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Effect of rTMS on cerebral functional hierarchical structure in schizophrenia

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Abstract

Background The hierarchical functional structure reflects the process of integrating primary information to form higher-order cognition in the human brain. Schizophrenia, as a severe chronic psychiatric illness, has been demonstrated to exhibit an abnormal cerebral functional hierarchical structure. Although repetitive transcranial magnetic stimulation (rTMS) of the dorsolateral prefrontal cortex (DLPFC) has shown therapeutic efficacy on schizophrenia, the mechanism of rTMS in schizophrenia is still unclear. Thus, this study attempted to reveal the modulation of rTMS on the cerebral functional hierarchy and its potential molecular mechanism in schizophrenia.

Methods A longitudinal study was performed on 53 patients with schizophrenia (randomly assigned to the rTMS group or drug treatment group). Additionally, 24 age- and gender-matched healthy adults were recruited. Functional gradient analysis was executed to depict the hierarchical organization of schizophrenia. The alteration of the functional gradients at baseline and after 4 weeks of treatment was evaluated in schizophrenia to detect the effect of rTMS on the cerebral hierarchical structure.

Results We found rTMS alleviated the compression of the fronto-parietal network, the core node of the salience network (SN), and the bilateral middle temporal areas in schizophrenia. Leveraging human brain gene expression data, we identified a spatial correlation between the expression of schizophrenia-related genes and impaired functional gradients, and further revealed a close association of the modulatory effects of rTMS with pathways related to neuroplasticity and neuroimmune processes. Furthermore, the alterations of gradient induced by rTMS were associated with the alleviation of clinical symptoms in schizophrenia, suggesting that the altered hierarchical structure may play an important role in the treatment of schizophrenia.

Conclusions Our findings indicate that the alteration of cerebral hierarchy induced by rTMS contributes to clinical symptom improvement in schizophrenia and provides new evidence for the efficacy of rTMS in the treatment of schizophrenia.

Keywords: schizophrenia, rTMS, hierarchical structure, functional gradient analysis, gene expression

Introduction

Schizophrenia is a severe chronic mental disorder characterized by multisensory integration deficits (Postmes et al. 2014). Currently, the clinical treatment of patients with schizophrenia mainly relies on antipsychotic drugs. Antipsychotics usually provide good relief from positive symptoms but have little effect on the cognitive deficits and negative symptoms (Hsu et al. 2018; Kay et al. 1987). Meanwhile, non-invasive brain stimulation, especially repetitive transcranial magnetic stimulation (rTMS), has been widely adopted as adjunctive therapy to improve clinical symptoms in schizophrenia (Sciortino et al. 2021). Accumulating evidence suggests that this technique has significant therapeutic potential in the clinical treatment of schizophrenia (Lefaucheur et al. 2020; Sale et al. 2015; Sciortino et al. 2021). For example, several studies have found that high-frequency rTMS (HF-rTMS) applied to the left DLPFC can significantly improve the negative symptoms of schizophrenia (Gan et al. 2021; Li et al. 2016). A meta-analysis also showed that rTMS on the left DLPFC with a total of pulses < 30,000 was more effective in improving working memory than pulses ≥ 30,000 (Jiang et al. 2019). However, the mechanisms underlying the effect of TMS on the improvement of schizophrenia symptoms remains unclear. Numerous studies found that rTMS can modulate local neural activity and functional connectivity between the brain regions as well as induce effects that spread across networks (Beynel et al. 2020; Chen et al. 2013), and improve the clinical symptoms (Gromann et al. 2012; Han et al. 2023; Sultana et al. 2023). For instance, a study using an activating TMS sequence (triplet at 10 Hz) over the left Brodmann area 9 resulted in a local "spill-over" in activation to neighboring ipsilateral regions (Webler et al. 2020). Thus, the rTMS may modulate both local and long-range functional connectivity of key brain regions to achieve therapeutic effects in schizophrenia.

The principle that brain functional topography plays a critical role in cognition is increasingly supported by evidence from neuroimaging (Huntenburg et al. 2018), microstructural differentiation (Beul et al. 2019; Paquola et al. 2019), transcriptomics (Hansen et al. 2021) and electrophysiology (Murray et al. 2014; Thomas Yeo et al. 2011). The cerebral hierarchical structure facilitates the integration of multimodal information from the primary sensorimotor system (Holmes et al. 2023; Mesulam 1998), and is helpful for high-order systems to process abstract cognitive information.

Functional gradient analysis is a powerful tool for revealing the abnormal hierarchical structure in neurological and psychotic disorders (Margulies et al. 2016; Vafaii et al. 2024). For example, the principal gradient in major depressive disorder showed lower gradient scores in default mode network (DMN) but higher scores mainly in the visual and sensorimotor cortices compared with the control group (Xia et al. 2022). Our previous studies proposed a framework to elucidate the interaction between low- and high-level functional abnormalities within cerebellar and cerebellar-cerebral cortical circuits in schizophrenia (Dong et al. 2019, 2022). These results reflect the impairment of hierarchical processing in mental disorders (Fornito et al. 2012; Holmes et al. 2023; Kebets et al. 2019; Zhang et al. 2023) and demonstrate that functional gradient may serve as a neuroimaging biomarker to identify the altered hierarchical processing in brain disorders. Despite these findings, little is known about the relationship between the genetic basis underlying brain functional gradients and their modulation by rTMS in schizophrenia.

Schizophrenia is characterized by high heritability and multiple genetic variations (Owen et al. 2016; Singh et al. 2022). A study using cell-resolution data from a standardized in situ hybridization (ISH) platform identified 67 risk loci for schizophrenia (Zeng et al. 2012). A previous study has shown that abnormalities in the morphological similarity network of patients with schizophrenia are significantly correlated with the expression patterns of schizophrenia-related genes (Morgan et al. 2019). Thus, the convergent expression of these schizophrenia-associated loci in neurons and their disruption of neuronal functionality may also underlie aberrations in brain functional hierarchical structure. The Allen Human Brain Atlas (AHBA) microarray dataset has provided an anatomically comprehensive transcriptomic map of the human brain (Hawrylycz et al. 2012; Sunkin et al. 2012). Concurrently, integrated imaging-transcriptomic analyses have uncovered potential biological pathways underlying regional brain alterations across multiple psychiatric disorders (Ardesch et al. 2023; Jiang et al. 2023; Li et al. 2021). Such cross-scale approaches offer a powerful framework to investigate how schizophrenia-associated microscale changes contribute to aberrant macroscale functional hierarchies.

Hence, to further understand the mechanism of rTMS in schizophrenia, we aimed to utilize the functional gradient analysis to investigate the effects of rTMS on the organization of hierarchical structure and to further explore the relationship with genetic factors. Here, we hypothesized that the

abnormalities in the brain functional hierarchy in schizophrenia may result from certain genetic factors, and rTMS could modulate the cerebral hierarchical structure to alleviate the clinical symptoms of schizophrenia. To verify this hypothesis, first, the functional gradient analysis was applied to the longitudinal data acquired before and after rTMS treatment to explore the modulation of HF-rTMS on the brain functional hierarchy organization in schizophrenia and its relationship with changes in clinical symptoms in this study. The first three functional gradient patterns were analyzed to reveal the altered hierarchical structure induced by HF-rTMS. Second, we assessed the relationship between the changes in these gradients and clinical symptom response in schizophrenia. Third, we established the relationship between abnormal functional hierarchies in schizophrenia and gene expression patterns. Finally, we performed enrichment analyses to identify the biological pathways related to alterations in functional hierarchies.

Materials and methods

Participants

In this study, 59 patients with schizophrenia as the experimental group and 24 age- and gender-matched healthy subjects as the control group (HC group) were recruited from the Clinical Hospital of Chengdu Brain Science Institute, UESTC, China. Psychiatric symptom severity of all patients was evaluated using the Positive and Negative Syndrome Scale (PANSS). The inclusion criteria for the schizophrenia subjects were as follows: 1) meeting the ICD-10 diagnostic criteria for schizophrenia; 2) having no obvious organic brain lesions; 3) the negative symptom sub-score of PANSS $\geq$ 21 and the positive symptom subscale-score of PANSS $\leq$ 24; 4) the absence of distinct auditory hallucinations or a history of other mental disorders; 5) having no drug abuse or suicidal tendencies; 6) having no neurological disorders in patients or their first-degree relatives; 7) having no contraindications to TMS or MRI. The detailed demographic information was described in supplementary materials (Table.S1) and our previous research (Huang et al. 2022). This study was carried out in accordance with the Declaration of Helsinki, and was reviewed and approved by the Ethics Committee of the Clinical Hospital of Chengdu Brain Science Institute (No. 106142021030920). All participants and/or their

guardians were conscious of the purpose and procedure of this study and signed written informed consent. The clinical trial number: ChiCTR2100049273.

Experimental design

The patients were randomly divided into two groups. The group that received both HF-rTMS treatment and a stable dosage of antipsychotics was designated the TMS stimulation group (TSZ group), while the group that only received antipsychotic medication was designated the drug group (DSZ group). Due to excessive head motion or failure to complete the experiment, 6 patients (3 patients in the TSZ group and 3 patients in the DSZ group) were excluded from the analysis. Therefore, 27 patients in the TSZ group and 26 patients in the DSZ group were included in the subsequent analysis. There were no significant differences between the two groups in age, gender, years of education, illness duration, or antipsychotic equivalent. For the TSZ group, HF-rTMS was administered using a YRDCCY-1 stimulator (Yiruide Medical Equipment New Technology Co., Ltd., Wuhan, China) with a figure-of-8 coil (B9076). The coil was positioned tangentially to the plane of the skull to provide stimulation, and the junction of the two loops was aligned with the stimulation target. In the period of treatment, 27 patients in the TSZ group received five sessions of HF-rTMS treatment per week for 4 weeks. The TMS coil was positioned on the F3 (a reference to the 10-20 EEG system), corresponding to the left DLPFC (Jiang et al. 2019; Shi et al. 2014). Each session delivered 1000 pulses/day (a total of 20 sets of pulse trains, each with 50 pulses, 5s per train, and an interval of 30s) at 10-Hz and an intensity of 110% of resting motor threshold. MRI scanning and PANSS assessments were conducted at baseline and post-treatment. All patients maintained the same stable medication throughout the study.

MRI data acquisition and pre-processing

MRI data were acquired on a 3-Tesla MRI scanner (GE DISCOVERY MR750) with an eight-channel phased-array head coil at the University of Electronic Science and Technology of China (UESTC). The resting-state fMRI data were acquired using a standard gradient-echo echo-planar imaging (EPI) sequence with the following parameters: repetition time(TR) = 2000 ms, echo time(TE) = 30 ms, flip angle(FA) = 90°, matrix size = 64×64, field of view(FOV) = 240×240 mm^2, slice thickness = 4 mm (gap = 0.4 mm), voxel size = 3.75×3.75×4.00 mm^3, and 35 axial slices in each

volume. The scan duration was 510 seconds, which yielded 255 whole-brain volumes. The high-resolution T1-weighted images were acquired using a three-dimensional fast spoiled gradient echo sequence. The parameters were as follows: TR = 6.008 ms, TE = 1.984 ms, FA = 9°, matrix size = 256 ×256, FOV = 25.6×25.6 cm^2, slice thickness = 1 mm(no gap). A total of 152 slices were acquired for each subject. During scanning, participants were instructed to remain still and be relaxed with their eyes while fixating on a cross on the screen, and their heads were immobilized with foam padding to minimize the effects of movement.

The preprocessing of fMRI data was carried out using the data processing and analysis for (resting-state) brain imaging (DPABI) software package (Yan et al. 2016), including: 1) the first 5 time points discarded due to the inhomogeneity of the magnetic field; 2) slice timing correction; 3) motion correction; 4) realignment; 5) normalization and resampled the voxel size into 5×5×5mm^3; 6) regressed out nuisance signals (including white matter (WM), cerebrospinal fluid (CSF), linear trend, global signal(GS), 24 head motion parameters (6 motion parameters (three translations and three rotations), and their first-order derivatives, and the squared terms of these 12 parameters); 7) band-pass filtered between 0.01-0.1 Hz.

Functional gradient and spatial dispersion analyses

In this study, we performed diffusion map embedding to identify the functional gradients for each participant. This nonlinear dimensionality reduction technique captures gradual transitions in functional connectivity patterns across the cortex by mapping high-dimensional similarity data into a low-dimensional space. It more effectively projects both local and long-distance connections into a common space compared to linear approaches (Margulies et al. 2016). The resultant components of this data-driven analysis are functional gradients that describe the connectome where each voxel is located along a gradient according to its connectivity pattern (Dong et al. 2022). Gradient values are unitless; higher or lower scores reflect the relative similarity changes in functional connectivity patterns in each dimension.

To investigate alterations in functional hierarchy as represented by functional gradients in schizophrenia, we computed the voxel-wise functional connectivity (of the cerebral cortex) for each

individual. Subsequently, we performed threshold processing on the connectivity matrix, retaining the top 10% of positive connectivity in each row. Cosine similarity was calculated to generate the individual affinity matrix, which served as the input of the diffusion embedding map. This algorithm identifies low-dimensional gradient component, ordering them by the proportion of variance explained. Each gradient was aligned to the group-level gradient component template, which was generated from an average connectivity matrix of the HC group. Since the first three gradients accounted for the vast majority of data variation, subsequent analysis focused on these three gradients.

Furthermore, to quantify the whole-brain connectivity separation captured by the multi-dimensional gradients, we calculated gradient eccentricity, a measure representing the 3D gradient dispersion property (Park et al. 2021). Gradient eccentricity was calculated as the Euclidean distance between the centroid of all vertices extracted from the HC template and all vertices in each 3D gradient point. Group-level differences in gradient eccentricity was then examined in the following statistical analysis to evaluate the effect of TMS on gradient dispersion.

Statistical analysis

First, to evaluate alteration in hierarchical functional topography in schizophrenia, we detected the difference in the principal gradient between the schizophrenia group at baseline and the HCs using the two-sample t-test (age and gender as the covariates). Then, the repeated measure analysis of variance (rANOVA) (between-group factor: treatment (TMS + Drug vs. only Drug), within-group factor: time (baseline vs. post-treatment)) was performed to investigate the effects of TMS on the principal gradient to the third gradient, controlling the age and gender. The post hoc tests were conducted using a two-sample t-test (between-group) or paired t-test (within-group).

Then, to identify the association between the altered brain functional gradient induced by the HF-rTMS and clinical symptom improvement in schizophrenia, we further examined the relationship between the altered gradients (post-treatment minus baseline) within regions showing significant interaction effects and the changes in PANSS scores (baseline minus post-treatment) by Spearman correlation. The significance of these correlations was assessed using permutation testing (5,000 permutations). Specifically, a null distribution was generated by randomly shuffling the pairing

between gradient values and PANSS scores across participants 5,000 times, recalculating the correlation coefficient each time. The observed correlation was then compared against this null distribution to obtain a permutation p-value (p_{perm}). The PANSS difference was calculated as (baseline minus post-treatment) so that a positive value indicated a reduction in symptoms (i.e., improvement).

In addition, to evaluate the influence of global signal regression (GSR), we also performed functional gradient analysis based on functional connectivity without global signal regression (without GSR).

Gene expression related to functional hierarchy

The AHBA dataset (http://human.brain-map.org) provides publicly available normalized gene expression data derived from bulk microarray analysis of tissue samples obtained from six healthy adult human donors. More than 20,000 genes across 3,702 brain regions in MRI-derived stereotactic space are provided. In present analysis, considering that the AHBA dataset contains only two right hemisphere data, only the left hemisphere was used. Following the preprocessing pipeline described by Arnatkevic et al., we assigned samples to Brainnetome atlas (210 cortical subregions) (Fan et al. 2016). Then we obtained the estimated expression values for each of the genes in regions of the left hemisphere, resulting in a regional transcription matrix (105 regions × 10,027 gene expression levels). More detailed steps for regional gene expression estimation are described in our previous research(Jiang et al. 2023). Next, to investigate gene expression associated with changes in brain functional hierarchy in patients with schizophrenia, partial least squares (PLS) analysis was performed separately on the patients versus healthy controls statistical map and the post-treatment versus pre-treatment statistical map in patients receiving TMS treatment. In each the PLS regression model, the left hemisphere T-statistic map served as the response variable, and the transcriptional activity data of 10027 genes served as the predictor variables. This yielded two distinct gene expression-weighted brain maps: one related to functional impairment and the other associated with TMS modulation effects. For the correlation between the first component of PLS (PLS1) and T-statistical maps, spin tests based on spherical rotations (5,000 iterations) was employed to eliminate spatial autocorrelation (Alexander-Bloch et al. 2018). And bootstrap method was used to estimate the variability of each gene's PLS1

weights, which resamples cortical regions with replacement. Then the weight of each gene was transformed to a Z-score reflecting the contribution of each gene to PLS1 by calculating the ratio of its weight to its bootstrap standard error. Based on the Z-score value, we ranked the genes and selected genes that significantly contributed to changes in brain functional hierarchy ($p < 0.005$, false discovery rate [FDR]-corrected).

In addition, to investigate the transcriptional correlation between disease-related gene and alterations in brain functional hierarchy, we overlapped the 10,027 background genes with 67 schizophrenia-related genes previously defined within the AHBA dataset. This dataset provides large-scale, cellular-resolution spatial expression patterns of 1,000 genes profiled using ISH (Zeng et al. 2012). Then, we estimated the associations between these 52 overlapped genes and gradient statistical maps using Spatial Spearman's correlation ($p_{spin}<0.05$, FDR corrected).

Enrichment analysis

Enrichment analyses were performed to further explore the molecular functions, cellular components, or biological processes implicated in the hierarchical changes of brain function in patients. Genes with higher loadings in the PLS1- and PLS1+ gene lists were selected for the enrichment analysis using Metascape website (https://metascape.org/gp/index.html#/main/step1). We conducted gene enrichment analyses on the results of the two PLS analyses respectively. For the genes related to baseline functional impairment, we selected 1748 PLS1+($Z>5$) genes and 658 PLS1-($Z<-5$) genes as the input for the enrichment analysis. Similarly, for the genes related to the TMS modulation effect, considering that the therapeutic effect of the four-week TMS treatment is relatively mild, we selected the 23 PLS1+ and 2,989 PLS1- weighted genes (all $p_{FDR} < 0.005$) as the input for the enrichment analysis.

Results

The effect of rTMS on the gradients of schizophrenia

Consistent with previous research (Margulies et al. 2016), the two ends of the principal gradient (referred to as gradient 1) corresponded to the sensorimotor cortex and the DMN. The two ends of the

second gradient (gradient 2) are the visual cortex and sensorimotor cortex. Besides, the third gradient (gradient 3) extends from the sensorimotor and the DMN to the fronto-parietal network (FPN) (Fig.1A, 1B; Supplementary Fig.S1). Similar distribution patterns were observed in gradients without GSR (Supplementary Fig.S2, Fig.S3). In addition, compared with the HC group, patients with schizophrenia showed an increased principal gradient in bilateral sensorimotor cortices (Fig.2A; Supplementary Fig.S4A).

For the principal gradient, a significant interaction between rTMS treatment and time was observed ($p<0.05$) (Fig.2B), involving the occipital lobe, the left inferior frontal gyrus, the left middle frontal gyrus, the superior frontal gyrus, and subcortical regions. Compared to the baseline condition, paired t-test revealed that rTMS significantly increased principal gradient values in the left DLPFC, left insula, and left inferior parietal lobule (IPL), while resulting in decreased principal gradient in the bilateral MT and frontoparietal regions (Fig.2C, 2D). For gradient 2, significant interaction effects were identified primarily in the occipital lobe, middle temporal gyrus and right inferior frontal gyrus. The paired t-test between post- and baseline showed a significant increase in the right superior temporal gyrus and right Heschl gyrus, and a decrease in the left inferior occipital gyrus (Supplementary Fig.S5). The rANOVA of the gradient 3 indicated significant interaction effects in the bilateral angular gyrus, the bilateral IPL, the right middle frontal gyrus, and the middle cingulate gyrus (Fig.3A). Compared to baseline, the paired t-test results revealed consistent increases in gradient 3 values across most of these areas (Fig.3B, 3C). Similar findings were observed in gradients without GSR (Supplementary Fig.S4B, S4C, S6, S7).

Additionally, we calculated the eccentricity of the three gradient components for each of the individuals. The results showed that the regions of increased eccentricity after rTMS treatment were mainly located in bilateral IPL (Supplementary Fig.S8).

Correlations between altered functional gradients and clinical variables

We extracted gradient scores from 9 regions of interest (ROIs) that showed significant interaction effects in both the principal gradient and the third gradient to examine the relationship between the altered functional gradients and clinical scale (PANSS positive, negative, general psychopathology

subscales and total scores). An increase in the principal gradient within the left insula induced by rTMS was significantly associated with the improvement of positive symptoms ($r=0.401$, $p_{perm}=0.035$) (Fig.4A). A decreased in the principal gradient within the left middle temporal area induced by TMS was significantly associated with improvement of general psychopathology symptoms ($r=-0.489$, $p_{perm}=0.011$) (Fig 4B). The results of the permutation tests are provided in Supplementary Fig. S9.

Correlations between altered functional gradients and genetic factors

To explore the contribution of the schizophrenia-related genes to alterations of functional hierarchy, we first obtained the overlapped genes from the 67 schizophrenia-related genes identified by ISH and 10,027 background genes. The complete list of overlapping genes is provided in supplementary materials (Table.S2). Sixteen of the 52 overlapping schizophrenia-associated genes showed significant correlations with changes in principal gradient ($p < 0.05$, FDR corrected) (Fig 5A). Six genes exhibited significant positive correlation (i.e., *ATF4*: $r = 0.58$, $p_{spin} = 0.003$; *NRG1*: $r = 0.55$, $p_{spin} = 0.005$; *PCNT*: $r = 0.46$, $p_{spin} = 0.037$; *PVALB*: $r = 0.57$, $p_{spin} = 0.008$; *FEZ1*: $r = 0.47$, $p_{spin} = 0.001$; *NDE1*: $r = 0.39$, $p_{spin} = 0.005$). Ten genes exhibited significant negative correlation (i.e., *BDNF*: $r = -0.56$, $p_{spin} <0.001$; *GRIN2B*: $r = -0.51$, $p_{spin} = 0.001$; *GRM7*: $r = -0.39$, $p_{spin} = 0.035$; *HTR2C*: $r = -0.53$, $p_{spin} = 0.006$; *KCNN3*: $r = -0.51$, $p_{spin} = 0.001$; *PRODH*: $r = -0.42$, $p_{spin} < 0.001$; *SYN2*: $r = -0.58$, $p_{spin} < 0.001$; *PDLIM5*: $r = -0.38$, $p_{spin} = 0.014$; *NPAS3*: $r = -0.44$, $p_{spin} = 0.005$; *PPP1R1B*: $r = -0.40$, $p_{spin} = 0.006$).

We performed two sets of PLS regressions to identify the spatial patterns of gene expression that were associated with the alteration of functional hierarchy in patients with schizophrenia. Subsequently, enrichment analyses were conducted separately for the lists of significantly expressed genes derived from each PLS regression analyses.

In the first PLS regression analysis, we investigated the correlation between gene expression and the principal gradient abnormalities in schizophrenia (Fig.5). The PLS1 explained 48.6% of the variance in the principal gradient abnormality (i.e., T-map comparing patients at baseline to the HC group). PLS1 gene expression weights were significantly positively correlated with the principal gradient T-map (Fig 5D, $r = 0.70$, $p_{spin} < 0.0001$). Then we converted the PLS1 scores to Z-scores and ranked the normalized PLS loadings (Fig.5E, $p_{FDR} < 0.005$). We found 1,748 PLS1+($Z > 5$) positively

weighted gene expressions were over-expressed as increased principal gradient value and 659 PLS1- ($Z < -5$) negatively weighted gene expressions were under-expressed as decreased principal gradient value (all $p_{FDR} < 0.005$). Next, we aligned the Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and Reactome (R-HAS) pathways with the above total 2,047 genes list. The enriched terms of top twenty significant pathways are shown in Fig 5F, which included seventeen GO biological processes, one KEGG pathway and two R-HSA pathways.

In the second PLS regression, we examined the correlation between gene expression and the principal gradient improvement induced by rTMS (Fig.6). The PLS1 explained 23.1% of the variance in the principal gradient improvement (i.e., T-map comparing the TSZ group at post-rTMS to baseline). PLS1 gene expression weights were significantly positively correlated with the principal gradient T-map (Fig.6C, $r = 0.48$, $p_{spin} = 0.0035$). Similarly, we ranked the normalized weights of PLS1 scores (Fig.6D, $p_{FDR} < 0.005$). We found 288 PLS1- ($Z < -5$) negatively weighted gene expressions were under-expressed as decreased principal gradient value, but no PLS1+ ($Z > 5$) positive weighted gene was observed. Given the relatively modest association between the short-term effects of rTMS and gene expression, we included 23 PLS1+ and 2,989 PLS1- weighted genes (all $p_{FDR} < 0.005$) in the enrichment analysis. The enriched terms of top twenty significant pathways are shown in Fig.6E, comprising sixteen GO biological processes, one KEGG pathway and three R-HSA pathways.

Discussion

This study utilized functional gradient analyses to explore the effects of HF-rTMS on functional hierarchical organization structure in patients with schizophrenia, and systematically compared the abnormal enriched pathways in schizophrenia as well as the biological pathway remodeling that accompanied the gradient reversal after rTMS treatment. The alleviated compression by rTMS mainly located in the FPN region (increased gradient scores in the prefrontal lobe, the top of the sensorimotor-to-association axis) and the visual network (decreased gradient scores in the occipital lobe, the bottom of the sensorimotor-to-association axis) in principal gradient. Meanwhile, the alteration of gradient scores was associated with the relief of clinical symptom. These findings suggest that HF-rTMS might relieve the clinical symptoms of schizophrenia to some degree by relieving the suppressed brain

hierarchical structure. This supports the view that the reduction of separation of the primary sensorimotor cortex from the higher-order cortex disrupts the macroscale network hierarchy organization in schizophrenia. Interestingly, this compression was alleviated by rTMS combined with medication rather than only drugs, highlighting the specific role of rTMS in modulating brain hierarchical organization.

The functional connectivity gradient mapping method provides a simplified representation of brain function in the lower-order to higher-order cognitive regions(Katsumi et al. 2023), which can reflect the macroscopic changes in the cortical hierarchy of schizophrenia. In both the HC and schizophrenia groups, the principal gradient depicted an axis extending from primary sensory areas (visual and sensorimotor) to the DMN, gradient 2 depicted an axis extending from visual to sensorimotor cortices, and gradient 3 depicted an axis extending from sensorimotor and DMN to the FPN. This pattern confirms that gradient analysis robustly captures the brain's hierarchical organization. Nonetheless, we observed a compressed principal gradient in schizophrenia, which is consistent with our previous studies(Dong et al. 2020). Together, these results indicate the cerebral functional hierarchical structure has been disrupted in schizophrenia although the inherent hierarchy is retained.

In our study, the regulatory effect of rTMS on functional connectivity gradient in schizophrenia was obvious. The DLPFC is the core hub of FPN (Menon et al. 2022). Extensive evidence indicates that rTMS targeted in the left DLPFC can alter the functional connectivity between DLPFC and other regions (Bation et al. 2021; Eldaief et al. 2011), which supported the excitement of targeted regions can modulate its distributed network connections. The rTMS targeted in the left DLPFC has shown beneficial effects on symptoms such as auditory verbal hallucinations, cognitive deficits, and schizophrenia, and has been associated with improvements in working memory (De Weijer et al. 2014; Hasan et al. 2016; Liu et al. 2025; Ragland et al. 2004). Our previous study found that the FC temporal variability between the DLPFC (target) and regions in the cortical-thalamic-cerebellar circuit is significantly reduced after HF-rTMS intervention (Huang et al. 2022). In this current study, the increased gradient scores in the left DLPFC reflected the changed hierarchical organization of the target region after rTMS treatment, and this shift may further help improve the abnormal cognitive control function of FPN in schizophrenia.

Although few studies have focused on the effect of rTMS on brain hierarchical structure, the previous studies demonstrated that the rTMS not only can modulate the functional connectivity of target regions but also can change the remote regions (Wang et al. 2024). The rTMS could also change the graph property of connectivity (Li et al. 2024; Olejarczyk et al. 2020), suggesting that the rTMS could reshape the whole brain organization. Mounting studies indicates that cognitive deficits in schizophrenia are related to abnormal mutual communication between networks. A dynamic causal modeling analysis has found that the cross-network interactions centered at salience network (SN) are most significantly reduced, and that dysregulation of triple-network interaction aggravates clinical symptoms in schizophrenia (Supekar et al. 2019; Xi et al. 2021). In this study, rTMS-induced increases in the principal gradient were observed in the left insula, which is a core area of SN acting as a switch to convert internally oriented cognition to externally oriented attention through activation of either the DMN or central executive network (Uddin 2015; Zhao et al. 2022). This change was associated with improvement in clinical symptoms. The insula-anchored SN plays a central role in the aberrant mapping of salient external and internal events in schizophrenia (Menon et al. 2023). This is consistent with the consensus that SN can activate the FPN to maximize attention to the task (Schimmelpfennig et al. 2023). From a theoretical perspective, increased functional hierarchy and enhanced function integration of the SN induced by rTMS could help identify relevant stimuli within a continuous stream of sensory input, which in turn could contribute to more efficient functional switching of the FPN in the triple-network framework and improving the clinical symptom in schizophrenia. However, this speculation should be further examined in future studies.

In addition, the altered functional gradient was also found in MT. As a highly specialized visual area, research on MT has focused on its role in visual information processing. The local information of the primary visual cortex (area striata, V1) must be integrated across a wider space and time into a global concept via high-order motion mechanisms in area V5 (i.e. middle temporal area, MT) in the dorsal pathway (Born et al. 2005; Tadin et al. 2011; Urbaniak et al. 2024). Studies have consistently revealed deficits in performance on multiple information-integration related tasks specific to the high-order motion area V5 in patients with schizophrenia, such as distinguishing stimuli of different velocities figure-ground segregation, and temporal order judgment (Kandil et al. 2013). Similar to these studies,

the principal gradient level of MT in schizophrenics showed a compression which represented a reduced degree of separation of primary sensory processing from the associated cortex. After four weeks of HF-rTMS treatment, this compression pattern was alleviated, and the change correlated with improvement in the general pathological symptoms of schizophrenia. Given the limited prior research on rTMS-induced modulation of MT, we propose that HF-rTMS might restore the bottom-up transmission of sensory information in the dorsal information pathway with MT as the hub by adjusting the functional connectivity gradient in the MT region. Moreover, HF-rTMS targeted left DLPFC might also modulate the top-down cognitive pathway, which further improved the inefficient integration between the two pathways to alleviate clinical symptoms. Together, these observations reinforce the importance of studying the abnormal information flow between primary sensorimotor and high-order association cortices in schizophrenia.

Among the correlations of overlapped schizophrenia-related genes, the expression of the *ATF4* gene and the *SYN2* gene respectively showed the strongest positive and negative correlations with the functional gradient changes. Oxidative stress is a convergence point for schizophrenia genetic and environmental risk factors (Cuenod et al. 2022), while *ATF4* is a key transcription factor in integrating stress responses(Costa-Mattioli et al. 2020). *ATF4* also affects trafficking of $GABA_B$ receptors, which in turn modulates the excitability properties of neurons (Corona et al. 2018). In addition, the *SYN2* gene has been reported to confer susceptibility to schizophrenia (Lee et al. 2005), and its abnormal expression in emotional disorders is highly associated with decreased DNA methylation (Cruceanu et al. 2016). Together with prior evidence on schizophrenia risk genes, our findings further support a genetic basis for the disorder. Besides, we identified the weighted combination of genes that significantly co-localized with functional gradient changes. Of the 16 overlapped schizophrenia-related genes correlated with functional gradient abnormalities in patients, two distinct patterns emerged regarding rTMS treatment response. Six genes with positive weights on PLS1 for the disease abnormality showed negative weights for the post-rTMS improvement. Conversely, the ten genes with negative weights for the disease abnormality showed no significant weighted contribution to the improvement. This divergence suggests that rTMS might primarily modulate genetic mechanisms governed by genes that are positively associated with the functional hierarchy aberrations in

schizophrenia (i.e., *ATF4*, *NRG1*, *PCNT*, *PVALB*, *FEZ1*, *NDE1*).

Furthermore, enrichment analysis identified the biological pathways that were highly associated with the alteration of functional hierarchy in schizophrenia. These pathways showed pronounced enrichment in biological pathways associated with ion homeostasis and transmembrane transport mechanisms, and cellular metabolic activities. These results are highly consistent with the classical hypothesis that schizophrenia is a disorder of neurotransmitter dysregulation (Eyles 2021; Howes et al. 2009). The patient group showed enrichment in multiple neurophysiological processes, suggesting that the abnormality of the brain's functional gradient stems not only from functional decoupling, but also from underlying molecular and cellular defects. The findings suggest that abnormal synaptic function, disorder in the integration of neuronal networks, and systemic metabolic imbalance together constitute the cross-scale circuit abnormalities which influenced cognitive and perceptual symptoms in schizophrenia. It is worth noting that after receiving rTMS treatment, patients showed significant enrichment of pathways related to neuroplasticity regulation, including “cell morphogenesis”, “microtubule cytoskeleton organization”, and “actin filament-based processes”. This indicates that rTMS may partially reverse the neural connectivity abnormalities by promoting neuronal structural reorganization and synaptic plasticity — a perspective supported by animal studies (Cirillo et al. 2017; Hoogendam et al. 2010). In particular, the *Rho GTPase* is a regulatory factor that plays a crucial role in maintaining metabolic homeostasis (Møller et al. 2019). The enrichment of *Rho GTPase* signaling pathway further supports that rTMS may affect the function of neural network by regulating the dynamics of cytoskeleton and signal transduction pathway (Sharbafshaaer et al. 2024). Furthermore, the neuroinflammation hypothesis has recently gained considerable attention in the field of psychiatric disorders (Melnikov et al. 2025). This study provides evidence supporting this hypothesis: the enrichment of pathways such as "response to oxidative stress" and "cellular response to cytokine stimulus" after treatment suggests that rTMS may exert its therapeutic effects by modulating neuroimmune mechanisms.

Limitations

There are several limitations associated with our study. First, the sample size of the current study

was relatively small, and future studies with larger cohorts are warranted. Second, although a drug control group was set up, our study lacked a sham TMS control group, which may confuse our results with a degree of placebo effect. Third, the stimulation target was based on a standardized location rather than individualized neuronavigation. Fourth, the use of left hemispheric transcriptomic data limited the generalizability of our spatial correlation analyses to whole-brain patterns. Despite these limitations, this study provides novel evidence linking alterations in cerebral functional hierarchical structure and clinical improvement following HF-rTMS to underlying gene expression patterns in schizophrenia.

Conclusion

Overall, this study demonstrates that HF-rTMS targeted left DLPFC combined with drug treatment can improve the clinical symptoms in patients with schizophrenia. This improvement is linked to the alleviation of abnormal compression pattern of functional hierarchy, as captured by the first three gradients of brain functional connectivity. The concrete manifestation is the regulation of the hierarchy of SN and FPN that may enhance their interaction and transmission of attention information. Our findings indicate that the mechanism by which rTMS ameliorates abnormal brain functional hierarchy in schizophrenia involves multiple biological processes such as ion transport, cellular metabolic and immune responses. The present study provides experimental evidence supporting the clinical validity of HF-rTMS therapy. This would be a necessary work to focus on the patterns of hierarchy variation between these specific brain regions of schizophrenia to promote the exploration of biomarker-potential of functional gradients and clarify the mechanisms of rTMS therapy. Future research should employ longitudinal designs to track gradient changes, thereby further verifying how TMS effects propagate through the cortical hierarchy in patients with schizophrenia.

Author contributions

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. All authors have approved the final manuscript.

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Data and code availability statement

The code of gradient analysis is openly available from the BrainSpace toolbox: http://brainspace.readthedocs.io. The data are not publicly available due to privacy or ethical restrictions. Sharing and re-use of data need the expressed written permission of the authors and clearance from the relevant institutional review.

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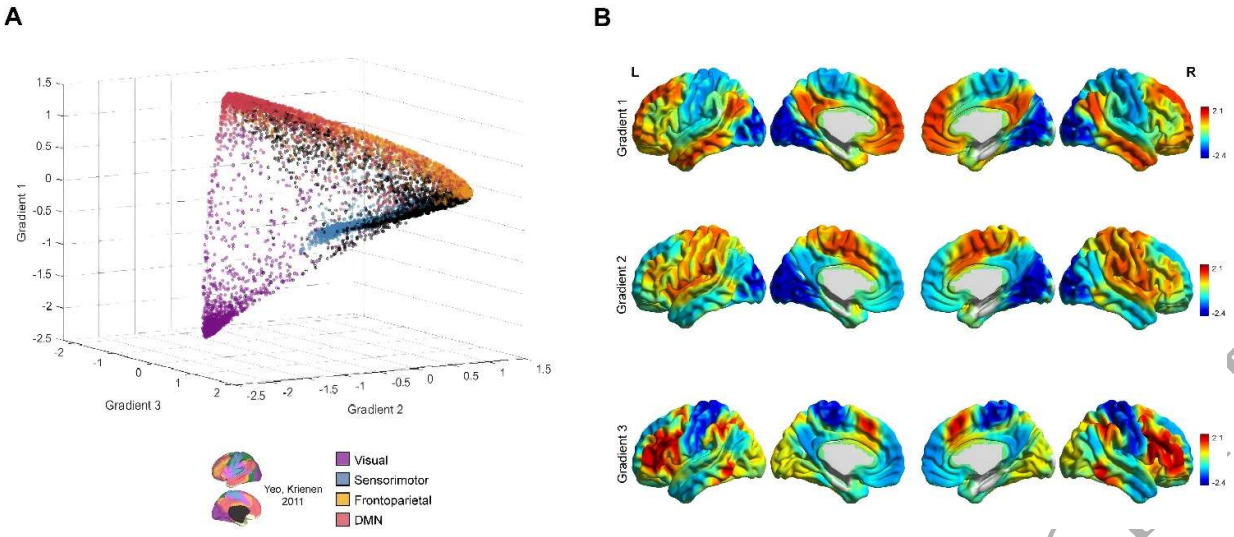

Fig.1. Gradients of HC group. **A)** Three-dimensional scatterplot of the first three gradient components from the HC group. **B)** The gradient map of the HC group. From top to bottom are the principal gradient (Gradient 1), the second gradient (Gradient 2), and the third gradient (Gradient 3).

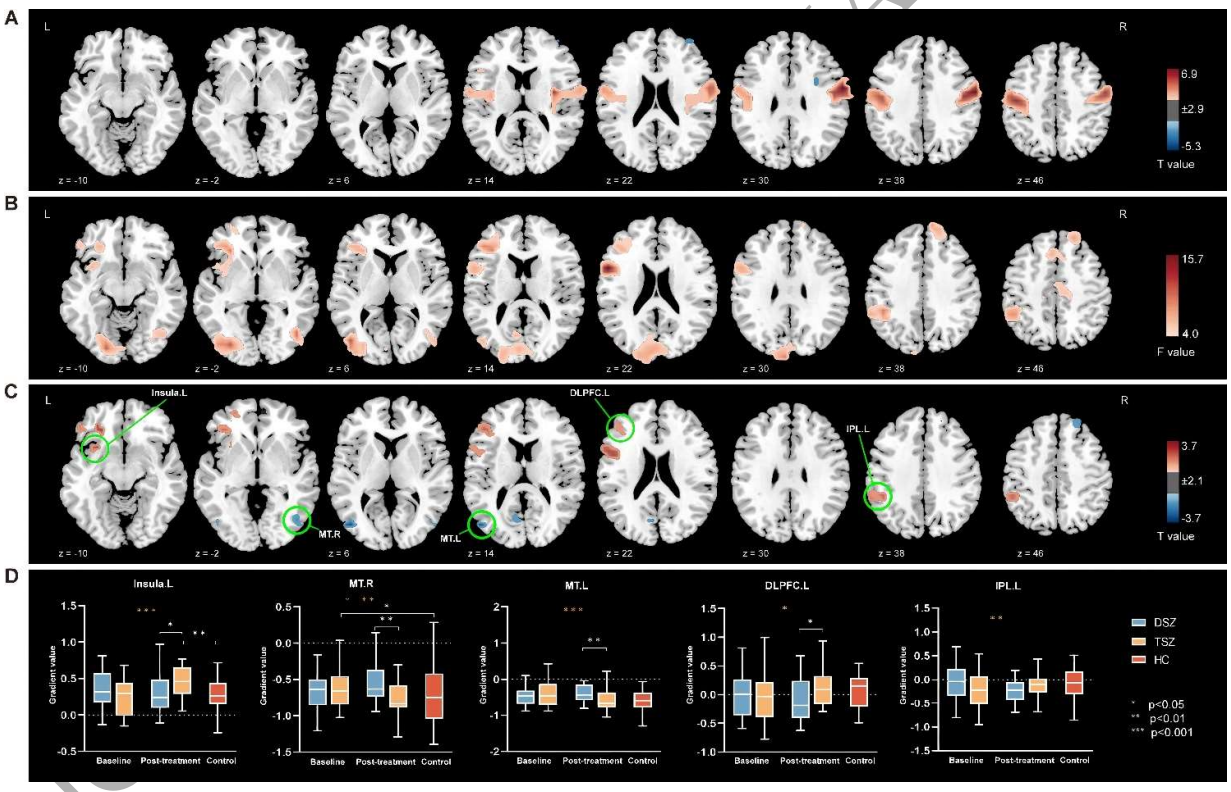

Fig.2. Differences between schizophrenia & HC at baseline, rANOVA and post hoc test results of the principal gradient. **A)** Regions with significant differences between schizophrenia (DSZ & TSZ at

baseline) and HC group ($p_{FDR} < 0.05$). **B)** Regions showed a significant interaction between TMS treatment and time ($p < 0.05$). **C)** Regions showed significant differences in the post hoc paired t-test of the TSZ group (post-rTMS minus baseline, $p < 0.05$). **D)** Gradient intergroup differences within region of interest (ROI). From left to right are the regions of the Insula.L, the MT.R, the MT.L, the DLPFC.L, and IPL.L. The number of * represents the level of significance (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$), and the color of * represents different tests (blue * represents paired t-test at baseline and after intervention in the DSZ group, orange * represent paired t-tests at baseline and after intervention in the TSZ group, and white * represents two-sample t-tests). MT: middle temporal visual area, DLPFC: the dorsolateral prefrontal cortex, MFG: middle frontal gyrus, IPL: inferior parietal lobule.

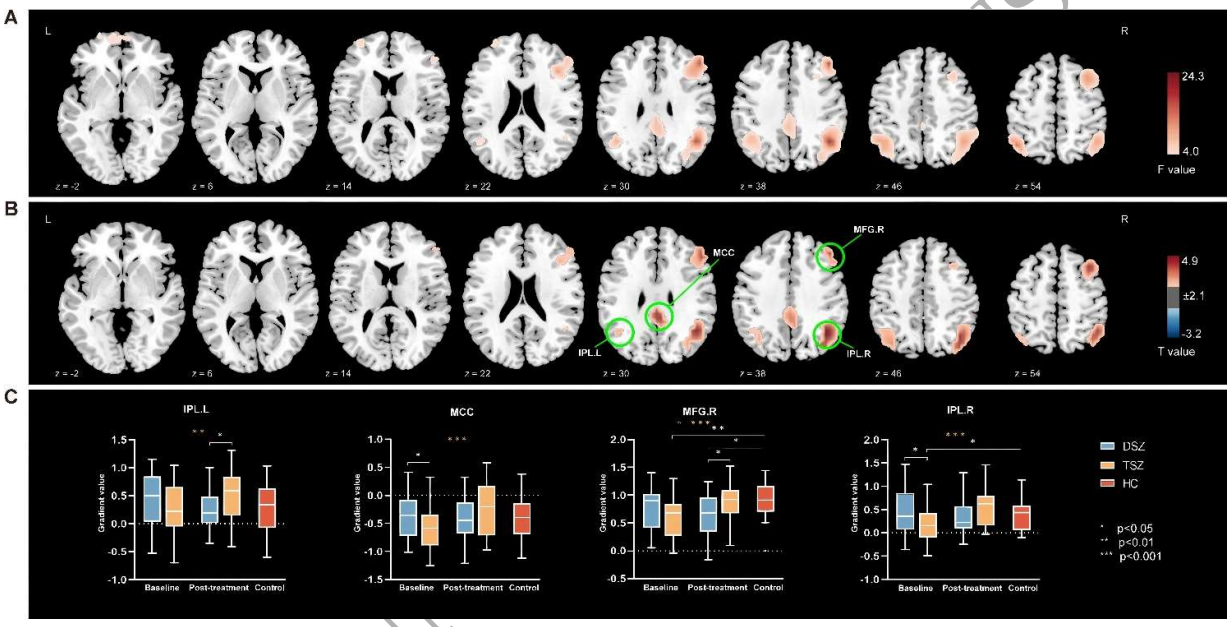

Fig.3. rANOVA and post hoc results of gradient 3. **A)** Regions showed a significant interaction between TMS treatment and time ($p < 0.05$). **B)** Regions showed significant differences in the post hoc paired t-test of the TSZ group (post-rTMS minus baseline, $p < 0.05$). **C)** Gradient intergroup differences within ROI. From left to right are the regions of the IPL.L, the MCC, the MFG.R, and the IPL.R. The number of * represents the level of significance (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$), and the color of * represents different tests (blue * represents paired t-test at baseline and after intervention in the DSZ group, orange * represents paired t-test at baseline and after intervention in the TSZ group,

and white * represents two-sample t-tests). MFG: middle frontal gyrus, IPL: inferior parietal lobule, MCC: middle cingulate cortex.

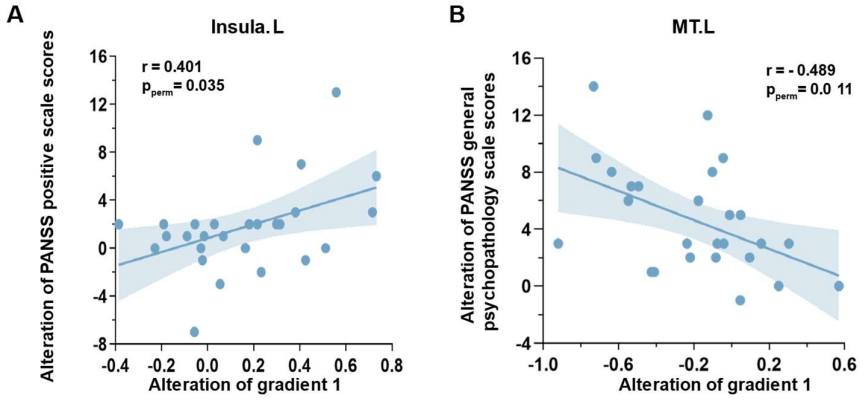

Fig.4. A) The alteration of principal gradient (post minus baseline) in the left insula was associated positively with the alteration of PANSS positive scale scores (baseline minus post) with positive values representing the improvement of symptoms. **B)** The alteration of the principal gradient (post minus baseline) in left MT was associated negatively with the alteration of PANSS general psychopathology scale scores (baseline minus post). Significance was assessed using permutation testing (n = 5,000). MT: middle temporal visual area.

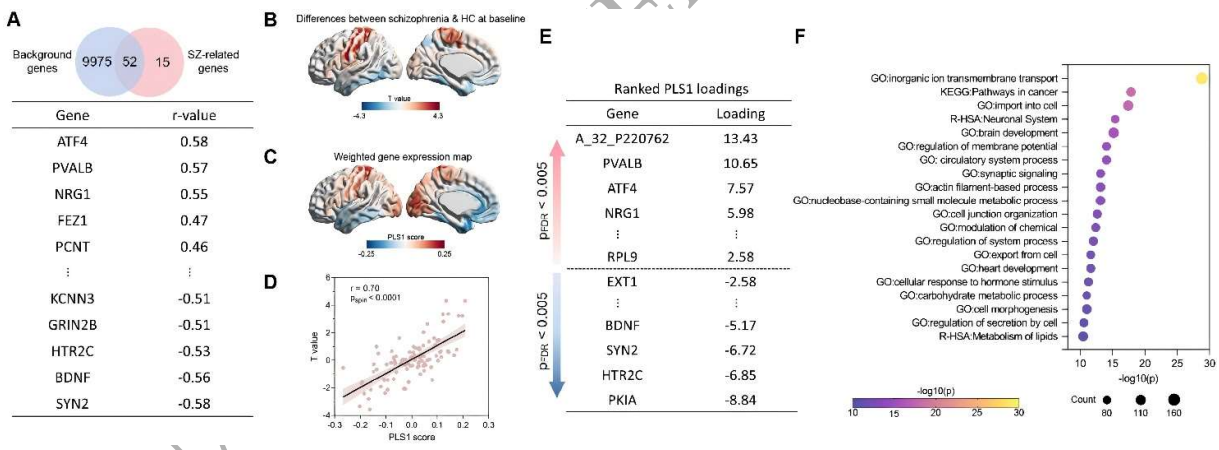

Fig.5. Schizophrenia-associated genetic profiles related to principal gradient differences. **A)** Schizophrenia-associated genes from in situ hybridization (ISH) overlapped with 10,027 background genes and were correlated with alteration of principal gradient in schizophrenia. **B)** Principal gradient differences between schizophrenia (DSZ & TSZ at baseline) and HC group. **C)** Cortical map of

regional PLS1 scores. **D)** Scatterplot showing the relationship between regional PLS1 scores and principal gradient differences. **E)** Ranked PLS1 scores. **F)** Functional enrichment ontology terms related to the alteration of principal gradient in the PLS+ (Z > 5) and PLS- (Z < -5) gene list (all p_{FDR} < 0.005). GO, gene ontology. KEGG, Kyoto Encyclopedia of Genes and Genomes.

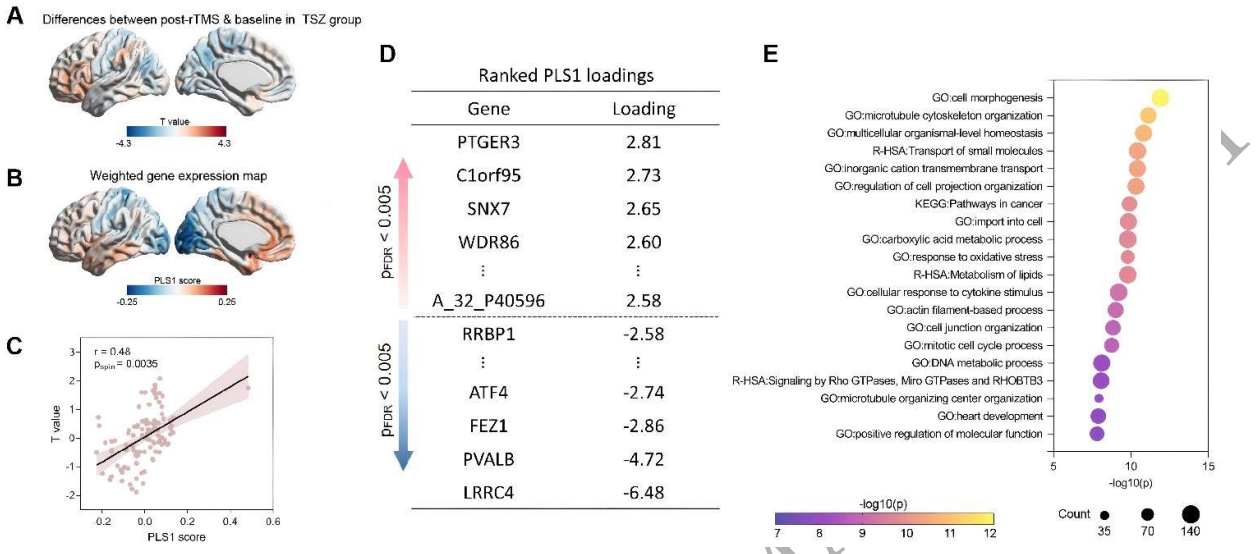

Fig.6. Genetic profiles related to the changes of principal gradient induced by rTMS. **A)** Principal gradient differences of TSZ group (post-rTMS minus baseline). **B)** Cortical map of regional PLS1 scores. **C)** Scatterplot illustrating the correlation between regional PLS1 scores and rTMS-induced gradient differences. **D)** Ranked PLS1 scores. **E)** Functional enrichment ontology terms related to the rTMS-induced alteration of principal gradient in the PLS+ and PLS- gene list (all p_{FDR} < 0.005). GO, gene ontology. KEGG, Kyoto Encyclopedia of Genes and Genomes.