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Short title: fMRI study of frontal tDCS in schizophrenia

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ABSTRACT:

Objective: Individuals with schizophrenia typically suffer a range of cognitive deficits, including prominent deficits in working memory (WM) and executive function (EF). These difficulties are strongly predictive of functional outcomes, but there is a paucity of effective therapeutic interventions targeting these deficits. Transcranial direct current stimulation (tDCS) is a novel neuromodulatory technique with emerging evidence of potential pro-cognitive effects; however there is limited understanding of its mechanism.

Method: A double-blind randomized sham controlled pilot study of tDCS on a WM (n-back) and EF (Stroop) task in 28 individuals with schizophrenia using fMRI. Study participants received 30 minutes of real or sham tDCS applied to the left frontal cortex.

Results: The 'real' and 'sham' groups did not differ in online WM task performance but the tDCS group demonstrated significant improvement in performance at 24 hours post-tDCS. tDCS was associated with increased activation in the medial frontal cortex beneath the anode; showing a positive correlation with consolidated WM performance 24 hours post-stimulation. There was reduced activation in the left cerebellum in the tDCS group, with no change in the middle frontal gyrus or parietal cortices. Improved performance on the EF task was associated with reduced activity in the anterior cingulate cortex.

Conclusions: tDCS modulated functional activation in local task-related regions, and in more distal nodes in the network. tDCS offers a potential novel approach to altering frontal cortical activity and exerting pro-cognitive effects in schizophrenia.

TEXT:

Introduction:

Individuals suffering from schizophrenia demonstrate persistent cognitive deficits, which impact on day-to-day functioning to a greater extent than the more widely recognized symptoms of hallucinations and delusions. Working memory (WM) and executive functioning (EF) are crucial to a range of essential neuropsychological functions including attention, goal directed behaviour, mental flexibility and conflict monitoring, all of which are significantly impaired in schizophrenia (Kerns, Nuechterlein, Braver, & Barch, 2008). Deficits in WM and EF have been linked with poorer functional outcomes indexed by occupational status and rates of independent living (Kerns et al., 2008). The neural network subserving WM and EF in healthy volunteers includes frontal cortical regions including the middle (MFG) and medial frontal gyri, anterior cingulate cortex (ACC), as well as the lateral temporal and parietal cortices, and cerebellum (Minzenberg, Laird, Thelen, Carter, & Glahn, 2009; Owen, McMillan, Laird, & Bullmore, 2005). Interestingly, recent meta-analyses have demonstrated dysfunctional WM and EF in individuals with schizophrenia to be related to aberrant brain activation in the frontal cortex, including the medial and MFG, the ACC (Forbes, Carrick, McIntosh, & Lawrie, 2009; Minzenberg et al., 2009), and within a network of structurally and functionally connected regions including the temporal lobe and cerebellum (Andreasen & Pierson, 2008; Repovs, Csernansky, & Barch, 2011).

There is an urgent need for effective therapeutic interventions given that both psychological and pharmacological interventions (Michalopoulou, Lewis, Wykes, Jaeger, & Kapur, 2013; Wykes, Huddy, Cellard, McGurk, & Czobor, 2011) have yielded very limited clinical benefits in improving cognitive dysfunction in schizophrenia. There

has been recent interest in combining the observed changes in regional prefrontal cortical activation in schizophrenia with the potential of mechanistic interventions such as transcranial direct current stimulation (tDCS) to modulate brain activation and improve cognition (Reinhart, Zhu, Park, & Woodman, 2015a). tDCS is a non-invasive brain stimulation technique where low intensity currents applied to the scalp through electrodes; anodal tDCS (AtDCS) demonstrate reduced neuronal firing thresholds with consequently increased rates of spontaneous firing, whereas cathodal stimulation increased thresholds and reduced tonic firing rates (M. A. Nitsche & Paulus, 2011). Although the mechanisms of action of tDCS are incompletely understood, pharmacological data suggest that *excitatory* effects are mediated by both reduction in GABAergic inhibition and modulation of glutamatergic NMDA receptors; whereas *inhibitory* effects are mediated primarily by reduction in excitatory glutamatergic neurotransmission (M. A. Nitsche & Paulus, 2011). Support for the use of tDCS comes from robust effects of AtDCS in improving cognitive performance in both healthy controls (HC) and in patients suffering from neurodegenerative and some psychiatric disorders (Reinhart et al., 2015a).

The neurophysiological effects of online AtDCS show behavioural changes to be accompanied by altered activation in task-related brain networks (Holland et al., 2011; Meinzer et al., 2014) in both healthy subjects and in patients with mild cognitive impairment. AtDCS over the left inferior frontal gyrus (IFG) improved verbal fluency in participants with mild cognitive impairment (MCI) accompanied by reductions in baseline hyperactivity of the prefrontal cortex, middle frontal gyrus, basal ganglia and thalamus (Meinzer et al., 2014). This suggests that modulating regional activation also

impacts on wider task related networks; with AtDCS applied to MFG altering connectivity between functionally associated brain regions (Keeser et al., 2011).

Interestingly, the improvements in cognitive performance in patients with schizophrenia are not always evident during the period of the online application of tDCS, but may be delayed in some tasks such as WM (Orlov et al., 2016) – suggesting a task specific effect on consolidation of learning - while immediate improvements were observed during a different EF task (Reinhart, Zhu, Park, & Woodman, 2015b; Takeuchi et al., 2012).

Here we describe the first functional magnetic resonance imaging (fMRI) examining the neural basis of online tDCS application in schizophrenia; during the performance of WM and EF tasks. We hypothesized that the AtDCS would improve online EF performance, and improve WM task performance after a consolidation period (Reinhart et al., 2015b; Stoodley, Valera, & Schmahmann, 2012; Takeuchi et al., 2012). We anticipated increased activation beneath the anode during task execution in the tDCS group relative to sham (M. Nitsche et al., 2003). At a systems level we hypothesized that there would be reduced activation in the task relevant WM and EF networks in the tDCS group (Meinzer et al., 2014) including the bilateral parietal cortex and ACC respectively (Küper et al., 2015; Minzenberg et al., 2009; Takeuchi et al., 2014).

Method:

Participants:

We recruited 49 right handed participants from outpatient services fulfilling criteria for a *DSM*-IV (American Psychiatric Association, 2000) diagnosis of schizophrenia or schizoaffective disorder from South London UK. Based on the study inclusion/exclusion

criteria, potential participants were identified and approached by their treating psychiatrists and contacted the researchers. The diagnosis and entry/exclusion criteria of patients who consented to participate were reviewed by the research team.

This analysis forms part of a larger behavioural study that investigated the longer-term effects of tDCS on cognition (Orlov et al., 2016). In brief, the participants completed eight sessions of cognitive training, delivered as two sessions per day over 4 days (on day 1, 2, 14 and 56 post-randomisation); and on each of these days, the 2 sessions were separated by 45 minutes. Each cognitive training session comprised the WM task along with an implicit learning and a stochastic learning task (Orlov et al., 2016 & supplementary info). Participants were randomly allocated to receive either real or sham tDCS during the second session of day 1 or the second session of day 14; 28 participants consented to undergo an fMRI scan during the tDCS stimulation at day 14. This fMRI session included the WM task that had been used in cognitive training, but also included the Stroop task – which had not been part of the cognitive training.

Medicated participants were on stable doses of antipsychotic medication for the three months prior to study enrolment. Participants' exclusion criteria included the recent or current use of benzodiazepines or other hypnotics; alcohol or substance dependence in the last three months; history of seizures, neurological disorder, or head injury. All participants provided written consent before the screening procedure and received a stipend for their involvement. This study was approved by the Stanmore National Research Ethics Committee (REC number 11/LO/0248).

Active tDCS was given continuously for 30 min (real) or 30 seconds (sham) at 2mA using an Edith stimulator (http://www.neuroconn.de/dc-stimulator_mr_en/) and magnetic field compatible electrodes pre-gelled with EEG paste. The anode ($35cm^2$) was placed over the F3 (Brodmann area (BA) 10/46), and the cathode ($35cm^2$) over the right supraorbital area, according to the 10-20 electrode placement system. All cognitive training tasks were optimised for and administered in fMRI environment. Here, we report results from two tasks that were completed with concomitant real/sham tDCS, a letter n-back task and colour-word inference Stroop task during fMRI imaging (see methods in supplementary info). The fMRI data were acquired on a Discovery MR750 3T at King's College London (see methods in supplementary info).

Behavioural data analysis:

The full data analysis has been reported elsewhere (Orlov et al., 2016). In short, for the WM the outcome measures were the *d'* and mean reaction times (RTs) during on-line tDCS and 1 day post-tDCS. The *d'* was calculated for the average of performance for monitoring (0-, 1-back) and manipulation (2-, 3-back) (Orlov et al., 2016). *d'* uses both the true and false positive responses (Haatveit et al., 2010). Analysis of the WM task was conducted by specification of full maximum likelihood-random effect multilevel models (MLREM), which included baseline n-back, online and next day retention data, group and interactions (Orlov et al., 2016). The task outcome measures for the EF, number of correct responses and mean RTs, were analysed using t-tests. Clinical and socio-demographic information was analysed by means of t- and Chi-squared tests, with the

real/sham tDCS being the grouping variable, using STATA (http://www.stata.com/stata12/).

fMRI analysis:

All data were pre-processed and analysed using Statistical Parametric Mapping 12 (SPM12) (www.fil.ion.ucl.ac.uk/spm) in MATLAB R2014a (https://uk.mathworks.com/). Functional data were spatially realigned to the mean image from the series, then resliced. Spatial normalization into Montreal Neurological Institute (MNI) stereotactic space was carried out by diffeomorphic anatomical registration using exponential lie algebra (DARTEL) using a study-specific template generated from all participants' structural images (Ashburner, 2007).

The subject-specific models for the WM task included regressors encoding the predicted BOLD response for two separate conditions: all the three WM loads combined and a regressor encoding button presses. A 1^{st} (i.e. linear) and 2^{nd} (quadratic) order polynomial expansion was applied to assess the WM load condition. The 0-back was left unmodelled and served as an implicit baseline. The motion parameters and button presses were modelled as nuisance regressors. Following parameter estimation, contrasts of beta coefficients for the three primary contrasts of interest (zero, 1^{st} and 2^{nd} order expansion of WM load) were generated, representing the mean activation, linear, and quadratic BOLD response with increasing WM load. The resultant parameter estimates were taken forward to a whole-brain random-effects analysis, a group (real/sham tDCS)-by-level (zero, 1^{st} and 2^{nd} order expansion of WM load) factorial ANOVA. Full whole brain multiple comparisons correction on the basis of response amplitude was carried out. For

the EF task, each correct response of the incongruent and congruent condition was modelled as a regressor, and the fixation cross was left unmodelled. Each participant's head movements and incorrect responses were modelled as nuisance regressors. The contrasts of parameter estimates for the condition of interest (congruent and incongruent) was taken forward to a whole-brain random-effect analysis, with a two-sample test (real/sham tDCS).

We used three regions of interest (ROI) analyses based on a meta-analysis of the EF task (Takeuchi et al., 2014) using small volume corrections with a volume of interest of 6 mm in the ACC (x=2 y=16 z=38), the left IFG (x=-44 y=4 z=33) and left parietal lobule (x=-40 y=-50 z=45) (converted to MNI space using Pickatlas within SPM). We also used an a priori defined ROI defining the likely region underneath the tDCS anode using a BA 10/46 mask (supplementary info) created in Pickatlas. Results were considered significant if they had a p-value of less than 0.05 following family-wise error correction (FWE). We assessed the relationship between any regional activation changes and behavioural performance on the WM and EF task using Pearson correlations.

Results:

Behavioural results:

There were no differences between the groups in terms of sociodemographic data, clinical functioning, or psychometric testing (Table 1). During tDCS applied during the WM task, the groups did not differ significantly in either monitoring (0-, 1-back) or manipulation (2-, 3-back) *d'* and mean RTs (Table 2.). After the consolidation period (1 day post-tDCS), there were significant between-group differences in manipulation of information

(b=0.68, CI 0.14 - 1.21; p=0.044) with the real tDCS performing significantly better than sham, controlled for baseline (b = −0.37, 95% CI -0.98–0.23; p = 0.226) (Orlov et al., 2016 and supplementary Table 3.).

In the EF task we observed significantly improved performance during the incongruent condition in the real stimulation group ($t_{(1, 24)}$ =1.71; p=0.05) (Table 2).

fMRI results:

Data from 6 participants were excluded: 1 with marked brain atrophy was excluded from both tasks and a further 4 participants were excluded from one or other of the tasks due to a technical problem with incomplete image acquisition (3 WM and 1 EF) leaving 24 participants (13 real tDCS, 11 sham stimulation) in the WM analysis and 26 participants (14 real tDCS, 12 sham stimulation) in the EF analysis (Table 1).

During the WM task, compared to the 0-back condition, the combined 1, 2, and 3-back conditions activated the verbal WM network, including: the bilateral MFG, cingulate gyrus, and the bilateral parietal cortices (supplementary info). The anodal ROI demonstrated a significantly increased activation in the medial frontal cortex (BA10) during the WM task with the real tDCS relative to the sham*; x, y, z = (-8, 66, 0); ($t_{1(66)}$ = 3.22 [t_{peak}=3.54]; K_E = 35, P_{FWE} = 0.01, z-score*$_{\text{peak}}$ = 3.38 FWE. The real tDCS, relative to sham, was also associated with reduced activation within the left cerebellum: (*x, y, z* = -40, -62, -32); main effect of group $F_{1, 66}$ = 11.86 [F_{peak} = 28.20]; K_{E} = 505; P_{FWE} = 0.028. However, contrary to our hypothesis, there were no reductions in BOLD response in the parietal cortices. Furthermore, we found no evidence for a significant treatment-by-WM-load interaction. In order to illustrate the direction of changes between the groups - the mean β for each WM load was extracted and plotted (Figure 1). We found a significant

correlation between the consolidation effect for manipulation and the increased activation underlying the tDCS anode (r=0.58, p<0.05), relative to sham (Figure 2).

The EF task demonstrated increased activation in a network of regions associated with inhibitory control, including the bilateral IFG, ACC, cingulate cortex and left parietal lobule (further details in results in supplementary info). The tDCS group demonstrated significant reduction in activation of the ACC, as compared to sham*; (x, y, z = 0, 10, 40); (*$t_{1(24)} = 2.49$ *[*$t_{peak}=3.11$*]; *$K_E = 23$, $P_{FWE} = 0.025$, $z\text{-score}_{\text{peak}} = 2.82$ FWE. An exploratory ROI analysis of the cerebellum demonstrated reduced activition of the cerebellum *(x, y, z = -40, -60, -26); (*$t_{1(24)} = 2.49$ *[*$t_{peak}=2.87$*]; *$K_E = 31$, $P_{FWE} = 0.037$, $z\text{-score}_{\text{peak}} = 2.63$ FWE. We found a significant correlation between performance in the incongruent condition activation in the ACC (r=-0.58, p<0.002, CI -0.84 ─ -0.30) (Figure 3).

Discussion:

This is the first study to use fMRI to examine the neurophysiological effects of tDCS during WM and EF assessment in individuals with schizophrenia. As hypothesised, there was increased activation underneath the site of the real tDCS anode in the medial frontal cortex during the WM task. This activation was positively correlated with the extent of the improved performance on this task after a consolidation period. While the real tDCS group demonstrated significantly reduced activation in the left cerebellum, there were no differences evident in the MFG or parietal cortices. During the EF task, the tDCS did not demonstrate an effect under the anode but showed significant reductions in activation in the ACC and cerebellum, and was associated with significantly lower errors in the incongruent condition. The data demonstrate a differential task dependent effects of tDCS on behavioural performance in individuals with schizophrenia, supporting previous

findings demonstrating that EF improvement can be immediate, whereas improvements in WM - perhaps reflecting increased demand on manipulation of information - are only observed after a consolidation period. Neurophysiologically, these data suggest that tDCS serves to bias the membrane potential of neuronal populations in the medial frontal cortex underlying the anode, and in more distal task specific regions including the ACC and cerebellum. Although the mechanism of action of tDCS has not yet been fully elucidated (Reinhart et al., 2015a), one proposal is that if the BOLD response represents synaptic activity (Attwell & Iadecola, 2002), then tDCS might increase the probability that a synaptic input will generate a response in an output neuron. It has been demonstrated that most energy is consumed synaptically, rather than by action potentials (Attwell & Iadecola, 2002), therefore it is likely that tDCS simply reduces the threshold for some of the output neurons and increases the effectiveness of processing - rendering the underlying neuronal populations more likely to respond in line with task related demands. Whilst the data demonstrate an increase in WM related activation underneath the site of AtDCS stimulation, there is a lack of a load dependent effect of tDCS directly on the MFG and parietal cortex; this variability in results is also evident in the literature in HC and MCI, and similarly we did not observe this neurophysiological effect during EF. Holland et al observed reduced activation underneath the anode (IFG) in HC after real AtDCS, but this was confounded by improved behavioural performance; they did not observe any effect on more distal regions (Holland et al., 2011). However, there is a report of reduced activation beneath the anode and in the distal task related network in MCI subjects (Meinzer et al., 2014). One suggestion to explain these differences is that the AtDCS impacts healthy brains/neuronal network systems in a more locally specific manner, whilst in pathological brains/neuronal networks this effect is evident across a wider task relevant neuronal network. Such differential task response might be explained

by task complexity, such that the EF response requires online monitoring and inhibitory control, whilst the WM task has a manipulation component and requires additional frontal activation for successful task execution.

The medial frontal cortex is considered to support the MFG during WM performance (Owen et al., 2005), with the MFG possessing a specific role in the allocation of demand led task performance (Fegen, Buchsbaum, & D'Esposito, 2015). Individuals with schizophrenia generally perform worse, and activate the MFG to a lesser extent, than HC during EF (Minzenberg et al., 2009) because information load demand is thought to exceed available computational resources (Braver et al., 1997). However, when task performance is matched, individuals with schizophrenia tend to recruit the WM network to a greater degree, including the MFG. Response inhibition, on the other hand, is thought to rely heavily on the activity of the ACC and IFG (Takeuchi et al., 2014). The meta-analysis of Minzenberg indicates that individuals with schizophrenia demonstrate increased activity in the ACC during EF when compared to HC (Minzenberg et al., 2009). Our results suggest that tDCS has reduced cortical activation during EF; reduced ACC activity was significantly correlated with improved performance in the incongruent condition. Reinhart demonstrated that tDCS was able to normalize the event related negativity (ERN), a brain response following behavioural errors (Reinhart et al., 2015b), in individuals with schizophrenia. Their results show that AtDCS to the medial frontal cortex induced an ERN response observed in HC during a stop signal EF task. In addition, real tDCS in individuals with schizophrenia improved task performance significantly making it indistinguishable from that of HC during sham stimulation.

The MFG has also been proposed as a coordinating hub for integration during both WM and EF (Jääskeläinen et al., 2015); thus, patients with schizophrenia demonstrated reduced connectivity between the MFG and the right cerebellum, suggesting that these neurointegrative deficits might be correlated with WM performance. There is significant white matter connectivity between the medial frontal and cingulate cortex via the cerebro-ponto-cerebellar loop (Jääskeläinen et al., 2015). Whilst the cerebellum has traditionally been associated with movement and motor learning, more recent data support a significant role in cognitive operations, including WM (Stoodley, 2012), where cerebellar activity has been demonstrated to increase in line with demand; and activity in bilateral cerebellum in participants with schizophrenia was also associated with increased load (Küper et al., 2015). Sapara et al demonstrated greater activation of bilateral cerebellum in individuals with schizophrenia during a WM task, relative to HC (Sapara et al., 2014). Our results suggest that tDCS may improve the efficiency of the network, reflected in decreasing requirement for this cerebellar recruitment. The association of cerebellar activity during EF suggests that greater grey matter volume in the cerebellum, as well as the ACC and IFG, were associated with reduced Stroop interference in HC (Wagner et al., 2015). Schizophrenia is associated with reduced grey matter volumes in the EF network (Buckner, 2013), and were associated with increased functional activation during task performance.

There are some limitations to this study; a pre-tDCS scan for the participants would have permitted a more powerful within-subject analysis of the effects of real tDCS. Nonetheless, we used a double-blind design and the blinding was robust as evidenced by participants not being able to discriminate reliably the real/sham tDCS group assignment. Some of the patients were receiving treatment with clozapine which carries a dose-related

risk of seizures risk and could therefore theoretically enhance cortical excitability and effects of tDCS if over represented in the active treatment group. However, the distribution of the clozapine treated patients was not significantly different across the treatment groups and any difference in the individual tasks actually revealed fewer clozapine treated patients in the AtDCS group. It is interesting that the changes in cerebellum activation were evident across both the WM and EF tasks, suggesting that the effect of the frontal AtDCS is evident on both familiar and novel frontal tasks, i.e. having prior exposure to the WM task through the cognitive training, does not mitigate the impact of AtDCS. However, there was a unique effect of AtDCS over the medial frontal cortex during the WM task, not evident during the EF task, and one cannot exclude this arising as a consequence of an interaction between the tDCS and prior training on this task; this could be examined in future studies through switching around these training and novel tasks. The sample size of this study is relatively modest, but as the first such study in schizophrenia suggests that tDCS can influence brain dynamics and which is related to behavioural change.

In summary, this study demonstrated that left MFG AtDCS resulted in increased activation in the cortex underlying the anode; and this correlated significantly with improved WM performance after a consolidation period. There was decreased activity in the cerebellum suggestive of an increase in efficiency in the wider WM network. AtDCS was also associated with improved performance on the EF inhibition task which was associated with reduced activation of both the ACC and cerebellum. Both WM and EF impairments are strongly related to poor functional outcomes in schizophrenia; tDCS offers a promising intervention based on neuromodulation of frontal activation warranting replication of these findings.

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TABLES AND FIGURES:

Table 1. Clinical and socio-demographic information of study participants per task

	n-back				Stroop			
	real tDCS	**sham tDCS**	***p***	***statistic***	**real tDCS**	**sham tDCS**	***p***	***statistic***
Participant N	13	11			14	12		
Age	33.3 (2.8)	37.4 (3)	0.32	1.02	33.1 (9.8)	37.5 (9.7)	0.28	1.1
Gender	3 Females	2 Females	0.77	0.09	3 Females	2 Females	0.91	0.11
Education	12.8 (0.9)	13.2 (0.9)	0.79	0.26	12.9 (2.8)	13.2 (2.9)	0.83	0.21
WASI	102.0 (4.0)	101.0 (3.0)	0.77	0.29	101.9 (11.0)	101.1 (14.0)	0.88	0.15
Years of FT Edu	12.8 (3.3)	13.2 (2.9)	0.79	0.26	13.0 (2.5)	13.2 (2.8)	0.87	0.16
PANSS Pos.	14.8 (1.1)	13.5 (1.1)	0.42	0.82	14.7 (3.5)	13.5 (3.9)	0.41	0.83
PANSS Neg.	15.0 (1.3)	16.0 (1.4)	0.61	0.52	15.2 (4.7)	15.6(4.5)	0.84	0.2
PANSS Gen.	28.1 (1.3)	27.1 (2.1)	0.66	0.53	28.4 (6.7)	26.8 (4.7)	0.48	0.71
Duration of illness	10.46 (7.5)	14.0 (9.45)	0.32	1.02	11.6 (6.9)	15.0 (9.7)	0.30	1.05
Med Chlor equi	368 (202)	460 (197)	0.27	1.14	395 (191)	470 (174)	0.31	1.04
1# gen antipsy	2	1	0.60	0.44	1	3	0.21	1.58
Clozapine	1	4	0.09	2.97	2	3	0.49	0.48

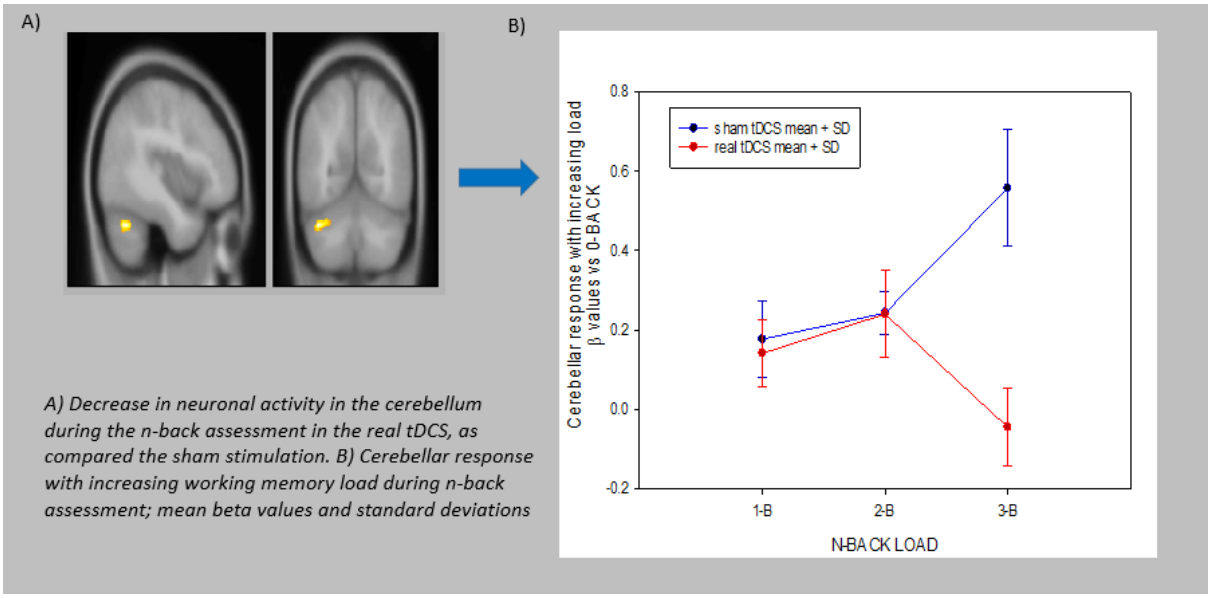
PANSS: Positive and Negative Syndrome Scale, Pos: Positive Syndrome; Neg: Negative Syndrome; Gen: General Psychopathology Syndrome; FT: full time; Edu: education; Med: medication; Chlor equi: chlorpromazine equivalent in mg; 1# gen antipsy: first generation antipsychotics; WASI: Wechsler Abbreviated Scale of Intelligence; statistic: test statistic, Students t-test for continuous and $\chi 2$ *for categorical data.*

Table 2. Behavioural performance of participants during concomitant tDCS and fMRI.

	real tDCS	**sham tDCS**	***p***	***t-statistic***
N-back RTs (ms)	648 (73)	587 (107)	0.63	0.48
d' 0-back	4.60 (0.2)	4.51 (0.8)	0.26	0.67
d' 1-back	3.87 (0.9)	4.04 (0.9)	0.68	0.49
d' 2-back	3.50 (1.0)	3.44 (1.1)	0.45	0.13
d' 3-back	2.14 (0.8)	2.41 (0.9)	0.77	0.77
Stroop RTs (ms)	1538 (242)	1516 (300)	0.42	0.21
RTs Congruent	1516 (135)	1448 (124)	0.29	0.56
RTs Incongruent	1560 (141)	1583 (140)	0.58	0.21
Accuracy	97.1% (3.0)	94.5% (5.6)	0.07	1.53
Accuracy Con	98.3% (2.5)	96.4% (5.3)	0.11	1.24
Accuracy Incon	96.2% (3.8)	92.7% (6.4)	0.05	1.71

RTs: reactions time in milliseconds; d': d-prime Con: congruent, Incon: incongruent; (): standard deviation from the mean; t-statistic-one sided test

Figure 1. Decrease in neuronal activity during n-back task in real tDCS as compared to sham stimulation. A) Main effects of group (sham> real) based on whole brain analysis contrasting 1, 2, 3 versus 0-back



A) Decrease in neuronal activity in the cerebellum during the n-back assessment in the real tDCS, as compared the sham stimulation. B) Cerebellar response with increasing working memory load during n-back assessment; mean beta values and standard deviations

Figure 2. Pearson's correlation between brain activity in the left prefrontal cortex within the tDCS template mask and averaged 2- and 3-back *d'* WM task performance after consolidation.

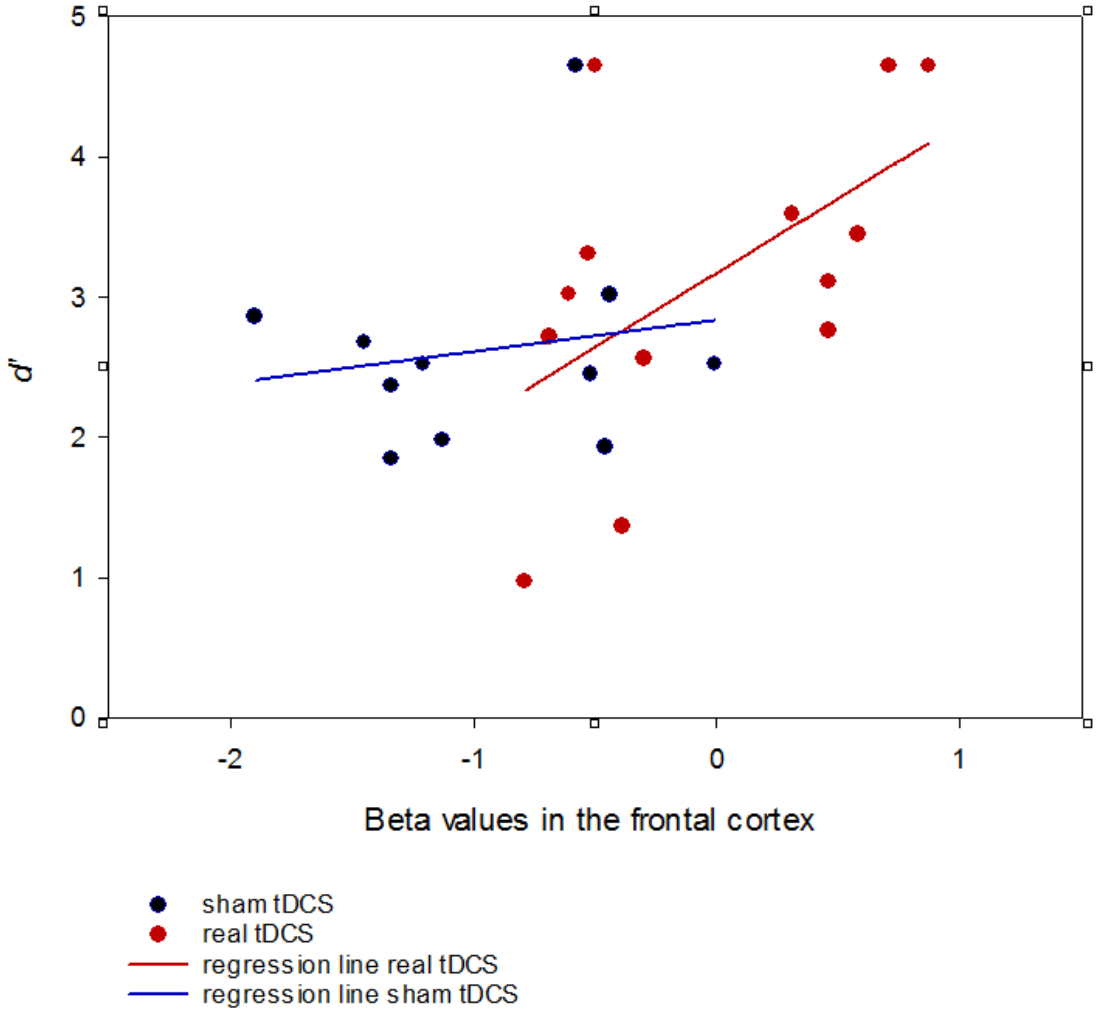
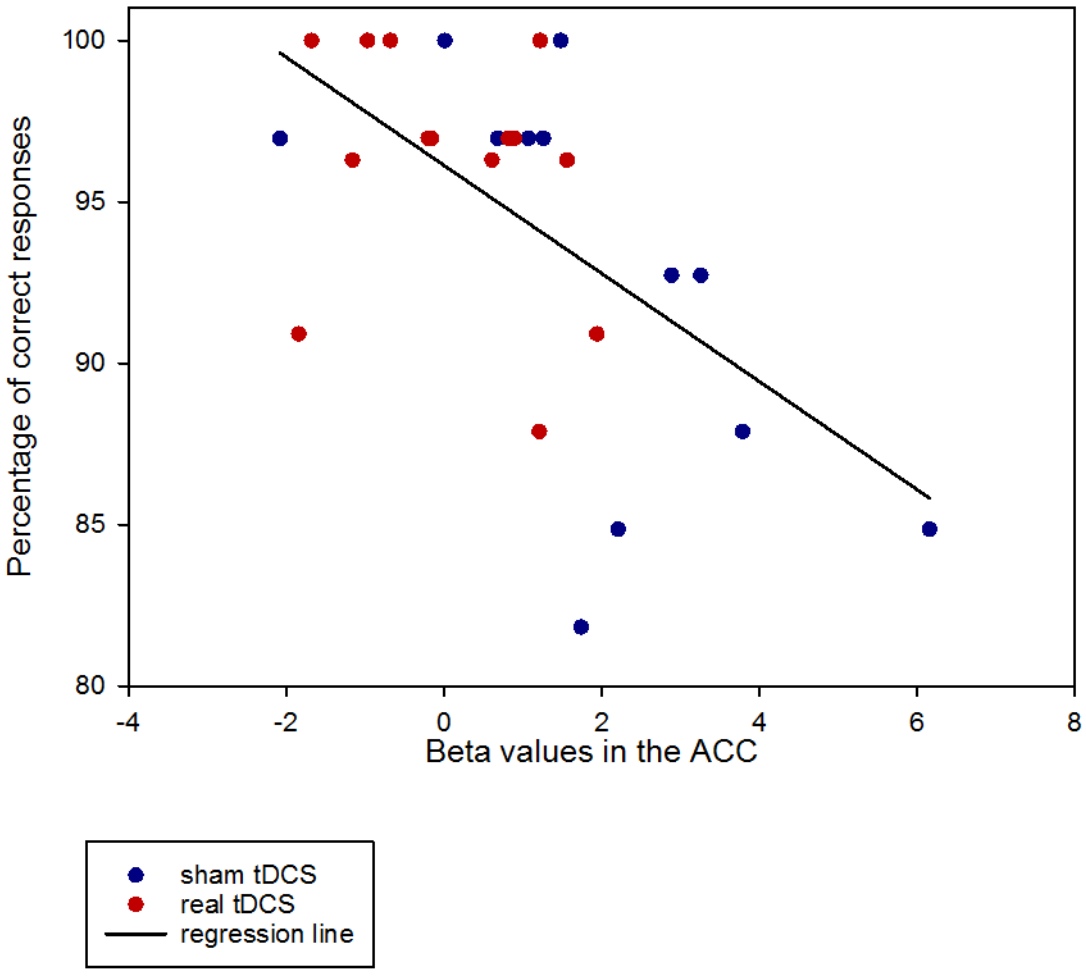


Figure 3. Pearson's correlation between brain activity in the anterior cingulate cortex (ACC) and behavioural performance in the incongruent condition in the executive functioning task.



Supplementary material:

Randomization and blinding:

Participants were randomly assigned to cognitive training and either real tDCS or sham stimulation using a 2:2 ratio randomization procedure stratified for two preselected factors, namely smoking status and sex using STATA 12.1. To ensure concealment of the tDCS randomization assignment, the stimulator's study mode software was used. The study mode software allows blinding of the individual applying the tDCS to the stimulation type through inputting of either real tDCS or sham stimulation assigned 5-digit codes. The codes key was available only to the investigator who conducted the randomization.

The tDCS was well tolerated and the most common side-effect observed was itching or tingling underneath the electrodes. In the sham stimulation group one participant reported a headache after the stimulation, but this did not result in distress and did not require any intervention. This tolerability was also evidenced through the lack of significant differences in participants' accuracy in identifying their treatment group post tDCS ($\chi2=0.3$; $p=0.85$; $\chi2=0.42$; $p=0.52$).

Cognitive training tasks:

In addition to the working memory task, participants trained on a probabilistic learning task and an implicit learning task.

Probabilistic learning task:

In the probabilistic learning task, participants learned a sequence of four button presses, using their left and right index fingers. At the beginning of each trail, participants were presented with an outline circle with a number inside (1, 2, 3, or 4) informing them which trial in the sequence it was. After each response, the outline circle filled green when they pressed the correct button, or red when the participant pressed the incorrect button. However, in 15% of the trials participants received incorrect feedback, i.e. the circle turned green when they executed an incorrect button press, or red when they executed a correct press in the sequence (Averbeck et al. 2011). Participants learned six sequences during each cognitive training session.

Implicit learning task:

In the implicit learning task 400 randomized picture-word pairings were presented in two blocks 90 s apart. The stimuli were 50 black and white drawings presented with 50 neologisms normalized in loudness and length. Participants indicated if a picture-word pairing was correct or incorrect, using their index fingers. The pace of the task was relatively rapid by design to prevent participants consciously rehearsing stimuli: the inter-stimuli-interval was 1.5 s; the response time was 1 s; the picture presentation commenced 200 ms after acoustic presentation of the neologism. Each neologism was repeated 4 times in each block. The randomisation was such that a correct pairing of a given picture-neologism combination appeared twice in a block, and that the same picture was incorrectly paired with two different neologisms. The incorrect pairing remained the same in the second block of the task (Flöel et al. 2008).

Both, the stochastic learning and implicit learning task were adapted for fMRI and completed during the fMRI, after the AtDCS.

Methods:

tDCS was applied during task competition (online stimulation) using an Eldith DC-stimulator (NeuroConn GmbH, Germany). Active tDCS was given continuously for 30 minutes at 2mA, with 30 seconds of “ramping up” and “ramping down” of the current. For the sham stimulation the stimulation was applied for 30 seconds with the same ramping parameters. The anode (35cm^2) was placed over the F3 (Brodmann area (BA) 10/46), and the cathode (35cm^2) over the right supraorbital area, in accordance with the 10-20 international system for electroencephalogram electrode placement. The magnetic field compatible electrodes pre-gelled with EEG paste and were held in place by cotton bands.

Data acquisition:

Functional magnetic resonance imaging (fMRI) was acquired on a Discovery MR750 3T scanner (T2* weighted gradient-echo echo-planar images (EPIs), TR = 2000 ms, TE = 30 ms, flip angle = 75°, 64 x 64 matrix) at King's College London. The functional images were resampled into 1.5mm3 voxels and spatially smoothed with an 8-mm full-with half-maximum Gaussian kernel.

Each whole-brain image contained 41 3-mm axial slices separated by a distance of 0.3 mm. After the behavioural portion of the experiment, a T1-weighted structural scan (TR = 9.356 ms, TE = 3.828 ms, flip angle = 75°) was acquired. The first four volumes were discarded to allow for transient effects.

The working memory task:

The WM task (0-, 1-, 2- and 3-back) varied the WM load incrementally (supplementary material) with participants responding using a button box using their right index finger. We used 168 capitalized letters separated into three blocks of each n-back condition. Participants were informed at the start of each 30-second block as to the nature of response required (N= 0, 1, 2, or 3). The inter-trial interval (ITI) was 2 seconds and each letter was presented for 0.5 second.

In the 0-back condition participants were asked to indicate whenever the letter 'X' appeared on the screen. In the 1-, 2- and 3-back conditions the participants were required to indicate when the current letter on the screen matched the 1-, 2- and 3-back previous letter respectively.

The executive function task:

100 stimuli were presented in the EF, consisting of one out of three colour words (RED, GREEN, and BLUE) written in congruent (33) or incongruent (33) inks (red, green, and blue), or a fixation cross (34). The stimuli were presented randomly, except that no stimulus was the same as the preceding one. The ITI was 6 seconds and each stimulus was presented for 1 second. Participants were instructed to name the colour of the ink, and their vocal responses were recorded with a microphone. The EF task was not part of the training regime and was only used during the fMRI scan.

The duration of the cognitive tasks in the MR scanner was 20 minutes and the order of presentation of the two tasks was counterbalanced within and between participants during online tDCS.

fMRI data acquisition:

300 and 180 scans were acquired for the Stoop and n-back task respectively.

Outcome measures:

The outcome measure for the WM, the d' was calculated as the inverse normal distribution function of true positive over the number all true positive responses, minus inverse normal distribution function of the number of false positive, over the number of false positive plus true negative.

Supplementary Figures

Figure 1. Brain regions significantly activated by the n-back task. The effect combined n-back as compared to 0-back.

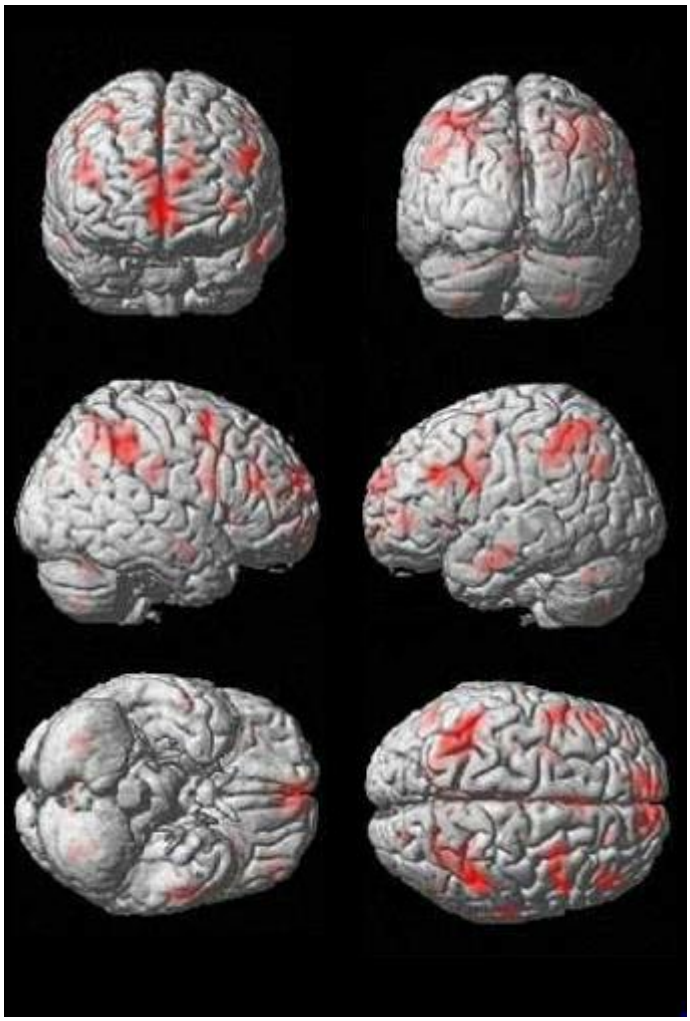


Figure 2. Brain regions significantly activated by the Stroop task. The effect of congruent and incongruent condition compared to the fixation cross.

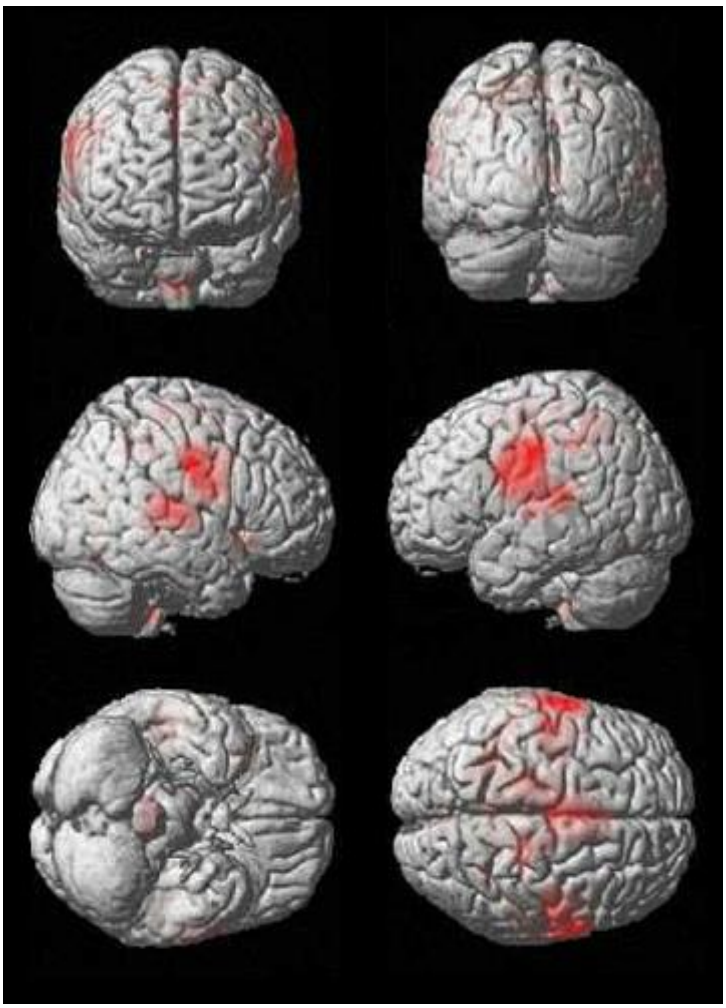


Figure 3. Mask for the ROI analysis underneath the anode.

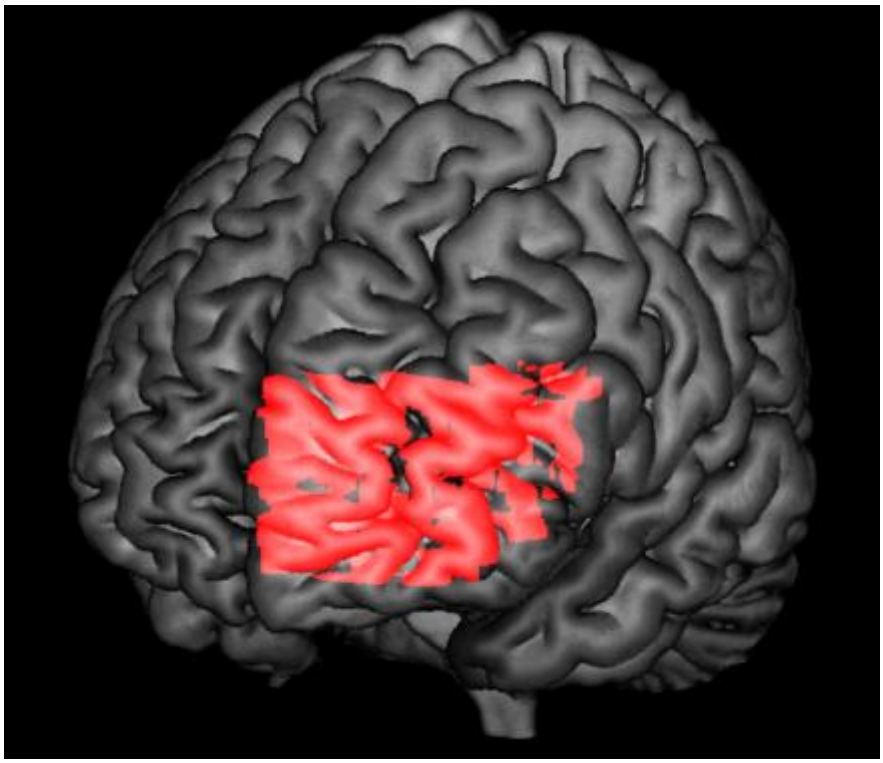


Table 1. Brain regions activated by the N-back task. The effect combined n-back as compared to 0-back. L-left, R-right FWE- family wise error

Brain Regions	BA	x	y	z	z-score	p(peak FWE corrected)	cluster size
R Parietal cortex	40/7	32	-42	34	7.5	0.001	2679
L Parietal cortex	40/7	-40	-39	38	6.8	0.001	2597
L &R Medial/Superior Frontal Gyrus	10/11	6	58	2	5.9	0.001	1243
Middle/Inferior Frontal Gyrus	46/9	-46	27	28	5.8	0.001	970
L Cerebellum		-27	-60	-32	5.7	0.001	170
Cingulate Gyrus	31/7	-4	-51	28	5.7	0.001	627
R Cerebellum		32	-62	-27	5.5	0.001	105
R Middle Frontal Gyrus	6	30	6	56	5.5	0.001	158
R Cingulate Gyrus	32	4	18	45	5.3	0.002	139
L Middle Temporal Gyrus	21	-56	-8	-21	4.9	0.003	202
R Insula	13	33	20	4	5.2	0.013	67
R Middle Frontal Gyrus	46	40	32	21	5.2	0.003	151
R Inferior Frontal Gyrus	9	44	6	30	5.1	0.004	251
R Cingulate Gyrus	24	2	-16	39	5	0.007	136
L Insula	13	-30	22	2	5	0.008	77
R Middle Temporal Gyrus	21	54	-6	21	4.9	0.01	32
L Cerebellum		-6	-75	-27	4.8	0.021	8
L Parahippocampal Gyrus	36	-24	-39	-16	4.7	0.025	12
L Inferior Frontal Gyrus	10	-44	46	0	4.7	0.028	12
R Cerebellum		30	-69	-50	4.6	0.035	4

Table 2. Brain regions activated by the Stroop task. The effect of the congruent and incongruent compared to fixation cross.

Brain Regions	BA	x	y	z	z-score	p(peak FWE corrected)	cluster size
L Precentral Gyrus	6/4/41/42/43	-44	-15	33	7.35	0.001	5988
R Precentral Gyrus	6/41/4/42/22	56	-9	38	7.73	0.001	4624
L &R Cingulate Gyrus	6/32/5/31	2	18	40	6.48	0.001	4802
		-2	-3	62	6.45	0.001	
L Cuneus	17	-10	-66	3	6.39	0.001	399
R Posterior Cingulate	1	16	-61.5	3	5.76	0.001	291
R Frontal Lobe	4	18	-26	60	5.78	0.001	588
L Inferior Parietal Lobule	40	-46	-36	40	5.56	0.001	268
L Insula	13	-39	-16	16	5.28	0.002	30
R Insula	13	30	-50	40	4.91	0.012	44
L Frontal Lobe	4	-18	-28	60	5.5	0.001	367
R Cingulate Gyrus	31	15	-34	44	4.91	0.012	70
L Parietal Lobe	7	-26	-50	38	4.8	0.019	69
L Precuneus	7	-30	-51	51	4.68	0.032	14

The effect of Stroop as compared to fixation cross. L-left, R-right FWE- family wise error

Table 3. Baseline performance of study participants on the n-back task.

	real tDCS	**sham tDCS**	***p***	***t-statistic***
Participant N	**13**	**11**		
d' 0-back	4.35 (0.38)	4.12 (0.89)	0.4	-0.85
d' 1-back	4.01 (0.75)	3.63 (1.11)	0.33	-0.63
d' 2-back	3.42 (1.34)	3.00 (0.88)	0.37	-0.91
d' 3-back	2.46 (1.37)	2.68 (1.11)	0.67	0.43

d': d-prime; (): standard deviation from the mean; t-statistic

References:

1). Averbeck, B. B., Evans, S., Chouhan, V., Bristow, E., & Shergill, S. S. (2011). Probabilistic learning and inference in schizophrenia. *Schizophrenia research*, *127*(1), 115-122.

2) Flöel, A., Rösser, N., Michka, O., Knecht, S., & Breitenstein, C. (2008). Noninvasive brain stimulation improves language learning. *Journal of Cognitive Neuroscience*, *20*(8), 1415-1422.