Responses to Reviewer 1

1)"Second-generation antipsychotics have limited effect on negative symptoms"? In the contrary: the impact on negative symptoms is one factor which differentiates second-generation antipsychotics from classic neuroleptics !

2)No information whether antipsychotic drugs have been changed before the study started and what was the effect of such a change? How the authors are able to exclude that those Pts who did not improve after the rTMS showed previously no satisfactory response to previous drugs treatment?

3)What was the explanation of the finding that there was no correlation between PRN and SANS score? The suggestion is that the correlation between Delta SANSS and Delta rTSM might be helpful. Authors suggestion on the need of the follow-up study should be underlined.

Answer: 1） This point is very excellent. As suggested by the reviewer, we have corrected this sentence as: "The success of second-generation antipsychotics in improving positive symptoms and partially improving negative symptoms differentiates second-generation antipsychotics from classical neuroleptics. However, second-generation antipsychotics have little effect on cognitive deficits [7, 29]."

2）These points are very excellent. As suggested by the reviewer, we have added

them as one of the limitations of this study to the Discussion section on page 15 and page 16, showing as: “Eleventh, according to our inclusion criteria, all participants were administrated with fixed dose of antipsychotics at least 12 months before entering this study. However, we had no information on whether antipsychotic drugs were changed before the study started and what the effect of such a change might have had. Furthermore, we were unable to exclude that those patients who did not improve after the rTMS showed had not previously responded satisfactorily to previous medication. Therefore, although antipsychotic drugs were used as covariates in statistical analysis, the effects of long-term, different doses and types of antipsychotics on the efficacy of rTMS cannot be avoided.”

3) This point is very excellent. In this study, we found that at baseline, there was significant correlation between PRM and SANS score, showing as: “At baseline, …… there was a significantly negative association between the PRM number correct or PRM percent correct and the SANS total and its 5 subscale scores ($p<0.05$~0.001) except the affect flattening subscale ($p>0.05$)” (please refer to the first paragraph of the Results section). Furthermore, after treatment, we found that “Correlation analysis showed that from baseline to week 8, increases in PRM-number correct were significantly associated with changes in the following parameters: the SANS total score ($r=0.34$, $df=38$, $p=0.034$; Figure 3), the SANS alogia sub score ($r=0.37$, $df=38$, $p=0.024$) and the SANS avolition/apathy subscore ($r=0.34$, $df=38$, $p=0.037$). Further multiple regression showed significant association between the increase in PRM- number correct and changes of SANS total score ($beta=0.42$, $t=2.53$, $p=0.017$)

from baseline to 8-weeks". Please refer to the Results section on page 7.

Then, we have discussed the possible reasons for these findings in the Discussion section on page 14, showing as:" It is suggested that the improvement of negative symptoms in patients with chronic schizophrenia is closely related to cognitive deficits. Moreover, at baseline we found a significantly negative association between the PRM number correct and the SANS total and its 5 subscale scores except for the affect flattening subscale, providing further support for this point. It is known that generalized DA deficits in cortical and extrastriatal regions are associated with cognitive deficits and negative symptoms of schizophrenia[58]. Recent studies have also shown that prefrontal hypodopaminergia itself can cause striatal dopamine disorders. In contrast, striatal dopaminergic dysfunction can lead to cognitive impairment [59, 60]. Previous studies found that increasing dopamine release, such as caused by low or moderate doses of psychostimulants could improve negative symptoms and cognitive deficits in schizophrenia[60]. As mentioned above, high-frequency rTMS applied over the left PFC increased the release of dopamine in the mesostriatal brain pathways[41] which may improve the negative symptoms and cognitive deficits simultaneously. In this study, we found that rTMS treatment was significantly associated with the improvement of negative symptoms and cognitive deficits in schizophrenia. However, the mechanism of how rTMS therapy affects the DA system and improves negative symptoms and cognitive deficits in patients with schizophrenia deserves further investigation".

Responses to Reviewer 2

Question 1. Abstract: Please proportionally present background, aim, methods, results, and conclusion, as the aim and the conclusions are not sufficiently described. Also, I think that the lack of an explanation of what "improvement of cognitive impairments" means in this study makes the reader unable to grasp the key aspects of this paper by consulting the abstract.

Answer: This point is very excellent. As suggested by the reviewer, we have proportionally presented background, aim, methods, results, and conclusion. Also, we have rewritten the aim and the conclusions, showing as:" Background： The main purpose of this study was to assess the effect of HF rTMS on visual memory performance in chronic schizophrenia patients with marked negative symptoms on stable treatment in a Chinese Han population.". "Conclusion: Our findings suggest that high-frequency rTMS improves visual memory function and reduces negative symptoms in patients with chronic schizophrenia. Furthermore, the increase in visual memory performance after rTMS is associated with a decrease in negative symptoms of schizophrenia".

In addition, we have written "improvement of cognitive impairments" to "the increase in visual memory performance".

Question 2. Keywords: Please consider adding 'Non-invasive brain stimulation (NIBS)' as keyword.

Answer: As suggested by the reviewer, we have add 'Non-invasive brain stimulation (NIBS)' as keyword.

Question 3. In general, I recommend authors to use more references to back their claims, especially in the Introduction of the manuscript, which I believe is lacking. Thus, I recommend the authors to attempt to expand the topic of their article, as the bibliography is too concise. Nevertheless, I believe that less than 60/70 articles are highly inadequate for a research paper. Currently authors cite only 45 papers, and in my opinion they too low. Therefore, I suggest the authors to focus their efforts on researching relevant literature: in my opinion, adding more citations will help to provide better and more accurate background to this study. In this review, I will try to help the authors by suggesting relevant articles that suit their manuscript.

Answer: This point is very excellent. As suggested by the reviewer, we have used more references to back our claims, especially in the Introduction of the manuscript. Also, we have attempted to expand the topic of our article. As a result, the number of references has increased from 45 to 60.

Question 4. Introduction: I suggest the authors to reorganize this section, which seems too thin, and yet, dispersive. I think that more organized and detailed information about schizophrenia would provide suitable background here. I suggest the authors to make an effort to provide a brief overview of the pertinent published literature that offer a perspective on definition, causes and symptoms of schizophrenia, because as it stands, this information is not highlighted in the text. The background should be presented in the following order: schizophrenia in general including brief descriptions of epidemiology, pathogenesis, symptoms, current treatment, and challenge in treatment, and finally the authors' hypothesis. Thus, I suggest presenting a short description of schizophrenia in general, risk, pathogenesis, prognosis, comorbidity, treatment, and current challenge of management in the first paragraph, leading to the indication and background of NIBS and rTMS (https://doi.org/10.3390/brainsci11111544; https://doi.org/10.3390/biomedicines9040403; . https://doi.org/10.3390/biomedicines9030235; https://doi.org/10.3390/biomedicines8080243; doi: 10.3389/fpsyt.2022.845493. Furthermore, I would suggest adding more information on neural substrates of schizophrenia, specifically on frontal lobe dysfunction, and on related effects

on patients' memory and learning impairments. Specifically, I would suggest exploring prefrontal cortex's key role and how its disrupted function may contribute to irregular behavioral responses and therefore to the development of many mood psychiatry disorders, including depression or anxiety, and those that are common in schizophrenia: evidence from a recent study conducted on patients with lesion in ventromedial portion of prefrontal cortex (https://doi.org/10.1523/JNEUROSCI.0304-20.2020) revealed that the ventromedial prefrontal cortex (vmPFC) is involved in the acquisition of emotional conditioning (i.e., learning), assessing how naturally occurring bilateral lesion centered on the vmPFC compromises the generation of a conditioned psychophysiological response during the acquisition of pavlovian threat conditioning (i.e., emotional learning). Also, in a recent theoretical review (https://doi.org/10.1038/s41380-021-01326-4) that focused on neurobiology of emotional conditioning, the role of ventromedial prefrontal cortex (vmPFC) was analyzed in the processing of safety-threat information and their relative value, and how this region is fundamental for the evaluation and representation of stimulus-outcome's value needed to produce sustained physiological responses. Secondary, authors also might to consider some studies that have focused on this topic

(https://doi.org/10.1162/NECO_a_00779;

https://doi.org/10.1111/cns.12835;

https://doi.org/10.1038/s41386-021-01101-7).

Answer: These points are very excellent. As suggested by the reviewer, we have reorganized this section and provide more organized and detailed information about schizophrenia. Especially, we have made an effort to provide a brief overview of the pertinent published literature that offer a perspective on definition, causes and symptoms of schizophrenia. As suggested by the reviewer, we have presented a short description of schizophrenia in general, risk, pathogenesis, prognosis, comorbidity, treatment, and current challenge of management in the first paragraph, leading to the indication and background of NIBS and rTMS, showing as follows:

"Schizophrenia is a major psychiatric disorder characterized by distorted thinking and cognition, from which those who suffer do not fully recover [1]. The median prevalence of schizophrenia is 15.2/100,000 individuals, and the central 80% estimate varies within a 5-fold range (7.7-43.0/100,000) [2]. Clinical symptoms of schizophrenia fall into three categories: positive, negative, and cognitive symptoms, and have significant public health implications [1, 3]. Currently, the main treatment for schizophrenia is antipsychotic drugs, but antipsychotic drugs have their limitations. Antipsychotic drugs mainly improve positive symptoms, but negative and cognitive symptoms remain untreated [3]. It is even more difficult to improve negative symptoms and cognitive function in patients with chronic schizophrenia who have been hospitalized for a long time [3]."

Furthermore, based on the suggestions of the reviewer, we have added more information on neural substrates of schizophrenia, specifically on frontal lobe dysfunction, and on related effects on patients' memory and learning impairments. Specifically, we have explored prefrontal cortex's key role and how its disrupted function may contribute to irregular behavioral responses and therefore to the development of many mood psychiatry disorders, showing as follows: "

"It has long been thought that the prefrontal cortex plays an important role in cognitive control and that its dysfunction may lead to irregular behavioral responses that contribute to the development of many emotional psychiatric disorders, including depression or anxiety disorders, and those that are common in schizophrenia [12,13]. Evidence from a recent study conducted on patients with lesion in ventromedial portion of prefrontal cortex [12] [13] revealed that the ventromedial prefrontal cortex (vmPFC) is involved in the acquisition of emotional conditioning (i.e., learning), assessing how naturally occurring bilateral lesion centered on the vmPFC compromises the generation of a conditioned psychophysiological response during the acquisition of pavlovian threat conditioning (i.e., emotional learning). Also, in a recent theoretical review [14] [15] that focused on neurobiology of emotional conditioning, the role of ventromedial prefrontal cortex (vmPFC) was analyzed in the processing of safety-threat information and their relative value, and how this region is fundamental for the evaluation and representation of stimulus-outcome's value needed to produce sustained physiological responses. Imaging studies of depression have shown that patients with impaired dorsolateral prefrontal circuits may exhibit executive

dysfunction [16] [17]. Furthermore, it has been demonstrated that antipsychotics have memory and attention impairment when administered to unimpaired subjects [18]. Previous studies have shown that atypical antipsychotics have superior efficacy on cognitive impairment in patients with schizophrenia compared to traditional antipsychotics. Despite the benefits of atypical antipsychotics on cognitive function, further efforts are needed to improve cognitive function [19]. In this context, non-pharmacological treatments, such as non-invasive brain stimulation may address this problem"

Question 5. Introduction: In according with the previous suggested literature, I would also recommend adding information from a very recent perspective manuscript that has focused on providing a deeper understanding of human learning neural networks, showed the crucial role of human PFC, giving interesting insights on the involvement of this important brain region in the advancement of alternative, more precise and individualized treatments for a variety of neurologic and psychiatric disorders (https://doi.org/10.17219/acem/146756).

Answer: This point is very excellent. As suggested by the reviewer, we have added information from a recent perspective manuscript, showing as follows: "A recent perspective manuscript focused on providing insight into human learning neural networks shows the critical role of the human PFC, providing interesting insights into

the involvement of this important brain region in advancing alternative, more precise and personalized treatments for a variety of neurological and psychiatric disorders [17].”

Battaglia S. Neurobiological advances of learned fear in humans. *Advances in Clinical and Experimental Medicine.* 2022; 31: 217–221

Question 6. The aims of this study are generally clear and to the point; however, I believe that there are some ambiguous points that require clarification or refining. I think that authors here need to be explicit regarding how they operationally determined the association between improvement in memory after rTMS treatment and improvement in negatives symptoms of schizophrenia, as it is the variable that is manipulated in the study.

Answer: This point is very excellent. As suggested by the reviewer, we have been explicit regarding how we operationally determined the association between improvement in memory after rTMS treatment and improvement in negatives symptoms of schizophrenia, showing as follows:

“Accordingly, the main purpose of this 4-week ra ndomized double-blind sham-controlled study was to assess the effect of high-frequency rTMS for left DLPFC with 5 sessions per week for 4 consecutive weeks on visual memory performance in chronic schizophrenia patients with marked negative symptoms on stable treatment in

a Chinese Han population, which, to our best knowledge, has not been reported yet. We hypothesized that memory performance would be improved better in rTMS than sham treatment. The secondary objective was to analyze the association between improvement in memory after rTMS treatment and improvement in negatives symptoms of schizophrenia. Since previous study has shown that generalized DA deficits in cortical and extrastriatal regions are associated with cognitive deficits and negative symptoms of schizophrenia[36], we hypothesized that the reduction of negative symptoms may be associated with improvement of cognitive deficits in patients with chronic schizophrenia".

Question 7. Design: I suggest Authors to reorganize/rewrite this paragraph because, as it stands, this section is way too much inhomogeneous and dispersive, and describes the research procedures in an excessively broad way. Also, I would ask the authors to provide an explanatory figure that clearly shows experiment design process.

Answer: This point is very excellent. As suggested by the reviewer, we have rewritten the Design section, showing as follows:

"The entire protocol of this investigation was disclosed in our prior report [35], which used a single-institution, randomized controlled, double-blinded design. During the 4-week treatment, participants were randomly assigned to undergo 20 sessions of either active or sham rTMS (Figure 1). During treatment, antipsychotic medicines and

all other medications remained unaltered. Two psychiatrists blinded to the treatment given completed clinical assessments at baseline, week 4, and week 8. One clinical psychologist assessed cognitive performance at baseline, week 4, and week 8. All experimental procedures were carried out with the appropriate IRB's approval".

In addition, we have provided an explanatory figure that clearly shows experiment design process. Please refer to Figure 1.

Question 8. Active and sham rTMS: Could the authors indicate proper reference for the number of trains, the stimulation intensity, the frequency, the stimulation site and the number of sessions utilized? May provide evidence for the parameters that they considered that could have represented the best protocol for schizophrenia treatment?

Answer: This point is very excellent. As suggested by the reviewer, we have provided these parameters of the rTMS stimulation, showing as follows:

The MAGPRO-R30 magnetic stimulator (Medtronic DantecNeuroMuscular, Skovlunde, Denmark) was used to provide rTMS (Figure-of-eight coils). Motor threshold (MT) was calculated before to each TMS administration by stimulating the left motor strip with the lowest possible energy to induce at least 5 evoked potentials ≥ 0.05 mV within 10 stimuli. In active rTMS, 10 Hz stimulations over the left DLPFC were performed at a power of 110 percent of MT for 5-s intervals with a 30-s inter-train interval. For 4 consecutive weeks, 30 trains were given out every day (Monday-Friday)

(total stimuli=30,000). The left DLPFC was chosen as the rTMS target since DLPFC was used in the majority of previous studies [5, 24, 25]. F3, a 10-20 electroencephalogram (EEG) device, was utilized to locate the prefrontal cortex on the body surface in this investigation.

All operations in sham rTMS were identical, except that instead of cylindrical magnets, non-magnetized steel cylinders were rotated. The sham stimulus was 180° rotation of the 8-word coil. The coil was thick enough and had a magnetic shielding feature because the rTMS equipment was used in a blinded method in this investigation. As a result, participants were unable to distinguish between active and sham rTMS".

Question 9. Discussion: In my opinion, this paragraph would benefit from some thoughtful as well as in-depth considerations by the authors, because as it stands, it is very descriptive but not enough theoretical as a discussion should be. Also, I believe that this study would be more compelling and useful to a broad readership if the authors could expand their examination of the efficacy of non-invasive brain stimulation (NIBS) for negative symptoms in schizophrenia, and investigate the effects of non-invasive brain stimulation (NIBS) on two forms of insight, clinical and cognitive, in patients with mood disorders. On this subject, I recommend citing recent evidence that revealed that the application of NIBS induces

long-lasting effects, noninvasively modulating the abnormal activity of neural circuits (i.e., amygdala-PFC-hippocampus) involved in mood psychiatry disorders, and modulates a variety of cognitive functions: results from a crucial study (https://doi.org/10.1016/j.cub.2020.06.091) showed causal evidence for the application of NIBS over DLPFC after memory reactivation in reducing responding to learned fear. Furthermore, a recent review acknowledged the implementation of NIBS to modulate in general emotional memories (https://doi.org/10.1016/j.neubiorev.2021.04.036). Similarly, another recent study illustrated the therapeutic potential of NIBS as a valid alternative in the treatment of abnormally persistent fear memories that characterized those patients with anxiety disorders that do not respond to psychotherapy and/or drug treatments (https://doi.org/10.1016/j.jad.2021.02.076). I may also recommend additional studies that have focused on this issue (https://doi.org/10.3390/biomedicines10010076; https://doi.org/10.3390/biomedicines9050517). These findings highlight how NIBS and are a valuable tool in research and have potential diagnostic and therapeutic applications for many mood psychiatry disorders, including depression or anxiety, and those that are common in schizophrenia.

Answer: These points are very excellent. As suggested by the reviewer, we have added these points to the Discussion section on page , showing as: "In addition, our study would be more convincing and useful if we could expand our examination of the efficacy of NIBS on both forms of insight (clinical and cognitive) in patients with mood disorders. Recent evidence shows that NIBS application induces lasting effects, non-invasively modulates abnormal activity in neural circuits involved in emotional psychiatric disorders (i.e., amygdala-PFC-hippocampus), and modulates a variety of cognitive functions [37], showing causal evidence of NIBS application to the DLPFC after memory reactivation to reduce responses to learned fear [37]. Furthermore, a recent review acknowledged that NIBS implementation modulates general emotional memory [38]. Similarly, another recent study illustrates the therapeutic potential of NIBS as an effective option for treating abnormal persistent fear memories in patients with anxiety disorders that do not respond to psychotherapy and/or medication [39,40]. Several studies have shown findings highlighting how NIBS can be a valuable tool in research and has potential diagnostic and therapeutic applications for many mood psychiatric disorders, including depression or anxiety disorders, as well as those common in schizophrenia [40]."

Question 10. I believe that the 'Conclusions' section would be useful to adequately indicate convey what the authors believe is the take-home message of their study, and therefore provide a synthesis of the data presented in the paper as well as possible keys to

advancing research and understanding of the prevalence of depression in post-stroke patients.

Answer: This point is very excellent. As suggested by the reviewer, we have rewritten the Conclusions section, showing as follow: "In conclusion, cognitive impairment is one of the core features of schizophrenia, which leads to social dysfunction, but is not well addressed by current treatments. The results of the present study suggest that high-frequency transcranial magnetic stimulation therapy is beneficial for cognitive dysfunction, especially for visual memory function in schizophrenia patients. Therefore, we believe that the take-home message from the present study is that rTMS may be a promising tool for cognitive enhancement in schizophrenia patients. However, despite our encouraging results, further studies are needed to demonstrate its effectiveness in treating cognitive deficits in first-episode and unmedicated schizophrenia patients with a large sample size and longitudinal design for long-term follow-up".

Question 11. In according to the previous comment, I would ask the authors to better define a proper 'Limitations and future directions' section before the end of the manuscript, in which authors can describe in detail and report all the technical issues brought to the surface.

Answer: This point is very excellent. As suggested by the reviewer, we have rewritten a proper '"Limitations and future directs' section, showing as follows: "

Several limitations of our present study should be pointed out here. First, our sample size is relatively small, which can lead to false positive or false negative results. Our findings should be confirmed in large samples from diverse ethnic populations. Second, it is worthy of mentioning that this study was a re-analyze of the same clinical data set as our previous published study[35], and only cognitive assessment were new unpublished data. This is a clear limitation that conduct to re-publish previous clinical data. Therefore, this study was exploratory sub-analysis from a RCT that could not be presented as a new study. Third, the 180° rotation did not prevent that no stimulation was delivered to the brain, and the use of real sham coil is more suitable to avoid any active effect in the sham arm. Fourth, in this study, although it is suitable to use intention-to-treat analysis, the use of last-observation-carrying- forward is questionable given the small sample size. Therefore, in future studies, a large sample size of schizophrenia patients should be recruited and appropriate statistical analysis should be carried out to make up for the shortcomings of this study. Fifth, an important limitation of our study is the limited 4-week follow-up period, which may not be sufficient to assess changes in cognitive function. Therefore, a longer follow-up period is needed to study the efficacy of rTMS for cognitive impairment in schizophrenia in order to rule out practical effects and establish a stable relationship between active intervention and outcomes[7]. Sixth, generalizing our study is limited by our sample of chronically hospitalized elder patients, who had more severe psychopathology and longer duration of illness than typical psychotic outpatients or first episode and drug-l patients with schizophrenia. Seventh, we only used the PRM task of the CANTAB to

test visual memory due to the clinical limit of patients, and did not capture other aspects of cognitive function, which may be more sensitive to rTMS treatment. Moreover, whether rTMS treatment can improve other domains of cognitive performance need to be investigated in the future. Eighth, although we have speculated about several possible mechanisms of rTMS for the treatment of cognitive impairment, our current study did not directly evaluate these possibilities. Further research should explore potential mechanisms by which rTMS improves visual or working memory performance in patients with schizophrenia. Nineth, it should be better to report some numeric data of blinding. For example, how many participants managed to correctly guess the group of treatment, especially when they did not compensate for somatosensory effects of rTMS (for example, using electrodes). Unfortunately, we did not collect this information, which should be remedied in future research. Tenth, we chose the left DLPFC as the rTMS target, since most of previous studies performed TMS on DLPFC; however, there is solid evidence as to the role of DLPFC in negative symptoms, and there is still no exact reason why DLPFC is a good target. In addition, we did not use neuronavigated TMS to determine the location of DLPFC for the rTMS treatment, which may lead to treatment bias. Eleventh, according to our inclusion criteria, all participants were administrated with fixed dose of antipsychotics at least 12 months before entering this study. However, we had no information on whether antipsychotic drugs were changed before the study started and what the effect of such a change might have had. Furthermore, we were unable to exclude that those patients who did not improve after the rTMS showed had not

previously responded satisfactorily to previous medication. Therefore, although antipsychotic drugs were used as covariates in statistical analysis, the effects of long-term, different doses and types of antipsychotics on the efficacy of rTMS cannot be avoided. Twelfth, we had partially explained why we used only one visual memory test, without testing other cognitive functions, because of low education and advanced age of the patients; however, this explanation is very reluctant. It is well known that there are various tests for almost every cognitive function adapted for various group ages, education and intelligence. Therefore, it should to use adequate additional tests before drawing a firm conclusion about generalization of this treatment".

Question 12. Figures: Please insert Figure 1 and Figure 2 into the main text close to their first citation, in this case in page 6, and provide a comprehensive explanatory title and caption. Overall, the manuscript contains 3 figures, 2 tables and 45 references. In my opinion, the number of references it is too low for an original research article, and this prevents the possibility of publishing it in this form. References should be more than 60/70 for original research articles. However, the manuscript might carry important value presenting effect of rTMS on visual memory in patients with schizophrenia. I hope that, after these careful revisions, the manuscript can meet the Journal's high standards for publication. I am available for a new round of revision of this article. I declare

no conflict of interest regarding this manuscript. Best regards,

Reviewer

Answer: As suggested by the reviewer, we have inserted Figure 1 and Figure 2 into the main text close to their first citation, and provided a comprehensive explanation title and caption.

In addition, based on the comments/suggestions of the reviewer, the number of references is increased from 45 to 61.