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Towards understanding rTMS mechanism of action: Stimulation of the DLPFC causes network-specific increase in functional connectivity

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ABSTRACT

Transcranial magnetic stimulation (TMS) is a powerful non-invasive technique for the modulation of brain activity. While the precise mechanism of action is still unknown, TMS is applied in cognitive neuroscience to establish causal relationships between stimulation and subsequent changes in cerebral function and behavioral outcome. In addition, TMS is an FDA-approved therapeutic agent in psychiatric disorders, especially major depression. Successful repetitive TMS in such disorders is usually applied over the left dorso-lateral prefrontal cortex (DLPFC) and treatment response mechanism was therefore supposed to be based on modulations in functional networks, particularly the meso-cortico-limbic reward circuit. However, mechanistic evidence for the direct effects of rTMS over DLPFC is sparse. Here we show the specificity and temporal evolution of rTMS effects by comparing connectivity changes within 20 common independent components in a sham-controlled study. Using an unbiased whole-brain resting-state network (RSN) approach, we successfully demonstrate that stimulation of left DLPFC modulates anterior cingulate cortex (ACC) connectivity in one specific meso-cortico-limbic network, while all other networks are neither influenced by rTMS nor by sham treatment. The results of this study show that the neural correlates of TMS treatment response are also traceable in DLPFC stimulation of healthy brains and therefore represent direct effects of the stimulation procedure.

1. Introduction

Within the last decades, transcranial magnetic stimulation (TMS) emerged as a powerful tool widely used in neuroscientific research and therapeutic settings. It allows for focused, non-invasive stimulation of cortical brain areas using rapidly switched extracranial magnetic fields.

Repetitive TMS (rTMS) is a variant applying trains of electromagnetic pulses to the brain for up to 20 min (Rossi et al., 2009), which results in depolarization of cortical neurons and subsequent behavioral changes (Barker et al., 1985; Rachid and Bertschy, 2006). Although the precise mechanisms of rTMS treatment effects are still unclear, rTMS is an FDA-approved treatment in treatment resistant major depressive disorder (MDD) (George et al., 2010; Janicak et al., 2010). Moreover, rTMS resulted in beneficial behavioral effects in a range of conditions including posttraumatic stress disorder, schizophrenia and addiction (Lefaucheur et al., 2014). Although response rates are increasing (Gross et al., 2007), many MDD patients do not respond to rTMS treatment, thus indicating the importance of further investigation into the precise rTMS treatment mechanism so to increase the number of treatment responders.

Successful rTMS treatment response in the above psychiatric disorders is linked to changes in meso-cortico-limbic and serotonergic systems (Dichter et al., 2015). While meso-cortico-limbic dopamine circuits are at the core of motivational actions and reward processing (Feil and Zangen, 2010), serotonergic neurotransmission plays a crucial role in mood regulation (Maes and Meltzer, 1995).

The left dorso-lateral prefrontal cortex (DLPFC) is one important hub in these circuits and involved in a broad range of executive function processes including planning, organization, set shifting, attention, response inhibition, working memory, reward processing and guiding behaviour (Feil et al., 2010). Thus, the DLPFC is an ideal stimulation

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target for rTMS when aiming for modulating these circuits.

The interplay between anterior cingulate cortex (ACC) and DLPFC has long been theorized to play a crucial role in the adjustment to cognitive conflicts (Mansouri et al., 2009) and therefore disrupted connectivity in this network is likely leading to maladjusted cognitive processes in neuro-psychiatric diseases. The ACC has also long been known to be a critical hub in depression and anxiety (Bench et al., 1992; Devinsky et al., 1995).

Although the effects of rTMS over left DLPFC were examined repeatedly, only very few studies were able to successfully demonstrate actual effects on ACC. Concerning combined rTMS/PET-studies, 10 Hz rTMS over DLPFC was shown to increase cerebral blood flow in the DLPFC and ACC (Paus et al., 2001) and modulate dopaminergic activity in ACC and orbitofrontal cortex (Cho and Strafella, 2009) as well as serotonergic activity in cingulate, insular and parahippocampal cortices (Sibon et al., 2007). In one fMRI study, Li et al. (2004) investigated 1 Hz rTMS effects over left DLPFC in depressed patients and found alterations in BOLD response at the stimulation site and in the ventromedial prefrontal cortex (VMPFC). These studies provide evidence for rTMS over DLPFC modulating ACC activity.

In addition to these PET and task-based fMRI approaches, resting-state (RS) fMRI seems to be a method ideally suited to capture changes in cerebral networks in a way unbiased by task instructions and performance differences (Biswal et al., 2010; Raichle, 2015; Raichle et al., 2001). We thus performed a sham-controlled, cross-over study to assess the effects of rTMS on resting-state functional connectivity patterns. In addition to RS-fMRI scans before and after rTMS, we performed a third, delayed RS-fMRI scan to capture the temporal evolution of rTMS-induced effects. Since EEG-studies have shown lasting effects of high-frequency rTMS of on average 30 min (Thut and Pascual-Leone, 2010), we chose the time delay for the second resting-state scan as 30 min post rTMS in order to investigate whether or not these effects are reproducible on a network level.

RS-fMRI data analysis is most often performed as seed voxel correlation where you define a priori network nodes that are related to the outcome; selection of these seeds is critical and the tempting possibility to perform hundreds of seed voxel correlations often lead to multiple comparison problems. On the other hand limiting the analyses to just a few seeds requires strong a priori assumptions and may lead to false negative results since (possibly much stronger) changes in networks not part of the seed regions might be missed. Approaches like independent component analysis (ICA) are ideal for an exploratory assessment of stimulus-induced changes, but suffers from the fact that generalization of its results are difficult as components are defined from the current sample and the chosen number of components may be inadequate.

Here, we use a different approach in RS-fMRI data analysis situated between seed-voxel and ICA methods. It is based on 20 independent components that were found consistent across a world-wide sample including over 1000 subjects (Biswal et al., 2010; Zuo and Xing, 2014). This allows us to compare whole-brain networks without bias regarding seed-voxel definition, component numbers and component selection. We decided in our approach to use components from an independent large sample in order to extract time courses of these spatial components on the subject level and use the time courses as seeds for seed voxel correlations. This allows for an assessment of connectivity changes across different studies, as network definitions are independent of the actual subject sample. We are then comparing changes in these components in our sample before and two times after rTMS over left DLPFC. We show that a single session of rTMS causes a temporary change in functional connectivity in one and only one of those 20 components, namely the component including the ACC, while sham rTMS did not lead to changes in any of the 20 components.

2. Methods

In this study we aimed to investigate the effect of rTMS over left DLPFC on a set of 20 common independent components of intrinsic connectivity (Biswal et al., 2010) using a sham-controlled, cross-over design (Fig. 1). During the verum session, rTMS was applied to the left DLPFC, whereas vertex stimulation was performed in sham sessions. The two stimulation conditions (verum/sham) were counterbalanced across subjects and sessions were separated by one week.

2.1. Participants

60 healthy right-handed subjects (age: 25.01 ± 4.6 years, f/m: 31/29) underwent rTMS over DLPFC (verum) and sham stimulation (over vertex). They had no medical, neurological, or psychiatric history (DSM IV) and no first-degree relatives with psychiatric or neurological diseases. Urine drug screening before fMRI measurements guaranteed inclusion with negative drug screening only. After a description of the study, written informed consent was obtained.

2.2. TMS

The TMS-coil used for this study was a figure-of-eight-shaped MR-compatible coil (MRi-B90 II; Magventure MagPro X 100 stimulator, Tonica Elektronik A/S, Denmark). Prior to each stimulation session, the motor threshold was defined for each participant. In order to define the motor threshold, three EMG-electrodes were placed in a belly-tendon application on the right hand. After locating the M1 target area via neuronavigation (Brainsight 2, Rogue Research Inc., Canada) including Polaris optical position sensor (Northern Digital, Waterloo, ON, Canada), single TMS-pulses were applied at an intensity of 80% stimulator output. The coil was positioned in a 45° angle in relation to the surface of the skull. The representational field of the first dorsal interosseus (FDI) muscle, which is placed at the outer edge of the hand-knob, served as target area. In an axial plane, the hand-knob resembles an omega or epsilon-shaped (Yousry et al., 1997) structure in the lateral precentral gyrus. The stimulation intensity was decreased until the lowest intensity was found, at which 3 of 5 pulses applied in a row resulted in a MEP response between 0.05 and 0.1 mV within a time window between 15 ms and 35 ms after application.

For the verum condition, the TMS coil was placed above the left DLPFC (−42,28,21 MNI), for sham above the vertex. Stimulation was at a frequency of 10 Hz with a stimulation time of 5s per train and 24 trains in total. The inter train interval (ITI) was 20s. Overall total stimulation time was 10 min, while the total number of pulses was 1200. The stimulator output was set to 90% of the motor threshold.

2.3. fMRI data acquisition

Imaging was performed using a whole-body 3 Tesla Tim Trio (Siemens Medical, Germany) whole-body MR-Tomograph combined with the manufacturer's default 32-channel head coil (Siemens Medical, Germany). 23 slices of 3 mm thickness were acquired through the sagittal axis centered above the fissura longitudinalis. The functional RS T2*-weighted scans were performed by means of a single-shot gradient-recalled EPI sequence (TE/TR = 38/1800 ms, 128 × 128210 × 250 1.5 mm, 23 axial slices parallel to the AC-PC-plane, thickness 3 mm, gap between slices: 4.8 mm). During RS fMRI, participants were asked to look at a fixation cross and let their mind wander. RS scans were acquired 10 min before and 15 and 30 mins after stimulation. We chose the first post-TMS time point as 15 min after rTMS as it was previously shown that TMS evokes robust aftereffects within this time window (Thut and Pascual-Leone, 2010) without direct effects of stimulation. To investigate if these effects would outlast a longer time period we performed a second measurement 30 min after stimulation since this was shown to be the average upper limit in TMS/EEG experiments (Thut and Pascual-Leone, 2010).

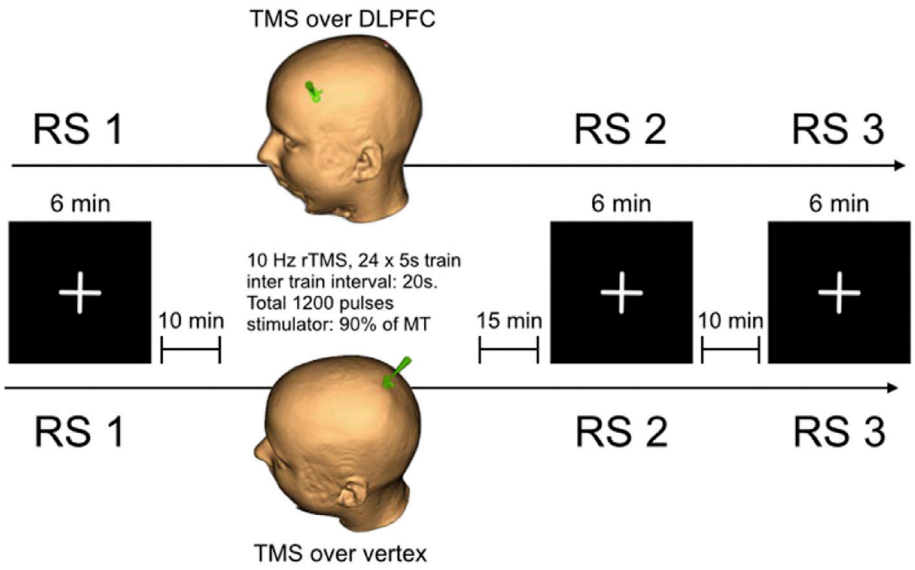

Fig. 1. Experimental procedure. Each subject participated in two sessions (verum DLPFC/sham vertex, counterbalanced) and within each session three resting-state scans were acquired: before rTMS, approximately 15 min after rTMS, approximately 30 min after rTMS.

2.4. Analyses

The acquired resting-state data were despiked using AFNI (http://afni.nimh.nih.gov/afni). In order to compensate for slice-timing differences between EPI slices we employed temporal interpolation of the MR signal, by shifting the signal of the misaligned slices back in time to the reference slice. It has been shown that slice-timing correction can successfully adjust time shift between the acquisitions of the different slices and therefore increase the robustness of the data analysis (Sladky et al., 2011). This step is implemented in our preprocessing pipeline using FSL 5 (FMRIB Software Library, Analysis Group, FMRIB, Oxford, http://fsl.fmrib.ox.ac.uk). Further preprocessing comprised bias-field correction using ANTs (http://stnava.github.io/ANTs), realignment using FSL 5, normalization to standard MNI space using ANTs in combination with a custom scanner-specific EPI-template resulting in a 1.5 mm^3 isotropic resolution and finally smoothing with a 6 mm FWHM Gaussian kernel using FSL 5. Further resting-state data processing was carried out using in-house applications which are mostly based on the GNU Scientific Library (http://www.gnu.org/software/gsl) and comprised the following steps:

(1) regressing out the cerebrospinal fluid and white matter signal to reduce physiological artifacts using the first 5 components of a temporal PCA and their mean for both regions as well as the global mean (Weissenbacher et al., 2009);
(2) FFT-based band-pass filtering in the frequency range of 0.009–0.08 Hz;
(3) motion-scrubbing following Power et al. (2012). Frames at, as well as directly before and after the exceedance of a framewise displacement threshold of 0.5 or DVARS >0.04 (D referring to temporal derivative of timecourses, VARS referring to variance over voxels; calculated as the root mean square value of the differentiated BOLD timeseries) were removed. Framewise displacement was calculated as the sum over all first-order translational and rotational FSL realignment parameter differences, compared to the previous frame. Rotational parameters where projected onto a sphere with a radius of 50 mm, reflecting the relative change on the skull surface.
(4) seed voxel correlation using the mean time-course of every independent component mask previously determined using large-sample ICA by Biswal et al. (2010) to re-establish RSN#1-20 in our sample. In more detail, we
    (a) Binarized the 20 IC correlation maps ($FWE_{whole\text{-}brain}$<0.05) from the 1000 Functional Connectomes Project (http://fcon_1000.projects.nitrc.org) to create seed masks.
    (b) Extracted the mean time course of all voxels within each seed mask (IC 1-20) for each of our preprocessed RS data-sets.
    (c) Performed whole-brain correlation analyses with network mean time courses to re-establish the 20 RSNs in our sample for each subject and run.

In order to identify networks that are influenced by rTMS, these 20 correlation maps were further analyzed using SPM12: For each of the 20 RSNs the z-transformed correlation maps from all subjects were included in flexible factorial design analyses to test for statistically significant ($p<0.05$ FWE-corrected) changes across sessions (verum/sham stimulation) and runs (pre/post1/post2).

In order to examine network specificity and possible sham effects not related to stimulation site we initially chose to account only for multiple comparisons within the networks. This showed statistically significant changes in a single network, and only in the verum condition. We then applied Bonferroni correction to the final result to account for testing across 20 networks.

In order to clarify the connectivity of our ACC significant region within the identified sensitive network, we subsequently extracted a 5 mm spherical seed from the ACC (centered around 8, 40, −6 [MNI]), to explore altered connectivity to other parts of RSN#17 in more detail by performing a seed voxel correlation.

3. Results

Flexible factorial design analyses (session: verum/sham; runs: pre/post1/post2) and comparisons (T-contrasts) between pre- and post-stimulation RSNs revealed that 19 out of a total of 20 RS networks remained unchanged, i.e. showed no statistically significant differences before and after rTMS irrespective of verum/sham stimulation (Fig. 2A). Only a single network (RSN#17) showed rTMS-related connectivity changes (Fig. 2B). This network incorporates the dorsal cingulate cortex, posterior dorso-medial prefrontal cortex, DLPFC, inferior parietal lobule, inferior frontal cortex and posterior temporal lobes. We found the ACC to have increased connection strength to this network in the first post-rTMS scan, i.e. 15 min after verum stimulation (peak: 7.5, 39.5, −5.5 mm [MNI], $T_{peak} = 5.44$, Bonferroni-adjusted $p_{FWE=}0.036$, $k = 35$; Fig. 2CD). Importantly, sham TMS did not show any significant changes in RS

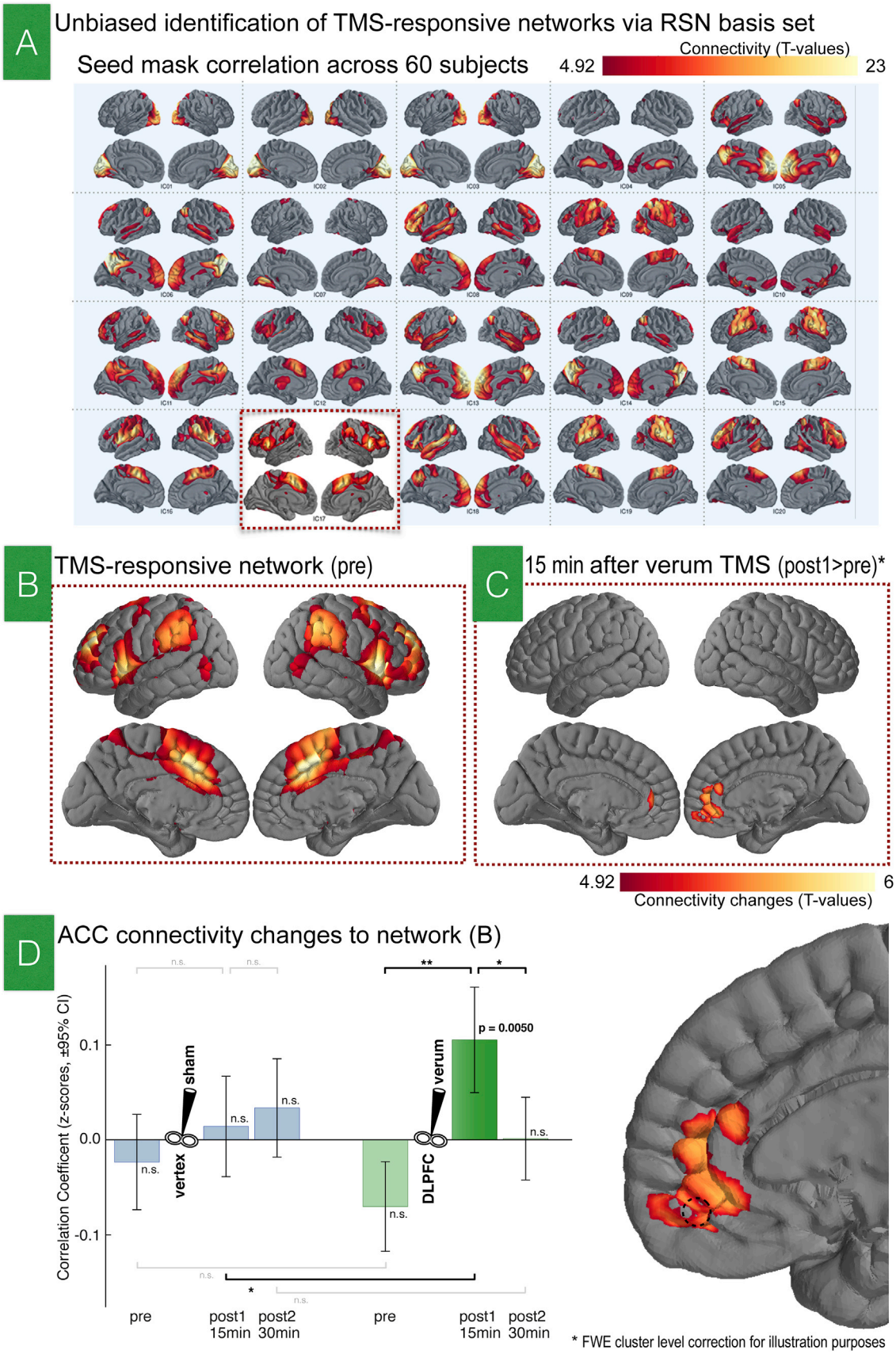

Fig. 2. Revealing specific effects of DLPFC rTMS. (A) To investigate how rTMS affects connectivity in networks within the whole brain we used maps from the seminal meta-analysis ($p < 0.05$) by Biswal et al. (2010). We used the mean time-course in masks based on independent components identified by this meta-analysis to replicate these networks (RSN#1-20) for the 6 acquired RS runs (sham/verum * 3runs) and further tested for changes pre and post verum (DLPFC) and sham (vertex) stimulation in all of the resulting 20 networks as displayed in (A). While 19 networks remain stable ($p<0.05$, FWE_{wb}) irrespective of stimulation site (sham/verum) and runs (before, 15 min and 30 min after stimulation), a single network (RSN#17,

highlighted) shows specific alterations. (B) Enlarged view of the single RSN (RSN#17, pre-TMS) that showed significant changes. Importantly, this RSN remained stable over the whole sham stimulation session. (C) Within RSN#17, verum rTMS on DLPFC increases connectivity to the anterior right ACC (verum post1 > pre is shown at $p < 0.05$, FWE_c. (D) This increase in ACC connectivity (z-scores) is only observed after verum stimulation (post1) but not in the sham condition. No significant changes were found after 30 min (post2) or between any RS run of sham session.

connectivity. Fig. 2D gives a more detailed insight on the changes in connectivity between ACC and RSN#17. TMS caused statistically significant increase in ACC-RSN#17 connectivity only in the first post-TMS resting-state scan (15 min after TMS). Connectivity in the second post-TMS scan (30 min after TMS) returned back to baseline level.

In a second step we aimed at revealing regions with higher connectivity to the ACC after verum stimulation. We therefore performed a correlation analysis with the ACC seed from our first analysis results (5 mm radius spheric ROI centered around 8, 40, −6 [MNI]). As shown in Fig. 3 and summarized in Table 1 this analysis revealed stronger functional connectivity of the ACC with the left DLPFC (peak: −36, 47, 32 mm [MNI], $T_{peak} = 5.14$), superior temporal/inferior frontal gyrus, BA47 (peak: −46, 18, −2.5 mm [MNI], $T_{peak} = 4.98$), bilateral insula (peak: 26, 11, 2 mm [MNI], $T_{peak} = 4.65$; peak: −32, 10, 6 [MNI], $T_{peak} = 4.45$), dorsal ACC (peak: −4, 23, 30 mm [MNI], $T_{peak} = 4.88$; peak: −8, 16, 46 mm [MNI], $T_{peak} = 3.45$), striatum, caudate nucleus and nucleus accumbens (NAcc) (peak: 15, 24, 4 mm [MNI], $T_{peak} = 4.71$). This was specifically observed in the second RS run, i.e. 15 min post verum stimulation; no significant changes were found comparing third RS (post2) with second (post1) or first (pre). No significant changes were found across RS runs in sham session.

4. Discussion

The results of this study show the high specificity of rTMS over left DLPFC in an unbiased, placebo-controlled large-sample approach. This study provides strong evidence that stimulation of left DLPFC influences the ACC within a specific functional network. We show that among all common resting-state networks only a single network was influenced by TMS, namely the network including ACC. Importantly, this TMS effect is observable only in the verum condition and does not exceed 30 min post stimulation; sham stimulation did not cause any statistically significant connectivity changes in any other functional network. As such, this study gives strong evidence for the possible ACC modulation mechanism on which rTMS depression therapy is based.

Given the large sample of 60 healthy subjects, the power of our analyses is high enough to demonstrate significant effects even at a strict statistical threshold with whole-brain family-wise error correction. Using a set of 20 common RS networks established in a large, independent sample (Biswal et al., 2010) allowed us to perform a comprehensive investigation of functional connections, revealing the high selectivity of rTMS over left DLPFC. Most importantly, this approach did not require a pre-selection of possible RSNs or seed regions. We might thus conclude that rTMS over DLPFC causes transient changes to only one RSN, namely the RSN#17 comprising DLPFC and ACC.

Fox et al. (2012b) indicate that to date no study was able to show the influence of rTMS over left DLPFC explicitly, as it remained inconclusive whether increased connectivity of one area with a specific network reflected functional connectivity or if this was just a result of local activity changes as a result of stimulation. Therefore it was argued that interpreters jumped to conclusions when they found activity changes after rTMS. The present study is the first attempt to solve this important question by applying an unbiased analysis approach involving all 20 RSNs extracted from an independent group of over 1000 subjects (Biswal et al., 2010).

A remarkable number of recent studies suggest connectivity between the ACC and frontal lobe nodes as an important factor in the application of rTMS over DLPFC: Dichter et al. (2015) have gathered evidence that DLPFC rTMS treatment response in MDD is associated with distinct functional connectivity changes within the meso-cortico-limbic dopamine circuit. Basically, rTMS responders showed significantly stronger anti-correlation between the subgenual ACC (sgACC) and prefrontal cortex (Baeken et al., 2014) and antidepressant treatment symptoms reduction was associated with selective modulation of aberrant sgACC hyperconnectivity to the default mode network (Liston et al., 2014). Salomons et al. (2014) reported higher rTMS treatment efficacy in major depressive disorder patients with higher baseline functional connectivity between sgACC and DLPFC. Moreover, stronger RS connections between different DLPFC targets and the ACC signify effective targets for stimulation (Fox et al., 2012a). Finally, ACC-DLPFC connectivity links invasive and noninvasive brain stimulation targets for effective depression treatment (Fox et al., 2014). All this evidence supports the idea that the

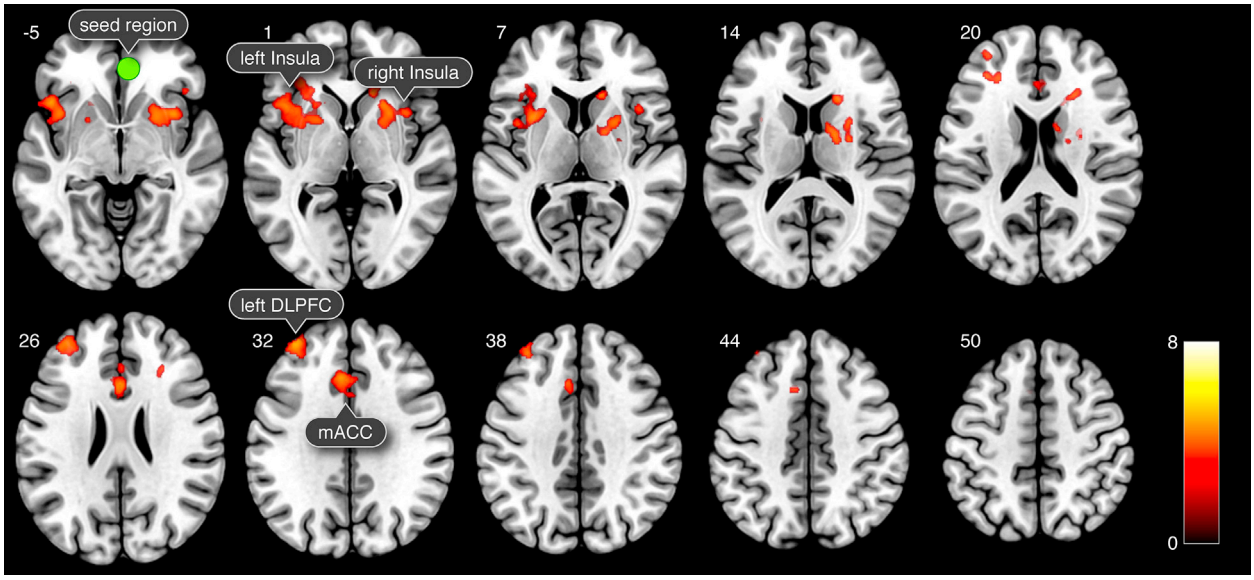

Fig. 3. Statistical parametric map of ACC POST1 > PRE. Time course correlations with a spheric 5 mm seed ROI around ACC (hotspot of Fig. 2C, green) reveals stronger correlation ($p < 0.05$, FWEc) post verum DLPFC stimulation between ACC and DLPFC, as well as insula, striatum and mACC.

Table 1
Changes in brain connectivity of ACC post1 > pre. This table gives an overview with cluster size (k), t-value and MNI coordinates of clusters showing increased connectivity with a spheric 5 mm seed ROI around ACC after DLPFC stimulation (see Fig. 1).

Cluster			Peak			MNI [mm]			Region
P (FWE-corr)	P (unc.)	k [vx]	P (FWE-corr)	P (unc.)	T	x	y	z	
0	0	790	0.026	0	5.14	−36	47	32	left DLPFC
			0.897	0	3.94	−34	36	18	left DLPFC
0	0	1 655	0.049	0	4.98	−46	18	−2	inferior frontal gyrus
			0.205	0	4.6	−40	12	−2	insula, superior temporal gyrus, BA47
			0.33	0	4.45	−32	10	6	insula
0	0	778	0.073	0	4.88	−4	23	30	dorsal ACC
			0.964	0	3.82	0	30	23	ACC
			1	0	3.45	−8	16	46	dorsal ACC, medial frontal gyrus
0.04	0.002	346	0.137	0	4.71	15	24	4	caudate nucleus, NAcc
			0.452	0	4.33	20	22	16	caudate nucleus
0	0	1584	0.17	0	4.65	26	11	−2	striatum
			0.187	0	4.62	36	11	−4	insula
			0.236	0	4.55	16	−2	11	putamen

treatment response following rTMS of the DLPFC could be explained by adjustment of abnormal connectivity as assessed by resting-state functional connectivity measures.

Notably the RSN#17 includes the dorsal cingulate cortex, posterior dorso-medial prefrontal cortex, DLPFC, inferior parietal lobule, inferior frontal cortex and posterior temporal lobes. Williams (2016) just proposed different networks that are involved in different biotypes of depression. She proposes a cognitive control network consisting of DLPFC, precentral gyrus, dorsal-pariatel cortex and anterior midline that is overlapped by increased default mode and attention network components, resulting in cognitive dyscontrol in patients. These overlapping nodes are the ACC and the inferior parietal lobules. She further claims disturbed cognitive control in these patients is best addressed by TMS and cognitive training. We therefore conclude for our work that TMS might allow the transition of ACC from default mode to an attentional state as a possible mechanism of action.

While the anterior midline structures are associated with attentional processes and the prefrontal areas with executive functions, inferior frontal cortex, inferior parietal lobule and temporal lobes are strongly involved in social cognition. Deregulation of the inferior parietal lobule was linked to depression (Müller et al., 2013) and altered connectivity between VMPFC and inferior parietal lobule and posterior temporal lobes was found as a marker of anhedonia in affective disorders (Young et al., 2016).

Former studies that found associations between rTMS stimulation over left DLPFC and altered activity or connectivity in the ACC either addressed depressive patient populations (Downar et al., 2014; Li et al., 2004; Liston et al., 2014; Salomons et al., 2014) and/or were not sham/placebo controlled (Cho and Strafella, 2009; Paus et al., 2001). Therefore it was not clear whether the changes were restricted to depressive populations and whether these changes were caused by direct, TMS-driven modulation of brain activity or by indirect placebo effects, i.e. noise, local skin irritations, facial muscle stimulation. This study overcomes the methodological issues associated with the use of rTMS over left DLPFC in depression treatment. First, the influence of inter-individual heterogeneity is addressed by scanning a large-sample of participants. Second, the causality and specificity of rTMS effects is assured by assessing resting-state fMRI activity in a placebo-controlled, cross-over design with one pre- and two post-rTMS scans for verum and sham session, respectively. Third, the influence of task requirements on brain activity is avoided by using a resting-state paradigm. Also, strong placebo responses, which may occur in studies including patients, are unlikely since the participants had no presumption about what effect they should expect during the resting-state paradigm.

One limitation of vertex stimulation as sham condition is that it leads to different somatosensory effects as compared to TMS over left DLPFC, since it is further away from facial muscles that can be co-stimulated by TMS. Furthermore, TMS over vertex leads to more symmetrical auditory sensations. Therefore, attention and corresponding network connectivity might be different during sham compared to verum stimulation. This issue becomes particularly critical in situations, where connectivity changes are assessed immediately after stimulation or even simultaneously (e.g., in concurrent TMS/imaging). However, since the design implemented in this study was based on investigating modulatory effects 15 min after stimulation, we consider vertex stimulation as an appropriate sham condition. In addition, our results show that sham stimulation had no significant influence on any prominent brain circuits that outlasts the stimulation procedure.

Finally, the inflated risk for false positive findings or false negatives is prevented by using an unbiased analysis investigating a specific number of stable Independent Components in network connectivity pre-established by Biswal et al. (2010).

Unlike previous evidence from EEG-studies, the RSN#17 connectivity changes did not outlast a time period of 30 min (Thut and Pascual-Leone, 2010). We conclude from our results that more superficial local effects as captured by EEG might be longer lasting than network effects as revealed by fMRI combined with our data analysis approach. We herein corroborate previous research and add to the understanding of the rTMS mode-of-action. When studying patient populations, differences in resting-state connectivity could be also caused by spontaneous symptom changes not directly related to rTMS treatment. Our results provide strong evidence that the observed changes in connectivity are caused by the neuromodulatory effects of rTMS.

The promising methodology of this study opens new possibilities in efficacy studies of rTMS and other treatment options in clinical populations, especially in subjects suffering from depressive disorders. In the future, RS connectivity analyses could be used to establish depressive connectivity states before treatment and remissive connectivity states after the first TMS treatment supporting classical neuropsychological test approaches, which rely on self-reported changes after several weeks of treatment. It thus seems feasible that short-term RSN changes could be used as indicators whether or not an individual patient might benefit from extended rTMS treatment.

Furthermore, our results show that rTMS over left DLPFC leads to higher connectivity of the ACC with structures of the meso-cortico-limbic dopamine system, namely the left DLPFC, caudate nucleus and NAcc. We extracted our specific network node out of the unbiased comparison of common RS networks, before investigating the relationship of connectivity changes of the resulting node to other structures of the network. Those regions show prominent axonal connectivity (Haber and Knutson, 2010) and are core regions of the so-called “reward-circuit”.

It remains a matter of debate why other RSNs including similar regions do not show any ACC connectivity changes. The inherent strictness of our analysis focusing on very specific independent components might have led to loss of some subthreshold connectivity changes. An other explanation might be that the detected RSN#17 is indeed very selective

for TMS-treatment as betoken by Williams (2016).

In conclusion, the results presented herein clearly demonstrate in an unbiased approach that rTMS over left DLPFC modulates functional connectivity within a *specific* network typically associated with attention, mood and reward processing and dysfunctions in psychiatric disorders. Therefore we suggest that this network-specific increase in ACC connectivity reflects a rTMS mechanism independent of subjective expectancy- and placebo effects. This strongly indicates that rTMS can be used to specifically target subcortical structures involved in neuro-psychiatric diseases. On a wider view it also suggests that the DLPFC rTMS treatment mechanism indeed relies on modulating meso-cortico-limbic networks. Together with evidence on the specific neural dysfunctions reported in other psychiatric conditions, this study indicates a mechanistic basis for using rTMS in the treatment of a much wider range of disorders as previously employed.

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Conflicts of interest

All authors declare no competing financial interests or potential conflicts of interest in relation to the work described.

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