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Test-retest reliability of prefrontal transcranial Direct Current Stimulation (tDCS) effects on functional MRI connectivity in healthy subjects

Jana Wörsching[a,*], Frank Padberg[a], Konstantin Helbich[a], Alkomiet Hasan[a], Lena Koch[a], Stephan Goerigk[b], Sophia Stoecklein[c], Birgit Ertl-Wagner[c], Daniel Keeser[a,c]

[a] Department of Psychiatry and Psychotherapy, Ludwig-Maximilians-University, Munich, Germany
[b] Department of Psychological Methodology and Assessment, Ludwig-Maximilians-University, Munich, Germany
[c] Institute for Clinical Radiology, Ludwig-Maximilians-University, Munich, Germany

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ABSTRACT

Transcranial Direct Current Stimulation (tDCS) of the prefrontal cortex (PFC) can be used for probing functional brain connectivity and meets general interest as novel therapeutic intervention in psychiatric and neurological disorders. Along with a more extensive use, it is important to understand the interplay between neural systems and stimulation protocols requiring basic methodological work. Here, we examined the test-retest (TRT) characteristics of tDCS-induced modulations in resting-state functional-connectivity MRI (RS fcMRI). Twenty healthy subjects received 20 minutes of either active or sham tDCS of the dorsolateral PFC (2 mA, anode over F3 and cathode over F4, international 10–20 system), preceded and ensued by a RS fcMRI (10 minutes each). All subject underwent three tDCS sessions with one-week intervals in between. Effects of tDCS on RS fcMRI were determined at an individual as well as at a group level using both ROI-based and independent-component analyses (ICA). To evaluate the TRT reliability of individual active-tDCS and sham effects on RS fcMRI, voxel-wise intra-class correlation coefficients (ICC) of post-tDCS maps between testing sessions were calculated. For both approaches, results revealed low reliability of RS fcMRI after active tDCS ($ICC_{(2,1)}$ = −0.09 – 0.16). Reliability of RS fcMRI (baselines only) was low to moderate for ROI-derived ($ICC_{(2,1)}$ = 0.13 – 0.50) and low for ICA-derived connectivity ($ICC_{(2,1)}$ = 0.19 – 0.34). Thus, for ROI-based analyses, the distribution of voxel-wise ICC was shifted to lower TRT reliability after active, but not after sham tDCS, for which the distribution was similar to baseline. The intra-individual variation observed here resembles variability of tDCS effects in motor regions and may be one reason why in this study robust tDCS effects at a group level were missing. The data can be used for appropriately designing large scale studies investigating methodological issues such as sources of variability and localisation of tDCS effects.

Introduction

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation (NIBS) technique that modifies cortical excitability by passing weak electrical current through the brain via two surface electrodes (Datta et al., 2009; Jackson et al., 2016). The current is flowing constantly from the anodal to the cathodal pole with an applied intensity of up to 4 mA and usually with 1–2 mA (Bikson et al., 2016; Edwards et al., 2013; Miranda et al., 2006). Depending on dose parameters, such as stimulation polarity, electrode positioning and applied current intensity, distinct current flow patterns as well as current density distributions are observable (Bai et al., 2014; DaSilva et al., 2015; Galletta et al., 2015; Mendonca et al., 2011; Neuling et al., 2012; Seibt et al., 2015; Woods et al., 2015). At the primary motor cortex, anodal tDCS (i.e. the anode is placed over the brain region of interest) is associated with increased motor-cortical excitability whereas the opposite is true for cathodal stimulation (Nitsche and Paulus, 2000, 2001). Such tDCS-induced excitability changes may originate from shifted resting membrane potentials towards de- or hyperpolarization (Jackson et al., 2016; Liebetanz et al., 2002; Nitsche et al., 2003). However, dose-response relations do not appear to be linear and measured responses may often be a function of the selected dose parameters (for review see Worsching et al., 2016). For example, Monte-Silva et al. (2013) found anodal stimulation of the prefrontal cortex (PFC, 1 mA intensity for 26 min) to decrease cortical excitability. Moreover, the position and size of the return electrode may influence neuromodulation within the region of the active electrode (Bikson et al., 2010). For example, for bipolar bilateral montages (Nasseri et al.,

* Corresponding author.
E-mail address: Jana.Woersching@med.uni-muenchen.de (J. Wörsching).

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2015) as often used for PFC stimulation, cortex regions close to the electrodes may receive both anodal facilitation and cathodal inhibition.

Based on the role of the PFC in cognitive domains and neuropsychiatric disorders (Mega and Cummings, 1994; Tandon, 2013) and in consideration of the potential of prefrontal tDCS to specifically modulate cognitive functions (for review see Tremblay et al., 2014), tDCS of PFC regions seems to be especially promising for therapeutic applications. Accordingly, the behavioural effects of prefrontal tDCS have been investigated in neurological (for review see Flöel, 2014; Schulz et al., 2013) and psychiatric disorders (for review see Kekic et al., 2016; Kuo et al., 2014). To elucidate the neural substrate of NIBS effects, tDCS can be combined with functional magnetic resonance imaging (fMRI). For instance, effects of prefrontal tDCS on the blood oxygenation level dependent (BOLD) signal in task fMRI can be observed in areas under or close to the electrode position as well as in regions distant from the electrode site (Hauser et al., 2016; Holland et al., 2011; Meinzer et al., 2012; Meinzer et al., 2013; Meinzer et al., 2014; Sacco et al., 2016; Weber et al., 2014). Instead of investigating activity in isolated brain regions, functional-connectivity MRI (fcMRI) provides the possibility to identify coordinated or integrated activity across regions (Beckmann et al., 2005; van den Heuvel and Hulshoff Pol, 2010), which is a central characteristic of healthy brain functions (Catani et al., 2013; Park and Friston, 2013). Such functional relations involve spatially distinct networks that can be extracted by analysis of the temporal coherence between spontaneous BOLD-signal fluctuations measured in different brain areas (Friston et al., 1993). In the resting state (RS), functional networks are reproducible across (Biswal et al., 2010; Damoiseaux et al., 2006) and within subjects (Blautzik et al., 2013; Braun et al., 2012; Laumann et al., 2015). Moreover, functional networks acquired under resting conditions – so called resting-state networks (RSN) – resemble functional networks during activation (i.e. task performance) (Smith et al., 2009) and are highly relevant for cognitive functions and behaviour (Laird et al., 2011; Tavor et al., 2016). For this reason, the impact of tDCS on RS fcMRI entails important information about the effectiveness of this method regarding its influence on cognition without requiring an active task. Previous studies examining the influence of prefrontal brain stimulation have shown that tDCS modulates RS fcMRI (Keeser et al., 2011; Meinzer et al., 2012; Meinzer et al., 2013; Meinzer et al., 2014; Palm et al., 2013a; Palm et al., 2016; Park et al., 2013; Pena-Gomez et al., 2012; Pereira et al., 2013; Volpato et al., 2013). For example, increased connectivity within the Frontal Parietal Network (FPN) was found following anodal tDCS of the PFC (Keeser et al., 2011; Pena-Gomez et al., 2012), potentially reflecting a cognitive state of enhanced alertness. Consequently, tDCS may bear the potential to restore altered connectivity patterns (Meinzer et al., 2013; Meinzer et al., 2014) which are often found in neuropsychiatric disorders (Buckholtz and Meyer-Lindenberg, 2012; Filippi et al., 2013; Fornito et al., 2015; Insel, 2010; Menon, 2011; Zhou et al., 2012). Though imaging stimulation, i.e. imaging the on- and offline effects of NIBS on RS fcMRI, may theoretically provide an ideal paradigm to investigate how tDCS affects neural integration and to test state, disorder and course dependency of tDCS effects, combined fMRI-tDCS investigations have methodologically not yet been developed to this point and the neurophysiological response to tDCS is still not completely understood (Parkin et al., 2015). One major issue is the intra- and inter-individual stability of tDCS effects. For both the therapeutic application of tDCS and the investigation of tDCS-induced neuromodulation and tDCS-related plasticity, it is essential to know whether the same stimulation protocol produces predictable effects across different treatment sessions. However, only few studies have tested the test-retest (TRT) reliability of tDCS effects and that only in motor-evoked potential (MEP) paradigms (Chew et al., 2015; Dyke et al., 2016; Horvath et al., 2016; Jamil et al., 2016; Lopez-Alonso et al., 2015). To our knowledge, this is the first study investigating the TRT reliability of prefrontal tDCS-induced modulation in RS fcMRI. For this purpose, effects of active or sham tDCS on RS fcMRI were measured on three different days in the same healthy subjects. In a first step, RS fcMRI at baseline and post tDCS was determined at an individual level. In a second step, reproducibility of intra-individual baseline and post-tDCS RS-fcMRI was tested using voxel-wise intra-class correlations, enabling comparisons between baseline RS-fcMRI reliability and reliability following active-tDCS or sham-tDCS intervention.

Methods

Participants and sociodemographics

We tested 20 healthy male participants (mean age: 23.85 years, age range: 18–32 years) in a total of 60 tDCS-fMRI sessions. They were all right-handed (M = 99, SD = 3.08, $range$ = 90 – 100) according to the Edinburgh Handedness Questionnaire (Oldfield, 1971). Exclusion criteria were a history of neurological and psychiatric diseases and the intake of neuroactive medication. Participant selection was also restricted to non-smokers and to people without drug consumption during the past 6 months. The study was approved by the local ethics committee (Department of Psychiatry and Psychotherapy, Ludwig-Maximilians-University Munich, Germany) and all patients gave their written informed consent for participation in this study.

Experimental procedure

This study followed a sham-controlled and double-blind design with parallel groups, such that neither participants nor investigators were aware of the stimulation condition. The 20 participants were pseudo-randomised into two groups: one active-tDCS and one sham group. Each participant received 20 min of either active or sham tDCS in the MRI scanner, always preceded, accompanied and ensued by a RS-fcMRI examination (combined on- and offline measurements, though only offline results are presented here). This procedure was conducted three times with at least seven days between each testing session. Within one participant, the stimulation condition (active vs. sham tDCS) was the same across all testing sessions. Daytime of measurement was kept constant for each participant across all testing sessions (see Fig. 1).

Participants were asked to abstain from alcohol the day before and from caffeine the morning before each testing session. At the beginning of each session and prior to fMRI scanning, participants filled in several questionnaires based on an in-house digital Android tablet system. Afterwards, participants went into the MRI scanner and were asked to keep their eyes closed, to not fall asleep, to think about nothing in particular and to avoid movements. During each session, RS scans were repeated three times, directly following each other: baseline/pre tDCS (10 min), during tDCS (20 min), post tDCS (10 min) (see Fig. 1). Instructions were repeated before each RS scan started and participants were informed before stimulation started. At the end of each session, participants again filled in several questionnaires in order to assess possible stimulation effects on mood and other variables.

Questionnaires

Several questionnaires were administered once at the overall baseline including the Edinburgh Handedness Questionnaire (Oldfield, 1971), the trait scale of the Positive And Negative Affect Schedule (PANAS, missing the item “enthusiastic” on the positive affect scale) (Krohne et al., 1996; Watson et al., 1988), the trait scale of the State Trait Anxiety Inventory (STAI) (Laux, 1981; Laux and Spielberger, 1981; Spielberger et al., 1970) and a questionnaire for sociodemographic data. Additionally, the PANAS state scale and the STAI state scale were completed prior to each stimulation. After each stimulation, the PANAS state scale was filled in again in addition to the Comfort Rating Questionnaire (CRQ) (Palm et al., 2014).

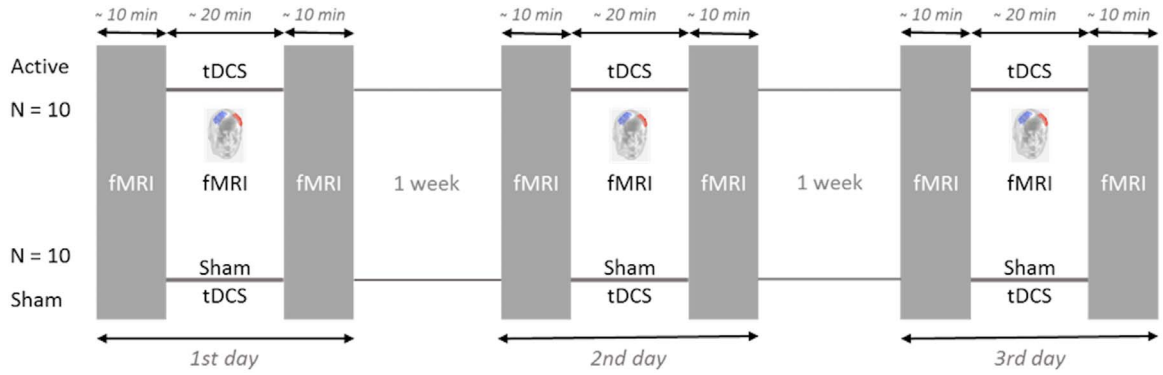


Fig. 1. Experimental protocol. Active- and sham-tDCS conditions were applied inside the MRI (online) after baseline fMRI scans according to a double-blind, between-subject design. The head model was created with Matlab/Comets (Jung et al., 2013).

Transcranial Direct Current Stimulation (tDCS)

TDCS was applied via two saline-soaked surface sponge electrodes (area = 7 × 5 cm^2) that were connected to an Eldith stimulator MR (neuroConn). In order to target the dorsolateral prefrontal cortex (DLPFC) bilaterally, the anode was placed over F3 and the cathode over F4 (according to the international 10–20 system). The impedance was kept below 10 kΩ. Distance between electrodes was at least 6 cm to avoid shunting effects (Miranda et al., 2006). TDCS was delivered for 20 min at an intensity of 2 mA (15 s ramp in and 15 s ramp out). For sham tDCS, the current was ramped up at the beginning and end of the stimulation period to mimic the somatosensory sensation of real tDCS, but turned off in between alternated with low-threshold direct-current impulses (Palm et al., 2013b). Operators and participants were kept blind to treatment conditions.

fMRI data processing

fMRI data acquisition

Subjects had to wear ear plugs and head phones for noise protection. FMRI was carried out at 3 T (SKYRA, Siemens) using a 20-channel head-coil. For functional imaging, an EPI sequence with the following parameters was used: repetition time (TR), 2000 ms; echo time (TE), 30 ms; flip angle (FA), 80°; spatial resolution, 3 × 3 × 3 mm^3; imaging matrix, 64 × 64; field-of-view (FoV), 192 × 192 mm^2; number of slices, 36; number of volumes, 250 (baseline), 620 (during tDCS), 250 (post tDCS). Functional images were acquired in axial orientation. For anatomical reference, a high-resolution MPRAGE was performed with the following specifications: FoV, 256 × 240 mm^2; spatial resolution, 0.8 × 0.8 × 0.8 mm^3; TR, 14 ms; TE, 7.61 ms; FA, 20°; number of slices, 160.

fMRI data pre-processing

Images were pre-processed using FSL 5.0.9 (http://www.fmrib.ox.ac.uk/fsl/index.html), AFNI (Analyses of Functional Images, http://afni.nimh.nih.gov/afni) and in-house scripts. Following brain extraction (BET; Smith, 2002), individual high-resolution T1-weightened images were reoriented to standard space, binarised, and segmented into grey matter (GM), white matter (WM), cerebrospinal fluid (CSF) using FAST (Smith, 2002). A linear and non-linear registration was applied using FLIRT and FNIRT (Jenkinson et al., 2002), a T1 atlas was generated and images were warped into MNI-standard space. Finally, calculations of the total amount of GM, WM and CSF, as well as of the volumetric proportion of all atlas regions were carried out. By means of a warping procedure, individual WM, GM and CSF deformation fields were created. The first ten volumes of functional MRI images were discarded to avoid non-steady-state effects. Functional image pre-processing comprised the following steps: (1) slice-time correction using 3dTshift to account for interleaved slice acquisition; (2) deobliquing using 3drefit and reorientation using 3dresample; (3) motion correction of time series using 3dTstat and 3dvolreg; (4) edge detection and removal of skull using 3dAutomask and 3dcalc; (5) linear and nonlinear spatial registration/normalisation to a standard EPI template in Montreal Neurological Institute (MNI) space using the T1 deformation field; (6) grand mean scaling; (7) de-trending; (8) calculation of motion outliers; (9) spatial smoothing using a 6 mm FWHM Gaussian kernel with high-pass temporal filtering (Gaussian-weighted, least-squares, straight-line fitting with σ = 50 s); (10) extraction of global signal, CSF and WM using 3dmaskave and creating a nuisance and motion parameter matrix; (11) obtaining residuals using 3dREMLfit; (12) demeaning of residuals using 3dTstat, 3dcalc and fslmaths; (13) band-pass filtering using 3dFourier (0.01 – 0.1 Hz, which are characteristic for RSNs according to: Boly et al., 2008; Damoiseaux et al., 2006; Fox et al., 2005; Greicius et al., 2003; Horovitz et al., 2009; Miller et al., 2009; Vincent et al., 2007); (14) smoothing; (15) warping of all fMRI images to the respective individual deformation template, resulting in normalised images in MNI space; (16) normalisation on segmented images (GM, WM and CSF); (16) censoring; (17) extraction of mean time courses in region-of-interest (ROI) masks using fslstats; (18) cross correlation using 3dfim, z-score normalisation using 3dcalc and normalisation to MNI space using applywarp.

ROI-based analysis

In a hypothesis-driven approach, ROIs were selected based on computational models that investigated current-flow patterns as generated with a F3-F4 electrode montage. Across studies, current distribution was widespread while current density clustered within the DLPFC (Bai et al., 2014; DaSilva et al., 2015; Nelson et al., 2014; Seibt et al., 2015; Woods et al., 2015). For this reason, ROIs were positioned within this region by means of the Sallet-atlas, which relies on a fMRI-based parcellation of the DLPFC (Sallet et al., 2013). Three different ROIs covering different parts of the DLPFC, in which an effect is to be expected, were chosen from the Sallet-atlas: area 46/9 dorsal, area 9 and area 10. These ROIs were drawn separately for the left and right hemisphere. The resulting masks are shown in inline supplementary figure 1. Additionally, by adding the beforementioned ROIs together, two hemisphere-specific total prefrontal ROIs were created for evaluation of group-tDCS effects. After converting them to a binary image, every mask was applied to each participant, each testing session and each measurement (baseline and post tDCS) using linear and non-linear registration (FLIRT, FNIRT). Connectivity values for each ROI and subject were generated by means of AFNI 3dfim command to cross correlate RS time-series within each ROI.

Independent component analysis (ICA)

In a complementary whole-brain approach, fcMRI data were analysed using the MELODIC (Multivariate Exploratory Linear

Optimized Decomposition into Independent Components) routine, version 3.14, implemented in FSL (Beckmann and Smith, 2004). Time courses of all participants, measurements (baseline and post tDCS) and testing sessions (t1, t2, t3), resulting in a total of 120 runs, were concatenated into a single 4D dataset. Decomposition into different functional networks was performed automatically by a dimensionality estimation of the MELODIC 3.14 tool. Four group-level components, which are known to involve brain regions within the DLPFC close to tDCS electrode sites, were selected for further analyses: the anterior Default Mode Network (DMN), the posterior DMN, the left FPN and the right FPN. An average z-score of $3 < z < 8$ was defined as the threshold for the resulting statistical group maps (see inline supplementary figure 2). Applying a threshold of $z = 3.0$, for each of these RSNs an independent-component (IC) mask was created. All ICA-derived group-level ICs containing the three RSNs of interest were reconstructed into individual ICs separately for each participant, measurement and testing session applying dual regression (Biswal et al., 2010; Filippini et al., 2009; Zuo et al., 2010).

Statistical analyses

Questionnaires

Group differences in scores for PANAS trait and STAI trait were analysed via independent t-tests using R (R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3–900051-07-0, URL http://www.R-project.org). Scores of STAI state were evaluated with two-way ANOVAS with a mixed effect design. Testing session was treated as repeated-measures factor with three stages (t1, t2, t3) and group as independent factor with two stages (active, sham). Scores of PANAS state were analysed with a three-way ANOVA including an additional pre-post comparison factor. Sphericity was examined for all statistical analyses and in case of non-sphericity, results were corrected according to Greenhouse Geisser. All effects are reported as significant at $p < .05$.

FMRI contrasts

Voxel-wise nonparametric statistical contrasts (with 5000 permutations) were determined using PALM alpha86 (Permutation Analysis of Linear Models; Winkler et al., 2014; Winkler et al., 2015; Linear Models, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/PALM/). Due to the exploratory character of our study changes in RS fcMRI from pre to post tDCS were considered significant at an uncorrected $p < .001$ (cluster size > 20 voxels). Effects of active tDCS on RS fcMRI at each testing session were summarised with two directional contrasts: brain regions that showed an increase (post active-tDCS > pre active-tDCS) or decrease (post active-tDCS < pre active-tDCS) in RS fcMRI with or within the ROI or ICA network (RSN). Changes in the sham group following the placebo intervention were analysed for each testing session using the same directional contrasts: positive effects (post sham-tDCS > pre sham-tDCS) and negative effects (post sham-tDCS < pre sham-tDCS). Hereby, each active-tDCS effect, i.e. post active-tDCS > pre active-tDCS or post active-tDCS < pre active-tDCS, could be compared to its corresponding sham effect (post sham-tDCS > pre sham-tDCS or post sham-tDCS < pre sham-tDCS), to test whether significant changes in each contrast arose from a tDCS-unrelated increase/reduction in RS fcMRI from baseline to post tDCS. All active-tDCS effects were checked for overlaps with direction-specific sham effects at each testing sessions. Overlaps were defined as visually observable intersections between direction-specific active-tDCS and sham effects. All active-tDCS effects were also checked for consistency across testing sessions. Consistent tDCS effects were defined as spatially proximal clusters covering a common anatomical structure.

Test-retest approach

To evaluate intra-individual TRT reliability, voxel-wise intra-class correlation coefficients (ICC) were calculated based on a script by Zou and colleagues (Zuo et al., 2010): https://de.mathworks.com/matlabcentral/fileexchange/22122-ipn-tools-for-test-retest-reliability-analysis. Activity within voxels of individual baseline or post-tDCS maps – resulting from individual normalized z-score maps (derived from the ROI-connectivity analyses) and reconstructed individual probabilistic ICs (derived by means of dual regression) – of each participant at each testing session was extracted and then consistency of the two-way random single measures was determined using $ICC_{(2,1)}$ across all testing sessions (t1 and t2 and t3) as well as across pairs of sessions: t1 to t2, t1 to t3 and t2 to t3. In this way, reliability maps were generated for both baseline and post-tDCS measurements separately for the seed- and the ICA-based approach and separately for each group (active and sham). TRT reliability maps were summarised by calculating the median ICC-value within each IC mask or ROI. According to Cicchetti (1994), ICC values were rated as follows: low (ICC values < 0.4), moderate (ICC values between 0.4 and 0.59), good (ICC values between 0.6 and 0.74) and excellent (ICC values ≥ 0.75). Negative ICC values indicate that the measure is not reliable (Lahey et al., 1983).

Results

TDCS effects: ROI-based and dual-regression analyses

Peak voxels of contrast-specific effects are given in Inline Supplementary tables, separately for the active (see Inline Supplementary table 1) and sham group (see Inline Supplementary table 2). To identify regions which change in active tDCS and do not change in sham, visual maps of direction-specific effects in the active and sham group are provided. Visualisations also provide the opportunity to compare one contrast across testing sessions and thus to identify repeatedly appearing tDCS effects (see Figs. 2 and 3).

ROI-based analysis

Functional connectivity within ROIs did not change appreciably and only once exhibited a significant active-tDCS effect, namely an increase in right-hemispheric functional connectivity during the first testing session (see Inline Supplementary table 1). There was no effect of sham tDCS on within-ROI connectivity.

ICA approach

Based on whole-brain dual-regression analyses, results indicate that active tDCS affected the magnitude of correlation in time series between voxels within a given RSN (see Inline Supplementary table 1). For active tDCS, neither positive nor negative effects on connectivity clustered within a specific brain region but were rather widespread. Consistent active-tDCS effects across testing sessions could be identified for each RSN (see Figs. 2 and 3). The anterior DMN exhibited an increase in correlated time series with the right superior temporal gyrus/insula as well as the left thalamus across two active-tDCS testing-sessions. For the posterior DMN, a positive active-tDCS effect appeared throughout all testing sessions within the right and left medial frontal gyrus. Increased correlations with the left FPN were found within the right postcentral gyrus, right parietal lobule, left middle and inferior frontal gyrus, left and right precentral gyrus and left middle temporal gyrus across two active-tDCS testing-sessions. The left inferior parietal lobule, right (pre-)cuneus and right thalamus showed increased correlations with the right FPN across two testing sessions. Repeatedly, decreased correlations across two active-tDCS testing-sessions appeared between the anterior DMN and right pre-central gyrus, posterior DMN and both the right inferior frontal gyrus and right cingulate gyrus, left FPN and right middle frontal gyrus, right FPN and both the right middle/superior frontal gyrus and left cingulate gyrus.

For sham tDCS, significant effects on RSNs were present as well

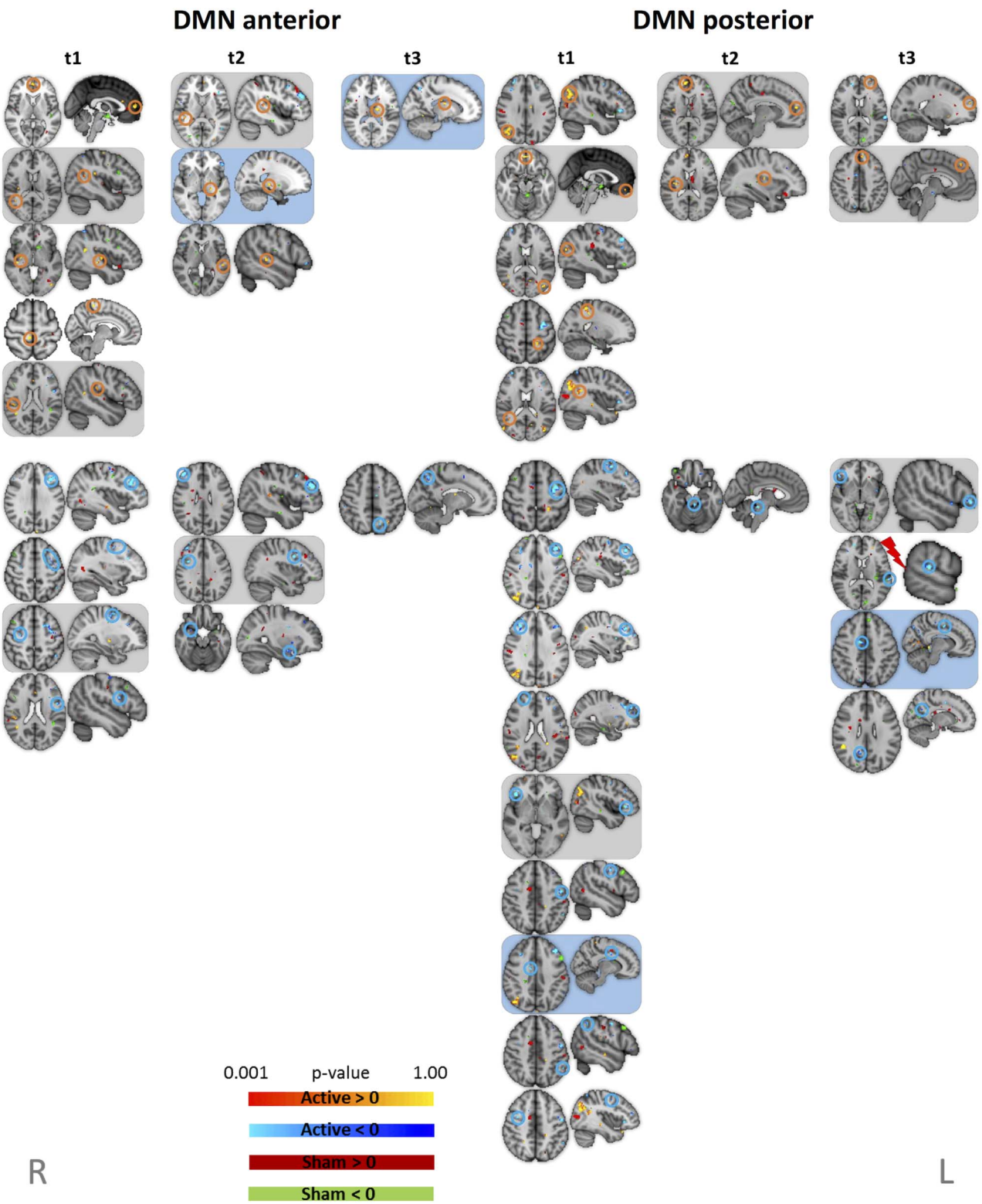


Fig. 2. Active-tDCS effects and sham effects on resting-state networks (RSN) at each testing session. Colours represent the following contrasts: orange = post active-tDCS > pre active-tDCS, blue = post active-tDCS < pre active-tDCS, dark red = post sham-tDCS > pre sham-tDCS, green = post sham-tDCS < pre sham-tDCS. Orange circles mark positive tDCS effects, i.e clusters that showed increased correlated time-series with the respective network after active tDCS as compared to baseline. Blue circles mark negative tDCS effects, i.e clusters that showed decreased correlated time-series with the respective network after active tDCS as compared to baseline. Framed effect clusters mark positive or negative tDCS effects in the active group that repeatedly appeared across different testing sessions ;colours mark effects that belong together. The flash indicates an overlapping negative effect between the active and sham group (orange and dark red). MNI coordinates (x, z) of each effect cluster is given in Inline supplementary table 1 and 2. Effects are sorted by cluster size. Correlated time series are shown at a threshold of $p < .001$, uncorrected, radiological convention. DMN = Default Mode Network, L/R = left/right, t1/2/3 = testing session 1/2/3.

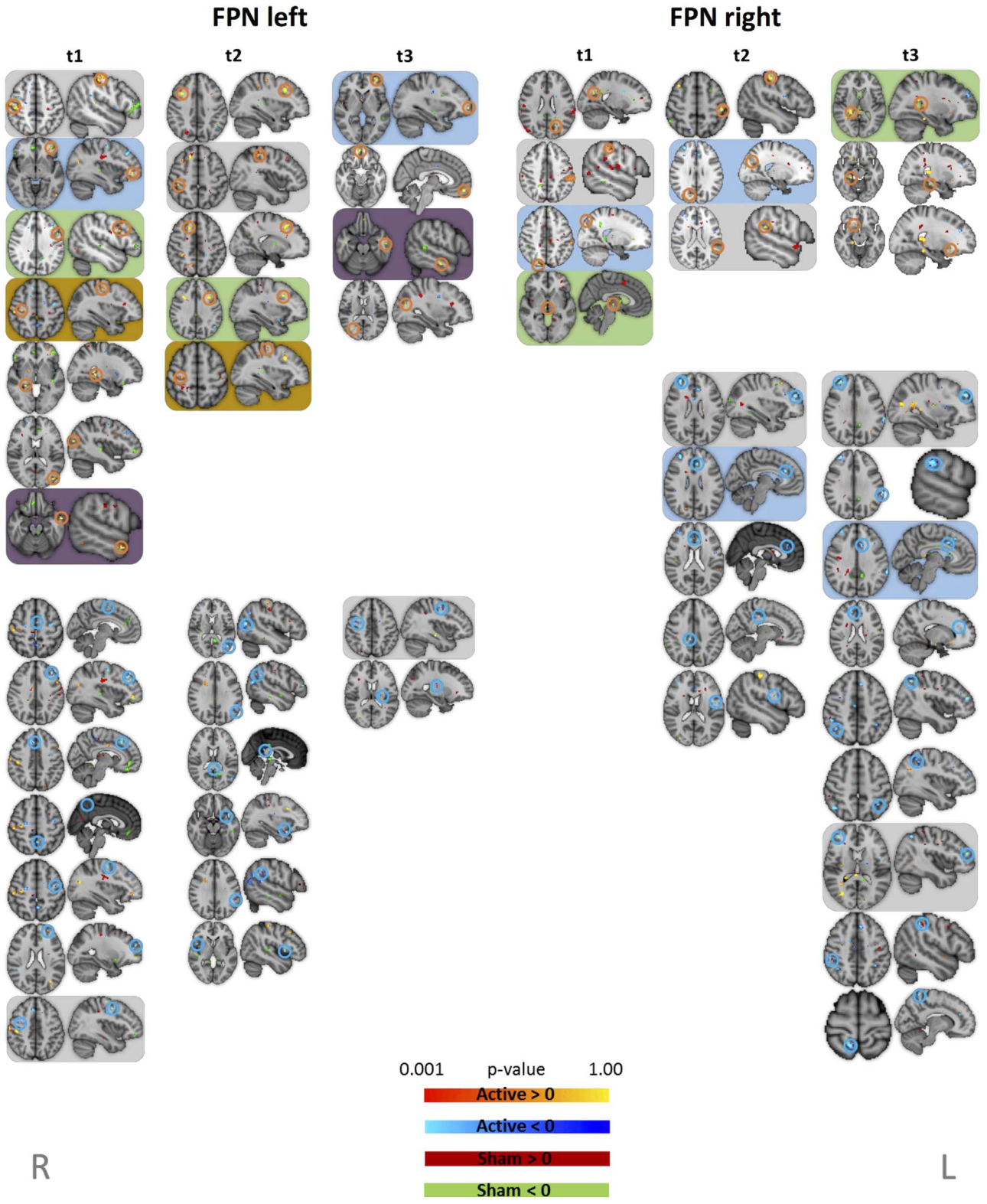


Fig. 3. Active-tDCS effects and sham effects on resting-state networks at each testing session. Colours represent the following contrasts: orange = post active-tDCS > pre active-tDCS, blue = post active-tDCS < pre active-tDCS, dark red = post sham-tDCS > pre sham-tDCS, green = post sham-tDCS < pre sham-tDCS. Orange circles mark positive tDCS effects. i.e. clusters that showed increased correlated time-series with the respective network after active tDCS as compared to baseline. Blue circles mark negative tDCS effects, i.e clusters that showed decreased correlated time-series with the respective network after active tDCS as compared to baseline. Framed effect clusters mark positive or negative tDCS effects in the active group that repeatedly appeared across different testing sessions; colours mark effects that belong together. MNI coordinates (x, z) of each effect cluster is given in Inline Supplementary table 1 and 2. Effects are sorted by cluster size. Correlated time series are shown at a threshold of $p < .001$, uncorrected, radiological convention. FPN = Frontal Parietal Network, L/R = left/right, t1//2/3 = testing session 1/2/3.

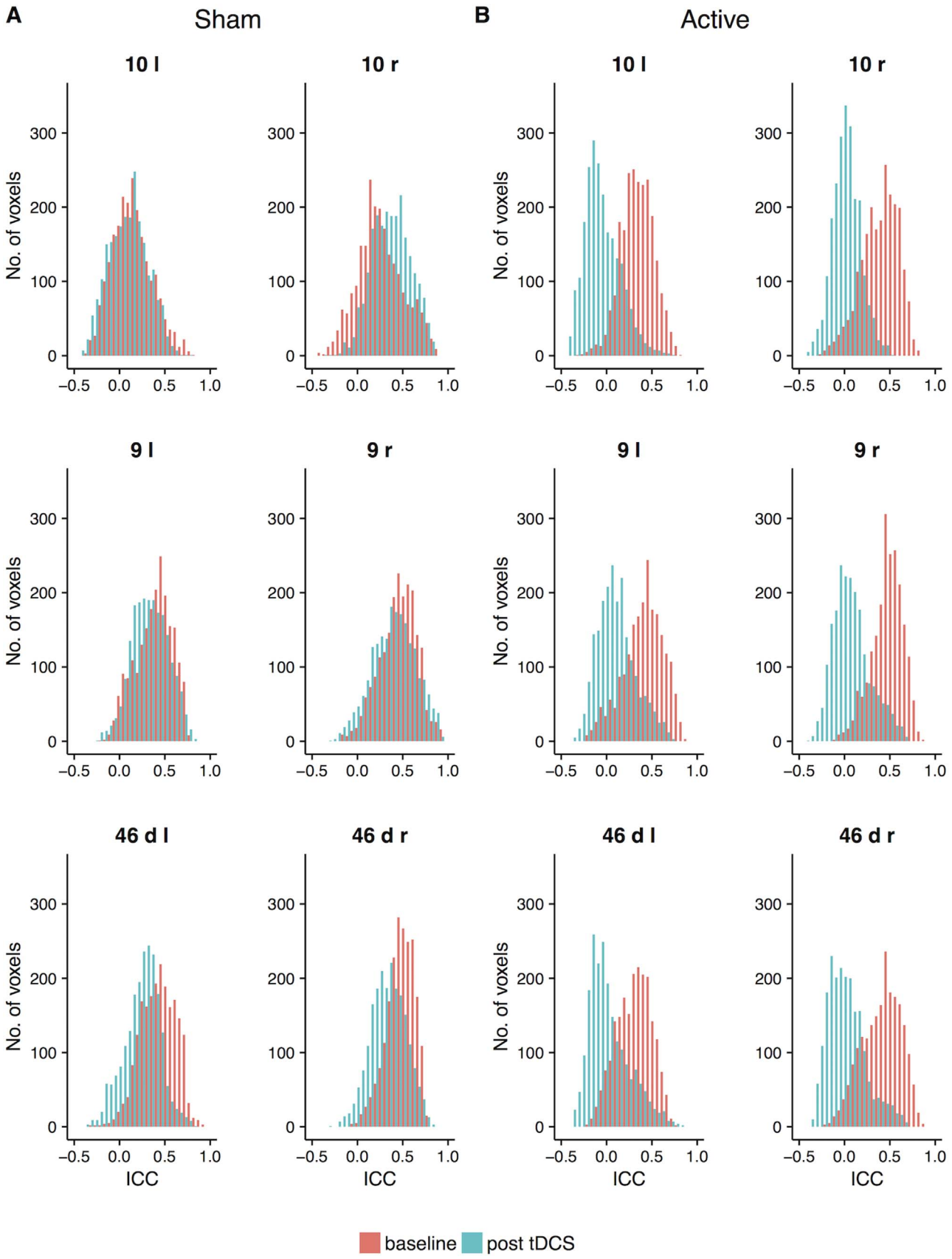


Fig. 4. Frequency distributions of voxel-wise ICC calculations within a region of interest (ROI). Colours represent frequency of ICC levels of baseline and post-tDCS measurements in a) the sham group. b) the active group. ICC = intra-class correlation coefficient, l/r = left/right, 10 = area 10 of the Sallet-atlas, 46d = area 46/9 dorsal of the Sallet-atlas, 9 = area 9 of the Sallet-atlas (Sallet et al., 2013).

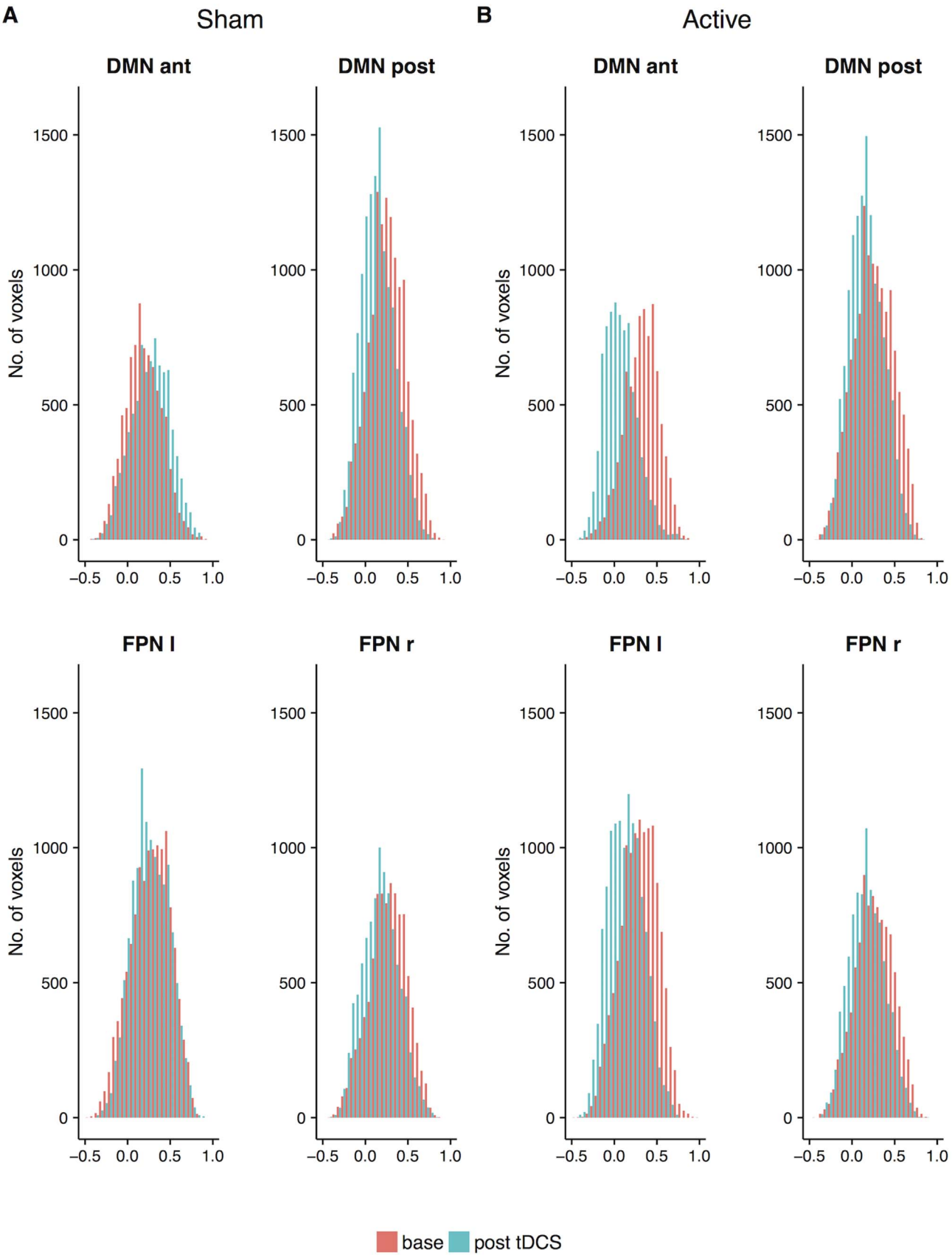


Fig. 5. Frequency distributions of voxel-wise ICC calculations within a resting-state network (RSN). Colours represent frequency of ICC levels of baseline and post-tDCS measurements in a) the sham group. b) the active group. ant = anterior, DMN = Default Mode Network, FPN = Frontal Parietal Network, ICC = intra-class correlation coefficient, l/r = left/right, post = posterior.

(see Inline Supplementary table 2). To visually identify regions for which changes from baseline to post-tDCS measurements were specific to active tDCS, flashes within contrast maps indicate overlapping direction-specific effects between the active and sham group (see Figs. 2 and 3). Only one overlap between direction-specific effects of active tDCS and sham tDCS occured: for the posterior DMN, both a negative active-tDCS effect and a negative sham effect were found within the left superior temporal gyrus at testing session 3.

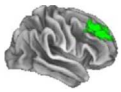
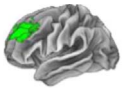
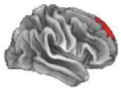
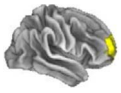
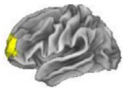
Reliability analyses

Inter-session TRT reliability was represented by the degree of consistency of single measurements (activity within voxels). To descriptively compare ICC levels between and within groups, the frequency-distributions of single-voxel ICC-values within a ROI or RSN are shown in histograms (see Figs. 4 and 5). Histograms also conduced to trace differences between ICC pairs (t1-t2, t1-t3, t2-t3). Here, values of the ICC-pair 23 were contrasted with values of both ICC-pair 12 and ICC-pair 13 (see Inline Supplementary figure 4 and 5).

TRT reliability of baseline RS-fMRI connectivity

For baselines as investigated with the ROI-based approach, ICCs were poor to moderate for both groups with median values ranging from $ICC_{(2,1)}$ = 0.13 to $ICC_{(2,1)}$ = 0.50 (see Table 1). Thereby, half of the median ICC-values were showing poor and the other half moderate TRT reliability. For the ICA-based approach, baseline connectivity patterns rather showed poor reliability across groups as indicated by median ICC-values ranging from $ICC_{(2,1)}$ = 0.19 to $ICC_{(2,1)}$ = 0.34 (see Table 2).

Table 1
Inter-session test-retest reliability of ROIs.

Surface ROI localisation	ROI and hemisphere	sham		active	
		baseline	post	baseline	post
	46 d l	0.44	0.26	0.33	−0.03
	46 d r	0.50	0.33	0.44	−0.02
	9 l	0.30	0.32	0.37	0.12
	9 r	0.46	0.49	0.50	0.06
	10 l	0.13	0.09	0.35	−0.09
	10 r	0.24	0.36	0.42	0.01

Note. Test-retest reliability of connectivity within ROIs, separately for both groups (active vs. sham) and both conditions (baselines and post tDCS). Test-retest reliability is expressed as median of voxel-wise intra-class correlation coefficients (ICC) between all testing sessions (t1, t2, t3). ROIs are illustrated in radiological convention. *d = dorsal, l/r = left/right, ROI = region of interest, 10 = area 10 of the Sallet-atlas, 46d = area 46/9 dorsal of the Sallet-atlas, 9 = area 9 of the Sallet-atlas* (Sallet et al., 2013) .

Table 2
Inter-session test-retest reliability of RSNs.

RSN localisation	RSN	sham		active	
		baseline	post	baseline	post
	DMN anterior	0.19	0.27	0.34	0.04
	DMN posterior	0.26	0.12	0.26	0.14
	FPN l	0.29	0.25	0.31	0.12
	FPN r	0.28	0.17	0.27	0.16

Note. Test-retest reliability of ICA-analyses-based RSN-connectivity, separately for both groups (active vs. sham) and both conditions (baselines and post tDCS). Test-retest reliability is expressed as median of voxel-wise intra-class correlation coefficients (ICC) between all testing sessions (t1, t2, t3). RSNs are illustrated in radiological convention. *DMN = Default Mode Network, FPN = Frontal Parietal Network, ICA = independent component analysis, l/r = left/right, RSN = resting-state network.*

TRT reliability of tDCS-related effects on RS-fMRI connectivity

For the ROI-based approach, reliability of individual post-tDCS maps differed depending on whether an active or sham tDCS intervention preceded the measurement. In the active group, median ICC-values ranged from $ICC_{(2,1)}$ = −0.09 to $ICC_{(2,1)}$ = 0.12 and in the sham group, median ICC-values ranged from $ICC_{(2,1)}$ = 0.09 to $ICC_{(2,1)}$ = 0.49 (see Table 1). Thereby, post-tDCS median ICC-levels of the active group can be rated as not reliable to poor, while median ICC-levels of the sham group are classifiable as poor to moderate. For the ICA-based approach, post-tDCS reliability could be classified as poor across groups as indicated by median ICC-values ranging from $ICC_{(2,1)}$ = 0.04 to $ICC_{(2,1)}$ = 0.16 in the active group and from $ICC_{(2,1)}$ = 0.12 to $ICC_{(2,1)}$ = 0.27 in the sham group (see Table 2). The described pattern is illustrated in Inline Supplementary figure 3.

Comparisons of TRT reliability

Based upon histograms, in the sham group, ICC frequency-distributions of baseline and post-tDCS measurements largely overlapped (see Figs. 4 and 5), pointing towards a close match in ICC levels between both RS-fcMRI examinations. By contrast, histograms of the active group indicate a distinction in ICC levels between baseline and post-tDCS measurements. Especially in the ROI approach, ICC frequency-distributions of baseline and post-tDCS measurements were so very far apart that the two peaks of each distribution were clearly discernible (see Fig. 4). This observation did not apply to ICA-based ICCs. Here, ICC frequency-distributions of the active group largely resembled those of the sham group, such that distributions were hardly

distinguishable. Only the anterior DMN exhibited a bimodal histogram (see Fig. 5).

Median ICC-values of ICC pairs ranged within the same values as median ICC-values resulting from overall ICC calculations across all three testing sessions (see Inline Supplementary table 3 and 4). Histograms of single-voxel ICCs show that the frequency distributions of different ICC pairs were quite similar, i.e. largely overlapping, across groups and measurements (see Inline Supplementary figure 4 and 5), again suggesting comparable ICC levels independent of the factor testing session.

Behavioural data

A significant difference was found between overall levels of positive affect (PANAS state), $F(1, 18) = 10.01$, $p < .01$, and overall levels of negative affect (PANAS state), $F(1, 18) = 9.80$, $p < .01$, before and after both active and sham tDCS, indicating that participants on average reported both higher positive and higher negative affects before measurement ($M_{POS} = 26.88$, $SD_{POS} = 6.54$; $M_{NEG} = 11.52$, $SD_{NEG} = 2.08$) than after ($M_{POS} = 24.27$, $SD_{POS} = 7.46$; $M_{NEG} = 10.67$, $SD_{NEG} = 0.90$). All other behavioural variables did not change significantly, neither between groups nor across testing sessions. There was also no difference in age between groups. Separate item analysis of CRQ showed that tDCS-related discomfort was low.

Discussion

In this study, we investigated the TRT reliability of effects that single prefrontal tDCS sessions exert on RS fcMRI and observed none or low reliability of responses (post-tDCS RS-fcMRI) in contrast to a more robust TRT reliability of baseline RS fcMRI. The underlying question is highly relevant as establishing imaging stimulation (tDCS) probes for exploring state, disorder or course dependency of tDCS effects would require a deeper understanding of the variability of tDCS-mediated RS-fcMRI changes. Moreover, the reliability of tDCS effects in general has recently been questioned by findings from three studies reporting variable inter- and intra-individual MEP responses to motor-cortex tDCS (Chew et al., 2015; Dyke et al., 2016; Horvath et al., 2016). Finally, varying RS-fcMRI response-patterns to NIBS may be related to the variation of responses observed in clinical applications of NIBS in psychiatry (for review see Lefaucheur et al., 2016). If neurophysiological effects of tDCS vary, variations in behavioural and clinical responses can be expected as well. And if significant variability of response-patterns to NIBS exist even at an intra-individual level, average measures of clinical responses should be replaced by intra-individual analyses.

In order to address the question of TRT reliability for prefrontal-tDCS RS-fcMRI effects, we chose a methodologically broad approach comprising both ROI-based as well as ICA-based analyses. Masks were seeded in prefrontal areas where a tDCS effect can be expected according to computational models. Next, voxel-wise ICCs were calculated for both individual baseline and individual post-tDCS RS-fcMRI separately for each group (active and sham). Mostly moderate TRT reliability was observed for RS-fcMRI measurements at baseline prior to tDCS using the ROI-based approach. For the ICA-based approach, low reliability of baseline RS fcMRI appeared. More reliable connectivity patterns in the ROI-based approach is in line with Craddock et al. (2012). According to this study, reliability increases as a function of the number of entities the brain is portioned out, which might be one reason why reliability within selected ROIs is slightly higher than in ICA-derived RS components. While the latter represents correlated time courses across the whole brain, our seeds comprised clearly defined small clusters within the DLPFC. The discrepancy between moderate to good RS-fcMRI ICC-values previously reported (Blautzik et al., 2013; Braun et al., 2012; Laumann et al., 2015; Zuo et al., 2010) and low to moderate baseline ICC levels may arise from our small sample size. Furthermore, low to moderate ICC values may be also due to network selection, which was based on anatomical proximity to stimulation sites. As a result, mainly prefrontal networks, which are associated with higher order cognitive functions, were considered stimulated networks. Because inter-subject variability of functional networks increases with their relevance for cognitive functions (Mueller et al., 2013), it is plausible that the networks selected here show lower reliability than networks associated with sensory or motor functions.

Regarding reliability of RS-fcMRI after tDCS, for the ROI-based approach, the ICC frequency distribution was shifted to lower TRT reliability in the active group. On the contrary, reliability of the post measurements following sham tDCS was low to moderate and, as suggested by ample overlap between frequency scales, did not seem to differ from baseline ICCs. On this descriptive level, active tDCS appears to induce additional variability and reduces TRT reliability in contrast to sham tDCS leaving TRT reliability largely on the baseline level. This supports the notion of active tDCS modulating RS-fcMRI, however, with variable and divergent effects. For the ICA-based approach, no clear difference between baseline and post-tDCS ICCs was observed, possibly due to the large spatial extend of IC-networks.

Test-retest reliability and sources of variability

The assumption that tDCS effects are highly variable at the individual level would be consistent with available data showing low TRT reliability of tDCS effects on MEPs. Similar to our results, in most studies investigating reproducibility of tDCS effects on MEPs, reliability values ranged from −0.50 to 0.28. Dyke et al. (2016) showed that effects of 2 mA anodal tDCS on MEPs, defined as ratio of post-tDCS and pre-tDCS slopes, were poorly reliable (ICC = 0.28). Poor intra-individual reliability was also found for MEPs over a 30-min interval following both anodal stimulation with 0.5 mA (ICC = −0.50) (Chew et al., 2015) and 1 mA (ICC = 0.06) (Horvath et al., 2016). For the same interval and stimulation intensity, Lopez-Alonso et al. (2015) detected fair reliability (ICC = 0.57) of anodal tDCS, but again poor reliability (ICC = −0.03) for MEPs obtained during the second half of the overall 60-min interval. Only in one recent motor-cortex study, intra-individual reliability over both early and late measurement periods following stimulation with 1 mA was reported to be satisfying (ICC = 0.74 and 0.64) (Jamil et al., 2016).

One may argue that ICCs must not be directly compared across MEP-tDCS and fMRI-tDCS studies, because different factors contribute to the overall variability in such paradigms, especially MEP/fMRI-related factors. Numerous studies have addressed TRT reliability of MEPs only and reported moderate to good reliability of different MEP measures (Carroll et al., 2001; Kamen, 2004; Livingston and Ingersoll, 2008; Malcolm et al., 2006). Though, to our knowledge, there is no single study directly comparing TRT reliability measures for MEPs alone and tDCS effects on MEPs. The above-mentioned studies have only investigated TRT reliability of MEPs following active and sham tDCS. Therefore, we can only speculate that ICCs of these measures may be different. In comparison with RS fcMRI, MEPs constitute an active intervention, i.e. measures cortical reactions to a single TMS (transcranial magnetic stimulation) pulse, that possibly influences baseline states and the mode of action of a subsequent intervention, namely tDCS. By contrast, RS fcMRI is task-free and represents a less controlled behavioural state. Besides, MEPs are specifically related to motor-cortex physiology, whereas tDCS-induced RS-fcMRI modulation can be also measured for other target regions (Callan et al., 2016; Krishnamurthy et al., 2015; Polania et al., 2012). Lastly, motor-cortex excitability can be neuropharmacologically modified (Ziemann, 2008), but very little is known about pharmacological effects on RS-fcMRI measures (Bartelle et al., 2016). If there were different contributions of MEPs and RS fcMRI to tDCS-related variability, it might be difficult to translate reliability estimations of tDCS effects on MEPs to tDCS effects

on RS fcMRI. However, the current debate in the literature is concerned with the reliability of tDCS and not with the reliability of MEP or fcMRI.

The variability of BOLD-responses has been investigated for task-fMRI studies. Here functional paradigms are used to evoke a BOLD signal in a certain brain region. In a study by Plichta et al. (2012), reliability of a task-fMRI battery targeting different systems (emotion, motivation and cognition) was assessed at both group and individual levels. While group-level activation maps were highly reliable independent of the task (whole brain level: ICC = 0.89 – 0.98, target ROIs: ICC = 0.66 – 0.97), within-subject reliability ranged from fair to good for the motivational and the cognitive task (ICC = 0.56 – 0.62 and ICC = 0.44 – 0.57, respectively) but remained low for the emotional task (ICC = −0.02 – 0.16) (Plichta et al., 2012). Furthermore, it has been shown that observed between-session variations in the spatial pattern of BOLD activation are mainly due to global effects that can be corrected by pre-processing steps such as spatial smoothing, for which reason the authors consider the localisation of fMRI signals as highly reliable. In contrast, variations in the amplitude of the activation were pronounced to a greater degree – especially for cognitive tasks – and may limit interpretations of the magnitude of brain activation (Raemaekers et al., 2012). Another study investigating variations in fMRI-task responses within subjects by means of within-subject variance-decompositions found that error in measurement contributes the most to the total variance (Gonzalez-Castillo et al., 2016). At the same time, within-subject variance across sessions and across runs or blocks within one session also constituted an important source of overall variance (Gonzalez-Castillo et al., 2016). Such natural within-subject variability in task-fMRI may reflect the potential of this method to capture intra-individual differences and point to the need for individual-subject analyses (Laumann et al., 2015; McGonigle, 2012; Shine et al., 2016). Consequently, sources of variability, which reside in intra- and inter-related factors other than natural differences in response to tDCS itself may limit reliability of individual measurements and hinder identification of the 'true' TRT level for tDCS. Therefore, it is essential to control for these factors.

Heterogenous groups and especially outliers add to the total variance and may affect the magnitude of ICCs. Consequently, it is important to ensure similar characteristics of the tested sample (Lopez-Alonso et al., 2015; Vaz et al., 2013). For this reason, behavioural control variables were carefully and elaborately reviewed and relevant individual covariates subsumed under 'sociodemographics' (see Results) were matched within and between groups. Importantly, there was no difference in trait and state aspects of both anxiety and affect between the active and the sham group, allowing for direct comparison between groups. Solely, changes in positive and negative affect were observed across groups, i.e. both positive and negative PANAS values were higher before each measurement compared to after. Importantly, PANAS scores that on average roughly changed to the same extend at each session (there was no interaction with the factor testing session), are unlikely to impact TRT reliability. Reduction in both positive and negative affect after as compared to before the beginning of each measurement (independent of stimulation condition) may reflect an overall tiring due to a one-and-a-half-hour testing session.

Apart from between-subject changes, we also took potential influences on intra-individual variability into account, such as attentional level, time of day and hormonal fluctuations (for review see Ridding and Ziemann, 2010). To exclude possible effects of hormonal cycles between sessions, we only included men in our study (De Bondt et al., 2015). In addition, we tried to keep day time of measurement constant between sessions within participants to avoid daily variations in vigilance and attention. Another possible source of within-subject variability is novelty. At first testing, most participants are naïve to MRI measurements and stimulation, implicating higher levels of anxiety, which in turn can affect cortical excitability (Wassermann et al., 2001). For example, heart rates have been shown to be especially high at the beginning of MRI investigations (van Minde et al., 2014) and equally sensing a stimulation for the first time may cause intensified physical and mental reactions. Our study design allows for verifying novelty as confounding factor and to ensure stable experiences with MRI and tDCS across participants. All participants attended three testing sessions, such that inter-session TRT reliability could be evaluated three times (testing session 1–2, 1–3 and 2–3), including one evaluation to which novelty aspects do not apply (testing session 2–3). Across groups and measurements, frequency distributions of novelty-unaffected ICC evaluations (ICC-pair 23) showed large overlap with ICC evaluations containing the first testing-session (ICC-pair 12 and ICC-pair13). Hence, ICC levels may be treated as comparable, suggesting no effect of the first measurement on neither baseline nor post-tDCS ICC values. Group-level analyses of active-tDCS effects at each time point also argue against an influence of the first testing session on ICC levels, because consistently appearing effects were not limited to the comparison between testing session 2 and 3, but already were present at first testing session. Taken together, novelty did not seem to influence ICC evaluations.

TDCS effect at each testing session

When discussing our results, we also must take into account the possibility that the main effect of tDCS on RS fcMRI has been missed. This consideration is presumably a methodical short-coming that is hardly avoidable because consistent ROIs across subjects are required for ICC calculations but are unsuitable for capturing individual responses to tDCS. However, analyses steps of our approach were considered thoroughly and relied on reviewed up-to-date literature. For example, ROIs were placed in regions where F3-F4 stimulation is most likely to exert an effect according to computational models (Bai et al., 2014; DaSilva et al., 2015; Nelson et al., 2014; Seibt et al., 2015; Woods et al., 2015). Additionally, our ROIs covered prefrontal regions where a tDCS effect was reported before (Keeser et al., 2011; Pena-Gomez et al., 2012). In order to account for local differences in the brain's responses to tDCS and thus to increase reliability, regions of expected effects were subdivided into clusters by means of the Sallet atlas, which provides a functional-connectivity-based parcellation of the DLPFC (Sallet et al., 2013). By evaluating a group-tDCS effect across ROIs in each hemisphere (i.e. the three different ROIs in each hemisphere were merged) at each testing session, we were provided with the possibility to roughly test our assumptions. Although comparisons between ICC levels and group-tDCS effects at each testing session is not optimal with this approach, approximations are possible.

With regard to the ROI-based approach, only the right-hemisphere ROI exhibited an effect after tDCS in terms of increased connectivity at testing session 1. Concurrently, within-ROI functional connectivity descriptively showed a clear difference in ICC frequency-distributions between baseline and post-tDCS measurements of the active group. This observation may reflect intra-individual variability of tDCS responses and fits to our group-level findings, showing only one tDCS effect at one testing session on within-ROI functional connectivity and thus missing any consistent pattern: When tDCS effects are highly variable already at an intra-individual level, it is likely that the effect is even less consistent at an inter-individual level averaged across groups. Altogether, in the ROI-based approach, reliability values may mirror the stability of tDCS effects observed at a group level. However, no definite comparison can be made between ICC levels and tDCS effects both based on coherent, yet different ROIs.

Therefore, we also followed a whole-brain approach by means of ICA. Here, more direct comparisons between tDCS effects and ICCs are possible, because group analyses of tDCS effects at all time points are available for each RSN separately. Following previous literature, RSNs showing a tDCS effect were selected (Keeser et al., 2011; Pena-Gomez et al., 2012). Again, this procedure keeps open the option of missed effects within another RSN. Still, within each RSN we were able to

investigate the whole brain in a hypotheses-free way. Here, group-level analyses revealed several active-tDCS effects. Most of these effects were unique to the active group (i.e. no overlapping directional contrasts between the active and sham group) with some of them even being repeatedly observable across two or three testing sessions. At an intra-individual level, no clear differences between ICC frequency-distributions appeared: in both groups distributions of baseline and post measurements were largely overlapping. Theoretically, tDCS may have exerted different effects on ICA- versus ROI-derived functional connections. In this case, it may be that comparable baseline and post-tDCS measurements in the active group, which range within the ICC levels of the sham group, may reflect a consistent tDCS effect. This speculation would be in accordance with repeatedly occurring tDCS effects observed at a group level. However, the uncorrected level, at which group-tDCS effects were reported, should not be overinterpreted. Therefore, it may also be that tDCS did not affect ICA networks at all, resulting in overlapping ICC-distributions for baseline and post-tDCS measurements similar to the sham group. Finally, it may also be possible, that low baseline TRT reliability undermined clear differences between baseline and post-tDCS measurements in the ICA approach.

Unspecific and placebo effects

Multiple factors may contribute to the considerable intra- and inter-subject variability observed here. For sham tDCS, baseline and post-tDCS TRT reliability were similar at an individual level. In contrast, group-level analyses showed differences between connectivity patterns before and after sham tDCS. Theoretically, it would be interesting to further analyse this placebo effect and its specific and non-specific compounds. Usually, an intervention, especially one that is physical sensible, triggers expectations (positive or negative ones), which contribute to the measured overall outcome (Gomm, 2009; Supino, 2012). Consequently, to receive the real or true effect induced by the intervention alone, it is necessary to subtract the specific placebo effect (including expectation and anxiety) from the effect as measured following an active intervention. However, we cannot further interpret our placebo effect because our design with two parallel groups does not allow discrimination between such placebo-specific effects, and non-specific overall effects of the intervention or setting. In order to analyse the effect of sham tDCS itself and its TRT reliability, a third arm including a second control group, i.e. a no-stimulation control, would be needed in future studies.

Limitations of the study

There are several methodological limitations of our study. Both inter- and intra-subject variability may be related to the stimulation itself. We did not employ a highly standardised or even MRI-guided electrode positioning system which would serve to minimalize variability of montages (Seibt et al., 2015). At the same time, tDCS with electrodes covering an area of 35 cm^2 is not focal and displacement of the electrodes by 1 cm may not have any significant impact on current flow (Bai et al., 2014). As we adhered to the EEG 10–20 system during positioning of the electrodes and always kept a distance of 6 cm between the sponges, we consider variability between montages within this range. Still, we cannot exclude influences of electrode positioning on TRT reliability, particularly because findings on small drifts in electrode positions are ambiguous and controversially discussed (Woods et al., 2015). Also, amount of NaCl was not standardised across participants and sessions, possibly, in the case of oversaturation of the sponges, leading to diverse stimulation targets due to irrepressible diffusion of the liquid and hence of the current.

Another limitation of our study is the small sample size that hampers inferences on the general population (Button et al., 2013). As this study was designed a pilot for further TRT experiments with larger sample sizes, only 10 subjects were investigated in each group. Consequently, our analyses should be considered as exploratory. Still, each subject was measured three times with two scans each time, resulting in a total of 120 RS scans.

Further, our study design, which followed practical needs on the one hand, faces statistical shortcomings on the other hand. We have chosen a parallel design to control potentially confounding factors. First, a parallel design allows to keep a sham-tDCS group free of any active tDCS across different time points and thus avoids carry-over effects from active to sham conditions. Secondly, subjects participating in a cross-over study can directly compare active and sham tDCS and may distinguish both based on subtly different skin reactions or sensations (Palm et al., 2014). Nevertheless, a crossover designs would have had statistical advantages, particularly at small sample sizes.

It is also important to emphasize that we investigated the main, i.e. group effect of tDCS by analysing changes from baseline to post tDCS, whereas we used only post-tDCS measurements for evaluating the TRT reliability of tDCS effects. This approach was chosen in order to assure a similar range of variability and allow comparisons between baseline reliability and reliability of tDCS responses. When individual contrast maps, i.e. differences between pre and post scans, are created, active-tDCS-related but also other variations, i.e. specific placebo as well as non-specific overall effects, may take effect and may artificially increase variability for both tDCS conditions. Similarities between post and baseline are subtracted out, leaving only non-specific and placebo-related variations in the sham group and a mixture of tDCS-related (if available) and non-specific changes in the active group. Consequently, as shown in Inline Supplementary table 5, no differences between groups in reliability of contrast maps could be found. To minimize the influence of these factors, we chose post-tDCS measurements as main output measure reflecting the tDCS response. Post active- and post-sham tDCS-effects – contrary to pre-post differences– showed rather distinct ICC values and can be regarded as independent, arguing in favour of our approach. Still, when comparing group tDCS effects with intra-individual TRT reliability of post-tDCS measurements, with this approach, we are examining different dimensions of tDCS responses.

The full extent of tDCS-related variability needs to be systematically addressed in future studies by including further control conditions (Atri et al., 2011; Plichta et al., 2012) into the experimental designs. Moreover, all efforts should be made to standardise the intervention (e.g. electrode positioning), individual predispositions (e.g. sleep, stress associated factors) and setting conditions (e.g. time of the day) as far as possible.

Conclusion

Reproducibility of data is an important issue in MRI research (Nichols et al., 2016), but also in combined neuroimaging-stimulation approaches in order to differentiate variable versus constant components of tDCS-induced modulation. Intra- as well as inter-individual variability in tDCS responses should be considered when evaluating tDCS as a therapeutic tool. Through identifying sources of this variability, possible responders could be distinguished from non-responders and effective treatment protocols with respect to time lag between stimulations and amount of treatment sessions could be designed.

This study investigated the TRT reliability of prefrontal tDCS-induced RS-fcMRI modulation for the first time. The analysis of individual responses to active tDCS across three testing sessions revealed none to low reliability, in comparison with baseline RS-fcMRI measurements and sham tDCS which did not reduce TRT reliability to such extend. Reduction in reliability from baseline to post tDCS was most notably for functional networks that also exhibited no consistent active-tDCS effect across testing sessions at a group level, suggesting that active tDCS induced additional variability and reduced TRT reliability. Further studies using a standardised positioning system and a higher sample size are warranted. Moreover, possible

sources of intra- and inter-subject variability need to be investigated in more detail.

Conflict of interest

F.P. has received speaker's honorarium from Mag & More GmbH and the neuroCare Group as well as support with equipment from neuroConn GmbH, Ilmenau, Germany, Mag & More GmbH and Brainsway Inc., Jerusalem, Israel. A. H. reports no conflicts of interest related to this work.

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We plan to make all future data (http://www.gcbs.network/gcbs/projects/human-models/Work-Package-5.html) publicly available and try to provide this possibility for previous data as well. A general ethical approval has been submitted for the Psychiatric Imaging Network Germany (PING): http://www.ping.rwth-aachen.de/ to publish all neuroimaging data of several BMBF funded projects.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.neuroimage.2017.04.052.

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