PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	The Balanced Growth project: a protocol of a single-center observational study on the involvement of the vestibular system in a child's motor and cognitive development.
AUTHORS	Van Hecke, Ruth; Deconinck, Frederik J. A.; Wiersema, Jan R.; Clauws, Chloe; Danneels, Maya; Dhooge, Ingeborg; Leyssens, Laura; Van Waelvelde, Hilde; Maes, Leen

VERSION 1 – REVIEW

REVIEWER	Delafield-Butt, Jonathan University of Strathclyde, Laboratory for Innovation in Autism
REVIEW RETURNED	19-Feb-2021

GENERAL COMMENTS	Review of The Balanced Growth project: the involvement of the vestibular system in a child's motor and cognitive development. For consideration in BMJ Open 19th February 2021 This is a particularly innovative study, especially in its attention linking vestibular pathways to higher cognitive function via subcortical integrative processing, which has great value in itself. Subcortical contribution to cognition and its role in conscious experience is a neglected topic in neuroscience and neuropsychology (see Merker, 2007). It is refreshing to see this line of enquiry extended here within a robust, very detailed and important clinical study that will add resolution to its function by way of pathology. As the authors state, "vestibulo-cognitive interactions have never been assessed in a typically developing cohort". This study will add value to neuropsychology, as well as in the development of new knowledge and technique in medicine that are its primary aims. The protocol manuscript is clear and well-written, describing succinctly a complex set of analyses of children with vestibular impairment and neurodevelopmental disorder. Study strengths are in its novelty and innovation, including inclusion of a heterogeneous NDD group that may yield important insight. Study weaknesses are in its lack of formal structures for study oversight, with data handling and analysis, not described in detail. This can be improved in the case of data handling and analysis (see below), and where formal structures may not be necessary, this can be explicitly stated in the manuscript. I have brought attention to this

	aspect within the detailed points below, especially in relation to the Protocol Checklist. This study is not funded by any agency, and is presumably supported by the University of Ghent. This study will otherwise become a valuable contribution to the field, and this protocol manuscript describes it well. Please find my detailed comments below for attention. 1. MS Page 16. Statistical analysis. There is no mention of a statistical analysis plan, or the workflow for how these data will be managed and analysed. 2. I have a number of questions left open about the data management and study design: a. How will the data be handled? Is there a data management plan that can be included or described? b. Where will they be stored, and what will happen to them after the study? Will they go into an open access data repository, for example? c. Will data collection be blind – I think it's not possible. So, will data analysis be blind? This relates to #17a and #17b in the checklist and should be made explicit in the protocol manuscript. 3. A number of items in the Protocol Checklist require attention: a. #5b and #5c are given in the MS and/or clinicaltrials.gov data and should be checked for completion in the MS. b. There is no formal data management, ethics, or other committee structure for this trial, and there is no funder. This is fine, c. #18a and #18b, should be described in MS. d. I could not find attention to data management (#19) in the MS. e. #21a there is no formal DMC, this is fine but should be noted. f. #29 data access, open access? 4. MS Page 4, line 14. Nuance of language is important. "In addition, together with centrally integrated proprioceptive and visual stimuli, the vestibular system gives an internal representation of the environment and movements through it". Can better read, "….the vestibular system contributes to a coherent representation of the environment and…." The vestibular system is one part of a system, as your Figure 1 shows. Further, it is worth avoiding the term 'representation', as it is arguable whether or not the CNS 'represents' the environment or not -- a philosophical psychological issue that can be avoided by not using the term. It becomes important when considering the role of subcortical processing. 5. MS Page 15. A comment on technique. Cognitive-Motor Task. The use of two different cameras operating at different sampling rates (50 and 30 Hz) is odd, and means that synchronisation of the motion data from the two cameras is not simple. A better way to do this would be to use an optical 3D motion capture system to record the gait kinematics during the tasks. It would be of some considerable benefit to collaborate with a biomechanical engineer to get proper measures of these gait parameters that can more sensitively test for gait differences.

	References Merker, B. (2007). Consciousness without a cerebral cortex: A challenge for neuroscience and medicine. Behavioral and Brain Sciences, 30, 63-134.

REVIEWER	Verrecchia, Luca Karolinska Institute
REVIEW RETURNED	13-Mar-2021

GENERAL COMMENTS	Well written study protocol, with appropriate objectives according to the ongoing clinical research on the field. I´m not particularly confident in reviewing the conceptual framework at the basis of cognitive tests used in this work. Excluding few raised concerns, which I have listed in the attached file, I consider the project important and appropriate in the research context. - The reviewer provided a marked copy with additional comments. Please contact the publisher for full details.

VERSION 1 – AUTHOR RESPONSE

COMMENTS/SUGGESTIONS OF REVIEWER 1:

 Remark 1: MS Page 16. Statistical analysis. There is no mention of a statistical analysis plan, or the workflow for how these data will be managed and analyzed.

o Answer: As the word limit was already exceeded in the first version of the manuscript, the authors decided to include only a concise overview of the statistical analysis plan. The authors support the comment, however, that a more extended paragraph on the envisaged statistical workflow is desirable in a protocol article. Therefore, following the new checklist (cfr. Editorial remarks), the paragraph on statistical analysis has been extended (methods, page 17-18, line 22-9). More information on how the data will be managed, was added to the manuscript as well (cfr. editorial remarks, and remark 2 & 3).

 Old description: “All data will be analyzed using SPSS software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, V.26.0. Armonk, New York). The level of significance will be set at p = 0.05. The normality of the data will first be assessed using the Kolmogorov-Smirnov test, QQ plots and histograms. Normally distributed data will be presented as mean (SD) and non-normally distributed data as median (IQR). Cross-sectional results of the audiovestibular group (objective 2) will be studied first using Fisher’s exact test for categorical data, the (Paired) Student’s t test and the Mann-Whitney U test or the Wilcoxon rank-sum test for normally and non-normally distributed data, respectively. In addition, correlation analyses will be performed. In order to verify the expected outcomes of objectives 1 and 3 (within the typically developing and NDD group), both univariate (ANOVAs) and multivariate (linear mixed models, multiple linear and logistic regressions), as well as correlation analyses will be applied.”

 New description: “All data will be analyzed using SPSS software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, V.26.0. Armonk, New York). The level of significance will be set at p = 0.05. The normality of the data will first be assessed using the Kolmogorov-Smirnov test, QQ plots

and histograms. Normally distributed data will be presented as mean (SD) and non-normally distributed data as median (IQR). General characteristics of all participants will be described quantitatively. The data derived from questionnaires will also be presented in a quantitative way. If participants would prematurely cease their participation and would not complete one of the two appointments, they will still be included in the analyses on the outcome parameters of the first appointment. If the latter would occur, the participant will be replaced via additional recruitment to maintain the required sample size for the overall research questions and the assessment on the relation between the outcomes of both appointments. Within the typically developing group (objective 1), visual investigation and analytical analyses will be performed along with multiple linear and logistic regression analyses to determine whether participant (motor and cognitive) characteristics may predict the vestibular outcome parameters, taking into account possible confounding factors (cfr. supra). Cross-sectional motor and cognitive results of the audiovestibular group (objective 2) will be studied using Fisher's exact test for categorical data, the (Paired) Student's t test and the Mann-Whitney U test or the Wilcoxon rank-sum test for normally and non-normally distributed continuous variables, respectively. In addition, correlation analyses will be performed to assess the

association between the motor and cognitive outcome measures on the one hand, and the audiovestibular data on the other hand. Additionally, adjustments for potential confounders and subgroup analyses will be executed, if possible. In order to assess the occurrence and vestibular characteristics in the NDD group compared to a typically developing group (objective 3), the (Paired) Student's t test and variance analyses or the non-parametric alternatives in case of violation of the assumptions for continuous variables (e.g. VEMP amplitude and vHIT gain) will be executed. Categorical variables (e.g. absence/presence of VEMPs) will be analysed using the chi-square test."

- Remark 2: I have a number of questions left open about the data management and study

design: [a] How will the data be handled? Is there a data management plan that can be included or described?; [b] Where will they be stored, and what will happen to them after the study? Will they go into an open access data repository, for example?; [c] Will data collection be blind – I think it's not possible. So, will data analysis be blind? This relates to

#17a and #17b in the checklist and should be made explicit in the protocol manuscript.

o Answer [a]: There is no formal data management plan registered for this observational study design. However, a new paragraph on data management was added to the manuscript (cfr. editorial remarks) (methods, page 16-17, line 36-20).

o Answer to [b]: Based on this rightful comment, the following sentences were added to the manuscript (methods, page 17, line 4-14):

- "After data collection, all outcome parameters will be organized and stored by the principal investigator (RVH) in a password-protected database. In addition, the answers of the questionnaires, which will be collected with an interactive PDF document, will automatically be stored in a password-protected Excel-file. Validation checks, such as range checks for data values, were programmed to minimize the number of errors. Personal information will be pseudonymized, of which only the principal investigator and the supervisor of this project (LM) know the coding system. The information collected in this study is kept strictly confidential, and will be stored for 20 years. The (coded) data will, if possible, and in accordance with the General Data Protection Regulation (GDPR) and rules and regulations of the ethical committee and the Ghent University, be shared and/or added as supplementary material if this

would be expected by the editorial board of a journal."

o Answer to [c]: Due to practical considerations, the data collection and analyses will not be blind. To minimize the potential observer bias and to optimize quality control of the collected data, however, the data collection and analyses will be frequently discussed by a guidance team consisting of experts in the five disciplines related to this project. As this is, indeed, important information to mention in the manuscript, the following sentences were added (methods, page 17, line 14-20):

 "The data collection, organization (, and analysis) procedures will not be blind since these will be performed by the same principal investigator. To optimize quality control of the collected data, a guidance team for this project was assembled, which supports the principal investigator in the data collection process, discusses the study progress, and will be consulted when problems

would arise. This team consists of experts in (pediatric) audiology/vestibulology (LM), otology (ID), movement (FD), rehabilitation (HVW), and cognitive/psychological sciences (JRW), and, therefore, covers all disciplines involved in this project."

 Remark 3: A number of items in the Protocol Checklist require attention: [a] #5b and #5c (sponsor information) are given in the MS and/or clinicaltrials.gov data and should be checked for completion in the MS. [b] There is no formal data management, ethics, or other committee structure for this trial, and there is no funder. This is fine, but should be noted. [c] #18a and #18b (information on data collection), should be described in MS. [d] I could not find attention to data management (#19) in the MS. [e] #21a there is no formal DMC, this is fine but should be noted. [f] #29 data access, open access?

o Answer: Based on the remarks of the editor, the SPIRIT checklist was deleted and the SPIROS checklist for observational studies was included. However, most of the items mentioned by the reviewer, are also included in the SPIROS checklist and have been clarified in the manuscript:

o Answer [a]: As this project is not funded/sponsored (cfr. funding statement), these items could not be completed in the previous (SPIRIT) checklist.

o Answer [b]: Although this observational study project was officially registered, this study is not a clinical trial. Therefore, no formal data management plan, data management committee etc. were composed. The fact that there is an ethical committee and no funder for this study, was already mentioned in the first version of the manuscript (ethics and dissemination & funding statement). The absence of a formal data management plan was now added to the manuscript as well (methods, page 17, line 16): "No formal data management plan has been registered."

o Answer [c & d]: More information on data management was added, such as information on the quality control, collection, storage, coding, blinding, and access of the data. To provide more clarity on data management, the following paragraph ('Data collection and management') was added to the manuscript (methods, page 16-17):

"The described outcome parameters will be collected by the principal investigator (RVH), who was trained to perform the pediatric motor, cognitive, and audiovestibular assessments. The research data will be gathered through observation and manual measurements during the M ABC II, visual, intelligence, and the traditional neuropsychological assessments. The outcome parameters of the audiovestibular, computerized cognitive and motor assessments will be obtained by automatic measurements of the used equipment and software. After data collection, all outcome parameters will

be organized and stored by the principal investigator (RVH) in a password-protected database. In addition, the answers of the questionnaires, which will be collected with an interactive PDF document, will automatically be stored in a password-protected Excel-file. Validation checks, such as range checks for data values, were programmed to minimize the number of errors. Personal information will be pseudonymized, of which only the principal investigator and the supervisor of this project (LM) know the coding system. The information collected in this study is kept strictly confidential, and will be stored for 20 years. The (coded) data will, if possible, and in accordance with

the General Data Protection Regulation (GDPR) and rules and regulations of the ethical committee and the Ghent University, be shared and/or added as supplementary material if this would be expected by the editorial board of a journal. The data collection, organization (, and analysis) procedures will not be blind since these will be performed by the same principal investigator. To optimize quality control of the collected data, a guidance team for this project was assembled, which supports the principal investigator in the data collection process, discusses the study progress, and will be consulted when problems would arise. This team consists of experts in (pediatric) audiology/vestibulology (LM), otology (ID), movement (FD), rehabilitation (HVW), and cognitive/psychological sciences (JRW), and, therefore, covers all disciplines involved in this project. No formal data management plan has been registered."

o Answer [e]: The absence of a formal data management committee was added to the

manuscript (methods, page 17, line 16): "No formal data management plan and/or

committee have been registered."

o Answer [f]: The following sentences were added to provide more clarity on data access

(methods, page 17, line 7-10): "The (coded) data will, if possible, and in accordance with the General Data Protection Regulation (GDPR) and rules and regulations of the ethical committee and the Ghent University, be shared and/or added as supplementary material if this would be expected by the editorial board of a journal.".

 Remark 4: MS Page 4, line 14. Nuance of language is important. "In addition, together with centrally integrated proprioceptive and visual stimuli, the vestibular system gives an internal representation of the environment and movements through it". Can better read, "….the vestibular system contributes to a coherent representation of the environment and…." The vestibular system is one part of a system, as your Figure 1 shows. Further, it is worth avoiding the term 'representation', as it is arguable whether or not the CNS 'represents' the environment or not -- a philosophical psychological issue that can be avoided by not using the term. It becomes important when considering the role of subcortical processing.

o Answer: Based on this rightful suggestion, the indicated sentence was rephrased accordingly (introduction, page 4, line 8-10):

 Old description: "In addition, together with centrally integrated proprioceptive and visual stimuli, the vestibular system gives an internal representation of the environment and movements through it."

 New description: “In addition, together with centrally integrated proprioceptive and visual stimuli, the vestibular system contributes to a coherent perception of the environment and movements through it.”

 Remark 5: MS Page 15. A comment on technique. Cognitive-Motor Task. The use of two different cameras operating at different sampling rates (50 and 30 Hz) is odd, and means that synchronization of the motion data from the two cameras is not simple. A better way to do this would be to use an optical 3D motion capture system to record the gait kinematics during the tasks. It would be of some considerable benefit to collaborate with a biomechanical engineer to get proper measures of these gait parameters that can more sensitively test for gait differences.

 Answer: The authors agree that 3D motion capture would be desirable, but for the current project the authors did not have access to such a system. The limited set-up with two cameras, however, does allow us to compute the relevant dependent variables without needing synchronization. The camera in the frontal plane (Canon) will be used to calculate the step width. Stride/step length and time and walking velocity will be derived from the sagittal view (Nikon). This has been clarified in the text.

o Old description: “…two cameras (D3300, Nikon, Tokyo, Japan – operating at 50 frames/second, and D500, Canon USA, Inc., Melville, NY, USA – operating at 30 frames/second) (Figure 8) information on a variety of spatiotemporal parameters will be collected: stride and step length (cm), step width (cm), step and stride time (s), and walking velocity (cm/s).”

o New description: “…two cameras (D3300, Nikon, Tokyo, Japan – operating at 50 frames/second for the sagittal plane, and D500, Canon USA, Inc., Melville, NY, USA – operating at 30 frames/second for the frontal plane) (Figure 8) information on a variety of spatiotemporal parameters will be collected: step width (cm), based on the frontal images, and stride and step length (cm) , step and stride time (s), and walking velocity (cm/s) based on the sagittal images.”

COMMENTS/SUGGESTIONS OF REVIEWER 2:

 Remark 1: I don´t really understand the need of (additional). It´s enough in my opinion to mention, as you have made before, the occurrence of vestibular-like dysfunction in NDD children to contrast with underlying vestibular dysfunction.

o Answer: In order to address this rightful remark, the authors removed ‘(additional)’ in the abstract (abstract, page 2, line 15) and introduction (introduction, page 6, line 2).

 Remark 2: Which kind of randomization do you use in this protocol? I suppose it deals with the test battery order randomization?

o Answer: Matching of the participants based on ‘randomization order’ reflects, indeed, the order of the test battery. More specifically, order of the cognitive test battery, as the vestibular and motor assessment will be performed in the order as they were represented in the manuscript. The cognitive test battery will, however, be presented to the participants in a randomized order (Latin square counterbalanced design) to minimize learning and order effects. As this was not clearly mentioned in the manuscript, the following adjustments were made:

 The following sentences were added (methods, page 9, line 22-25): “During the cognitive test session, the eight neurocognitive tests will be performed in a randomized order (Latin square

counterbalanced design) in order to minimize learning and order effects. The vestibular and motor assessments will be performed in the order as described below.“

 “When group differences will be analyzed, all participants will be matched for the following variables: age, gender, handedness, (hearing loss) and randomization order.” was rephrased by “When group differences will be analyzed, all participants will be matched for the following variables: age, gender, handedness, (hearing loss) and randomization order of the cognitive test battery.” (methods, page 7, line 15-16).

 “When group differences will be analyzed, all participants will be matched for the following variables: age, gender, handedness, (hearing loss) and randomization order” was rephrased by “When group differences will be analyzed, all participants will be matched for the following variables: age, gender, handedness, (hearing loss) and randomization order of the cognitive test battery.” (methods, page 16, line 25).

 Remark 3: Given the type of stimuli, it may be more correct to use the dB sound pressure level. Normally the used device has a convertion table

o Answer: Based on the conversion of the neurosoft software, 95 dB nHL corresponds to 119 dB SPL. This was added to the manuscript (methods, page 10, line 18).

 Remark 4: two important informations are lacking here: [1] the cVEMP amplitude must be corrected for the muscle activity level, commonly measured just before the delivery of stimulus; [2] the number of stimulus repetitions used (Sweeps) for curve averaging during the cVEMP assessment + also here give the number of sweeps (oVEMP).

o Answer [1]: The authors of the current study indeed use corrected peak-to-peak (or ‘rectified’) amplitude as important outcome parameter of the cVEMP results, as this is the internationally recommended method of performing valid inter- and intra-subject comparisons (Dlugaiczyk, 2020; Lee et al., 2008; Papathanasiou et al., 2014; Rosengren et al, 2019). In accordance with the used software (Neurosoft), the algorithm to compute the peak-to-peak amplitude first calculates the averaged EMG level (in the pre-stimulus area), after which the whole response waveform is scaled according to this average EMG level (raw peak-to-peak amplitude/averaged EMG level). To provide more clarity on the calculation and use of the corrected peak-to-peak amplitude, this information was added to the manuscript (methods, page 10, line 24-28):

 Old description: “Contraction of the SCM muscle, necessary for this examination, will be achieved by lifting and rotating the child’s head to the non-stimulus side in supine position. Outcome measures that will be included in the database are the absolute latencies of P1 and N1 (ms), rectified interpeak amplitude, asymmetry ratio (%), and absence/presence of the cVEMP-response.”

 New description: “Contraction of the SCM muscle, necessary for this examination, will be achieved by lifting and rotating the child’s head to the non-stimulus side in supine position. Additionally, a pre-stimulus EMG measurement of at least 20 ms will be conducted for calculation of the background EMG activity. Outcome measures that will be included in the database are the absolute latencies of P1 and N1 (ms), rectified interpeak amplitude (raw peak-to-peak amplitude/averaged EMG level; according to the

Neurosoft software), asymmetry ratio (%), and absence/presence of the cVEMP-response.”

o Answer [2]: Based on these rightful suggestions, to provide more details on the cVEMP and oVEMP procedures, the amounts of sweeps have been clarified and the following sentences were added accordingly:

- “A minimum of 100 sweeps will be presented per trial, and at least two trials will be administered to ensure reproducibility of the response.” (methods, page 10, line 21-23).

- “For the oVEMP measurement, a minimum of 60 sweeps will be presented per trial.” (methods, page 10, line 36).

- Remark 5: Why add a test only in a group of study? I understand that the rotatory chair has been used before in NDD children, but if you will test the rotatory chair on NDD children you should add the same procedure in vestibular impaired and normal groups, to show significant differences. Otherwise, as mentioned before you may use the rotatory chair results already achieved in vestibular impaired children before the inclusion in the study. In this case, please confirm that in the text

o Answer: The authors would like to thank the reviewer for this critical remark, as this was erroneously described in the manuscript and, therefore, resulted in misinterpretation of the study design. In order to address this comment, the following sentence was rephrased (methods, page 11, line 9-11):

- Old description: “In the NDD group (objective 3), rotatory chair testing including a visual suppression test will be included as well.”

- New description: “To assess the occurrence and characteristics of vestibular dysfunctions in children with a NDD compared to a typically developing group (objective 3), rotatory chair testing including a visual suppression test will be included as well.”

- Remark 6: Please define the velocity and axis of head movement. Personally I do not consider the DVA a confounding factor in your project, as this parameter has a direct relation with one of the tested conditions (vestibular failure). So, it may be rather intended as outcome than confounding factor.

o Answer: In order to address this remark, more information on the DVA assessment was added to the manuscript:

- “The DVA will be completed with passive head movements; i.e. the examiner will stand behind the child and move the head of the participant in the horizontal plane with a velocity of 2Hz.” (methods, page 16, line 9-11).

o Answer: Additionally, in accordance with this rightful remark, the DVA measurement was added to the vestibular assessment paragraph as outcome measure. However, due to practical reasons (as the execution of the SVA and DVA is nearly identical), the information on the test administration is still described in the ‘confounding factors’ section. The authors hope that this compromise is capable of meeting the requirement of the reviewer:

- “Lastly, in order to assess the contribution of the vestibulo-ocular reflex during head movements, a dynamic visual acuity (DVA) test will be performed as well.

The execution and stimulus parameters are described in the ‘confounding factors’ section (cfr. visual screening).” (methods, page 11, line 25-27).

VERSION 2 – REVIEW

REVIEWER	Delafield-Butt, Jonathan University of Strathclyde, Laboratory for Innovation in Autism
REVIEW RETURNED	26-Apr-2021

GENERAL COMMENTS	All of my concerns have been addressed. The protocol manuscript is fine, and the study an interesting. The study team could improve the motor analysis method better with synchronised cameras. Good luck with the study.