ORIGINAL STUDY PROTOCOL (as submitted to and approved by the Institutional Review Board – translated version)

Treatment of negative symptoms of schizophrenia with transcranial direct current stimulation (tDCS): clinical, randomized, sham-controlled, double-blind

Researchers:

Wagner Farid Gattaz

Leandro da Costa Lane Valiengo

Maurício Serpa

José Gallucci Neto

Martinus Theodorus van de Bilt

Acioly Lacerda

Andre Russowsky Brunoni
Introduction

Negative symptoms of schizophrenia include blunted affect, apathy, and anhedonia.\textsuperscript{1,2} Several studies have shown that these symptoms are related to low premorbid functioning, low IQ\textsuperscript{3}, and poorer clinical prognosis.\textsuperscript{4-8} It has also been suggested that negative symptoms are "semi-independent" of positive symptoms, as they increase over time in severity and prognosis.\textsuperscript{7} Although antipsychotic medications are effective in managing positive symptoms, negative symptoms often persist or even worsen in some cases with these treatments.\textsuperscript{9}

Some studies have consistently suggested that prefrontal dysfunction, particularly in the dorsolateral prefrontal cortex (DLPFC), is involved in the pathophysiology of negative symptoms.\textsuperscript{10-12} Neuroimaging studies have shown reduced metabolism in the prefrontal cortex in patients with both medicated and unmedicated schizophrenia,\textsuperscript{13,14} and do not seem to relate to performance on task or duration of the disorder. In addition, some studies have suggested an inverse correlation involving the severity of the negative symptoms and cerebral blood flow in the frontal region\textsuperscript{15} and cerebral perfusion as a whole.\textsuperscript{16}

Repetitive transcranial magnetic stimulation (rTMS) uses alternating magnetic fields applied at the same frequency to induce electrical currents in cortical brain tissue.\textsuperscript{17} In one study, rTMS has been shown to induce changes in dopamine in ipsilateral prefrontal cortical regions in healthy people, in a study with rTMS and PET it was demonstrated that 10Hz rTMS in the DLPFC resulted in an increase in the extracellular levels of dopamine for 9 minutes after an application.\textsuperscript{18}

Protocols with rTMS have been used in two major brain areas.\textsuperscript{19-30} Hypoactivation in the prefrontal regions seems to be related to the presence of negative symptoms in schizophrenia.\textsuperscript{31} Thus, it was hypothesized that increasing activity in DLPFC with high frequency rTMS could improve negative symptoms.\textsuperscript{30} Another area studied in schizophrenia is the temporo-parietal cortex, where some studies have suggested that auditory hallucinations would be related to hyperactivity in this region on the left.\textsuperscript{32,33} At least two meta-analyses\textsuperscript{34,35} have demonstrated the efficacy of low-frequency stimulation in left temporoparietal cortex for the treatment of auditory hallucinations in schizophrenia. However, a clinical trial has also demonstrated efficacy when stimulated by the same region in the right hemisphere.\textsuperscript{27}
Recent meta-analyses evaluating the efficacy of rTMS in randomized clinical trials have shown either efficacy or a tendency of small to medium effects on negative symptoms with statistically significant results.\textsuperscript{36}

Transcranial direct current stimulation (tDCS) is another non-invasive brain stimulation method that has been used in recent years to treat different neuropsychiatric conditions.\textsuperscript{37-39} This consists of the application of a direct electric current that flows between two relatively large electrodes (cathode and anode). During tDCS, a low intensity electrical current is applied through the scalp, penetrating the skull and reaching the cerebral cortex, which may modify the resting potential of the neuronal membrane,\textsuperscript{40,41} and therefore modulate the rate of neuronal firing.

Another important aspect of tDCS is that its effects are polarity-dependent, that is, there is an increase in cortical activity with anodic stimulation and a decrease in cortical activity with cathodal stimulation,\textsuperscript{42} and that this stimulation technique increases cortical excitability without inducing action potential.\textsuperscript{42} It is worth mentioning that tDCS is a technique different from TMS, whose principle is the generation of electromagnetic pulses to induce electric currents in the brain. The tDCS offers some advantages when compared to transcranial magnetic stimulation, including: (1) greater portability: the tDCS device is small and portable, which would allow home treatment - an aspect of great importance for patients with difficulty locomotion; (2) tDCS effects have a longer duration: 10 minutes of rTMS can modulate cortical excitability by no more than 10 minutes,\textsuperscript{43} whereas a 13-minute of tDCS session has cortical excitability effects for up to 2 hours;\textsuperscript{44} (3) the cost of a tDCS device (less than one thousand BRL) is significantly lower than one magnetic stimulation device (around one hundred thousand BRL), making tDCS an interesting option for different socioeconomic levels.

According to this model, the stimulation of the left prefrontal dorsolateral cortex would imply an increase in the activity of this area, leading to the improvement of the negative symptoms, similar to that observed in relation to the symptomatology of depression.\textsuperscript{45} There is only one study with tDCS and improvement of negative symptoms in schizophrenia,\textsuperscript{46} which showed a decrease of total PANSS from 76.9 to 66.9 in the active group compared to the sham group (ranging from 82.8 to 80.5). The PANSS size that modified with the treatment was that of negative symptoms only ($d = 1.07$, 95% CI $= 0.30$-1.84, $p = 0.01$), and the positive and depressive dimensions did not show differences between sham and active treatments.
Therefore, tDCS is a noninvasive method that can be used to stimulate DLPFC, in which there is evidence of hypoactivity related to the origin of negative symptoms in schizophrenia. In addition, it is a technique that allows stimulation of one area of the cortex concomitantly inhibiting another area. Thus, an interesting way of using this technique would be to stimulate left DLPFC (which showed more results when stimulated with rTMS in the negative symptoms of schizophrenia) along with an inhibition of the temporo-parietal cortex (which showed more results in hallucinations in studies with rTMS and schizophrenia), we thought of assembling the electrodes in this way to optimize the treatment: anodal stimulation in DLPFC (area F3 according to the EEG 10-20 system) and cathodal stimulation in the temporo-parietal cortex on the left (area TP 3, according to EEG system 10-20).

Methods

Objectives

The primary objective is to evaluate if tDCS will be statistically superior to the sham treatment for the symptomatology of negative symptoms in patients with schizophrenia through the PANSS negative symptoms subscale after 6 weeks of trial onset.

Secondary Objectives

To evaluate:

1. The response rate, defined as a reduction of $\geq 20\%$ in the intensity of the negative symptomatology, will be higher in the active group than in the sham.
2. The tDCS will be superior to sham in the treatment of hallucinations as measured by AHRS.
3. The tDCS will be superior to sham in the treatment of depressive symptoms associated with schizophrenia evaluated by MADRS.
4. The tDCS shall be higher than sham in the global functioning as measured by the CGI.
5. The tDCS will be superior to sham in improving cognitive deficits associated with schizophrenia.
6. Clinical efficacy of tDCS after 12 weeks of trial onset (secondary timepoint).
Design and study population

This is a randomized, double-blind clinical trial in which eligible patients will be recruited from the Bairral Institute of Psychiatry (Instituto Bairral de Psiquiatria - IBP), Clinics Hospital – University of São Paulo Medical School (Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo – HCFMUSP), and the primary care network in the region, designed to evaluate the efficacy of tDCS in the treatment of symptomatology of schizophrenia. Subjects will be allocated to one of the groups: sham group or active tDCS group. Participants who meet the inclusion / exclusion criteria and agree to participate in the survey will receive five consecutive days (excluding weekends) of active stimulation or sham and will return periodically for evaluation of negative symptoms, cognition and overall functioning. The trial will have a phase of stability of the medications for 4 weeks, followed by 5 consecutive days of stimulation, twice a day, with sham or active treatment. The PANSS will be applied before the stimulation in the second and fourth weeks, and after 6 and 12 weeks of the beginning of the study. Type of medication and dose will be monitored. Changes in medication or psychosocial interventions will not be allowed. Concomitant treatment with anticonvulsants or benzodiazepines will not be allowed, as well as electroconvulsive therapy.

At the end of the double-blinded phase (12 weeks), volunteers who have not had symptom improvement and have been allocated to the sham group may choose to receive 5 days of active partial cross-over.

Randomization and allocation

The randomization will be done in block, in which there will be permutation in the order and size of the blocks. The randomization will be generated through the website www.randomization.com by a statistician who will not be directly linked to the survey and therefore will remain unblinded in relation to the allocated treatment. Patient allocation will be done through sealed, opaque, and standardized envelopes. After the patient signs the informed consent form, the envelope will be opened and the envelope will be allocated to the treatment, in a coded form. The envelope will be identified with a random number that will be assigned to each patient.

Inclusion and exclusion criteria
Patients between 18 and 55 years old of both genders with the diagnosis of schizophrenia according to DSM-IV-TR criteria and confirmed by the Structured Clinical Interview for DSM-IV (SCID) will be included, which will be applied by a psychiatrist. The PANSS (positive and negative symptom score) scale will be also applied and patients with a minimum score of 20 on the negative subscale and with stable antipsychotic medications for at least 4 weeks in any dose will be selected. Patients will also need to present stable positive and negative symptoms for 4 weeks, according to psychiatrist’s evaluation.

Other drugs that may interfere with negative symptoms such as: antidepressants, modafinil, erythropoietin and minocycline will not be allowed, and they will be washed out prior to study entry. There is a need for at least one trial with at least one antipsychotic at the appropriate dose and time to enter the study.

Among the exclusion criteria we have: unstable or uncontrolled clinical diseases, previous treatment with rTMS or tDCS, psychiatric comorbidities, use of benzodiazepines in doses greater than 10mg of diazepam or equivalent.

**Blinding**

The study will be double-blinded; that is, researchers, evaluators, and patients will not be aware of the treatment administered until the end of the study. The stimulation appliers will not be able to evaluate patients. The tDCS technique is particularly advantageous for ensuring blinding when compared to other non-invasive brain stimulation techniques. This is because the tDCS generates a tinnitus effect in the first minutes of the intervention, generating no additional effect after this initial effect. In this way, sham stimulation consists in positioning a switch in the off position, so that the machine cuts off the electric current after one minute of stimulation, thus generating the same effects of a true intervention.47

The evaluation of the depressive symptomatology will be performed by psychiatrists or psychologists properly trained in the application of psychometric scales who will be blind to the intervention performed.

**Interventions**

In the tDCS will be used two electrodes that will be placed in the skull of the patient; specifically, the anode will be positioned over the dorsum-lateral area of the left
prefrontal cortex (F3) and the cathode will be positioned over the ipsilateral temporoparietal area (TP3). The stimulus will be 20 minutes a day for 5 days in a row, 2 times a day, with a minimum interval of 3 hours between the two stimulations. The applied current will be 2mA with ramp-up and ramp-down periods of 40 seconds. Sham stimulation will be performed for 30 seconds only at 2mA, with similar ramp-up and ramp-down periods.

**Measurement of variables and outcomes**

The demographic and clinical profile of the patients will be evaluated through the following variables: gender, age, schooling, socioeconomic condition, presence of clinical and psychiatric comorbidities, refractoriness of the psychotic symptoms, duration of current psychotic episode, number of previous hospitalizations for episodes psychotics and previous treatment with electroconvulsive therapy. The main outcome will be measured with the PANSS scale. This is a continuous scale of points. The main evaluation will be done at the end of 6 weeks. The PANSS scale will also be used categorically, defining the treatment as unresponsive (less than 20% improvement in scale) or responsive (more than 20% reduction in scale. We will also use the scales of MADRS, GAF and the scale clinical impression (CGI).

<table>
<thead>
<tr>
<th>Measurements/Day</th>
<th>Day 0</th>
<th>Day 14</th>
<th>Day 28</th>
<th>Day 42</th>
<th>Day 84</th>
</tr>
</thead>
<tbody>
<tr>
<td>PANSS</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>CGI</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>MADRS</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>GAF</td>
<td>X</td>
<td></td>
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<td>X</td>
<td>X</td>
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<tr>
<td>Blood</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>SCID</td>
<td>X</td>
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</table>

**Biological markers**

Blood will be collected the first day and in the sixth week after entry into the study to check for changes in biomarkers such as: inflammatory cytokines and BDNF. In addition, genetic polymorphisms related to tDCS response will be evaluated.

**Sample Size Calculation**
The sample calculation is based on the negative symptoms of PANSS, which is our primary outcome on day 42. This score has a mean variation in this group of patients from 20 to 24 points in previous studies. A three-point difference is considered clinically significant. Standard deviations from previous studies showed a range of 4 to 5 points. Therefore, a study with 80% of power and alpha of 0.05 requires a sample of 44 patients in each group. A drop-out rate of 15% is expected, which results in about 50 patients in each group.

Statistical analysis

Statistical analysis will be done using the Stata 12 SE and SPSS version 17 program for Windows. In general, all analyses will be made on the intention-to-treat principle (i.e., all patients will be included in the analysis) in which lost data will be imputed according to the last observation carried forward principle. Analyses will be considered significant at p <0.05.

For the main outcome we will use a general linear model with a continuous dependent variable (PANSS negative symptom score).

The secondary analyses will use the same model above, having as a dependent variable the scores of the other scales used.

We will also analyse how other socio-demographic characteristics influence the therapeutic effect. Schooling, number of previous admissions, presence of clinical comorbidities will be analyzed as ordinal data. Refractoriness will be a variable analyzed as dichotomous (presence or not of refractoriness).

Expected Results

We expect that active stimulation will be superior to sham stimulation to reduce negative symptoms in the PANSS.

References

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### Summary of changes in the study protocol

<table>
<thead>
<tr>
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<tbody>
<tr>
<td><strong>Active stimulation will be superior to sham stimulation to reduce negative symptoms in the PANSS after 6 weeks of trial onset</strong></td>
<td>Participants receiving active tDCS will present a reduction of negative symptoms as measured by the PANSS negative symptoms subscale scores 6 weeks after beginning the treatment, compared to patients receiving sham tDCS.</td>
<td>No changes</td>
<td></td>
</tr>
<tr>
<td><strong>Randomization</strong></td>
<td>Randomization in block, with permutation in the order and size of the blocks, generated through the website <a href="http://www.randomization.com">www.randomization.com</a> by a statistician not directly linked to the survey.</td>
<td>Randomization generated through the website <a href="http://www.randomization.com">www.randomization.com</a> and performed in blocks to allow permutation of block size and order.</td>
<td>No changes</td>
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<tr>
<td><strong>Allocation</strong></td>
<td>Sealed, opaque envelopes that contained codes for the assigned groups.</td>
<td>Sealed, opaque, patterned envelopes, labeled with a random number code assigned to the participant.</td>
<td>No changes</td>
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<tr>
<td><strong>Blinding</strong></td>
<td>Double-blind</td>
<td>Double-blind</td>
<td>No changes</td>
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<tr>
<td><strong>tDCS interventions (parameters)</strong></td>
<td>Two electrodes placed in the skull of the patient; specifically, the anode positioned over the dorsolateral area of the left prefrontal cortex (F3) and the cathode positioned over the ipsilateral temporo-parietal area (TP3). Stimulus for 20 minutes a day for 5 days in a row, 2 times a day, with a minimum interval of 3 hours between the two stimulations. The applied current will be 2mA with ramp-up and ramp-down periods of 40 seconds. Sham stimulation will be performed for 30 seconds only at 2mA, with similar ramp-up and ramp-down periods.</td>
<td>Two electrodes over the scalp. The anode positioned over the area corresponding to the left DLPFC, between F3 and FP1, and the cathode positioned over the area of the left temporoparietal junction (CP5). The minimum distance between electrodes is 7 cm, the electrode dimensions are 5 × 7 cm, and the applied current in the active group will be 2mA. The device used is the DC Stimulator tDCS (Neuroconn®, Ilmenau, Germany) in study mode for double-blind trials. Each session lasts 20 minutes, and participants receive two sessions a day over 5 consecutive days (Monday to Friday).</td>
<td>No changes</td>
</tr>
<tr>
<td><strong>Primary Objective</strong></td>
<td>To evaluate if tDCS will be statistically superior to the sham treatment for the symptomatology of negative symptoms in patients</td>
<td>To evaluate the efficacy of active tDCS for the treatment of the negative symptoms of schizophrenia, as measured by</td>
<td>No changes</td>
</tr>
<tr>
<td><strong>Secondary Objectives</strong></td>
<td><strong>Eligibility Criteria</strong></td>
<td><strong>Adverse Events</strong></td>
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<td>To evaluate: 1) The response rate, defined as a reduction of $\geq 20%$ in the intensity of the negative symptomatology, if it will be higher in the active group than in the sham; 2) If tDCS will be superior to sham in the treatment of hallucinations as measured by AHRS; 3) If tDCS will be superior to sham in the treatment of depressive symptoms associated with schizophrenia evaluated by MADRS; 4) If tDCS shall be higher than sham in the global functioning as measured by the CGI; 5) If tDCS will be superior to sham in improving cognitive deficits associated with schizophrenia; 6) Clinical efficacy of tDCS after 12 weeks of trial onset (secondary timepoint).</td>
<td>Inclusion criteria: Patients between 18 and 55 years old of both genders with the diagnosis of schizophrenia according to DSM-IV-TR criteria and confirmed by the Structured Clinical Interview for DSM-IV (SCID) and with a minimum score of 20 on the negative subscale of PANSS and with stable antipsychotic medications for at least 4 weeks in any dose. Patients will also need to present stable positive and negative symptoms for 4 weeks, according to psychiatrist’s evaluation. There is a need for at least one trial with at least one antipsychotic at the appropriate dose and time to enter the study. Exclusion criteria: Use of drugs that may interfere with negative symptoms such as: antidepressants, modafinil, erythropoietin and minocycline; unstable or uncontrolled clinical disease; previous treatment with rTMS or rTMS.</td>
<td>Not described</td>
<td>Use of a tDCS questionnaire for adverse effects to assess treatment tolerability.</td>
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</table>

| | | (1) The CDSS substituted the MADRS, since the former is specific for schizophrenia; (2) SANS was added as a complementary assessment of negative symptoms; (3) cognition and blood biomarkers are not reported here, since they are currently under analysis. |

Inclusion criteria: Males and females between 18 and 65 years old, diagnosed with schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) confirmed by Structured Clinical Interview for DSM-IV (SCID). Patients must have a minimum score of 20 points in the sum of negative symptoms subscale, to be under proper antipsychotic treatment, and with stable positive symptoms. Patients should be under stable psychotropic medication and dosage for at least 6 weeks. Other drugs that may interfere with the assessment of negative symptoms, including antidepressants, modafinil, erythropoietin, and minocycline should be washed-out for at least 4 weeks before trial onset. | Inclusion criteria was 18-55 years old. It was clarified that tobacco use disorder was not an exclusion criterion. It was clarified that patients who used ECT previously than 6 months could have been included. No other changes. |
| tDCS; psychiatric comorbidities; use of benzodiazepines in doses greater than 10mg of diazepam or equivalent. | Exclusion criteria: unstable medical illness; pre-treatment with rTMS or tDCS; psychiatric comorbidities (such as mood disorders, personality disorders, abuse or dependence on alcohol or drugs, or use of any illicit drug during the last 6 months); current or previous electroconvulsive therapy during the last 6 months; use of benzodiazepines in doses equal to or higher than 10 mg of diazepam or the equivalent; presence of specific contraindications to tDCS such as electronic or metal implants in the cephalic segment. |
## Summary of changes in SAP (Statistical Analysis Plan)

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<tbody>
<tr>
<td>Sample Size Calculation</td>
<td>Sample calculation is based on the PANSS negative symptoms score, which is the primary outcome on day 42. A study with 80% of power and alpha of 0.05 requires a sample of 44 patients in each group. A drop-out rate of 15% is expected, which results in about 50 patients in each group.</td>
<td>Sample calculation is based on the PANSS negative symptoms score, which is the primary outcome on day 42. A study with 80% of power and alpha of 0.05 requires a sample of 44 patients in each group. A drop-out rate of 15% is expected, suggesting an enrollment of about 50 patients per group.</td>
<td>No changes</td>
</tr>
<tr>
<td>Primary Outcome</td>
<td>General linear model with a continuous dependent variable. Measured with the PANSS scale at the end of 6 weeks.</td>
<td>General linear model with a continuous dependent variable (PANSS negative symptoms subscale).</td>
<td>No changes; NNTs were also obtained.</td>
</tr>
<tr>
<td>Secondary outcomes</td>
<td>PANSS also used categorically, defining the treatment as unresponsive (less than 20% improvement in scale) or responsive (more than 20% reduction in scale). Secondary analyses were performed as the primary outcome</td>
<td>PANSS also used categorically to separate subjects into responders (≥ 20% reduction in scale) and non-responders (&lt; 20% improvement in scale). Secondary analyses were performed as the primary outcome</td>
<td>No changes; NNTs were also obtained.</td>
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<tr>
<td>Post-hoc analyses</td>
<td>***</td>
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<tr>
<td>Missing Data</td>
<td>Performed intention-to-treat principle. Missing data imputed according to the last observation carried forward principle</td>
<td>Performed intention-to-treat principle.</td>
<td>No changes</td>
</tr>
<tr>
<td><strong>Statistical Analysis</strong></td>
<td>Use of Stata 12 SE and SPSS version 17 program for Windows. Analyses made on the intent-to-treat principle (i.e., all patients included in the analysis) in which lost data will be imputed according to the last observation carried forward principle. Analyses considered significant at p &lt; 0.05.</td>
<td>Use of Stata 12 SE programs and the Statistical Package for the Social Sciences (SPSS) version 17 for Windows. All analyses performed according to the intention to treat principle (all patients will be included in the analysis). Analyses considered significant at p &lt; 0.05.</td>
<td>Analyses were performed using R, version 3.5.2 (lme4 package; R Foundation)</td>
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