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.[1,2] Several studies have shown that these symptoms are related to low pre-morbid functioning, low IQ[3], and poorer clinical prognosis.[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.[7] Although antipsychotic medications are effective in managing positive symptoms, negative symptoms often persist or even worsen in some cases with these treatments.[9]

Some studies have consistently suggested that prefrontal dysfunction, particularly in the dorsolateral prefrontal cortex (DLPFC), is involved in the pathophysiology of negative symptoms.[10-12] Neuroimaging studies have shown reduced metabolism in the prefrontal cortex in patients with both medicated and unmedicated schizophrenia,[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[15] and cerebral perfusion as a whole.[16]

Repetitive transcranial magnetic stimulation (rTMS) uses alternating magnetic fields applied at the same frequency to induce electrical currents in cortical brain tissue.[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.[18]

Protocols with rTMS have been used in two major brain areas.[19-30] Hypoactivation in the prefrontal regions seems to be related to the presence of negative symptoms in schizophrenia.[31] Thus, it was hypothesized that increasing activity in DLPFC with high frequency rTMS could improve negative symptoms.[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.[32, 33] At least two meta-analyses[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.[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.[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.[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,[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,[42] and that this stimulation technique increases cortical excitability without inducing action potential.[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,[43] whereas a 13-minute of tDCS session has cortical excitability effects for up to 2 hours;[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.[45] There is only one study with tDCS and improvement of negative symptoms in schizophrenia,[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),[27] 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 ≥ 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 temporo-parietal 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-um 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.[48] 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[50], GAF[51] and the scale clinical impression (CGI).

Measurements/Day	Day 0	Day 14	Day 28	Day 42	Day 84
PANSS	X	X	X	X	X
CGI	X	X	X	X	X
MADRS	X			X	X
GAF	X			X	X
Blood	X			X	
SCID	X				

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.[28,52,53] 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

1. Andreasen NC, Olsen S. Negative v positive schizophrenia. Definition and validation. Archives of general psychiatry. 1982; 39(7): 789-94.
2. Ananth J, Djenderdjian A, Shamasunder P, Costa J, Herrera J, Sramek J. Negative symptoms: psychopathological models. J Psychiatry Neurosci. 1991; 16(1): 12-8.

3. Tamminga CA, Buchanan RW, Gold JM. The role of negative symptoms and cognitive dysfunction in schizophrenia outcome. International clinical psychopharmacology. 1998; 13 Suppl 3: S21-6.
4. Crow TJ. Brain changes and negative symptoms in schizophrenia. Psychopathology. 1995; 28(1): 18-21.
5. Anthony WA, Jansen MA. Predicting the vocational capacity of the chronically mentally ill. Research and policy implications. Am Psychol. 1984; 39(5): 537-44.
6. Bowen L, Wallace CJ, Glynn SM, Nuechterlein KH, Lutzker JR, Kuehnel TG. Schizophrenic individuals' cognitive functioning and performance in interpersonal interactions and skills training procedures. J Psychiatr Res. 1994; 28(3): 289-301.
7. Fenton WS, McGlashan TH. Antecedents, symptom progression, and longterm outcome of the deficit syndrome in schizophrenia. Am J Psychiatry. 1994; 151(3): 351-6.
8. Gasquet I, Haro JM, Novick D, Edgell ET, Kennedy L, Lepine JP. Pharmacological treatment and other predictors of treatment outcomes in previously untreated patients with schizophrenia: results from the European Schizophrenia Outpatient Health Outcomes (SOHO) study. International clinical psychopharmacology. 2005; 20(4): 199-205.
9. Schneider AL, Schneider TL, Stark H. Repetitive transcranial magnetic stimulation (rTMS) as an augmentation treatment for the negative symptoms of schizophrenia: a 4-week randomized placebo controlled study. Brain Stimul. 2008; 1(2): 106-11.
10. Potkin SG, Alva G, Fleming K, Anand R, Keator D, Carreon D, et al. A PET study of the pathophysiology of negative symptoms in schizophrenia. Positron emission tomography. Am J Psychiatry. 2002; 159(2): 227-37.
11. Hill K, Mann L, Laws KR, Stephenson CM, Nimmo-Smith I, McKenna PJ. Hypofrontality in schizophrenia: a meta-analysis of functional imaging studies. Acta Psychiatr Scand. 2004; 110(4): 243-56.
12. Wolkin A, Sanfilipo M, Wolf AP, Angrist B, Brodie JD, Rotrosen J. Negative symptoms and hypofrontality in chronic schizophrenia. Archives of general psychiatry. 1992; 49(12): 959-65.
13. Kishimoto H, Yamada K, Iseki E, Kosaka K, Okoshi T. Brain imaging of affective disorders and schizophrenia. Psychiatry Clin Neurosci. 1998; 52 Suppl: S212-4.
14. Cohen JD, Perlstein WM, Braver TS, Nystrom LE, Noll DC, Jonides J, et al. Temporal dynamics of brain activation during a working memory task. Nature. 1997; 386(6625): 604-8.
15. Sabri O, Erkwoh R, Schreckenberger M, Cremerius U, Schulz G, Dickmann C, et al. Regional cerebral blood flow and negative/positive symptoms in 24 drug-naive schizophrenics. J Nucl Med. 1997; 38(2): 181-8.

16. Molina Rodriguez V, Montz Andree R, Perez Castejon MJ, Gutierrez Labrador R, Ferre Navarrete F, Carreas Delgado JL, et al. Cerebral perfusion correlates of negative symptomatology and parkinsonism in a sample of treatment-refractory schizophrenics: an exploratory 99mTc-HMPAO SPET study. Schizophr Res. 1997; 25(1): 11-20.
17. Burt T, Lisanby SH, Sackeim HA. Neuropsychiatric applications of transcranial magnetic stimulation: a meta analysis. Int J Neuropsychopharmacol. 2002; 5(1): 73-103.
18. Eisenegger C, Treyer V, Fehr E, Knoch D. Time-course of "off-line" prefrontal rTMS effects--a PET study. Neuroimage. 2008; 42(1): 379-84.
19. Le YY, Qian GZ, Sui LM, Gong QZ, Xu RB. [Low-affinity binding sites of glucocorticoid]. Zhongguo Yao Li Xue Bao. 1991; 12(4): 358-62.
20. Rollnik JD, Huber TJ, Mogk H, Siggelkow S, Kropp S, Dengler R, et al. High frequency repetitive transcranial magnetic stimulation (rTMS) of the dorsolateral prefrontal cortex in schizophrenic patients. Neuroreport. 2000; 11(18): 4013-5.
21. Sachdev P, Loo C, Mitchell P, Malhi G. Transcranial magnetic stimulation for the deficit syndrome of schizophrenia: a pilot investigation. Psychiatry Clin Neurosci. 2005; 59(3): 354-7.
22. Sommer IE, de Weijer AD, Daalman K, Neggers SF, Somers M, Kahn RS, et al. Can fMRI-guidance improve the efficacy of rTMS treatment for auditory verbal hallucinations? Schizophr Res. 2007; 93(1-3): 406-8.
23. Schonfeldt-Lecuona C, Gron G, Walter H, Buchler N, Wunderlich A, Spitzer M, et al. Stereotaxic rTMS for the treatment of auditory hallucinations in schizophrenia. Neuroreport. 2004; 15(10): 1669-73.
24. Poulet E, Brunelin J, Bediou B, Bation R, Forgeard L, Dalery J, et al. Slow transcranial magnetic stimulation can rapidly reduce resistant auditory hallucinations in schizophrenia. Biol Psychiatry. 2005; 57(2): 188-91.
25. McIntosh AM, Semple D, Tasker K, Harrison LK, Owens DG, Johnstone EC, et al. Transcranial magnetic stimulation for auditory hallucinations in schizophrenia. Psychiatry Res. 2004; 127(1-2): 9-17.
26. Loo CK, Sainsbury K, Mitchell P, Hadzi-Pavlovic D, Sachdev PS. A shamcontrolled trial of left and right temporal rTMS for the treatment of auditory hallucinations. Psychol Med. 2010; 40(4): 541-6.
27. Lee SH, Kim W, Chung YC, Jung KH, Bahk WM, Jun TY, et al. A double blind study showing that two weeks of daily repetitive TMS over the left or right temporoparietal cortex reduces symptoms in patients with schizophrenia who are having treatment-refractory auditory hallucinations. Neurosci Lett. 2005; 376(3): 177-81.

28. Hajak G, Marienhagen J, Langguth B, Werner S, Binder H, Eichhammer P. High-frequency repetitive transcranial magnetic stimulation in schizophrenia: a combined treatment and neuroimaging study. Psychol Med. 2004; 34(7): 1157- 63.
29. Blumberger DM, Fitzgerald PB, Mulsant BH, Daskalakis ZJ. Repetitive transcranial magnetic stimulation for refractory symptoms in schizophrenia. Curr Opin Psychiatry. 2010; 23(2): 85-90.
30. Cohen E, Bernardo M, Masana J, Arrufat FJ, Navarro V, Valls S, et al. Repetitive transcranial magnetic stimulation in the treatment of chronic negative schizophrenia: a pilot study. J Neurol Neurosurg Psychiatry. 1999; 67(1): 129- 30.
31. Andreasen NC, O'Leary DS, Flaum M, Nopoulos P, Watkins GL, Boles Ponto LL, et al. Hypofrontality in schizophrenia: distributed dysfunctional circuits in neuroleptic-naive patients. Lancet. 1997; 349(9067): 1730-4.
32. Shergill SS, Brammer MJ, Williams SC, Murray RM, McGuire PK. Mapping auditory hallucinations in schizophrenia using functional magnetic resonance imaging. Archives of general psychiatry. 2000; 57(11): 1033-8.
33. Silbersweig DA, Stern E, Frith C, Cahill C, Holmes A, Grootoonk S, et al. A functional neuroanatomy of hallucinations in schizophrenia. Nature. 1995; 378(6553): 176-9.
34. Aleman A, Sommer IE, Kahn RS. Efficacy of slow repetitive transcranial magnetic stimulation in the treatment of resistant auditory hallucinations in schizophrenia: a meta-analysis. J Clin Psychiatry. 2007; 68(3): 416-21.
35. Slotema CW, Blom JD, Hoek HW, Sommer IE. Should we expand the toolbox of psychiatric treatment methods to include Repetitive Transcranial Magnetic Stimulation (rTMS)? A meta-analysis of the efficacy of rTMS in psychiatric disorders. J Clin Psychiatry. 2010; 71(7): 873-84.
36. Dlabac-de Lange JJ, Knegtering R, Aleman A. Repetitive transcranial magnetic stimulation for negative symptoms of schizophrenia: review and metaanalysis. J Clin Psychiatry. 2010; 71(4): 411-8.
37. Fregni F, Pascual-Leone A. Technology insight: noninvasive brain stimulation in neurology-perspectives on the therapeutic potential of rTMS and tDCS. Nat Clin Pract Neurol. 2007; 3(7): 383-93
38. Nitsche MA, Boggio PS, Fregni F, Pascual-Leone A. Treatment of depression with transcranial direct current stimulation (tDCS): a review. Exp Neurol. 2009; 219(1): 14-9.
39. Boggio PS, Rigonatti SP, Ribeiro RB, Myczkowski ML, Nitsche MA, PascualLeone A, et al. A randomized, double-blind clinical trial on the efficacy of cortical direct current stimulation for the treatment of major depression. Int J Neuropsychopharmacol. 2008; 11(2): 249-54.

40. Miranda PC, Lomarev M, Hallett M. Modeling the current distribution during transcranial direct current stimulation. Clin Neurophysiol. 2006; 117(7): 1623-9.
41. Wagner T, Fregni F, Fecteau S, Grodzinsky A, Zahn M, Pascual-Leone A. Transcranial direct current stimulation: a computer-based human model study. Neuroimage. 2007; 35(3): 1113-24.
42. Bindman LJ, Lippold OC, Redfearn JW. The Action of Brief Polarizing Currents on the Cerebral Cortex of the Rat (1) during Current Flow and (2) in the Production of Long-Lasting after-Effects. J Physiol. 1964; 172: 369-82.
43. Romero JR, Anschel D, Sparing R, Gangitano M, Pascual-Leone A. Subthreshold low frequency repetitive transcranial magnetic stimulation selectively decreases facilitation in the motor cortex. Clin Neurophysiol. 2002; 113(1): 101-7.
44. Nitsche MA, Paulus W. Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology. 2001; 57(10): 1899-901.
45. Kalu UG, Sexton CE, Loo CK, Ebmeier KP. Transcranial direct current stimulation in the treatment of major depression: a meta-analysis. Psychol Med. 2012: 1-10.
46. Brunelin J, Mondino M, Gassab L, Haesebaert F, Gaha L, Suaud-Chagny MF, et al. Examining transcranial direct-current stimulation (tDCS) as a treatment for hallucinations in schizophrenia. Am J Psychiatry. 2012; 169(7): 719-24.
47. Gandiga PC, Hummel FC, Cohen LG. Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. Clin Neurophysiol. 2006; 117(4): 845-50.
48. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull. 1987; 13(2): 261-76.
49. Buchanan RW. Persistent negative symptoms in schizophrenia: an overview. Schizophr Bull. 2007; 33(4): 1013-22.
50. Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. Br J Psychiatry. 1979; 134: 382-9.
51. Endicott J, Spitzer RL, Fleiss JL, Cohen J. The global assessment scale. A procedure for measuring overall severity of psychiatric disturbance. Archives of general psychiatry. 1976; 33(6): 766-71. 52. Cordes J, Thunker J, Agelink MW, Arends M, Mobascher A, Wobrock T, et al. Effects of 10 Hz repetitive transcranial magnetic stimulation (rTMS) on clinical global impression in chronic schizophrenia. Psychiatry Res. 2010; 177(1- 2): 32-6. 53. Lewis SW, Barnes TR, Davies L, Murray RM, Dunn G, Hayhurst KP, et al. Randomized controlled trial of effect of prescription of clozapine versus other second-generation antipsychotic drugs in resistant schizophrenia. Schizophr Bull. 2006; 32(4): 715-23.

Summary of changes in the study protocol

	Original Protocol (Institutional Review Board Protocol)	Published Protocol (Valiengo et al., Trends In Psychiatry and Psychotherapy, 2019)	Present Report
Main Hypothesis	Active stimulation will be superior to sham stimulation to reduce negative symptoms in the PANSS after 6 weeks of trial onset	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.	No changes
Randomization	Randomization in block, with permutation in the order and size of the blocks, generated through the website *www.randomization.com* by a statistician not directly linked to the survey.	Randomization generated through the website *www.randomization.com* and performed in blocks to allow permutation of block size and order.	No changes
Allocation	Sealed, opaque envelopes that contained codes for the assigned groups.	Sealed, opaque, patterned envelopes, labeled with a random number code assigned to the participant.	No changes
Blinding	Double-blind	Double-blind	No changes
tDCS interventions (parameters)	Two electrodes placed in the skull of the patient; specifically, the anode positioned over the dorsum-lateral 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-um 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.	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).	No changes
Primary Objective	To evaluate if tDCS will be statistically superior to the sham treatment for the symptomatology of negative symptoms in patients	To evaluate the efficacy of active tDCS for the treatment of the negative symptoms of schizophrenia, as measured by	No changes

	with schizophrenia through the PANSS negative symptoms subscale in 6 weeks of treatment.	reduction of the Positive and Negative Syndrome Scale (PANSS) negative symptoms subscale scores, 6 weeks after beginning the treatment, relative to sham tDCS.	
Secundary Objectives	To evaluate: 1) The response rate, defined as a reduction of ≥ 20% in the intensity of the negative symptomatology, if it will be higher in the active group than in the sham; 2) If the 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).	To evaluate: 1) The efficacy of tDCS for the treatment of auditory hallucinations, as measured by the Auditory Hallucination Rating Scale (AHRS); 2) response rate, defined as a reduction of ≥ 20% in PANSS negative symptoms subscale scores; 3) efficacy in treating positive symptoms, as measured by the PANSS positive symptoms subscale; 4) efficacy in treating depressive symptoms associated with schizophrenia, as measured by the Calgary Depression Scale for Schizophrenia; 5) change in overall functioning, as measured by the Global Assessment of Functioning (GAF); 6) changes in several blood biomarkers; 7) changes in cognitive measurement.	(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.
Adverse Events	Not described	Use of a tDCS questionnaire for adverse effects to assess treatment tolerability.	No changes
Eligibility Criteria	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	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)

	Original Protocol (Institutional Review Board Protocol)	Published Protocol (Valiengo et al., Trends In Psychiatry and Psychotherapy, 2019)	Present Report
Sample Size Calculation	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.	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.	No changes
Primary Outcome	General linear model with a continuous dependent variable. Measured with the PANSS scale at the end of 6 weeks.	General linear model with a continuous dependent variable (PANSS negative symptoms subscale).	No changes. NNTs were also obtained.
Secondary outcomes	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	PANSS also used categorically to separate subjects into responders (≥ 20% reduction in scale) and non-responders (< 20% improvement in scale). Secondary analyses were performed as the primary outcome	No changes. NNTs were also obtained.
Post-hoc analyses	---	---	Performed during the review process: (1) similar LMM analyses, corrected for multiple comparisons (False Discovery Rate Method), for each individual symptom of the PANSS negative subscale; and for the (2) PANSS-Factor Score for Negative Symptoms (PANSS-FSNS), which is more specific for negative symptoms; (3) response rates were analyzed using a 25% cutoff.
Missing Data	Performed intention-to-treat principle. Missing data imputed according to the last observation carried forward principle	Performed intention-to-treat principle.	No changes

Statistical Analysis	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 $p < 0,05$.	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 $p < 0,05$.	Analyses were performed using R, version 3.5.2 (lme4 package; R Foundation)