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ORIGINAL PAPER

# Transcranial Direct Current Stimulation Modulates Neuronal Networks in Attention Deficit Hyperactivity Disorder

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**Abstract** Anodal transcranial direct current stimulation (tDCS) of the prefrontal cortex has been repeatedly shown to improve working memory (WM). Since patients with attention deficit hyperactivity disorder (ADHD) are characterized by both underactivation of the prefrontal cortex and deficits in WM, the modulation of prefrontal activity with tDCS in ADHD patients may increase their WM performance as well as improve the activation and connectivity of the WM network. In the present study, this hypothesis was tested using a double-blind sham-controlled experimental design. After randomization, sixteen adolescents with ADHD underwent either anodal tDCS over the left dorsolateral prefrontal cortex (DLPFC, 1 mA, 20 min) or sham stimulation with simultaneous fMRI during n-back WM task. Both in one-back and two-back conditions, tDCS led to a greater activation (compared with sham stimulation) of the left DLPFC (under the electrode), left premotor cortex, left supplementary motor cortex, and precuneus. The effects of tDCS were long-lasting and influenced resting state functional connectivity even 20 min after the stimulation, with patterns of strengthened DLPFC connectivity after tDCS outlining the WM network. In summary, anodal tDCS caused increased neuronal activation and connectivity, not only in the brain area under the stimulating electrode (i.e. left DLPFC) but also in other, more remote brain regions. Because of moderate behavioral effects of tDCS, the significance of this technique for ADHD treatment has to be investigated in further studies.

**Keywords** ADHD · Transcranial direct current stimulation · Working memory · fMRI

## Introduction

Attention deficit hyperactivity disorder (ADHD) is a highly prevalent and disabling disorder characterized by inattentiveness, hyperactivity, and impulsiveness (American Psychiatric Association 1994). Different pharmacological and psychotherapeutic treatment options have been proven to be highly effective in ADHD (Feldman and Reiff 2014). However, about 30% of patients do not respond well to pharmacological treatment. Moderate effects of behavioural therapy, high withdrawal rates to stimulant medication due to side effects, and critical attitude of parents to pharmacotherapy (Clavenna and Bonati 2014; Evans et al. 2014; Gajria et al. 2014) emphasize the urgent need to develop alternative treatment strategies for patients with ADHD.

Transcranial direct current stimulation (tDCS) represents a new treatment option for neuropsychiatric disorders. The tDCS technique is based on the application of weak, direct electrical currents to the brain via relatively large electrodes placed over the scalp. Anodal and cathodal stimulation may increase and decrease neuronal activity, respectively, and, in such a way, influence brain function (Nitsche and Paulus 2000). Effects of tDCS depend not only on polarity, but also on stimulation intensity, electrode montages, duration of stimulation as well as baseline level of neuronal activity (Horvath et al. 2014). Recent animal and human studies have confirmed that tDCS induces significant and long-lasting neuroplastic effects, establishing the potential of

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this technique for therapeutic purposes (Stagg and Nitsche 2011). This method has been successfully used to treat depression, tinnitus, chronic pain conditions, Parkinson's disease, and has been employed in neurorehabilitation (Kuo et al. 2014). It has also been established that tDCS can influence cognitive function (Elmasry et al. 2015; Kuo and Nitsche 2015), particularly 1 and 2 mA anodal tDCS over the left dorsolateral prefrontal cortex (DLPFC) have been repeatedly shown to influence working memory (WM) in a number of blind, randomized, sham-controlled, and single-session studies using a n-back paradigm. In these studies, tDCS stimulation reduced the number of omission and commission errors and shortened the reaction time (RT) compared with sham stimulation, especially in runs with high memory load (Brunoni and Vanderhasselt 2014).

This study was motivated by the following findings: (1) children and adolescents with ADHD show more omission errors, false alarms, slower RT, and higher RT variability in n-back tasks compared with healthy age-matched control subjects (Klein et al. 2006; Chamberlain et al. 2011; Feige et al. 2013). (2) The lower performance of ADHD patients in n-back tasks may be explained by diminished activation of the prefrontal cortex, especially of the DLPFC, as evidenced by a number of fMRI and neurophysiological studies (Valera et al. 2010; Bedard et al. 2014; Cubillo et al. 2014; McCarthy et al. 2014). (3) Anodal tDCS over the left DLPFC significantly improves the performance in n-back tasks in healthy subjects, as well as in patients with depression, Parkinson's disease, and in those recovering from stroke (Fregni et al. 2005; Boggio et al. 2006; Jo et al. 2009; Keeser et al. 2011a; Mulquiney et al. 2011; Teo et al. 2011; Berryhill and Jones 2012; Mylius et al. 2012; Oliveira et al. 2013; Martin et al. 2014). (4) Anodal tDCS causes neurophysiological and hemodynamic changes under the anodal electrode, both in the motor cortex and the DLPFC; these changes are indicative of increased neuronal activity (Baudewig et al. 2001; Lang et al. 2005; Antal et al. 2011; Keeser et al. 2011a; Polania et al. 2011; Zheng et al. 2011; Stagg et al. 2013). (5) 1 mA anodal tDCS causes a significant and long-lasting increase in cortical excitability in children and adolescents which is comparable with the excitability changes observed in adults (Moliadze et al. 2015a). (6) The group of adolescents seems to be adequate in order to investigate effects of tDCS on WM and related neuronal networks in patients with ADHD and, at the same time, minimize influence of brain development on these effects. Indeed, studies indicate that the span capacities of WM develop in a linear fashion from about 2–12 years of age. In adolescents, cognitive performance on WM tasks peaks and reaches levels which held constant through adulthood (Thaler et al. 2013). Moreover, neuronal networks underlying WM (superior frontal cortex, dorsolateral prefrontal cortex, precuneus) are well developed in children and adolescents and resemble those in adults. Especially adolescents are characterized by minimal immaturity in the WM network (Klingberg 2006; Geier et al. 2009; Luna et al. 2010; Darki and Klingberg 2015).

These combined observations led us to hypothesize that 1 mA anodal tDCS applied over the left DLPFC might be able to improve performance in WM tasks and cause a significant activation of relevant neuronal networks in patients with ADHD. To prove this hypothesis, anodal tDCS and sham stimulation were applied simultaneously with functional MRI during an n-back WM task in adolescents with ADHD using a double-blind, sham-controlled experimental design. In contrast to other studies demonstrating effects of tDCS on n-back WM (Brunoni and Vanderhasselt 2014; Hill et al. 2016), a new electrode montage with the anode over the left DLPFC and cathode over the vertex was used. The choice of this montage was justified by the following aspects: 1) one of the the most frequent and reliable neuropsychological abnormalities in ADHD is the high RT variability in cognitive tasks (Castellanos et al. 2005; Klein et al. 2006; Uebel et al. 2010; Chamberlain et al. 2011). In patients with ADHD, the RT variability has been associated with the activity in the premotor cortex (Suskauer et al. 2008). Because modeling the electrical current distribution revealed the greatest stimulation current densities between the primary stimulating and return electrodes with the global maxima at the edges of electrodes (Salvador et al. 2015; Opitz et al. 2015), it could be suggested that by using the described montage we would effectively stimulate not only the left DLPFC but also the left premotor cortex and could expect not only effects of tDCS on accuracy in a WM task but also on motor function, especially on the RT variability.

## Methods and Materials

### Participants

For ethical reasons, only adolescents were included in the study. Sixteen patients with ADHD (age $14.33 \pm 1.32$ years, age range 12–16 years; 13 boys, 3 girls) were recruited from the outpatient unit of the Department for Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, Philips-University of Marburg. The inclusion criteria were as follows: (a) ADHD without conduct disorders or tic disorders as diagnosed by an experienced child and adolescent psychiatrist; (b) no other neuropsychiatric (Child Behaviour Checklist global score and anxiety depression scale $T < 70$) or paediatric disorders; (c) sufficient compliance of the child and his/her family; (d) normal school achievement; and (e) no MRI exclusion criteria (i.e. ferromagnetic body objects, pregnancy, or a history of claustrophobia).

According to DSM IV, all ADHD children met the criteria for combined type or hyperactive-impulsive type (314.01). The diagnosis of ADHD was supported by the parents' version of a German adaptive Diagnostic Checklist, DCL-HKS (Döpfner et al. 2004). The number of items in this questionnaire is equal to the number of DSM-IV items, and it also provides a severity score for each ADHD symptom. Moreover, all participants underwent neurocognitive and neuropsychological testing before inclusion in the study (see Table 1). All of them had average cognitive abilities (IQ > 85) and abnormal performance in a number of neuropsychological tests. The performance in the WM test was measured using the Qb Test (Q-Tech Stockholm, Sweden). Qb Test is a commercial neuropsychological test that combines the n-back WM paradigm with apparative measurement of motor activity (using an infrared camera) and aims at assessing all three core ADHD symptoms (i.e. inattention, hyperactivity and impulsivity). In Qb Test, a combination of the n-back task with the no-go component is used: as in the classical n-back task, subjects were asked to press a button as soon as possible, if a figure (circle or square) corresponded with a previous figure (1-back) in terms of both shape and colour (target stimulus). The subjects had to keep track of the two features and refrain from responding, if only one feature matched. The performance in Qb Test was abnormal in each of the included patients as compared with Qb normative data (Brocki et al. 2010; Reh et al. 2015). Patients receiving stimulant treatment (n = 5) were to have discontinued medication at least 96 h before the first fMRI recording. All subjects had normal or corrected to normal vision as assessed by the Schnellen chart and were right-handed as determined by the Edinburgh handedness inventory. None presented with any neurological symptoms during neurological examination before MRI sessions. All adolescents were normally developed and had normal structural MRI and EEG. None of them took any additional medication or presented with any history of developmental disorders or language problems. All participants were German native speakers. All participants and their parents were instructed about the study, and written informed consent according to the Declaration of Helsinki was obtained. The study was approved by the local Ethic Committee.

In the course of the study, three of the 16 adolescents with ADHD were excluded: two because of excessive movement artefacts (>2 mm in any of the estimated realignment parameters), and one because of ferromagnetic orthodontic material. The 13 remaining adolescents with ADHD (age 14.21 ± 1.28 years, 3 girls, IQ = 95 ± 8.7) were considered for final analysis. There were no statistically significant differences between the excluded and included adolescents in terms of age, gender, clinical variables (ADHD score in DCL-HKS), performance in the n-back tasks outside or inside the scanner, and IQ (tested using the non-parametric Wilcoxon test). Thirteen adolescents underwent both tDCS and sham stimulation.

**Table 1** Clinical and neuropsychological characteristics of the sample

| Clinical/neuropsychological measure | Mean ± SD |
|---|---|
| IQ | 99.5 ± 12.15 |
| DCL-HKS inattention (scores) | 1.84 ± 0.81 |
| DCL-HKS impulsivity (scores) | 1.39 ± 0.81 |
| DCL-HKS hyperactivity (scores) | 0.98 ± 0.61 |
| DCL-HKS total score (scores) | 1.87 ± 1.04 |
| CBCL internalizing score (T-value) | 59.64 ± 8.81 |
| CBCL externalizing score (T-value) | 64.42 ± 7.00 |
| CBCL total score (T-value) | 65.14 ± 6.07 |
| Qb-test RT (percentile) | 68.0 ± 31.8 |
| Qb-test variability of RT (percentile) | 76.8 ± 31.3 |
| Qb-test best omission errors (percentile) | 83.9 ± 19.7 |
| Qb-test commission errors (percentile) | 74.0 ± 32.7 |
| Qb-test movement time (percentile) | 47.5 ± 22.1 |
| Qb-test movement distance (percentile) | 95.1 ± 8.55 |
| Qb-test movement simplicity (percentile) | 83.1 ± 14.7 |
| Qb-test movement area (percentile) | 95.1 ± 6.27 |

For CBCL, T-values are given (t > 60 demonstrates abnormal values compared with healthy population of children and adolescents). For Qb-test, percentile values are presented demonstrating a pronounced deviation of the most of given measures in our sample from values of the healthy age-matched control subjects (Brocki et al. 2010)

*CBCL* child behavior checklist, *DCL-HKS* German adaptive diagnostic checklist for attention deficit hyperactivity disorder, *RT* reaction time

## Procedure

The study was performed using a double-blind, sham-controlled protocol. After recruitment, all adolescents were randomized between two groups: the first group was firstly treated with tDCS and then with sham stimulation; the second group received treatment in the inverse order. The time between both stimulation sessions was at least 2 weeks. The protocols for every patient were preprogrammed in the stimulation device, so that both patient and investigators were blind to stimulation conditions. The experimental design is shown in the Fig. 1a. A short practice session of the WM paradigm was carried out outside the scanner to ensure the instructions were understood correctly. After this, the tDCS electrodes were placed on the subjects' scalp who were then introduced into the bore of the MRI scanner. Scanning, visual stimulation, and tDCS stimulation were synchronized so that all started simultaneously. After the WM protocol with

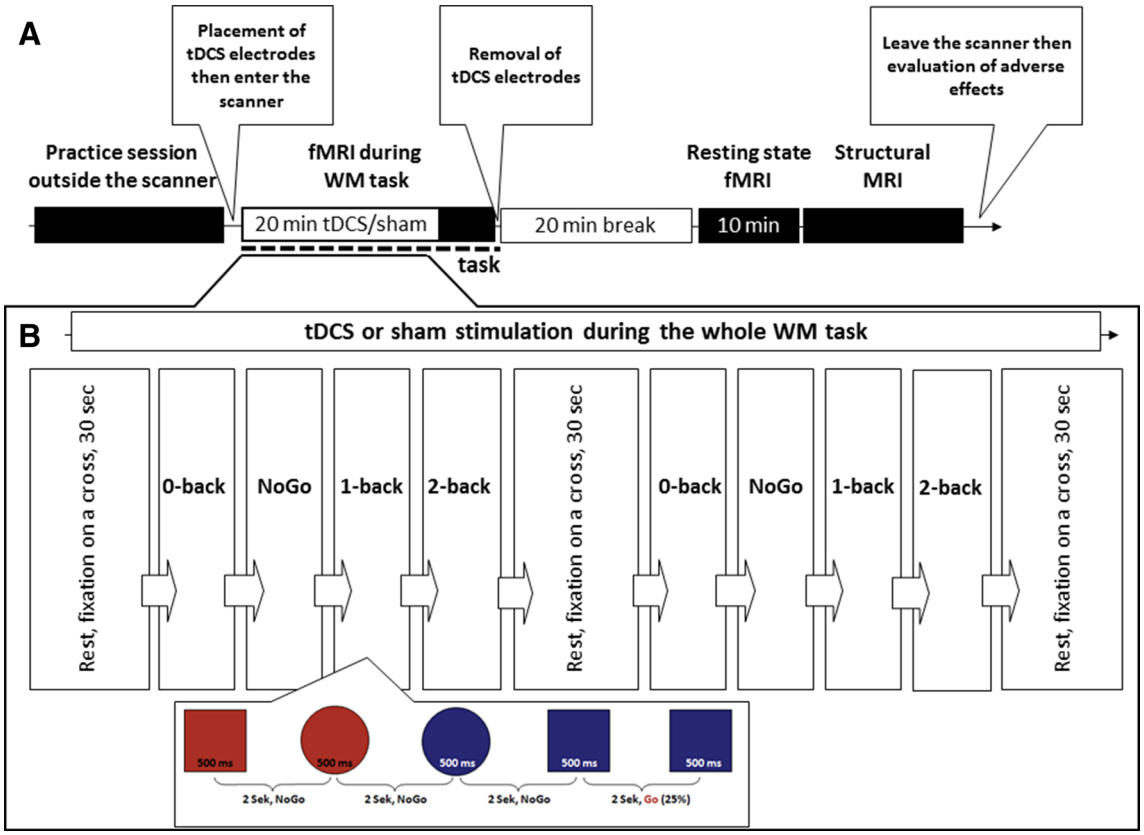


**Fig. 1** Experimental design (**a**) and paradigm (**b**) which was applied inside the MR scanner. The experiment started with a short practice session then continued with tDCS or sham stimulation during the WM task simultaneously with fMRI (please note that the task lasted 11 min longer that tDCS). After the WM task was finished, stimulation electrodes were removed. Then, after a pause of 20 min, resting state fMRI and structural MRI were carried out. The working memory (WM) task was structured in blocks with four tasks: 0-back (subjects were to press a button as soon as possible when stimuli appeared), go/no-go task (subjects were to react to all frequent *blue* stimuli and refrain from response to all rare *red* stimuli), 1-back (subjects were to press a button as soon as possible if the appearing figure corresponded with the previous figure in terms of both *shape* and *color*) and 2-back (subjects were only to respond if the appearing figure corresponded with the one before the previous figure). (Color figure online)

simultaneous tDCS/sham stimulation, a 10 min resting state fMRI session and structural neuroimaging for superimposition with functional MRI were carried out. The time between the WM session and resting state session was about 20 min. Voice contact with participants was maintained throughout the whole experiment in order to interrupt the protocol in case of any adverse effect. After the experiment, any adverse effects were evaluated via a semi-structured interview based on previous published interviews (Poreisz et al. 2007). All recordings were carried out in the afternoon between 2 and 6 p.m. At each appointment, the current well-being of the participants was assessed by recording the quality and duration of sleep the night before investigation, assessing mood (subjective evaluation on a digital scale from 1 to 7), intake of medication, drugs, alcohol and caffeine. After the experiment, the subjects were encouraged for participation with cinema vouchers.

## Working Memory Paradigm

Following many reports using the n-back paradigm with increasing memory load for the study of the effects of tDCS on WM (Fregni et al. 2005; Boggio et al. 2006; Jo et al. 2009; Keeser et al. 2011a; Mulquiney et al. 2011; Teo et al. 2011; Berryhill and Jones 2012; Mylius et al. 2012; Oliveira et al. 2013; Martin et al. 2014), we adapted a modified version of this paradigm for the present study. Besides WM, impulsivity is the most robust neuropsychological feature characterizing patients with ADHD (Klein et al. 2006). We used a combination of the n-back task with the go-nogo-component. As in the classical n-back task, subjects had to press a button as soon as possible, if the figure (circle or square) appearing corresponded with the previous figure (1-back) or with one before the previous figure (2-back) in terms of both shape and color (red or blue). The subjects had to keep track of the two features and refrain from responding, if only one feature matched.

This paradigm is integrated in the test battery of Qb Test (Q-Tech Co., Stockholm, Sweden) and has been evaluated repeatedly in several ADHD studies, showing robust discrimination between patients and age-matched healthy subjects on the individual level (Brocki et al. 2010; Reh et al. 2015). A 0-back task (response to the incoming stimuli) and a go/no-go task (requiring quick reaction in response to the red square and no response to other stimuli) were used as control conditions for fMRI analysis in order to model button press (0-back) and no-go effect in the first level analysis and differentiate these effects later from the effect of WM. A detailed overview of the experimental paradigm is presented in Fig. 1b. The paradigm was divided into six blocks. A fixation cross was shown for 20 s before each block. All blocks consisted of 0-back, go/no-go, 1-back and 2-back tasks. Written instructions, announcing the type of upcoming task, appeared on the screen for 10 s prior to each task. Then, the tasks were carried out with 24 stimuli in every task. Thirty percent of the trials (n = 8) contained matched stimuli in the n-back tasks (i.e. 30% of the stimuli required reaction) or no-go stimuli in the go/no-go task. Stimuli were presented on the screen for 200 ms. The interstimulus interval was 1500 ms, and 144 stimuli were presented throughout all blocks for each task using the Presentation software (Neurobehavioral Systems, Albany, CA). The duration of the entire WM experiment was 31 min.

### Resting State fMRI

After the WM experiment, tDCS electrodes were removed, and the resting state recordings were started. Children were instructed to stay quiet in the scanner for 10 min with eyes open (in order to avoid fluctuations in vigilance). During the resting state fMRI, no stimulation was carried out.

### Transcranial Direct Current Stimulation

Direct current was applied through a pair of rubber MR-compatible electrodes (rectangular cathode, 35 $cm^2$ and round anode, 13 $cm^2$) and delivered using an MR-compatible, battery-driven and constant-current stimulator (NeuroConn GmbH, Ilmenau, Germany). Electrodes were fitted with 5 kΩ resistors to be compatible with the MR magnetic field. For a detailed description of the tDCS set-up inside the scanner, see Antal et al. (2011). The electrode set-up in this study was the following: anodal active electrode over the left DLPFC (F3 according to the International 10–20 system) and cathodal active electrode over the Cz. TDCS was applied for 20 min, with current ramped up and down to and from 1 mA during a period of 8 s. The stimulation started with the beginning of the WM experiment inside the scanner and with the first scan of fMRI. During sham stimulation, the current was ramped up for 8 s, followed by 5 s of 1 mA stimulation and then ramped down for 8 s. The impedance was controlled by the device throughout each tDCS session, staying <10 kΩ and limited by the voltage. Exceeding these limits (e.g., increase of impedance due to dried up or shifting electrodes) would have resulted in automatic termination of the stimulation (see instruction manual of the NeuroConn stimulator). Both subject and experimenter were blind to the type of stimulation.

### MRI Data Acquisition

BOLD-sensitive MRI was performed with a 3 Tesla MR scanner (Siemens Trio, Erlangen, Germany) and a 32-channel head coil. A single-shot, T2*-weighted gradient-echo planar imaging sequence was used (TR = 2500 ms, TE = 35 ms, 30 slices, 64 × 64 matrix, slice thickness = 3.5 mm, FOV = 200 mm, flip angle = 90°, interleaved order), allowing whole-brain (i.e. including cerebellum and midbrain) volume acquisition. An anatomical MRI for superimposition with functional images was acquired using a high-resolution, whole-head, T1-weighted, 3-D MPR sequence (1 mm slice thickness, 208 × 208 matrix, 150 slices, FOV = 208 mm, TE = 3.6 ms, TR = 7.8 ms, flip angle = 8°, NSA = 2).

### Behavioral Measurements During the fMRI Session

The neuropsychological effects of tDCS were assessed for the following dependent variables from all tasks: RT (in ms), RT variability (standard deviation of RT), omission errors (number of matches ignored in the n-back tasks or number of go-trials omitted in the go/no-go task), and false alarms (number of mismatches in the n-back task or number of no-go trials which elicited a reaction). Moreover, performance accuracy was calculated for Go-Nogo as well as both WM tasks using the following formula: Accuracy = Hits + Correct rejections/Total number of stimuli, where Hits = number of targets (where the button press is required) − omission errors, and Correct rejections = Number of distractors (no-go stimuli) − false alarms (Jacola et al. 2014). All behavioural variables were normally distributed (Kolmogoroff–Smirnov test) and characterized by homogeneous variances (F-test). Differences between the stimulation conditions were estimated using parametric statistics. For the estimation of general effects, ANOVA with main within-subjects effects STIMULATION (anodal tDCS vs. sham) and CONDITION (0-back vs. go/no-go vs. 1-back vs. 2-back) was carried out for each dependent variable. Differences between and within stimulation conditions were assessed using pair-wise two-tailed *t* tests. The significance level was kept at $p < 0.05$ after Bonferroni alpha adjustment for multiple comparisons. All statistical

analyses were performed using SPSS Version 17.0 (SPSS Co., Chicago, US).

### Task fMRI Data Analysis

The fMRI data were analysed using SPM8 software (Wellcome Department of Imaging Neurosciences, UCL, UK, http://www.fil.ion.ucl.ac.uk/spm). The first five images were discarded to ensure steady-state longitudinal magnetization. All volumes were realigned to the first volume, slice-time corrected, and spatially normalized to the template of the Montreal Neurological Institute (MNI) (voxel size 3×3×3 mm). Images were then smoothed with a 3-D isotropic Gaussian kernel (8 mm full-width-half-maximum) and high-pass filtered at a cut-off of 128 s. The preprocessed fMRI time series were statistically analysed at an individual level using the general linear model. The block design (blocks of 0-back, go/no-go, 1-back, and 2-back) was modelled by stick functions convolved with the canonical hemodynamic response function (Friston et al. 1995). The following regressors were included in the analysis: (a) 0-back, (b) go/no-go task; (c) 1-back task; (d) 2-back task, and (e) six realignment parameters for possible movement effects as a covariate of no interest. Trials with omission errors or false alarms were disregarded due to low statistical power and were modelled as regressors of no interest. Our preliminary analyses of these trials did not reveal any significant results.

Using the parameters estimated from the single-subject analyses, we performed second-level random effect group analyses with a two-factor ANOVA with STIMULATION (anodal tDCS vs. sham) and CONDITION (0-back, go/no-go, 1-back, and 2-back) as the within-subject factors. Afterwards, we performed post-hoc *t* tests to evaluate effects at the population level. The statistical significance threshold was set at $p < 0.001$ (uncorrected), with the resulting statistical maps overlaid into a standard anatomical template in MNI space. The following two contrasts were analysed for each condition: (1) tDCS > sham and (2) sham > tDCS. The labels of activated regions were defined using the Anatomy toolbox for SPM (Eickhoff et al. 2005).

### Resting State fMRI Functional Connectivity Analysis

Data from resting sessions was preprocessed with FSL 6.0 (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) using the same steps as those applied to the task fMRI data, including motion correction with MCFLIRT, non-brain removal with BET, spatial smoothing with a Gaussian kernel of FWHM 8 mm, grand-mean intensity normalisation of the entire 4D data by a single multiplicative fact, high-pass temporal filtering with 0.01 Hz cut-off (Gaussian-weighted least-squared straight line fitting, with sigma = 50 s). We first studied the global connectivity of each voxel with the rest of the brain by means of the functional connectivity density (FCD) (Tomasi and Volkow 2010). FCD is obtained by averaging the linear correlations between the BOLD signal at each voxel and the signals at all remaining grey matter voxels (as determined by the Automated Anatomical Labelling template, Tzourio-Mazoyer et al. 2002). Formally, this corresponds to the sum of the columns/rows of the correlation matrix, obtained as follows:

$$C_{ij} = \frac{< \left(X_i - < X_i >\right)\left(X_j - < X_j >\right) >}{\sigma_{X_i}\sigma_{X_j}} \quad (1)$$

where $X_i$ represents the BOLD time series of the i-th voxel; $\sigma_{X_i}$, its standard deviation; and <,>, temporal averaging. After obtaining the correlation matrix between all grey matter voxels, the FCD of the i-th voxel was derived by summing all entries in the i-th column (or row) of $C_{ij}$, i.e.:

$$FCD_i = \sum_j C_{ij} = \sum_j C_{ji} \quad (2)$$

High FCD values correspond to well-connected voxels (in the functional sense); low FCD values correspond to voxels weakly connected with the rest of the brain. As such, FCD can be interpreted as indexing the global connectivity of each individual voxel. Individual FCD maps were obtained for each participant under two different conditions: resting state after tDCS stimulation and resting state after sham stimulation. These two sets of maps were compared using a paired *t* test with a significance threshold of $p < 0.05$ corrected for multiple comparison at the cluster level. After identifying clusters with different degrees of global functional connectivity between real and sham stimulation (by means of the FCD), seed correlation analyses were conducted to identify which networks were more or less engaged with the aforementioned brain regions. For this purpose, the average BOLD signal was extracted from the clusters exhibiting significant differences in FCD between tDCS and sham, and the functional connectivity between this average signal and all brain voxels was computed (using Pearson's linear correlation coefficient, as in Eq. 1). The seed correlation maps of individual participants were compared across both conditions (tDCS and sham) using a paired *t* test with a significance threshold of $p < 0.05$ corrected for multiple comparisons at the cluster level.

## Results

### Adverse Effects

A mild tingling and itching sensation under the electrodes was the most commonly reported adverse effect.

This sensation was reported by 46% of the subjects during anodal tDCS and by 46% during sham stimulation. None of the subjects reported fatigue, burning, pain or other uncomfortable sensations during stimulation. Also, none found the stimulation procedure to be unpleasant or reported difficulties in concentrating during the experiment. Headache after anodal stimulation was reported only by one subject. Only one subject felt nervous or overexcited during experiments with tDCS and sham. These behavioral changes may be attributed to anxiety related to the MR scanner environment. None of the participants reported changes in visual perception or were hyperactive during or after the stimulation.

### Behavioral Changes Under tDCS

Changes in the behavioural parameters with respect to the kind of stimulation and experimental condition are shown in the Fig. 2. The means of RT, its variability, false alarms and omission errors increased and of accuracy decreased with the difficulty of the task (from 0-back, go/no-go, 1-back, and, finally, 2-back). Accordingly, the effect CONDITION was significant for RT [$F(3,36)=33.67$; $p<0.001$], omission errors [$F(3,36)=13.35$; $p<0.001$] as well as accuracy [$F(3,36)=55.32$; $p<0.001$] and demonstrated a tendency towards significance for RT variability [$F(3,36)=2.88$; $p=0.095$]. As for the interaction STIMULATION x CONDITION, increases in RT and RT variability were significantly less pronounced during anodal tDCS than during sham stimulation [$F(3,36)=2.81$; $p=0.05$ and $F(3,36)=5.8$; $p=0.017$ accordingly]. There were no significant main effects and interactions for the false alarms. Surprisingly, the effect STIMULATION was significant for omission errors [$F(1,12)=8.07$; $p=0.014$] and accuracy [$F(1,12)=8.39$; $p=0.013$], resulting in more errors and less accuracy under tDCS stimulation than with sham. This effect was task unspecific as the interaction STIMULATION × CONDITION was non-significant (therefore, for omission errors and accuracy no post-hoc tests were carried out). Without correction for multiple comparisons, the post-hoc *t* tests revealed differences ($p<0.05$) between stimulation modalities demonstrating for the 2-back condition that the RT [$t(12)=2.58$; $p=0.026$] and the variability of the RT [$t(12)=2.68$; $p=0.021$] were lower under tDCS compared with sham. However, these differences did not survive Bonferroni alpha adjustment.

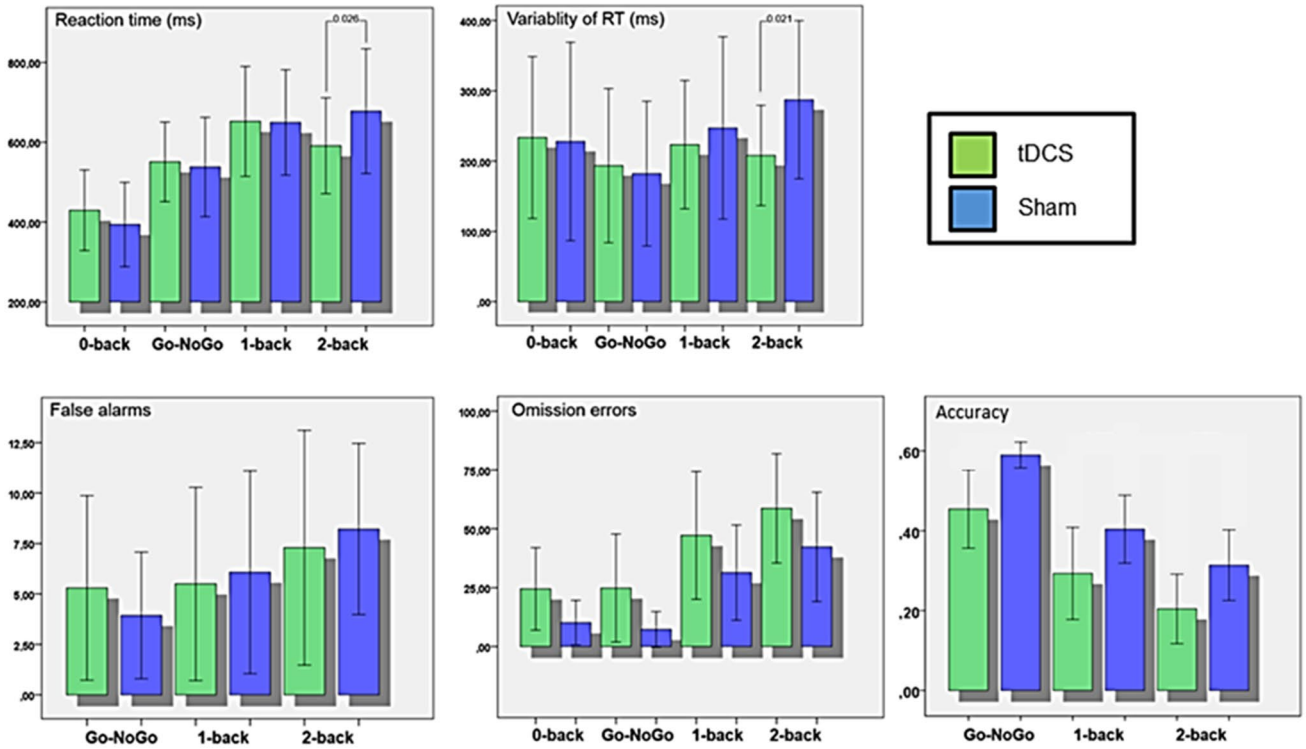


**Fig. 2** Behavioral measures (reaction time RT, variability of the reaction time RT, false alarms, omission errors, and accuracy) obtained in the working memory experiment which was carried out during simultaneous fMRI recording and transcranial stimulation with either anodal tDCS or sham (means and standard deviations). Note that for the 2-back task the difference between tDCS and sham stimulation was significant for RT and variability of RT without correction for multiple comparisons. These differences did not survive the Bonferroni alpha adjustment. Therefore, these differences have to be interpreted with caution

## Task fMRI Results

Figure 3 and Table 2 summarize the hemodynamic changes associated with tDCS compared to sham stimulation. The ANOVA analysis of the STIMULATION × CONDITION interaction revealed a significant effect in the left DLPFC, left premotor cortex and SMA and in the parietal cortex bilaterally ($p < 0.001$, uncorrected). There were no significant main effects. Post-hoc comparisons revealed the following results: (1) there were generally no significant differences between tDCS and sham for any task by comparing sham in advance to tDCS (sham > tDCS); (2) by analysing tDCS in advance to sham, there were no significant BOLD signal changes for the condition 0-back and go/no-go; (3) for the 1-back paradigm: tDCS caused more pronounced BOLD signal increases in the left DLPFC (under the stimulating electrode) as well as in the left premotor cortex, left SMA, medial prefrontal cortex, posterior insula and precuneus bilaterally compared with sham stimulation; (4) for the 2-back paradigm: tDCS was associated with more activations in the left DLPFC, left premotor cortex, left SMA and right precuneus compared with sham stimulation. Note that the differences between the tDCS and sham conditions

**Table 2** Results of fMRI analysis (n-back task during transcranial stimulation)

| Brain area | x | y | z | Max. t value | Cluster size |
|---|---|---|---|---|---|
| 1-back tDCS > sham | | | | | |
| Precuneus left | −18 | −74 | 20 | 4.76 | 2120 |
| Precuneus right | 22 | −58 | 16 | 4.00 | 1007 |
| Anterior cingulate cortex | −3 | 27 | 19 | 3.75 | 353 |
| Premotor cortex left | −48 | 16 | 18 | 3.69 | 517 |
| SMA left | −14 | −10 | 42 | 3.23 | 127 |
| DLPFC left | −39 | 27 | 17 | 3.54 | 42 |
| DLPFC right | 50 | 22 | 14 | 3.39 | 88 |
| Posterior insula right | 40 | −16 | 20 | 3.54 | 179 |
| 2-back tDCS > sham | | | | | |
| Premotor cortex left | −36 | −8 | 20 | 4.36 | 352 |
| SMA left | −16 | −4 | 38 | 3.29 | 15 |
| DLPFC left | −30 | 30 | 18 | 3.62 | 18 |
| Precuneus right | 22 | −58 | 18 | 3.46 | 168 |

*DLPFC* dorsolateral prefronral cortex, *SMA* supplimentary motor area

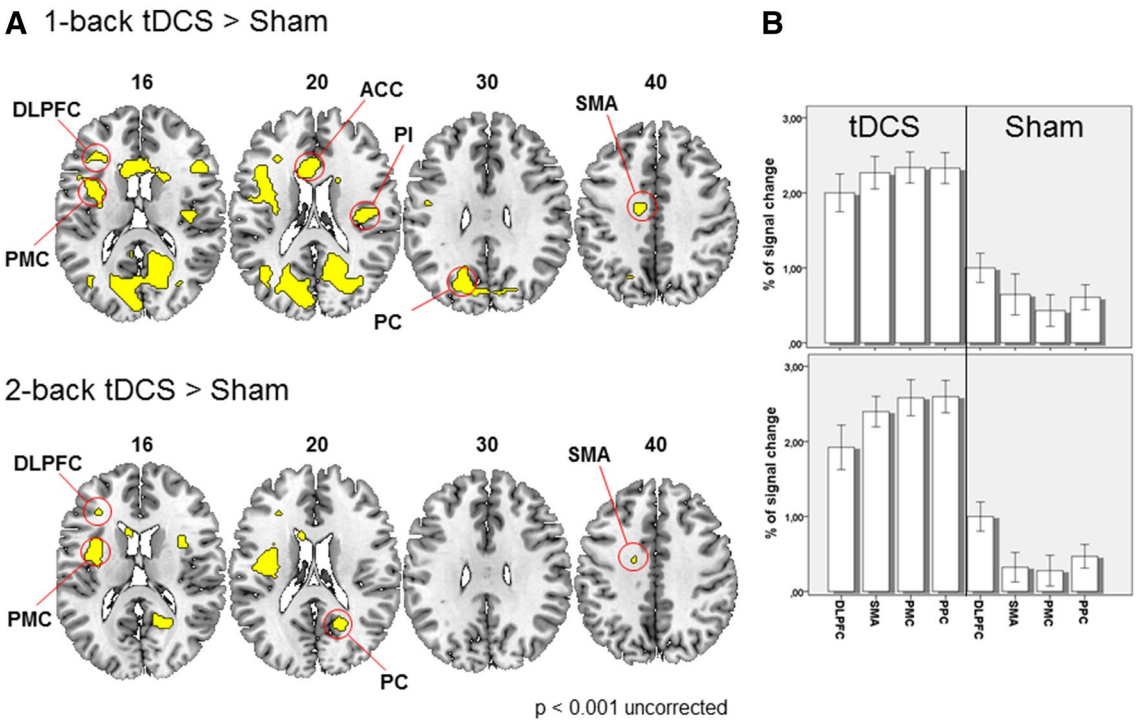


**Fig. 3** Results of fMRI analysis of the working memory paradigm. During tDCS, there was a significantly greater activation in the depicted brain regions compared with sham condition (**a**). The increase in BOLD signal (percent of signal change) in the left DLPFC, SMA, left premotor cortex, and precuneus was significantly more pronounced during anodal tDCS compared to sham condition (**b**). Comparisons of the percent of signal change for the defined regions between tDCS and sham condition: $p < 0.05$. *ACC* anterior cingulate cortex, *DLPFC* dorsolateral prefrontal cortex, *PMC* premotor cortex, *PI* posterior insula, *PC* precuneus, *SMA* supplementary motor area

were also significant for the means of percent of BOLD signal change obtained from regions of interest (ROI: left DLPFC, left premotor cortex, left SMA, precuneus, significance testing with two-tailed *t* tests, $p < 0.05$).

### Results of Resting State fMRI

The FCD analysis revealed increased global connectivity located on the left DLPFC under the stimulation electrode (contrast of tDCS stimulation vs. sham stimulation). Results of this analysis are shown in Fig. 4. This cluster of increased connectivity was used as a seed for a correlation analysis in order to identify the network of regions becoming more engaged with DLPFC after tDCS stimulation (see Fig. 5 for results). This analysis revealed increased DLPFC connectivity with regions associated with WM function, including the bilateral middle frontal gyrus, angular gyrus, inferior temporal gyrus, and middle cingulate gyrus. If no correction for multiple comparisons will be done (see Fig. 5), additional regions appear having increased connectivity with the DLPFC after tDCS stimulation included the precuneus, the middle occipital gyrus, the middle temporal gyrus, the fusiform gyrus and the paracentral lobule. These regions were compared with those obtained from a meta-analysis of studies involving WM function, as performed with Neurosynth (Yarkoni et al. 2011). It is already apparent to the naked eye that regions with increased connectivity with DLPFC after tDCS stimulation match with the Neurosynth-derived WM network. We formalized this observation by computing the mean connectivity with the DLPFC after tDCS and sham inside masks based on Neurosynth's WM map as well as inside 5 well-established resting state networks (RSN): visual, auditory, sensorimotor, DMN, and executive control (Beckmann et al. 2005). Significantly different DLPFC connectivity between real and sham stimulation was found in the WM map and in the executive control network but not in the other resting state networks, highlighting the specificity of the observed effect (Fig. 6).

## Discussion

### Tolerability of tDCS in Adolescents

TDCS was tolerated well by adolescents in our study. A mild tingling and itching sensation under the electrodes was the most common adverse effect and only reported by a minority of subjects. None of the participants found the stimulation procedure unpleasant. Our experience of tDCS tolerability corresponds well with previous reports (Krishnan et al. 2015). For example, we recently investigated the side effects of tDCS systematically in different ages and demonstrated that the 1 mA direct current

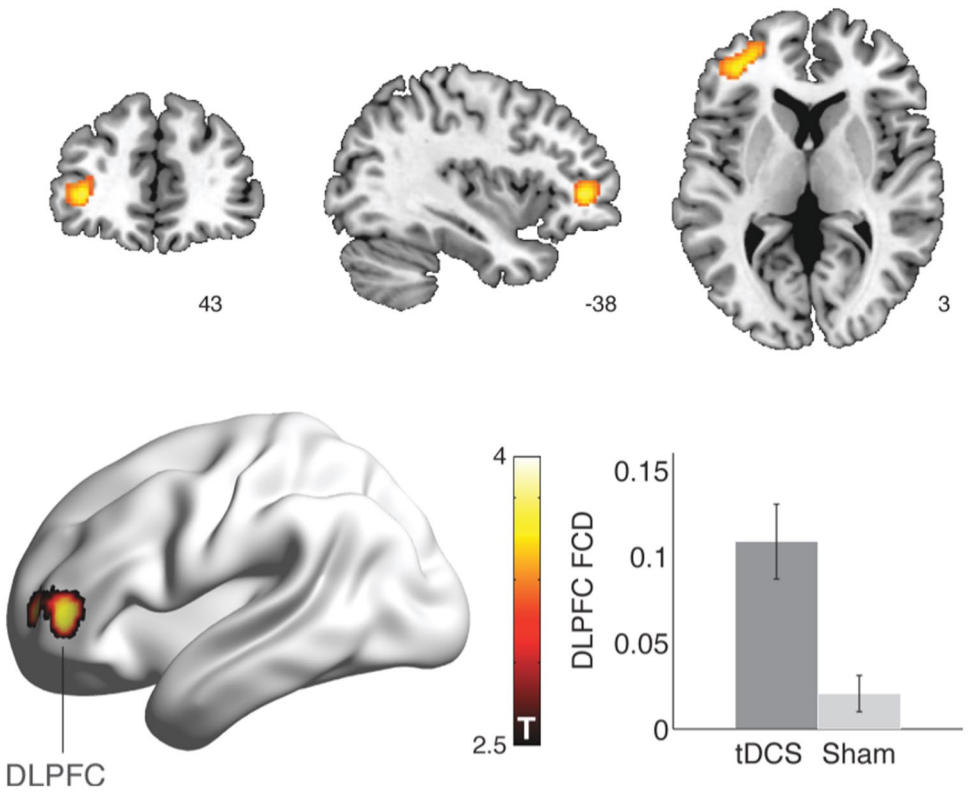


**Fig. 4** tDCS stimulation increases the global functional connectivity density (FCD) of the left dorsolateral prefrontal cortex (DLPFC). Statistical significance maps of increased FCD (resting state after tDCS stimulation vs. resting state after sham stimulation) are displayed, both as volumetric and 3D-rendered maps. A significant cluster ($p < 0.05$, corrected for multiple comparisons at the cluster level) was found in the left DLPFC, the site of the anodal stimulation

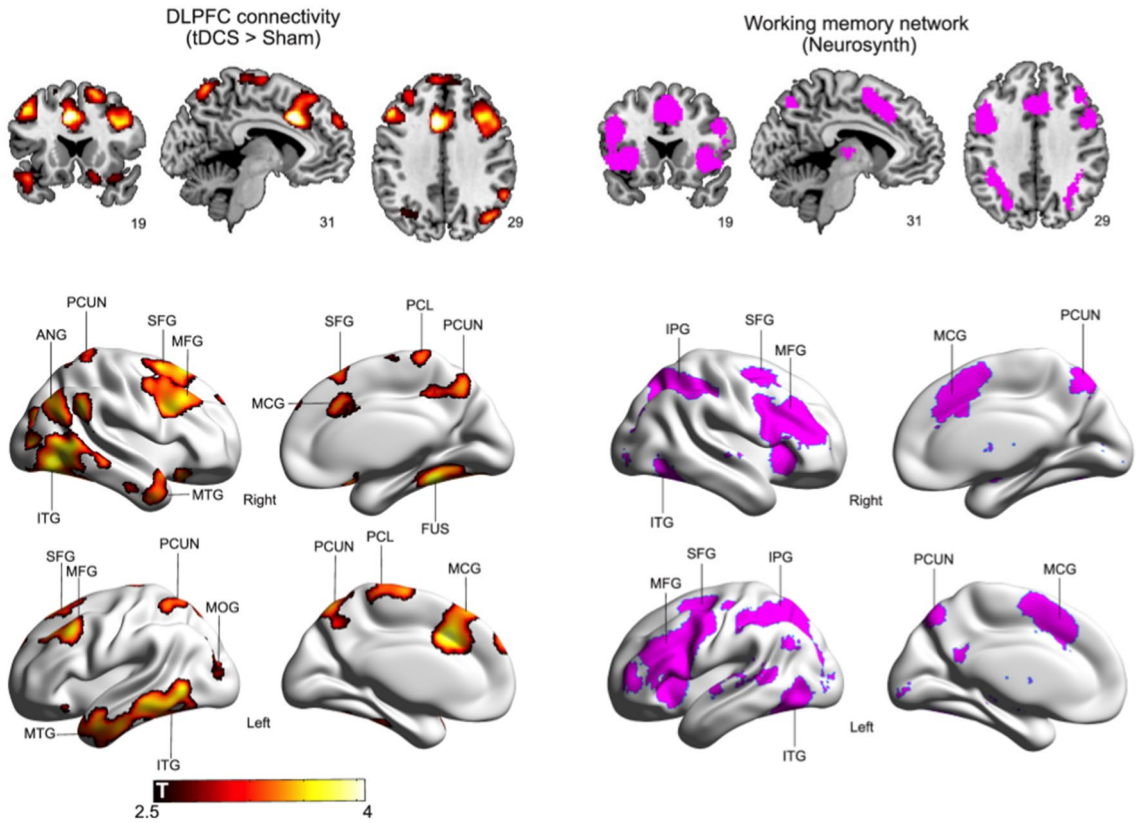


**Fig. 5** Anodal tDCS stimulation increases the functional connectivity of the dorsolateral prefrontal cortex (DLPFC) and the working memory network. *Left* volumetric and 3D rendering of statistical significance maps for the increase of DLPFC functional connectivity (resting state after tDCS stimulation vs. resting state after sham stimulation). The threshold of significance for presentation of results is set on 0.05 non-corrected. Activated brain areas after correction for multiple comparisons are given in Table 3. *Right* for comparison, the working memory network obtained from the meta-analysis of a large number of published studies (from neurosynth). *ANG* angular gyrus, *FUS* fusiform gyrus, *IPG* inferior parietal gyrus, *ITG* inferior temporal gyrus, *MCG* medial cingulate gyrus, *MFG* medial frontal gyrus, *MTG* medial tempotal gyrus, *PCL* paracentral lobule, *PCUN* precuneus, *SFG* superior frontal gyrus

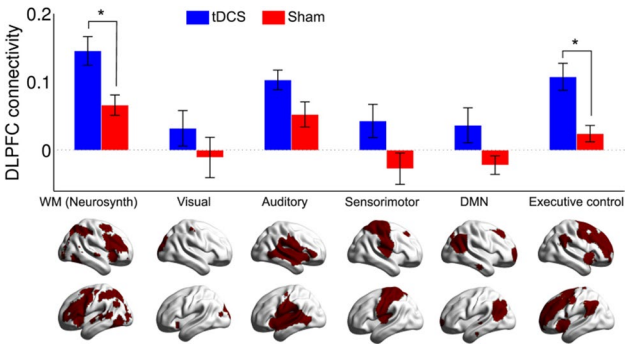


**Fig. 6** Increased dorsolateral prefrontal cortex (DLPFC) connectivity after tDCS stimulation is specific to working memory and regions of the brain responsible for executive function. Mean DLPFC connectivity after tDCS (*blue*) and sham stimulation (*red*) computed within Neurosynth's working memory map and 5 canonical resting state networks (visual, auditory, sensorimotor, default mode network, and executive control) from Beckmann et al. 2005. *DLPFC* dorsolateral prefrontal cortex, *DMN* default mode network, *WM* working memory. (Color figure online)

**Table 3** Differences in the functional connectivity density (FCD) and dorsolateral prefrontal cortex (DLPFC) resting state functional connectivity between tDCS and sham stimulation

| Brain area | x | y | z | Max. t value | Cluster size |
|---|---|---|---|---|---|
| FCD tDCS > sham | | | | | |
| Left DLPFC | −31 | 33 | 6 | 4.9 | 665 |
| DLPFC connectivity tDCS > sham | | | | | |
| Right angular gyrus | 58 | −50 | 29 | 7.5 | 1485 |
| Left middle cingulate gyrus | −2 | 20 | 36 | 7.12 | 27 |
| Left inferior temporal gyrus | −46 | 22 | −20 | 6.63 | 1524 |
| Right middle frontal gyrus | 40 | 22 | 40 | 6.18 | 1098 |
| Left middle frontal gyrus | −34 | 38 | 40 | 6.12 | 889 |

polarization is safe and tolerated well by young healthy subjects (Moliadze et al. 2015b). Also, tDCS does not cause serious adverse effects in young clinical populations, even when applied daily over a number of consecutive days (Mattai et al. 2011; Schneider and Hopp 2011; Siniatchkin et al. 2012a, b). Thus, all published studies support that tDCS may be safely applied in children and adolescents. Note, however, that tDCS in the present study was performed inside MR scanner; indeed, the prevalence of unpleasant sensations in children was comparable to that reported for within-scanner tDCS stimulation of adults (Baudewig et al. 2001; Antal et al. 2011; Stagg et al. 2013). Thus, our study may open the way for the application of tDCS inside MR scanners to investigate the effects of neuromodulation on the developing brain.

### Changes in Working Memory Performance

TDCS exerts a significant effect on WM performance in adolescents with ADHD (significant interaction STIMULATION × CONDITION for RT and variability of RT and significant main effect STIMULATION for accuracy and omission errors). Without tDCS (in sham condition), the increasing task complexity and memory load are associated with the increasing RT, RT variability and larger number of omission and commission errors as well as with the decreasing accuracy. These changes in behavioural parameters correspond well with changes in the same measures in healthy subjects and ADHD patients obtained without concurrent stimulation (Klein et al. 2006). Anodal tDCS applied over the left DLPFC, however, may influence WM performance, especially for tasks with high complexity and high memory load as shown in our study. In the 2-back task and under tDCS, RT was shorter and its variability was smaller compared to sham stimulation. It seems likely that tDCS prevents the increase of mental effort with increasing memory load and task complexity. Note that this effect was mostly related to the motor aspects of performance during the WM task. Surprisingly, the anodal tDCS over the left DLPFC was associated with more omission errors and less accuracy than sham stimulation. It seems likely that in adolescents with ADHD anodal tDCS improves motor performance and worsens accuracy.

The effects of anodal tDCS over the left DLPFC on the n-back WM task have been demonstrated repeatedly in a large number of studies (for a review see Brunoni and Vanderhasselt 2014 and; Hill et al. 2016). Most studies have revealed increases in the number of correct responses as well as a reduction in the number of errors and in the RT (Fregni et al. 2005; Boggio et al. 2006; Jo et al. 2009; Keeser et al. 2011a; Mulquiney et al. 2011; Teo et al. 2011; Berryhill and Jones 2012; Mylius et al. 2012; Oliveira et al. 2013; Martin et al. 2014). Although these studies may indicate a clear effect of tDCS on n-back WM tasks, the results are inhomogeneous. In some studies, the main effect of tDCS was related to the reduction of errors (false alarms in most studies) with no or little effect on the RT (Fregni et al. 2005; Jo et al. 2009; Berryhill and Jones 2012; Mylius et al. 2012; Oliveira et al. 2013). Some studies have demonstrated no or little effect of tDCS on the errors and a clear effect on RT (Boggio et al. 2006; Teo et al. 2011). Finally, others have reported that tDCS influences both the error rate and the RT (Keeser et al. 2011a; Mulquiney et al. 2011). In our study, we demonstrated a significant effect of tDCS on the RT and its variability and on accuracy. Such inhomogeneity in the results is likely related to methodological inconsistencies.

We suppose that the improved motor function and the worsened accuracy under tDCS in our study may be explained by a special montage with the anode placed over the left DLPFC and cathode over the vertex (Cz). This montage differs from montages used in previous studies which put the reference electrode on the right supraorbital area or on the contralateral cheek (Hill et al. 2016). Especially the montage with the reference on the right supraorbital area was associated with very moderate effects of tDCS on motor function (in the recent meta-analysis of Hill et al. 2016 the effect of anodal tDCS on RT was significant only for offline application of tDCS and no significant effects on RT were observed in clinical populations). Studies which have performed modeling of electrical current distribution have demonstrated the greatest stimulation current densities between the primary stimulating and return electrodes with the global maxima at the edges of electrodes (Salvador et al. 2015; Opitz et al. 2015). It could be suggested that by using the montage with the cathode over the right supraorbital area we would effectively stimulate not only the left DLPFC but also the medial frontal cortex (brain area which is involved in the error processing and correction, Mazaheri et al. 2009) and could expect more pronounced effect of tDCS on accuracy. By using the montage with the cathode over the vertex we would stimulate, among the left DLPFC, also the left premotor cortex and could expect effects of tDCS on motor function, especially on the RT variability (Suskauer et al. 2008). This might be the case in our study. It could be hypothesized that the chosen montage caused a typical speed/accuracy trade-off effect: increase of speed accompanying by an increase of errors. Therefore, it seems likely that, within the same task, tDCS improves one neuropsychological function and causes "adverse effects" by worsening the other. On the one hand, one may assume that the reduced accuracy provides an argument against the use of tDCS in the treatment of ADHD, at least by application of the described montage. On the other hand, the anodal tDCS over the left DLPFC in our study led to a reduction of RT and its variability.

Different studies on ADHD have shown the high RT variability as a key neuropsychological abnormality in ADHD closely related to clinical symptoms (Castellanos et al. 2005; Klein et al. 2006; Uebel et al. 2010; Chamberlain et al. 2011). The improvement of RT variability may be associated with the improvement of the clinical course of ADHD. And finaly, the cathode over the vertex is not just a passive reference but produces cathodal stimulation of the motor cortex. However, how this cathodal tDCS would influence RT, its variability and other behavioural measures is difficult to predict because of the following reasons: (1) in previous studies done in adults, the cathodal tDCS has caused a significant reduction of the motor cortex excitability and RT (Nitsche and Paulus 2000, 2001). These studies, however, have placed the cathode over the hand area (C3 according to the international 10–20 system). In our study, the cathode was situated over Cz (leg area), this placement was far away from the hand area. Whether the stimulation of Cz would cause similar cathodal effects as the stimulation of C3 remains to be studied. (2) The adolescents in our study were of the similar age as subjects from the study of Moliadze et al. (2015a). This study demonstrated paradoxical effects of the 1 mA cathodal tDCS over the left motor cortex as a function of brain development. In adolescents, the cathodal tDCS exerts an excitatory effect because of developmental deviation in the baseline levels of excitability and preactivation (Moliadze et al. 2015a). Whether similar paradoxical effects of the cathode have occurred in our study and explain shorter RT and reduced RT variability, is difficult to say because Moliadze and colleagues studied healthy subejcts (the effect of the disorder in our study can not be excluded) and used other montage (cathode over C3 and anode over the right supraorbital area). In order to prove which montage would cause the most pronounced clinical effects, controlled clinical trials comparing different montages are necessary.

Moreover, inconsistencies between our and previous studies may be also explained by differences in the sample. All previous studies have investigated healthy adults; only three studies have used tDCS in clinical populations (Boggio et al. 2006; Jo et al. 2009; Oliveira et al. 2013). According to these studies, patients differ from healthy subjects in terms of the tDCS effects. However, no previous studies investigated the effects of tDCS on WM in children or ADHD patients. Additionally, the region-specific developmental effects of tDCS cannot be excluded. When applied over the DLPFC, anodal tDCS may cause no or even paradoxical effects on WM performance in young subjects (Moliadze et al. 2015a). Moreover, patients suffering from neurological or psychiatric disorders such as ADHD may present with abnormal levels of cortical excitability and preactivation (Barry et al. 2003; Johnstone et al. 2013). Functional changes of baseline excitability may influence the results of transcranial stimulation (Siebner et al. 2004; Batsikadze et al. 2013; Moliadze et al. 2015a). And finaly, the restricted MR environment, intensive and distracting noise, and situational anxiety may have influenced both performance and the effects of tDCS.

### Effects of tDCS on Neuronal Networks During the Working Memory Task

In both the 1-back and 2-back conditions and in comparison to sham stimulation, tDCS caused greater activation of the left DLPFC as well as of the left premotor cortex, left supplementary motor area, and precuneus. These results correspond with previous studies demonstrating increased activation under the anodal tDCS electrode as well as in brain areas remote from the stimulation site (Baudewig et al. 2001; Lang et al. 2005; Antal et al. 2011; Keeser et al. 2011a; Polania et al. 2011; Zheng et al. 2011; Stagg et al. 2013). Even if applied locally, tDCS likely influences the whole neuronal network associated with WM performance. Note that tDCS caused significant BOLD signal increases in the left DLPFC as well as in the left motor network contralateral to the dominant hand. These BOLD signal increases in the left SMA and left premotor cortex correspond with improved motor performance in the WM task, i.e. with changes in RT and its variability. Both the DLPFC and the motor network seem to play a role in the regulation of RT and its variability. For instance, Suskauer and colleagues (2008) showed abnormal prefrontal and premotor activation underlying abnormalities in RT variability in children with ADHD. Using a classical go/no-go task, we have previously demonstrated that successful behavioral treatment increases BOLD signals in DLPFC, SMA and parietal cortex and reduces RT variability, and that these changes parallel an improvement of the clinical symptoms of ADHD (Siniatchkin et al. 2012a, b; Sotnikova et al. 2012). In these studies, clinical improvement was associated with activation of similar network of regions reported on in the present study. We suggest, therefore, that tDCS may be a potentially effective form of treatment in children with ADHD which results in the modulation of activity in a network of regions directly implicated with clinical improvement and in terms of restoration of behavioural measures such as RT variability.

Although the cortical excitability changes induced by tDCS are virtually indistinguishable during stimulation and in the poststimulation period, based on human and animal studies it can be suggested that the effects of tDCS during stimulation are largely driven by direct effects on membrane polarity while changes after stimulation involve modulation of GABAergic and glutamatergic synapses (Stagg and Nitsche 2011). Both the effects of membrane polarity and changes in the described neurotransmission may result

in BOLD signal changes as demonstrated here (Keeser et al. 2011b; Stagg et al. 2013). We suppose that the anodal tDCS increases neuronal activity in the left DLPFC and, in such a way, strengthens connectivity in the WM network. This neurophysiological effect may be used to treat patients with ADHD. Indeed, as demonstrated in a great number of neurophysiological and neuroimaging studies, clinical symptoms in ADHD and associated neuropsychological deficits (for example, deficits in WM) can be attributed to a reduced activity in prefrontal brain regions, basal ganglia, and the cerebellum (Rubia 2011; Rubia et al. 2014). Our study shows that the increase of activity in the left DLPFC can be achieved using anodal tDCS. The high potential of anodal tDCS over the DLPFC for the treatment of ADHD has been supported in different recent studies. Leffa et al., (2016) stimulated spontaneous hypertensive rats (an animal model of ADHD) over 8 consecutive days using tDCS over the prefrontal cortex and caused a significant improvement of WM. Soltaninejad et al. (2016) and Bandeira et al., (2016) applied anodal tDCS over the left DLPFC in children and adolescents with ADHD and demonstrated an increase in correct responses during a task for sustained attention, improved signal detection, ability to switch between an ongoing activity and a new one, and more efficient processing speed. Now the clinical efficacy of tDCS in ADHD has to be proven in future controlled clinical trials.

### After-Effect of tDCS on Resting State Functional Connectivity

An important prerequisite for the clinical application of non-invasive brain stimulation is the presence of after-effects, including the possibility to induce long-lasting positive changes of brain function as a basis of sustained clinical improvement. Minutes of stimulation with both anodal and cathodal tDCS may elicit changes of cortical excitability lasting for hours, depending on the site of stimulation and of the associated brain function (Nitsche and Paulus 2001). The duration of after-effects depends on stimulation intensity, baseline level of cortical excitability, changes in neurotransmitter function as well as the association of stimulation with a particular task or training (Liebetanz et al. 2002). Here we demonstrated that 20 min of anodal stimulation of the left DLPFC influences resting state functional connectivity, even after 20 min of stimulation cessation. We observed increased global connectivity of the left DLPFC in the position of the anodal electrode, with these increases in connectivity being associated with augmented interaction between DLPFC, WM, and executive function networks. Increased connectivity of the DLPFC has been shown to correlate with improved WM function in healthy subjects (Hampson et al. 2010); conversely, impaired DLPFC connectivity has been implicated with WM dysfunction in clinical populations (Meyer-Lindenberg et al. 2005). Therefore, it may be hypothesized the observed after-effect may cause sustained improvement in the behavioural metrics underlying clinical symptoms in ADHD patients. Future work will need to address the efficacy of the treatment by following patients and their performance over more extended periods of time.

Note that the anodal tDCS during a WM task caused a task-specific activation of WM network which lasted over time. This finding corresponds with previous results providing evidences that the task during tDCS primes activation of neuronal networks which are closely related to the task (Wörsching et al. 2016). For example, anodal stimulation of the inferior frontal cortex (IFC) during a picture naming task resulted in more efficient naming and specifically reduced BOLD signal in the left IFC and ventral premotor cortex (Holland et al. 2016). Anodal tDCS of the left parietal cortex during different arithmetic tasks revealed task specific behavioural effects corresponding with an increased activity in the bilateral angular gyri and medial and lateral prefrontal cortices during solving of repeated arithmetic problems and with an increase BOLD signal in the intraparietal sulci and dorsomedial prefrontal cortex during solving novel problems (Hauser et al. 2016). Accordingly, anodal stimulation of the left inferior frontal gyrus (IFG) during word generation led to an improved word retrieval paralleled by selectively reduced task-related activation in the left ventral IFG and in additional major hubs overlapping with the language network (Meinzer et al. 2012). These and other studies point to the significance of the task for the specificity of the neuronal network activation under tDCS.

### Limitations

This study is characterized by several limitations which have to be discussed. Electrical currents can affect fMRI recordings as demonstrated in simultaneous tDCS session and echo-planar imaging (EPI) on two post-mortem subjects (Antal et al. 2014). tDCS induced signals in both superficial and deep structures. The signal was specific to the electrode montage, with the strongest signal near cerebrospinal fluid and scalp. Also in our study, tDCS may have influenced activation pattern and biased results. However, the effect of tDCS on fMRI is rather weak. The magnitude of the artifact signal resulting from the electrical current alone is approximately 1/2 of a typical physiological BOLD response (Antal et al. 2014). In resting state fMRI studies, neither anodal nor cathodal tDCS induced a detectable BOLD signal change in normal subjects (Kwon et al. 2008; Antal et al. 2011). Also in different activation studies, tDCS produced network specific activations which can not

be simply attributed to passive tDCS effects on EPI signal (Holland et al. 2011, 2016; Polania et al. 2011; Weber et al. 2014). In our study, if effects of tDCS on EPI signal are strong enough, we have to be able to observe similar significant differences in activation pattern between tDCS and sham stimulation for all conditions. However, no significant differences were found for 0-back and go/no-go conditions. In 1-back and 2-back conditions, the activation pattern was astounding specific for motor and WM networks. Although the concurrent effects of tDCS on EPI signal can not be excluded, their influence on results of our study seems to be minimal. Another problem of the study is related to the sample size. Although it is difficult to calculate sample size based on results of previous studies because of a number of novel interfering factors (this is the first study on tDCS inside the MR scanner in adolescents with ADHD), in a very optimistic case with medium effect sizes a required sample size becomes larger than 20 patients. This insufficient power may explain why many post-hoc tests for behavioural data were non-significant. However, our sample size seems to be large enough in order test main hypothesis of the study: also with 13 patients it was possible to demonstrate significant and specific BOLD signal changes under tDCS compared with sham stimulation for both activity during the working memory task and resting state functional connectivity. Out sample size is in line with a number of previous studies which used tDC stimulation inside the scanner (n = 12–15, see Antal et al. 2011; Keeser et al. 2011b; Polania et al. 2011). Another limitation is related to control conditions: it is still unclear whether the described effects of tDCS on neuronal networks polarity and region specific (in order to control for that a cathodal stimulation and stimulation of other non-relevant brain areas are necessary). However, for testing the hypothesis of the study sham as a control condition is sufficient. In this proof-of-concept study, application of all possibly control conditions is difficult from ethical point of view. Now, next studies may investigate specificity of the described effects in more detail. And finally, one of interesting findings of this study is the discrepancy between behavioral and neural data. For the 0-back and Go/NoGo tasks, higher omission error rates were not accompanied by specific BOLD signal changes, and the same is true for worse accuracy for anodal tDCS in the Go/NoGo task. These and other results (for example increased activities in several brain regions during the 1-back and 2-back tasks that were accompanied by faster responses and more errors) are difficult to interpret without analyzing data of healthy controls. The lack of data from a healthy control group is a further limitation of the study that should be overcame in the course of further research.

Despite of limitations, this is a first study which provides an evidence for effects of anodal tDCS over the left DLPFC on neuronal networks of working memory and executive control in adolescents with ADHD. The study suggests that anodal tDCS may be proven in further clinical studies as a possible option for treatment of neuropsychological deficits in patients with ADHD.

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**Compliance with Ethical Standards**

**Conflict of interest** The authors reported no biomedical financial interests or potential conflicts of interest.

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