PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Efficacy of MRI-guided rTMS for posttraumatic stress disorder by modulating amygdala activity: study protocol for a randomized controlled trial
AUTHORS	Zhang, Yaochi; Peng, Zhengwu; Tang, Nailong; Zhang, Yuyu; Liu, Nian; Lv, Runxin; Meng, Yumeng; Cai, Min; Wang, Hua-Ning

VERSION 1 – REVIEW

REVIEWER	Mikellides, Georgios University of Nicosia Medical School
REVIEW RETURNED	16-Nov-2023

GENERAL COMMENTS	Please expand further the limitations of this study in more detail. Also highlight how this paper will be valuable to the current literature, Why is your age limited to 65? How will you access the amygdala, you have said that is directly reached, is this true? Which coils will you be using brand specify the exact model of coil and machine? Some coils have around 2-3 cm penetration.

REVIEWER	Qi, Mingming Liaoning Normal University
REVIEW RETURNED	06-Dec-2023

GENERAL COMMENTS	This paper presents a protocol for examining the effectiveness of repetitive transcranial magnetic stimulation (rTMS) in the treatment of post-traumatic stress disorder. The right ventrolateral prefrontal cortex (VLPFC) was stimulated by high-frequency rTMS, and the activity of the amygdala was supposed to be inhibited. The topic and design was interesting. there are still some major concerns in the current manuscript. My specific comments are listed below. (1)Why the activation of right VLPFC can suppress that of amygdala should be introduced in detail. The studies on functional connectivity between these brain regions should be introduced in detail. The left-DLPFC, right-DLPFC and other brain regions have been associated with the suppression of amygdala activation. Why the authors chose the right VLPFC as the target region. (2)The relationship between amygdala dis-function and PTSD should be introduced in detail. (3)Provide a power calculation and a justification of the effect size used for the calculation. I recommend the authors to use the Morepower for the sample size calculation [Campbell, J.I.D.,

	Thompson, V.A. MorePower 6.0 for ANOVA with relational confidence intervals and Bayesian analysis. Behav Res 44, 1255–1265 (2012). https://doi.org/10.3758/s13428-012-0186-0].

REVIEWER	Oestreich, Lena K.L. The University of Queensland
REVIEW RETURNED	15-Apr-2024

GENERAL COMMENTS	This study protocol outlines a procedure for targeting dlPFC that is most strongly connected to the amygdala in individuals with PTSD. For most of the manuscript it is unclear, that the dlPFC will be targeted. Instead, it seems like any cortical area connected to amygdala will be targeted. This needs to be made clearer and the link to individualise or precision treatments should be made. Stimulation of deep brain regions with TMS is not based on functional connectivity but physiological connectivity through white matter tracts, that connect the stimulated area and the subcortical region. The first paragraph of the “Study Design” section is repetitive – information provided previously should be excluded. Please provide more information about the MRI acquisition. I.e. What b-values does the DWI series include? Is there reverse phase-encoding? Why is the T1 series not a regular MP2RAGE to make it comparable to other studies? How long it the rs-fMRI series and will participants have eyes closed or open? Why are PTSD patients undergoing TMS treatments simultaneously taking SSRI medications? This will most likely bias the results. SSRIs are known to improve PTSD symptoms. Giving a known and effective treatment simultaneously to testing a new treatment makes it impossible to determine which one is effective.

VERSION 1 – AUTHOR RESPONSE

Response to Reviewer 1
We appreciate your careful review of our paper. Our answers are as follows.
1. Please expand further the limitations of this study in more detail. Also highlight how this paper will be valuable to the current literature
Response: Thanks to your suggestion, we have added a "STRENGTHS AND LIMITATIONS" section at the end of the manuscript to describe the limitations and potential value of this study. (Page 11)
2. Why is your age limited to 65?
Response: The age cut-off for the elderly in different countries is either 60 or 65 years, and the age limit of 65 years in this study may appropriately reduce the difficulty of recruiting patients without excessive age differences between patients.
3. How will you access the amygdala, you have said that is directly reached, is this true? Which coils will you be using brand specify the exact model of coil and machine? Some coils have around 2-3 cm penetration.
Response: Thank you for your comments. This study will select direct stimulation targets for TMS intervention in the vlPFC through connectivity between the amygdala and the vlPFC, so we are not

directly intervening in amygdala activity, but rather toward indirectly modulating amygdala activity through connectivity between brain regions. This approach has been proven effective in several studies (https://pubmed.ncbi.nlm.nih.gov/35731882/; https://pubmed.ncbi.nlm.nih.gov/32252538/). The coils used in this study are common figure-of-eight coils, and relevant information has been added to the manuscript (page 7).

Response to Reviewer 2

We appreciate your careful review of our paper. Our answers are as follows.

1.Why the activation of right VLPFC can suppress that of amygdala should be introduced in detail. The studies on functional connectivity between these brain regions should be introduced in detail. The left-DLPFC, right-DLPFC and other brain regions have been associated with the suppression of amygdala activation. Why the authors chose the right VLPFC as the target region.

Response: Thank you for your observations, which are the critical issues of our study. We do not choose the dlPFC as the target for several reasons: first, the effect of rTMS-dlPFC on amygdala activity is not stable, and our retrospective analyses did not find that rTMS-dlPFC effectively modulates local activity in the amygdala. Second, the vlPFC has a large number of amygdala projections relative to the dlPFC, and thus rTMS-vlPFC may regulate amygdala activity more stably. The study by Sydnor et al. gives us important support for this idea (https://pubmed.ncbi.nlm.nih.gov/35731882/) ; Third, rTMS to dlPFC often elicits activity changes in many brain regions, when changes in amygdala activity may be secondary to changes in other brain regions, which prevents us from further investigating the exact mechanism of MRI-guided rTMS; Finally, the right hemisphere is chosen as the target of intervention because a large number of previous clinical studies in PTSD have found that the right hemisphere is more effective relative to the left hemisphere (https://pubmed.ncbi.nlm.nih.gov/31901449/).

More detailed information between vlPFC and amygdala has been added in the manuscript. (page 4)

2.The relationship between amygdala disfunction and PTSD should be introduced in detail.

Response: Thanks to your suggestion, we have described the relationship between the amygdala and PTSD in more detail in the background section (page 3 and 4).

3.Provide a power calculation and a justification of the effect size used for the calculation. I recommend the authors to use the Morepower for the sample size calculation [Campbell, J.I.D., Thompson, V.A. MorePower 6.0 for ANOVA with relational confidence intervals and Bayesian analysis. Behav Res 44, 1255–1265 (2012). https://doi.org/10.3758/s13428-012-0186-0].

Response: Thank you for your suggestion, but regretfully we were not successful in obtaining the use of Morepower. We have reconfirmed the calculation process of our sample size. Here is the specific basis for the calculation: the effect size refers to our previous study and is set at 0.82 (doi:10.3969/j.issn.2095-9346.2016.02.003), with a significance level of 0.05. Meanwhile, we would like to be able to detect a difference with 80% certainty. Ultimately, the above data are entered into PASS 2021 to obtain the need for 38 subjects, and the sample size is ultimately increased to 48 subjects to take into account the 20% dropout rate.

Response to Reviewer 3

We appreciate your careful review of our paper. Our answers are as follows.

1.This study protocol outlines a procedure for targeting dlPFC that is most strongly connected to the amygdala in individuals with PTSD. For most of the manuscript it is unclear, that the dlPFC will be targeted. Instead, it seems like any cortical area connected to amygdala will be targeted. This needs to be made clearer and the link to individualise or precision treatments should be made.

Response: Thank you for the reminder. Actually the direct stimulation target area that will be targeted in this study is not the dlPFC, but the vlPFC, and we have added more information about the vlPFC in the introduction to make it clearer in the manuscript. (page 3 and 4).

Previous studies have suggested that vlPFC has more amygdala projections, while the study by Sydnor et al. demonstrates that TMS targeting vlPFC can modulate amygdala activity in real time (https://pubmed.ncbi.nlm.nih.gov/35731882/). Therefore, we believe that the vlPFC is the brain region that can more accurately and consistently modulate the amygdala relative to other brain regions. In addition, the mPFC is actually more structurally connected to the amygdala, and we do not choose the mPFC as a target area because the mPFC is also located at a deeper location, and currently the H-coil (dTMS) would be needed to intervene the mPFC definitively. However, this coil has a wider stimulation range, so it is not possible to make precise interventions, and if the H-coil is used with the stimulation pattern of our study, it may have poor tolerance and safety.

2.Stimulation of deep brain regions with TMS is not based on functional connectivity but physiological connectivity through white matter tracts, that connect the stimulated area and the subcortical region.

Response: In this study, we will select target sites based on functional connectivity and structural connectivity. Specifically, we will first select the top 20 most connected sites based on amygdala-vlPFC functional connectivity. Then the degree of white matter connection of each site to the amygdala will be calculated. Finally, functional connectivity and structural connectivity will be combined to determine the final stimulation target.

3.The first paragraph of the “Study Design” section is repetitive – information provided previously should be excluded.

Response: Thank you for the reminder, we will remove the duplicate information from the manuscript.

3.Please provide more information about the MRI acquisition. I.e. What b-values does the DWI series include? Is there reverse phase-encoding? Why is the T1 series not a regular MP2RAGE to make it comparable to other studies? How long it the rs-fMRI series and will participants have eyes closed or open?

Response: Thank you for your suggestion. More detailed information about the MRI scan has been added to the manuscript.(Page 6 and 7)

The b-value is 1000 s/mm² and reverse phase-encoding is included in the MRI acquisition. In this study, MRI data will be acquired with a 3T uMR 780 scanner using the 3D GRE_fsp sequence, which is also commonly used to acquire T1-weighted images. This sequence has been used in previous studies by our team, and this setup is followed in this study to enable a better comparison with previous studies. The rs-fMRI scan lasts 8 min 6 sec, during which participants will be asked to close their eyes, remain awake, and not think intentionally.

4.Why are PTSD patients undergoing TMS treatments simultaneously taking SSRI medications? This will most likely bias the results. SSRIs are known to improve PTSD symptoms. Giving a known and effective treatment simultaneously to testing a new treatment makes it impossible to determine which one is effective.

Response: Thank you for your comments, which is an issue that we have considered many times in our trial design. The population of this study is first-episode untreated PTSD patients, because of the ethical requirements, we can not just give subjects MRI-guided rTMS which has not been proved effective, so the subjects will receive medication at the same time. However, a control group is also set up in this study, which will receive sham-stimulation along with medication, so if there is a difference in efficacy between the two groups, it can be attributed to MRI-guided rTMS. In addition, it is generally believed that it takes 4-6 weeks for medications to begin to be effective in the treatment of PTSD, and more than 8 weeks for its full therapeutic effect to be realized. In this study, MRI-guided rTMS will be performed for only 10 days, and the primary outcome will be the scale score on day 10, so we believe that medication will have limited disturbance on the difference in efficacy between the two groups at this time. Further, the current treatment of PTSD is still based on medication, but the effect of medication is slow, and whether MRI-guided rTMS combined with medication can bring patients to clinical remission more quickly is also a question that can be explored in this study.

We look forward to your response regarding our submission. Please do not hesitate to contact us if there are any further questions or comments.

VERSION 2 – REVIEW

REVIEWER	Qi, Mingming Liaoning Normal University
REVIEW RETURNED	03-Jun-2024

GENERAL COMMENTS	The authors addressed my concerns adequately.

REVIEWER	Oestreich, Lena K.L. The University of Queensland
REVIEW RETURNED	16-May-2024

GENERAL COMMENTS	The authors gave adequately addressed my concerns.

VERSION 2 – AUTHOR RESPONSE