

# Parietal-Hippocampal rTMS Improves Cognitive Function in Alzheimer's Disease by Inducing Increased Dynamic Functional Connectivity of Default Mode Network

**Lili Wei**

Zhejiang University School of Medicine Sir Run Run Shaw Hospital

**Yingchun Zhang**

Zhejiang University School of Medicine Sir Run Run Shaw Hospital

**Jintao Wang**

Zhejiang University School of Medicine Sir Run Run Shaw Hospital

**Luoyi Xu**

Zhejiang University School of Medicine Sir Run Run Shaw Hospital

**Kehua Yang**

Zhejiang University School of Medicine Sir Run Run Shaw Hospital

**Xinghui Lv**

Zhejiang University School of Medicine Sir Run Run Shaw Hospital

**Zhenwei Zhu**

Zhejiang University School of Medicine Sir Run Run Shaw Hospital

**Qian Gong**

Zhejiang University School of Medicine Sir Run Run Shaw Hospital

**Weiming Hu**

People's Hospital of Quzhou

**Xia Li**

Shanghai Mental Health Center

**Mincai Qian**

Huzhou Third People's Hospital

**Yuedi Shen**

Hangzhou Normal University

**Wei Chen** (✉ [srrcw@zju.edu.cn](mailto:srrcw@zju.edu.cn))

Shao Yifu Hospital: Zhejiang University School of Medicine Sir Run Run Shaw Hospital

**Keywords:** Repetitive transcranial magnetic stimulation, Alzheimer's disease, default mode network, functional magnetic resonance imaging, parietal lobe, hippocampus

**Posted Date:** August 3rd, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-746528/v1>

**License:**  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# **Parietal-hippocampal rTMS Improves Cognitive Function in Alzheimer's disease by inducing Increased Dynamic Functional Connectivity of Default Mode Network**

Lili Wei<sup>1#</sup>, Yingchun Zhang<sup>1#</sup>, Jintao Wang<sup>1#</sup>, Luoyi Xu<sup>1</sup>, Kehua Yang<sup>1</sup>, Xinghui Lv<sup>1</sup>, Zhenwei Zhu<sup>1</sup>, Qian Gong<sup>1</sup>, Weiming Hu<sup>2</sup>, Xia Li<sup>3</sup>, Mincai Qian<sup>4\*</sup>, Yuedi Shen<sup>5\*</sup>, Wei Chen<sup>1,6,7\*</sup>

# These authors contributed equally to this work.

<sup>1</sup> Department of psychiatry, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou, China

<sup>2</sup> Third People's Hospital of Quzhou, Quzhou, Zhejiang, China

<sup>3</sup> Shanghai Mental Health Center, Shanghai, China

<sup>4</sup> Third People's Hospital of Huzhou, Huzhou, Zhejiang, China

<sup>5</sup> Hangzhou Normal University Medical School, Hangzhou, Zhejiang, China

<sup>6</sup> Department of Psychology and Behavioral Sciences, Zhejiang University, Hangzhou, China

<sup>7</sup> Key Laboratory of Medical Neurobiology of Zhejiang Province, Hangzhou, China

Correspondence to: Wei Chen

Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University

East Qingchun Road 3#, Hangzhou, Zhejiang, China

E-mail: [srrcw@zju.edu.cn](mailto:srrcw@zju.edu.cn); Tel: +86-571-86006375; Fax: +86-571-87887801

Correspondence may also be addressed to:

Yuedi Shen

Hangzhou Normal University Medical School, Hangzhou, Zhejiang, China

E-mail: [shenyd5@126.com](mailto:shenyd5@126.com)

Mincai Qian

Third People's Hospital of Huzhou, Huzhou, Zhejiang, China

E-mail: [1311956613@qq.com](mailto:1311956613@qq.com)

## **Abstract**

**Background:** Alzheimer's disease, a neurodegenerative disease with significant social and economic impact, is mainly treated by focusing on decelerating cognition decline. Parietal-hippocampal repetitive transcranial magnetic stimulation (rTMS) improves memory and cognitive function in Alzheimer's disease, however, the underlying therapeutic mechanism has not been elucidated.

**Methods:** A double-blind, randomized, sham-controlled parietal-hippocampal rTMS trial of mild-to-moderate Alzheimer's disease patients was conducted in the current study. High-frequency rTMS was applied to a subject-specific left lateral parietal region with the highest functional connectivity with the hippocampus based on resting-state fMRI. Patients were randomized to either rTMS or sham treatment (five sessions/week for a total of 10 sessions). A multimodal MRI scan and a complete neuropsychological battery of tests were conducted at baseline, immediately after the intervention and 12-week follow-up after the rTMS treatment. Primary outcomes were differences in the Mini Mental State Examination (MMSE) and Philadelphia Verbal Learning Test (PVL) scores between the groups and between pre- and post-treatment. Moreover, flexible least squares (FLS) method was used to calculate the dynamic functional connectivity (dFC) of the default mode network (DMN), and dFC changes were compared between the groups and between pre- and post-treatment.

**Results:** Patients undergoing active rTMS treatment (n=31) for two weeks showed higher MMSE, PVL-Immediate recall, and PVL-Short Delay recall scores, whereas those who underwent sham rTMS (n=27) treatment did not show significant changes in

these measures. Dynamic functional connectivity (dFC) magnitude of the default mode network (DMN) was significantly higher after two weeks of rTMS treatment in the patients who underwent active-rTMS treatment, however, no significant changes were observed in patients who received sham-rTMS treatment. dFC magnitude reduced to baseline level at 12-week follow-up, which resembled the trajectory of the cognitive measures. A significant positive correlation was observed between changes in MMSE and changes in the dFC magnitude of DMN in patients who underwent active-rTMS treatment, but not in those who received sham-rTMS treatment.

**Conclusions:** The findings of the current study indicate that fMRI-guided rTMS treatment improves memory and cognitive function of Alzheimer's disease patients. In addition, the findings indicate that the DMN functional connectivity contributes to therapeutic effectiveness of rTMS.

**Trial registration:** China National Medical Research Platform (<http://114.255.48.20/login>, No:MR-33-20-004217), retrospectively registered 2020-12-23.

**Keywords:** Repetitive transcranial magnetic stimulation; Alzheimer's disease; default mode network; functional magnetic resonance imaging; parietal lobe; hippocampus

## **Background**

Alzheimer's disease is a highly prevalent brain disorder with significant social and economic impact. It is projected that more than 100 million people globally will have Alzheimer's disease by the year 2050 [1]. Currently, treatment of Alzheimer's disease is mainly through pharmacological therapy. Repetitive transcranial magnetic stimulation (rTMS) therapy is a novel promising treatment protocol for psychiatric and neurological disorders [2-4], including mild cognitive impairment (MCI) and Alzheimer's disease [5-8]. Previous studies report that rTMS improves language performance [9-11], attentional capacity [12], executive function [13-15], as well as verbal memory and episodic memory [16-19]. Despite its therapeutic efficacy, there is no consensus on the most effective target region for rTMS-based clinical treatment of Alzheimer's disease. Notably, previous studies report varying spatial regions, including dorsolateral prefrontal cortex (DLPFC) [9,20], inferior frontal gyrus [12,13], precuneus [21], and posterior temporal gyrus [16]. Moreover, the neurophysiological mechanism underlying rTMS's action in treatment of Alzheimer's disease has not been fully elucidated [22,23], thus hindering adoption and application of the technology.

Alzheimer's disease is a neurodegenerative disease that affects functional connections in the brain, and is characterized by default mode network (DMN) changes in patients [24-26]. Alzheimer's patients mainly present with a reduction in functional connection of DMN, mainly those between the posterior precuneus, posterior cingulate gyrus and the anterior medial prefrontal cortex (MPFC), anterior cingulate gyrus; and show DMN-related left and right hippocampus changes [27-29]. Previous studies report

that changes in DMN functional connectivity are correlated with those in patients' cognitive function, implying that they represent high-risk factors for development of dementia [30,31]. Notably, Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), auditory vocabulary and delayed recall, reportedly change following medication, cognitive therapy, and acupuncture treatment, whereas DMN's functional connectivity increases after treatment in Alzheimer's disease patients [32-34]. These findings indicate that DMN plays an important role in development and treatment of Alzheimer's disease, and is a potential therapeutic target for development of treatment therapies.

In the present study, a double-blind, randomized, sham-controlled clinical trial was conducted using the functional magnetic resonance imaging (fMRI)-guided rTMS technique [35,36] for treatment of patients with Alzheimer's disease. rTMS showed efficacy in treatment of major depression [37-39] and targeted an individualized left lateral parietal region with highest functional connectivity in each patient's hippocampus using resting-state fMRI for two weeks. Thereafter, each patient was subjected to several fMRI sessions and a complete neuropsychological series of tests. Our previous research [40] reported that high specificity of rTMS to the left parietal cortex improves cognitive function, mainly memory in Alzheimer's disease. The aim of the current study was to explore whether improvement of rTMS-induced memory and cognitive function is associated with perturbation of DMN.

## **Methods**



## **Participants**

A total of 103 patients with mild-to-moderate Alzheimer's disease patients were recruited to the current study from outpatient and inpatient section of the Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou, China. Each patient underwent standard examination. Patients were screened from an ongoing follow-up project, which sought to treat Alzheimer's disease through personalized fMRI-guided rTMS of the parietal lobe. The current study was registered on the China National Medical Research Platform (<http://114.255.48.20/login>), No:MR-33-20-004217. Patients were included if they; (1) were diagnosed with probable Alzheimer's disease based on the Diagnostic and Statistical Manual of Mental Disorders, 5<sup>th</sup> edition; (2) had a Clinical Dementia Rating (CDR) score between 0.5 and 2; (3) were aged between 55-85 years old; and (4) were right-handed. Conversely, patients that met the following criteria were excluded: (1) patients who were diagnosed with severe liver, kidney, heart, and lung diseases; (2) patient who had a history of significant head trauma or neurological disorders; (3) patients whose T1 or T2 images showed presence of focal brain lesions; (4) patients who exhibited any MRI contraindications, such as medical implants or devices, metals in the body or claustrophobia. This study was approved by the Ethics Committee of the Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University (Approval No: 20170228-1), and all patients signed an informed consent form prior to participation in the study.

## **Procedures**

The current study was a double-blind, randomized, sham-controlled trial. Patients were randomly assigned to groups, using a single random sequence number in a series of opaque and sealed envelopes. The envelope of each patient was opened by the rTMS operators prior to the first treatment session. Patients were placed either into the active-rTMS group in which subjects underwent a 2-week rTMS treatment, or a sham group in which subjects were subjected to 2 weeks of sham treatment. Each session was performed once daily, 5 days/week. Patients underwent a complete neuropsychological series of tests and a multimodal MRI scan at baseline (T0), immediately (T1) and at 12-week follow-up after the end of rTMS treatment (T2).

Prior to randomization, 10 patients were excluded from the study due to presence of brain lesions and psychiatric disorders, 5 withdrew due to personal reasons, whereas 2 were lost to follow up. Therefore, a total of 86 patients were randomized into either rTMS or sham treatment groups. 58 patients completed the 2-week treatment and corresponding assessments, including neuropsychological tests and fMRI scan. Notably, 8 and 9 patients in the rTMS and sham groups, respectively, did not complete neuropsychological assessments at baseline or rTMS/ sham treatment. Moreover, 4 and 7 patients in the rTMS and sham groups, respectively, did not complete fMRI scan at T1. Therefore, 31 and 27 patients were assigned to the rTMS and sham groups, respectively. Although 9 and 7 patients in the rTMS and sham groups, respectively, are yet to complete the 12-week follow-up, their data at baseline and immediately after the end of rTMS treatment were included in analyses. Notably, 4 patients in both the rTMS and sham groups were lost to follow-up, whereas 5 and 3 in the treatment and sham

groups, respectively, have not yet reached the follow-up period.

### **Acquisition of MRI data**

T1-weighted and resting-state fMRI data were acquired using a 3.0 T MR scanner (GE Discovery MR750, GE Medical Systems, Milwaukee, Wisconsin, USA), equipped with an 8-channel head coil array at the Affiliated Hospital of Hangzhou Normal University. Patients were instructed to remain still in the scanner during scanning, with their eyes open and were requested not think about anything specific. Functional images were obtained, axially, using an echo-planar imaging (EPI) sequence using the following parameters: volumes=240; repetition time (TR)=2,000 ms; echo time (TE) = 30 ms; flip angle (FA) = 90°; field of view (FOV) = 220 × 220 mm<sup>2</sup>; matrix = 64 × 64; and slice thickness = 3.2 mm with no gap. High-resolution anatomic 3-dimensional T1-weighted images were obtained using the following parameters: 176 axial slices; TR = 8.1 ms; TE = 3.1 ms; FA = 8°; FOV = 250 × 250 mm<sup>2</sup>; matrix = 250 × 250; and slice thickness = 1.0 mm with no gap.

### **Identification of the stimulus target**

Stimulus target of each patient was identified using personal maps of hippocampal resting-state functional connectivity obtained at baseline. For each patient, fMRI performed during the resting state was used to generate seed-based connectivity maps, with a hippocampal target as the seed. The voxel at the middle of the hippocampal body (MNI coordinate x=-24, y=-18, z=-18) was chosen as the hippocampal target voxel [35],

whereas a left lateral parietal location that showed high functional connectivity with the left hippocampal seed was chosen as the stimulation site.

Functional and structural MRI data were preprocessed using Data Processing Assistant for Resting-State fMRI tool (DPARSF; <http://rfmri.org/DPARSF>) [41], in the SPM12 software (<http://www.fil.ion.ucl.ac.uk/spm>) and a Resting-State fMRI Data Analysis Toolkit (REST; [www.restfmri.net](http://www.restfmri.net)) [42]. Preprocessing steps included: slice timing after removing first 10 volumes; realignment; motion correction; functional/structural co-registration; segment and affixer regularization following the International Consortium for Brain Mapping European brain template; resampling to a resolution of  $1.5 \times 1.5 \times 1.5$  mm; normalization into the standard Montreal Neurological Institute (MNI) space using T1 image unified segmentation; spatial smoothing using a 6-mm full-width-at-half-maximum Gaussian kernel; removal of the linear trend; and filtering at 0.01–0.08 Hz. The resting-state functional connectivity between the hippocampal target and the entire brain was then computed, and the local maxima connectivity within a 15 mm radius of the MNI coordinates ( $x=-47$ ,  $y=-68$ ,  $z=+36$ ; area region encompassing the inferior parietal lobule, Brodmann areas 39 and 40) was identified, and was designated as the stimulation target region. This stimulation location was marked in stereotactic space and was overlaid onto the structural MRI to provide localization during rTMS.

### **rTMS stimulation**

Application of rTMS treatment to a personalized left lateral parietal target was

performed following an online neuronavigation system (Brainsight 2, Rogue Research, Montreal, Quebec, Canada). rTMS was applied to the stimulation location during daily treatment sessions using a Magstim Rapid2 stimulator, with a 70mm air-cooled figure eight coil (Magstim Company, Whitland, Wales, United Kingdom). The stimulation location was located through the structural MRI using a frameless infrared stereotactic system. Motor threshold of each patient was defined as the minimum TMS intensity that triggered a motor evoked potential (MEP) of at least 50  $\mu$ V for at least 5 of 10 consecutive pulses at baseline. For stimulation, rTMS was applied at a motor threshold of 100-110% to the stimulation location, for 20 minutes of consecutive blocks of 10 Hz pulses for two seconds followed by 28 seconds of no stimulation. The TMS coil was held tangential to the scalp of the stimulation location. For the sham stimulation, the coil was rotated by a 45° along the handle axis of the coil and the distance between the stimulation side and the scalp was more than 5 cm, and the coil's stimulation side was kept away from the scalp.

## **Blinding**

Patients in the sham group produced the same noise and sensation, and were made aware that scalp discomfort as well as transient fatigability could occur during rTMS or sham sessions. Only the rTMS operators were aware of the randomized treatment, whereas patients and neuropsychologists administering clinical assessments were not aware whether patients received rTMS or sham treatment. After every treatment session, patients were asked how they felt about the treatment to confirm that they did not know

which treatment they received.

## **Neuropsychological assessments**

All measurements for neuropsychological assessments were repeated three times, at baseline, immediately after intervention and after 12-week follow-up after the end of rTMS treatment. These assessments were performed by a trained neuropsychologist who was blinded to the randomized treatment. The main neuropsychological test questionnaire comprised the Chinese version of Mini Mental State Examination (MMSE), 12-Word Philadelphia Verbal Learning Test (PVLTL), CDR, Lawton-Brody Activities of Daily Living (ADL) scale and Patient Health Questionnaire-9 (PHQ-9).

## **Processing of neuroimaging data**

Functional and structural MRI data were preprocessed using DPARSF, which is based on SPM12 software. Preprocessing steps included: slice timing after removing the first 10 volumes; realignment; functional/structural co-registration; normalization of data into the standard MNI space using T1 image unified segmentation; resampling to 3-mm isotropic voxels; and spatial smoothing with a 6-mm FWHM Gaussian kernel. The preprocessed data were used for subsequent analyses. Notably, a maximum head motion criterion of 3 mm and 3° was used. Two patients in the active-rTMS group exceeded this criterion, and their scans were excluded from further analyses.

The preprocessed data for all patients was concatenated and data were subjected to group independent component analysis (ICA) using GIFT toolbox

(<http://icatb.sourceforge.net>). Approximately 34 independent components based on the minimum description length criteria was used. Common components for all participants were acquired using the ICA decomposition based on the Infomax algorithm [43], whereas ICASSO procedure with 10 runs of ICA was used to ensure stability [44]. Notably, two trained investigators (J.W. and Y.S.) focused on the DMN, and identified 6 DMN subnetworks based on inspection of their spatial locations and time courses. A DMN subnetwork was selected if it was spatially located in canonical DMN regions, including the posterior cingulate cortex, medial prefrontal cortex, anterior cingulate cortex, and bilateral parietal lobule, as well as power spectrum of its associated time course which was primarily located in the range of 0.01-0.1 Hz. In addition, the flexible least squares (FLS) method was used to calculate the dynamic functional connectivity (dFC) within each patient's DMN through the dynamic brain connectivity (DynamicBC) toolbox [45]. A time-varying parameter regression equation was used to describe the dynamic interactions between the 6 DMN subnetworks as shown below:

$$y(t) = x(t) \times \beta(t) + \varepsilon(t)$$

where  $x(t)$  and  $y(t)$  represent the time series of two DMN subnetworks, whereas  $\varepsilon(t)$  represents the approximation error.  $\beta(t)$  represents the coefficient for estimating covariance of the two networks and reflects the connectivity between  $x(t)$  and  $y(t)$  at time  $t$ . To obtain the value of  $\beta$  at each time point, a cost function was defined as follows:

$$C(\beta, \varepsilon, T) = \mu \times r_d^2(\beta, T) + r_m^2(\beta, T)$$

where  $r_d^2(\beta, T)$  is the sum of the squared residual dynamic error  $r_d^2(\beta, T) =$

$\sum_{t=1}^{T-1} (\beta(t+1) - \beta(t))^T (\beta(t+1) - \beta(t))$  where FLS indicates that the coefficients vector evolves slowly over time with  $\beta(t+1) - \beta(t) \approx 0$ ;  $r_m^2(\beta, T)$  is the sum of squared residual measurement errors  $r_m^2(\beta, T) = \sum_{t=1}^T (y(t) - x(t) \times \beta(t))^2$  that satisfy  $y(t) - x(t) \times \beta(t) \approx 0$ ; whereas  $\mu$  represents the weighting parameter for controlling these two parts of the cost function. In the present study, this parameter was set at 100 as previously described [45]. FLS was used to extract time courses of the DMN subnetworks, then the dynamic functional connectivity (dFC) magnitude and dFC variance between each pair of subnetworks were calculated. For inter-network connectivity, DMN's dFC magnitude was defined as the average of the  $\beta$  values between each pair, whereas the average standard deviation of  $\beta$  values across all inter-network pairs was defined as the dFC variance of DMN.

### **Sample size and power analysis**

In the pilot study, the mean increment in PVLt score from baseline to two weeks was 6.10 (SD = 4.80) and 1.80 (SD = 3.58) points in the rTMS and sham groups, respectively. A relatively conservative difference of 4.00 (SD = 5.00) points, between the two groups, was used to estimate the sample size. Statistical analysis showed that a total of 68 patients were enough to provide a power of approximately 90% (at a 5% significance level). The sample size was increased by 25%, to 43 patients per group to cater or possible study dropouts.

### **Statistical analysis**



Chi-squared test was used to determine gender and medication distribution, and an independent *t*-test was used to analyze differences in other continuous variables between the rTMS and sham groups at baseline. Differences in dFC magnitude, dFC variance, and neuropsychological assessments, between the rTMS and sham groups, across different time points were evaluated using paired *t*-tests. Moreover, correlational analysis was performed to determine the relationship between changes in MMSE, PVLV and those of DMN's dFC magnitude and variance (baseline phase T0 and immediate after treatment phase T1). All statistical analyses were performed using SPSS for Windows (v 20.0, IBM). Two-tailed probability values of  $p < 0.05$  were considered statistically significant for paired *t*-test results. For correlations, significance level was set as a one-tailed  $p < 0.05$ , since a significant correlation was expected in the active-rTMS group.

## **Results**

### **Patient characteristics**

A total of 31 and 27 patients were assigned to the rTMS and sham groups, respectively, prior to analysis. Analysis showed no statistically significant differences in baseline characteristics between the two groups (Table 1).

### **rTMS treatment improves cognitive function in patients**

Patients in the rTMS group showed a 1.2 increase in MMSE scores immediately after 2 weeks of treatment ( $p = 0.009$ ), whereas those in the sham group exhibited a 0.6

increase over the same period although the difference was not statistically significant ( $p = 0.273$ ). PVLТ was used to further explore memory performance of Alzheimer's disease patients, with higher values indicating greater memory performance. The finding showed that patients in the treatment group had significantly improved PVLТ scores for Immediate ( $p < 0.001$ ) and Short Delay ( $p = 0.044$ ) recall, after 2 weeks of rTMS treatment compared with those in the sham group. Notably, 9 and 7 patients in the rTMS and sham groups, respectively, have not yet completed the 12-week follow-up, thus 22 and 20 participants in the treatment and sham groups, respectively were included in the 12-week follow-up analyses. Analysis showed that baseline characteristics were not significantly different between the 22 participants in the treatment group and 20 in the sham group (Supplementary Table S1). Moreover, analysis showed no significant improvement in MMSE and PVLТ scores in patients in both groups at 12-week follow-up, as shown by insignificant changes in the CDR, ADL, PQH-9 and PVLТ Long Delay recall scores. These findings indicate that rTMS treatment significantly improved cognitive function in patients, over a 2-week period, although the benefits were not observed at 12-week follow-up (Table 2).

### **Changes in DMN's dFC magnitude and their correlation with MMSE**

A total of 6 DMN subnetworks were successfully identified (Fig. 1A; Fig. S1 in the supplement) with group ICA. The findings showed a significantly higher DMN dFC magnitude in patients in the rTMS group compared with that of the sham group, after 2 weeks of treatment ( $p=0.003$ ). However, this magnitude decreased at 12-week follow-

up (Fig. 1B). Notably, changes in MMSE showed a significant positive correlation with those of DMN dFC magnitude, immediately after 2 weeks of rTMS treatment ( $r=0.291$ ,  $p=0.015$ ) across all Alzheimer's disease patients (Fig. 1C). Moreover, improved MMSE scores were correlated with higher DMN dFC magnitudes in the rTMS group ( $r=0.325$ ,  $p=0.042$ ), but not in the sham group ( $r=0.207$ ,  $p=0.151$ ) (Fig. 1C). Analysis showed no correlation between the changes in PVLt and DMN dFC magnitude. The findings showed no statistically significant differences in dFC variance measures between the two groups.

### **Adverse effects**

Two patients in the rTMS, and one in the sham group manifested adverse effects. One patient in the rTMS group reported local scalp discomfort which persisted for more than 15 minutes after the first treatment session. One patient from the treatment group and one from the sham group reported transient fatigue. The two subjects could not tolerate the effects and requested to withdraw from the study. Notably, no severe adverse effects were observed in the study.

### **Discussion**

The current study explored whether applying subject-specific hippocampal-targeted rTMS treatment over the lateral parietal lobule improves cognitive functions of Alzheimer's disease patients. Further, the study explored whether the resulting improvement was associated with change in the intra-DMN functional connectivity.

The findings showed that rTMS administration significantly increased MMSE, PVL-Immediate recall, PVL-Short Delay recall scores and the dFC magnitude of the DMN after two weeks, compared with the sham group. Moreover, improvement in MMSE scores was significantly correlated with changes in DMN's dFC magnitude in the rTMS treatment group.

### **Parietal-hippocampal targeted rTMS improves cognitive function in Alzheimer's disease patients**

Memory impairment in Alzheimer's disease patients is mainly associated with the hippocampus, which is hardly accessible through the conventional rTMS technique. Previous studies on healthy adult participants reported that memory performance can be enhanced by subject-specific fMRI-guided rTMS treatment targeting cortical-hippocampal networks [35,46]. In the present study, fMRI-guided rTMS protocol was used for treatment of patients with Alzheimer's disease, and the findings showed that patients in the rTMS group had significantly higher MMSE, PVL Immediate recall and Short Delay recall scores compared with those in the sham group. Prevalence of depression in patients with Alzheimer's disease is approximately 50% [47], and even mild depressive symptoms have been associated with significant functional impairment. Therefore, it is important to control for the possibility that patients might benefit from TMS secondary to stimulation effects on comorbid depressive symptoms. The findings of the current study did not show significant change in PHQ-9, a module used in evaluation of depression in primary care [48,49]. This implies that the cognitive

improvements in patients under rTMS treatment were not due to alleviation of depressive symptoms. Notably, the findings of the current study showed that rTMS-induced cognitive improvement was reduced at 12-week follow-up in subjects in the treatment group which is consistent with findings from a previous study [50] which reported that 30 sessions comprising 20Hz rTMS treatment over a six-week period in the DLPFC significantly improved cognitive function of patients, although this improvement could not be maintained during a three-month follow up period. This can be attributed to rTMS dosage, and its combination with other therapeutic approaches. For example, Rabey and Dobronevsky [51] combined rTMS (consisting 30 sessions of 1-hour daily, over a 5-day period per week, for 6 weeks) with cognitive training to treat patients with mild-to-moderate Alzheimer's disease, and reported that this regimen improved cognitive function, and the effect lasted for a minimum of 9 months. The short time of symptomatic improvement, observed in the present study, may be due to application of a single treatment modality (rTMS) over a short period of time (20 minutes daily session, 5 days per week for 2 weeks). The efficacy of rTMS can be increased if the treatment time is extended or the regime is combined with other non-drug therapies, such as cognitive stimulation and exercise. Further studies are needed to explore the longevity of the effects of rTMS treatment on patients with Alzheimer's disease.

### **Parietal-hippocampal targeted rTMS enhances DMN neural activity in Alzheimer's disease**

In the present study, DMN's dFC magnitude was calculated by summarizing the dynamic functional connectivity strength across all pairs of the six DMN subnetworks. Therefore, it can be considered as a measure of the intra-DMN functional connectivity. The findings showed a significantly higher dFC magnitude in patients treated with rTMS compared with those in the sham group. In addition, improved MMSE scores showed a significant positive correlation with higher DMN's dFC magnitude changes in patients treated with rTMS. The dFC magnitude was reduced to baseline level at 12-week follow-up, which was similar to the trajectory of the cognitive measures. Alzheimer's disease is a generalized disconnection syndrome that causes functional impairments in resting state networks mainly in DMN [52-54]. Although previous studies report that rTMS improves cognitive performances of Alzheimer's disease patients [55], the actual neural substrates underlying this therapeutic efficacy have not been fully elucidated. A previous study combined rTMS and electroencephalogram techniques and reported that rTMS treatment on precuneus improved long-term memory in prodromal Alzheimer's disease patients by modulating neural activity of the precuneus, as well as its connections with medial parietal and frontal areas in the DMN [21]. Moreover, a recent study used the fMRI technique and reported that rTMS-induced functional connectivity changes within the DMN were associated with clinical cognitive improvements in patients with amnesic MCI [56]. The findings from the current study and from previous studies show effectiveness of rTMS in treating patients with Alzheimer's disease. Further, the findings indicate that intra-DMN functional

connectivity may be a neuroimaging target for therapeutic effectiveness of rTMS during recovery of cognitive impairment in this group of patients.

## **Limitations**

A major limitation of this study was the relatively heterogeneous sample of Alzheimer's disease patients ( $0.5 \leq \text{CDR} \leq 2.0$ ). However, analysis showed no significant differences in baseline characteristics between participants in the treatment group and sham groups. Stimulation parameters and study protocols reported in previous studies varied significantly [57], thus the current study adopted a protocol which has demonstrated efficacy in enhancing memory ability in healthy participants [35]. However, further studies using larger sample sizes should be conducted to identify optimal parameters in this protocol by comparing other stimulus targets and validate its application in clinical practice. Moreover, the findings of the current study should be replicated in studies with larger sample size.

## **Conclusion**

In summary, the findings of the current study show feasibility and efficacy of fMRI-guided rTMS treatment over the lateral parietal lobule in treatment of patients with Alzheimer's disease. The neuroimaging results provide evidence that the intra-DMN functional connectivity may be a neural target for rTMS's therapeutic efficacy in this group of patients. These findings provide new insights to guide future prospective clinical trials.

## **List of abbreviations**

ADL=Activities of Daily Living scale; CDR=Clinical Dementia Rating; dFC=dynamic Functional Connectivity; DLPFC=Dorsolateral Prefrontal Cortex; DMN=Default Mode Network; MCI= Mild Cognitive Impairment; MMSE=Mini-Mental State Examination; PHQ-9=Patient Health Questionnaire-9; PVL=12-Word Philadelphia Verbal Learning Test; rTMS=repertive Transcranial Magnetic Stimulation.

## **Declarations**

### ***Ethical approval and consent to participate***

This study was approved by the Ethics Committee of the Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University (No: 20170228-1). All participants signed an informed consent form prior to inclusion in the study.

### ***Consent for publication***

Written informed consent for publication of their clinical details was obtained from each patient or their relative. A copy of the consent form is available for review by the Editor of this journal.

### ***Availability of data and materials***

Data that support the findings of the current study are available upon reasonable request.



### ***Competing interests***

Authors declare that they have no competing interests to declare.

### ***Funding***

This work was supported by grants from the National Key Research & Development Program of China (2017YFC1310502), the Natural Science Foundation of China (82071181), and Key Research & Development Program of Zhejiang Province (2020C03021, 2018C03023), and the Science and Technology Program of Hangzhou Municipality (20190101A10).

### ***Author contributions***

LW carried out data collection and analysis. JW performed neuropsychological assessment and data analysis. YZ, LX, QG, WH and MQ carried out data collection. KY and XL performed rTMS treatments. ZZ performed neuropsychological assessment. WC, YS, and XL participated in study conceptualization and design. All authors read and approved the final manuscript.

### ***Acknowledgements***

We thank Professor Li Su for assistance during the final preparation of this manuscript.

### **References**

- [1] Barnes DE, Yaffe K. The projected effect of risk factor reduction on Alzheimer's disease prevalence. *Lancet Neurol.* 2011; 10: 819-28.
- [2] Fregni F, Pascual-Leone A. Technology insight: noninvasive brain stimulation in neurology-perspectives on the therapeutic potential of rTMS and tDCS. *Nat Clin Pract Neurol.* 2007; 3: 383-93.
- [3] Slotema CW, Blom JD, Hoek HW, Sommer IE. Should we expand the toolbox of psychiatric treatment methods to include Repetitive Transcranial Magnetic Stimulation (rTMS)? A meta-analysis of the efficacy of rTMS in psychiatric disorders. *J Clin Psychiatry.* 2010; 71: 873-84.
- [4] Elder GJ, Taylor JP. Transcranial magnetic stimulation and transcranial direct current stimulation: treatments for cognitive and neuropsychiatric symptoms in the neurodegenerative dementias? *Alzheimers Res Ther.* 2014; 6: 74.
- [5] Nardone R, Bergmann J, Christova M, Caleri F, Tezzon F, Ladurner G, et al. Effect of transcranial brain stimulation for the treatment of Alzheimer disease: a review. *Int J Alzheimers Dis.* 2012; 2012: 687909.
- [6] Chou YH, Ton That V, Sundman M. A systematic review and meta-analysis of rTMS effects on cognitive enhancement in mild cognitive impairment and Alzheimer's disease. *Neurobiol Aging.* 2020; 86: 1-10.
- [7] Koch G, Martorana A, Caltagirone C. Transcranial magnetic stimulation: Emerging biomarkers and novel therapeutics in Alzheimer's disease. *Neurosci Lett.* 2020; 719: 134355.

- [8] Chu CS, Li CT, Brunoni AR, Yang FC, Tseng PT, Tu YK, et al. Cognitive effects and acceptability of non-invasive brain stimulation on Alzheimer's disease and mild cognitive impairment: a component network meta-analysis. *J Neurol Neurosurg Psychiatry*. 2021; 92: 195-203.
- [9] Cotelli M, Calabria M, Manenti R, Rosini S, Zanetti O, Cappa SF, et al. Improved language performance in Alzheimer disease following brain stimulation. *J Neurol Neurosurg Psychiatry*. 2011; 82: 794-7.
- [10] Trebbastoni A, Raccach R, de Lena C, Zangen A, Inghilleri M. Repetitive deep transcranial magnetic stimulation improves verbal fluency and written language in a patient with primary progressive aphasia-logopenic variant (LPPA). *Brain Stimul*. 2013; 6: 545-53.
- [11] Devi G, Voss HU, Levine D, Abrassart D, Heier L, Halper J, et al. Open-label, short-term, repetitive transcranial magnetic stimulation in patients with Alzheimer's disease with functional imaging correlates and literature review. *Am J Alzheimers Dis Other Demen*. 2014; 29: 248-55.
- [12] Eliasova I, Anderkova L, Marecek R, Rektorova I. Non-invasive brain stimulation of the right inferior frontal gyrus may improve attention in early Alzheimer's disease: a pilot study. *J Neurol Sci*. 2014; 346: 318-22.
- [13] Anderkova L, Eliasova I, Marecek R, Janousova E, Rektorova I. Distinct Pattern of Gray Matter Atrophy in Mild Alzheimer's Disease Impacts on Cognitive Outcomes of Noninvasive Brain Stimulation. *J Alzheimers Dis*. 2015; 48: 251-60.

- [14] Drumond Marra HL, Myczkowski ML, Maia Memoria C, Arnaut D, Leite Ribeiro P, Sardinha Mansur CG, et al. Transcranial Magnetic Stimulation to Address Mild Cognitive Impairment in the Elderly: A Randomized Controlled Study. *Behav Neurol*. 2015; 2015: 287843.
- [15] Padala PR, Padala KP, Lensing SY, Jackson AN, Hunter CR, Parkes CM, et al. Repetitive transcranial magnetic stimulation for apathy in mild cognitive impairment: A double-blind, randomized, sham-controlled, cross-over pilot study. *Psychiatry Res*. 2018; 261: 312-8.
- [16] Zhao J, Li Z, Cong Y, Zhang J, Tan M, Zhang H, et al. Repetitive transcranial magnetic stimulation improves cognitive function of Alzheimer's disease patients. *Oncotarget*. 2017; 8: 33864-71.
- [17] Turriziani P, Smirni D, Mangano GR, Zappala G, Giustiniani A, Cipolotti L, et al. Low-Frequency Repetitive Transcranial Magnetic Stimulation of the Right Dorsolateral Prefrontal Cortex Enhances Recognition Memory in Alzheimer's Disease. *J Alzheimers Dis*. 2019; 72: 613-22.
- [18] Zhang F, Qin Y, Xie L, Zheng C, Huang X, Zhang M. High-frequency repetitive transcranial magnetic stimulation combined with cognitive training improves cognitive function and cortical metabolic ratios in Alzheimer's disease. *J Neural Transm (Vienna)*. 2019; 126: 1081-94.
- [19] Bagattini C, Zanni M, Barocco F, Caffarra P, Brignani D, Miniussi C, et al. Enhancing cognitive training effects in Alzheimer's disease: rTMS as an add-on treatment. *Brain Stimul*. 2020; 13: 1655-64.

- [20] Rutherford G, Lithgow B, Moussavi Z. Short and Long-term Effects of rTMS Treatment on Alzheimer's Disease at Different Stages: A Pilot Study. *J Exp Neurosci.* 2015; 9: 43-51.
- [21] Koch G, Bonni S, Pellicciari MC, Casula EP, Mancini M, Esposito R, et al. Transcranial magnetic stimulation of the precuneus enhances memory and neural activity in prodromal Alzheimer's disease. *Neuroimage.* 2018; 169: 302-11.
- [22] Weiler M, Stieger KC, Long JM, Rapp PR. Transcranial Magnetic Stimulation in Alzheimer's Disease: Are We Ready? *eNeuro.* 2020; 7.
- [23] Mimura Y, Nishida H, Nakajima S, Tsugawa S, Morita S, Yoshida K, et al. Neurophysiological biomarkers using transcranial magnetic stimulation in Alzheimer's disease and mild cognitive impairment: A systematic review and meta-analysis. *Neurosci Biobehav Rev.* 2021; 121: 47-59.
- [24] Sorg C, Riedl V, Muhlau M, Calhoun VD, Eichele T, Laer L, et al. Selective changes of resting-state networks in individuals at risk for Alzheimer's disease. *Proc Natl Acad Sci U S A.* 2007; 104: 18760-5.
- [25] Buckner RL, Sepulcre J, Talukdar T, Krienen FM, Liu H, Hedden T, et al. Cortical hubs revealed by intrinsic functional connectivity: mapping, assessment of stability, and relation to Alzheimer's disease. *J Neurosci.* 2009; 29: 1860-73.
- [26] Ng ASL, Wang J, Ng KK, Chong JSX, Qian X, Lim JKW, et al. Distinct network topology in Alzheimer's disease and behavioral variant frontotemporal dementia. *Alzheimers Res Ther.* 2021; 13: 13.
- [27] Wang L, Zang Y, He Y, Liang M, Zhang X, Tian L, et al. Changes in hippocampal

connectivity in the early stages of Alzheimer's disease: evidence from resting state fMRI. *Neuroimage*. 2006; 31: 496-504.

[28] Zhou J, Greicius MD, Gennatas ED, Growdon ME, Jang JY, Rabinovici GD, et al. Divergent network connectivity changes in behavioural variant frontotemporal dementia and Alzheimer's disease. *Brain*. 2010; 133: 1352-67.

[29] Grieder M, Wang DJJ, Dierks T, Wahlund LO, Jann K. Default Mode Network Complexity and Cognitive Decline in Mild Alzheimer's Disease. *Front Neurosci*. 2018; 12: 770.

[30] Celone KA, Calhoun VD, Dickerson BC, Atri A, Chua EF, Miller SL, et al. Alterations in memory networks in mild cognitive impairment and Alzheimer's disease: an independent component analysis. *J Neurosci*. 2006; 26: 10222-31.

[31] Agosta F, Pievani M, Geroldi C, Copetti M, Frisoni GB, Filippi M. Resting state fMRI in Alzheimer's disease: beyond the default mode network. *Neurobiol Aging*. 2012; 33: 1564-78.

[32] Lorenzi M, Beltramello A, Mercuri NB, Canu E, Zoccatelli G, Pizzini FB, et al. Effect of memantine on resting state default mode network activity in Alzheimer's disease. *Drugs Aging*. 2011; 28: 205-17.

[33] Sole-Padullés C, Bartres-Faz D, Llado A, Bosch B, Pena-Gomez C, Castellvi M, et al. Donepezil treatment stabilizes functional connectivity during resting state and brain activity during memory encoding in Alzheimer's disease. *J Clin Psychopharmacol*. 2013; 33: 199-205.

- [34] Blautzik J, Keeser D, Paolini M, Kirsch V, Berman A, Coates U, et al. Functional connectivity increase in the default-mode network of patients with Alzheimer's disease after long-term treatment with Galantamine. *Eur Neuropsychopharmacol.* 2016; 26: 602-13.
- [35] Wang JX, Rogers LM, Gross EZ, Ryals AJ, Dokucu ME, Brandstatt KL, et al. Targeted enhancement of cortical-hippocampal brain networks and associative memory. *Science.* 2014; 345: 1054-7.
- [36] Balderston NL, Roberts C, Beydler EM, Deng ZD, Radman T, Luber B, et al. A generalized workflow for conducting electric field-optimized, fMRI-guided, transcranial magnetic stimulation. *Nat Protoc.* 2020; 15: 3595-614.
- [37] O'Reardon JP, Solvason HB, Janicak PG, Sampson S, Isenberg KE, Nahas Z, et al. Efficacy and safety of transcranial magnetic stimulation in the acute treatment of major depression: a multisite randomized controlled trial. *Biol Psychiatry.* 2007; 62: 1208-16.
- [38] Blumberger DM, Vila-Rodriguez F, Thorpe KE, Feffer K, Noda Y, Giacobbe P, et al. Effectiveness of theta burst versus high-frequency repetitive transcranial magnetic stimulation in patients with depression (THREE-D): a randomised non-inferiority trial. *Lancet.* 2018; 391: 1683-92.
- [39] Ge R, Downar J, Blumberger DM, Daskalakis ZJ, Vila-Rodriguez F. Functional connectivity of the anterior cingulate cortex predicts treatment outcome for rTMS in treatment-resistant depression at 3-month follow-up. *Brain Stimul.* 2020; 13: 206-14.
- [40] Jia Y, Xu L, Yang K, Zhang Y, Lv X, Zhu Z, et al. Precision repetitive transcranial magnetic stimulation over the left parietal cortex improves memory in Alzheimer's

disease: A randomized, double-blind, sham-controlled study. *Front Aging Neurosci.* 2021; 13: 693611.

[41] Yan CG, Zang YF. DPARSF: A MATLAB Toolbox for "Pipeline" Data Analysis of Resting-State fMRI. *Front Syst Neurosci.* 2010; 4: 13.

[42] Song XW, Dong ZY, Long XY, Li SF, Zuo XN, Zhu CZ, et al. REST: a toolkit for resting-state functional magnetic resonance imaging data processing. *PLoS One.* 2011; 6: e25031.

[43] Lee TW, Girolami M, Sejnowski TJ. Independent component analysis using an extended infomax algorithm for mixed subgaussian and supergaussian sources. *Neural Comput.* 1999; 11: 417-41.

[44] Himberg J, Hyvarinen A, Esposito F. Validating the independent components of neuroimaging time series via clustering and visualization. *Neuroimage.* 2004; 22: 1214-22.

[45] Liao W, Wu GR, Xu Q, Ji GJ, Zhang Z, Zang YF, et al. DynamicBC: a MATLAB toolbox for dynamic brain connectome analysis. *Brain Connect.* 2014; 4: 780-90.

[46] Hermiller MS, Karp E, Nilakantan AS, Voss JL. Episodic memory improvements due to noninvasive stimulation targeting the cortical-hippocampal network: A replication and extension experiment. *Brain Behav.* 2019; 9: e01393.

[47] Rutherford G, Gole R, Moussavi Z. rTMS as a Treatment of Alzheimer's Disease with and without Comorbidity of Depression: A Review. *Neurosci J.* 2013; 2013: 679389.

[48] Wang W, Bian Q, Zhao Y, Li X, Du J, Zhang G, et al. Reliability and validity of



the Chinese version of the Patient Health Questionnaire (PHQ-9) in the general population. *Gen Hosp Psychiatry*. 2014; 36: 539-44.

[49] Levis B, Benedetti A, Thombs BD. Accuracy of Patient Health Questionnaire-9 (PHQ-9) for screening to detect major depression: individual participant data meta-analysis. *BMJ*. 2019; 365: 11476.

[50] Li X, Qi G, Yu C, Lian G, Zheng H, Wu S, et al. Cortical plasticity is correlated with cognitive improvement in Alzheimer's disease patients after rTMS treatment. *Brain Stimul*. 2021; S1935-861X (21)00017-6.

[51] Rabey JM, Dobronevsky E. Repetitive transcranial magnetic stimulation (rTMS) combined with cognitive training is a safe and effective modality for the treatment of Alzheimer's disease: clinical experience. *J Neural Transm (Vienna)*. 2016; 123: 1449-55.

[52] Sheline YI, Raichle ME. Resting state functional connectivity in preclinical Alzheimer's disease. *Biol Psychiatry*. 2013; 74: 340-7.

[53] Dillen KNH, Jacobs HIL, Kukulja J, Richter N, von Reutern B, Onur OA, et al. Functional Disintegration of the Default Mode Network in Prodromal Alzheimer's Disease. *J Alzheimers Dis*. 2017; 59: 169-87.

[54] Yildirim E, Soncu Buyukiscan E. Default Mode Network Connectivity in Alzheimers Disease. *Turk Psikiyatri Derg*. 2019; 30: 279-86.

[55] Lin Y, Jiang WJ, Shan PY, Lu M, Wang T, Li RH, et al. The role of repetitive transcranial magnetic stimulation (rTMS) in the treatment of cognitive impairment in patients with Alzheimer's disease: A systematic review and meta-analysis. *J Neurol Sci*.

2019; 398: 184-91.

[56] Cui H, Ren R, Lin G, Zou Y, Jiang L, Wei Z, et al. Repetitive Transcranial Magnetic Stimulation Induced Hypoconnectivity Within the Default Mode Network Yields Cognitive Improvements in Amnesic Mild Cognitive Impairment: A Randomized Controlled Study. *J Alzheimers Dis.* 2019; 69: 1137-51.

[57] Holczer A, Nemeth VL, Vekony T, Vecsei L, Klivenyi P, Must A. Non-invasive Brain Stimulation in Alzheimer's Disease and Mild Cognitive Impairment-A State-of-the-Art Review on Methodological Characteristics and Stimulation Parameters. *Front Hum Neurosci.* 2020; 14: 179.

**Table 1. Baseline characteristics of patients with Alzheimer's disease**

<b>Characteristic</b>	<b>rTMS group (n=31)</b>	<b>sham group (n=27)</b>	<b>p-value</b>
<b>Age (years)</b>	70.39 ± 8.47	71.67 ± 7.16	0.540
<b>Female (%)</b>	71.0%	74.1%	0.792
<b>Education (years)</b>	7.06 ± 5.51	6.63 ± 4.99	0.755
<b>Medication (%)</b>	67.7%	70.4%	0.829
<b>MMSE</b>	14.36 ± 6.94	13.74 ± 7.16	0.742
<b>CDR</b>	1.13 ± 0.56	1.17 ± 0.54	0.796
<b>ADL</b>	29.07 ± 7.54	32.26 ± 9.73	0.165
<b>PHQ-9</b>	1.81 ± 3.35	1.41 ± 2.06	0.594
<b>PVLT</b>			
Immediate	12.45 ± 9.16	10.30 ± 9.52	0.384
Short Delay	2.52 ± 3.27	2.30 ± 3.62	0.809
Long Delay	1.61 ± 3.09	1.07 ± 2.18	0.453

Notes: Means ± standard deviation tested by two-sample t-tests (2-tailed).

Abbreviations: MMSE: Mini-Mental State Examination; CDR: Clinical Dementia Rating; ADL: Activities of Daily Living scale; PHQ-9: Patient Health Questionnaire-9; PVLT: 12-Word Philadelphia Verbal Learning Test.

**Table 2. Baseline characteristics of Alzheimer's disease patients and changes after rTMS treatment (Baseline, Immediately after treatment and at 12-week follow-up)**

Characteristic	rTMS group (n=31)			p-value		sham group (n=27)			p-value	
	Baseline [B]	Immediate	12-week	[I]	[T]	Baseline [B]	Immediate	12-week follow-	[I]	[T]
		after treatment	follow-up [T]	vs.	vs.		after treatment	up [T]	vs.	vs.
	[I]		[B]	[B]		[I]		[B]	[B]	
MMSE	14.36 ± 6.94	15.52 ± 7.23	13.82 ± 7.82	0.009**	0.892	13.74 ± 7.16	14.30 ± 7.76	14.75 ± 7.55	0.273	0.406
CDR	1.13 ± 0.56	1.11 ± 0.57	1.14 ± 0.58	0.768	0.815	1.17 ± 0.54	1.19 ± 0.57	1.05 ± 0.54	0.713	0.804
ADL	29.07 ± 7.54	29.36 ± 8.00	31.50 ± 9.48	0.53	0.126	32.26 ± 9.73	32.44 ± 9.36	30.35 ± 10.24	0.811	0.383
PHQ-9	1.81 ± 3.35	2.32 ± 4.50	2.36 ± 4.48	0.29	0.339	1.41 ± 2.06	0.70 ± 0.99	1.65 ± 2.60	0.103	0.212
PVLT										
Immediate	12.45 ± 9.16	15.58 ± 10.27	13.18 ± 10.02	<0.001***	0.129	10.30 ± 9.52	11.56 ± 11.03	13.65 ± 11.65	0.124	0.340
Short Delay	2.52 ± 3.27	3.38 ± 3.68	3.77 ± 4.93	0.044*	0.064	2.30 ± 3.62	2.48 ± 3.25	3.20 ± 3.74	0.686	0.137
Long Delay	1.61 ± 3.09	2.07 ± 3.88	2.18 ± 4.59	0.080	0.126	1.07 ± 2.18	1.63 ± 3.21	1.50 ± 2.80	0.146	0.748

Notes: Means ± standard deviation tested by paired t-tests (2-tailed).

Twelve weeks follow-up was completed for 22 (71%) in the rTMS group and 20 (74%) in the sham group.

\*p<0.05; \*\*p<0.01; \*\*\*p<0.001.

Abbreviations: MMSE: Mini-Mental State Examination; CDR: Clinical Dementia Rating; ADL: Activities of Daily Living scale; PHQ-9: Patient Health Questionnaire-9; PVLT: 12-Word Philadelphia Verbal Learning Test.

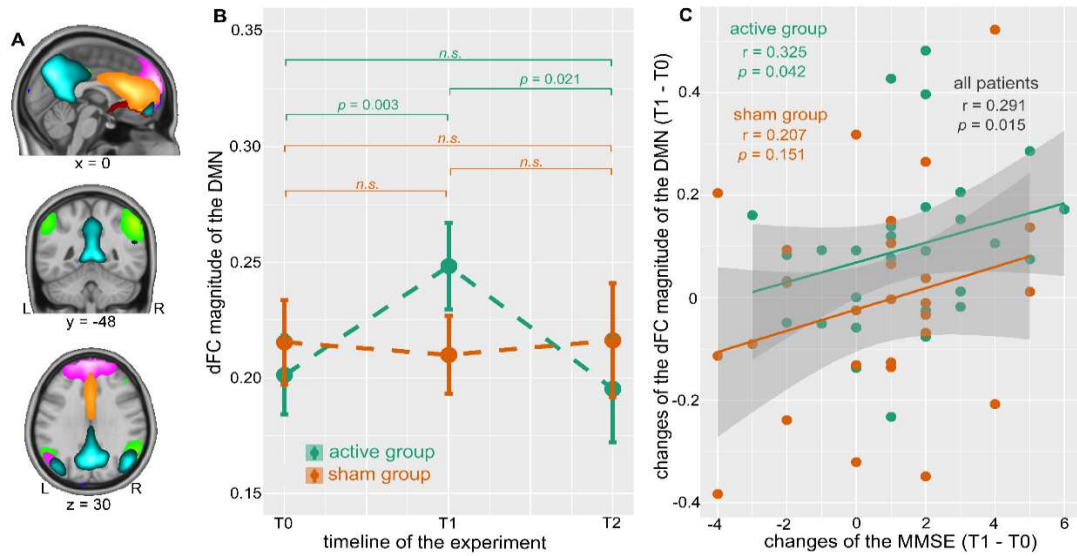


Fig. 1. (A) Overview of the DMN in all patients based on the ICA group. (B) Differences in DNM's dFC magnitude at each experimental timeline (baseline, immediately after, and 12-week follow-up following treatment). (C) A scatter plot showing the correlation between changes in MMSE and those of the DNM's dFC magnitude immediately after 2 weeks of treatment. Abbreviations: DMN: default mode network; dFC: dynamic functional connectivity; MMSE: Mini-Mental State Examination; T0: baseline; T1: immediately after the treatment; T2: 12-week follow-up after the treatment; L: left; R: right.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [AppendixA.Supplementarydata.docx](#)