Author’s response to reviews

Title: Evaluating the effect and mechanism of upper limb motor function recovery induced by immersive virtual reality-based rehabilitation for subacute stroke subjects: study protocol for a randomized controlled trial

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Author’s response to reviews:

Reviewer #1: This manuscript presents an interesting and useful question within the area of rehabilitation research after stroke. The study is well systematized and presented as well as details important aspects of the research. I have minor comments that I believe could strengthen the value of the study.

Thank you very much for your time and helpful comments. We revised our paper according to your suggestions.

1. - Your SPIRIT figure is incomplete. Please insert all information about the timeline phases of your study in the SPIRIT figure. This figure should be detailed and filled with all information pertinent to the study. In addition, in accordance with the Trials Journal submission guidelines, the figure should be included in the main body of the text and referenced in the text.
Response: The SPIRIT figure has been updated and its reference is included. Please see Fig. 2: Schedule of enrollment, intervention, and assessments.

2. - The form you entered as SPIRIT checklist is not correct. The correct file is named "Fillable Checklist" and is available in http://www.spirit-statement.org/publications-downloads/ (in accordance with Trials Journal submission guidelines). Please insert the correct SPIRIT checklist as additional file and filled with all study information.

Response: Thank you for the correction. The SPIRIT checklist was updated.

3. - Study design section: Please insert more information about the randomization aspect, i.e inserts the allocation ratio, block size and how the randomization sequence will be generated.

Response: The randomization section of "Study design" was revised as follows:

“60 subjects diagnosed with stroke in its subacute stage (defined as more than 1 week and less than 12 weeks after stroke onset) from an in-patient stroke rehabilitation unit in China will be enrolled in a single-blinded randomized controlled trial for 15 weeks. They will be randomized in a 1:1 fashion into: (1) a new 3-week rehabilitation training program with an immersive VR system, (2) a 3-week conventional rehabilitation program. The random allocation will follow a covariate-adaptive randomization procedure(1, 2). Each subject was randomly assigned a code based on a computer-generated stratified permuted block, randomization with block size of 8 and balanced by age and location of stroke.”

4. - In the Figure 1: in order to improve reader's understanding, please change the word "randomize" to "randomization".

Response: The word was changed to "randomization" in Figure 1.

5. - Sample size considerations section: please clearly state the rate of dropout of patients expected in your study and whether you will be considering any strategy to reduce this rate during conduct of the study.

Response: "Sample size consideration" section was revised as follows:
"As a conservative estimate (dropout rate = 25%), we presume that 60 subjects will complete the study. To reduce the dropout rate, we will employ two strategies to keep participants engaged: regular communication via phone or social media and clinician visits."

6. - Statistical analysis section: Please report how you will prevent or treat missing information.

Response: "Statistical analysis" section was revised as follows:

"To help prevent missing data, all questionnaires are user friendly and collected electronically, and all personnel related to the study are trained to identify and engage the subjects who are at the greatest risk of dropout during follow-up. In cases of missing data, the baseline-observation-carried-forward method(3) will be used to impute these data from all enrolled subjects."

Reviewer #2: Huang et. al propose a randomized controlled trial to investigate the "effect and mechanism of upper limb motor recovery induced by immersive virtual reality-based rehabilitation for subacute stroke subject." In this proposal, the investigators aim to: 1) Assess the efficacy of immersive VR based upper extremity rehabilitation in subacute stroke patients 2) Explore underlying brain mechanisms of VR-based rehabilitation. Task specific motor learning is an area of much interest in Neurology and more trials are certainly needed in this area, however there are areas in the current proposal that warrant improvement prior to undertaking.

Thank you very much for your time and helpful comments. We revised our paper according to your suggestions.

Below are specific comments for the investigators by sections of the grant as well as a general summary of the study at the conclusion of the review.
Abstract:

1) The investigators should use caution when utilizing the word efficacy, particularly in the setting of clinical trials as the reader will immediately think of efficacy vs. effectiveness trials, and given that efficacy trials normally reflect a highly selective homogeneous population with several exclusion criteria, this term wouldn't be the best reflection of the proposed study.

Response: "efficacy" was replaced by "effectiveness", which correctly reflects the nature of the study.

2) It would be helpful to define non-immersive in the abstract, as those not familiar with this area will not understand the difference

Response: The definition of non-immersive systems was included in the "Abstract" section.

"There is compelling evidence of beneficial effects of non-immersive virtual reality (VR)-based intervention on rehabilitation for patients with stroke, where patients experience both the real world and the virtual environment."

3) The investigators would do well to include the timing of intervention (specifically when the intervention will begin post-stroke and when it will conclude post-stroke, the enrollment window and the treatment window) in the abstract. As written, "over a 3-week time period" feels vague.

Response: We have added additional information on the timing of the intervention, per your suggestion.

"60 subacute stroke subjects (defined as more than 1 week and less than 12 weeks after stroke onset) will be recruited to participate in a single-blinded, randomized controlled trial. Subjects will be randomized 1:1 to either: 1) an experimental intervention group, or 2) a conventional group (control). Over a 3-week time-period immediately following baseline assessments and randomization, ..."
4) The term brain reorganization should be defined in the abstract as it is an outcome of interest

Response: We have now defined brain reorganization.

"To trace brain reorganization in which upper extremity functions previously performed by ischemic-related brain areas are assumed by other brain areas, ..."

5) Again, it would be helpful to define the timing of the scans. Does before rehabilitation mean that a scan one year before rehabilitation would be permissible?

Response: We have clarified the timing of the scans.

"brains of subjects will be scanned immediately following enrollment but before randomization, immediately following the conclusion of rehabilitation, and 12 weeks after rehabilitation has concluded."

6) The discussion focuses on the 2nd aim, but does not mention the 1st aim. How will efficacy be defined?

Response: The discussion was revised as follows to clarify this point:

"The effectiveness is assessed by evaluating motor improvement using the arm motor section of the Fugl-Meyer Assessment. The study utilizes a cutting-edge brain neuroimaging approach to trace longitudinally the effectiveness of both VR-based and conventional trainings on stroke rehabilitation, which will hopefully describe brain mechanisms of intervention effects on stroke recovery. Findings from the trial will greatly contribute to evidence for the use of immersive VR-based training for stroke rehabilitation."

Introduction:
1) Regarding stroke incidence, it is not increasing in all areas across the globe. One would recommend caution in making such statements


Response: The statement about stroke incidence was revised and the above article was referenced.

"Its incidence is decreasing in the US(4) but rising in China(5)."

2) It would be helpful to cite current literature that discussed the outcomes of what is the current norm. For example, how effective is current traditional upper limb rehabilitation?

Response: We have added additional information about effectiveness of current traditional upper limb rehabilitation.

"Conventional rehabilitation techniques are effective in improving upper limb function but are resource-intensive and costly, often requiring specialized facilities not always widely available(6-8)."

3) There has been some controversy surrounding constraint induced movement therapy and its true improvement in upper limb function. This literature should be acknowledged.

Response: We have now acknowledged this literature(9).

4) The summary on page 4 is well written and helps set the stage for the study. It could be further improved upon by defining what neural mechanisms of recovery are specifically being targeted in this approach.

Response: We will focus on the motor execution network and the corresponding paragraph was revised as follows:
"Functional magnetic resonance imaging (fMRI) has played an important role in exploring the neural mechanisms of recovery after brain disease, for instance, exploring the motor execution network of patients after a stroke(10, 11)."

5) Why are subacute stroke patients being targeted? This should also inform some of the background information for the proposal.

Response: One paragraph was added in the "Background" section to provide background and clarify why subacute stroke patients are targeted in our study.

"There are two reasons for choosing subacute stoke subjects rather than other stages post-stroke in the clinic trial. First, in this subacute stage, patients have been shown to have the best and most rapid functional recovery(12), which benefits the observations of critical brain reorganization. Secondly, in China most stroke patients would be hospitalized during this stage, which makes recruitment and MRI scanning easier."

6) What is the hypothesis for this clinical trial?

Response: It is presumably assured motor learning and motor control plays a pivot role to the development of sensorimotor interventions for post-stroke recovery and VR-based intervention may enhance motor learning and motor control(6). This paragraph was added in the "Aim" section.

" It is presumably assured motor learning and motor control plays a pivot role to the development of sensorimotor interventions for post-stroke recovery and VR-based intervention may enhance motor learning and motor control(6)."

Methods:

1) Please define how efficacy is being assessed or defined in the study. This should be information that is included early in the proposal and is essential when considering power calculations for a study.

Response: The "Aim" section was rewritten as follows to define effectiveness:
"The aim of the study is to assess the effectiveness of immersive VR-based upper extremity rehabilitation on patients with subacute stroke and explore the underlying brain mechanisms of immersive VR-based rehabilitation. The arm motor section of the Fugl-Meyer Assessment (FMA)(13, 14) will be used to assess the effectiveness, and MRI techniques will be applied to investigate the brain mechanisms. The details will be discussed in sections "Primary outcome measure" and "Secondary outcome measures"."

2) When writing a trial protocol, it is helpful to use proper clinical trial terminology. Specifically, when mentioning that randomization will occur evenly, does that mean 1:1?

Response: "Evenly" was changed to "1:1".

3) How was the number for 60 subacute stroke subjects determined? What was the literature that formed this power calculation?

Response: The number 60 was determined by two factors: 1. power calculation 2. budget and availability of subacute stroke subjects at the research site. The power analysis was calculated using G*Power software. We have added this additional information to the manuscript:

"After conducting a power analysis based on the aforementioned statistical parameters using software GPower3.1.9.2(15), the effect size is calculated as 0.74, which is between a medium (0.5) and large (0.8) effect size(16)."

4) What will form the basis of the 3-week conventional rehabilitation program? As this is the control arm, it is important to define this carefully. Later it is mentioned that 60-minutes of conventional rehab training per day is specified, but will this be the same in all 30 patients? Will it be only targeting the affected upper limb? How much time will be spent doing each activity?

Response: A more thorough description about the conventional rehabilitation program was added to the manuscript.
This conventional rehabilitation delivered by a therapist at the hospital includes physical and occupational therapy (upper extremities flexion and extension training) which comprise task-related practice for gross movements and dexterity, including different grips and selective finger movements, strength training, stretching, and training in daily life activities. Conventional rehabilitation will be designed with similar intensity and complexity to simulate the skills required in the immersive VR group. Researchers in the study will supervise and encourage all participant to fully participate in the training to guarantee the quality of the training…

Yes, the rehabilitation will be only targeting the affected upper limb. The total time is same for all 30 patients but the time for each activity is different, dependent on the status and progress of each patient.”

5) What is the self/clinician filled questionnaire? Will there be two questionnaires? The participant and the clinician may have very different view of the progress of a participant.

Response: The patient's healthy history was entered by the patient or his/her family member into REDCap. All assessments were performed by clinicians. For clarification, the word "self" was removed from the manuscript.

6) The flow diagram is helpful but needs to be improved upon by defining the post-training assessment. As this is a main outcome of interest, the definition by this point in the grant is still unclear. Later it is defined as the Fugl-Meyer score.

Response: Fig.2 was created, which now lists the timing of all questionnaires.

7) Inclusion criteria state that Brunnstrom stages 1-3 will be considered eligible for enrollment. Stage 1 is flaccid paralysis. How will participants be able to participate in VR if they are completely flaccid?

Response: Thank you for your thoughts on this aspect of the study. Only Brunnstrom stages 2-4 will be considered eligible in the upcoming study.

8) Given the small sample size, it does not appear prudent to allow for participants to use the unaffected side limb to help with participation in the tasks. This would form an area of great concern when assessing the outcome.
Response: Only subjects with Brunnstorm stage 1 were to be asked to use the unaffected side limb, due to flaccidity. Since we have now changed the inclusion criteria to only include subjects with Brunnstrom stages 2-4, this description was removed from the manuscript.

9) There are numerous outcome measures on such a small group of patients with multiple time points. Once again, the power calculations should reflect this if all outcome measures are kept. The data that appears to have been used by the team is those with chronic back pain, is this a fair comparison to those with stroke, who will already have some degree of brain damage?

Response: When we do statistical analysis, Bonferroni correction will be used to counteract the problem of multiple comparisons. The brain parameters obtained by MRI will be assessed and correlated with the clinical outcome measures. Other stroke-associated measures will also be evaluated as covariates in regard to the primary outcome measure.

From our observation, the brain reorganization will be more significant for subjects with stroke than those with chronic back pain. But the data of patients with chronic back pain will never be used in this study. To avoid confusion, the description about chronic back pain was removed and a new reference was added.

"Moreover, there was evidence from a small sample (8 subjects) that virtual reality-enhanced treadmill training for 5 sessions per week over 3 weeks induced significant cerebral reorganization(17)."

10) Size and location of stroke will be a great determinant when considering the outcome of the trial. Was stratification considered? How will this be accounted for in adjustment models?

Response: Size and location of stroke was considered during randomization.

"The random allocation will follow a covariate-adaptive randomization procedure(1, 2). Each subject was randomly assigned a code based on a computer-generated stratified permuted block, randomization with block size of 8 and balanced by age and location of stroke."
Discussion:

1) In the discussion, the authors appropriately recognize sample size as a limitation and the need for multicenter, large-scale trials. Yet this is followed by a statement regarding feasibility and efficacy, while the proposed trial does not include feasibility as an outcome.

Response: "Feasibility" was removed in the manuscript.

Summary:

The proposed study is an area of interest and much active research, particularly given task based learning following stroke and the unique utilization of functional MRI. However, the trial is limited in its description of its outcome measures and would be greatly improved upon by carefully defining the outcomes; specifically efficacy, self/clinician questionnaires and changes in neuronal network. The sample size proposed for the trial, particularly when considering multiple outcome measures and the literature considered, does not seemingly compute and should be revisited.

Response: Thank you for your helpful comment with regards to the sample size. As mentioned in the "limitation" section, we plan to contact another local Stroke Rehabilitation Institute to recruit additional stroke patients to this study within the proposed time frame.

Reviewer #3: This is a very interesting article and generally well written, however, below please see some comments / suggestions:

Thank you very much for your time and helpful comments. We revised our paper based on your suggestions.

Abstract - well written and concise, there are some spelling and language errors in the text.

Response: Spelling and language was double-checked.
Background - minor language editing needs to be done for clarification (for example in paragraph 1 - lines 33-36 needs restructuring to make the content easier to read)

Response: For clarification, paragraph 1 - lines 33-36 was restructured.

"Stroke is a major cause of death and long-term disability across the globe (18, 19) and its incidence is decreasing in the US (4) but rising in China (5). A common disabling consequence of stroke is upper limb dysfunction (20), which significantly affects patients' activities of daily life. Therefore, one of the main goals of stroke rehabilitation is to improve upper limb function."

I would also like to suggest that some of the information pertaining to non-immersive VR therapy is either omitted or more extensively compared to immersive VR therapy (or the lack of evidence for immersive VR therapy must come out clearly) - the depth of information on the non-immersive VR therapy creates the impression that the researchers intend to study it.

Response: The description order of non-immersive VR and immersive VR in the manuscript was adjusted to clarify that immersive VR is the focus of the research.

"For the past several years, non-immersive VR systems have been widely used in stroke rehabilitation, with the aim to improve motor function (6, 21). Most of these studies have indicated that the non-immersive VR-based rehabilitation was effective for upper limb functional improvement in individuals following stroke (22-25) but not significantly more beneficial compared with conventional rehabilitation, likely due to lack of relevant tasks provided by non-immersive VR (6). Although it was reported that physical exercises through VR programs were effective for functional improvement in subjects with neurologic disorders (26) and that immersive virtual reality systems may enhance motor learning and motor control (6), only one study demonstrated that the immersive VR-based rehabilitation can improve fine hand motion rehabilitation training effectiveness (27). However, what is the most appropriate frequency, intensity, and type of immersive VR-based rehabilitation to promote motor recovery and critical brain reorganization in this early post-stroke stage remains unknown. Functional magnetic resonance imaging (fMRI) has played an important role in exploring the neural mechanisms of recovery after brain disease, for instance, exploring the motor execution network of patients post-stroke (10, 11). Therefore, a longitudinal Magnetic Resonance Image (MRI) study (similar to ones performed previously in subjects with subacute back pain (28, 29)) will be implemented. Using MRI techniques, brain mechanisms related to strategies that enable the rehabilitation of repetitive, relevant, and skilled activities in the early stage post-stroke can be explored."
Aim - i suspect the aim has been altered to suit the article, but rephrase the aim for clarity (the aim is to test the efficacy of the intervention, not to compose an article / proposal for publication)...the aim in the discussion should be presented in this section.

Response: The aims paragraph has been revised.

"The aim of the study is to assess the effectiveness of immersive VR-based upper extremity rehabilitation on patients with subacute stroke and explore the underlying brain mechanisms of immersive VR-based rehabilitation. The arm motor section of the Fugl-Meyer Assessment (FMA)(13, 14) will be used to assess the effectiveness, and MRI techniques will be applied to investigate the brain mechanisms. The details will be discussed in the sections "Primary outcome measure" and "Secondary outcome measures"."

Methodology - the flow chart is excellent and gives a very clear indication of the study procedure. However, the eligibility criteria is very broad - maybe a motivation for the wide age range (and the comparability of patients within this wide age range) should be provided. If the participants are randomly allocated and the one group has patients ins the 65 - 85 age range and the other group has patients in the 30 - 50 age range (by chance), will the groups still be comparable?

Response: In order to accommodate more subjects, the inclusion age range is set wide (30-85 years old). However, the randomization scheme will consider two factors: age and location of stroke.

"The random allocation will follow a covariate-adaptive randomization procedure(1, 2). Each subject will be randomly assigned a code based on a computer-generated stratified permuted block, randomization with block size of 8 and balanced by age and location of stroke."

The intervention period is very short (although a follow-up period is planned) - is this due to the information provided in the background that indicated that upper limb rehabilitation is usually a tedious and long process OR is there sufficient evidence in the literature to indicate that improvement can be observed after three weeks of intervention? The short intervention period may adversely affect the outcome of the study.
Response: Motor function is mostly recovered within 10 weeks post-stroke(30) and the recovery is relatively rapid during the first 4-weeks of intervention(31). Moreover, there was evidence from a small sample (8 subjects) that virtual reality-enhanced treadmill training for 5 sessions per week for 3 weeks induced significant cerebral reorganization(17).

In the VR intervention procedure it is stated that participants can either sit in a 'regular' chair or a wheelchair - will the environment be altered to provide similar seating options for patients who chose either of these options - my concern is that patients in a 'regular' chair will not have arm rests, as patients in wheelchairs would, that could potentially influence the extent to which they can move their arms while participating in the VR intervention.

Response: We have clarified that all patients will be in a wheelchair.

I would also like to recommend that a second outcome measure to determine upper limb function is added - so that there is a clear differentiation between gross- and fine motor improvement (or not) after the intervention.

Although this is not an aim of the study, the researchers may like to consider adding an additional tool (such as the star cancellation test) to track hemispatial neglect - this influences upper limb recovery tremendously and could inadvertently improve due to the head movements that the participants will perform during the VR therapy.

Response: Unfortunately, there is no verified Chinese-version of the star cancellation test. We anticipate many of our participants to not be familiar with English letters, so we are unable to add this outcome measure.

Throughout the article, please ensure that the time frames indicated remains the same - the study will be 15 weeks in total and the follow-up will be 12 weeks after the 3 week intervention (if I understand the information correctly) - this is not clear throughout the study, at times it seems as if the follow-up will be after 15 weeks of the intervention.

Please move the sample size calculation to the sampling description (just after the eligibility criteria) - this will provide greater clarity on the information provided in the sampling section.

Response: The time points in the paragraph of explaining the flow chart of the study (Fig.1) were revised to match Fig. 1. Section "Sample size considerations" was moved after section "Participants".

Discussion - well written and a good summary of the study.
Good luck with the study - looking forward to the results!

Reviewer #4: Virtual reality-based rehabilitation is an inmerging rehabilitation choose for both stroke patients and therapists but its effect needs to be verified. The author aimed to assess the efficacy of immerse VR training and find the most appropriate frequency, intensity, and type of immersive VR-based rehabilitation for stroke patients via a longitudinal fMRI study.

Thank you very much for your time and helpful comments. We revised our paper in response to your suggestions.

Some suggestions are as follow:

1. Where will you carry out your study, in hospital or anywhere? Which kind of patients will you select, outpatients or inpatients?
   
   Response: This study will be carried out in the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University. All participants are inpatients. The paragraph about this information in the "Study design" section was revised as follows:

   "60 subjects diagnosed with stroke in its subacute stage (defined as more than 1 week and less than 12 weeks after stroke onset) from an in-patient stroke rehabilitation unit in China will be enrolled in a single-blinded randomized controlled trial for 15 weeks."

2. Give more details about interventions and care options that are permitted or prohibited during the study.

   Response: The description of the conventional rehabilitation program was added to the manuscript.

   "This conventional rehabilitation delivered by a therapist at the hospital includes physical and occupational therapy (upper extremities flexion and extension training) which comprise task-
related practice for gross movements and dexterity, including different grips and selective finger movements, strength training, stretching, and training in daily life activities."

Care options that are permitted or prohibited during the study are the same as inclusion or exclusion criteria for the participants. Please see the "Participants" section.

Reviewer #5: Thank you for the opportunity to review this manuscript. ON the whole this a generally well written manuscript and the study is an interesting look at improving rehabilitation in stroke patients. However, reading through I felt there were several areas where there was a lack of detail surrounding several aspects of the study. Further, many of the items on the SPIRIT checklist have not been detailed with no explanation of their omission. I have specified a number of areas below where further detail would improve the robustness of this manuscript as a protocol paper.

Thank you very much for your time and helpful comments. We revised our paper following your suggestions.

1) The title of the manuscript suggests that the use of immersive VR for sub acute stroke recovery is already effective. It may be worth re-phrasing the title to highlight that the effectiveness of VR is being investigated

   Response: The title was rephrased as

   "Evaluating the effect and mechanisms of immersive virtual reality-based rehabilitation on upper limb motor function recovery in subacute stroke patients: study protocol for a randomized control trial"

2) Items 3-5d on the SPIRIT checklist have not been completed and no reason for non-completion has been given. For transparency, these items should be described in the protocol.

   Response: The SPIRIT checklist was updated.
3) The study objective could be made more concise. It should not be described as an aim because aims and objectives are different. I would also advise removing the 'design' aspect from the objective as this is written as a trial of efficacy and not of feasibility.

Response: The "objective" was changed to "aim" and the "design" was removed. The "aim" section was revised as follows:

"The aim of the study is to assess the effectiveness of immersive VR-based upper extremity rehabilitation on patients with subacute stroke and explore the underlying brain mechanisms of immersive VR-based rehabilitation. The arm motor section of the Fugl-Meyer Assessment (FMA)(13, 14) will be used to assess the effectiveness, and MRI techniques will be applied to investigate the brain mechanisms. The details will be discussed in the sections "Primary outcome measure" and "Secondary outcome measures"."

4) It is not clear from the manuscript why the comparator group has been chosen to be 'rehabilitation as usual' only. This does not control for the use of a novel intervention (VR) and so the design is not purely testing the use of immersive VR, but is also testing the effect of an additional intervention. Did the authors consider controlling for this by providing the control group with a non-specific VR experience/ session? This would help reduce bias in the control group. It would also be helpful if the authors could explain why they chose for the experimental group to have a combination of immersive VR and normal rehabilitation.

Response: Adding a control group with a non-specific VR experience/ session to the study is an excellent suggestion and we will plan to do so in a follow-up investigation.

The reason that those in the experimental group will have a combination of immersive VR and normal rehabilitation is due to immersive VR technology limitations, as in it is still unable to simulate all rehabilitation trainings. However, in clinical practice, it is shown that patients who receive comprehensive rehabilitation training rather than only single training have better clinical outcomes. As a compromise, for those in experimental group, they will have a combination of immersive VR and normal rehabilitation with similar intensity and complexity to ensure a comprehensive 60-minute training.

The above mentioned limitation was added to "Discussion" section.
Another limitation is that current immersive VR technology is still unable to simulate all rehabilitation trainings. As a compromise, for subjects in the experimental group, they will experience a combination of 30-minute normal rehabilitation and 30-minute immersive VR rehabilitation.

5) The study setting (Item 9 SPIRIT checklist) is not adequately described on p.5 Where is the intervention taking place? Is it in clinic? A rehabilitation centre? Please clarify

Response: The paragraph about this information in "Study design" section was revised as follows:

"60 subjects diagnosed with stroke in its subacute stage (defined as more than 1 week and less than 12 weeks after stroke onset) from an in-patient stroke rehabilitation unit in China will be enrolled in a single-blinded randomized controlled trial for 15 weeks."

6) There is not enough detail concerning who will deliver the conventional rehabilitation and what constitutes this. I would suggest reviewing the TIDiER guidelines for the reporting of complex non-pharmacological interventions and using this framework to report the intervention.

Response: The paragraph regarding conventional rehabilitation was revised as follows:

"This conventional rehabilitation delivered by a therapist at the hospital includes physical and occupational therapy (upper extremities flexion and extension training) which comprise task-related practice for gross movements and dexterity, including different grips and selective finger movements, strength training, stretching, and training in daily life activities."

7) How will the amount of conventional rehabilitation (not just length of session) be measured? If one participant puts in a lot of effort into their exercises and another participant puts in very little effort, how will that be recorded, if at all?

Response: The following paragraph was added to the "Intervention design" section.
"Conventional rehabilitation will be designed with similar intensity and complexity to simulate the skills required in the immersive VR group. Researchers in the study will supervise and encourage all participant to fully participate in the training to guarantee the quality of the training."

8) For the primary outcome measure- as there are multiple domains within the Fugl-Meyer assessment of upper limb motor function, will the primary outcome be a composite score of all the domains, or will a single domain be selected as the primary outcome prior to analysis?

Response: The primary outcome will be a composite score of all the domains. We have clarified this in the manuscript.

9) The sample size calculation sample appears a little simplistic. It does not seem to take into account what would be considered a clinically relevant change in the Fugl-Meyer assessment and to what degree it would be expected for the control group to improve with conventional rehabilitation alone. I think this would benefit from some expert input from a trials statistician and should be revised accordingly.

I also struggle with the use of a sample size from an MRI study into neural mechanisms of chronic back pain to determine the required sample size for a study into the neural mechanisms of post stroke rehabilitation, although this may come from a lack of understanding of imaging studies on my part. Even if the imaging techniques have demonstrable change in the back pain study for that number of subjects; that does not automatically mean that you will be able to detect imaging changes in the same number of people recovering from stroke; the likely white/grey matter changes will be fundamentally different between the two cohorts. The authors need to explain the similarities in far greater detail to be understood by non-imaging expert audience.

Response: The "Sample size calculation" section was revised as follows to provide additional details:

" This randomized controlled trial is a two-group independent design examining the effects of immersive VR on rehabilitation of subjects with subacute stroke. We assumed a two-tailed comparison and set Type I error rate at 0.05 with 80% power. We plan to screen approximately 100 individuals with subacute stroke. After screening, 80 subjects will be recruited and randomized to the experimental group or the control group. As a conservative estimate (dropout rate = 25%), we presume that 60 subjects will complete the study. To reduce the dropout rate, we will employ two strategies to keep participants engaged: regular communication via phone or
social media and clinician visits. After conducting a power analysis based on the aforementioned statistical parameters using software GPower3.1.9.2(15), the effect size is calculated as 0.74, which is between a medium (0.5) and large (0.8) effect size(16). Moreover, there was evidence from a small sample (8 subjects) that virtual reality-enhanced treadmill training for 5 sessions per week over 3 weeks induced significant cerebral reorganization(17). Thus, 30 subjects for one group is sufficient to assess the effectiveness of immersive VR training in post-stroke rehabilitation in eliciting upper-limb motor recovery post-stroke compared to traditional rehabilitation training and investigate the underlying brain mechanisms of immersive VR-based rehabilitation using three MRI modalities.

10) Why is item 15 of the SPIRIT checklist not included? Is there a high enough throughput of eligible patients at the dingle site to allow recruitment to target within the recruitment period? Is this because this trial does not have any funding and as such is not subject to the usual time/ funding constraints usually expected in a clinical trial?

Response: Item 15 of the checklist was included. Since the stroke rehabilitation unit where we recruit and scan participants has an excellent reputation locally, we have enough eligible patients for the study. In order to expedite recruitment, we have contacted another local stroke rehabilitation unit for helping in recruitment, which is now mentioned in the "Discussion" section. One of concerns is that participant might miss the third MRI scan after conclusion of the rehabilitation program. A paragraph about prevention of missing data has been added.

"To help prevent missing data, all questionnaires are user friendly and collected electronically, and all personnel related to the study are trained to identify and engage the subjects who are at the greatest risk of dropout during follow-up. In cases of missing data, the baseline-observation-carried-forward method(3) will be used to impute these data from all enrolled subjects."

11) There is not enough information on how the random sequence will be generated by the computer. Are there any balancing factors? Will the randomisation control for factors such as age/ functional ability/ indices measured by MRI?

Response: The paragraph about randomization was revised as follows to provide additional details:
"The random allocation will follow a covariate-adaptive randomization procedure(1, 2). Each subject was randomly assigned a code based on a computer-generated stratified permuted block, randomization with block size of 8 and balanced by age and location of stroke."

12) There is no mention in the manuscript of any monitoring or auditing methods, either for monitoring data or oversight of the trial in general.

Response: A section "Study oversight and participant confidentiality" was added to the manuscript.

"Study oversight will be at the direction of an Independent Safety Monitor (ISM), composed of experts in clinical trials, medical ethics, statistics, and data management. The ISM is independent of the study and the sponsor, responsible for monitoring data and participant safety."

13) There is no indication of who within the study team will be taking informed consent form the participants, or who will be making the assessment of eligibility with regards to capacity. This should be included.

Response: The research coordinator on the study team will obtain informed consent. The assessment of eligibility will be performed by a physiotherapist who has at least 2-years of experience in physical therapy. The description about the assessment in "Study design" section was revised as follows to provide these additional details:

"The assessment includes inclusion and exclusion of subjects, clinician-filled questionnaires and MRI scans. To avoid assessment bias, all inclusion and exclusion assessments and clinician-filled questionnaires will be completed by physiotherapists who are blinded to this study and with at least 2-years of experience in physical therapy."

14) The authors should also include a statement about how participant's confidentiality is protected (if at all) via, for example, anonymisation of data.

Response: A statement about participant confidentiality was added as follows:
"We are committed to respect participant privacy and to keep personal information confidential. All participants' personal and health information and brain imaging data will be maintained on a secure server. Access to these data are password protected, and all data are anonymized and coded prior to uploading to the server."


