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Inter-individual variability in cortical excitability and motor network connectivity following multiple blocks of rTMS

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Abstract

The responsiveness to non-invasive neuromodulation protocols shows high inter-individual variability, the reasons of which remain poorly understood. We here tested whether the response to intermittent theta-burst stimulation (iTBS) – an effective repetitive transcranial magnetic stimulation (rTMS) protocol for increasing cortical excitability – depends on network properties of the cortical motor system. We furthermore investigated whether the responsiveness to iTBS is dose-dependent.

To this end, we used a sham-stimulation controlled, single-blinded within-subject design testing for the relationship between iTBS aftereffects and (i) motor-evoked potentials (MEPs) as well as (ii) resting-state functional connectivity (rsFC) in 16 healthy subjects. In each session, three blocks of iTBS were applied, separated by 15 min. We found that non-responders (subjects not showing an MEP increase of ≥10% after one iTBS block) featured stronger rsFC between the stimulated primary motor cortex (M1) and premotor areas before stimulation compared to responders. However, only the group of responders showed increases in rsFC and MEPs, while most non-responders remained close to baseline levels after all three blocks of iTBS. Importantly, there was still a large amount of variability in both groups.

Our data suggest that responsiveness to iTBS at the local level (i.e., M1 excitability) depends upon the pre-interventional network connectivity of the stimulated region. Of note, increasing iTBS dose did not turn non-responders into responders. The finding that higher levels of pre-interventional connectivity precluded a response to iTBS could reflect a ceiling effect underlying non-responsiveness to iTBS at the systems level.

Keywords

Cortical plasticity; Variability; Dose-dependency; dPMC; SMA

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Introduction

Theta-burst stimulation (TBS) is an effective repetitive transcranial magnetic stimulation (rTMS) protocol, which allows modulation of cortical excitability upon a rather short period of stimulation (Huang et al., 2005). However, a growing number of studies report that the responsiveness to rTMS/TBS shows high inter-individual variability, sometimes even resulting in no overall alteration of cortical excitability (Hamada et al., 2013; Hinder et al., 2014; López-Alonso et al., 2014). Recent studies suggest that 50% – 73% of subjects are non-responders to rTMS/TBS (Hamada et al., 2013; Hinder et al., 2014).

To date, the reasons for this inter-individual variability remain poorly understood. Hamada et al. (2013) suggested that the differential recruitment of subtypes of cortical interneurons embedded in different cortico-cortical circuits may account for about 50% of the inter-individual variability. Based on a combined functional magnetic resonance imaging (fMRI)-TMS study, we recently demonstrated that the differential recruitment of these interneuron networks by TMS correlates with the functional connectivity between premotor areas and the primary motor cortex (M1) (Volz et al., 2014). This implies a relationship between responsiveness to TBS and motor network connectivity. However, in that study no aftereffects of iTBS on cortical excitability (motor-evoked potentials, MEPs) were investigated. Other studies also suggested a tight relationship between rTMS-induced aftereffects and network connectivity of the stimulated region (Cárdenas-Morales et al., 2014; Andoh and Zatorre, 2011, 2013; Downar et al., 2014; Salomons et al., 2014). For instance, the amount of pre-interventional premotor-M1 connectivity in the activated motor system was strongly related to the individual susceptibility to cortical excitability enhancing intermittent TBS (iTBS) (Cárdenas-Morales et al., 2014). Furthermore, decreased levels of baseline connectivity have been associated with non-responsiveness to rTMS in clinical treatments (Salomons et al., 2014).

Moreover, we could recently show that increases in cortical excitability after iTBS are paralleled by increases in resting-state functional connectivity (rsFC) (Nettekoven et al., 2014). Both, increases at the local (MEPs) as well as at the systems level (rsFC) were found to be dose-dependent with an additional increase after three blocks of iTBS (3 × 600 pulses). However, in that study we did not address the question of individual differences in iTBS responsiveness and relationships with motor network connectivity. Therefore, it is unclear whether the group-level effect observed after the application of a higher iTBS dose in the first study stems from non-responders showing responsiveness after repeated stimulation, which would suggest that responsiveness is dose-dependent (i.e., a lacking MEP increase in the first block followed by MEP increases after additional blocks of stimulation). Alternatively, the group-level effect might be driven by an amplification of iTBS effects exclusively in a subgroup of subjects (i.e., “responders”), indicating that individual factors determine responsiveness (Hamada et al., 2013). Furthermore, it is still open whether responders and non-responders also differ in their response to iTBS at the level of motor network connectivity, i.e., in the increase of rsFC after iTBS as well as in their rsFC at baseline.

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We, therefore, re-analyzed the entire data set of our previous study (Nettekoven et al., 2014) with respect to individual responsiveness at the MEP level as well as fMRI network level, a question that we did not address in the original publication. To assess changes in MEP size and rsFC, 16 healthy subjects received three blocks of iTBS applied over left M1 and a control stimulation over the vertex (Nettekoven et al., 2014). We assigned subjects to two groups: responders and non-responders. Assignment was based upon subjects' increase in MEP amplitudes after one iTBS block. We hypothesized that (i) responders show decreased rsFC between premotor areas and M1 compared to non-responders at baseline (Hamada et al., 2013; Salomons et al., 2014; Volz et al., 2014) and that (ii) a higher dose of iTBS will primarily modulate cortical excitability and rsFC in responders rather than in non-responders (Hamada et al., 2013; Nettekoven et al., 2014).

Methods

Subjects

All data have previously been included in a publication on general dose-dependent effects of iTBS on MEPs and resting-state connectivity (Nettekoven et al., 2014). We here re-analyzed the entire data set with respect to individual responsiveness at the MEP level as well as fMRI network level. Accordingly, data from 16 healthy, right-handed subjects were included (7 males, mean ± SD age: 27 ± 3 years, range: 23–35 years; no history of neurological or psychiatric diseases). Right-handedness was verified using the Edinburgh Handedness Inventory (Oldfield, 1971). All subjects provided informed written consent. The study was carried out according to the declaration of Helsinki (1969, last revision 2008) and had been approved by the local ethics committee.

Experimental design

A detailed description of the procedure has been previously published (Nettekoven et al., 2014). We here summarize the important steps. Fig. 1 illustrates the experimental design. We used a single-blind, vertex-stimulation controlled, cross-over within-subject design to test for the effects of multiple serially applied iTBS blocks on (i) cortical excitability (MEP sessions) and (ii) rsFC (resting-state fMRI sessions) to further elucidate mechanisms underlying the individual responsiveness to iTBS. Each subject participated in two MEP sessions (A, B) and two resting-state fMRI sessions (C, D). In each of the four sessions iTBS was repeated three times separated by 15 min, leading to a total of 1800 pulses (i.e., iTBS600, iTBS1200, iTBS1800) per session to examine the effect of dose (please cf. Nettekoven et al., 2014; Volz et al., 2013). Respectively, MEPs were measured at baseline and after each block of iTBS in the MEP sessions (A, B), and resting-state fMRI was measured at baseline and after each block of iTBS in the resting-state fMRI sessions (C, D). In two of the four sessions stimulation was applied over the left M1 (A: M1-iTBS_MEPs, C: M1-iTBS_rs-fMRI), and in the other two sessions over the parieto-occipital vertex (B: sham-iTBS_MEPs, D: sham-iTBS_rs-fMRI) (Herwig et al., 2007, 2010). Sessions were separated by at least one week to avoid carry-over effects. The order of M1- and sham-iTBS was randomized across subjects.

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All subjects performed a simple finger tapping task with their dominant (right) hand in order to test whether the subjects had similar levels of performance (i.e., whether one subject showed “abnormal” motor behavior). Accordingly, subjects were asked to perform vertical fingertapping movements with their right index finger at maximum speed in three five-second trials (separated by a few seconds break to prevent fatigue). We did not assess finger-tapping frequencies (or other behavioral measures) between the stimulation blocks in order not to interfere with the stimulation after effects, which can be significantly altered by motor activity (Gentner et al., 2008; Huang et al., 2008).

Neuronavigated transcranial magnetic stimulation

The position of the TMS coil was tracked and recorded using a Brain-Sight2 computerized frameless stereotaxic system ensuring a reliable positioning of the stimulation site across all sessions and subjects (Rogue Research Inc., Montreal, Canada). The head of the subject was coregistered with an individual high-resolution anatomical MR image. MEPs were recorded from the abductor pollicis brevis (APB) muscle of the right hand with Ag/AgCl surface electrodes (Tyco Healthcare, Neustadt, Germany) placed in a belly-tendon montage. The electromyographic (EMG) signal was amplified, filtered (0.5 Hz high pass and 30–300 Hz band pass) and digitized with a Powerlab 26 T device and LabChart software package (version 5, ADInstruments, Ltd., Dunedin, New Zealand).

Theta-burst stimulation

iTBS was delivered over the left M1 using a Magstim SuperRapid 2 with a figure-of-eight coil (70-mm standard coil, Magstim Co., Whitland, Dyfed, UK) according to Huang et al. (2005). As previoulsy described (and evaluated), iTBS was applied during M1- and sham-iTBS at 70% of the resting motor threshold (RMT) instead of 80% of the active motor threshold (AMT) (Cárdenas-Morales et al., 2014; Gentner et al., 2008; Sarfeld et al., 2012) due to the following: We wished to prevent voluntary preactivation of the target muscle, which is necessary for AMT determination but may impact on TBS aftereffects (Gentner et al., 2008; Huang et al., 2008) and may increase inter-subject variability (Goldsworthy et al., 2014).

MEPs

Neuronavigated single-pulse TMS was applied over the same location as used for iTBS using a Magstim 200^2 stimulator (Magstim Co., Whitland, Dyfed, UK). At baseline and after each iTBS-application (iTBS600, iTBS1200, iTBS1800), stimulus-response curves of MEPs evoked with intensities ranging from 90% to 150% of the RMT were assessed in steps of 10%. Two blocks of five pulses were recorded in a randomized order for each intensity, except for 120%, which was assessed in six blocks at five pulses adding up to a total number of 90 MEPs.

Data analysis – MEPs

In line with previous experiments, we used “normalized” MEP amplitudes to assess changes in cortical excitability rather than absolute MEP amplitudes to account for variance in RMTs at different stimulation days (i.e., M1- and sham-iTBS) (Cárdenas-Morales et al., 2014;

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Huang et al., 2005; Nettekoven et al., 2014). Therefore, mean MEP amplitudes acquired after each block of iTBS were normalized to mean baseline values of the respective intensity (i.e., 90–150% of the RMT). To test for differences between responders and non-responders in MEP amplitudes at baseline absolute values were used. In contrast, normalized MEP amplitudes were used to test for differences between responders and non-responders in the dose-dependent modulation after iTBS in order to account for differences in baseline values between different days.

Finally, stimulus-response curves were plotted for each subject using the absolute MEP amplitudes to test for iTBS effects on the slope of the stimulus-response curves. The steepness of each curve was computed by means of a linear regression analysis and R^2 values were calculated to assess the quality of model-fit using SPSS 21 (Statistical Package for the Social Sciences, IBM, New York/USA).

Definition of responders

The scope of this paper was to investigate whether inter-individual differences in the electrophysiological response to iTBS (i.e., in MEP amplitudes) are related to the different connectivity profiles of the stimulated region before and after stimulation. Therefore, responders and non-responders were classified according to their increase in MEPs after the first stimulation block (M1-iTBS600): Subjects showing an increase of ≥10% in MEPs compared to baseline were defined as responders (Hinder et al., 2014). This threshold criterion ensured that responders had a clear stimulation aftereffect, possibly accounting for random fluctuations around the baseline-level. In addition, we also tested 15% and 20% cut-off values in order to evaluate the stability of the results at different response thresholds.

To test for differences between responders and non-responders in the dose-dependent modulation of normalized MEP amplitudes, we set up a four-factorial repeated measures analysis of variance (ANOVA) with the within-subject factors INTERVENTION (2 levels: M1-iTBS, sham-iTBS), DOSE (3 levels: iTBS600, iTBS1200, iTBS1800), INTENSITY (7 levels: 90–150% of the RMT) and the between-subject factor GROUP (2 levels: responders, non-responders) using SPSS 21. The Greenhouse-Geisser alpha-correction was used in case of a violation of the non-sphericity assumption. Post-hoc Student's t-tests were performed to compare the iTBS response between responders and non-responders and to test for iTBS effects within groups. False discovery rate (FDR)-correction was used to correct p-values derived from the post-hoc Student's t-tests for multiple comparisons (Benjamini and Hochberg, 1995). To test whether M1-iTBS differentially modulated the slope of stimulus-response curves in responders and non-responders in a dose-dependent fashion, the curve-steepness was entered into a three-way repeated measures ANOVA with the within-subject factors INTERVENTION (2 levels: M1-iTBS, sham-iTBS), DOSE (3 levels: iTBS600, iTBS1200, iTBS1800) and the between-subject factor GROUP (2 levels: responders, non-responders).

Magnetic resonance imaging

After assessing the TMS hotspot and the RMT subjects were transported in an MR-compatible wheel chair between the anteroom of the scanner where stimulation was applied

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and the scanner room. This was to minimize any further movement since pre- or post-interventional neuronal activity can strongly impact on TBS aftereffects (Gentner et al., 2008; Goldsworthy et al., 2014; Huang et al., 2008) and thereby also on the susceptibility to iTBS. Moreover, we aimed to obtain comparable conditions between the resting-state scans. In the scanner, subjects were instructed to lie motionless with open eyes fixating a red cross (resting-state fMRI), which was presented on a TFT screen visible through a mirror attached to the MR head coil. After the baseline fMRI, subjects were transported from the scanner to the anteroom of the MR console. After coregistration with the neuronavigation system, iTBS was applied followed by another 8 min resting-state fMRI. This procedure was repeated three times in total (three blocks of iTBS), always separated by 15 min.

In addition, we used a simple motor task as a functional localizer to identify the location of core motor regions for the subsequent resting-state analysis. The localizer task consisted of rhythmic thumb ab- and adductions with the right or left hand activating the same muscle as used for TMS recordings (APB). Left hand movements were necessary in order to also localize motor regions of the hemisphere contralateral to stimulation. Written instructions displayed for 2 s indicated separate movements of the left or right thumb for the following block of trials. Abduction-adduction movements were triggered by a blinking circle at the frequency of 1.0 Hz for 15 s until a black screen indicated to rest for 15 s. Six blocks for each hand resulted in an acquistion time of approximately 7 min. Motor performance was visually controlled during the whole assessment by the experimentator. The motor task was acquired after the first resting-state scan to prevent motor activity to bias rsFC.

Image acquisition and preprocessing

fMRI images were acquired on a Siemens Trio 3.0 T scanner (Siemens Medical Solutions, Erlangen, Germany) using a gradient echo planar imaging (EPI) sequence with the following parameters: TR = 2070 ms, TE = 30 ms, FOV = 200 mm, 31 slices, voxel size: 3.1 × 3.1 × 3.1 mm^3, 20% distance factor, flip angle = 90°, resting-state: 225 volumes (3 dummy images), localizer task: 202 volumes (3 dummy images). Acquisition planes and slice orientation were identical for the four fMRI assessments (i.e., 1 × baseline, 3 × post iTBS sessions). The slices covered the whole brain extending from the vertex to the lower parts of the cerebellum. fMRI data (resting-state and motor task) were analyzed using Statistical Parametric Mapping (SPM8, http://www.fil.ion.ucl.ac.uk/spm/). The first three volumes (dummy images) of each session were discarded from further analyses to allow for magnetic field saturation. All remaining EPI volumes were realigned to the mean image of each time series and coregistered with the structural T1-weighted image. In a next step, all images were spatially normalized to the standard template of the Montreal Neurological Institute (MNI, Canada) using the unified segmentation approach (Ashburner and Friston, 2005) and smoothed with an isotropic Gaussian kernel of 8 mm full-width at half-maximum.

To exclude the possibility that head movements during the resting-state scans contributed to group differences in rsFC we tested for differences in head motion parameters acquired from image realignment by comparing the framewise displacement (FD) and the root mean squared error (RMSE) (Power et al., 2012; Satterthwaite et al., 2012).

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Importantly, there was no difference between groups neither in the FD nor in the RMSE ($p > 0.2$ for all comparisons, FDR-corrected). Likewise, FD and RMSE were not significantly different between M1- and sham-iTBS within the responder and non-responder group. Furthermore, there was no significant difference within groups between FD and RMSE at baseline and after iTBS (iTBS600, iTBS1200, iTBS1800) as well as between stimulation blocks (iTBS600/iTBS1200, iTBS1200/iTBS1800 and iTBS600/iTBS1800) ($p > 0.2$ for all comparisons, FDR-corrected, Supplementary Table 1). Therefore, neither between nor within group differences in head movements are likely to have biased the rsFC results.

Statistical analysis — functional localizer task

In the functional localizer task, the two experimental conditions (movements of the left or right thumb) were modeled using boxcar stimulus functions convolved with a canonical hemodynamic response function. The time series of each voxel were high-pass filtered at 1/128 Hz. The six head motion parameters, as assessed by the realignment algorithm, were treated as covariates to remove movement-related variance from the image time series. Simple main effects for each experimental condition were calculated for each subject by applying appropriate baseline contrasts. Voxels were identified as significant on the single-subject level if their T-values passed a height threshold of $P \leq 0.001$ ($T = 3.14$). The individual M1-coordinates of the stimulated hemisphere were then used as seed regions for the resting-state whole-brain analysis (see below). For the group analysis, the parameter estimates of all conditions (main effect RIGHT THUMB MOVEMENTS, main effect LEFT THUMB MOVEMENTS) were subsequently entered into a full factorial ANOVA. Voxels were considered significant when passing a height threshold of $P \leq 0.05$, FWE-corrected ($T = 5.72$).

Statistical analysis — resting-state fMRI

For the statistical analysis of the resting-state data, variance that could be explained by known confounds was removed from the smoothed fMRI time-series. Confound regressors included the tissue-class-specific global signal intensities and their squared values, the six head motion parameters, their squared values and their first-order derivatives (Satterthwaite et al., 2012). A band-pass filter was used to preserve only frequencies between 0.01 and 0.08 Hz in the time-series data.

We computed a seed-based whole-brain analysis. Here, the time-course within a sphere of 10 mm-diameter centered on the seed voxel (left M1, single-subject coordinates derived from localizer task) was correlated with the time course of every other voxel in the brain by means of linear Pearson's correlation (Eickhoff and Grefkes, 2011; zu Eulenburg et al., 2012). Correlation coefficients were converted to Fisher's Z-scores using the formula $Z = (1/2) \times \ln(1 + r)/(1-r) = \operatorname{atanh}(r)$ to yield approximately normally distributed data.

In order to determine changes in functional connectivity following iTBS, individual baseline functional connectivity maps were subtracted from the respective maps post iTBS for each subject (Nettekoven et al., 2014). For group-level analysis, the individual subtraction maps were entered into a "full factorial" general linear model (GLM) analysis as implemented in SPM8 with the factors GROUP (2 levels: responders, non-responders), INTERVENTION (2

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levels: M1-iTBS, sham-iTBS) and DOSE (3 levels: iTBS600, iTBS1200, iTBS1800). Differential contrast were computed (i) between M1- and sham-iTBS for iTBS600, iTBS1200 and iTBS1800 (separately for responders and non-responders), (ii) between the different stimulation blocks (i.e., iTBS1800-iTBS1200, iTBS1800-iTBS600, iTBS1200-iTBS600; separately for responders and non-responders), and (iii) between responders and non-responders for M1- versus sham-iTBS for different stimulation blocks (i.e., iTBS600, iTBS1200, iTBS1800). The resting-state maps were masked by cytoarchitectonic probability maps of frontoparietal sensorimotor areas (Brodmann areas 6, 4 a/b, 3 a/b, 2, 1) as provided by the SPM Anatomy Toolbox (Eickhoff et al., 2005) to focus inference on rsFC within the cortical sensorimotor network. Changes in rsFC after iTBS as well as baseline differences between groups were tested across these cortical sensorimotor regions. The statistical threshold was set to $p < 0.05$, family-wise error (FWE) corrected at the cluster-level. MNI coordinates (x, y, z) derived from the fMRI localizer task were used to a-priori delineate the following motor regions: right M1 (30, −28, 57), left SMA (−4, −9, 64), right SMA (6, −3, 69), left dPMC (−32, −9, 60) and right dPMC (36, −9, 60).

To test whether there was a spatial overlap between areas showing different rsFC between responders and non-responders at baseline and areas showing significant increases in rsFC, the former contrast (non-responders > responders, baseline) was used as an “inclusive mask” in the conjunction of the following contrasts: (i) responder > non-responder, real > sham, iTBS600; responder > non-responder, real > sham, iTBS1200; responder > non-responder, real > sham, iTBS1800 and (ii) responder, real > sham, iTBS600; responder, real > sham, iTBS1200; responder, real > sham, iTBS1800.

Mean rsFC values from the clusters showing a significant increase in rsFC after each block of iTBS over the whole group (n = 16, Nettekoven et al., 2014) were extracted and entered into SPSS to test for a linear correlation between increases in MEP amplitudes and increases in rsFC (Pearson’s correlation).

Results

We here exclusively report findings related to iTBS responsiveness at the MEP and the fMRI network level. General effects have been reported elsewhere (Nettekoven et al., 2014). Seven of the subjects were classified as responders (i.e., increase in MEP amplitudes of ≥10% after the first iTBS block; Hinder et al., 2014) and nine as non-responders. This ratio is similar to what has been found in other studies (Hamada et al., 2013). Likewise, cut-offs of 15% or 20% resulted in similar response rates within our cohort of subjects (15%: 6 responders, 10 non-responders; 20%: 5 responders, 11 non-responders).

Baseline measures

There were no significant differences in RMT at baseline and baseline MEPs when directly comparing M1-iTBS as well as sham-iTBS between responders and non-responders (Table 1). Likewise, there were no differences in maximum fingertapping frequencies between the two groups (responders: 6.7 ± 0.7 Hz, non-responders: 6.6 ± 0.7 Hz, $p > 0.5$).

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However, non-responders (relative to responders) featured a significantly higher baseline rsFC before M1-iTBS between the stimulated (left) M1 and bilateral premotor areas ($p \leq 0.05$, FWE-corrected at the cluster-level; Fig. 2). The localizer task confirmed that the local maxima within the cluster shown in Fig. 2 were situated in right M1, left and right dPMC as well as left and right SMA. The reverse contrast yielded no significant effects. That is, in responders (compared to non-responders) no area featured significantly stronger rsFC at baseline with left M1.

Within groups, RMTs at baseline were not significantly different between M1- and sham-iTBS for responders ($p = 0.365$, FDR-corrected for multiple comparisons) and non-responders ($p = 1.000$, FDR-corrected). Likewise, baseline MEP amplitudes did not differ between sham- and M1-iTBS neither for responders ($p = 0.685$, FDR-corrected) nor non-responders ($p = 0.753$, FDR-corrected).

iTBS-induced plasticity: MEP amplitudes

A four-way repeated measures ANOVA assessing MEP differences after iTBS between responders and non-responders revealed a significant main effect of GROUP ($F_{1,14} = 10.362$, $p = 0.006$) as well as an interaction effect of the factors INTERVENTION × GROUP ($F_{1,14} = 10.246$, $p = 0.006$). However, there was no interaction effect of the factor INTENSITY so that we averaged MEPs across all intensities for further analysis. Post-hoc Student's t-tests on averaged MEP amplitudes revealed a significantly higher increase of MEP amplitudes in responders compared to non-responders after all three blocks of M1-iTBS. Increases between blocks (iTBS600/iTBS1200, iTBS1200/iTBS1800, iTBS600/iTBS1800) were not significantly different between groups (no dose-dependent differences between groups). Of note, we included the first block of iTBS in the ANOVA (although responders and non-responders were grouped according to their initial increase after the first block) to verify that our criterion defining responsiveness (MEP increase >10%) resulted in two statistically different groups. However, when performing the same ANOVA without the first block of iTBS results were highly similar. Therefore, the findings were not driven or biased by the first block of iTBS. Moreover, there was no significant difference between responders and non-responders in the steepness of stimulus-response curves.

Within the non-responder group, post-hoc Student's t-tests on MEP amplitudes revealed a dose-dependent decrease between sham-iTBS1200 and sham-iTBS1800 ($p = 0.039$, FDR-corrected) (Fig. 3A). Importantly, no significant differences were found after sham-iTBS between groups. Furthermore, there were no significant increases neither for M1-iTBS compared to baseline nor between M1-iTBS and sham-iTBS for non-responders. By contrast, in responders MEP amplitudes significantly increased after M1- compared to sham-iTBS (iTBS600: $p = 0.032$, iTBS1200: $p = 0.018$, iTBS1800: $p = 0.030$, FDR-corrected) as well as after M1-iTBS compared to baseline (iTBS600: $p = 0.010$, iTBS1200: $p = 0.015$, iTBS1800: $p = 0.023$, FDR-corrected) (Fig. 3B). Statistical trends suggesting dose-dependent increases in responders could be found between iTBS600 and iTBS1800 ($p = 0.076$, FDR-corrected) as well as between iTBS1200 and iTBS1800 ($p = 0.076$, FDR-corrected). Moreover, there were no significant effects on the steepness of the stimulus-response curves within the responder and the non-responder group.

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For the cut-off of >15% a significant main effect of GROUP ($F_{1,14}$ = 18.948, p = 0.001) as well as an interaction effect of the factors INTERVENTION × GROUP ($F_{1,14}$ = 13.407, p = 0.003) could be replicated. Similarly, the cut-off >20% revealed a significant main effect of GROUP ($F_{1,14}$ = 18.025, p = 0.001) as well as an interaction effect of the factors INTERVENTION × GROUP ($F_{1,14}$ = 43.034, p = 0.000). Hence, statistics were very similar across different cut-off values defining iTBS response.

Fig. 4 depicts the individual response profiles for all subjects and sessions. Accordingly, despite robust effects at the group level, a large variability of effects is evident for the different subgroups. Two-thirds of subjects within the non-responder group (6/9) exhibited decreased MEP amplitudes (1% or more below baseline level) after the first block. Only two subjects showed a decrease of more than 10%, which rater represents an “inverse” response (Hamada et al., 2013). Furthermore, with respect to dose-dependent effects, only one subject out of nine non-responder subjects showed an increase of >10% after the second block of iTBS. This subject already showed an increase after the first block of iTBS (+8%), which was close to our cut-off criterion. Moreover, three of the subjects showed an increase of >10% (compared to baseline) after the third block of iTBS. However, Fig. 4 also shows that in these subjects showing a somewhat delayed iTBS effect, increases were only moderate (max. +42% after three iTBS blocks) and clearly different from those showing a strong response (max. +62% in the responder group).

iTBS-induced plasticity: resting-state functional connectivity

When comparing rsFC after iTBS between responders and non-responders for different iTBS doses, we found a significantly higher increase in rsFC for M1-iTBS vs. sham-iTBS for iTBS600 (p = 0.006, FWE-corrected at the cluster-level) as well as iTBS1800 ($p \leq 0.001$, FWE-corrected at the cluster-level) and a statistical trend after iTBS1200 (p = 0.071, FWE-corrected at the cluster-level) in responders compared to non-responders (Fig. 5A). Here, rsFC was significantly enhanced between M1 and a bilateral network comprising premotor areas, parts of the somatosensory cortex as well as the contralateral M1 (right M1, left and right SMA, left and right dPMC). Between the different stimulation blocks, responders showed a significantly higher, dose-dependent increase from iTBS1200 to iTBS1800 compared to non-responders in right M1 and dPMC ($p \leq 0.05$, FWE-corrected at the cluster-level, Fig. 6A). Within groups we found a significant increase in rsFC after M1-iTBS compared to sham-iTBS after each block only in the responder group ($p \leq 0.05$, FWE-corrected at the cluster-level, Fig. 5B). Functional connectivity was enhanced between M1 and bilateral SMA and dPMC as well as parts of the somatosensory cortex and contralateral M1. No significant decreases could be found within the responder group. In contrast, non-responders showed no significant changes in rsFC after M1-iTBS compared sham-iTBS. Moreover, a dose-dependent increase in rsFC could be observed between iTBS1200 and iTBS1800 in responders for the right M1 and dPMC (Fig. 6B). In contrast, no significant dose-dependent increases or decreases were evident in the non-responder group.

Response cut-offs of 15% and 20% revealed highly similar connectivity changes compared to a cut-off of 10%. However, changes in rsFC for a cut-off of 20% were only significant at

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an uncorrected level, probably due to decreased statistical power (small number of subjects in the responder group, n = 5).

Interestingly, we found that areas showing different rsFC between responders and non-responders at baseline (non-responders > responders, Fig. 2) overlapped with areas showing a higher increase in rsFC after all three iTBS blocks (conjunction iTBS600-1800) for responders compared to non-responders ($p \leq 0.05$, uncorrected). As shown in Fig. 5C this overlap was present in the SMA. A similar overlap was evident for the increase in rsFC after all three M1-iTBS blocks compared to sham-iTBS (conjunction iTBS600-1800) in the responder group ($p \leq 0.05$, uncorrected, Fig. 5D).

Finally, we found a significant positive correlation between the mean increase in rsFC across the whole group (n = 16; real > sham, increase compared to baseline) with increases in MEP amplitudes after iTBS600 (Pearson: r = 0.619, p = 0.010; Spearman: Rho = 0.632, p = 0.009; Fig. 5). However, similar effects were absent for the equivalent correlation after iTBS1200 and iTBS1800.

In summary, only responders featured a significant increase in rsFC after iTBS as seen for MEP amplitudes. Likewise, rsFC increased dose-dependently between the second and third block only in the responder group. Moreover, rsFC was higher (significantly or statistical trend) in the responder group compared to the non-responder group for all three blocks of iTBS.

Discussion

Summary of findings

iTBS non-responders compared to responders featured higher pre-interventional levels of M1-connectivity with a cortical network comprising bilateral premotor areas. Furthermore, responders and non-responders differed in iTBS-induced aftereffects on MEP amplitudes as well as rsFC: only responders showed an increase in MEP amplitudes and in rsFC in a bilateral motor network comprising premotor areas as well as the contralateral M1 and somatosensory areas. Likewise, dose-dependent increases were found exclusively in responders. Of note, the network in which connectivity was significantly modulated by iTBS overlapped with areas showing baseline differences in connectivity between responders and non-responders.

Inter-individual variability in iTBS-responses (MEPs)

Recent studies reported no group-level effect of TBS on cortical excitability (Hamada et al., 2013; López-Alonso et al., 2014; Martin et al., 2006). Although in our cohort of subjects ~56% were classified as non-responders, we still observed a significant increase in MEP amplitudes across the entire sample (please cf. Nettekoven et al., 2014). We here found that this effect was driven by 44% of our subjects that featured strong canonical responses, i.e., increases in cortical excitability already after one block of iTBS. Note that responsiveness to iTBS in our cohort was slightly lower but similar compared to previous studies reporting 50%–73% of their subjects to respond as expected, i.e., with an increase in MEPs after iTBS (Hamada et al., 2013; Hinder et al., 2014). Importantly, response rates observed between

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studies are similar, although different cut-offs regarding changes in MEP amplitudes were used to define responders and non-responders: above or below 100%, 110% or 120% (Goldsworthy et al., 2014; Hamada et al., 2013; Hinder et al., 2014; López-Alonso et al., 2014). In the present study, we confirmed that the choice of the cut-off value (i.e., 10%, 15% or 20%) was not critical with respect to the stability of the results. Variance in response rates between studies may result from, e.g., different time points and durations of MEP-assessment after iTBS as well as intensities used to obtain MEPs. We chose our criterion (at least 10%) in accordance to Hinder et al. (2014) since changes in MEP amplitudes less than 10% might be due to variance of MEP assessment or represent rather negligible effects regarding behavioral or clinical implications. However, whether or not a MEP increase of 10% actually leads to a relevant improvement in motor function needs to be investigated in future studies.

Plotting the individual responses to iTBS for the responder and the non-responder group illustrates that responders and non-responders are not homogenous groups, but show a considerable amount of inter-individual variability (Hamada et al., 2013). On average, non-responders cannot be turned into responders by increasing the stimulation dose. Those few non-responders with an increase after the second or third block of iTBS still remained at the lower bound of responsiveness, confirming that the predisposition of showing excitability changes after iTBS seems to be individually determined, and cannot be fundamentally changed by increasing the stimulation dose.

Taken together, the further enhancement of cortical excitability after iTBS1800 observed in our previous study (Nettekoven et al., 2014) was basically driven by an amplification of aftereffects in responders, and not from non-responders turning into responders. This finding is of high relevance not only for rTMS/TBS experiments in healthy subjects but also with respect to therapeutic interventions in patients.

Relationship between baseline measures and increases in cortical excitability

A number of factors have been discussed to contribute to the high inter-individual variability observed in the response to iTBS (and other rTMS protocols) such as daytime, previous history of activity, or genetic polymorphisms (for a review see Ridding and Ziemann, 2010). In line with other studies, we here did not find a significant effect of RMT or MEP amplitudes on changes in cortical excitability after iTBS (Hamada et al., 2013; López-Alonso et al., 2014).

We recently showed that fMRI connectivity between the premotor cortex and the stimulated M1 (in the activated motor system, i.e., when subjects performed an unimanual task) correlated with iTBS-induced increases in cortical excitability after 10 min (Cárdenas-Morales et al., 2014). However, in that study, rsFC did not correlate with MEP changes. Here, we could show that the average increase of the cluster showing a significant increase in rsFC after iTBS600 correlated positively with the increase in MEP amplitudes after iTBS600, suggesting a linear relationship between aftereffects on MEPs and rsFC. Moreover, when dividing subjects into responders and non-responders according to their increase in MEPs after the first stimulation we found a significantly higher baseline rsFC between M1 and premotor areas in non-responder compared to responders (Fig. 2). One

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interpretation is that high baseline levels of rsFC (as found in the non-responder group) could preclude a further increase in rsFC and MEPs, hence constituting a ceiling effect. Indeed, other groups have suggested that ceiling effects with respect to the ability of modulating neural connectivity might underlie absent intervention effects (Huang et al., 2010; Koch et al., 2008; Quartarone et al., 2003; Salomons et al., 2014). For example, Salomons et al. (2014) showed that a high baseline resting-state cortico-thalamic, cortico-striatal and cortico-limbic connectivity was associated with poorer rTMS treatment outcome in patients with major depressive disorder. Of note, a successful intervention effect was also associated with an increase in rsFC as seen for our group of responders.

Taken together, our data suggest that the responsiveness to iTBS (in terms of changes in MEP amplitudes) depends – at least in part – on the baseline level of rsFC between premotor areas and M1, possibly representing a biomarker for the individual responsiveness to iTBS.

Mechanisms underlying responsiveness to iTBS

Recent evidence from human and animal studies suggests that the individual response to TBS might derive from the stimulation of distinct subpopulations of interneurons (Benali et al., 2011; Funke and Benali, 2011; Hamada et al., 2013). High intensity (suprathreshold) single-pulse TMS with a latero-medial oriented current (LM-TMS) directly activates the axons of the corticospinal neurons resulting in direct waves (D-waves) as shown by epidural recordings (Di Lazzaro et al., 2012). In contrast, anterior-posterior (AP) TMS tends to evoke indirect waves (I-waves) with longer latencies resulting from the transsynaptic (hence indirect) activation of corticospinal neurons. Hamada et al. (2013) found that the response to AP-TMS (recruitment of I-waves) varies between subjects, accounting for a part of the inter-individual differences in stimulation aftereffects: Subjects showing the “expected” response to TBS tended to recruit late I-waves (high MEP-latency after AP-TMS relative to LM-TMS), whereas non-responders tended to recruit earlier I-waves (low MEP-latency after AP-TMS relative to LM-TMS). There is evidence that I-wave recruitment (i.e., responsiveness to iTBS) is related to premotor-M1 connectivity (Volz et al., 2014): Functional connectivity between premotor areas and M1 as well as SMA and M1 (highly similar to the network obtained here, Fig. 2) is lower in subjects preferentially recruiting late I-waves following AP-TMS, resembling responders as described by Hamada et al. (2013). These findings fit our present data revealing that subjects, who featured a lower connectivity between M1 and premotor cortex as well as between M1 and SMA show a significant response to iTBS (increase in MEPs and rsFC). In contrast, cortical excitability and rsFC did not increase after iTBS in subjects with higher M1-premotor as well as SMA-M1 connectivity at baseline.

Premotor input to M1, which has been found to be related to iTBS responsiveness, excites some of the same circuitry that participate in I-wave generation (Di Lazzaro and Ziemann, 2013; Lemon, 2008; Shimazu et al., 2004), providing further evidence for the relationship between responsiveness to iTBS/I-wave recruitment and premotor-M1 connectivity.

Therefore, our data suggest that responsiveness to iTBS not only underlies local M1 excitability, but also the connectivity strength between M1, SMA and lateral premotor areas (which is associated with the recruitment of I-waves). This interpretation is further supported by our post iTBS data as well as other studies reporting that rTMS/TBS does not only lead

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to changes in cortical excitability of the stimulated region but also in connectivity of the stimulated and remote areas (Grefkes et al., 2010; Nettekoven et al., 2014; Suppa et al., 2008; van der Werf et al., 2011; Vercammen et al., 2010).

We found a linear relationship between increases after the first block of iTBS in rsFC and MEPs. Although no linear correlation was evident for the second and third block of iTBS, increases in MEP amplitudes are paralleled by changes in rsFC. Increases in both MEPs and rsFC could only be observed in the responder group, whereas neither MEPs nor rsFC increased in non-responders. Therefore, aftereffects on the level of MEPs and functional connectivity seem to be related to each other. Note that in our previous paper (Nettekoven et al., 2014) we did not find a correlation between MEP changes and iTBS response when performing a voxel-wise analysis. Only when taking the average response (first eigenvariate) across the entire cluster showing stimulation aftereffects, a correlation with MEP changes became significant. This finding implies that a linear relationship between MEP and rsFC is rather found at the global level considering average changes in motor system connectivity. Interestingly, Watanabe et al. (2014) reported a relationship between the effects of quadripulse TMS (QPS) on MEPs and rsFC. The magnitude of changes between the stimulated and the contralateral M1 was significantly correlated with the magnitude of changes in MEPs. Hence, iTBS exerts widespread changes in intra- and interhemispheric connectivity in parallel to changes at the local (excitability) level. Moreover, a study in stroke patients could show that patients with lesions affecting the premotor cortex but not M1 were less responsive to rTMS over M1 in terms of behavioral changes (Ameli et al., 2009), suggesting that the effectiveness of rTMS depends on the functional integrity of the stimulation site (recruitment of corticospinal connections, “network shaping effect” by propagating rTMS-effects to other nodes of the network). Non-responsiveness, therefore, seems to result from a missing propagation of facilitation caused by the missing connection between M1 and premotor regions or a high baseline M1-rsFC (limited capacity for modification) as found here. Hence, the excitability state of premotor-M1 as well as SMA-M1 connections and the increase in rsFC between these areas could represent one mechanism underlying responsiveness to iTBS also at the behavioral level.

Future prospects

In this study, we did not observe a behavioral correlate of the resting-state difference at baseline between responders and non-responders with respect to maximum finger tapping frequencies. However, it might well be that more complex motor task (relying on, e.g., SMA activity like movement sequences) behavioral differences between responders and non-responders might have become evident. Such a question should be addressed in a future study.

Moreover, studies using a brain-state dependent stimulation (BSDS) set up, where TMS is combined with a simultaneous EEG measurement, showed that BSDS significantly increased the excitability of the stimulated motor cortex (Gharabaghi et al., 2014). Therefore, it might well be that iTBS responses observed in the present study also depend on the current electrophysiological state of the stimulated brain, which could also explain the variability observed in the data.

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Conclusion

Responsiveness to iTBS seems to be strongly linked to motor network connectivity. Responders revealed increased MEP amplitudes as well as increased connectivity following a higher number of stimuli. Our findings might also hold implications for the clinical use of non-invasive brain stimulation, i.e., highlighting the necessity to identify patients that might benefit from TMS interventions (and thereby from multiple applications). Finding reliable biomarkers for responsiveness also in patient populations represents an important aim for future research.

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Abbreviations

AP	anterior–posterior
APB	abductor pollicis brevis
AMT	active motor threshold
ANOVA	analysis of variance
dPMC	dorsal premotor cortex
EPI	echo planar imaging
FD	framewise displacement
FDR	false discovery rate
fMRI	functional magnetic resonance imaging
FOV	field of view
FWE	family wise error
GLM	general linear model
iTBS	intermittent theta-burst stimulation
LM	latero-medial
MEP	motor-evoked potential
M1	primary motor cortex
RMSE	root mean squared error

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RMT	resting motor threshold
rsFC	resting-state functional connectivity
rTMS	repetitive transcranial magnetic stimulation
SMA	supplementary motor area
TBS	theta-burst stimulation
TE	echo time
TR	repetition time

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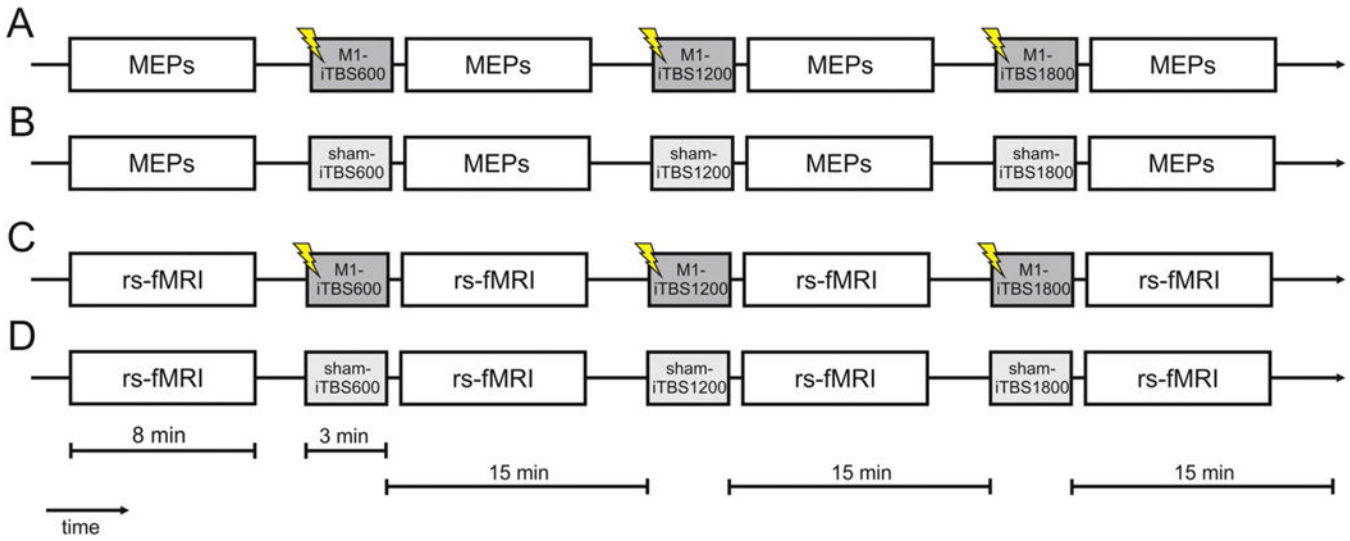

Fig. 1.

Experimental design. Using a within-subjects design each subject took part in four sessions to assess (i) MEPs before and after **(A)** M1-iTBS and **(B)** sham-iTBS as well as to assess (ii) rs-fMRI before and after **(C)** M1-iTBS and **(D)** sham-iTBS. In each session three iTBS blocks were applied separated by 15 min. Each iTBS block consisted of 600 pulses, leading to a total of 1800 pulses. MEPs/rs-fMRI measurements were started approximately 3 min after the end of a given iTBS block.

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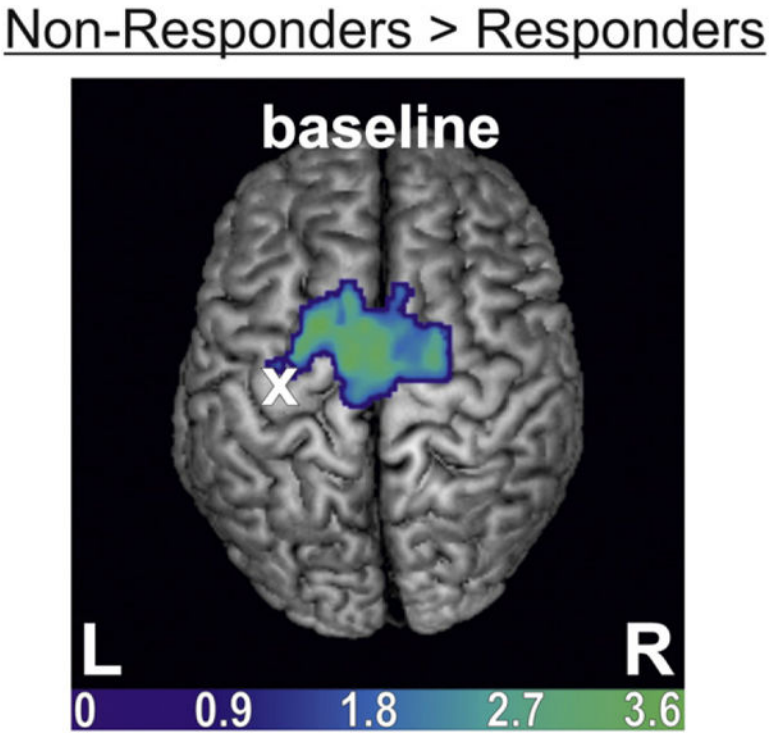

Fig. 2.

Baseline rsFC. Non-responders featured a higher baseline rsFC between M1 and a bilateral network including premotor areas (SMA, dPMC) compared to responders. Color bar represents t-values. Only clusters surviving a cluster level FWE-correction ($p \leq 0.05$) are shown. The X indicates the stimulated M1.

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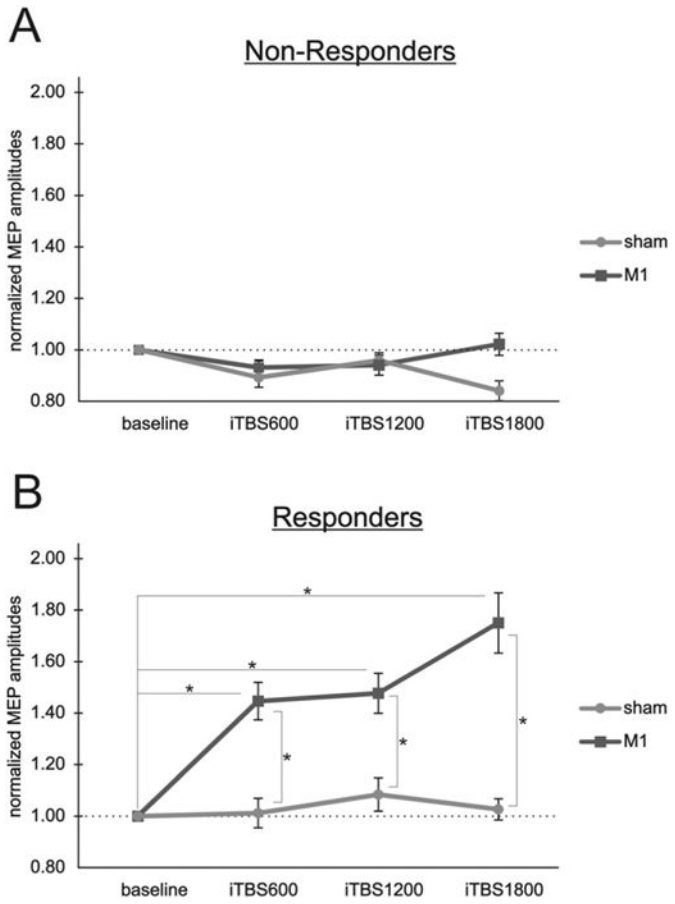

Fig. 3.

Changes in normalized MEP amplitudes after iTBS. **A.** Non-responders. M1-iTBS did not lead to changes in normalized MEPs compared to sham-iTBS or baseline. MEP amplitudes decreased significantly between sham-iTBS1200 and sham-iTBS1800. **B.** Responders. M1-iTBS led to a significant increase in MEPs compared to baseline and sham-iTBS after all three iTBS blocks. Sham-iTBS did not lead to changes in MEPs compared to baseline. For M1-iTBS a statistical trend was evident for the increase between iTBS600 and iTBS1800 as well as between iTBS1200 and iTBS1800. *$p \leq 0.05$ (post-hoc Student's *t*-test, FDR-corrected).

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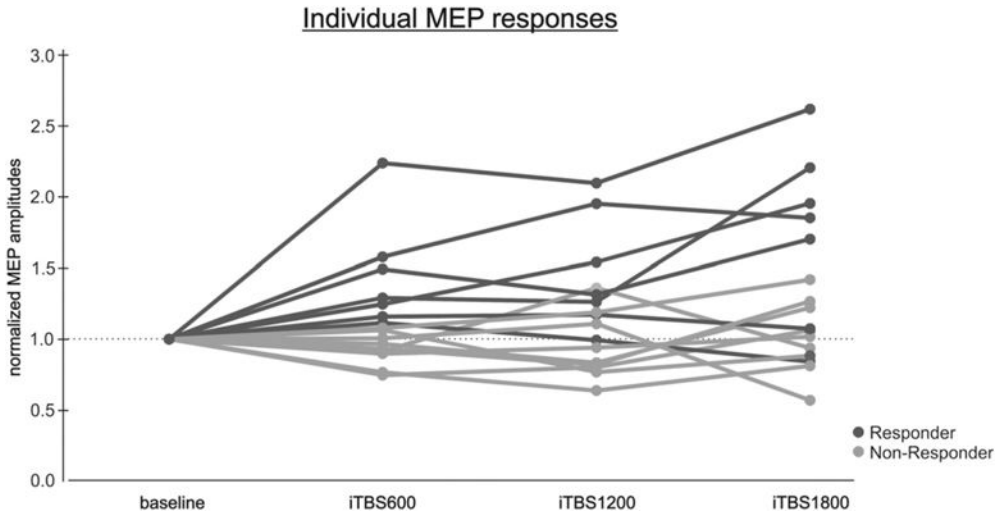

Fig. 4.

Changes in MEP amplitudes after M1-iTBS, single-subject level. Taken together, on average the repeated application of iTBS in non-responders did not lead to a significant increase of cortical excitability compared to baseline.

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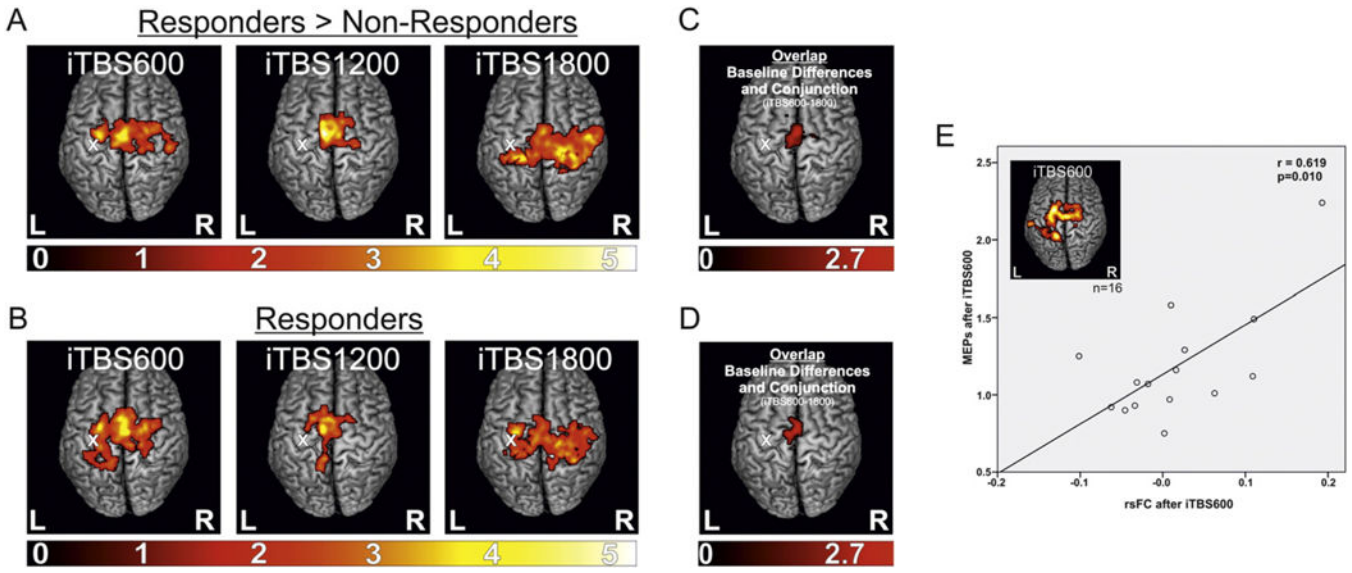

Fig. 5.

Changes in rsFC after iTBS. **A.** Responders > non-responders. Responders (compared to non-responders) featured a significantly higher increase in rsFC for M1-iTBS > sham-iTBS after iTBS600 and iTBS1800. Likewise, a statistical trend was evident for iTBS1200. **B.** Responders. A significant increase in rsFC after M1-iTBS compared to sham-iTBS was found for responders after all three blocks of iTBS. In contrast, non-responders did not show a significant increase in rsFC. Color bar represents t-values. Only clusters surviving a cluster level FWE-correction ($p \leq 0.05$) are shown. The X indicates the stimulated M1. Overlap with baseline differences. **C.** Responders > non-responders. The areas showing baseline differences between responders and non-responders (Fig. 2) overlapped with areas showing a stronger increase in rsFC after all three blocks of iTBS (conjunction iTBS600-1800) in responders compared to non-responders ($p \leq 0.05$, uncorrected). The overlap is present in the SMA. **D.** Responders. A similar overlap with baseline differences was found for the conjunction of the increase in rsFC after M1-iTBS600-1800 compared to sham-iTBS in the responder group ($p \leq 0.05$, uncorrected). **E.** Correlation. Mean increases in rsFC after iTBS600 across the whole group correlated significantly with increases in MEP amplitudes after iTBS600.

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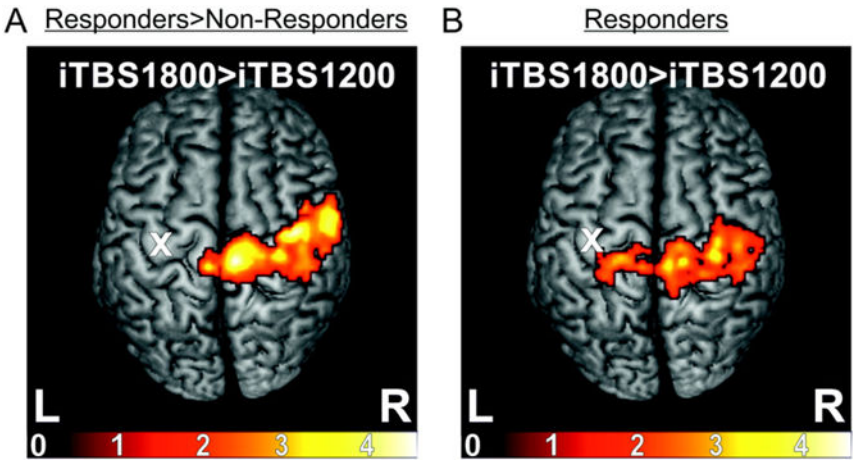

Fig. 6.

Dose-dependent increase in rsFC. **A.** Responders > non-responders. The dose-dependent increase in rsFC between iTBS1200 and iTBS1800 was significantly higher in responders compared to non-responders **B.** Responders. rsFC significantly increased after iTBS1800 compared to iTBS1200 (M1-iTBS > sham-iTBS) only in the responder group. $p \leq 0.05$, FWE-corrected on the cluster. The X indicates the stimulated M1.

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Table 1

Differences between responders (10% cut-off criterion) and non-responders in RMTs before iTBS and MEPs at baseline[a].

	Responders	Non-responders	p-value
Baseline RMTs			
Sham-iTBS	36.71 ± 8.79	30.78 ± 4.84	0.286
M1-iTBS	35.14 ± 7.40	30.00 ± 4.44	0.551
Baseline MEPs			
Sham-iTBS	0.97 ± 0.68	0.87 ± 0.48	0.757
M1-iTBS	0.93 ± 0.72	0.83 ± 0.42	1.000

[a]p-values are FDR-corrected for multiple comparisons.