PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	The Effects of Priming Intermittent Theta Burst Stimulation on Upper Limb Motor Recovery After Stroke: Study Protocol for a Proof-of-Concept Randomized Controlled Trial
AUTHORS	ZHANG, Jack; Fong, Kenneth

VERSION 1 – REVIEW

REVIEWER	Fayaz Khan King Abdulaziz University Saudi Arabia
REVIEW RETURNED	11-Dec-2019

GENERAL COMMENTS	The authors deals with an innovative neurorehabilitative approach, which is the priming intermittent theta burst stimulation (iTBS) on post-stroke upper limb motor recovery. To achieve this goal, they propose a three-arm randomized controlled trial, with all participants randomly allocated to receive 10 sessions of repetitive transcranial magnetic stimulation (rTMS) with different TBS protocols (cTBS+iTBS, sham cTBS+iTBS, and sham cTBS+sham iTBS) and 60 minutes of robot-assisted training (RAT) after each stimulation session. Primary outcomes will be assessed using Fugl-Meyer Assessment – Upper Extremity scores and Action Research Arm Test, whereas secondary outcomes using kinematic outcomes generated during RAT and electroencephalography (EEG). Overall, the study is nicely conceived and designed; the protocol is adequately described and illustrated; therefore, it might disclose interesting translational findings. However, there are several issues needing attention and revision. MAJOR - Title: as the authors themselves state in the main text (i.e. “Ethics and dissemination”), this is actually a “proof-of-concept” study and, therefore, the title should be changed accordingly. - Introduction: at the end of the first sentence, in addition to the reference n. 1, a more recent review of the rTMS in stroke rehabilitation should be included (i.e. Repetitive transcranial magnetic stimulation in stroke rehabilitation: review of the current evidence and pitfalls. Ther Adv Neurol Disord 2019). - Introduction: it has been demonstrated that metaplasticity is significantly involved also in neuropsychiatric disorders, such as major depression (Repetitive transcranial magnetic stimulation in patients with drug-resistant major depression: A six-month clinical follow-up study. Int J Psychiatry Clin Pract 2015), and that metaplastic effects can be probed and measured by different TMS techniques (Cortical Plasticity in Depression. ASN Neuro 2017).

	Given the relevance of this topic within the proposed study, a short mention should be included. - Introduction: the EEG is not a “brain imaging technique” but rather a “brain electrophysiological technique” (or a “neurophysiological technique”); please rephrase accordingly. - Introduction: a protocol “delivered across three to five sessions per week for two to three weeks” seems to confer too much variability to the study and, therefore, to affect its reliability and repeatability; please refer to similar study protocols already available in the literature and revise both abstract and main text. - Inclusion and exclusion criteria: the inclusion of both ischemic and hemorrhagic stroke may be not appropriate given that they represent two very different disease model, with different etiology, location, severity, and outcome. This variability can be even enhanced when the authors propose to include patients with “stroke onset of one year to six years before the study” and subjects “between 18 and 75 years old”. I would suggest more strict and homogeneous inclusion criteria. - Inclusion and exclusion criteria: please note that “significant aphasia or difficulty understanding the instructions given by the investigators” and “does not consent to TBS intervention” are not rTMS contraindications; please revise. - Inclusion and exclusion criteria: the sentence “All participants will undergo a safety screening for the potential risks of TMS to ensure they are eligible to participate in this study” needs citation (i.e. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol 2009). - TBS session: the latest guidelines of the International Federation of Clinical Neurophysiology on the clinical and research use of TMS (Rossini PM, et al. Clin Neurophysiol 2015) recommend to define the resting motor threshold (RMT) as the minimum stimulation intensity over the hot spot that could elicit a motor evoked potential (MEP) of no less than 50 μv in 5 out of 10 trials over the contralesional target muscle. - TBS session: the authors state that “Sham cTBS will be delivered with the same coil, but the intensity will be reduced to 20% of the individual RMT”. Is this a standardized procedure? Are there other studies using this sham stimulation modality? If so, please add citation(s). Although the stimulation intensity is clearly subthreshold, I think that it is not possible to exclude that this “sham” procedure may actually induce some minimal electrophysiological and/or neurochemical effects. Ideally, the authors should use a “sham coil” to ensure a proper fictitious stimulation. - Secondary outcomes: why do not assess and compare specific TMS measures (i.e. RMT, contralateral silent period, ipsilateral silent period, central motor conduction time, MEP amplitude and latency, short-latency intracortical inhibition, intracortical facilitation, short-latency afferent inhibition) at baseline and after the different TBS modalities? This would allow to objectively estimate any electrophysiolgical change in cortical excitability and synaptic plasticity, also with translational insights on the neurochemical basis underlying these changes, as demonstrated by some TMS studies in different disease models (Transcranial magnetic stimulation in the assessment of motor cortex excitability and treatment of drug-resistant major depression. IEEE Trans Neural Syst Rehabil Eng 2013; Clinical and electrophysiological impact of repetitive low-frequency transcranial magnetic stimulation on the sensory-motor network in patients with restless

	legs syndrome. Ther Adv Neurol Disord 2018; Impaired short-term plasticity in restless legs syndrome: a pilot rTMS study. Sleep Med 2018). - Secondary outcomes: it is stated “We expect that around five patients from each group will voluntarily take part in the EEG examinations before and after the intervention.” Why voluntarily? It seems that the EEG is part of the study protocol. The following sentence (“For participants who participate the EEG part, 400 Hong Kong dollars will be paid as an incentive”) is not necessary and probably not ethic. - Ethics and dissemination: why do the authors state that “We will not perform interim analyses until the completion of this study”? Actually, they cannot exclude, at this stage, that additional patients have to be recruited and, therefore, an interim data analysis would be helpful to obtain more robust final results. MINOR - Abstract: the comma after “preceding iTBS, …” should be removed. Few lines below, please change in “The aim of this study protocol is to investigate…”. Finally, please specify that a sample size analysis has been done. - Article Summary: “The present study is the first…” should be “The present study protocol is the first…” - Introduction: the sentence “…no study has investigated the effects of priming iTBS protocols in patients with stroke to date” should be “…to date no study has investigated the effects of priming iTBS protocols in patients with stroke.” Few lines below: “Therefore, our study has two objectives: First, …” should be “Therefore, our study has two objectives. First, …” - Inclusion and exclusion criteria: please replace “pregnant” with “pregnancy”. - TBS session: please add the word “muscle” after “…contralesional first dorsal interosseous (FDI)” - Primary outcomes: it is not necessary to specify that “Upon the follow-up assessment, participants will be paid 100 Hong Kong dollars as the travel allowance.” - Patient and public involvement: “Patients will not be involved in participant recruitment”. Why? Please clarify or remove.

REVIEWER	Smriti Agarwal Neurology Unit Addenbrooke's Hospital, Cambridge UK
REVIEW RETURNED	27-Jan-2020

GENERAL COMMENTS	This is an interesting study aimed at investigating whether a priming TBS protocol improves response to motor rehabilitation after stroke. While the study background is well described and methods are clearly stated and informed, the work would benefit from addressing a few issues. Major points: A major issue with this study is that the inclusion criteria do not encompass a detailed measure of motor weakness. Degree of motor weakness at baseline is a major determinant of outcome, affects cortical excitability measurements and neuroplasticity. The authors measure FMA-UE score at baseline. It would also be worthwhile having a cut off for inclusion based on this rather than

	a functional score alone (FTHUE). Since the study has alreay been recruiting, authors could potentially retrospectively assess the baseline hand power scores. Alternatively, they could also match the scores between groups at baseline. Including chronic stroke patients from a convenience sample is reasonable and is common practice. But given the spread of timelines after stroke (inclusion criteria state that patients will be included 1-6 years after stroke and this seems rather arbitrary), it is possible that the change from TBS protocol on response to rehab may not be adequately captured. The authors acknowledge this and state that acute studies would be needed in the future. Given EEG as one outcome measure, the authors need to specify whether strokes are cortical or subcortical as the baseline EEG will differ in these two groups. The sample size may preclude correcting for stroke type which is likely to confound interpretation of results. Drugs may affect CNS plasticity. Ideally any centrally acting drugs should be omitted before TBS sessions. As a minimum, their administration should be documented clearly. Other factors like age also need to be factored into the statistical analysis. Post stroke fatigue, depression and degree of engagement due to pre existing deficits need to be documented clearly. Ideally these should be matched between groups. At an early stage of recruitment, an additional measure of these such as HADS score could be considered. Minor points EEG is mentioned as a 'brain imaging' modality which is strictly speaking not correct (page 7). Please modify Handedness and side of stroke should be recorded and ideally balanced between the groups. If not, should be mentioned as limitations of the study when results are available.

VERSION 1 – AUTHOR RESPONSE

Response to Reviewer 1

The authors have come up with a good protocol to test the effect of iTBS on patients with stroke. Existing literature doesn't give a satisfactory results for the use of iTBS. Moreover none of the guidelines stipulates the use of TBS or any kind of non invasive brain stimulation techniques as a treatment intervention for rehabilitation of patients with stroke.

In this protocol I would suggest minor modification and justification for few points and outcomes used.

1. In the strength and limitations the authors have mentioned the intended study as the first randomised controlled trial to explore the effects of priming iTBS in regard to facilitating hemiparetic upper limb.

The authors could have referred "Fayaz Khan, Chaturbhuj Rathore, Mahesh Kate, Josy Joy, George Zachariah, P C Vincent, Ravi Prasad Varma, Kurupath Radhakrishnan. The comparative efficacy of theta burst stimulation or functional electrical stimulation when combined with physical therapy after stroke: A randomised controlled trial. Clin Rehabil. 2019; 33(4):693–703" where the authors of the mentioned study has used iTBS and cTBS combined in a RCT.

Response: We thank the reviewer for the suggestion. However, we noted that there was a difference between our intended study and the study by Khan et al. In Khan et al., the iTBS was applied to the ipsilesional hemisphere and cTBS was applied to the contralesional hemisphere. Khan et al.'s study is based on the interhemispheric imbalance model after stroke, however, in our study, cTBS was applied to the same hemisphere at ipsilesional M1 first and then iTBS subsequently. Our study rationale is based on metaplasticity, i.e., a priming session of inhibitory cTBS may make the brain more amendable to the subsequent excitatory iTBS stimulation. This stimulation paradigm is named as priming protocol in the literature. To the best of our knowledge, no published study has adopted this protocol before in stroke rehabilitation.

2. Blinding should have been mentioned separately as a heading for the better understanding of readers, however it warrants more detailing of the blinding in randomization as well in assessment of outcome. The authors should mention whether its a single blind or double blind RCT.

Response: Thanks for your advice. We have highlighted that the present study was "subjects- and assessors-blinded", in our abstract and methodology.

Revisions:

Page 2, Abstract:

A three-arm, subjects- and assessors-blinded, randomized controlled trial (RCT) will be performed, with an estimated total of 36 patients with chronic stroke.

Page 10, Methods:

This study is designed as a three-arm, parallel group, subjects- and assessors-blinded, sham-controlled RCT.

3. Authors should justify, why robotic therapy was chosen as the default treatment for all the groups rather than the conventional function based rehabilitation techniques, however the primary objective was to find the effectiveness of iTBS. Moreover the robotic therapy is not accessible for most of the stroke rehabilitation centres so the question of generalising the outcome is questionable.

Response: The reasons for our study to choose robot-assisted therapy (RAT) as the default motor training were: (1) The present study will be carried out in a neuroscience laboratory, rather than a hospital, we will not provide any conventional rehabilitation for patients routinely there; (2) We used the robot-assisted training (RAT), because the training duration, number of movement, grading of training difficulty are standardized and can be easily controlled/adjusted via the robotic device. The aim of this study is to find the potentially differential effects of different protocols of iTBS, therefore, we want to use a standardized motor training for stroke patients with different levels of hemiplegia. We understand that the study outcome may not be generalized to hospital-based clinical settings.

4. Justification needed for not using any of the biomarkers which are more significant in stroke for assessing neuroplasticity like (MEP, intra cortical inhibition and facilitation) with TMS and other outcome measures like fMRI/DTI which is more objective

Response: We added our justifications of using EEG outcomes for this study in our revised manuscript. We have experiences of using sensorimotor desynchronization (ERD) from EEG as a physiological marker for stroke recovery before, hence, we believe that ERD might be more useful then MEP as an outcome.

We usually assessed the RMT/MEP of contralesional M1 to determine the stimulation intensity for patients with stroke, since some patients do not have observable MEP from the ipsilesional M1. Hence, we do not have the TMS-based metrics of ipsilesional M1 for the analysis.

Revisions:

Page 8, Introduction:

Sensorimotor ERD will be be used to probe cortical oscillatory activities of large number of neurons in different rhythms, during a given task (movement or movement observation). A previous study comparing the effects of TBS on MEPs and movement-related rhythmic oscillations showed that the modulatory effect of TBS was more reliable on movement-related ERD than that on MEPs.[33] The potential explanations may be that TMS-based metrics may not represent all cortical responses, reflecting exclusively those destined to the spinal cord,[33] and the magnitude of TMS-based metrics is also contaminated by the neuronal responses at subcortical and spinal levels, as well as the peripheral MEP,[34] when a suprathreshold stimulation intensity is used for the measurements.

Hence, we decided to use sensorimotor ERD in this study, which may provide new insight about the sensorimotor neuroplasticity in association with priming iTBS.

5. Explanation of drop out data analysis needed.

Response: We appreciate the reviewer for the suggestion. We plan to use the mixed-effect model instead of using dropout data analysis for the missing data. This model can be fitted with the dataset with missing observations.

Revisions:

Page 23, Methods:

A mixed-effects model with random intercepts and slopes will be used to detect any significant differences in the rate of change in motor outcomes and sensorimotor ERD among the three groups, because of its superiority in analyzing repeated measures data and dataset with missing values.

Response to Reviewer 2

The authors deals with an innovative neurorehabilitative approach, which is the priming intermittent theta burst stimulation (iTBS) on post-stroke upper limb motor recovery. To achieve this goal, they propose a three-arm randomized controlled trial, with all participants randomly allocated to receive 10 sessions of repetitive transcranial magnetic stimulation (rTMS) with different TBS protocols (cTBS+iTBS, sham cTBS+iTBS, and sham cTBS+sham iTBS) and 60 minutes of robot-assisted training (RAT) after each stimulation session. Primary outcomes will be assessed using Fugl-Meyer Assessment – Upper Extremity scores and Action Research Arm Test, whereas secondary outcomes using kinematic outcomes generated during RAT and electroencephalography (EEG). Overall, the study is nicely conceived and designed; the protocol is adequately described and illustrated; therefore, it might disclose interesting translational findings. However, there are several issues needing attention and revision. MAJOR - Title: as the authors themselves state in the main text (i.e. “Ethics and dissemination”), this is actually a “proof-of-concept” study and, therefore, the title should be changed accordingly.

Response: Thanks for the suggestion, and we have modified our study title.

Revisions:

Page 1, Research title:

The Effects of Priming Intermittent Theta Burst Stimulation on Upper Limb Motor Recovery After Stroke: Study Protocol for a Proof-of-Concept Randomized Controlled Trial

- Introduction: at the end of the first sentence, in addition to the reference n. 1, a more recent review of the rTMS in stroke rehabilitation should be included (i.e. Repetitive transcranial magnetic stimulation in stroke rehabilitation: review of the current evidence and pitfalls. Ther Adv Neurol Disord 2019).

Response: Thanks for the suggestion from the reviewer, we have now used your updated reference.

Revisions:

Page 27, References:

1. Fisicaro F, Lanza G, Grasso AA, et al. Repetitive transcranial magnetic stimulation in stroke rehabilitation: review of the current evidence and pitfalls. Ther Adv Neurol Disord. 2019;12:1756286419878317.

- Introduction: it has been demonstrated that metaplasticity is significantly involved also in neuropsychiatric disorders, such as major depression (Repetitive transcranial magnetic stimulation in patients with drug-resistant major depression: A six-month clinical follow-up study. Int J Psychiatry Clin Pract 2015), and that metaplastic effects can be probed and measured by different TMS techniques (Cortical Plasticity in Depression. ASN Neuro 2017). Given the relevance of this topic within the proposed study, a short mention should be included.

Response: Thanks for the suggestion from the reviewer, we have now added a brief introduction about the studies of using priming TMS in neuropsychiatric disorders.

Revisions:

Page 7, Introduction:

"Metaplasticity is also significantly involved in rTMS studies for patients with major neuropsychiatric disorders.[24 25]"

- Introduction: the EEG is not a "brain imaging technique" but rather a "brain electrophysiological technique" (or a "neurophysiological technique"); please rephrase accordingly.

Response: We appreciate your suggestion, we have now rephrased the wordings.

Page 7, Introduction:

Revisions: "…Electroencephalography (EEG), a non-invasive measure of cortical neuronal oscillation, is of great interest, because it is a relatively convenient and well-tolerated neurophysiological technique for patients with stroke…"

- Introduction: a protocol "delivered across three to five sessions per week for two to three weeks" seems to confer too much variability to the study and, therefore, to affect its reliability and repeatability; please refer to similar study protocols already available in the literature and revise both abstract and main text.

Response: We appreciate this suggestion from the reviewer. As our patients are mostly from community-dwellings, most of them could not visit our laboratory everyday. Hence, we adopt a more flexible schedule for motor training. We found that there are many studies using similar training protocol for people with chronic stroke, and we have now added them to the references. We also acknowledged this limitation and it might affect the generalization into hospital-based clinical settings, as they can provide motor training on a daily basis.

Revisions:

Page 13, Methods:

Participants will receive 10 sessions of TBS intervention combined with RAT, delivered three to five sessions per week, for two to three weeks. We decide to use a more flexible training schedule, because most community stroke survivors are unable to visit our laboratory on a daily basis. Similar schedule for motor training has been used in some previous studies for patients with chronic stroke.[40 41]

- Inclusion and exclusion criteria: the inclusion of both ischemic and hemorrhagic stroke may be not appropriate given that they represent two very different disease model, with different etiology, location, severity, and outcome. This variability can be even enhanced when the authors propose to include patients with "stroke onset of one year to six years before the study" and subjects "between 18 and 75 years old". I would suggest more strict and homogeneous inclusion criteria.

Response: We appreciate the suggestion from the reviewer. We revised our inclusion criteria as the reviewer has suggested. We decided to recruit adults aged between 18 to 64 years (i.e., excluding older adults). The time after stroke from 1 to 6 years seems to be arbitrary. As we aimed to patients with chronic stroke who have limited potential of spontaneous recovery, we followed the definition of chronic phase of stroke (≥6 months). Potential cofounding effect of age/time after stroke will be included in the mixed-effects model, in case of any baseline difference among the 3 groups.

Revisions:

Page 10, Methods:

"..Participants must meet all of the following criteria: (1) have a diagnosis of a unilateral ischemic or hemorrhagic first-ever stroke; (2) time after stroke onset ≥ 6 months;[36] (3) adults between 18 and 64 years old…"

Page 23, Methods:

Demographic and baseline characteristics will be compared using analysis of variance (ANOVA; continuous and ordinal data) or Chi-square tests (categorical data). Any factor with significant between-group difference in the baseline will be included in the mixed-effects model as covariates.

- Inclusion and exclusion criteria: please note that "significant aphasia or difficulty understanding the instructions given by the investigators" and "does not consent to TBS intervention" are not rTMS contraindications; please revise.

Response: Thanks for your advice. We have now removed them in our revision.

Revisions:

Page 11-12, Methods:

In this study, patients who meet any of the following rTMS contraindications will not be included: (1) unstable medical condition; (2) history of epileptic seizures, unconsciousness, or intracranial hypertension; (3) serious heart disease; (4) pregnancy; (5) with metal implants in vivo, such as a pacemaker, artificial cochlear, or implant brain stimulator; (6) history of receiving a craniotomy.[2] To ensure safety, the participants will be under the supervision of at least one investigator who has completed training in TMS. All participants will undergo a safety screening for the potential risks of TMS to ensure they are eligible to participate in this study.[2]

In addition to TMS contraindications, participants who meet any of the following criteria will be also excluded: (1) previous diagnosis of any neurological disease excluding stroke; (2) presence of any sign of cognitive problems (Abbreviated Mental Test, Hong Kong Cantonese version < 6/10);37 (3) patients with extreme spasticity over the elbow or wrist in the hemiparetic upper limb (Modified Ashworth score > 2),38 or severe pain that hinders upper limb movement; (4) other notable impairments of the upper limb not affected by stroke (e.g., a recent fracture, severe osteoarthritis, congenital upper limb deformity); (5) significant aphasia or difficulty understanding the instructions given by the investigators; and (6) concurrent participation in upper limb rehabilitation training in a hospital, university laboratory or other rehabilitation settings, or active participation in another clinical trial.

- Inclusion and exclusion criteria: the sentence "All participants will undergo a safety screening for the potential risks of TMS to ensure they are eligible to participate in this study" needs citation (i.e. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol 2009).

Response: Thanks for your advice. We have now added back the reference.

Revisions:

Page 11, Methods

To ensure safety, the participants will be under the supervision of at least one investigator who has completed training in TMS. All participants will undergo a safety screening for the potential risks of TMS to ensure they are eligible to participate in this study.[2]

- TBS session: the latest guidelines of the International Federation of Clinical Neurophysiology on the clinical and research use of TMS (Rossini PM, et al. Clin Neurophysiol 2015) recommend to define the resting motor threshold (RMT) as the minimum stimulation intensity over the hot spot that could elicit a motor evoked potential (MEP) of no less than 50 μv in 5 out of 10 trials over the contralesional target muscle.

Response: Thank you for your suggestion. We have now amended this point in our methodology.

Revisions:

Page 14, Methods

Resting motor threshold (RMT) is defined as the minimum stimulation intensity over the hot spot that could elicit a motor evoked potential (MEP) of no less than 50 μv in five out of ten trials over the contralesional first dorsal interosseous (FDI) muscle.

- TBS session: the authors state that "Sham cTBS will be delivered with the same coil, but the intensity will be reduced to 20% of the individual RMT". Is this a standardized procedure? Are there other studies using this sham stimulation modality? If so, please add citation(s). Although the stimulation intensity is clearly subthreshold, I think that it is not possible to exclude that this "sham" procedure may actually induce some minimal electrophysiological and/or neurochemical effects. Ideally, the authors should use a "sham coil" to ensure a proper fictitious stimulation.

Response: We acknowledged that intensity reduction is not a perfect sham procedure. The use of a special sham coil will be better, however, it is not available in our setting. Coil flipping is not a good way as the subjects can easily recognize the difference between real and sham stimulations. We choose using the method of reduced intensity in our sham protocol. This procedure has been used in our previous work as well as other studies. We have now added back the references accordingly. We will mention the limitation of our sham procedure in our final paper

Revisions:

Page 14, Methods

Sham cTBS will be delivered with the same coil, but the intensity will be reduced to 20% of the individual RMT. Intensity reduction has been used as sham stimulation in some previous clinical studies,[5 44] and our pilot study.[32]

- Secondary outcomes: why do not assess and compare specific TMS measures (i.e. RMT, contralateral silent period, ipsilateral silent period, central motor conduction time, MEP amplitude and latency, short-latency intracortical inhibition, intracortical facilitation, short-latency afferent inhibition) at baseline and after the different TBS modalities? This would allow to objectively estimate any electrophysiolgical change in cortical excitability and synaptic plasticity, also with translational insights on the neurochemical basis underlying these changes, as demonstrated by some TMS studies in different disease models (Transcranial magnetic stimulation in the assessment of motor cortex excitability and treatment of drug-resistant major depression. IEEE Trans Neural Syst Rehabil Eng 2013; Clinical and electrophysiological impact of repetitive low-frequency transcranial magnetic stimulation on the sensory-motor network in patients with restless legs syndrome. Ther Adv Neurol Disord 2018; Impaired short-term plasticity in restless legs syndrome: a pilot rTMS study. Sleep Med 2018).

Response: We add the justification for using EEG as our secondary outcome. We also acknowledged that we cannot use all kinds of neural investigations in a preliminary study. Using EEG outcomes also follows our previous work in healthy adults.

For patients with stroke, we follow the common practice in stroke studies that they usually assessed the RMT of contralesional M1 to determine the stimulation intensity, since the RMT of ipsilesional M1 may be abnormally elevated and the amplitude MEP of ipsilesional M1 in some patients may become very low. Hence, we will not include these outcomes.

Revisions:

Page 8-9, Introduction

Sensorimotor ERD can be used to probe cortical oscillatory activities of large number of neurons in different rhythms, during a given task (movement or movement observation). A previous study comparing the effects of TBS on MEPs and movement-related rhythmic oscillations showed that the modulatory effect of TBS was more reliable on movement-related ERD than that on MEPs.[33] The potential explanations may be that TMS-based metrics may not represent all cortical responses, reflecting exclusively those destined to the spinal cord,[33] and the magnitude of TMS-based metrics is also contaminated by the neuronal responses at subcortical and spinal levels, as well as the peripheral MEP,34 when a suprathreshold stimulation intensity is used for the measurements. Hence, we decide to use sensorimotor desynchronization in this study, which may provide new insight about the sensorimotor neuroplasticity in association with priming iTBS.

- Secondary outcomes: it is stated “We expect that around five patients from each group will voluntarily take part in the EEG examinations before and after the intervention.” Why voluntarily? It seems that the EEG is part of the study protocol. The following sentence (“For participants who participate the EEG part, 400 Hong Kong dollars will be paid as an incentive”) is not necessary and probably not ethic.

Response: Thank you for your advice, we have now removed this sentence on incentives and the word ‘voluntary’. As EEG is a timely assessment, we will not require every subject to undergo the EEG assessment. The EEG part is used as a preliminary exploration of the neural mechanism.

- Ethics and dissemination: why do the authors state that “We will not perform interim analyses until the completion of this study”? Actually, they cannot exclude, at this stage, that additional patients have to be recruited and, therefore, an interim data analysis would be helpful to obtain more robust final results.

Response: Thanks for your suggestion. We will perform an interim data analysis.

Revisions:

Page 27, Ethics and dissemination

We will perform an interim analysis when 50% of patients have been included and have completed the follow-up assessment.

MINOR

- Abstract: the comma after “preceding iTBS, …” should be removed. Few lines below, please change in “The aim of this study protocol is to investigate…”. Finally, please specify that a sample size analysis has been done.

Response: Done. Thank you.

- Article Summary: “The present study is the first…” should be “The present study protocol is the first…”

Response: Done. Thanks.

- Introduction: the sentence "…no study has investigated the effects of priming iTBS protocols in patients with stroke to date" should be "…to date no study has investigated the effects of priming iTBS protocols in patients with stroke." Few lines below: "Therefore, our study has two objectives: First, …" should be "Therefore, our study has two objectives. First, …"

Response: Done. Thanks.

- Inclusion and exclusion criteria: please replace "pregnant" with "pregnancy".

Response: Done. Thanks.

- TBS session: please add the word "muscle" after "…contralesional first dorsal interosseous (FDI)"

- TBS session: please add the word "muscle" after "…contralesional first dorsal interosseous (FDI)"

Response: Done. Thanks.

- Primary outcomes: it is not necessary to specify that "Upon the follow-up assessment, participants will be paid 100 Hong Kong dollars as the travel allowance."

Response: We have removed this sentence. Thanks.

-Patient and public involvement: "Patients will not be involved in participant recruitment". Why? Please clarify or remove.

Response: We have removed this sentence. Thanks.

Response to Reviewer 3

This is an interesting study aimed at investigating whether a priming TBS protocol improves response to motor rehabilitation after stroke.

While the study background is well described and methods are clearly stated and informed, the work would benefit from addressing a few issues.

Major points:

A major issue with this study is that the inclusion criteria do not encompass a detailed measure of motor weakness. Degree of motor weakness at baseline is a major determinant of outcome, affects cortical excitability measurements and neuroplasticity. The authors measure FMA-UE score at baseline. It would also be worthwhile having a cut off for inclusion based on this rather than a functional score alone (FTHUE). Since the study has already been recruiting, authors could potentially retrospectively assess the baseline hand power scores. Alternatively, they could also match the scores between groups at baseline.

Response: We appreciate the suggestion from the reviewer. The FTHUE is an assessment of functional upper extremity use that is commonly used in local occupational therapy practice. Our previous papers showed that FTHUE is a suitable screening tool for triage of the hemiplegic upper limb motor functions. We have now added the citations. We will compare the baseline differences in other variables, e.g., FMA-UE, ARAT, and mixed-effects model will be used for analysis if the baseline is not balanced. We think that hand power may not be a good assessment as most patients have limited voluntary control over their hemiplegic hand.

Revisions:

Page 10 Method:

FTHUE is a fast screening tool for upper limb functional movement, which has been used as a screening tool in our previous RCTs.[37 38] FTHUE levels two to four are defined as low upper limb functioning poststroke, and levels five and seven are defined as high upper limb functioning poststroke;[37]

Including chronic stroke patients from a convenience sample is reasonable and is common practice. But given the spread of timelines after stroke (inclusion criteria state that patients will be included 1-6 years after stroke and this seems rather arbitrary), it is possible that the change from TBS protocol on response to rehab may not be adequately captured. The authors acknowledge this and state that acute studies would be needed in the future.

Response: We acknowledged that a very strict and homogeneous inclusion criteria was not used in the present study. We also cannot use blocked randomization for each potential confounding factors due to limited sample size of this study. However, no study has used this priming iTBS protocol before, and we first tested the protocol using a RCT design. As a proof-of-concept study with a small sample size, we will analyze the factors when the results are available, to explore the effect of those

factors on the response to priming stimulation. The future study may be inspired by the preliminary results of this pilot study. As you suggested, we acknowledge this and state that acute studies would be needed in the future.

> Given EEG as one outcome measure, the authors need to specify whether strokes are cortical or subcortical as the baseline EEG will differ in these two groups. The sample size may preclude correcting for stroke type which is likely to confound interpretation of results.

Response: Your suggestion is appreciated. The cofounding effect caused by stroke types whether they are cortical or subcortical will be analyzed by a subgroup analysis, when the final data is ready.

> Drugs may affect CNS plasticity. Ideally any centrally acting drugs should be omitted before TBS sessions. As a minimum, their administration should be documented clearly.
>
> Other factors like age also need to be factored into the statistical analysis.

Response: We have already documented the routine drug use, via self-reporting and hospital discharge summary reports from the patients. As an exclusion criteria, patients who take any centrally acting drugs will not be included in this study. We have added this information in our manuscript. For other factors, like age, we will put it as covariate in the mixed effects model to account for the confounding effect of the factor with significant between-group differences.

Revisions:

Page 11 Methods:

"….and (7) taking any centrally acting drugs in the recent three months…"

Page 24 Methods:

Demographic and baseline characteristics will be compared using analysis of variance (ANOVA; continuous and ordinal data) or Chi-square tests (categorical data). Any factor with significant between-group difference will be included in the mixed-effects model as the covariable.

> Post stroke fatigue, depression and degree of engagement due to pre existing deficits need to be documented clearly. Ideally these should be matched between groups. At an early stage of recruitment, an additional measure of these such as HADS score could be considered.

Response: Your suggestion is appreciated. We have sought the medical history of each patient from the hospitals (e.g., whether they have ever been diagnosed as major depression before). We further include HADS as a baseline screening to rule out patients with any sign of anxiety or depression.

Revisions:

Page 12 Methods

"…(6) any sign of anxiety and/or depression screened by Hospital Anxiety and Depression Scale (HADS), using a cut-off value of 8 in both subscales…"

Minor points EEG is mentioned as a 'brain imaging' modality which is strictly speaking not correct (page 7). Please modify

Response: Done, thanks.

Revisions: Electroencephalography (EEG), a non-invasive measure of cortical neuronal oscillation, is of great interest, because it is a relatively convenient and well-tolerated neurophysiological technique for patients with stroke.

Handedness and side of stroke should be recorded and ideally balanced between the groups. If not, should be mentioned as limitations of the study when results are available.

Response: We cannot use block randomization to account for each potential confounds since our estimated sample size is not large. If any baseline difference was found, we will analyze the potential confounding effects of those factors by including them as covariates.

Revisions:

Page 24 Methods:

Demographic and baseline characteristics will be compared using analysis of variance (ANOVA; continuous and ordinal data) or Chi-square tests (categorical data). Any factor with significant between-group difference will be included in the mixed-effect model as the covariable.

VERSION 2 – REVIEW

REVIEWER	Fayaz Khan King Abdulaziz University Saudi Arabia
REVIEW RETURNED	12-Feb-2020

GENERAL COMMENTS	The authors have addressed the reviewers comments in an acceptable manner. Its now acceptable for publication

REVIEWER	Giuseppe Lanza University of Catania, Italy
REVIEW RETURNED	13-Feb-2020

GENERAL COMMENTS	The authors have addressed my concerns, thus improving the quality of this manuscript. I do not have further comments.

REVIEWER	Smriti Agarwal Neurology Unit Addenbrooke's Hospital Cambridge UK
REVIEW RETURNED	16-Feb-2020

GENERAL COMMENTS	The authors have considered the comments from initial review and amended the manuscript satisfactorily.