Effect of Transcranial Direct Current Stimulation associated with aerobic exercise on the autonomic modulation of hemiparetic individuals due to stroke: a study protocol for a double-blind randomized controlled trial

[version 2; peer review: 1 approved, 1 not approved]

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Abstract

Background: Individuals after a stroke have an imbalance in the autonomic nervous system, which increases the risk of death or recurrent episodes of stroke. Transcranial Direct Current Stimulation (tDCS) combined with aerobic exercise has shown an effect on the modulation of this system.

Objective: The Heart Rate Variability (HRV) and the distance traveled on the exercise bike will be assessed to verify the additional impact of tDCS combined with aerobic exercise on individuals with chronic stroke sequelae.

Methods: The 34 adult individuals with diagnoses of chronic stroke will be randomized into two aerobic exercise intervention groups: G1 (with active tDCS) and G2 (with sham tDCS), three times a week, for 12 weeks.

Procedures: tDCS will be implemented during the aerobic exercise with the anodal electrode positioned over the lateral dorsal prefrontal left cortex, and the cathodal electrode over the contralateral supraorbital region, with 2mA, for 20 minutes. Assessments will be

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1
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2. Brenton Hordacre, Innovation, IMPlmentation And Clinical Translation in
carried out pre, immediately after the intervention, and on the 12th, 24th, 36th interventions, and 30 days later. The HRV data that are pulse interval (PI), square root of the mean of the squares of the differences between adjacent normal RR intervals (rMSSD), absolute high frequency (HF), absolute low frequency (LF), high and low frequency ratio (LF / HF) will be collected using a cardio frequency meter. The analysis of the distance traveled on the exercise bike before and after interventions will be analyzed in meters.

**Discussion:** The autonomic control via brain networks after a stroke can be altered and can promote an increase in sympathetic tone, and a higher risk of sudden death or relapse of stroke. It is crucial to demonstrate the effectiveness of available treatments to improve the autonomic function.

**Trial registration:** The study is registered as a **BRAZILIAN CLINICAL TEST RECORD (ReBEC): U1111-1222-4588** on the 2018/10/16

**Keywords**
Stroke, Autonomic Modulation, Transcranial Direct Current Stimulation, Physical Exercise, Hemiplegia

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**Competing interests:** No competing interests were disclosed.

**Grant information:** The Brazilian Agency for the Promotion of the Coordination for the Improvement of Higher Education Personnel (CAPES) supported the research by granting a scholarship to the first author of this study.

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HRV dysfunction is related to poor outcome in patients with acute brain injury and chronic cerebrovascular event. Heinz et al. observed a decrease in HRV in individuals with a 65-month average duration of injury. These findings therefore suggest that monitoring heart rate behavior may become a helpful indicator of the outcome of cerebral injury and show the importance of treatments aimed at modulating this system.

Thus far, studies have investigated the effect of Transcranial Direct Current Stimulation (tDCS) as adjuvant therapy for modulating cardiac autonomic function. In a systemic review, Rossi et al. showed that tDCS is a therapeutic option in autonomic modulation permitting instantaneous (‘online’) and lasting (‘offline’) modulation of cortical excitability. Clancy et al. showed that anodic tDCS on the motor cortex of healthy individuals modifies the autonomic nervous system’s balance, leading to sympathetic dominance; Heinz et al. observed that application of tDCS associated with aerobic exercise in individuals with stroke sequelae tends to modulate parasympathetic action. However, a contradictory result was found by Nguyen et al., who did not observe the efficacy of tDCS in the population having had a stroke.

Justification
There are few studies that have investigated the effects of tDCS on HRV in connection with stroke sequelae. Transcranial direct current stimulation (tDCS) is a non-invasive technique of brain stimulation (NIBS) that consists of applying a constant direct current through electrodes placed on the head. In a recent meta-analysis Makovac et al. investigated the effects of NIBS on blood pressure (BP), heart rate (HR), and HRV, and indicated that NIBS can affect the autonomic nervous and cardiovascular systems’ activity, with mandatory reductions in heart rate (HR) and increased HRV. Farinatti et al. investigated the effect of tDCS applied before an aerobic exercise session in normotensive men; as a result, they observed decreased parasympathetic activity while sympathovagal balance increased after tDCS. These results demonstrated that NIBS applied over the prefrontal cortex (PFC) can indeed modulate the autonomic nervous system’s activity.

Thus, this study aims to investigate the effects on HRV of tDCS applied on the prefrontal cortex, associated with aerobic exercise.

The choice of the prefrontal cortex for stimulation was due to it being an area related to the neural networks that control heart rhythm, as shown by Ter Horst and by Ter Horst and Postema, who identified direct and indirect pathways through which the frontal cortex modulates parasympathetic activity by subcortical inputs, and by Thayer and Lane, who were the first to link this circuit to HRV.

Benarroch and Benarroch identified functional units within the central nervous system, called the central autonomic network (CAN), through which the brain controls visceromotor, neuroendocrine, and behavioral responses. The CAN includes several structures, including the ventromedial prefrontal
cortex and orbitofrontal cortex. CAN’s primary output is mediated by sympathetic and parasympathetic preganglionic neurons that innervate the heart through the stellate ganglia and the vagus nerve, respectively. Thus, CAN output is directly linked to HRV and is under tonic inhibitory control via prefrontal cortical areas, including the frontal orbital cortex26,27.

The proposed study
• The main objective is to investigate the effect of tDCS associated with aerobic exercise on heart rate variability (HRV) in chronic stroke immediately after the 1st, 2th, 24th, and 36th interventions, and 30 days after the last intervention.
• The secondary objectives are to assess the distance covered during aerobic training on an exercise bike, assess quality of life and cognition after the 12th, 24th, and 36th days of interventions, and 30 days after the last intervention.

Hypotheses
The study hypothesis is that anodic tDCS applied to the left dorsal prefrontal cortex (DLPFC) combined with aerobic exercises will enhance the effects of aerobic training on vagal modulation, since the left DPPC has the function of inhibiting the amygdala’s sympathetic excitatory circuit, helping in vagal autonomic regulation26.

Both tDCS in DLPFC, Fregni et al.29 and Ohn et al.30, and aerobic exercise (AE), Memorris2 can stimulate cognition. Recent studies have combined tDCS and AE in different clinical contexts, including for cognitive training, showing superior effects compared to single technique applications23,31–34. In a randomized controlled trial, tDCS and AE resulted in greater improvements in multiple cognitive domains in cognitive training with healthy people than did using either technique alone31. Ward et al.31 and Céspın et al.35 propose that multimodal approaches, that is, with more than one intervention technique, can elicit synergistic or additive effects and increase efficacy. Thus, this study’s hypothesis is that aerobic training will improve study participants’ cognition and that TDCS added to exercise will enhance these effects.

Methods
Study design
This is a protocol for a double-blind (evaluator and participants), sham controlled, randomized study following the recommendations of the Consolidated Standards of Reporting Trials (CONSORT) (Figure 1) and the recommendations of the standard protocol items for clinical trials (SPIRIT) (Figure 2). The study was approved by the ethics committee of Nove de Julho University, São Paulo, Brazil (CAAE: 97475718.5.0000.5511) and registered in the Brazilian Clinical Trials Registry (ReBEC) (U1111 -1222-4588). Participants will be informed about the research, procedures, risks, and benefits. If they agree, they will sign an informed consent form (Extended data: Appendix 1F).

Sample recruitment and selection
Thirty-four participants will be enrolled according to the sample calculation of both sexes at Nove de Julho University’s physiotherapy clinics, in São Paulo.

Inclusion and Exclusion Criteria
Inclusion criteria: individuals of both sexes, between 21 and 74 years of age with a minimum of six months since stroke, medical authorization to participate in the study, and having lower limb functional capacity that allows them to pedal an exercise bike, even if needing help from the therapist. The participants who usually ingest beta-blockers will not be excluded, but after the end of the research, analysis will be carried out to compare the HRV of individuals who use this medication with those who do not . Exclusion criteria are individuals with cognitive impairment (≤17) assessed by the mini-mental status exam (MMSE)36, severe heart problems, use of a pacemaker, Parkinson’s disease, diabetes, hypertension, non-controlled hypercholesterolemia, kidney disease, and contraindications to the use of tDCS18.

Withdrawal and discontinuation
Participants may freely withdraw from assessment and therapy at any time. Criteria for termination in the study include participants who are absent more than a week after the start date, and those who became ill or acquire any injury making it impossible for them to perform physical activity.

Sample size
The sample size for this study was established using the results of the rMSSD index of a pilot study with eight subjects (4 for the active tDCS group and 4 for the sham tDCS group) in which the sample power was considered α 0.05 and β of 0.80.

Using the website calculoamostral.bauru.usp.br’s sample calculation tool, the difference between the two HRV averages of the rMSSD index with independent groups was calculated (t test), resulting in a total N=15 individuals. Considering possible losses, 10% was added, totaling N=17 individuals for each group. Therefore, we considered 34 individuals, with effect size d= 0.000077 by Cohen. (Figure 2).

Randomization
The allocation of individuals to Group 1 (active tDCS combined with aerobic exercise on the stationary bike) and Group 2 (tDCS sham combined with aerobic exercise on the stationary bike) will take place using the website www.randomized.com by a researcher not involved in the evaluation and intervention.

Study assessments schedule
A study evaluation schedule with standard protocol items is provided in Table 1.

Intervention
Assessments and interventions will be carried out in the morning, always at the same time, to minimize the effects of the circadian cycle. Recommendations will be to continue using
medications according to their regular schedule, have a light
diet on test days, abstain from caffeine or alcoholic bever-
ages, and smoking, and avoid moderate or excessive efforts
on the day before the test day.

Transcranial Direct Current Stimulation. The tDCS
DC-Stimulator Plus (NeuroConn) therapy (active or sham)
will be combined with aerobic exercise on the stationary
bike. The anode electrode will be placed over the left dorsola-
teral prefrontal cortex (F3), and the return electrode (cathode)
will be placed over the contralateral supra-orbital region,
defined by the 10/20 electroencephalogram system. A current of
2mA, will be applied for 20 minutes, 10-second linear ramp
up / down.

The electrodes used will be of conductive rubber, anode
5x5 cm, and cathode 5x7 cm, wrapped in a cellulose sponge
moistened in 0.9% saline solution.

For sham stimulation, all electrode placement procedures
will be performed equal to the active tDSCS. Nevertheless, the stimu-
lator will only be switched on for 30 seconds, considered
a valid control method in tDSCS studies.

Blinding
The NeuroConn DC-STIMULATOR PLUS device has
settings that allow selection of the active or sham stimulation
mode by inserting codes. A researcher not involved in the
procedures will assign the participants as sham or active. The

Figure 1. Flowchart of the study.
Figure 2. Representation of the sample calculation by the site: calculoamostral.bauru.usp.br from Test t: by the difference between the two averages with independent groups, with α of 0.05 and a β of 0.80 between 2 groups in 4 periods and an effect size by Cohen’s d.

After using the tDCS is carried out, participants and researchers will be asked to complete questionnaires about blinding (Extended data: Appendix 2\textsuperscript{1}), adverse effects (Extended data: Appendix 3\textsuperscript{2}), and satisfaction of therapy (Extended data: Appendix 4\textsuperscript{3}).

Aerobic activity
The participant will perform the aerobic activity on a Reebok® RT 445 model N° RBEX49021 exercise bike, device’s external functioning does not reveal the stimulus mode. Therefore, neither the researcher who applies the intervention nor the individual will know what treatment is being used (double-blind).
30 minutes a day, with the initial 5 minutes of warm-up, 20 minutes of aerobic activity associated with active tDCS or sham, and the final 5 minutes of cooling down.

The treatment will be carried out three times a week, every other day, over 12 weeks, for a total of 36 sessions. The initial intensity will be 50% of the reserve heart rate, as tolerated, and will be rising until 5% of the reserve heart rate each week. The target aerobic intensity will be 50% to 70% of the reserve heart rate.

The following formula [\( \text{% reserve HR} = \left( \frac{\text{maximum HR} - \text{resting HR}}{\text{resting HR}} \right) \times \% \) + resting HR] will be used to obtain the reserve heart rate (HR). If an individual uses β-blockers, the maximum corrected HR should be calculated with the following formula [(the dosage taken with the drug + 95.58) / 9.74 =% that should be removed from the maximum HR]. The maximum heart rate (HRmax) will be estimated using the Karvonen formula.

HR and oxygen saturation (SpO₂) will be monitored as a protective measure by a portable pulse oximeter UT-100 Polar V800 frequency meter every two minutes of exercise; as well as blood pressure (BP) and the perception of dyspnea and fatigue of the lower limbs by the modified Borg questionnaire.

### Assessments

Evaluations will be carried out before the 1st and after the 12th, 24th, and 36th interventions, as well as 30 days after the last intervention. Personal data about individuals and the disease will be collected.

### Table 1. Standard protocol items: recommendations for interventional trials of this study.

<table>
<thead>
<tr>
<th>TIME POINT</th>
<th>Enrollment (T1)</th>
<th>Allocation (T0)</th>
<th>Pre-intervention (T1)</th>
<th>Intervention</th>
<th>Post-intervention</th>
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<tr>
<td>ENROLLMENT:</td>
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<td>Eligibility screen</td>
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<td>Informed consent</td>
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<td>Demographic information</td>
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<td>Allocation</td>
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<td>INTERVENTION:</td>
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<td>active (anodal) tDCS associate aerobic training</td>
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<td>Sham tDCS associate aerobic training</td>
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<td>ASSESSMENTS:</td>
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<td>Neurological and cardiovascular assessments</td>
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<td>Primary outcome: HRV</td>
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<td>SECONDARY OUTCOMES:</td>
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<td>Cognition assessments</td>
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<td>Depression assessments</td>
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<td>Quality of life assessments</td>
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<td>Type of stimulation, real or sham tDCS and adverse effects</td>
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Note: Heart Rate Variability (HRV); T1: one week before intervention; T0: one day before intervention; T1: 20 minutes before intervention; T2: 1st-day session; T3: 12th-day session; T4: 24th-day session; T5: 36th-day session; T6: immediately after the session; T7: 30th-day follow-up after the last training session.
**Evaluation of heart rate variability (HRV).** CardioSeries software (http://www.danielpenteado.com/cardioseries) will be used to identify correct premature ectopic beats, and undesirable transients will be removed using linear interpolation that alters the signal stationarity. Variances of pulse interval (PI) will be evaluated in the domain of time and frequency using the linear method.

HRV will be measured using the Polar® V800 heart rate monitor device. The evaluation of cardiac modulation will be performed by recording the RR interval, processed using the Flow software (https://flow.polar.com), calculating the transducer indices of cardiac cycle fluctuation, high-frequency waves (0.15 and 0.4 Hz), low frequency (0.04 to 0.15 Hz), and the interrelation between low frequency and high frequency (0.15 and 0.4 Hz). The raw, unfiltered data will be exported, converted, and stored in an Excel file, used later for the domain of time and frequency.

The indexes obtained by analyzing the RR intervals in the time domain will be the average of the RR pulse interval utilizing absolute variance and the square root of the squared mean of the difference between the normal adjacent RR intervals (rMSSD), expressed in ms. As for the frequency domain, data will be examined through analysis of absolute high frequency (HF) expressed in ms², low absolute frequency (LF) expressed in ms², and the vagal sympathetic balance between low frequency and high frequency (LF/HF).

**Distance Traveled.** The distance covered (meters) by the participant will be measured by the exercise bike’s digital marker at the end of 30 minutes of aerobic exercise.

The results of distance covered will be compared intragroup (active and sham tDCS) for each moment before the 1st intervention; after the 12th, 24th, and 36th interventions; and 30 days after the last intervention to verify evolution in both.

**Cognitive Performance.** Cognitive performance of chronic stroke patients will be assessed using Addenbrooke’s Cognitive Exam (ACE) questionnaire. The evaluator will apply the questionnaire before and after the 1st, 12th, 24th, and 36th interventions and 30 days after the last intervention.

**Quality of life assessment.** Quality of life will be measured by the Stroke Specific Quality of Life questionnaire (SSQOL). The evaluator will apply this questionnaire before and after the 1st, 12th, 24th, and 36th interventions, and 30 days after the last.

**Determination of potential confounding factors**

**Depressive symptoms.** The Beck Depression Inventory (BDI) will be used to assess depressive symptoms. The evaluator will apply the questionnaire before and after the 1st, 12th, 24th, and 36th interventions, and 30 days after the last intervention.

Results will be correlated with the performance of the physical activity.

**Statistical analysis.** The program SPSS Statistic version 17.0 will be used for statistical analysis.

For measures of central tendency and dispersion, descriptive statistical analysis will be used. To measure the parametric variables, the mean and standard deviation will be used. To measure non-parametric variables, the median and the interquartile range will be used, and to measure the categorical variables, frequency and percentage will be used.

The Shapiro-Wilk normality test, using the unpaired t-test for parametric data and Mann-Whitney for non-parametric data, will be applied to the date.

HRV data (linear methods) will be analyzed in the time domain with variance and rMSSD; the frequency-domain, with the absolute, low-frequency band, high-frequency band; and the low frequency to high-frequency ratio. Acute data will be submitted to the Shapiro-Wilk normality test, using the unpaired t-test for parametric data and Mann-Whitney for non-parametric data, considering the significance level p<0.05 for all conditions.

Heart Rate Variability data between the periods after the 1st, 12th, 24th, and 36th of interventions, and 30 days after the last intervention will be submitted to the Shapiro-Wilk normality test and unpaired repeated measures ANOVA will be used for parametric data. Friedman test with a general linear model for non-parametric data, considering the significance level p<0.05 for all conditions.

The correlation between the root mean square of successive differences between normal heartbeats (rMSSD) index, distance performed in the ergometer bike, the Beck Depression Inventory (BDI), Stroke Specific Quality of Life (SSQOL), and Addenbrooke’s Cognitive Examination (ACE) questionnaire over the three months of interventions will be submitted to the Shapiro-Wilk normality test, using Person’s correlation for parametric data and Spearman’s Correlation for non-parametric data.

**Discussion**

This article provides a detailed description of a prospective, randomized, controlled, double-blind clinical trial designed to demonstrate the effects of combining tDCS and aerobic training with an exercise bike on the autonomic modulation of individuals with hemiparesis due to chronic stroke.

Post-stroke cardiovascular complications are related to derangement of the brain-heart axis, which can be observed by cardiac autonomic dysregulation. As a consequence, there is sympathetic hyperactivity, a reduction in cardiac vagal modulation associated with a decrease in HRV. These changes can last more than six months after the acute cerebrovascular event, increased up to four times as secondary cardiac complications, and mortality.

It has also been observed that patients with stroke sequelae show a decrease in aerobic capacity due to prolonged inactivity which causes greater damage to autonomic cardiac
control. Thus, cardiovascular rehabilitation for these patients is important and can be achieved through aerobic training. The average time for aerobic training to result in an adaptive response of the autonomous system is approximately two to three months, with moderate intensity, which makes the rehabilitation process costly both in terms of time and money, causing many individuals to give up during this process.

The use of tDCS in combination with aerobic exercise can be a very important tool, as its ability to stimulate autonomic modulation, as well as its ability to enhance the outcome of therapies, has been shown in literature. In this context, multimodal interventions provide the possibility of initiating neuromodulatory processes and superior performance gains compared to monomodal interventions. Aerobic exercise may be able to interact positively with tDCS in inducing synaptic plasticity. Though not tested so far, it may very well be that the combination of these techniques could improve synaptic processing. This reinforcement pathway is potentially mediated by catecholamines, the result of which may extend to clinical cardiovascular and cognitive improvement.

Furthermore, the advantage of using tDCS is that it is a simple, safe resource, which can be performed in a direct combination, that is, stimulation during exercise. It is relatively easy to apply, cost-effective, and has no known serious side effects. Thus, this feature can easily be applied in almost all real-life environments: health, sports, functional performance, and the treatment of diseases (except in patients with contraindications) without any significant restrictions on body movement.

The evaluation of HRV to monitor the autonomic nervous system is reliable (CV = 5.3-11.5%), Koskinen et al., and has high reproducibility, Dietrich et al., Gujit et al., Mukherjee et al., in addition to being simple and non-invasive, low cost and having high clinical applicability.

If this study presents positive data, it will allow for better prognosis in the rehabilitation of patients with stroke sequelae, with a possible reduction in the time of the cardiac rehabilitation protocol and better clinical adherence, reducing the probability of recurrence of a more severe stroke.

Evaluation status
At this point, participants have been enrolled, and allocation is being done with the perspective of completing collections in June 2021.

Dissemination of results
Results will be communicated to the public through publication as a data set and original research in the relevant scientific journals.

Study limitations
We consider some topics as being possible limitations to our study:

The possible difficulty in recruiting patients due to their limited mobility.
A possible difficulty in analysis due to decreased vagal withdrawal and use of β-blockers.
Absence of control over modifiable risk factors.
Difficulty getting complimentary examinations for accurate diagnosis of the location and extent of lesions.

Data availability
Underlying data
No underlying data are associated with this article

Extended data
Harvard Dataverse: Effect of Transcranial Direct Current Stimulation associated with aerobic exercise on the autonomic modulation of hemiparetic individuals due to stroke: a study protocol for a double-blind randomized controlled trial, https://doi.org/10.7910/DVN/MUNWDB

This project contains the following extended data:
- Appendix 1: Clarified Free Consent Term
- Appendix 2: Blinding Questionnaire tDCS Researcher
- Appendix 3: Adverse effect tDCS
- Appendix 4: Evaluation of treatment satisfaction
- Appendix 5: Personal data about the individual
- Register approved by the ethics committee
- Registered in the Brazilian Registry of Clinical Trial (ReBEC)

Reporting guidelines

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

Acknowledgments
The authors thank the Brazilian fostering agency Coordination for the Improvement of Higher Education Personnel (CAPES) for granting a scholarship to the first author and the University Nove de Julho. Funding provided by CAPES.
References


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Open Peer Review

Current Peer Review Status: ✔️ ✗

Version 2

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Rita Khadka
Department of Basic and Clinical Physiology, B.P. Koirala Institute of Health Sciences, Dharan, Nepal

It is well written. All the comments previously suggested are well incorporated in the manuscript. The rationale of the study, objectives, and methods are appropriately explained. It is worth indexing now from my end.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Assessment of cardiovascular autonomic function, heart rate variability, Blood pressure variability, baroreflex sensitivity, exercise physiology, yoga, electroencephalography

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 23 June 2021

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Brenton Hordacre
Innovation, IMPlmentation And Clinical Translation in Health (IIMPACT in Health), Allied Health
Thank you for the opportunity to review this study. It is an interesting concept and I have raised a few points the authors should consider below.

1. In general, this manuscript will need language editing. There are sections which do not make sense and paragraphs that are not well formulated. For example, the first two paragraphs of the introduction are 1 sentence long. These could be combined to a single paragraph. Equally, it is highly unusual to see dot points within an academic manuscript. I'd suggest they are removed from the introduction and discussion.

2. The introduction lacks the relevant background information. As a reader, I am unaware why tDCS or aerobic exercise as therapies delivered over several sessions can modulate the autonomic nervous system. I suggest the authors expand the introduction, possibly utilising references 13-15, rather than just saying the evidence has been noted by some researchers.

3. I am unclear what the primary and secondary aims of the study are. Is the first dot point the primary aim?

4. The hypotheses do not align with the aims. Why is there a hypothesis that you are stimulating the left DLPFC? Similarly, why is there a hypothesis that it is possible to observe effects on cognition – the hypothesis should be directional. Eg. we hypothesise that anodal TDCS to the left DLPFC combined with aerobic exercise will increase cognitive function compared to sham tDCS and aerobic exercise.

5. I am unclear why the consort diagram is provided if no data is collected. Interestingly, it appears 34 participants have already been randomised.

6. Why is there an upper age limit of 74 years for inclusion criteria?

7. The cohens D value for the power calculation is extremely low – raising concerns. Please have a biostatistician review. In fact, I cannot replicate the power calculation. I have had a biostatistician perform the calculation and they determined 56 participants are required based on the data provided.

8. Who is performing the outcome measures?

9. What is the primary outcome measure(s) and why. What is the reliability/validity and sensitivity of these measures?

10. The statistical analysis section is inadequate. If there are multiple outcome measurement times and two groups, a repeated measures anova or linear mixed model is appropriate. T-tests are going to increase your error.

11. The discussion is inadequate. What gap in the literate is this study likely to address and what is the clinical value of running this study. What potential implications could it have for the field. How do you see this study informing clinical practise of scientific knowledge?

Is the rationale for, and objectives of, the study clearly described?
Is the study design appropriate for the research question?
Partly

Are sufficient details of the methods provided to allow replication by others?
Partly

Are the datasets clearly presented in a useable and accessible format?
Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Stroke recovery, neuroplasticity, brain stim

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 07 April 2021

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Rita Khadka
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The present study has aimed to investigate the effect of transcranial direct current (tDCS) stimulation associated with aerobic exercise on the autonomic modulation of hemiparetic individuals due to stroke. It is a double-blind randomized controlled trial. The control group will receive sham tDCS. Both groups of patients will do aerobic exercise on an exercise bike for 30 min a day, 3 days a week for 12 weeks. Autonomic modulation will be assessed using heart rate variability (HRV).

I find the study protocol interesting and noble. It has both research and clinical significance. The title of the study protocol is well written, clearly summarizing the main objective of the study. Similarly, the rationale and some portions of the methods of the study are well written/explained. However, I have some comments that need to be discussed for the further improvement of the study.

Comments
Related to the Objectives of the study:
It would be better to modify the objectives of the study (page 3) for better clarity. It can be modified as:

1. To investigate the effect of transcranial direct current (tDCS) stimulation associated with aerobic exercise on the autonomic modulation of chronic stroke survivors immediately after the 1st, 12th, 24th, and 36th interventions, and 30 days after the end of the intervention.

2. To evaluate the effect of tDCS on the distance covered during aerobic training, quality of life, and cognition after the 12th, 24th, and 36th interventions and 30 days after the end of the intervention.

Related to the methods of the study:

1. In the inclusion and exclusion criteria of the study, nothing is mentioned about patients with chronic stroke along with other complications like diabetes mellitus, chronic kidney disease, Parkinsonism, and some other complications (page 4, last para & page 5, first para).

2. If patients with chronic stroke along with other complications (like diabetes mellitus, chronic kidney disease, Parkinsonism, and some other complications) are enrolled in the study, it needs to be noted down because these complications decrease HRV. If possible patients with these complications can be excluded from the study. Otherwise, a similar number of such patients enrolled in both the groups may nullify the effects.

3. For the intra-group comparisons of related data, it would be better to use repeated measure ANOVA for the parametric data and Friedman test for the non-parametric data (under statistical analysis page 7).

4. It is mentioned that the results of the depressive symptoms will be correlated with the performance of the physical activity (page 7). However, no statistical tool has been discussed under the statistical analysis. Please mention it.

5. Whether participants will perform aerobic exercise 3 times/days a week successively or on an alternate day (page 7, para 4). It would be better to mention it.

6. Please mention how you will measure distance traveled (page 7).

7. When will the quality of life and depressive symptoms be assessed? Not mentioned clearly in the protocol. It would be better to mention the time point for the assessment. Please check for the assessment of the other parameters also and improve them.

8. Evaluation of heart rate variability is written well. However, it needs some minor modifications. It is written that "rMSSD, expressed in ms^2" (page 7). It needs to be corrected as "rMSSD is expressed in ms." It would be better to use the standard abbreviations for the absolute high-frequency and absolute low-frequency powers and their ratios (page 7). Absolute powers are expressed in ms^2. It can be mentioned.

9. It would be better to add two more time-domain parameters of HRV; SDNN (standard deviation of R-R intervals) and pNN50 (percentage of the number of pairs of adjacent R-R intervals differing by more than 50 ms). Adding these parameters may strengthen the
results.

Is the rationale for, and objectives of, the study clearly described?
Yes

Is the study design appropriate for the research question?
Yes

Are sufficient details of the methods provided to allow replication by others?
Yes

Are the datasets clearly presented in a useable and accessible format?
Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Assessment of cardiovascular autonomic function, heart rate variability, Blood pressure variability, baroreflex sensitivity, exercise physiology, yoga, electroencephalography

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 29 Jun 2021
Solange Zilli Lo Presti Heinz, Nove de Julho University, São Paulo/SP, Brazil

Dear Reviewer
We are grateful for yours comments and suggestions. We believe that we could improve our manuscript substantially based on these comments. We are submitting the revised version of our manuscript entitled "Effect of Transcranial Direct Current Stimulation (tDCS) associated with aerobic exercise on the autonomic modulation of hemiparetic individuals due to stroke - Clinical trial protocol, Controlled, Randomized, Double-blind". We address reviewer points in a point-by-point fashion as shown below.

1. It would be better to modify the study objectives (page 3) for clarity. It can be modified as:
Reviewer's suggestion:
a) To investigate the effect of transcranial direct current stimulation (tDCS) associated with aerobic exercise on the autonomic modulation of chronic stroke survivors immediately after the 1st, 12th, 24th and 36th interventions and 30 days after the end of the intervention.
b) To evaluate the effect of tDCS on the distance covered during aerobic training, quality of life, and cognition after the 12th, 24th, and 36th interventions and 30 days after the end of the intervention.
Answer:
Thank you for your suggestion. Your considerations make our goals better described and clear. Suggested are in lines 93 to 98.
2. Related to the methods of the study:
   a) In the inclusion and exclusion criteria of the study, nothing is mentioned about patients with chronic stroke along with other complications like diabetes mellitus, chronic kidney disease, Parkinsonism, and some other complications (page 4, last para & page 5, first para).
   b) If patients with chronic stroke along with other complications (like diabetes mellitus, chronic kidney disease, Parkinsonism, and some other complications) are enrolled in the study, it needs to be noted down because these complications decrease HRV. If possible, patients with these complications can be excluded from the study. Otherwise, a similar number of such patients enrolled in both the groups may nullify the effects.

   Answer:
   Thank you for your suggestion.
   We agree with your comments. We insert this information in the eligibility criteria. However, some sequelae are very common among Stroke patients, like Diabetes, Hypertension, Hyperlecosterolemia, because of this these comorbidities will be not excluded, but they must be under the control of medication. However, after the end of the research, will be performed the regression analysis to verify if these variables may have interfered with the results. Lines 139 to 140.

3. For the intra-group comparisons of related data, it would be better to use repeated measure ANOVA for the parametric data and Friedman test for the non-parametric data (under statistical analysis page 7).

   Answer:
   Thank you for your suggestion.
   We agree with your comments, and we inserted this information. For HRV (linear methods) the data analyzed between the periods after the 1st, 12th, 24th, and 36th interventions, and 30 days after the end of the tDCS associated aerobic exercise will be analyzed in the time domain with the index of variance, rMSSD and in the frequency domain with absolute, normalized high frequency and the ratio of LF to HF power. The data will be submitted to the Shapiro-Wilk normality test, using the unpaired ANOVA with repetition parametric data and Friedman test for non-parametric data, considering the significance level $p \leq 0.05$ for all conditions. Line 267 to 276.

4. It is mentioned that the results of the depressive symptoms will be correlated with the performance of the physical activity (page 7). However, no statistical tool has been discussed under the statistical analysis. Please mention it.

   Answer:
   Thank you for your suggestion.
   The results of the depression symptoms (BDI) will be correlated with the distance performed on the ergometer bike. The data will be submitted to the Shapiro-Wilk normality test, using the Person’s correlation for parametric data and Sperman’s Correlation for non-parametric data.
   Lines: 272 to 274.
5. Whether participants will perform aerobic exercise 3 times/days a week successively or on an alternate day (page 7, para 4). It would be better to mention it.

**Answer:**
Thank you for your suggestion.
The treatment will be carried out three times a week, every other day, for 12 weeks, totaling 36 sessions.

Lines: 44 and 195.

6. Please mention how you will measure distance traveled (page 7).

**Answer:**
Thank you for your suggestion.
The distance covered (meters) performed by the participant will be measured by the device's digital marker, at the end of the 30 minutes of aerobic exercise, on the exercise bike. Line 233 to 235.

7. When will the quality of life and depressive symptoms be assessed? Not mentioned clearly in the protocol. It would be better to mention the time point for the assessment. Please check for the assessment of the other parameters also and improve them.

**Answer:**
Thank you for your suggestion.
The cognitive performance of chronic stroke patients will be assessed using the Addenbrooke Cognitive Exam (ACE) questionnaire. The evaluator will apply the questionnaire before and after the 1st, 12th, 24th, and 36th interventions, and 30 days after the end of the intervention. Line 240 to 243.

Quality of life will be measured by the Stroke Specific Quality of Life questionnaire (SSQOL). The evaluator will apply the questionnaire before and after the 1st, 12th, 24th, and 36th interventions, and 30 days after the end of the intervention. Line 245 to 247.

The Beck Depression Inventory (BDI) will be used to assess the depressive symptoms. The evaluator will apply the questionnaire before and after the 1st, 12th, 24th, and 36th interventions, and 30 days after the end of the intervention. Line 250 to 252.

8. Evaluation of heart rate variability is written well. However, it needs some minor modifications. It is written that "rMSSD, expressed in ms\(^2\)" (page 7). It needs to be corrected as "rMSSD is expressed in ms." It would be better to use the standard abbreviations for the absolute high-frequency and absolute low-frequency powers and their ratios (page 7). Absolute powers are expressed in ms\(^2\). It can be mentioned.
Answer: Thank you for your comments. We correct it. Line: 228 to 231.

9. It would be better to add two more time-domain HRV parameters; SDNN (standard deviation of RR intervals) and pNN50 (percentage of the number of pairs of adjacent RR intervals that differ by more than 50 ms). Adding these parameters can strengthen the results.

Answer: We appreciate your suggestion. However, the software we will use is the *CardioSerie*, so it will not be possible to analyze the SDNN and pNN50 data as suggested by the reviewer. But we can add the time-domain index of variance, which equals the SDNN index, and the rMSSD, which equals pNN50. Line 261 to 265.

**Competing Interests:** I declare that I don't have any competing interests.