PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Protocol study for a randomized, controlled, double-blind, clinical trial involving virtual reality and anodal transcranial direct current stimulation for the improvement of upper limb motor function in children with Down syndrome
AUTHORS	Lopes, Jamile; Grecco, Luanda; Moura, Renata; Lazzari, Roberta; Duarte, Natalia; Miziara, Isabela; melo, Gileno Edu; Dumont, Arislander; Galli, Manuela; Santos Oliveira, C

VERSION 1 - REVIEW

REVIEWER	Dr Alicia Goodwill Institute for Health and Ageing, Australian Catholic University, Australia
REVIEW RETURNED	26-Mar-2017

GENERAL COMMENTS	This protocol paper presents an interesting trial investigating the effect of TDCS concurrent with virtual reality to improve motor function in children with Down syndrome. However, there are some methodological considerations that should be addressed. Further, given the lack of research on the use of TDCS in children with Down syndrome, the authors should provide greater rationale as to how augmenting motor cortical excitability via TDCS may improve motor function in this population. Minor comments: 1. Abstract: page 3, line 30-31. I suggest adding the length of the intervention (total no. of sessions) into the methods. 2. Strengths and limitations: line 21. Please elaborate on the outcome measures used to define ‘satisfactory results’ in the literature (i.e. cognition, motor function, learning etc.). Line 6. Please define the abbreviation for transcranial direct current stimulation. Line 48. Use abbreviation ‘VR’ for virtual reality. Introduction: 3. page 5, line 48 & 53. Please place virtual reality ‘(VR)’ parenthesis on line 48 and remove on line 53. 4. Page 5, line 60. Please insert references to support the claim “……promising results achieved with regard to motor learning.” 5. Page 6, lines 16-21 please insert references to support the results

	of clinical trials to treat neurological disorders and modulation of cortical excitability. Objectives: 6. Page 7, line 28. Please abbreviate Down syndrome to DS as used previously in the manuscript. 7. Page 7. Please add hypotheses after the primary objective. Methods: 8. Please add intervention duration to the virtual reality training protocol. 9. Please detail the recruitment strategy in the main document. 10. Discussion: page 13, line 10. Please abbreviate virtual reality as previously used in the manuscript. Major comments: 1. Strengths and limitations: This section highlights the rationale for the proposed protocol. Please also address the limitations of this study in this section. Introduction: 2. Page 5. The opening paragraphs emphasise the mental and cognitive impairments in individuals with Down syndrome. Please also comment on the motor deficits (and brain regions involved) as the intervention is specifically targeting the primary motor cortex and primary outcome measure of motor function. 3. As there is no group that receives only TDCS without virtual reality motor training, the authors should make clear in the intro that the aim of the TDCS, in this instance, is to augment the effects from virtual reality motor training. Currently the introduction reads as though the aims are to investigate the benefits of TDCS in this population, which cannot be quantified without a group receiving TDCS only. The authors should consider also adding a group that receives only TDCS to address this question. 4. Please provide more discussion around virtual reality motor training and cortical excitability/neuroplasticity, and the potential mechanisms in which concurrent TDCS and motor training may upregulate plasticity and improve motor function. Methods: 5. TDCS protocol: As there are two target electrodes, I suggest using the term multiple-monopolar anodal TDCS in the first instance (see Nasseri et al. 2015, Front Hum Neurosci), as traditional anodal TDCS typically refers to unilateral stimulation. 6. TDCS protocol: As the described electrode montage is less frequently used within the literature, could the authors please provide justification (and reference) for the chosen electrode montage to improve motor function.

	7. Please insert the TDCS current density in addition to the 1mA intensity. 8. TDCS protocol: Please comment on how the sham stimulation conducted will be blinded from the principal researcher. Please also add a reference after the statement ….”a valid control procedure in studies involving TDCS”. 9. TDCS protocol: As the benefits of TDCS have not been established in this population, it may be worthwhile powering the study to include a group that receives only TDCS without virtual reality, to identify the changes in brain activation and motor function from the TDCS alone. 10. Please comment on the rationale for a 10-day intervention, and whether this will be a sufficient dose to detect clinically meaningful differences in motor function variables and brain activation.

REVIEWER	Chakrabarty, Samit University of Leeds, UK
REVIEW RETURNED	02-May-2017

GENERAL COMMENTS	An interesting and important study. I think the article could do with a good going over by someone more conversant in English. some sections seem to be worse than others. Have flagged some and raised queries, which are presented as comments in the attached text. The reproducibility is in doubt as some of the methods are not detailed enough, and similarly, some tools described are not incorporated in the training and testing protocols outlined. Figures are not incorporated in the text, so not sure why they are in the document. The reviewer also provided a marked copy with additional comments. Please contact the publisher for full details.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Minor comments:
ABSTRACT:
1) Page 3, line 30-31. I suggest adding the length of the intervention (total no of sessions) into the methods.
We appreciate the comment. The text has been altered as follows:
“Virtual reality training will be performed three times a week (one 20-minute session per day) for a total of ten sessions. During the protocol, transcranial stimulation will be administered concomitantly to upper limb motor training.”

STRENGTHS AND LIMITATIONS:
2) Line 21. Please elaborate on the outcome measures used to define ‘satisfactory results’ in the literature (cognition, motor function, learning etc.).
We appreciate the comment. The following paragraph has been added for a more satisfactory description of the results:
“The literature reports positive effects with the use of tDCS on upper limb movements in children with

cerebral palsy. However, no scientific data were found regarding the use of tDCS during upper limb training in the population of the proposed study (children with DS). Optimizing such movements has a direct impact on improving one's performance of activities of daily living and functional independence."

 Lazzari RD, Politti F, Belina ST, Grecco LAC, Santos CA, Dumont AJL et al. Effect of Transcranial Direct Current Stimulation Combined With Virtual Reality Training on Balance in Children With Cerebral Palsy: A Randomized, Controlled, Double-Blind, Clinical Trial. Journal of Motor Behavior. 2016. 1940-1027.

 Wuang PY, Chiang SC, Su YC et al., Effectiveness of virtual reality using Wii gaming technology in children with Down syndrome Science Direct. 2011; 32: 312-321

"The literature also reports promising results with the use of VR regarding improvements in cognitive aspects of the population in question, as this intervention constitutes multisensory therapy that optimizes one's concentration and assists in the anticipation of movements, thereby exerting an impact on learning aspects in children submitted to this intervention."

 Chamovitz YS e Weiss PL Virtual reality as a leisure activity for young adults with physical and intellectual disabilities Science Direct. 2008;29:273- 287

 Wuang PY, Chiang SC, Su YC et al., Effectiveness of virtual reality using Wii gaming technology in children with Down syndrome Science Direct. 2011; 32: 312-321

a) Line 6. Please define the abbreviation for transcranial direct current stimulation.

We appreciate the comment. The text has been changed to include a description of the abbreviation:

"The proposed project involves the combination of virtual reality (RV) activities for upper limb motor training and multiple-monopolar anodal transcranial direct current stimulation (tDCS) over the primary motor cortex with the aim of optimizing motor control and upper limbs function in children with Down syndrome (DS)."

b) 2. b Line 48. Use abbreviation 'VR' for virtual reality.

We appreciate the comment. The text has been changed, as follows:

"We believe that the administration of multiple-monopolar anodal transcranial direct current stimulation over the primary motor cortex, specifically the areas that correspond to upper limb motor control (C3 and C4 of the 10-20 electroencephalogram system) during upper limb motor training with the use of VR activities will enhance the cortical excitability of motor regions and optimize cerebral activity, thereby potentiating the effects of upper limb motor therapy".

INTRODUCTION:

3) Page 5, line 48 & 53. Please place virtual reality '(VR)' parenthesis on line 48 and remove on line 53.

We appreciate the comment. We have added the abbreviation, as suggested:

"The positive results achieved with virtual reality (VR) are believed to be related to training in an interactive environment that provides a broad range of activities and scenarios with multiple sensory channels, enabling the creation of exercises at an intensity that is promising for the needs of individuals with DS"

4) Page 5, line 60. Please insert references to support the claim "……promising results achieved with regard to motor learning."

We appreciate the important comment. The following references have been added.

 Lazzari RD, Politti F, Belina ST, Grecco LAC, Santos CA, Dumont AJL et al. Effect of Transcranial Direct Current Stimulation Combined With Virtual Reality Training on Balance in Children With Cerebral Palsy: A Randomized, Controlled, Double-Blind, Clinical Trial. Journal of Motor Behavior. 2016. 1940-1027.

 Moura RFC, Santos C, Grecco LCA, Albertini G, Cimolin V, Galli M, Oliveira C Effects of a single

session of transcranial direct current stimulation on upper limb movements in children with cerebral palsy: A randomized, sham controlled study. Developmental Neurorehabilitation. 2017. 19:18.
- Gillick T, Feyma T, Menk J, Usset M, Vaith A, Wood J, Worthing R et al., Safety and feasibility of transcranial direct current stimulationin pediatric hemiparesis: randomized controlled preliminarystudy. Physical Therapy 2015;95(3):337–349.

5) Page 6, lines 16-21 please insert references to support the results of clinical trials to treat neurological disorders and modulation of cortical excitability.
We appreciate the important comment. The following references have been added:
- Kaufmann, W.E.; Moser, H.W. Dendritic anomalies in disorders associated with mental retardation. Cerebral Cortex, v.10, n.10, p.981-991, 2000.
- Pandilla, M.M. Pyramidal cell abnormalities in the motor cortex of a child with Down's Syndrome: a golgi study. Journal of Comparative Neurology, v.67, p.63-81, 1976.
- Rosenbaum, D. Human motor control. New York, Academic Press, 1991.
- Block, M.E. Motor development in children with Down Syndrome: a review of the literature. Adapted Physical Actvity Quaterly, v.8, p.179-209, 1991
- Minhas P, Bikson M, Woods AJ, Rosen AR, Kessler SK. Transcranial direct current stimulation in pediatric brain: a computational modeling study. Conf Proc IEEE Eng Med Biol Soc. 2012;2012:859–62.
- Nitsche MA, Paulus W. Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology. 2001; 27(10):1899–901.

OBJECTIVES:
6) Page 7, line 28. Please abbreviate Down syndrome to DS as used previously in the manuscript.
We appreciate the comment. The change appears below:
“Primary objective: The aim of the proposed study is to evaluate and compare the effect of multiple-monopolar anodal tDCS and sham stimulation over the primary motor cortex during upper limb motor training involving VR on motor control (spatiotemporal variables and kinematics of a reaching task), activity of the elbow flexors and extensors, cerebral activity and functional independence in children with DS”.

7) Page 7. Please add hypotheses after the primary objective.
We appreciate the comment. The hypotheses have been added, as follows:
“2.1.1 HYPOTHESES
Null hypothesis: Ten sessions of anodal transcranial direct current stimulation over the motor cortex concomitantly to upper limb motor training involving the use of virtual reality activities will result in the same effects as motor training with the use of virtual reality combined with sham transcranial stimulation in children with Down syndrome.
Alternative hypothesis: Ten sessions of anodal transcranial direct current stimulation over the motor cortex concomitantly to upper limb motor training involving the use of virtual reality activities will result in the better effects than motor training with the use of virtual reality combined with sham transcranial stimulation in children with Down syndrome.”

METHODS:
8) Please add intervention duration to the virtual reality training protocol.
We appreciate the comment. The following has been added to the text:
“Intervention protocol
The therapeutic intervention will consist of a combination of tDCS and VR during reaching movements. The protocol will follow safety procedures described in the literature for the use of tDCS on the pediatric population.[29,58,59] Three 20-minute sessions of combined therapy (tDCS concomitantly to upper limb motor training) will be held for a total of ten sessions.”

⎱ Minhas P, Bikson M, Woods AJ, Rosen AR, Kessler SK. Transcranial direct current stimulation in pediatric brain: a computational modeling study. Conf Proc IEEE Eng Med Biol Soc. 2012;2012:859–62.

9) Please detail the recruitment strategy in the main document.

We appreciate the comment. The following has been added to the manuscript:

Methods and analysis: The sample will be composed of children with DS recruited from the physical therapy clinics of Universidade Nove de Julho, São Paulo, Brazil. Letters and emails will be sent to pediatricians, physiotherapists and pediatric neurologists to divulge the study.

DISCUSSION:

10) Page 13, line 10. Please abbreviate virtual reality as previously used in the manuscript.

We appreciate the comment. The following has been added to the manuscript:

“Upper limb motor control enables individuals to perform functional activities. VR will be used as a therapeutic tool to enhance motor control.[29-30] Moreover, a noninvasive brain stimulation method (tDCS) will be employed to facilitate motor cortical excitability in the areas subjacent to stimulation to enhance the effects of motor control and learning.[67] Lazzari et al. (2016) demonstrated the efficacy of the combination of tDCS and VR in potentiating motor effects on balance and functional mobility in children with cerebral palsy.[30] This document offers a detailed description of a randomized, controlled, double-blind, clinical trial designed to determine the effectiveness of VR training combined with tDCS on upper limb movements in individuals with DS.”

Major comments:

STRENGTHS AND LIMITATIONS:

1) This section highlights the rationale for the proposed protocol. Please also address the limitations of this study in this section.

We appreciate the comment. The limitations of the study have been added to the manuscript, as follows:

“The limitations of the proposed study regard the lack of scientific data from previous studies involving children with DS for the purposes of comparison with the findings obtained in the proposed study. However, this aspect also demonstrates the importance of the data that will be generated in the proposed study. ”

INTRODUCTION:

2) Page 5. The opening paragraphs emphasise the mental and cognitive impairments in individuals with Down syndrome. Please also comment on the motor deficits (and brain regions involved) as the intervention is specifically targeting the primary motor cortex and primary outcome measure of motor function.

We appreciate the important comment. The following text has been added to the manuscript:

“According to Flórez and Troncoso (1997), the brain of individuals with DS is smaller in volume in comparison to individuals without this condition. Hypoplasia of the frontal and occipital lobes is a common finding. A unilateral or bilateral reduction in the temporal lobe occurs in up to 50% of cases and reductions in the corpus callosum, anterior commissure, and hippocampus are found.[6-7] Such individuals also have a smaller number of secondary sulci in comparison to individuals without this syndrome, the temporal gyri are underdeveloped and differences in nerve cells are also reported. For instance, Pandilla (1976) reports differences in the axons and dendrites of pyramidal neurons in the motor cortex.[8] Such differences are highly correlated with fragmentation problems and necrosis of these branches as well as differences in the electrical activity of the brain.[9] This problem leads to limitations with regard to synaptic connections and the neural transmission of nerve impulses.

The literature also reports atrophied nerve cells, which are likely associated with lags during the integration of visual and spatial information. According to Block (1991), individuals with DS also have a smaller cerebellum and base ganglia, which are related to the control of coordination, timing, and

balance. Such problems imply limitations with regard to the acquisition of motor skills.[10] According to Bomono & Rosetti (2010), neuromotor abnormalities in DS include hypotonia, diminished primitive reflexes, delayed motor and cognitive development, and lower levels of learning.[11].
Seaman and DePauw (1982) propose a model in which reaching phases of fundamental movements and culturally determined movements is conditioned by the achievement of previous development phases.[9] As this population exhibits problems with regard to systems of early-onset and late-onset maturation, children with DS could encounter difficulties reaching the phase of sensory-motor responses and even acquiring motor skills. According to Connolly (1970), the mechanisms or systems that offer support to development and the acquisition of motor skills can be understood using the concepts of “hardware” and “software”, in which changes in “hardware” regard structure, such as the myelinization that occurs in axons, whereas changes in “software” regard function, such as a gain in information processing speed as a result of myelinization; thus, individuals with DS have problems with their “hardware” that have repercussions on their “software”.[12] “Hardware” problems lead to limitations with regard to physical and motor aspects, which is an important problem, as both physical proficiency and perceptive-motor proficiency contribute to the acquisition and performance of motor skills. In other words, it is possible that problems with balance, timing, and agility constitute a hindrance to the acquisition of fundamental patterns or specialized skills.[13]
The population with DS exhibits abnormal muscle coordination, difficulty processing sensory information and functional limitations. The upper limb dysfunctions in this population (muscle weakness and hypotonus, slow reflexes, abnormal biomechanics, sensory deficiency) exert a negative impact on the performance of activities of daily living, independence and quality of life. ”
Gimenez, R; Stefanoni, F.F & Farias, P.B. Relação entre a capacidade de sincronização temporal e as habilidades motoras rebater e receber em indivíduos portadores da síndrome de Down. Revista Brasileira de Ciência e Movimento, no prelo.

Block, M.E. Motor development in children with Down Syndrome: a review of the literature. Adapted Physical Actvity Quaterly, v.8, p.179-209, 1991.
Connolly, K.J. Skill development: Problems and Plans. In: CONNOLLY, K.J. (ed.) Mechanisms of motor skill development. London, Academic Press,
1970.
Kaufmann, W.E.; Moser, H.W. Dendritic anomalies in disorders associated with mental retardation. Cerebral Cortex, v.10, n.10, p.981-991, 2000.
Flórez BJ, Troncoso VM. Síndrome de Down y educacíon. 3. reimp. Barcelona: Masson – Salvat Medicina y Santander, 1997.
Bomono LMM, Rosseti CB. Aspects in perceptual-motor development and sensory-motor intelligence in Down syndrome. Rev bras crescimento desenvolv hum. 2010; 3: 723-734.
Pandilla, M.M. Pyramidal cell abnormalities in the motor cortex of a child with Down's Syndrome: a golgi study. Journal of Comparative Neurology, v.67, p.63-81, 1976.
Seaman, J.; Depauw, K.P. The new adapted physical education. California, Mayfield, 1982.
Schwartzman, J.S. Síndrome de Down. São Paulo, Editora Mackenzie, 1999.

3) As there is no group that receives only TDCS without virtual reality motor training, the authors should make clear in the intro that the aim of the TDCS, in this instance, is to augment the effects from virtual reality motor training. Currently the introduction reads as though the aims are to investigate the benefits of TDCS in this population, which cannot be quantified without a group receiving TDCS only. The authors should consider also adding a group that receives only TDCS to address this question.
The reviewer’s comment is pertinent, but the aim of the study is to analyze the ability of tDCS to potentiate the effects of motor training during VR activities. Therefore, no group submitted to tDCS alone is included. However, we have analyzed this suggestion together with the research group and have decided to include such a group in a future study.
The following has been added to attend the reviewer’s suggestion:

“The proposed study could be used as the basis for the development of further projects conducted to broaden knowledge on this technique, enabling a novel intervention option for the optimization of motor control in individuals with DS.”

4) Please provide more discussion around virtual reality motor training and cortical excitability/neuroplasticity, and the potential mechanisms in which concurrent TDCS and motor training may upregulate plasticity and improve motor function.
We appreciate the important comment. We understand the need for a reference that demonstrates the expected effect of the use of these two therapies. Thus, we have added a study to the Discussion section that employed tDCS combined with motor training involving VR in children with cerebral palsy (see below), since there are no scientific data on tDCS for children with Down syndrome. We only know of the promising results described in the study cited:
“Moreover, a noninvasive brain stimulation method (tDCS) will be employed to facilitate motor cortical excitability in the areas subjacent to stimulation to enhance the effects of motor control and learning.[67] Lazzari et al. (2016) demonstrated the efficacy of the combination of tDCS and VR in potentiating motor effects on balance and functional mobility in children with cerebral palsy.”
⎫ Lazzari RD, Politti F, Belina ST, Grecco LAC, Santos CA, Dumont AJL et al. Effect of Transcranial Direct Current Stimulation Combined With Virtual Reality Training on Balance in Children With Cerebral Palsy: A Randomized, Controlled, Double-Blind, Clinical Trial. Journal of Motor Behavior. 2016. 1940-1027.
⎫ Wuang PY, Chiang SC, Su YC et al., Effectiveness of virtual reality using Wii gaming technology in children with Down syndrome Science Direct. 2011; 32: 312-321

METHODS:
5) TDCS protocol: As there are two target electrodes, I suggest using the term multiple-monopolar anodal TDCS in the first instance (see Nasseri et al. 2015, Front Hum Neurosci), as traditional anodal TDCS typically refers to unilateral stimulation.
We appreciate the important comment. Our research group has analyzed this suggestion and the suggested references. The following has been added to the manuscrip:
“This montage will enable the child to receive multiple-monopolar anodal tDCS over the primary motor cortex, specifically the area that manages upper limb motor control, while minimizing the effect of cathodal stimulation in the brain. Sham stimulation will consist of the same electrode montage and the stimulator will be switched on for 30 seconds, giving the child the initial sensation of stimulation, but no current will be administered during the remainder of the session.”

6) TDCS protocol: As the described electrode montage is less frequently used within the literature, could the authors please provide justification (and reference) for the chosen electrode montage to improve motor function.
We appreciate the important comment. As one of the objectives of the study is to analyze whether anodal stimulation is capable of optimizing the effects of motor training with VR for both upper limbs, we have decided to test a montage that involves two anodal electrodes positioned over the primary motor cortex in regions of motor cortical representation of the upper limbs. Thus, we will analyze the effects obtained with tDCS administered with one anode positioned over C3 (cortical representation of the right upper limb) and another positioned over C4 (cortical representation of the left upper limb). We believe that the simultaneous stimulation of both these areas will facilitate motor cortical excitability on both sides of the brain, thereby contributing to the optimization of the improvement in voluntary motor control of the upper limbs. Therefore, the children will perform upper limb motor activities through VR games with the modulation of an important area of voluntary motor control, specifically upper limb motor control, thereby contributing to the improvement in upper limb function. The cathode will be positioned over the deltoid muscle to minimize/eliminate the effect of this pole of the current, which results in hyperpolarization with a consequent increase in the difficulty of the firing of the action potential. We did not find any previous studies demonstrating the effects of this specific

montage, but the effects of the multiple-monopolar montage with one electrode positioned over each hemisphere and the cathode positioned over the deltoid muscle have been demonstrated in previous studies, specifically in studies addressing components:

 Front Hum Neurosci. 2015 Feb 6;9:54. doi: 10.3389/fnhum.2015.00054. eCollection 2015.A framework for categorizing electrode montages in transcranial direct current stimulation.

 Boggio P. S., Ferruci R., Mameli F., Martins D., Martins O., Vergari M., et al. . (2012). Prolonged visual memory enhancement after direct current stimulation in Alzheimer's disease. Brain Stimul. 5, 223–230. 10.1016/j.brs.2011.06.006 [PubMed] [Cross Ref]

 Lapenta O. M., Fregni F., Oberman L. M., Boggio P. S. (2012). Bilateral temporal cortex transcranial direct current stimulation worsens male performance in a multisensory integration task. Neurosci. Lett. 527, 105–109. 10.1016/j.neulet.2012.08.076 [PubMed] [Cross Ref]

7) Please insert the TDCS current density in addition to the 1mA intensity.
We appreciate the important comment. The following change has been made to the text:
“A current of 1 mA (current density 0.029 mA/cm2) will be administered over the primary motor cortex for 20 minutes during the upper limb motor training activity.”

8) TDCS protocol: Please comment on how the sham stimulation conducted will be blinded from the principal researcher. Please also add a reference after the statement”a valid control procedure in studies involving TDCS”.
We appreciate the important comment. The blinding of the main researcher will be ensured with the use of the DC-Stimulator (NeuroConn, Germany), which has active and sham modes that function based on encrypted code, with three configurations to choose so that the more complex conditions of the study can be achieved. The parameters are adjusted individually and the activated mode can only be altered by the programmer.

9) TDCS protocol: As the benefits of TDCS have not been established in this population, it may be worthwhile powering the study to include a group that receives only TDCS without virtual reality, to identify the changes in brain activation and motor function from the TDCS alone.
This is an important observation. The literature offers studies that demonstrate the greater effectiveness of tDCS combined with motor training than tDCS alone as well as studies that demonstrate the effectiveness of VR in motor rehabilitation protocols. Thus, we decided to combine the two techniques to determine whether tDCS potentiates the effects of motor training with VR for this population.

 Duarte, N. A. C., Grecco, L. A., Galli, M., Fregni, F., & Oliveira, C. S. (2014). Effect of Transcranial Direct-Current Stimulation Combined with Treadmill Training on Balance and Functional Performance in Children with Cerebral Palsy: A Double-Blind Randomized Controlled Trial. PLoS One, 9(8), e105777. doi:10.1371/journal.pone.0105777

 Grecco, L. A. C., Duarte, N. A. C., Mendonca, M. E., Pasini, H., ¸ Costa de Carvalho Lima, L., Franco, R. C., ... Oliveira, C. S. (2013). Effect of transcranial direct current stimulation combined with gait and mobility training on functionality in children with cerebral palsy: study protocol for a double—blind randomized controlled clinical trial. BMC Pediatrics, 13, 168.

 Lazzari RD, Politti F, Belina ST, Grecco LAC, Santos CA, Dumont AJL et al. Effect of Transcranial Direct Current Stimulation Combined With Virtual Reality Training on Balance in Children With Cerebral Palsy: A Randomized, Controlled, Double-Blind, Clinical Trial. Journal of Motor Behavior. 2016. 1940-1027.

 Moura RFC, Santos C, Grecco LCA, Albertini G, Cimolin V, Galli M, Oliveira C Effects of a single session of transcranial direct current stimulation on upper limb movements in children with cerebral palsy: A randomized, sham controlled study. Developmental Neurorehabilitation. 2017. 19:18.

 Gillick T, Feyma T, Menk J, Usset M, Vaith A, Wood J, Worthing R et al., Safety and feasibility of transcranial direct current stimulationin pediatric hemiparesis: randomized controlled preliminarystudy.

Physical Therapy 2015;95(3):337–349.

10) Please comment on the rationale for a 10-day intervention, and whether this will be a sufficient dose to detect clinically meaningful differences in motor function variables and brain activation.
We appreciate the important comment. Articles in the literature that involve the pediatric population follow a margin of safety for the use of tDCS (see references below) and studies involving motor training demonstrate that 6 to 12 sessions are sufficient to achieve promising results (see references below).

 Duarte, N. A. C., Grecco, L. A., Galli, M., Fregni, F., & Oliveira, C. S. (2014). Effect of Transcranial Direct-Current Stimulation Combined with Treadmill Training on Balance and Functional Performance in Children with Cerebral Palsy: A Double-Blind Randomized Controlled Trial. PLoS One, 9(8), e105777. doi:10.1371/journal.pone.0105777

 Grecco, L. A. C., Duarte, N. A. C., Mendonca, M. E., Pasini, H., ¸ Costa de Carvalho Lima, L., Franco, R. C., ... Oliveira, C. S. (2013). Effect of transcranial direct current stimulation combined with gait and mobility training on functionality in children with cerebral palsy: study protocol for a double—blind randomized controlled clinical trial. BMC Pediatrics, 13, 168.

 Lazzari RD, Politti F, Belina ST, Grecco LAC, Santos CA, Dumont AJL et al. Effect of Transcranial Direct Current Stimulation Combined With Virtual Reality Training on Balance in Children With Cerebral Palsy: A Randomized, Controlled, Double-Blind, Clinical Trial. Journal of Motor Behavior. 2016. 1940-1027.

 Chamovitz YS e Weiss PL Virtual reality as a leisure activity for young adults with physical and intellectual disabilities Science Direct. 2008;29:273- 287

 Wuang PY, Chiang SC, Su YC et al., Effectiveness of virtual reality using Wii gaming technology in children with Down syndrome Science Direct. 2011; 32: 312-321

 Lin HC e Wuang PY Strength and agility training in adolescents with Down syndrome: A randomized controlled trial. Science Direct 2012;33:2236-2244.

Reviewer:
1) An interesting and important study. I think the article could do with a good going over by someone more conversant in English. some sections seem to be worse than others. Have flagged some and raised queries, which are presented as comments in the attached text. The reproducibility is in doubt as some of the methods are not detailed enough, and similarly, some tools described are not incorporated in the training and testing protocols outlined. Figures are not incorporated in the text, so not sure why they are in the document.
We appreciate the comments and the important suggestions. The manuscript has been revised by a native English-speaking text editor and the Methods section has been altered to include greater details, as suggested. The figures were sent separately, following the norms of the journal.
We are at your disposal should any further changes be deemed necessary.

VERSION 2 – REVIEW

REVIEWER	Alicia Goodwill Institute for Health and Ageing, Australian Catholic University
REVIEW RETURNED	18-Jun-2017

GENERAL COMMENTS	The authors have improved this manuscript and appreciate their consideration in addressing the feedback provided. Before acceptance/publication, I suggest a final proofread, as there are some abbreviation inconsistencies and typographical errors throughout (I have highlighted these in the attached document).

	Some additional comments to consider: Introduction: page 8, line 51: Although this comment was addressed in the author responses, there doesn't appear to be references next to the statement: "The results of clinical trials have demonstrated its considerable potential in the treatment of neurological disorders and the investigation of processes of cortical excitability modulation". You may want to double check this. Page 8, line 26-8: For clarity, you may want to comment that these referenced studies are in other paediatric populations such as Cerebral Palsy. The reviewer also provided a marked copy with additional comments. Please contact the publisher for full details.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

Introduction:
Page 8, line 51: Although this comment was addressed in the author responses, there doesn't appear to be references next to the statement: "The results of clinical trials have demonstrated its considerable potential in the treatment of neurological disorders and the investigation of processes of cortical excitability modulation". You may want to double check this.
We appreciate the comment. New references were cited on studies that used tdcs in neurological disorders, for different variables.
• Lazzari RD, Politti F, Belina ST, Grecco LAC, Santos CA, Dumont AJL et al. Effect of Transcranial Direct Current Stimulation Combined With Virtual Reality Training on Balance in Children With Cerebral Palsy: A Randomized, Controlled, Double-Blind, Clinical Trial. Journal of Motor Behavior. 2016. 1940-1027.

• Grecco LA1, Duarte Nde A, de Mendonça ME, Pasini H, Lima VL, Franco RC, de Oliveira LV, de Carvalho Pde T, Corrêa JC, Collange NZ, Sampaio LM, Galli M, Fregni F, Oliveira CS. et al. Effect of transcranial direct current stimulation combined with gait and mobility training on functionality in children with cerebral palsy: study protocol for a double-blind randomized controlled clinical trial. BMC Pediatr. 2013:168.

• Dumont AJL, Araujo M, Lazzari RD, Santos CA, Carvalho DB, Moura RCF. Effects of a single session of transcranial direct current stimulation on static balance in a patient with hemiparesis: a case study. J. Phys. Ther. Sci. 2015. 27: 955–958.

Page 8, line 26-8: For clarity, you may want to comment that these referenced studies are in other pediatric populations such as Cerebral Palsy.
We appreciate the comment. The text has been altered as follows:
´´Non-invasive brain stimulation methods have been employed in physical rehabilitation protocols due to the promising results achieved with regard to motor learning, in the pediatric population with cerebral palsy since it was never used in DS.´´

Comments in text
All suggested comments regarding the abbreviations have been corrected and highlighted in the text.