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Full title: Stimulating thought: an fMRI study of transcranial direct current stimulation in schizophrenia.

Short title: fMRI study of frontal tDCS in schizophrenia

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Abstract:

Importance: Individuals with schizophrenia typically suffer a range of cognitive deficits, including prominent deficits in working memory and executive function. These difficulties are strongly predictive of patient outcomes, but there are a lack of effective therapeutic interventions.

Objective: Transcranial direct current stimulation is a novel neuromodulatory technique with emerging evidence of potential pro-cognitive effects; however there is limited understanding of its mechanism in patients.

Setting: This study was conducted in a UK university between March 2011 and July 2013.

Design: Design: A double-blind randomized placebo controlled pilot study of tDCS on a working memory and executive function task in 28 individuals with schizophrenia using functional magnetic resonance imaging.

Intervention: Study participants received 30 minutes of real or sham tDCS applied to the left frontal cortex.

Main outcome measure: Brain activation change beneath the anodal electrode and in the working memory and executive function network. Full factorial ANOVA, Pearson's correlation and d'.

Results: The 'real' and 'sham' groups did not differ in on-line working memory task performance but the tDCS group demonstrated significant improvement in performance at 24 hours post tDCS. Participants demonstrated task-related activation within the working memory network including the bilateral middle frontal gyrus, cingulate, and the parietal cortex. tDCS was associated with increased activation in the medial frontal cortex beneath the anode; showing a positive correlation with consolidated 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 Stoop task associated with reduced activity in the anterior cingulate cortex.

Conclusions: The neuroimaging changes, observed in the frontal cortex underneath the anode and the correlation with consolidation to task performance data, suggest that tDCS renders affected neuronal populations more likely to respond in line with task-related demands, and it may also impact on more distal nodes in the network, such as the anterior cingulate cortex and cerebellum. tDCS offers a potential novel approach to modulating frontal cortical activity exerting pro-cognitive effects in schizophrenia.

Introduction:

Individuals suffering from schizophrenia (Sz) demonstrate consistent cognitive deficits, that impact on day to day functioning possibly to a greater extend that the more widely recognized positive psychotic symptoms such as hallucinations and delusions. Working memory (WM) and executive functioning (EF) dysfunction represent core cognitive impairments in Sz underlying several other higher order neuropsychological functions [1, 2] including attention, goal directed behavior, planning, metal flexibility and conflict monitoring [3], all of which are impaired in schizophrenia. Deficits in WM and EF have been linked with decrements in functional outcomes such as occupational status and lower rates of independent living [4, 5]. Neuroimaging in healthy participants during WM and EF tasks demonstrate activation within the middle (MFG) and medial frontal gyri and a network of related areas, including anterior cingulate cortex (ACC) lateral temporal and parietal cortices, and cerebellum [6-8]. Recent meta-analyses demonstrate dysfunctional WM and EF in individuals with schizophrenia to be related to aberrant brain activation in frontal cortex, including the medial and MFG, the ACC [7, 9, 10], as well as structurally and functionally connected regions including the medial temporal lobe, cerebellum, thalamus and the striatum [11-16].

Unfortunately, both psychological and pharmacological interventions [17-19] have yielded limited clinical benefits in treating cognitive dysfunction. This has renewed interest in the potential of mechanistic interventions focused on modulating specific brain regions to influence brain function; the key technologies are repetitive transcranial magnetic stimulation (rTMS), and transcranial direct current stimulation (tDCS). There is little evidence of benefit of rTMS on cognitive dysfunction. tDCS is a promising neuromodulatory tool with emerging

evidence suggesting it may improve working memory performance [20]. It is a non-invasive brain stimulation technique; low intensity currents are applied to the scalp through two electrodes, which render neuronal populations more or less ready to fire in response to additional inputs. The brain regions underlying the anodal stimulation demonstrate reduced firing thresholds with consequently increased rates of spontaneous firing, whereas cathodal stimulation reduces tonic firing rates [21, 22]. Although the mechanisms of action are incompletely understood, pharmacological data suggest that excitatory effects are mediated partly by both reduction in GABAergic inhibition and are NMDA receptor dependent; whereas inhibitory effects are mediated by reduction in excitatory glutamatergic neurotransmission [23, 24].

The systems level consequences of tDCS suggest that tDCS applied to MFG and motor cortex alters connectivity between functionally associated brain regions [25-27]. For example 20 minutes of 2mA offline tDCS to the left MFG influenced both proximal and distant networks, including the bilateral frontal-parietal network and the para- and midcingulate cingulate cortex, suggesting that tDCS alters the integrity and strength of connected networks (Stagg paper).

The handful of studies investigating the neurophysiological effects of online tDCS on task performance show behavioral changes to be accompanied by activation in task-related brain networks [28-30]. 20 minutes 1mA anodal tDCS to the left inferior frontal gyrus (IFG) improved verbal fluency in participants with mild cognitive impairment (MCI) accompanied by reductions in baseline hyperactivity of the bilateral prefrontal cortex, right middle frontal gyrus, left basal ganglia and thalamus [30]. Similarly 20 min of online 2mA to the IFG resulted in improved performance on a picture-naming task accompanied with reduced activation of

Broca's area [28]. Overall, behavioral changes during anodal online tDCS applied to the frontal cortex are associated with a reduced brain activity under the stimulation site and in task relevant networks.

Data have shown robust effects of tDCS improving cognitive performance in both healthy participants and in patient samples suffering from stroke, neurodegenerative and psychiatric disorders [for recent reviews see 31, 32]. Interestingly, in schizophrenia a delayed improvement in WM has been observed after online stimulation, [33, 34]; wherein the effects of anodal tDCS to the left MFG improved performance after consolidation of 20 and 40 minutes [33, 34]; whereas immediate effects were observed on a top-signal task investigating executive control [35, 36]. However, to date no work has investigated the effects of online tDCS on the brain's neurophysiological response in individuals with schizophrenia.

In this study, we examined the effects of tDCS on two tasks, a previously trained WM and a novel EF task, and the related brain response in schizophrenia using fMRI; we hypothesized that the online administration of tDCS will only impact EF performance, and not impact the immediate behavioral performance on a WM task, as earlier studies have noted differential improvements in these domains [34, 36]. However, we anticipated increased activation beneath the anode during WM and EF in the real tDCS group; in the former task activation change would correlate with task performance after consolidation [37, 38]. At the system level we expected reduced activation in the task relevant WM and EF networks in the real tDCS group [30]; the bilateral parietal cortex and ACC respectively [39-43].

Method:

Participants:

49 dexterous participants with *DSM*-IV [44] diagnosis of schizophrenia or schizoaffective disorder were enrolled; 28 of these consented to undergo an fMRI scan during the tDCS stimulation. Participants were randomly allocated to either real tDCS or sham stimulation.

Medicated participants were required to be on stable doses of antipsychotic medication for the three months prior to study enrolment. Participants' exclusion criteria included the use of benzodiazepines or other hypnotics; alcohol or substance dependence within three months before study procedures; history of 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, with 30 seconds of ramping up and down of the current using a DC- stimulator MR (NeuroConn GmbH Germany). The anode (35cm^2) was placed over the F3 site (Brodmann area (BA) 10/46), and the cathode (35cm^2) was placed over the right supraorbital area, at FP2 according to the 10-20 international system for electroencephalogram electrode placement. The electrodes used were manufactured to be compatible with a magnetic field and were pre-gelled with EEG paste, and held in place by cotton bands.

Whilst lying in the scanner participants completed two tasks with concomitant real/sham tDCS, a letter n-back task and colour-word inference Stroop task. The n-back task (0-, 1-, 2- and 3-back) varied the working memory load incrementally. 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 (see Figure 1). 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 was 2 seconds and each letter was presented for 0.5 second.

ADD baseline

In the Stroop the stimuli consisted of one out of three colour words (RED, GREEN, and BLUE) that were written in one out of three colour inks (red, green, and blue) and presented on the screen, or a fixation cross. Stimuli could be congruent (word and ink matched) or incongruent (word and ink did NOT match). Both congruent and incongruent stimuli were presented randomly, except that no stimulus was the same as the preceding one. A total number of 100 were presented, 33 congruent, 33 incongruent and 34 fixation crosses. Each stimulus was presented for X seconds, with an inter-trial interval of 6 seconds. The total task execution in the scanner lasted 10 minutes. Participants' vocal responses were recorded with a microphone. Participants were instructed to name the colour of the ink.

The total length of tasks execution in the MR scanner was ~20 minutes. The both tasks were randomised within and between participants during online tDCS.

The 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). A 12 channel head coil was used over the whole head for RF transmission and reception. Each whole-brain image contained 41 3-mm axial slices separated by a distance of 0.3 mm. 300 and 180 scans were acquired for the Stoop and n-back task respectively. After the behavioral portion of the experiment, a T1-weighted structural scan (TR = 9.356 ms, TE = 3.828 ms, flip angle = 75°) was acquired for reference purposes. The first four volumes were discarded to allow for transient effects.

Behavioral data analysis:

For the n-back the outcome measures were the *d'* and mean reaction times (RTs) during tDCS and 1 day post-tDCS. The *d'* of the average of performance for monitoring (0-, 1-back) and manipulation (2-, 3-back) was calculated [45]. *d'* was chosen as the outcome measure, as it takes into account the range of both true and false positive responses and 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 [46]. Data analysis of the WM task was conducted by specification of full maximum likelihood-random effect multilevel models (MLREM). A MLREM including the task relevant outcome scores (*d'*) during the tDCS administration; next day retention at the session following tDCS administration; controlled for baseline performance with fixed categorical effects for group (1-real tDCS/0-sham stimulation) and time (0-2); an interaction of time (0-2) and group (1-real tDCS/0-sham stimulation).

The task outcome measures for the Stoop, number of correct responses and mean reaction times (RTs), were analyses were conducted by means of independent - tests. Clinical and socio-demographic information was analyzed by means of t- and Chi-squared tests (for continuous and categorical variables respectively), with the real tDCS and sham stimulation being the grouping variable, using STATA 12.1 (StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP). The assumption of normality was confirmed using the Shapiro-Wilks and the skewness tests.

fMRI analysis:

All data were pre-processed and analyzed using Statistical Parametric Mapping 12 (SPM12) (Wellcome Department of Imaging Neuroscience, London, UK. www.fil.ion.ucl.ac.uk/spm) in MATLAB R2014a (MathWorks Inc. Sherbon, MA, USA). 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 [47]. The functional images were resampled into 1.5mm^3 voxels and spatially smoothed with an 8-mm full-with half-maximum Gaussian kernel.

In the n-back the subject-specific models included regressors encoding the predicted BOLD response for two separate conditions: all three WM loads combined and a final regressor encoding button presses. For the WM load condition and first (i.e. linear) and 2nd (quadratic) order polynomial expansion was employed). Furthermore, the attentional control condition (0 back) was left unmodelled and served as an implicit baseline. The model also included the six motion parameters generated at during realignment as nuisance regressors. Following parameter estimation, contrasts of beta coefficients for the three primary contrasts of interest were generated, specifically separate mean activation (i.e. zeroth expansion), linear change in BOLD response with increasing WM load and quadratic WM load-related change in response amplitude. The resultant contrasts of parameter estimates were taken forward to a whole-brain random-effects analysis, specifically a group (sham stimulation and real tDCS)-by-level (zero, 1st and 2nd order expansion of WM load) factorial ANOVA. Full whole brain multiple comparisons correction on the basis of response amplitude was carried out. Results

were only considered significant if they had a p-value of less than 0.05 following family-wise error correction.

At a single-subject, each correct responses of the incongruent and congruent condition was modelled as a regressor, and the fixation cross was left unmodelled. Each participant's vocal response and incorrect responses were modelled as a conditions of no interested. Additionally, six nuisance regressors encoding participant volume-to-volume head movements from the realignment stage of pre-processing were included. Following parameter estimation, contrasts of beta coefficients for the conditions of interest (congruent and incongruent) were generated. The resultant contrast of parameter estimates was taken forward to a whole-brain random-effect analysis, with a two-sample test (sham vs real). Full whole brain multiple comparisons correction on the basis of response amplitude was carried out. Results were only considered significant if they had a p-value of less than 0.05 following family-wise error correction. . In addition, we have completed three regions of interest (ROI) analyses based on the Laird et al. (2005) meta-analysis of the verbal Stroop using small volume corrections, with a volume of interest of 6 mm; specifically the anterior cingulate gyrus (x=2 y=16 z=38), the left inferior frontal gyrus (x=-44 y=4 z=33) and left parietal lobule (x=-40 y=-50 z=45) converted to MNI space using WFU (Wake Forest University) Pickatlas toolbox within SPM . Results were considered to be significant if they had a p value of less or equal of 0.05 FWE at a voxel threshold of $p<0.01$.

In order to focus on the region showing strongest evidence for the tDCS effect, we conducted a region of interest analysis (ROI) to compare mean frontal and prefrontal activation between the two groups during tDCS. The analysis was restricted to a priori defined region of interest drawn from underneath the anode and a p-value of 0.01 or less was considered to be significant, following family wise-error correction (FWE). We created a Broadmann area 10/46 mask (see supplementary material) using the WFU toolbox within SPM [48].

After observing changes in the ROIs, we assessed the relationship between those changes and performance on the WM and Stroop task by calculating Pearson correlations between the ROI and improved behavioral performance across participants.

Results:

In the n-back analysis three participants' were excluded due to a technical problem with incomplete image acquisition. Additionally, in both the Stroop and n-back, one participant's data was excluded due prefrontal brain atrophy and consequently, the n-back analysis included 24 participants (13 real tDCS, 11 sham stimulation), and the Stroop analysis included 26 participants (14 real tDCS, 12 sham stimulation).

Overall, the tDCS and sham stimulation groups did not differ significantly on any of the clinical and socio-demographic characteristics (see Table 1.)

Behavioral results:

During the application of stimulation, the tDCS and sham stimulation groups did not differ significantly in either monitoring (0-, 1-back) or manipulation (2-, 3-back) *d'* and mean RTs (see table 2.). After the consolidation (1 day post-tDCS), there were significant between group differences in manipulation of information with the real tDCS performing significantly better relative to sham, controlled for baseline (b=0.68, CI 0.14 - 1.21; p=0.044). As predicted we found significantly better performance during the incongruent condition in the real stimulation (see table X). The real tDCS and sham groups did not differ in their performance on the congruent condition and in RTs (see table X).

fMRI results:

The WM and EF task completion were associated with activation of the task relevant networks.

Overall the combined 1, 2, and 3-back conditions activated the verbal working memory network; including bilateral MFG, cingulate gyrus, bilateral parietal cortex compared to the 0-back condition (see table 3 and Figure 2.) The ROI demonstrated a significantly increased

activation in the medial frontal cortex (BA10) during the working memory task with the real tDCS*; x, y, z = (-8, 66, 0); (t $_{1(66)}$ = 3.22 [t_{peak}=3.54]; K_E = 35, P_{FWE} = 0.01, z-score*$_{peak}$ = 3.38 FWE.

The real tDCS, relative to sham, was 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 hypotheses, 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 investigate the directions of the effect mean *β* for each n-back load were extracted and plotted (Figure 5).

The exploratory analysis demonstrated a significant correlation between the consolidation effect for manipulation and the increased activation underlying the anode (r=0.58, p<0.05), relative to sham (Figure 5).

The Stroop task activated regions relevant to inhibitory control, including the. There tDCS group demonstrated significant less activation in 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-score*$_{peak}$ = 2.82 FWE. An exploratory ROI analysis of the cerebellum demonstrated, similarity in the reduced action in 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-score*$_{peak}$ = *2.63* FWE. -

Discussion:

This is the first study to examine the neurophysiological effects of tDCS during WM and EF assessment in individuals with schizophrenia using fMRI.

As predicted, the ROI analysis demonstrated increased activation underneath the site of the anode in the medial frontal cortex during real tDCS, but only during WM. This was associated

with improved performance after a consolidation period. Further, real tDCS group demonstrated significantly reduced activation in the left cerebellum, with no differences evident in the MFG or parietal cortices. The ROI Stroop results demonstrate that tDCS induced a reduced ACC and cerebellar response and was associated with significantly less errors in the incongruent condition, when real tDCS was compared with sham stimulation. Our results suggest that tDCS impacts behavioral responses in individuals with schizophrenia, replicating previous findings demonstrating that EF improvement can be immediate, whereas improvements on more complex task that require manipulation of information, are dependent on a consolidation period. Neurophysiologically this data suggests that tDCS biases the membrane potential of neuronal populations in the medial frontal cortex, ACC and cerebellum. Although the mechanism of action of tDCS is not clear yet [49], one suggestion is that if the BOLD response represents synaptic activity [50], 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 [51], therefore it is conceivable that tDCS simply reduced the threshold for some of the output neurons and increased 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 anodal tDCS 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 healthy subjects and MCI, similarly we did not observe this neurophysiological effect during EF. Holland et al, observed reduced activation underneath the anode (IFG) in healthy volunteers after real tDCS, but this was confounded by improved behavioral performance; they did not

observe any effect on more distal regions [28]. However, there is a report of reduced activation beneath the anode and in the distal task related network in MCI subjects [30]. One suggestion to explain these differences is that the tDCS impacts healthy brains/neuronal network systems in a locally specific manner, whilst in pathological brains/neuronal networks this effect is evident on a wider task relevant neuronal network. The differential task response might be explained by task complexity, such that the Stroop response only requires 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 [6]; with the MFG possessing a specific role in the allocation of demand led task performance [52]. Individuals with schizophrenia perform worse and activate the MFG to a lesser extent than healthy subjects during executive functioning [7]; because information load demand is thought to exceed available computational resources [53, 54]. However, when task performance is matched, individuals with schizophrenia tend to recruit the WM network, including the MFG, to a greater degree. Response inhibition, on the other hand, is thought to rely heavily on the activity of the ACC and IFG (Laird and new meta-analysis of IGF and inhibitory control). The meta-analysis of Minzenberg indicates that individuals with schizophrenia demonstrate increased activity in the ACC during EF, when comparted to healthy controls. Our result, thus suggest that tDCS has normalized the brain response during EF (mention correlation). In addition, in our sample, we found a significantly negative correlation between performance and activity in the ACC. This is supported by evidence from a study by (Reinhart), which demonstrated that tDCS ameliorated the typical for schizophrenia lack of event related negativity (ERN), a brain response following behavioral

errors relative to correct response. Their results demonstrate the 20 minutes of anodal tDCS to the medial frontal cortex induced an ERN response to a level observed in HC during the a EF task, the stop signal 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 (Wagner et al. 2015 Structural and functional dysconnectivity of the fronto-thalamic system in schizophrenia: A DCM-DTI study); for example individuals with schizophrenia demonstrating reduced connectivity between the MFG and the right cerebellum, suggesting that these neurointegrative deficits might be correlated with WM performance. The cerebrocerebellar system is connected through one of the largest white matter pathways of the brain, in which the medial frontal and cingulate cortex is connected with the cerebellum through the cerebro-ponto-cerebellar loop via the pons [55] (add here neuron review the cerebellum and cognitive function 25 years of insight from anatomy and neuroimaging; Functional topography of the cerebellum for motor and cognitive tasks: an fMRI study and Wagner).

Whilst the cerebellum has traditionally been associated with movement and motor learning, more recent data support a significant role in cognitive operations, including WM [56] where cerebellar activity (in addition to the medial and middle frontal gyri) increases with demand. Similarly, bilateral cerebellum showed increased activity with load for both verbal and abstract stimuli,[43] and in participants with schizophrenia, Sapara demonstrated greater activation of bilateral cerebellum during a WM task, relative to healthy controls [57]. These data support the concept of a necessary compensatory activation in regions such as the

cerebellum in schizophrenia to perform at the equivalent level to healthy controls. Our results suggest that tDCS may improve the efficiency of the network, decreasing the requirement for this cerebellar recruitment. The investigations of cerebellar involvement in the Stroop inference are sparse, but the available data demonstrate that larger grey matter volume in the cerebellum, as well as the ACC and IFG, was associated with reduced Stroop interference in HC, due to involvement in attention and cognitive flexibility {Takeuchi, 2012 #743}. It has been demonstrated that schizophrenia is associated with reduced grey matter volumes executive functioning network {Han, 2012 #746}, and that grey matter volumes are associated with increased functional activity during task performance; although this does not always hold true for border grey matter regions {Takeuchi, 2014 #744}. Nonetheless, the accompanying reduction in the ACC activity and reduced Stroop interference in the real tDCS suggests that tDCS increased network efficiency during Stoop.

There are some limitations to this study. Firstly, we do not have a pre- tDCS scan for our participants, which would have permitted 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. The sample size of this study is relatively modest, but as the first pilot study in schizophrenia, this suggests that this technique is capable of influencing brain dynamics and the proposal that it improves efficiency offers a mechanism to explore in further work.

In summary, our results demonstrate that left MFG anodal tDCS resulted in the increase of activation in the cortex underlying the anode; this correlated significantly with improved performance WM after a consolidation period. There was also decreased action in the

cerebellum suggestive of an increase in efficiency in the wider WM network. Anodal tDCS was associated with improved performance on the Stroop interference and associated with reduced action in the ACC and cerebellum. Given that WM and EF impairments are strongly related to poor functional outcomes in schizophrenia, and the lack of effective therapies, tDCS offers a promising intervention with further studies with larger sample sizes necessary to replicate these preliminary findings.

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