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Cognitive Improvement is Correlated with Increased Dynamic Functional Connectivity of Default Mode Network by Personalized rTMS in Alzheimer's disease

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Abstract

Background: Repetitive transcranial magnetic stimulation (rTMS) is thought to be a promising therapeutic approach for Alzheimer's disease patients.

Methods: In the present report, a double-blind, randomized, sham-controlled rTMS trial was conducted in mild-to-moderate Alzheimer's disease patients. High-frequency rTMS was delivered to a subject-specific left lateral parietal region that demonstrated highest functional connectivity with the hippocampus using resting-state fMRI. The Mini Mental State Examination (MMSE) and Philadelphia Verbal Learning Test (PVL) were used to evaluate patients' cognitive functions.

Results: Patients receiving active rTMS treatment (n=31) showed a significant increase in the MMSE, PVL-Immediate recall, and PVL-Short Delay recall scores after two weeks of rTMS treatment, whereas patients who received sham rTMS (n=27) did not show significant changes in these measures. Dynamic functional connectivity (dFC) magnitude of the default mode network (DMN) in the active-rTMS group showed a significant increase after two weeks of rTMS treatment, and no significant changes were found in the sham-rTMS group. There was a significantly positive correlation between changes of the MMSE and changes of the dFC magnitude of DMN in the active-rTMS group, but not the sham-rTMS group.

Conclusions: Our findings are novel in demonstrating the feasibility and effectiveness of the fMRI-guided rTMS treatment in Alzheimer's disease patients, and DMN might play a vital role in therapeutic effectiveness of rTMS in Alzheimer's disease.

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 that is predicted to affect more than 100 million people by the year 2050[1]. It has tremendous social and economic impact worldwide. Pharmacological regimen has so far dominated the clinical practice in treating Alzheimer's disease. In recent years, repetitive transcranial magnetic stimulation (rTMS) therapy has been considered to be a promising treatment protocol for psychiatric and neurological disorders [2-4], including mild cognitive impairment (MCI) and Alzheimer's disease [5-8]. Previous studies have demonstrated that rTMS improves language performance [9-11], attentional capacity [12], executive function [13-15], verbal memory and episodic memory [16-19]. Despite its therapeutic effectiveness, there is no consensus on the most effective target region for clinical treatment of Alzheimer's disease with rTMS, with varying spatial regions in different studies, including dorsolateral prefrontal cortex (DLPFC) [9,20], inferior frontal gyrus [12,13], precuneus [21], and posterior temporal gyrus [16]. Moreover, neurophysiological mechanism underlying rTMS on Alzheimer's disease treatment is unclear [22-24]. This knowledge gap hinders faster application of rTMS in Alzheimer's disease.

Alzheimer's disease is a neurodegenerative disease that affects the functional connections of the brain, which can be proven by the default mode network (DMN) changes in Alzheimer's disease patients [25,26]. Alzheimer's disease patients mainly manifest the reduction of functional connection of DMN, especially the connections between the posterior precuneus, posterior cingulate gyrus and the anterior medial

prefrontal cortex (MPFC), anterior cingulate gyrus; there are also DMN-related left and right hippocampus changes [27-29]. Changes in DMN functional connectivity are related to changes of patients' cognitive function, and are considered to be a high risk factor of patients to develop dementia [30,31]. After medication, cognitive therapy, and acupuncture treatment, Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), auditory vocabulary and delayed recall will change, and the functional connectivity of DMN increase after treatment in Alzheimer's disease patients [32-34]. In summary, DMN plays an important role in the development and treatment of Alzheimer's disease. Therefore, this network might serve as a promising therapeutic target for Alzheimer's disease.

In the present study, we conducted a double-blind, randomized, sham-controlled clinical trial using an functional magnetic resonance imaging (fMRI)-guided rTMS technique [35,36] to treat patients with Alzheimer's disease. Specifically, therapeutic high-frequency rTMS, which has been demonstrated effective in major depression [37-39], was delivered to an individualized left lateral parietal region that demonstrated highest functional connectivity with the each patient's hippocampus using resting-state fMRI for two weeks, and several fMRI sessions and a complete neuropsychological battery of tests were administered in this trial. The hypotheses of this study are (1) the cognitive functions of Alzheimer's disease patients can be improved by stimulating a key brain region of DMN (lateral parietal lobule) with the fMRI-guided personalized rTMS technique, and (2) the rTMS-induced cognitive function improvement is correlation with the perturbation of DMN.

Materials and methods

Participants

A total of 103 patients with mild-to-moderate Alzheimer's disease patients were recruited from outpatients and inpatients of the Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou, China, and underwent a standardized evaluation. The patients were screened from an ongoing follow-up project, which aimed to treat Alzheimer's disease by personalized fMRI-guided rTMS of the parietal lobe. This project has been 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) met the diagnostic criteria for probable Alzheimer's disease according to the Diagnostic and Statistical Manual of Mental Disorders, 5th edition; (2) had a Clinical Dementia Rating (CDR) score between 0.5 and 2; (3) were from fifty-five to eighty-five years old, and right-handed. The exclusion criteria included: (1) severe liver, kidney, heart, lung diseases; (2) history of significant head trauma or neurological disorders; (3) focal brain lesions on T1 or T2 images; (4) any MRI contraindications (e.g., medical implants or devices, metal 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 (No: 20170228-1), and all patients signed an informed consent form.

Procedures

A double-blind, randomized, sham-controlled trial was conducted in patients (The clinicians, neuropsychologists and patients did not know whether the patients received the real or sham treatment, only the rTMS operators knewed the randomized treatment). The active-rTMS group underwent a 2-week real rTMS treatment, while the sham group underwent a 2-week sham treatment. Sessions occurred once daily, 5 days/week. Patients underwent a complete neuropsychological battery of tests and a multimodal MRI scan at baseline (T0), immediately (T1) and 12-week follow-up after the end of rTMS treatment (T2).

Among the 103 patients, before randomization, ten patients were excluded due to the brain lesion and psychiatric disorder, five patients withdrew due to personal reasons, and two patients were not contactable. Finally, eighty-six patients were randomized to either rTMS or sham treatment. A total of fifty-eight patients completed 2-week treatment and assessments including neuropsychological tests and fMRI scan (Eight patients in the rTMS group and nine patients in the sham group did not complete neuropsychological assessments at baseline or rTMS/ sham treatment. Four patients in the rTMS group and seven patients in the sham group did not finish the fMRI scan at T1). Thirty-one of them were assigned to the rTMS group, and twenty-seven were assigned to the sham group. In addition, another nine patients in rTMS group and seven patients in sham group have not completed the 12-week follow-up up to now (four patients in rTMS group and four patients in sham group refused the follow-up, while five patients in rTMS group and three patients in sham group have not reached the follow-up period), but their data of baseline and

immediately after the end of rTMS treatment were kept for analyses.

MRI data acquisition

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 eight-channel head coil array in the Affiliated Hospital of Hangzhou Normal University. During scanning, patients were instructed to keep still in the scanner with their eyes open and not think about anything specific. The functional images were obtained axially using an echo-planar imaging (EPI) sequence with the following parameters: 240 volumes; repetition time (TR)=2,000 ms; echo time (TE)=30 ms; flip angle (FA)=90°; field of view (FOV)=220 × 220 mm²; matrix = 64 × 64; slice thickness = 3.2 mm with no gap. High-resolution anatomic three-dimensional T1-weighted images were obtained with the following parameters: 176 axial slices; TR = 8.1 ms; TE = 3.1 ms; FA = 8°; FOV = 250 × 250 mm²; matrix = 250 × 250; slice thickness = 1.0 mm with no gap.

Identification of stimulus target

The stimulus target of each patient was identified by using personal maps of hippocampal resting-state functional connectivity obtained at the baseline. For each patient, fMRI collected during resting state was used to generate seed-based connectivity maps using a hippocampal target as the seed. We chose the voxel in the middle of the hippocampal body (MNI coordinate x=-24, y=-18, z=-18) as

hippocampal target voxel [35]. The stimulation site was a left lateral parietal location that showed high functional connectivity with the left hippocampal seed.

Functional and structural MRI data were preprocessed using Data Processing Assistant for Resting-State fMRI (DPARSF; <http://rfmri.org/DPARSF>) [40], which is based on SPM12 software (<http://www.fil.ion.ucl.ac.uk/spm>) and a Resting-State fMRI Data Analysis Toolkit (REST; www.restfmri.net) [41]. The preprocessing steps included: slice timing after removing first 10 volumes; realign; motion correction; functional/structural co-registration; segment and affixer regularization according to European; resampling to a resolution of 1.5 mm × 1.5 mm × 1.5mm; normalizing into the standard Montreal Neurological Institute (MNI) space using T1 image unified segmentation; spatial smoothing with a 6-mm full-width-at-half-maximum Gaussian kernel; removing linear trend; and filter at 0.01–0.08 Hz. Finally, the resting-state functional connectivity between the hippocampal target and the entire brain were computed. We identified 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), and this local maxima was designated as stimulation target region. This stimulation location was marked in stereotactic space and overlaid onto the structural MRI to provide localization during rTMS.

rTMS Stimulation

We applied rTMS treatment at personalized left lateral parietal target, guided by an online neuronavigation system (Brainsight 2, Rogue Research, Montreal, Quebec,

Canada). rTMS was applied to the stimulation location during daily treatment sessions using Magstim Rapid2 stimulator with a 70mm air-cooled figure eight coil (Magstim Company, Whitland, Wales, United Kingdom). The stimulation location was located via the structural MRI using a frameless infrared stereotactic system. Motor threshold of each patient was defined as the minimum TMS intensity that evoked a motor evoked potential (MEP) of at least 50 μ V for at least 5 of 10 consecutive pulses at baseline. For the stimulation, rTMS was applied at 100%-110% motor threshold 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, we rotated the coil by a 45° along the handle axis of the coil and the distance between the stimulation side and the scalp was larger than 5 cm, so that the stimulation side of the coil keep away from the scalp.

Neuropsychological assessments

All measurements of neuropsychological assessments were repeated three times: baseline, immediately and 12-week follow-up after the end of rTMS treatment. The trained neuropsychologist, blinded to the randomized treatment, performed the assessments. The main neuropsychological test questionnaire included 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).

Neuroimaging Data processing

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

We concatenated the preprocessed data of all patients to perform group independent component analysis (ICA) using the GIFT toolbox (<http://icatb.sourceforge.net>). The number of independent components was estimated as 34 using the minimum description length criteria. The common components were acquired over all participants with ICA decomposition using Infomax algorithm [42], and ICASSO procedure with 10 runs of ICA was used to assure the stability [43]. In the present study, we focused on the DMN, and identified 6 DMN subnetworks based on inspection of their spatial locations and time courses by two trained investigators (J.W. and Y.S.): a DMN subnetwork was selected if it spatially located in canonical DMN regions (including posterior cingulate cortex, medial prefrontal cortex, anterior cingulate cortex, and bilateral parietal lobule) and power spectrum of its associated time course primarily located in the range 0.01-0.1 Hz. The flexible least squares

(FLS) method was applied to calculate the dynamic functional connectivity (dFC) within the DMN for each patient via the dynamic brain connectivity (DynamicBC) toolbox [44]. Specifically, a time-varying parameter regression equation was employed to describe the dynamic interactions between 6 subnetworks of the DMN:

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

where $x(t)$ and $y(t)$ are the time series of two DMN subnetworks, $\varepsilon(t)$ is the approximation error. The $\beta(t)$ is the coefficient to estimate the covariance of the two networks and reflects the connectivity between $x(t)$ and $y(t)$ at time t . To obtain the β value at each time point, a cost function was defined as:

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

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 declares 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 $y(t) - x(t) \times \beta(t) \approx 0$; μ is the weighting parameter to control these two parts of the cost function and in the present study, this parameter was set as 100 according to [44]. With FLS, we extracted the time courses of the DMN subnetworks and calculated the dynamic functional connectivity (dFC) magnitude and dFC variance between each pair of the subnetworks. For the inter-network connectivity, dFC magnitude of the DMN was defined as the average of the β values between each pair; the average standard deviation of the β values of all the inter-network pairs was defined as the dFC variance of DMN.

Statistical analysis

The Chi-squared test was performed for the gender and medication distribution, and the independent t-test was used to analyze differences of other continuous variables between the rTMS group and the sham group at baseline. The dFC magnitude, dFC variance, and neuropsychological assessments were analyzed via paired t-tests to evaluate the changes between different time points in each group. Moreover, we performed correlational analysis between changes of the MMSE and changes of the dFC magnitude and variance of the DMN (baseline phase T0 and immediate after treatment phase T1). The analyses were performed using SPSS for Windows (v 20.0, IBM). Two-tailed probability values of $p < 0.05$ were considered statistically significant for the paired t-test results. For the correlational analysis, the significance level was set as one-tailed $p < 0.05$ since we expected significant correlation in the active-rTMS group.

Results

Patient characteristics

In the present study, there were totally thirty-one patients in rTMS group and twenty-seven patients in sham group for the analyses. As shown in Table 1, statistically significant differences between the treatment and sham groups in baseline characteristics were not found. Specifically, the baseline characteristics include age ($p = 0.540$), male-to-female ratio ($p = 0.792$), education level ($p = 0.755$), medication

rate ($p = 0.829$), MMSE ($p = 0.742$), CDR ($p = 0.796$), ADL ($p = 0.165$), PHQ-9 ($p = 0.594$), PVLТ ($p > 0.05$).

Neuropsychological assessment improvements after rTMS

The rTMS group showed a significant increase by 1.2 MMSE scores immediate after 2 weeks of rTMS treatment ($p = 0.009$). Whereas, the MMSE scores in the sham group showed no significant increase by 0.6 immediate after 2 weeks of sham treatment ($p = 0.273$). We used the PVLТ to further assess memory performance of Alzheimer's disease patients, with higher values indicating greater memory performance. Compared with the sham group, the scores of PVLТ Immediate recall ($p < 0.001$) and Short Delay recall ($p = 0.044$) in the treatment group had significant improvement immediate after 2 weeks of rTMS treatment. Nine patients in rTMS group and seven patients in sham group had not completed the 12-week follow-up up to now, there were twenty-two patients in rTMS group and twenty patients in sham group for the 12-week follow-up analyses. Similar to the total sample, the baseline characteristics had no significant differences between groups (Supplementary Table S1). There was no significant improvement on MMSE and PVLТ scores in both groups at 12-week follow-up. Performance of both group on the CDR, ADL, PQH-9 and PVLТ Long Delay recall did not change significantly. These results showed that 2 weeks of rTMS treatment significantly improved cognitive function in patients of rTMS group compared to the sham group, but the benefits disappeared at 12-week follow-up. Details are shown in Table 2.

Changes of the dFC magnitude of the DMN and correlation results

As shown in Fig. 1A, we identified 6 DMN subnetworks (Fig. S1 in the supplement) with group ICA. The dFC analysis revealed that the dFC magnitude of the DMN in the rTMS group showed a significant increase immediate after 2 weeks of rTMS treatment ($p=0.003$), and the dFC magnitude then decreased at 12-week follow-up (Fig. 1B). The dFC magnitude of the DMN showed no significant changes in the sham group (Fig. 1B). Across all Alzheimer's disease patients, there was a significantly positive correlation between changes of the MMSE and changes of the DMN dFC magnitude immediate after 2 weeks of rTMS ($r=0.291$, $p=0.015$) (Fig. 1C). Moreover, the better improvement in MMSE scores were correlated with the higher dFC magnitude of the DMN in rTMS group ($r=0.325$, $p=0.042$), but not in sham group ($r=0.207$, $p=0.151$) (Fig. 1C). No significant results were found for the dFC variance measure.

Discussion

In this study, we examined whether the subject-specific hippocampal-targeted rTMS treatment over the lateral parietal lobule could improve the cognitive functions of Alzheimer's disease patients. In the meanwhile, we examined whether this improvement was related to the change of the intra-DMN functional connectivity. After two weeks of rTMS treatment, we found that the active-rTMS group showed a significant increase in the MMSE, PVL-Immediate recall, PVL-Short Delay recall

scores and the dFC magnitude of the DMN, whereas the sham-rTMS group did not show significant changes in these measures. Moreover, we found that better improvement in MMSE scores in the active-rTMS group was correlated with more DMN's dFC magnitude changes.

Parietal-hippocampal targeted rTMS can improve cognitive function in Alzheimer's disease

Memory impairment in Alzheimer's disease patients is mainly related to the hippocampus, which is hardly be accessible by the conventional rTMS technique. Prior literature with healthy adult participants has confirmed that memory performance could be enhanced by the subject-specific fMRI-guided rTMS treatment targeted in cortical-hippocampal networks [35,45]. In the present study, we successfully adopted this fMRI-guided rTMS protocol for treating Alzheimer's disease. The active-rTMS group showed a significant increase in MMSE, PVLT Immediate recall and Short Delay recall scores after two weeks of rTMS treatment. The prevalence of depression in Alzheimer's disease may be as high as 50% [46], and even mild depressive symptoms are associated with significant functional impairment. It is important to control for the possibility that patients might benefit from TMS secondary to stimulation effects on comorbid depressive symptoms. It is notable in our results that PHQ-9, which is a validated module for depression in primary care [47,48], did not change significantly. Therefore, the cognitive improvements in the active-rTMS group were not results from alleviating depressive symptoms. Our

results showed that the rTMS-induced cognitive improvement in the active-rTMS group disappeared at 12-week follow-up. It was similar to a recent study [49], which reported that 20Hz rTMS treatment for 30 sessions over six weeks in the DLPFC resulted in a significant improvement in cognition, but this improvement was not maintained in the three-month follow up. rTMS dose and its combination with other therapeutic approaches were important issues in this case. Rabey and Dobronevsky [50] combined rTMS of 1-hour daily session, 5 days per week, for 6 weeks (30 sessions) with cognitive training to treat mild-to-moderate Alzheimer's disease patients, and they found the cognitive improvement lasted for a minimum of 9 months. Our study employed a single treatment modality (i.e., rTMS) and a shorter treatment period (20 minutes daily session, 5 days per week for 2 weeks), therefore potentially have a shorter time of symptomatic improvement. We speculate that the efficacy of rTMS will be consolidated if extended the treatment time or combined with other non-drug treatments such as cognitive stimulation and exercise. The lasting effect of rTMS treatment on Alzheimer's disease deserves further investigation.

Parietal-hippocampal targeted rTMS can enhance DMN neural activity in Alzheimer's disease

In the present study, the dFC magnitude of the DMN was calculated by summarize 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. We found that the dFC magnitude of the DMN in the active-rTMS group

showed a significant increase after two weeks of active rTMS treatment, and better improvement in MMSE scores was correlated with higher DMN's dFC magnitude change in the active-rTMS group. The dFC magnitude resumed to baseline level at 12-week follow-up, which resembled the trajectory of the cognitive measures. Alzheimer's disease is a generalized disconnection syndrome that causes functional impairments in resting state networks, particularly in DMN [51-53]. Prior literature provided evidence that rTMS improves cognitive performances of Alzheimer's disease patients [54], however there is still a knowledge gap in understanding the neural substrates underlying the therapeutic effectiveness of rTMS in Alzheimer's disease treatment. A study combined rTMS and electroencephalogram techniques [21] found that rTMS treatment on precuneus improved long-term memory in prodromal Alzheimer's disease patients by modulating neural activity of the precuneus and its connections with medial parietal and frontal areas in the DMN. A recent study with fMRI technique [55] demonstrated that rTMS-induced functional connectivity changes within the DMN was associated with clinical cognitive improvements in patients with amnesic MCI. The present study, together with these two studies, highlighted the effectiveness of rTMS in the treatment of Alzheimer's disease patients, and further revealed that intra-DMN functional connectivity might be a neuroimaging substrate for the therapeutic effectiveness of rTMS in the recovery of cognitive impairment in Alzheimer's disease patients.

A major limitation of this study was the relatively heterogeneous Alzheimer's disease sample ($0.5 \leq \text{CDR} \leq 2.0$). However, significant differences between the

active-rTMS treatment and sham-rTMS treatment groups in baseline characteristics were not found. Stimulation parameters and study protocol designs varied considerably in previous studies [56], in the present study, we adopted a protocol which has been demonstrated effective in enhancing healthy participants' memory ability [35]. However, larger trials are essential to determine the optimal parameters in this protocol for its utility in clinical practice, and the findings need to be replicated in studies with larger sample size.

Conclusions

In summary, our findings may provide the foundation for a prospective clinical trial. We demonstrated the feasibility and effectiveness of the fMRI-guided rTMS treatment over the lateral parietal lobule in Alzheimer's disease patients, and the neuroimaging results provided evidence that the intra-DMN functional connectivity might be a neural substrate for the therapeutic effectiveness of rTMS in the treatment of Alzheimer's disease patients.

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=repetitive Transcranial

Magnetic Stimulation.

Appendix A. Supplementary data

Declarations

Ethics 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), and all patients signed an informed consent form.

Consent for publication

Written informed consent for publication of their clinical details was obtained from the patient or relative of the patient. 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 this study are available upon reasonable request.

Competing interests

The authors report no competing interests.

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Authors' contributions

LW contributed to the data collections and data analysis. JW contributed to the neuropsychological assessments and data analysis. YZ, LX, QG, WH and MQ contributed to the data collections. KY and XL contributed to the rTMS treatment. ZZ contributed to the neuropsychological assessments. WC, YS, and XL were responsible for the study concept and design. All authors read and approved the final manuscript.

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Table 1. Baseline characteristics of Alzheimer's disease patients

Characteristic	rTMS group (n=31)	sham group (n=27)	p-value
Age (years)	70.39 ± 8.47	71.67 ± 7.16	0.540
Female (%)	71.0%	74.1%	0.792
Education (years)	7.06 ± 5.51	6.63 ± 4.99	0.755
Medication (%)	67.7%	70.4%	0.829
MMSE	14.36 ± 6.94	13.74 ± 7.16	0.742
CDR	1.13 ± 0.56	1.17 ± 0.54	0.796
ADL	29.07 ± 7.54	32.26 ± 9.73	0.165
PHQ-9	1.81 ± 3.35	1.41 ± 2.06	0.594
PVLT			
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 the improvements after the rTMS (Baseline, Immediate after treatment and Twelve weeks follow-up)

Characteristic	rTMS group (n=31)			p-value		sham group (n=27)			p-value	
	Baseline [B]	Immediate	Twelve weeks	[I]	[T]	Baseline [B]	Immediate	Twelve weeks	[I]	[T]
	after treatment			vs.	vs.	after treatment			vs.	vs.
	[I]		[T]	[B]	[B]	[I]		[T]	[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 group ICA. (B) Differences in the dFC magnitude of the DMN at each timeline of the experiment (baseline, immediately after, 12-week follow-up after the treatment). (C) A scatter plot shows the correlation between changes of the MMSE and changes of the dFC magnitude of the DMN 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.

Figures

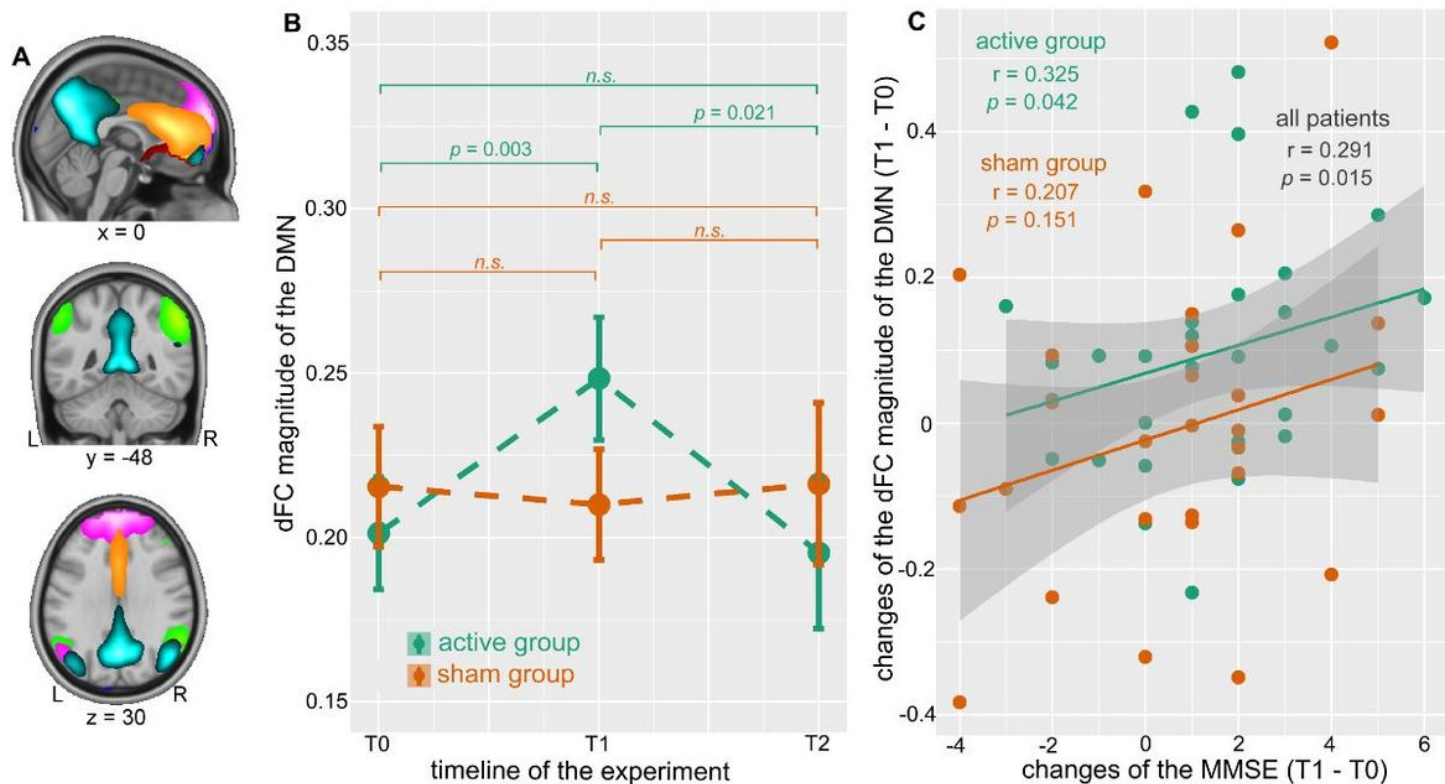


Figure 1

(A) Overview of the DMN in all patients based on the group ICA. (B) Differences in the dFC magnitude of the DMN at each timeline of the experiment (baseline, immediately after, 12-week follow-up after the treatment). (C) A scatter plot shows the correlation between changes of the MMSE and changes of the dFC magnitude of the DMN 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

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- [AppendixA.Supplementarydata.docx](#)