excitability, the applied θ-β tACS protocol may explain the increase in cortical excitability observed in the second experiment. Further studies have to examine whether changing the order of stimulation frequency yields different effects.

In addition, β activity is a well-studied brain rhythm of the primary motor cortex. Previous work has shown the presence of β oscillations correlates to cortical excitability levels and control processes in the motor system that involves bilateral hemispheres. A recent study found that midrange-β (15-18 Hz) oscillation amplitudes differed between small and large MEPs, with weak midrange-β activity preceding large MEPs. This present study extends the relation between β oscillations and MEPs to the contralateral hemisphere. Oscillations in the β range have been repeatedly linked to processes associated with active inhibition of neural activity, suggesting that the presently observed increase in cortical excitability may be linked to the induction of ipsi- and contralateral inhibitory postsynaptic currents on inhibitory interneurons rather than excitatory neurons. Finally, despite frequency-dependent tACS proving effective in the modulation of cortical excitability, low intensity, and brief stimulation period could explain the short duration of the effect.

In sum, ipsi- and contralateral θ and β oscillations may signify different electrophysiologic properties involved in the balance between inhibitory and excitatory processes in the cerebral cortex underlying neural excitability.

The obvious question then is why we did not study the contributions of each frequency separately? The tACS protocol in the second pilot experiment was based on the outcome of the linear regression analysis described in the first experiment. The regression analysis identified two frequency bandwidths involving both motor cortices. Particularly, it is the unique combination of dependent variables that is associated with cortical excitability. We speculate that the currently observed increase in cortical excitability is the net outcome of the combined frequency stimulation approach. For example, high-frequency stimulation superimposed on a θ rhythm has proven effective in increasing cortical excitability levels, and suggests that more complex oscillation patterns may be more powerful in changing cortical excitability. A logical next step is to look more closely at the contributions of each frequency and to see whether combining frequencies as was done in the second experiment yield additive or possibly even opposite results as compared with the effects of single-frequency tACS on cortical excitability levels (for a comparison see ref. 14).

Finally, as a result of the alternating current stimulation setup, we could presently not study the functional relevance of the signs of the regression coefficients with respect to the contributions of θ and β oscillations in explaining cortical excitability. Further research is needed to determine whether the positive or negative relations to cortical excitability carry any physiologic significance.

In conclusion, the current results provide preliminary evidence that intra- and interhemispheric cortical circuits resonating at distinct frequencies contribute to local excitability levels and suggest that mimicking natural brain rhythms using weak oscillating electric currents may be an alternative approach for modulating cortical excitability. These findings may contribute to the development of EEG-based stimulation protocols to study cortical physiology and treat psychiatric disorders using weak alternating electric currents.

References


