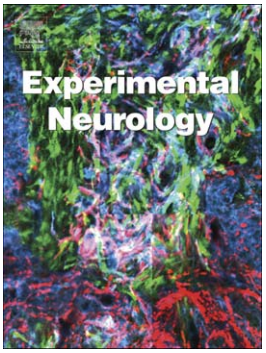

A neurophysiological insight into the potential link between transcranial magnetic stimulation, thalamocortical dysrhythmia and neuropsychiatric disorders

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Title page

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A neurophysiological insight into the potential link between transcranial magnetic stimulation, thalamocortical dysrhythmia and neuropsychiatric disorders

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Abstract

Altered neural oscillations and their abnormal synchronization are crucial factors in the pathophysiology of several neuropsychiatric disorders. There is increasing evidence that the perturbation with an abnormal increase of spontaneous thalamocortical neural oscillations lead to a phenomenon termed Thalamocortical dysrhythmia (TCD) which underlies the symptomatology of a variety of neurological and psychiatric disorders including Parkinson's disease, schizophrenia, epilepsy, neuropathic pain, tinnitus, major depression and obsessive-compulsive disorder. In addition, repetitive transcranial magnetic stimulation (rTMS) is a non-invasive neurophysiological tool that has been shown to both induce a modulation of neural oscillations and alleviate a wide range of human neuropsychiatric pathologies. However, little is known about the precise electrophysiological mechanisms behind the therapeutic effect of rTMS and its potential to improve abnormal oscillations across diverse neuropsychiatric disorders. Here we show, using combined rTMS and surface electroencephalography (EEG), a short lasting frequency-dependant rTMS after-effect on thalamocortical rhythmic interplay of low-frequency oscillations in healthy humans at rest. In particular, high-frequency rTMS (10 Hz) induces a transient synchronised activity for delta and theta rhythms thus mimicking the pathological TCD-like oscillations. In contrast, rTMS 1 and 5 Hz have the opposite outcome of de-synchronising low-frequency brain rhythms. These results lead to a new neurophysiological insight of basic mechanisms underlying neurological and psychiatric disorders and a probable electrophysiological mechanism underlying the therapeutic effects of rTMS. Thus, we propose the use of rTMS and EEG as a platform to test possible treatments of TCD phenotypes by restoring proper neural oscillations across various neuropsychiatric disorders.

Keywords: resting-state neural oscillations; brain stimulation; oscillatory brain activity; pathophysiology; frequency dependent effects of rTMS; cortical excitability

Introduction

Neural oscillations are thought to be an essential mechanism that enables the coordination of neural activity in normal brain functioning (Buzsaki and Draguhn, 2004). An emerging electrophysiological approach is considering the fundamental role of abnormal neural oscillations in the low delta (δ) and theta (θ), and high beta (β) and gamma (γ) frequency ranges observed in patients at rest or while performing a task, to explain the pathophysiology of neuropsychiatric disorders (Jeanmonod, et al., 2003, Llinas, et al., 1999, Schulman, et al., 2011, Uhlhaas and Singer, 2010). Evidence from single cell physiology, Electroencephalography (EEG) and Magnetoencephalography (MEG) studies demonstrate that a common link among a wide range of neuropsychiatric disorders is the perturbation of the thalamocortical resonance known as Thalamocortical dysrhythmia (TCD) (Jeanmonod, et al., 2003, Llinas, et al., 1999, Sarnthein and Jeanmonod, 2007, Sarnthein and Jeanmonod, 2008, Sarnthein, et al., 2003, Schulman, et al., 2011). The idea behind TCD is that persistent abnormal internally-generated δ and θ oscillations in thalamic neurons disrupt the normal, state-dependent, flow of information within the thalamo-cortico-thalamic network. This increased spontaneous activity at low-frequency oscillations (LFOs), while awake and at rest, leads to disturbances of sensation, motor performance and cognition observed in a number of disorders including Parkinson's disease, schizophrenia, epilepsy, neuropathic pain, tinnitus, major depression and obsessive-compulsive disorder (Jeanmonod, et al., 2003, Jones, 2010, Llinas, et al., 1999, Schulman, et al., 2011, Walton, et al., 2010, Whitwell, et al., 2011, Zhang, et al., 2009).

Transcranial magnetic stimulation (TMS), with rapidly oscillating magnetic fields administered by a coil positioned on the scalp, allows non-invasive stimulation

of the human brain. It is well documented that repetitive TMS (rTMS) is relatively safe with a few cases of seizures associated primarily with its use at higher frequencies (i.e., 10 Hz or above, (Rossi, et al., 2009). Moreover, rTMS has proved to have a therapeutic role improving symptoms of various neuropsychiatric disorders (Dell'osso, et al., 2011, George, et al., 1999, Groppa, et al., 2012, Hallett and Rothwell, 2011, Rossini and Rossi, 2007, Wassermann and Zimmermann, 2012, Ziemann, 2011). However, the optimal parameters of magnetic stimulation remain elusive (Hallett and Rothwell, 2011, Ridding and Ziemann, 2010, Wu, et al., 2008), and little is known about the electrophysiological mechanisms that underlie the beneficial effect of rTMS and the exact nature of neural effects induced by TMS (Allen, et al., 2007, Hoogendam, et al., 2010, Miniussi and Thut, 2010). Finally, to our knowledge, there is no study which has investigated the probable link between rTMS after-effects and the TCD phenomenon.

Repetitive TMS can be used in basic research to study how perturbations in activity in a focal brain area affect the neural network oscillations (Hampson and Hoffman, 2010). Previous electrophysiological studies, that investigated the functional role of brain rhythms modulated through brain stimulation in humans, have consistently shown a synchronization of high-frequency alpha (α) and β rhythms, but they could not differentiate any effects for low- and high-frequency rTMS on cortical oscillations (Brignani, et al., 2008, Fuggetta, et al., 2008, Veniero, et al., 2011). It is possible that a differential rTMS frequency-dependent effect on the modulation of neural oscillations could be better reflected by the other low frequency bands as delta (δ) and theta (θ) rhythms. Thus, investigating the functional role of oscillatory brain activity in specific frequency bands after magnetic stimulation can provide insight

into the physiology of brain rhythms, and the possible role of brain oscillations in neuropsychiatric disorders (Buzsaki and Draguhn, 2004).

The general purpose of this electrophysiological study was to gain new insight into the basic neural circuitry that sustains the abnormal alteration of dynamic brain oscillations observed in neurological and psychiatric disorders under the umbrella term of TCD. The results of this investigation will also contribute to enhance the understanding of the probable electrophysiological mechanism underlying the therapeutic effects of rTMS in a variety of neuropsychiatric patients. To achieve this, the effect of rTMS induced modulation of oscillatory activity was compared with the abnormal synchrony of LFO's observed in TCD. The hypothesis that, in healthy participants at rest, a transient effect of synchronisation or de-synchronisation of LFO's can be externally induced depending on the frequency of rTMS used will be tested. The resolution of this matter is of considerable interest because it may suggest new ways to use combined EEG/TMS techniques as both a diagnostic test and therapeutic tool to restore proper neural oscillations in neuropsychiatric disorders. To address this issue, we manipulated different frequencies of rTMS (1 Hz, 5 Hz and 10 Hz) and used surface EEG to quantify the cortical oscillatory activity post-rTMS.

Several lines of evidence support the use of the aforementioned frequencies of rTMS in the current study. Firstly, a review of clinical trials which have assessed the therapeutic effects of rTMS in Parkinson's disease (PD), shows that rTMS parameters have been varied widely in terms of cortical target, frequency, intensity, and duration, with a common clinical benefit measured with the Unified Parkinson's Disease Rating Scale (UPDRS) motor subscale scores (Wu, et al., 2008). However, how to adjust rTMS parameters individually for the most beneficial effects on symptoms of PD remains unclear. While holding other parameters constant and varying the frequency

of rTMS over M1, previous studies have demonstrated beneficial effects of rTMS, with both low (0.5 Hz, 5 Hz) and high (10 Hz, 25 Hz) frequencies, on motor symptoms in PD patients (Lefaucheur, et al., 2004, Wu, et al., 2008).

Recently a quantitative review was conducted in order to examine the efficacy of slow-frequency rTMS to the frontal cortex in major depressive (MD) disorder and to compare slow and fast-frequency stimulation. The results did not show any significant difference between the two procedures. The author's concluded that slow-frequency rTMS was as effective as fast frequency and generally better tolerated, allowing for longer and safer stimulation periods (Schutter, 2010).

Given that several rTMS frequencies have been shown to have positive effects in clinical studies of both PD and MD patients (Dell'osso, et al., 2011, Wu, et al., 2008), it is important to begin to distinguish among those frequencies commonly used in clinical research. To this end, we have chosen to focus on 1 Hz, 5 Hz, and 10 Hz in an attempt to determine which is most effective in modulating the neural oscillations of the motor cortical-subcortical loop, which are abnormal in TCD.

Secondly, our chosen parameters are supported by a few studies where single cell physiology recording from the human thalamus was executed during stereotactic surgery, in patients suffering from chronic severe therapy-resistant neurological and neuropsychiatric disorders (Jeanmonod, et al., 1996, Jeanmonod, et al., 2003, Sarnthein, et al., 2003). These studies have demonstrated the presence of low threshold calcium spikes (LTS) which activate a series of recurring rhythmic bursting of action potentials at frequencies ranging from 2.5 Hz to 5.6 Hz (Average 3.8± 0.7 Hz). This slows down and locks the related thalamocortical circuit into low-frequency resonance oscillations (4-7 Hz) (Jeanmonod, et al., 1996, Llinas, et al., 1999). It is this abnormal synchrony of LFO's, observed in TCD, that we intend to explore with

rTMS. Thus, we decided to deliver the rTMS at frequencies of 1 Hz and 5 Hz which correspond to the EEG rhythms of δ and θ, respectively.

Lastly, a series of studies using positron emission tomography (PET) provide evidence that short trains of 10 Hz rTMS can stimulate subcortical dopamine release in the striatum (specifically, the caudate nucleus and putamen) in healthy subjects and PD patients (Strafella, et al., 2005, Strafella, et al., 2001, Strafella, et al., 2003). Additionally, the release of dopamine in the putamen was greater in the more affected hemisphere in mild hemiparkinsonian patients (Strafella, et al., 2005). The reason for including 10 Hz in our study is that it has been demonstrated that this frequency of stimulation can act indirectly on the subcortical level via stimulation at connected cortical areas (Strafella, et al., 2005). Moreover, it has been proposed that high-frequency stimulation has sufficiently short time intervals between pulses to build up a large "summation" of neural activity, which consequently results in greater recruitment and activation of cortico-thalamic descending pathways (Veniero, et al., 2011).

Material and methods

Subjects

Forty-four healthy volunteers (19 males, 25 females; mean age 22.3 years ± 2.6) with no reported neurological history participated in the study. Participants were randomly divided into four experimental groups (rTMS 1Hz, rTMS 5Hz, rTMS 10Hz and sham rTMS 10Hz) of eleven subjects each. Gender was counterbalanced among the four different groups. The participants were naive to rTMS prior to the study and were unfamiliar with the differences between sham and active rTMS regarding its

acoustic and tactile artifacts. Written informed consent was obtained from all participants and the Local Ethics Committee approved the study.

Experimental paradigm

Subjects were tested in a quiet and dimly light room. They were seated in a comfortable armchair in a "resting wakefulness state" with eyes open. Twenty intermittent trains of 20 pulses (400 stimuli), were delivered for each of the four groups of frequency of rTMS: 1Hz, 5Hz, 10Hz or sham rTMS 10Hz. The duration of each train for 1 Hz rTMS was 20 seconds, the intertrain interval (ITI) was 30 seconds, and the rTMS was applied for 16 min 40 seconds. As for 5 Hz rTMS, the duration of each train was 4 seconds, the ITI was 30 seconds and the rTMS was applied for 11 min 20 seconds. At last for 10 Hz rTMS groups the duration of each train was 2 seconds, the ITI was 30 seconds and the rTMS was applied for 10 min and 40 seconds.

Transcranial magnetic stimulation

TMS was performed using a high-power Magstim-Rapid stimulator (Magstim, Whitland, UK).The rTMS was applied over the left primary motor cortex (M1) (in the proximity of the C3 electrode) simultaneously with EEG data collection. TMS was delivered through a figure-of-eight coil (70 mm standard coil; Magstim), oriented so that the induced electric current flowed in a posterior–anterior direction over the underlying motor cortex. The coil was placed tangentially to the scalp with the handle pointing backward and laterally at a 45° angle away from the midline perpendicular to the line of the central sulcus to achieve the lowest motor threshold. A sham rTMS condition was carried out to control for the air and bone-conducted auditory stimuli that could contaminate EEG oscillations in the target motor system. The sham rTMS

condition was performed with the coil tilted at 90˚ to the skull in order to avoid real stimulation of the motor cortex.

Motor Evoked Potentials (MEPs) were recorded from the right thenar eminence (TE) muscle using Ag/AgCl surface electrodes in a bellytendon montage. The amplified and bandpass-filtered (50 Hz–5 kHz) EMG signal was fed into a Basis Esaote Machine (Esaote Company, Florence, Italy) at a sampling rate of 5000 Hz. The optimal position for right TE activation was determined by moving the coil in 0.5-cm steps around the motor hand area of the left motor cortex. The optimal position was defined as the site where stimuli of slightly suprathreshold intensity consistently produced the largest MEPs with the steepest negative slope in the target muscle (referred to as "motor hot spot"). The intensity of rTMS in all three frequencies of rTMS at 1 Hz, 5Hz and 10 Hz was set to 100% of individual Resting Motor Threshold (RMT), and is defined as the lowest value of TMS intensity able to produce in each individual a minimum of five MEPs of at least 50 μV in ten successive stimuli.

Electroencephalographic acquisition and analysis

Continuous EEG was recorded with a MR compatible EEG amplifier (SD MRI 32, Micromed, Treviso, Italy). Electrode montage and placement was according to the 10/10 system (Jurcak, et al., 2007). The EEG was continuously recorded from 30 Ag/AgCl electrodes sites (Fp1, AF3, AF4, F7, F3, Fz, F4, F8, FC5, FC1, FC2, FC6, T3, C3, Cz, C4, T4, CP5, CP1, CP2, CP6, T5, P3, Pz, P4, T6, PO3, PO4, O1, O2). According to the 10/10 system, the reference electrode was at AFz site, whereas the ground electrode was at FCz site as in previous studies using the same system (Fuggetta, et al., 2005, Fuggetta, et al., 2008). The impedance was kept below 10 kΩ. The activities in the right TE muscle and in the right eye vertical electroculogram

(vEOG) were bipolarly registered from two surface electrodes in two EMG channels. To ensure the subjects' safety, the wires were carefully arranged to avoid loops and physical contact with the subject. To avoid electrical saturation of EEG channels induced by TMS, the EEG amplifier had a resolution of 22 bits with a range of ± 25.6 mV. An anti-aliasing hardware band-pass filter was applied with a bandwidth between 0.15 and 269.5 Hz. EEG data were sampled at a frequency of 1024 Hz using the software package SystemPlus (Micromed, Treviso, Italy).

To demonstrate the transient rTMS frequency-dependant effect on modulation of EEG oscillations, the following nine electrodes were selected for analyses: F3, Fz, F4, C3, Cz, C4, P3, Pz, and P4 using a commercial software (Vision Analyser, Brain Vision, Munich, Germany). For each group of participants, the first 20-seconds after each of the 20 intermittent trains of rTMS were considered for analysis. This time window was divided, post rTMS, into four non-overlapping epochs of four seconds each (first epoch, 1-5 seconds; second epoch, 6-10 seconds; third epoch, 11-15 seconds and forth epoch, 16-20 seconds). Each epoch was also divided into 2 identical non-overlapping segments of 2 seconds (2048 data points). Thus a total amount 80 seconds (40 segments) of EEG raw data was available for each epoch for further analyses. A semi-automatic segment inspection-rejection procedure was applied to remove TMS artefacts, muscle or eye movements/blinks (rejecting segments with values outside the range of ±70 μV). A mean of 50.0 ±12.2 of artefact-free seconds, corresponding to 25.0 ± 6.1 of clean segments, were extracted for each of the four epochs post rTMS and each of the four experimental groups. These data lengths were considered sufficient to achieve reliable spectral estimates (Fuggetta, et al., 2008). For each subject, epoch and frequency condition used, a discrete Fast Fourier Transform

(FFT) of the segments was computed for all electrodes and then averaged. Recordings were non-overlapping Hamming-windowed to control spectral leakage.

In order to reduce the effects of inter-subject and inter-electrode variation in absolute spectral power values, the relative changes of EEG power at an electrode x (ERPowx), was calculated as in previous studies of TMS-EEG co-registration (Brignani, et al., 2008, Fuggetta, et al., 2005, Fuggetta, et al., 2008, Veniero, et al., 2011) by using the following accepted event-related desynchronisation/ synchronisation (ERD/ERS) procedure (Pfurtscheller and Lopes da Silva, 1999), according to the equation:

$$ERPow_x = [(Pow_{x\ event} - Pow_{x\ reference}) / Pow_{x\ reference}] \times 100$$

ERPow (or ERD/ERS) transformation in this study represents the rTMS after effects on regional oscillatory activity of neural assemblies and is defined as the percentage of decrease/increase of instant power density at the 'event' (epoch 1, 2 and 3) compared to a 'reference' period of time (epoch 4). ERPow decreases/increases are expressed as negative/positive values and imply a reduction/augmentation in synchrony of the underlying neuronal populations. Broadband power changes were acquired by averaging the power values (with 0.5 Hz bin width) of δ (1-3 Hz), θ (4-7 Hz), μ (10-12 Hz) and β (13-30 Hz) frequency bands chosen for analysis.

Statistical analyses

Data were analysed using SPSS for Windows version 18. First, we examined the acute perturbation of regional oscillatory activity induced by different frequencies of intermittent short trains of rTMS. Thus spectral analysis of mean ERPow was submitted to four repeated measures ANOVAs for δ (1-3 Hz), θ (4-7 Hz), μ (10-12 Hz) and β (13-30 Hz) frequency bands. Three-way ANOVAs were applied with the factor: "rTMS frequency" (1 Hz, 5 Hz, 10 Hz and sham 10 Hz); "epoch" (first epoch,

1-5 seconds; second epoch, 6-10 seconds; third epoch, 11-15 seconds); and "electrode" (F3, Fz, F4, C3, Cz, C4, P3, Pz and P4). For each ANOVA, the sphericity assumption was assessed with Mauchly's test. Greenhouse-Geisser epsilon adjustments for non-sphericity were applied where appropriate. Post-hoc paired t-test adjusted for multiple comparisons with Bonferroni method was used.

Second, to assess whether the rTMS artificially induced (de)synchronisation of neural oscillations in the δ and θ frequency ranges is accompanied by a strong correlation between low-frequencies, as well as between low- and high-frequencies in the β band as in TCD phenomenon (Llinas, et al., 1999), we conducted correlation analyses for each of the four experimental groups (rTMS 1Hz, rTMS 5Hz, rTMS 10Hz and sham rTMS 10Hz). Thus Pearson product-moment correlation coefficients were computed using mean ERPow values for each EEG frequency in the 1-30 Hz range for all electrodes at first epoch (1-5 seconds) post rTMS. For all statistical tests, $p < .05$ was considered significant.

Results

No adverse side effects were reported by any of the participants during all experimental conditions. The acute perturbation of brain rhythms induced by rTMS delivered at different frequencies over the left M1 was assessed by ERPow, which reflected the regional oscillatory activity of neural assemblies. In the overall frequency bands analysed in the range between 1-30 Hz, a dichotomy was observed between low- and high-frequency rTMS modulating EEG rhythms. In particular, the group receiving active rTMS 10 Hz showed an acute synchronisation of neural oscillations (increased the ERPow), whereas groups receiving active rTMS 1 Hz and active rTMS 5 Hz exhibited an opposite de-synchronisation of brain oscillations

(decreased the ERPow) compared with sham rTMS up to 10 seconds post rTMS. The rTMS frequency dependant modulation of neural oscillations was greater for low frequency rhythms of δ and θ (< 8 Hz) compared with μ and β (Figure 1).

< Figure 1 about here >

Table 1 summarises the ANOVA results for mean ERPow transformations and the four frequency bands analysed.

< Table 1 about here >

ERPow δ (1-3 Hz)

Post-hoc comparisons for the significant interaction of "rTMS frequency x epoch" showed that there was a significant EEG synchronisation of ERPow modulation for rTMS 10 Hz compared with sham for 10 seconds after magnetic stimulation [epoch one: rTMS 10 Hz (127.5%) vs. sham (42.3%); epoch 2: rTMS 10 Hz (53.6%) vs. sham (7.7%). In contrast, there was an opposite decrease of ERPow modulation (de-synchronisation) for rTMS 1 Hz and rTMS 5 Hz compared with sham for 10 seconds post rTMS [epoch one: rTMS 1 Hz (-53.7%), rTMS 5 Hz (-48.6%) vs. sham (42.3%), epoch two: rTMS 1 Hz (-52.6%), rTMS 5 Hz (-48.7%) vs. sham (7.7%)]. Figure 2A illustrates the percentage of ERPow modulation of "rTMS frequency x epoch" for δ rhythm.

< Figure 2 about here >

Post-hoc comparisons for the significant interaction of "rTMS frequency x epoch x electrode" showed EEG synchronisation in rTMS 10 Hz and de-synchronisation in rTMS 1 Hz and rTMS 5 Hz versus sham across all electrodes for 10 seconds after magnetic stimulation. C3 was the most sensitive electrode affected by the experimental manipulations [epoch one: rTMS 10 Hz (270.9%), rTMS 1 Hz (-

63.3%), rTMS 5 Hz (-51.7%), sham (73.9%); epoch two: rTMS 10 Hz (73.1%), rTMS 1 Hz (-63.7%), rTMS 5 Hz (-47.5%), sham (11.3%)]. Figure 3A1-2 illustrates the percentage of ERPow modulation of "rTMS frequency x epoch x electrode" for δ rhythm.

< Figure 3 about here >

ERPow θ (4-7 Hz)

Post-hoc comparisons for the significant interaction of "rTMS frequency x epoch" showed that there was a significant EEG synchronisation for rTMS 10 Hz (56.5%) for 5 seconds after magnetic stimulation. In contrast, there was a de-synchronisation of neural oscillations for rTMS 1 Hz and 5 Hz for 10 seconds after magnetic stimulation [epoch one: rTMS 1 Hz (-46.4%), rTMS 5 Hz (- 37.6 %) vs. sham (24.2%); epoch two: rTMS 1 Hz (-38.7%), rTMS 5 Hz (-32.5%) vs. sham (5.1%)]. Figure 2B illustrates the percentage of ERPow modulation of "rTMS frequency x epoch" for θ rhythm.

Post-hoc comparisons for the significant interaction of "rTMS frequency x epoch x electrode" showed EEG synchronisation in rTMS 10 Hz and desynchronisation in rTMS 1 Hz and rTMS 5 Hz versus sham across all 10 seconds after magnetic stimulation. C3 was the most sensitive electrode affected by the experimental manipulations [epoch one: rTMS 10 Hz (115.9%), rTMS 1 Hz (-55.3%), rTMS 5 Hz (-38.4%), sham (39.8%); epoch two: rTMS 10 Hz (32.9%), rTMS 1 Hz (-49.3%), rTMS 5 Hz (-33.7%), sham (5.2%)]. Figure 3B1-2 illustrates the percentage of ERPow modulation of "rTMS frequency x epoch x electrode" for θ rhythm.

ERPow μ (10-12 Hz)

Post-hoc comparisons for the significant interaction of "rTMS frequency x epoch" showed that there was a significant EEG de-synchronisation for rTMS 1 Hz compared with sham for 10 seconds after magnetic stimulation [epoch one: rTMS 1 Hz (-20.7%) vs. sham (2.6%); epoch two: rTMS 1 Hz (-20.0%) vs. sham (1.8%)]. Figure 2C illustrates the percentage of ERPow modulation of "rTMS frequency x epoch" for μ rhythm.

ERPow β (13-30 Hz)

Post-hoc comparisons for the significant interaction of "rTMS frequency x epoch" showed that there was a significant EEG synchronisation for rTMS 10 Hz for 5 s after magnetic stimulation [epoch one: rTMS 10 Hz (17.1%) vs. sham (1.8%)]. However, there was a significant de-synchronisation for rTMS 1 Hz and rTMS 5 Hz for 10 seconds post magnetic stimulation [epoch one: rTMS 1 Hz (-18.6%), rTMS 5 Hz (-10.9%) vs. sham (1.8%), epoch two: rTMS 1 Hz (-15.8%), rTMS 5 Hz (-12.1%) vs. sham (2.0%)]. Figure 2D illustrates the percentage of ERPow modulation of "rTMS frequency x epoch" for β rhythm.

Correlation Analyses (1-30 Hz)

Pearson product-moment correlation coefficients show that the artificially induced neural synchronisation in the low-frequencies rhythms of δ and θ observed after the application of high frequency rTMS 10 Hz is accompanied by a strong association between low- and high-frequencies in the β band as observed in TCD phenomenon (Llinas, et al., 1999). Furthermore, the correlations between low- and high-frequencies are stronger in the rTMS 10 Hz group compared with rTMS 1Hz, rTMS 5Hz, and sham rTMS 10Hz groups, respectively (Figure 4).

< Figure 4 about here >

Discussion

The present research was initiated as an attempt to contribute to the understanding of electrophysiological mechanisms sustaining dysfunctional synchronized oscillatory activity of neural populations in humans, which play a central role in the pathophysiology of different neuropsychiatric disorders (Llinas, et al., 1999, Schulman, et al., 2011, Uhlhaas and Singer, 2010). The main objective was to relate the transient increase of LFOs in healthy subjects, which were externally generated by the rTMS, at awake and rest state with the TCD phenomenon observed in a variety of neuropsychiatric disorders. It is important to remember that physiological propagation of activation between brain regions under natural conditions may not be precisely reflected by the patterns elicited during the artificial brain stimulation throughout rTMS. Despite this limitation, the combination of rTMS with EEG can be very informative in investigate brain systems (Hampson and Hoffman, 2010). Since our study was specifically designed to examine the possible short-lasting interference of the thalamocortical oscillatory network resulting from rTMS, we chose healthy subjects rather than patients. The advantage of using healthy subjects is that the effects of short trains of rTMS are transient; therefore, it is unlikely to result in cortical reorganisation or compensatory plasticity, which becomes more widespread the longer the patients have the neuropsychiatric deficits (Johnston, 2009).

The main finding of this research using a combined rTMS with EEG approach was the acute short-lasting (10 seconds) rTMS frequency-dependent neuromodulation effect on LFOs, after short intermittent trains (20 pulses) of magnetic stimulation in

healthy subjects. Among the main findings, a mild synchronization of EEG oscillations was observed at epoch 1 (1-5 sec) post sham rTMS in all electrodes and in both low frequency rhythms of δ and θ analysed. This result for sham rTMS at 10 Hz could be a cumulative effect of the rapid sequence of auditory coil-click sounds produced during each train of high-frequency rTMS. This finding emphasizes the need to carefully control for external influences on cortical oscillations due to concomitant auditory stimulation. Similar to our study, additional confounds related to unwanted activation of the auditory system caused by rTMS have been seen in some neuroimaging and electrophysiological studies in which the motor system was investigated. (Bestmann, et al., 2004, Fuggetta, et al., 2008, Takano, et al., 2004).

Overall, the post high-frequency rTMS 10 Hz acute synchronisation of neural oscillations results of the present study mimicked the observation of abnormal, internally generated LFOs of TCD seen in previous investigations with clinical populations where a strong correlation has been found between the internally generated persistent synchrony of amplitude of δ and θ frequency range with that of β or γ band activity in TCD states of neuropsychiatric disorders including Parkinson's disease, schizophrenia, epilepsy, neuropathic pain, tinnitus, major depression and obsessive-compulsive disorder (Jeanmonod, et al., 2003, Llinas, et al., 1999, Sarnthein and Jeanmonod, 2007, Sarnthein and Jeanmonod, 2008, Sarnthein, et al., 2003, Schulman, et al., 2011).

The idea behind the TCD states is that abnormal, internally generated and persistent thalamic δ and/or θ frequency range activity serves as the trigger for thalamo-cortical resonance oscillatory network dysfunction, with a consequent increase in the amplitude in both low-frequency cortical oscillatory activity of δ and θ rhythms, as well as high-frequency β or γ band activity (Llinas, et al., 2005, Llinas, et

al., 1999, Llinas and Steriade, 2006, Schulman, et al., 2011, Whitwell, et al., 2011). Indeed the TCD theory is based on either diminished excitatory or exaggerated inhibitory input at the thalamic level, which leads to a shift from tonic to burst firing of the thalamocortical neurons and subsequently with low-frequency pathological oscillations (Llinas and Steriade, 2006). The relatively long intervals of hyperpolarisation between bursts in the thalamo-cortico-thalamic network will disrupt the normal, state-dependent, flow of information between thalamus and cortex (Zhang, et al., 2009). The increase in δ and θ power at rest and awake state accords with the presence of low-threshold spike (LTS) bursting activity, with δ and θ rhythmicity in the medial thalamus of patients with TCD, as demonstrated by MEG and single-unit recordings during stereotactic surgery (Jeanmonod, et al., 2003). The rTMS 10 Hz in the present study appears to be able to modulate the rhythmic thalamocortical interplay by entraining the resonance between the thalamus and cortex at low frequency thus generating a state that mirrors the pathological TCD. This finding is supported by clinical studies using high-frequency rTMS 10 Hz on Parkinson's disease and tinnitus patients, whose symptoms worsened (Boylan, et al., 2001, Langguth, et al., 2008, Langguth, et al., 2010). Overall these results support the hypothesis that electrical brain stimulation like TMS can trigger an oscillation, or reset the ongoing rhythmic activity, of a local thalamic pacemaker (Fuggetta, et al., 2005, Van Der Werf, et al., 2006).

The insight into the cellular mechanisms of repeated stimulation could be derived from animal studies investigating the thalamic short-term plasticity. The rhythmic stimulation with pulse-trains at 10 Hz on anaesthetised cats with dual EEG intracellular recordings from the thalamocortical (TC) and cortical neurons demonstrated that the TC neurons remained hyperpolarised during repeated

stimulations due to the influence of GABAergic thalamic reticular (RE) neurons (Steriade, 2006, Steriade and Timofeev, 2003). The hyperpolarisations of TC neurons result in the generation of oscillatory activity at about 2 Hz (the frequency range of δ) lasted for 8 seconds after the cessation of repetitive stimulations (Steriade, 2006, Steriade and Timofeev, 2003). In a groundbreaking study on the physiology of rTMS (Allen, et al., 2007), a tight coupling has been demonstrated between the short rTMS trains (1-4 s) at the stimulation frequencies of 1-8 Hz and TMS-evoked neural responses and synchronisation of neural firing in the cat visual cortex (Allen, et al., 2007). The authors found that the effects of the neural oscillations and haemodynamic signals were stimulation dependent, scaling linearly with low and high stimulation frequencies and duration (Allen, et al., 2007). Overall, the results of the present study on the neurophysiologic effects of rTMS extended the findings in previous animal models to humans.

Recently, TMS has been combined with EEG to derive inhibitory measurements directly from the cortex through a single pulse and paired pulse TMS paradigms. The synchronised δ and θ LFOs and the modulation of α and β frequency rhythms in the current study suggest the involvement of cortical γ-aminobutyric acid (GABA) inhibitory interneurons which selectively attenuate the activity of other neurons in the cortex, after TMS perturbation of the human motor cortex in healthy individuals (Manganotti, et al., 2012). Manganotti et al., by using single pulse TMS and paired-pulse TMS for the measurement of short intracortical inhibition (SICI), which is related to $GABA_A$ receptor-mediated inhibitory neurotransmission within the motor cortex (Ziemann, et al., 1996), found that SICI is able to induce different patterns of oscillatory activities up to 5 seconds after the application of TMS. There was a transitory decrease in α and β activity (< 1 sec.) and short-lasting (5 sec.)

increase in δ and θ activity. The authors suggested that the early pattern could be related with $GABA_A$, circuits, while the later effect could be related with $GABA_B$, receptor activity (Manganotti, et al., 2012). Other studies have used a paired pulse TMS paradigm known as long interval cortical inhibition (LICI), which reflects $GABA_B$ receptor-mediated inhibitory neurotransmission (Radhu, et al., 2012). LICI using TMS-EEG is defined using the area under rectified single pulse and paired pulse evoked waveforms for averaged EEG recordings between 50-150 ms post-test stimulus. A recent study found a significant suppression in the TMS evoked EEG cortical oscillations following the application of LICI to the dorsolateral prefrontal cortex (DLPFC) and the motor cortex of healthy individuals. It has been shown that $GABA_B$ inhibitory post synaptic potentials contribute to the modulation by reducing the evoked cortical oscillations from δ to α rhythms for motor cortex stimulation and from δ to γ rhythms for DLPFC stimulation (Farzan, et al., 2009).

Several lines of evidence suggest that deficits in GABA inhibitory neurotransmission are implicated in the pathophysiology of schizophrenia, bipolar disorder, major depressive disorder, and ossessive-compulsive disorder. Single or double pulse TMS combined with EEG is becoming a standard paradigm for diagnosing neuropsychiatric disorders, linking dysfunctional GABAergic neurotransmission to various disease states (Daskalakis, et al., 2012, Radhu, et al., 2012). A recent study used combined TMS-EEG to examine LICI on oscillatory frequencies in patients with schizophrenia, bipolar disorder and healthy subjects (Farzan, et al., 2010). It was demonstrated that only schizophrenic patients had significant deficits in inhibition of γ oscillations that were specific to DLPFC stimulation (Farzan, et al., 2010).

With this study we also observed with the application of short trains of rTMS 1 Hz and 5 Hz an induced short-lasting de-synchronisation of EEG oscillations. The reason why the effects of rTMS 5 Hz are similar of those of rTMS 1 Hz, could be due to the insufficient time interval between pulses in rTMS 5 Hz to cause large "summation" of the neural activity, which consequently results in lesser recruitment and activation of cortico-thalamic descending pathways (Fuggetta, et al., 2008, Veniero, et al., 2011). The EEG de-synchronisation observed in rTMS 1 Hz and 5 Hz points to the potential of rTMS to prevent the overly-rhythmic low-frequency thalamic bursting and may provide a new strategy for reducing or even reversing TCD and alleviating the numerous symptoms that are coming to be recognized as having their basis in TCD (Jeanmonod, et al., 2003, Llinas, et al., 1999, Schulman, et al., 2011, Uhlhaas and Singer, 2010). We therefore suggest that the beneficial effect of rTMS could arise from reversing patterns of TCD-like electrophysiology and that rTMS parameters can be configured by referencing the relationship between stimulation setup and their effect on TCD-like EEG patterns. It is tempting to associate the transient acute de-synchronisation induced by rTMS 1 and 5 Hz, which may reverse the persistent abnormal alterations of thalamocortical resonance phenomenon in a variety of neuropsychiatric disorders. However, the limitation of this current research, where only short conditioning trains of rTMS have been delivered in healthy subjects, make us cautious in doing so. Future research using both clinical and non-clinical populations should extend the frequencies of rTMS used in the current investigation (i.e. 4 Hz, 20 Hz) and especially increase the total number of pulses applied in order to obtain longer-lasting effects of rTMS trains on modulation of LFO. However, we could not exclude the possibility that the transient

effects noted in the current study could produce the more prolonged effects observed in other rTMS combined with EEG studies.

Indeed, a couple of studies have looked at electrophysiological measures pre and post rTMS on modulation of γ rhythm evoked activity for schizophrenic and healthy subjects, albeit not immediately after stimulation (Barr, et al., 2011, Barr, et al., 2009). In one of these studies, 20 Hz rTMS delivered as 25 trains of 30 pulses per train over the DLPFC resulted in the increase of frontal γ oscillatory activity during a working memory task involving the on-line maintenance and manipulation of information in healthy subjects and an opposite effect of reduction of frontal γ oscillations in schizophrenic patients. These results have been associated with the concept of homeostatic plasticity involving regulation of GABAergic inhibitory mechanisms that maintain neuronal excitability within a useful physiological range (Barr, et al., 2011).

The low and high rTMS frequencies in humans often result in opposite physiological effects as measured by MEP; low frequency stimulation (1 Hz or lower) decreases cortical excitability whereas high frequency stimulation (more than 1 Hz) increases cortical excitability (Hallett and Rothwell, 2011). However, previous EEG and TMS investigations were unable to detect this differential modulation of low and high frequency rTMS on of cortical oscillations because they were focusing on α and β bands (Brignani, et al., 2008, Fuggetta, et al., 2008, Veniero, et al., 2011). Here, we show for the first time that not only can rTMS induce synchrony of δ and θ bands but it may also reverse the oscillatory phenomena depending on the frequency of stimulation used.

Conclusions

In summary, this is the first study which investigates the probable link between rTMS aftereffects and the TCD phenomenon to gain new insight into the basic mechanisms underlying neurological and psychiatric disorders. With this basic neuroscience research we induced rTMS frequency dependant transient perturbation of spontaneous thalamocortical oscillatory dynamics in the healthy human brain. Thus, we provided the baseline for researchers and clinicians to distinguish the oscillatory patterns that may be disrupted in patients of various neuropsychiatric disorders. The ability of combined rTMS-EEG to modulate frequency-specific oscillations and measure the dysrhythmic thalamocortical oscillatory activity offers exciting possibilities for carefully designed clinical trials as a diagnostic or therapeutic tool for characterising the functional networks underlining pathological conditions (Hampson and Hoffman, 2010, Kobayashi and Pascual-Leone, 2003, Thut and Miniussi, 2009, Thut and Pascual-Leone, 2010). Future applied clinical research promises to provide advances not only to further elucidate the pathophysiology of a variety neurological and psychiatric disorders but may develop therapeutic strategies of using non-invasive brain stimulation to reverse abnormal neural oscillations and synchrony in these disorders.

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Figures captions

Figure 1: Superimposition of Grand average Event-Related Power (ERPow) transformation from 1 to 30 Hz, at 'epoch' 1 (1-5 seconds) post rTMS for all the electrodes analysed and the four groups of "rTMS frequency": 1 Hz (n=11), 5 Hz (n=11), 10 Hz (n=11), and sham 10 Hz (n=11). The graph shows the frequency-dependant effect of rTMS on modulation of neural oscillations especially for low frequency oscillations of δ (1-3Hz) and θ (4-7Hz) rhythms.

Figure 2: Grand average of Event-related power (ERPow) transformation for (A) δ (1-3 Hz), (B) θ (4-7 Hz), (C) μ (10-12 Hz) and (D) β (13-30 Hz) frequency ranges as a function of "rTMS frequency" and "epoch of time" post rTMS. Overall there was a neural synchronisation for rTMS 10 Hz and an opposite neural de-synchronisation for rTMS 1 Hz and 5 Hz compared to sham rTMS up to 10 seconds after magnetic stimulation. The symbol "●" indicates a significant difference between rTMS 1Hz vs. sham rTMS; the symbol "◆" indicates a significant difference between rTMS 5Hz vs. sham rTMS; and the symbol "★" indicates a significant difference between rTMS 10Hz vs. sham rTMS ($p < .05$; Bonferroni corrected).

Figure 3 Grand average of Event-related power (ERPow) transformation for (A1-2) δ (1-3 Hz), (B1-2) θ (4-7 Hz) frequency ranges as a function of "frequency of rTMS" and "electrodes" at 'epoch' 1 (1-5 seconds) and 2 (6-10 seconds) post rTMS. Overall the graphs show a neural synchronisation for rTMS 10 Hz and an opposite desynchronisation for rTMS 1 Hz and 5 Hz compared to sham rTMS across all electrodes up to 10 seconds after magnetic stimulation. A1-B1, show that C3 is the most sensitive electrodes affected by high frequency rTMS 10 Hz at epoch 1. The

symbol "●" indicates a significant difference between rTMS 1Hz vs. sham rTMS; the symbol "◆" indicates a significant difference between rTMS 5Hz vs. sham rTMS; and the symbol "★" indicates a significant difference between rTMS 10Hz vs. sham rTMS ($p < .05$; Bonferroni corrected).

Figure4 Correlation plots of Event-related power (ERPow) transformation from 1 to 30 Hz at epoch 1 (1-5 seconds) post rTMS. A shows correlation plots for the two groups of rTMS 1 Hz (n=11) and rTMS 5 Hz (n=11). B shows correlation plots for the two groups of rTMS 10 Hz (n=11) and sham rTMS 10 Hz (n=11). Note in particular the wide range of greater and significant correlation coefficients (from δ to β) in the rTMS 10 Hz group compared with the sham rTMS 10 Hz group.

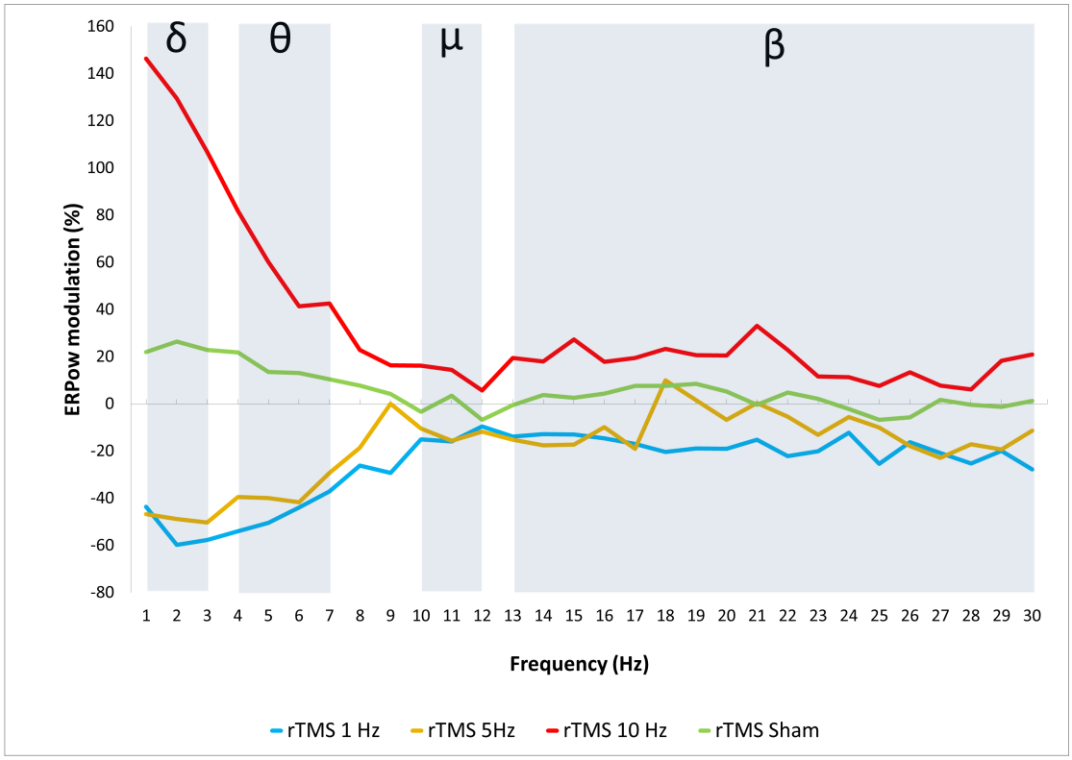

Fig. 1

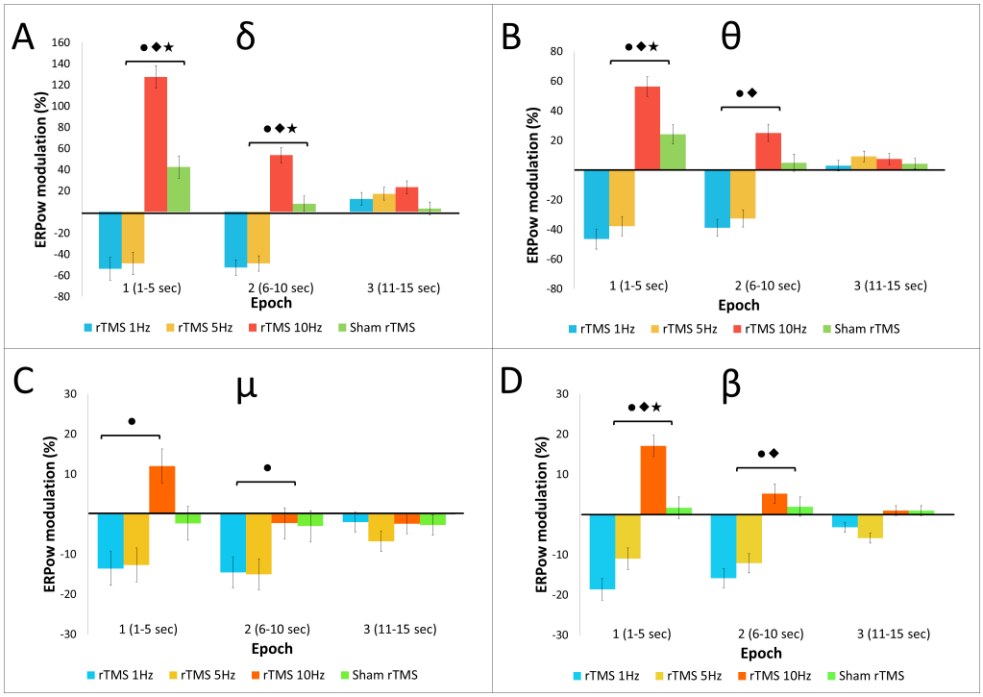

Fig. 2

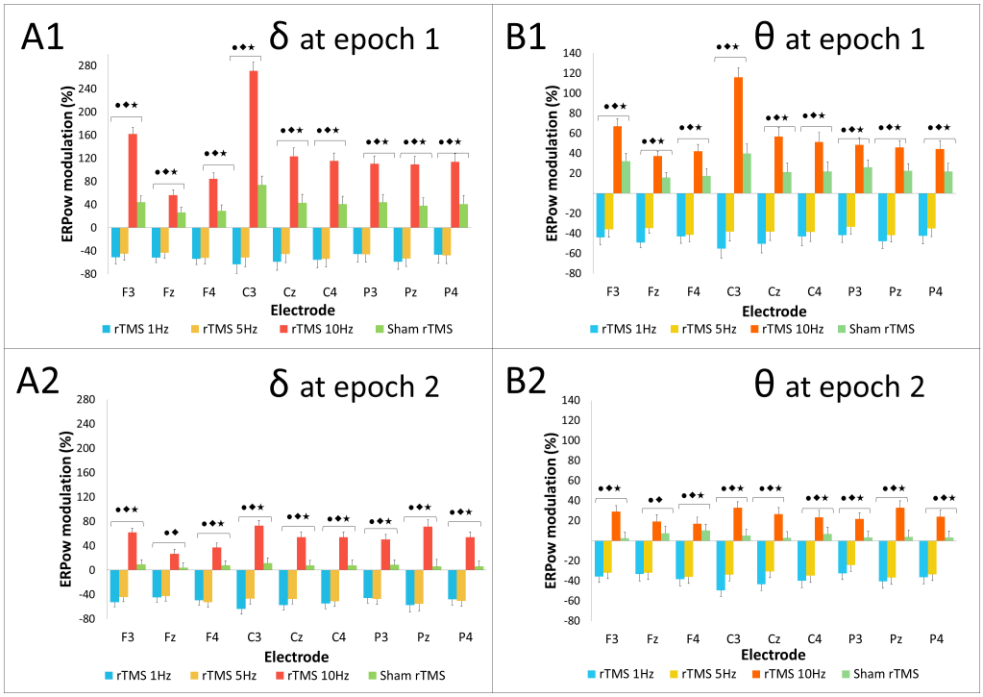

Fig. 3

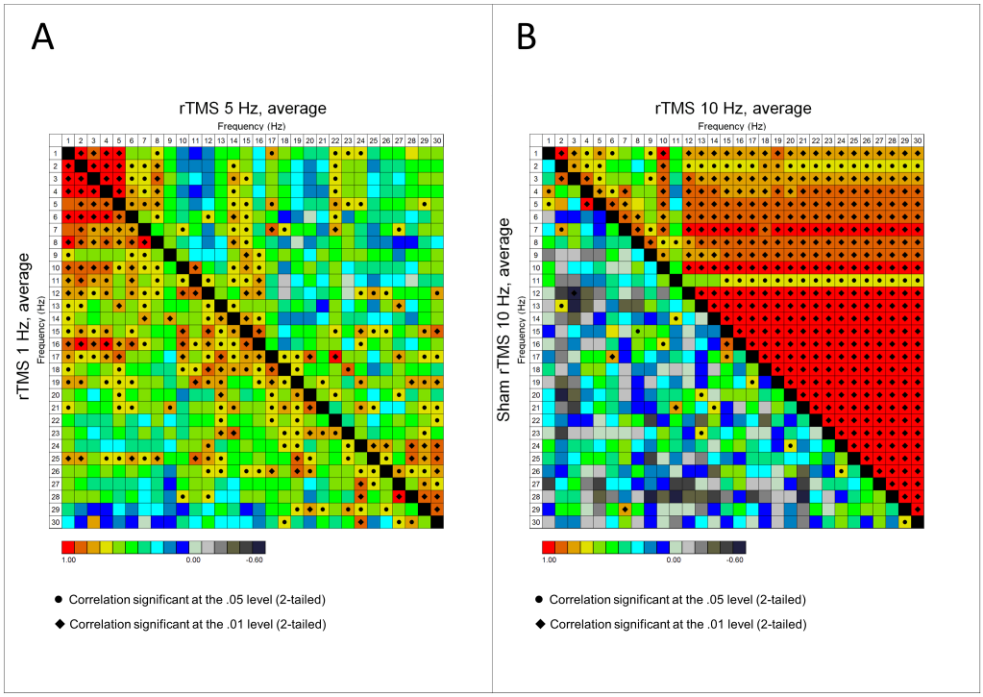

Fig. 4

Table 1

Statistical results of Event-Related Power (ERPow) for δ, θ, μ and β frequency bands

Factors	δ	θ	μ	β
rTMS Frequency	$F_{3,40}$ = 45.05*** $p < .001$; η_p^2 = .77	$F_{3,40}$ = 33.36*** $p < .001$; η_p^2 = .71	$F_{3,40}$ = 9.56*** $p < .001$; η_p^2 = .42	$F_{3,40}$ = 27.53*** $p < .001$; η_p^2 = .67
Epoch	$F_{1.6,63.7}$ = 29.6*** $p < .001$; η_p^2 = .43	$F_{2,80}$ = 21.61*** $p < .001$; η_p^2 = .35	$F_{1.6,64.4}$ = 3.71* $p < .05$; η_p^2 = .09	$F_{1.7,66.8}$ = 3.51* $p < .05$; η_p^2 = .08
Electrode	$F_{4.5,179}$ = 12.07*** $p < .001$; η_p^2 = .23	$F_{4.9,194.4}$ = 3.76** $p < .01$; η_p^2 = .09	$F_{4.7,187.4}$ = 2.54* $p < .05$; η_p^2 = .06	$F_{4.7,188}$ = 3.67* $p < .05$; η_p^2 = .08
rTMS Frequency x Epoch	$F_{4.8,63.7}$ = 60.33*** $p < .001$; η_p^2 = .82	$F_{6,80}$ = 49.25*** $p < .001$; η_p^2 = .79	$F_{4.8,64.4}$ = 11.09*** $p < .001$; η_p^2 = .45	$F_{5,66.8}$ = 19.81*** $p < .001$; η_p^2 = .6
rTMS Frequency x Electrode	$F_{13.4,179}$ = 10.32*** $p < .001$; η_p^2 = .4	$F_{14.6,194.4}$= 5.62*** $p < .001$; η_p^2 = .3	$F_{14.1,187.4}$ = 3.38*** $p < .001$; η_p^2 = .2	$F_{14.1,188}$ = 3.65*** $p < .001$; η_p^2 = .22
rTMS Frequency x Epoch x Electrode	$F_{16.3,217}$ = 9.05*** $p < .001$; η_p^2 = .4	$F_{21.7,289.6}$ = 4.7*** $p < .001$; η_p^2 = .26	$F_{26.5,353.2}$ = 2.74*** $p < .001$; η_p^2 = .17	$F_{22.1,294.1}$ = 4.38*** $p < .001$; η_p^2 = .25

*$p < .05$; **$p < .01$;

***$p < .001$

Highlights

- rTMS frequency-dependent neuromodulation effect on low frequency oscillations
- rTMS transient interference of the thalamocortical oscillatory network in humans
- insight into basic neural circuitry sustaining abnormal brain oscillations in TCD
- insight into neurophysiological mechanism underlying therapeutic effects of rTMS