PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	EFFECTS OF ACTUAL AND IMAGINED MUSIC-CUED GAIT TRAINING ON MOTOR FUNCTIONING AND BRAIN ACTIVITY IN PEOPLE WITH MULTIPLE SCLEROSIS: PROTOCOL OF A RANDOMISED PARALLEL MULTICENTRE TRIAL
AUTHORS	Seebacher, Barbara; Helmlinger, Birgit; Pinter, Daniela; Ehling, Rainer; Hegen, Harald; Ropele, Stefan; Reishofer, Gernot; Enzinger, Chris; Brenneis, Christian; Deisenhammer, Florian

VERSION 1 – REVIEW

REVIEWER	Solari, Alessandra Foundation IRCCS Neurological Institute C. Besta, Unit of Neuroepidemiology
REVIEW RETURNED	06-Sep-2021

GENERAL COMMENTS	This is a protocol of a multicenter, double blind RCT to assess the acceptability, safety and efficacy of motor imagery (MI) on gait function in people with multiple sclerosis. The patients will be randomized in 3 parallel arms (groups): Group 1 (MI with music and verbal support; 30 min, 4 times a week, for 4 weeks), Group 2 (MI with music and verbal support plus gait training with music; 15 + 15 min, 4 times a week, for 4 weeks), Group 3: (gait training with music alone and verbal support; 30 min, 4 times a week, for 4 weeks). A range of outcome measures will be assessed at baseline, post-intervention and 3-month follow-up: Walking speed (T25FW) and distance (2MWT) co-primary outcome measures; MS-related fatigue (NFI-MS); HRQOL (MusiQol); Kinesthetic and visual Imagery (KVIQ-10); motor imagery (comparison of the duration of imagined and actual walking a distance of six meters); mood (HADS); Cognitive function (MoCA, SDMT); music-induced motivation (BMRI-2); music-induced enjoyment (SAM); MS-specific self-efficacy (USE-MS-G). In addition, brain activation patterns at fMRI (baseline and post-intervention) of patients and healthy controls will be compared. The topic of the study is of interest, and the authors have previous experience on it. The fMRI sub-study further increases the importance of the study. I have a number of points requiring clarification: • (Page 5) In the trial registration, the name of the register should be given (here, the German Clinical Trials Register). • (Pages 6 and 9) 'The study intervention was developed based on previous study results and patient involvement' Please describe in what consisted the patient involvement.

	• (Page 11) 'Stratification …. age (<40, ≥40), gender (female, male) and disability (EDSS 2.0–3.5, 4.0–5.0).' Please explain the rationale of stratifying by gender. • (Page 12) Please give the order of administration of the patient-reported outcome measures at baseline and follow-up visits (see also SPIRIT- (see also SPIRIT- 20c PRO Elaboration table) • (Page 14) DMTs are categorized as 'lowly (?) effective' and 'highly effective'. Please operationalize and reference this categorization. • (Page 21) The Authors state that the analysis will be intention-to-treat. However, they do not report the method they will use to handle missing data (see also SPIRIT- 13c PRO Elaboration table).

REVIEWER	Crosby, Lucas University of Toronto, Rehabilitation Sciences Institute
REVIEW RETURNED	01-Nov-2021

GENERAL COMMENTS	Introduction My only comments are that the purpose and hypothesis statements could be more direct. Terms like 'explore the effects' and 'investigate to what extent' are somewhat vague. Since this is a multicentre RCT, and it is not exploratory, use direct terms like 'to determine the effect'. In terms of the hypotheses, again be more specific: - By effective for walking, do you mean all training groups will reach clinical significance (e.g., 20% change in walking distance), or just statistical significance over pre-test measurement? - Regarding changes in brain activation, do you mean increased or decreased activation? There is some evidence to suggest that increased brain activation to detrimental for attention and memory (see Bast, Pezze, & McGarrity, 2017), which is a key mental component of auditory cueing. This may not be the case for this investigation, but just to caution and be clear with what changes you expect to see. You begin to touch on this in the discussion of this protocol paper, but better to be clear upfront. Methods Line 133: cite the "previous study results" Line 138: cite the "previous study data" Line 352: change mid-sentence period to a comma Line 377: Aside from the footnote in Table 2, this is the first mention of DMT categories, and here this categorization is being included as a covariate in the analysis. This categorization should be explained (and referenced properly by supporting documentation) earlier in the methods. Figure 1 is not clear. It could be interpreted as a single group receiving three interventions. Add which groups are receiving which intervention, and clearly label which elements are shared by all three groups. Figure 2 – Consider including (perhaps in an appendix) some examples of the planned semi-structured, open-ended questions for complete methodological transparency. Discussion Lines 430-31: How could it impact? The sentence is not clear, please revise. Lines 444-47: There seems to be some conflict in the concluding statements. On one hand, the home-based intervention can be

	used independently, but on the other hand the intervention this study finds most effective can be put into practice, provided therapists are there to guide training? Please clarify. Potential limitations of this study protocol and/or limitations in achieving the objectives are not discussed.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Alessandra Solari, Foundation IRCCS Neurological Institute C. Besta Comments to the Author:
This is a protocol of a multicenter, double blind RCT to assess the acceptability, safety and efficacy of motor imagery (MI) on gait function in people with multiple sclerosis.
The patients will be randomized in 3 parallel arms (groups): Group 1 (MI with music and verbal support; 30 min, 4 times a week, for 4 weeks), Group 2 (MI with music and verbal support plus gait training with music; 15 + 15 min, 4 times a week, for 4 weeks), Group 3: (gait training with music alone and verbal support; 30 min, 4 times a week, for 4 weeks).
A range of outcome measures will be assessed at baseline, post-intervention and 3-month follow-up: Walking speed (T25FW) and distance (2MWT) co-primary outcome measures; MS-related fatigue (NFI-MS); HRQOL (MusiQol); Kinesthetic and visual Imagery (KVIQ-10); motor imagery (comparison of the duration of imagined and actual walking a distance of six meters); mood (HADS); Cognitive function (MoCA, SDMT); music-induced motivation (BMRI-2); music-induced enjoyment (SAM); MS-specific self-efficacy (USE-MS-G). In addition, brain activation patterns at fMRI (baseline and post-intervention) of patients and healthy controls will be compared.
The topic of the study is of interest, and the authors have previous experience on it. The fMRI sub-study further increases the importance of the study.
Dear Dr. Solari,
Many thanks for reviewing our study protocol and providing helpful comments. Please see our point-by-point response as follows.
I have a number of points requiring clarification:
• (Page 5) In the trial registration, the name of the register should be given (here, the German Clinical Trials Register).
We have added the name of the register.
• (Pages 6 and 9) 'The study intervention was developed based on previous study results and patient involvement' Please describe in what consisted the patient involvement.
We have added the following information: An MS advisory group was consulted to clarify any questions, for example, with respect to their music preference and suggestions for the duration of the imagined and actual gait training.
• (Page 11) 'Stratification …. age (<40, ≥40), gender (female, male) and disability (EDSS 2.0–3.5, 4.0–5.0).' Please explain the rationale of stratifying by gender.
We will use stratification by gender because the differences in gait patterns1 and muscle strength between males and females, reflected by walking tests.2 We have added the references to the manuscript.
• (Page 12) Please give the order of administration of the patient-reported outcome measures at baseline and follow-up visits (see also SPIRIT- (see also SPIRIT- 20c PRO Elaboration table)
We have added the following information to the Data collection section: […] with the order of the patient-reported outcome measures being randomised for each participant and visit to minimise order effects.
• (Page 14) DMTs are categorized as 'lowly (?) effective' and 'highly effective'. Please operationalize and reference this categorization.

Thank you for this suggestion. We have replaced ‘lowly’ by ‘moderately’, operationalised and updated the DMTs and provided a relevant reference for the categorisation. Responding to a request from Reviewer 2, we have added some DMT related information in the Data collection section. The manuscript text now reads:
Data collection: Three categories of disease modifying treatment (DMT) will be operationalised according to the disease activity and course (1) no DMTs; (2) moderately effective and (3) highly DMTs (active substances are detailed below Table 2). DMTs will be recorded handled as a covariate in the data analysis because they may affect the primary and secondary outcomes.3 4
Table 2 legend: 1Three categories of disease modifying treatment (DMT): (1) no DMTs; (2) moderately effective DMTs: interferon-b 1a and 1b, pegylated interferon-b 1a, glatiramer acetate, dimethyl fumarate, teriflunomide, azathioprine, intravenous immunoglobulins; (3) highly effective DMTs: alemtuzumab, cladribine, fingolimod, natalizumab, ocrelizumab, cyclophosphamide, mitoxantrone, rituximab, siponimod, ofatumumab, and ozanimod.4
• (Page 21) The Authors state that the analysis will be intention-to-treat. However, they do not report the method they will use to handle missing data (see also SPIRIT- 13c PRO Elaboration table).
We indeed missed to mention the method for handling missing data and have now added the following information to the Statistical analysis section: Using Little’s test of missing completely at random (MCAR) the assumption of missing completely at random will be tested, signified by a p-value >0.05.5 With data missing (completely) at random, multiple imputation will be used for handling missing data, or other strategies as appropriate.6

Reviewer: 2
Dr. Lucas Crosby, University of Toronto
Dear Dr. Crosby,
Thank you very much for reviewing our study protocol and advice. Please see our point-by-point response as follows.
Comments to the Author:
Introduction
My only comments are that the purpose and hypothesis statements could be more direct. Terms like ‘explore the effects’ and ‘investigate to what extent' are somewhat vague. Since this is a multicentre RCT, and it is not exploratory, use direct terms like ‘to determine the effect’.
We have expressed the study purpose more directly: Therefore, the purpose of this study is to determine the effects of actual and imagined rhythmic-cued gait training versus their combination on walking, cognitive and emotional functioning in pwMS. Further aims are to compare brain activation changes during a motor or MI task between groups and determine which changes are specifically associated with improvements in gait function.
In terms of the hypotheses, again be more specific:
- By effective for walking, do you mean all training groups will reach clinical significance (e.g., 20% change in walking distance), or just statistical significance over pre-test measurement?
Thank you for this comment. By effective for walking, we mean statistical significance, but we will also determine the percentage of participants who reached a clinically significant improvement. We have clarified the hypothesis accordingly: All trainings will significantly improve walking, fatigue, QoL, emotional and cognitive functioning, and normalise brain activation (i.e., a more focal activation of the sensorimotor network as observed in healthy controls) in pwMS.
- Regarding changes in brain activation, do you mean increased or decreased activation? There is some evidence to suggest that increased brain activation to detrimental for attention and memory (see Bast, Pezze, & McGarrity, 2017), which is a key mental component of auditory cueing. This may not be the case for this investigation, but just to caution and be clear with what changes you expect to see. You begin to touch on this in the discussion of this protocol paper, but better to be clear upfront.
We thank you for suggesting a clear specification of our expectations regarding brain activation changes underlying beneficial gait training in pwMS. We therefore adapted the paragraph in the introduction accordingly. We furthermore agree that, considering the complex interplay of primary and

secondary brain regions associated with motor performance, not only increased brain activation (e.g., in the primary motor cortex), but also decreased brain activation (e.g., in supporting frontal brain areas) might be expected due to beneficial training. We have now included this important notion in the discussion.

Methods
Line 133: cite the “previous study results”
We have added the references accordingly.7-10
Line 138: cite the “previous study data”
We had already cited our previous study with the effect size and have now moved the reference according to your suggestion.
Line 352: change mid-sentence period to a comma
We did accordingly.
Line 377: Aside from the footnote in Table 2, this is the first mention of DMT categories, and here this categorization is being included as a covariate in the analysis. This categorization should be explained (and referenced properly by supporting documentation) earlier in the methods.
Thank you for this suggestion. Responding to your recommendation and a request from Reviewer 1, we have replaced ‘lowly’ by ‘moderately’, operationalised and updated the DMTs and provided a relevant reference for the categorisation. We have not explained the DMT categorisation because of the space restrictions, but this is explained in detail in the cited white paper.
We have also added DMT related information in the Data collection section:
Three categories of disease modifying treatment (DMT) will be operationalised according to the disease activity and course (1) no DMTs; (2) moderately effective and (3) highly DMTs (active substances are detailed below Table 2). DMTs will be recorded and handled as a covariate in the data analysis because they may affect the primary and secondary outcomes.3 4
Figure 1 is not clear. It could be interpreted as a single group receiving three interventions. Add which groups are receiving which intervention, and clearly label which elements are shared by all three groups.
We have revised Figure 1 accordingly and hope that the intervention is clearly presented now.
Figure 2 – Consider including (perhaps in an appendix) some examples of the planned semi-structured, open-ended questions for complete methodological transparency.
Thank you for this advice. We have included examples of the planned semi-structured, open-ended questions in Supplemental File 3.
Discussion
Lines 430-31: How could it impact? The sentence is not clear, please revise.
We agree and have revised the sentence. We have moved the sentence slightly to enhance the clarity: Pleasurable, motivating music is known to induce highly enjoyable emotions, motivation and arousal.11 Music-based interventions have been found to improve motor performance, mood and cognition in healthy people and patients with neurological disorders including MS.12 13 This may be relevant because studies have further shown that depression14 and cognitive or higher levels of motor impairment15 16 reduce the MI ability in pwMS.
Lines 444-47: There seems to be some conflict in the concluding statements. On one hand, the home-based intervention can be used independently, but on the other hand the intervention this study finds most effective can be put into practice, provided therapists are there to guide training? Please clarify.
We again agree that these statements were somewhat contradictory as we had not expressed ourselves clearly. The revised statement reads as follows: Advantages of a home-based intervention are that pwMS can practise independently, provided that specifically trained physiotherapists familiarise them with the programme and guide their initial training phases.
Potential limitations of this study protocol and/or limitations in achieving the objectives are not discussed.

Thank you for this comment. We have discussed potential limitation as follows: The absence of a physiotherapist during the homebased intervention could be a potential limitation of this study. Using a thorough familiarisation to the music-supported MI and gait training, as well as regular telephone support, this limitation should be overcome. A further limitation could be a lack of motivation and adherence in participants, which we aim to counterbalance using weekly support phone calls and further support calls upon request. A potential limitation in achieving the study objectives may be patients' hesitancy to undergo two extra MRI investigations at Centre 3. Patients will be explained that they will be provided with the examination results at their request, which their treating doctors may include in their consultation and treatment planning.

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VERSION 2 – REVIEW

REVIEWER	Solari, Alessandra Foundation IRCCS Neurological Institute C. Besta, Unit of Neuroepidemiology
REVIEW RETURNED	06-Dec-2021

GENERAL COMMENTS	I am fine with the revised manuscript.

REVIEWER	Crosby, Lucas University of Toronto, Rehabilitation Sciences Institute
REVIEW RETURNED	22-Dec-2021

GENERAL COMMENTS	Upon review of the author's response and revised manuscript, the authors have addressed all prior concerns and questions. I have no further questions/concerns and I will be recommending the publication of this study protocol paper.