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Effect of ZNF804A gene polymorphism (rs1344706) on the plasticity of the functional coupling between the right dorsolateral prefrontal cortex and the contralateral hippocampal formation

Wan Zhao[a], Xiongying Chen[b], Qiumei Zhang[a,c], Boqi Du[a], Xiaoxiang Deng[a], Feng Ji[c], Yu-Tao Xiang[d], Chuanyue Wang[b], Qi Dong[a], Chuansheng Chen[e], Jun Li[a,*]

[a] State Key Laboratory of Cognitive Neuroscience and Learning & IDG/McGovern Institute for Brain Research, Beijing Normal University, PR China
[b] The National Clinical Research Center for Mental Disorders & Beijing Key Laboratory of Mental Disorders & Beijing Institute for Brain Disorders Center of Schizophrenia, Beijing Anding Hospital, Capital Medical University, Beijing 100088, PR China
[c] School of Mental Health, Jining Medical University, 45# Jianshe South Road, Jining 272013, Shandong Province, PR China
[d] Faculty of Health Sciences, University of Macau, Avenida da Universidade, Taipa, Macau, PR China
[e] Department of Psychological Science, University of California, Irvine, CA 92697, United States

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ABSTRACT

ZNF804A has now been recognized as a schizophrenia risk gene by multiple genome-wide association studies with its intronic polymorphism rs1344706 being reported as the first genome-wide significant risk variant for schizophrenia. Although the functional impact of this gene is still unknown, rs1344706's contribution to the functional coupling between the right dorsolateral prefrontal cortex (DLPFC) and the contralateral hippocampal formation (HF) has been reported by several studies. The current study tested whether the right DLPFC-left HF functional coupling showed plasticity during cognitive training (Study I) and whether rs1344706 affected the plasticity (Study II). In Study I, we conducted a randomized controlled trial with 30 subjects receiving 20 sessions of adaptive training on a memory span task (the training group) and 30 subjects practicing on a non-adaptive easy version of the same memory span task for 20 sessions (the control group). All subjects were scanned using fMRI before and after the training. Analyses of resting-state and task-state fMRI data consistently showed that the adaptive memory span training significantly strengthened the right DLPFC-left HF functional coupling. In Study II, we conducted a genetic association study with 101 subjects (combining the data from the training group in Study I with those from an additional subsequent sample of 71 subjects who received the same training and fMRI scans). Results showed that rs1344706 was significantly associated with training-induced changes in functional coupling. Subjects carrying the non-risk allele (C) of rs1344706 showed greater training-induced plasticity than the risk allele (A) homozygotes. These findings expanded our current understanding of the functional impact of the schizophrenia risk variant of *ZNF804A* gene and suggested that the *ZNF804A* gene could be used as a prospective target for future antipsychotic drugs and clinical research.

1. Introduction

Schizophrenia is a severe and complex neurodevelopmental disorder with a worldwide lifetime prevalence rate of about 1% (Saha et al., 2005) and high heritability of about 81% (Sullivan et al., 2003). Previous studies have identified *ZNF804A* gene (2q32.1) as the first genome-wide significant susceptibility gene for schizophrenia (Donohoe et al., 2010; O'Donovan et al., 2008). This finding was confirmed by the Schizophrenia Working Group of the Psychiatric Genomics Consortium (PGC2 release) in the largest meta-analysis of schizophrenia genome-wide association studies (GWASs) in the world (Schizophrenia Working Group of the Psychiatric Genomics, 2014). Recently, the largest GWAS of schizophrenia in the Han Chinese population also identified *ZNF804A* gene as a schizophrenia risk gene (Li et al., 2017). ZNF804A contains a C_2H_2-type zinc finger domain and may play a role in DNA binding and transcription. Although the exact function of this gene is still unknown, some studies have suggested that this gene regulates multiple neurodevelopmental processes such as neurite formation (Deans et al., 2017).

From the studies that explored the functional impact of the

* Corresponding author.
E-mail address: lijundp@bnu.edu.cn (J. Li).

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ZNF804A gene polymorphisms, the most reproducible result is that the single-nucleotide polymorphism (SNP) rs1344706 contributes to the functional coupling between the right dorsolateral prefrontal cortex (DLPFC) and the contralateral hippocampal formation (HF). This association has been established based on data from both working memory tasks (Esslinger et al., 2011, 2009; Paulus et al., 2013; Rasetti et al., 2011; Zhang et al., 2016) and resting state (Cousijn et al., 2015; Zhang et al., 2018). DLPFC is activated when performing a working memory task but is suppressed during the resting state (Cieslik et al., 2013; Fox et al., 2005). In contrast, the HF is suppressed when performing a working memory task but is activated during the resting state (Buckner et al., 2008; Greicius et al., 2003). Evidence from both animal and human studies has suggested that the negative coupling between the two regions is crucial for working memory because it links two important networks for working memory, the central executive and the default mode networks (Axmacher et al., 2008; Benchenane et al., 2010; Gordon, 2011; Jones and Wilson, 2005). Studies of schizophrenia have considered this functional coupling as a potential intermediate phenotype (Meyer-Lindenberg et al., 2005; Rasetti et al., 2011).

Although lacking evidence on this functional coupling, some researchers have found functional coupling within the central executive network is responsive to training (Jolles et al., 2013; Takeuchi et al., 2013; Thompson et al., 2016). An animal study found that working memory training strengthened the negative coupling between the prefrontal cortex and the HF (Sigurdsson et al., 2010). The authors also found that the functional coupling increased more after the training in wild-type mice than in mice that modeled a microdeletion on human chromosome 22 (22q11.2), one of the largest known genetic risk factors for schizophrenia. However, it is still unknown whether working memory training in human could also increase the coupling between the right DLPFC and the contralateral HF and whether the genetic risk variant of *ZNF804A* rs1344706 could contribute to the plasticity of the functional coupling.

In the current study, we first performed a randomized controlled trial (RCT) to investigate the effect of working memory training on the functional coupling between the right DLPFC and the left HF. We hypothesized that the training would increase (in the negative direction) this functional coupling based on the previous animal study (Sigurdsson et al., 2010). With a larger training sample, we performed a genetic association study to investigate the effect of *ZNF804A* gene rs1344706 on the training-related plasticity of this functional coupling. We hypothesized that subjects carrying the "A" allele (with an increased risk for schizophrenia) would show less increase in the functional coupling between the right DLPFC and the left HF after the training.

2. Materials and methods

The protocols of both of the randomized controlled study (Study I) and the genetic association study (Study II) were reviewed and approved by the Institutional Review Board of the Institute of Cognitive Neuroscience and Learning at Beijing Normal University. Study I has been registered at the Chinese Clinical Trial Registry (http://www.chictr.org.cn; chiCTR-INR-17011728).

2.1. Subjects

Participants in Study I included 60 healthy undergraduate student volunteers. Subjects were randomly allocated to either the training group (receiving adaptive training on a memory span task, N = 30) or the control group (repeatedly practicing on a non-adaptive easy version of the same memory span task, N = 30). All subjects were Han Chinese and gave written informed consent for this study. All subjects received the same set of fMRI scans at both pretest and posttest. CONSORT diagram for randomized controlled trials is shown in the Supplement Fig. S1.

For Study II, we recruited an additional sample of 71 undergraduate students who received the same training as the subjects in the training group of Study I. The combined 101 subjects were used to examine the effect of *ZNF804A* gene rs1344706 polymorphism on brain plasticity induced by the training.

2.2. Genotyping

Genomic DNA for subjects who received adaptive training (N = 101) was extracted using the standard method. *ZNF804A* gene rs1344706 polymorphism was genotyped using Taqman allele-specific assays on the 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The sample success rate for this SNP was 100%. The reproducibility of the genotyping was 100% according to a duplicate analysis of 40% of the genotypes.

2.3. Tasks for the training and the fMRI scans

The working memory training task was a visual-spatial span task (see Supplement Fig. S2A). Stimuli were green-colored squares presented sequentially in a 5 × 5 empty grid on a computer screen. After an intermission, subjects were required to recall the sequence of locations by clicking on the empty grid. Each stimulus was presented for 500 ms, the interstimulus interval was also 500 ms, and the intermission was 1000 ms. Difficulty level was determined by the number of the stimuli. The task difficulty was adaptive to the participants' performance for the adaptive training group. In the first training session, the training started with 3 stimuli and the number of stimuli automatically increased by 1 if the subjects made 5 continuous correct responses at the current level. In subsequent training session, the training started with 2 stimuli less than the maximum level in the previous session. For the non-adaptive training control group, the task difficulty was kept at a low level with 3 stimuli in all the training sessions. The training consisted of 20 sessions and was conducted in a neuropsychological laboratory over the course of 4 weeks (5 sessions per week, no > 1 session per day). Each training session consisted of 80 trials (lasting 30 ~ 40 min). Then we revised the training task and created an assessment version, which included two trials for each span. Testing ended when subjects failed both trials. The length of the longest correct span was used to reflect the memory span.

The fMRI task was similar to the training task, which included the working memory condition (72 trials) and the baseline condition (36 trials) (see Supplement Fig. S2B). The stimuli were presented in cue-probe pairs. For the working memory condition, the green-colored cue stimuli were presented in the same way as in the training task and the number of the stimuli was set at 5. The probe stimulus was an empty grid. Subjects were asked to judge if the Arabic number indicated the correct order of the cue stimuli by pressing the button (left button for the correct number and right button for the incorrect number). For the baseline condition, the 5 red-colored cue stimuli were presented in a fixed order (top left corner → top right corner → bottom right corner → bottom left corner → center), and the probe stimuli were the same in all trials. Each trial started with a centrally placed fixation cross for 500 ms, followed by 5 cue stimuli. Each cue stimulus was presented for 500 ms, followed by a 500 ms interstimulus interval. After a 1000 ms intermission screen, probe stimuli were presented for 2000 ms.

2.4. fMRI data acquisition

The imaging data were acquired at the Brain Imaging Center of Beijing Normal University. All subjects were scanned both before and after the training. Subjects lay supine in a Siemens TIM Trio 3T scanner (Siemens, Erlangen, Germany) with their head snugly fixed with straps and foam pads to restrict head movement. Resting-state images were acquired first, followed by a T1-weighted image scan. Subjects were then moved out of the scanner and given practice on the working memory task. After the practice, subjects went back into the scanner

and were scanned while performing the working memory task.

Both resting-state and task-related fMRI were acquired axially using the same echo-planar imaging (EPI) sequence: repetition time (TR) = 2000 ms; echo time (TE) = 30 ms; flip angle (FA) = 90°; field of view (FOV) = 200 × 200 mm^2; matrix size = 64 × 64; axial slices = 31; 4.0 mm slice thickness without gap (i.e. interleaved scan); voxel size = 3.125 × 3.125 × 4.0 mm^3. During the resting-state fMRI data collection (240 volumes, lasting about 8 min), all subjects were required to keep their eyes closed, to stay still but relaxed, and not to think of anything in particular and not to fall asleep. During the task-related fMRI data collection (432 volumes, lasting about 14.4 min), all subjects were required to make their responses using a fiber-optic response box.

After the task scan, structural images were acquired using a T1-weighted sagittal 3D magnetization-prepared rapid gradient echo (MPRAGE) sequence: TR = 2530 ms; TE = 3.45 ms; FA = 7°; FOV = 256 × 256 mm^2; matrix size = 256 × 256; slices = 176; thickness = 1.0 mm; voxel size = 1 × 1 × 1 mm^3.

2.5. Resting-state fMRI data processing and functional connectivity analysis

Preprocessing and analysis of resting-state fMRI data were implemented using Statistical Parametric Mapping software (SPM12.0, Wellcome Department of Cognitive Neurology, UCL, London, UK; https://www.fil.ion.ucl.ac.uk/spm/). Preprocessing of the resting-state fMRI data included: (1) discarding the first 10 volumes for scanner stabilization to allow the participant to adapt to the scanner; (2) slice timing to correct the acquisition time differences between slices; (3) realigning the images to correct for head movement (any image with more than 2 mm displacement or 2° angular rotation in any direction was excluded); (4) segmenting the T1 image into cerebrospinal fluid (CSF), gray matter and white matter; (5) normalizing to the Montreal Neurological Institute (MNI) space using the transformation parameters estimated from each participant's T1 unified segmentation; (6) resampling to 3 mm isotropic voxels; (7) spatial smoothing with 8 mm full-width at half maximum (FWHM) of the Gaussian smoothing kernel; and (8) removing linear trends using a temporal filter (0.01–0.1 Hz) to reduce low-frequency drifts and high frequency physiological noise (Chen et al., 2019).

In the resting-state fMRI data analysis, we first calculated the functional connectivity between the right DLPFC and the left HF at the resting-state following the previous studies (Esslinger et al., 2009; Rasetti et al., 2011; Zhang et al., 2016). The most significantly activated voxel in the right DLPFC (right BA9 + right BA46, without dilation, defined by the Wake Forest University PickAtlas toolbox; http://fmri.wfubmc.edu/software/PickAtlas) during the memory span task under fMRI scanning for each subject was used as the center to create a 6 mm sphere as the region of interest (ROI). The seed for the next analysis was the activated voxels ($P < 0.05$) within the sphere. Then the time series of the seed was extracted as the first eigenvariate and the functional connectivity of this seed was measured after regressing out the confounding factors of the signals from the mask of the CSF and the white matter, and six head movement parameters (three parameters of the displacement and three parameters of the angular rotation). Next, we used time point (posttest minus pretest) as a predictor to produce brain functional connectivity changes for each subject after training.

2.6. Task-state fMRI data processing and psychophysiological interaction (PPI) analysis

Preprocessing and analysis of task-related fMRI data were conducted using SPM12.0. Preprocessing steps were similar to those used to preprocess resting-state fMRI data, including (1) realignment (subject with > 2 mm translation or 2° rotation would be excluded); (2) segmentation of the T1 image; (3) normalizing to the MNI space using the transformation parameters estimated from each participant's T1 unified segmentation; (4) resampling to voxel size of 3 × 3 × 3 mm; and (5) spatial smoothing with 8 mm FWHM of the Gaussian smoothing kernel.

In this analysis, we focused our analysis on the cue phase of the correct trials which covered cognitive processing of both encoding and maintenance during the working memory task. Contrast (working memory condition minus baseline condition) image for each subject at each time point was produced. We conducted the PPI analysis to investigate the task-modulated functional coupling between the right DLPFC and left HF. The seed was the same as the above resting-state fMRI data analysis. A general linear model was constructed using the following regressors: the time course of signal in the seed (the physiological variable), the task-related predictor (working memory condition minus baseline condition, the psychological variable), and the interaction of the first two regressors (the PPI variable). In addition, white matter and cerebrospinal fluid signals, and six head motion parameters were also controlled as confounding factors. Then we used time point (posttest minus pretest) as a predictor to produce brain PPI changes for each subject after training. In these analyses, a high-pass filter at 128 s was used to remove noise associated with low-frequency confounds.

2.7. Data analysis

In Study I, we first conducted two-sample T tests to investigate whether the right DLPFC-left HF coupling of at pretest was comparable between the two groups. Group was entered as an independent factor, and resting-state functional connectivity or task-state PPI at pretest was entered as the dependent factor. Afterwards, we used two-sample T tests to examine the group differences in the training effect. Group was entered as an independent factor, training-induced changes in coupling (posttest minus pretest) at resting state or task state were entered as the dependent factor.

In Study II, we first conducted multiple regression analyses to test the association between rs1344706 and the right DLPFC-left HF coupling at pretest to replicate previous finding (Cousijn et al., 2015; Esslinger et al., 2011, 2009; Paulus et al., 2013; Rasetti et al., 2011; Zhang et al., 2018, 2016). Genotype (AA vs. AC vs. CC) was entered as an independent factor, resting-state functional connectivity or task-state PPI images at pretest were entered as the dependent factor. Then we used multiple regression analysis to test the genotypic effect on the training-induced coupling changes. Genotype (AA vs. AC vs. CC) was entered as an independent factor, and training-induced coupling changes (posttest minus pretest) at resting state or task state were entered as the dependent factor.

All the above analyses were limited to the left HF (defined by the Wake Forest University PickAtlas toolbox; http://fmri.wfubmc.edu/software/PickAtlas). Significance level was set at $P < 0.05$ after family-wise error (FWE) correction at the peak-level. Significant results were followed up by *post-hoc* analysis using repeated measures ANOVA in SPSS 22.0.

3. Results

3.1. Study I: Randomized controlled study

In the resting-state fMRI data analysis, 8 subjects in the training group and 8 subjects in the control group were excluded due to their excessive head motions (> 2 mm or 2°) at either pre- or posttest. As shown in Table 1, the two groups did not differ significantly in terms of demographic factors, behavioral performance, and functional connectivity between the right DLPFC and the left HF (FWE corrected $P > 0.05$) at pretest.

The two groups differed significantly in training-induced changes in their right DLPFC-left HF coupling (peak voxel MNI coordinate: x = −33, y = −39, z = −9; cluster size = 32, FWE corrected $P = 0.002$) (see Fig. 1A). *Post hoc* analysis showed that the training

Table 1
Demographic variables and behavioral performance across groups in Study I.

	Mean ± SD		F or χ^2	*P*
	Training group	Control group		
Resting-state analysis				
Number of subjects	22	22		
Gender (male/female)	2/20	4/18	0.77	0.383
Age (years)	21.18 ± 2.68	21.82 ± 1.99	0.80	0.377
Education (years)	14.68 ± 1.70	15.45 ± 1.47	2.60	0.115
IQ[a]	129.73 ± 6.28	128.45 ± 5.45	0.52	0.477
Task-state analysis				
Number of subjects	24	24		
Gender (male/female)	3/21	4/20	0.17	0.683
Age (years)	21.54 ± 2.43	21.75 ± 1.89	0.11	0.742
Education (years)	15.08 ± 1.66	15.29 ± 1.30	0.23	0.632
IQ[a]	130.42 ± 5.73	128.67 ± 5.72	1.12	0.295
fMRI task performance at pretest[b]	0.90 ± 0.07	0.87 ± 0.09	1.69	0.200
fMRI task performance at posttest[b]	0.92 ± 0.06	0.88 ± 0.08	0.35	0.068

[a] Full scale IQ, as measured by Wechsler Adult Intelligence Scale.
[b] The accuracy of the fMRI task was used to index the performance.

group showed significantly increased (in the negative direction) functional connectivity between the right DLPFC and the left HF after the training ($F = 27.02$, $P < 0.001$), while the control group showed less change ($F = 4.12$, $P = 0.055$).

We then used PPI analysis on the task-state fMRI data to see if the training could also strengthen task-modulated functional coupling between the right DLPFC and the left HF. For this analysis, 6 subjects in the training group and 6 subjects in the control group were excluded due to their excessive head motions (> 2 mm or 2°) at either pre- or posttest. The two groups were matched in all background information (see Table 1). The two-sample T test on the PPI changes again showed significantly greater increases in functional coupling (in the negative direction) between the right DLPFC and left HF in the training group than in the control group (peak voxel MNI coordinate: $x = -33$, $y = -12$, $z = -18$; cluster size = 46, FWE corrected $P = 0.001$) (see Fig. 1B). *Post hoc* analysis showed that the training group showed significantly increased functional coupling after the training ($F = 23.09$, $P < 0.001$), while the control group showed no significant changes ($F = 0.46$, $P = 0.504$).

3.2. Study II: Genetic association study

No deviation from Hardy-Weinberg Equilibrium (HWE) was found ($P > 0.05$) for the polymorphism.

In the resting-state fMRI data analysis, 84 subjects (AA = 23; AC = 40; CC = 21) were included in the final fMRI analysis (17 subjects were excluded due to their excessive head motions at either pretest or posttest). No significant differences between rs1344706 genotypes were found for demographic factors and behavioral performance at the pretest (*Ps* > 0.05, Table 2). We first conducted a regression analysis on the pretest fMRI data to replicate the association between rs1344706 and the right DLPFC-left HF functional connectivity that has been reported previously (Cousijn et al., 2015; Zhang et al., 2018). Such an association was confirmed (see Fig. 2A, peak voxel MNI coordinate: $x = -18$, $y = -33$, $z = 0$; cluster size = 294, FWE corrected $P < 0.001$). Specifically, the C allele was associated with more negative right DLPFC-left HF functional connectivity. We then conducted a regression analysis on the training-induced functional connectivity changes to further test if rs1344706 was associated with the plasticity of the right DLPFC-left HF functional connectivity. This analysis also showed a significant result (peak voxel MNI coordinate: $x = -21$, $y = -33$, $z = 0$; cluster size = 33, FWE corrected $P = 0.011$) (see

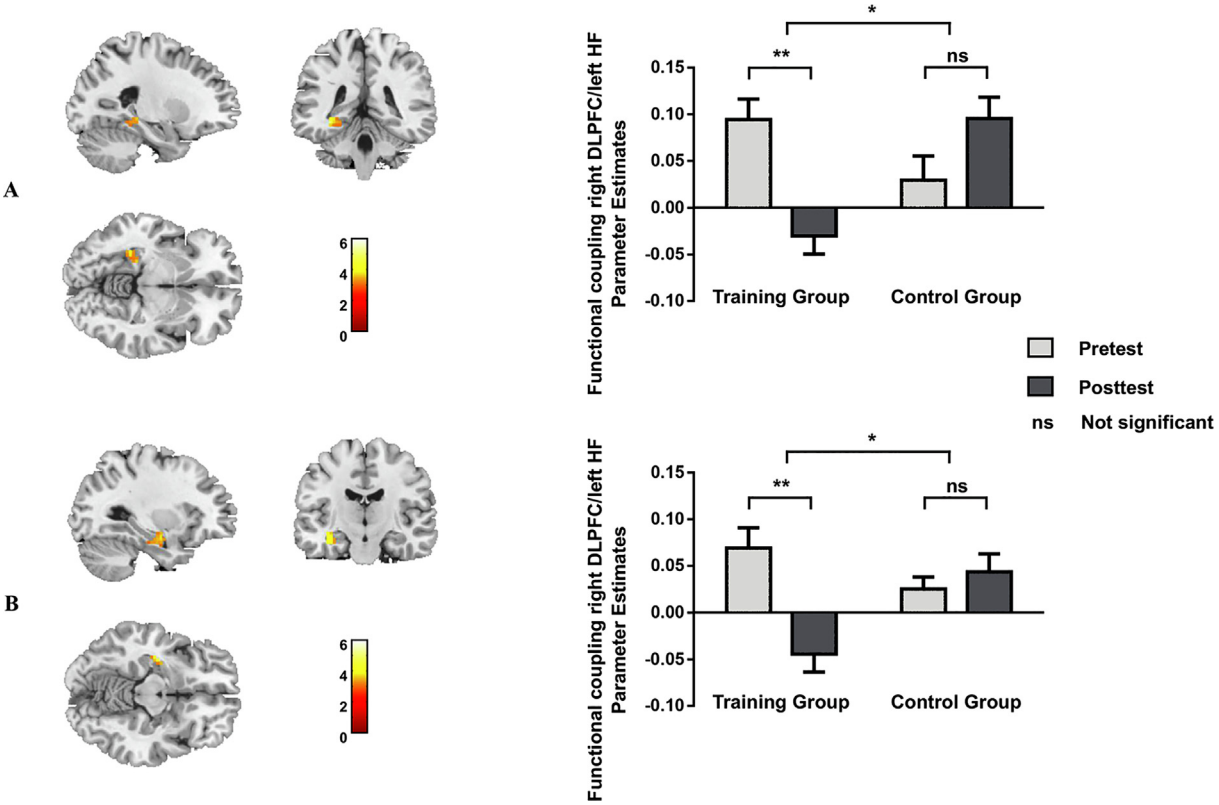

Fig. 1. Significant group differences in the training effects. Functional coupling between the right DLPFC and the left HF increased (in the negative direction) significantly in the training group but did not change significantly in the control group for both the resting state (Panel A) and the working memory task state (Panel B) (FWE corrected $P < 0.05$). NS stands for Not Statistically Significant.

Table 2
Demographic variables and behavioral performance across rs1344706 genotypes in Study II.

	Mean ± SD			F or χ^2	*P*
	AA	AC	CC		
Resting-state analysis					
Number of subjects	23	40	21		
Gender (male/female)	5/18	9/31	5/16	0.03	0.871
Age (years)	21.65 ± 2.71	21.90 ± 2.23	22.67 ± 2.44	1.07	0.349
Education (years)	15.13 ± 2.42	15.70 ± 1.74	16.19 ± 1.94	1.56	0.216
IQ[a]	129.09 ± 5.62	128.10 ± 6.21	131.48 ± 4.85	2.40	0.098
Task- state analysis					
Number of subjects	20	41	24		
Gender (male/female)	4/16	8/33	5/19	0.17	0.992
Age (years)	21.90 ± 2.67	21.83 ± 2.27	22.46 ± 2.36	0.56	0.575
Education (years)	15.30 ± 2.41	15.66 ± 1.74	16.00 ± 1.91	0.70	0.501
IQ[a]	129.45 ± 5.88	128.05 ± 6.22	131.38 ± 4.55	2.58	0.083
fMRI task performance at pretest[b]	0.92 ± 0.05	0.88 ± 0.08	0.91 ± 0.06	2.72	0.072
fMRI task performance at posttest[b]	0.94 ± 0.07	0.92 ± 0.07	0.95 ± 0.04	0.91	0.405
Spatial span at pretest[c]	5.70 ± 0.73	5.20 ± 0.81	5.33 ± 0.92	2.52	0.087
Spatial span at posttest[c]	7.35 ± 0.81	6.93 ± 1.23	7.46 ± 1.69	1.49	0.231

[a] Full scale IQ, as measured by Wechsler Adult Intelligence Scale.
[b] The accuracy of the fMRI task was used to index the performance.
[c] The length of the longest correct span of the spatial span task was used to index the performance.

Fig. 3A). *Post hoc* analysis showed that the functional connectivity increased significantly (in the negative direction) after the training in subjects carrying the C allele (for CC genotype, F = 6.98, $P = 0.015$; for CA genotype, F = 12.91, $P = 0.001$), however, subjects with the AA genotype did not improve much (F = 2.27, $P = 0.147$).

In the task-state fMRI data analysis, 85 subjects (AA = 20; AC = 41; CC = 24) were included in the final analysis (16 subjects were excluded due to their excessive head motions at either pretest or posttest). No significant difference between the three genotypes was found in terms of demographic information and behavioral performance at the pretest (*Ps* > 0.05, Table 2). Our regression analyses on the pretest fMRI data also showed a significant association between rs1344706 and the task-

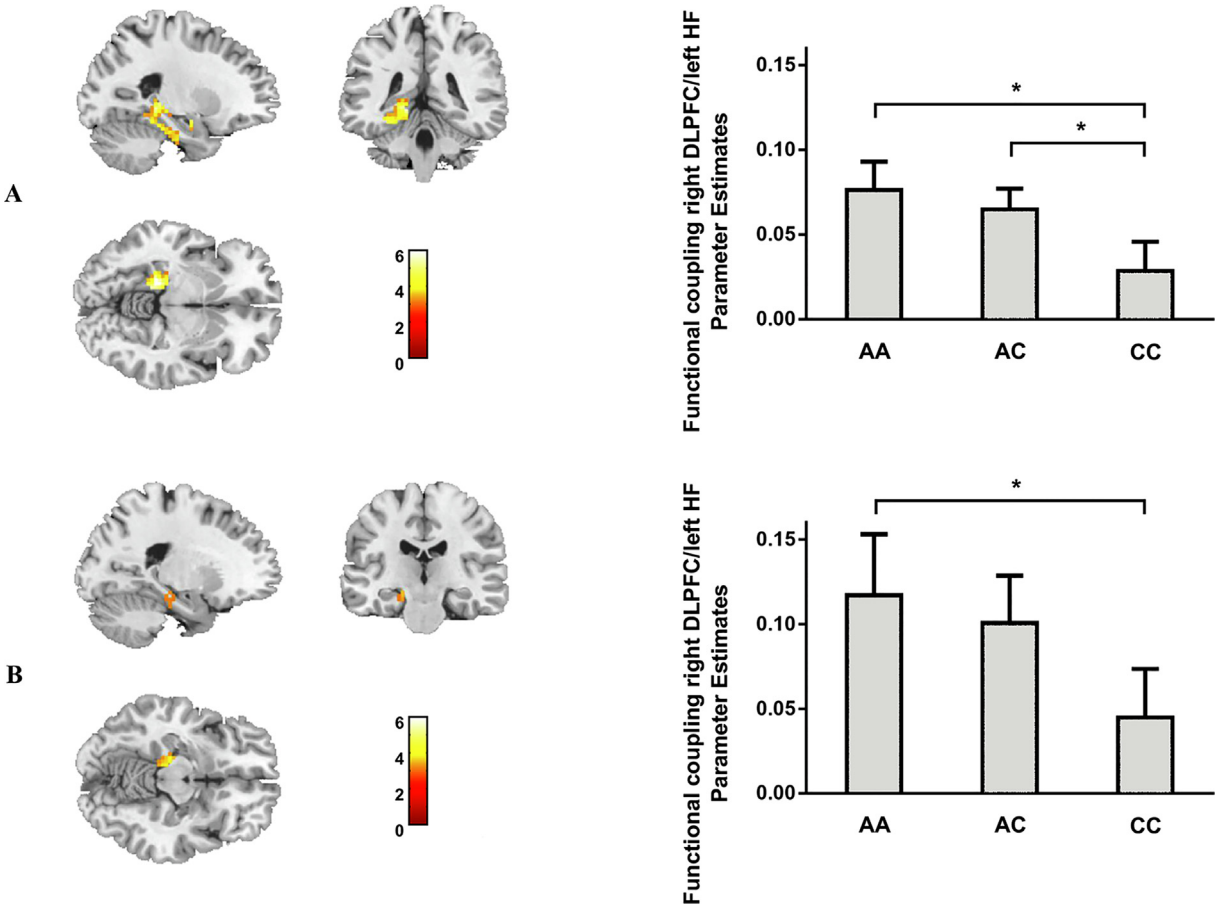

Fig. 2. Significant genotypic effects on right DLPFC-left HF functional coupling at pretest (FWE corrected $P < 0.05$). The C allele was associated with more negative functional coupling for both the resting state (Panel A) and the working memory task state (Panel B).

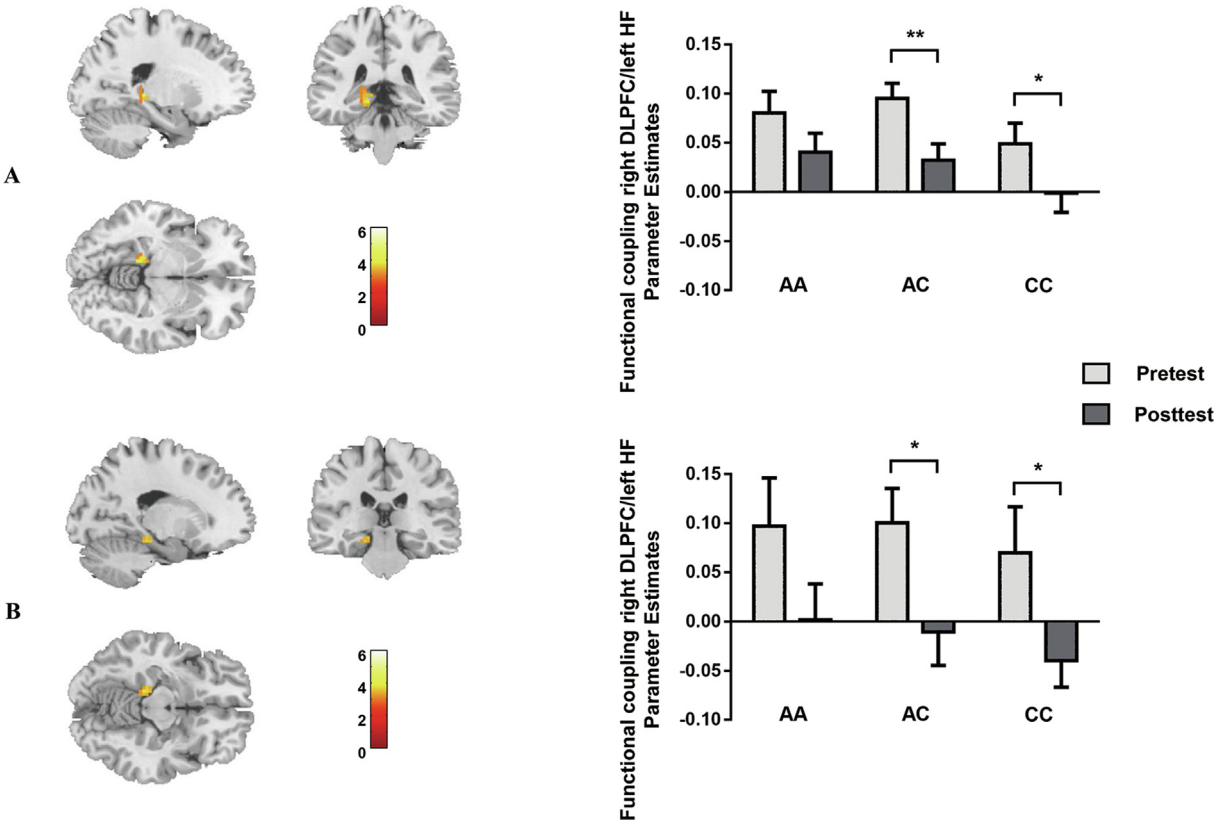

Fig. 3. Significant genotypic effects on the training-induced plasticity of the right DLPFC-left HF functional coupling (FWE corrected $P < 0.05$). The C allele was associated with more plastic changes for both the resting state (Panel A) and the working memory task state (Panel B) (FWE corrected $P < 0.05$).

modulated functional coupling between the right DLPFC and the left HF (see Fig. 2B, peak voxel MNI coordinate: x = −12, y = −30, z = −9; cluster size = 50, FWE corrected $P = 0.023$). Consistent with previous studies (Esslinger et al., 2009; Paulus et al., 2013; Zhang et al., 2016), the C allele was associated with more negative functional coupling between the right DLPFC and the left HF. Our regression analysis further identified a significant cluster within the left HF whose change of PPI with the right DLPFC was associated with rs1344706 (peak voxel MNI coordinate: x = −15, y = −33, z = −15; cluster size = 24, FWE corrected $P = 0.036$) (see Fig. 3B). *Post hoc* analysis showed a similar pattern as the resting-state analysis, with that the functional coupling increased significantly (in the negative direction) after the training in subjects carrying the C allele (for CC genotype, F = 5.10, $P = 0.034$; for CA genotype, F = 6.90, $P = 0.012$), but not for subjects with the AA genotype (F = 1.68, $P = 0.210$).

4. Discussion

To our knowledge, the current fMRI study is the first to provide evidence for the effect of cognitive training on the plasticity of the right DLPFC-left HF functional coupling and the first to report the contribution of *ZNF804A* gene polymorphism (rs1344706) to the plasticity of the same coupling.

In our randomized controlled trial (Study I), the training specifically strengthened the negative functional coupling between the right DLPFC and the left HF. This effect was significant based on data from the resting state and the task state. Our result was also consistent with a previous animal study (Sigurdsson et al., 2010) that found strengthened negative coupling between the prefrontal cortex and the HF as a results of a spatial working memory training. In human studies, the right DLPFC-left HF functional coupling has been commonly used to reflect the coupling between the central executive network and the default mode network, the two important networks for working memory (Buckner et al., 2008; Cieslik et al., 2013; Fox et al., 2005; Greicius et al., 2003). Liu et al. (2014) have further found that right DLPFC-left HF negative coupling was associated with higher working memory performance. Several studies also reported similar results between the prefrontal cortex and the HF (Axmacher et al., 2008; Benchenane et al., 2010; Gordon, 2011; Jones and Wilson, 2005). Consistent with previous studies suggesting that functional connectivities within the frontoparietal central executive network are prone to the effect of working memory training (Jolles et al., 2013; Takeuchi et al., 2013; Thompson et al., 2016), our study provided direct RCT evidence for the effect of cognitive training on the plasticity of the coupling between the two important networks.

Our Study II first confirmed previous research linking a schizophrenia risk gene polymorphism (rs1344706) to the right DLPFC-left HF functional coupling at pretest (based on data from both the resting state and the task state). To our knowledge, there are at least seven studies that have tested the relationship between rs1344706 and the right DLPFC-left HF functional coupling (equivalent to that at pretest in this study) (Cousijn et al., 2015; Esslinger et al., 2011, 2009; Paulus et al., 2013; Rasetti et al., 2011; Zhang et al., 2018, 2016), with five of them (Cousijn et al., 2015; Esslinger et al., 2011, 2009; Paulus et al., 2013; Zhang et al., 2016) reporting a significant association.

More importantly, our Study II used the training-related changes in the right DLPFC-left HF functional coupling as a phenotype to test the functional impact of a schizophrenia risk gene polymorphism (rs1344706). We found significant genotype × time interaction effects based on data from both the resting state and the task state. Schizophrenia genetic risk factor carriers and non-carries were different at their neuroplastic responses to an environmental factor (cognitive training), showing less neuroplasticity for individuals with the risk allele. Our result added to the limited animal literature on the effect of

genetic risk factors for schizophrenia on the plasticity of the right DLPFC-left HF functional coupling (Sigurdsson et al., 2010).

Finally, it needs to be mentioned that the significant HF locations in both Study I and Study II were more posterior in the resting-state functional connectivity analyses than that in the task-state PPI analyses. There are a couple of possible explanations for this pattern of results. First, the functions of the anterior and posterior HF were similar regarding their contributions to working memory based on a most recent meta-analysis (Grady, 2020) on brain activation and functional connectivity. An earlier structural MRI study also found that the anterior and posterior HF were similarly correlated with working memory (Voineskos et al., 2015). Within this current study, the left HF was deactivated along the anterior and posterior axis which covered the varied HF coordinates that were found in our resting-state functional connectivity analyses and task-state PPI analyses. In other words, despite their differences in specific coordinates, all of the significant HF locations were involved in working memory. The second plausible explanation is methodological. The varied HF coordinates may be related to data from different states (resting state vs. task state) and the use of different functional coupling measurements (PPI vs. functional connectivity) (Archer et al., 2018; Di et al., 2020; Rasetti et al., 2011). Future research is needed to investigate potential biophysical mechanisms.

Taken together, our genetic association findings suggested that the functional coupling between the central executive network and the default mode network was impaired and its plasticity lost in schizophrenia risk allele (A) homozygotes. Due to their impaired functional coupling between the two important networks and its lack of plasticity, the risk allele (A) homozygotes could not withstand high cognitive load as non-carriers of the C allele. As a result, any high cognitive load becomes a stressful event that exacerbates the risk of schizophrenic symptoms in the risk allele (A) homozygotes.

5. Conclusion

The current study using a randomized controlled trial found that visual-spatial span working memory training induced plastic changes of the right DLPFC-left HF functional coupling, providing experimental evidence in support of this functional coupling as a neural marker of working memory. Our genetic association study further found that schizophrenia risk variant of *ZNF804A* rs1344706 affected the right DLPFC-left HF functional coupling and its plasticity, in support of this functional coupling as one of the most valuable intermediate phenotypes of schizophrenia (Meyer-Lindenberg and Tost, 2014). Our results suggest a possibility of using the *ZNF804A* gene as a prospective target in future antipsychotics and clinical research.

CRediT authorship contribution statement

Wan Zhao: Methodology, Software, Investigation, Resources, Formal analysis, Data curation, Writing - original draft, Visualization. **Xiongying Chen:** Resources, Software, Investigation, Data curation, Validation. **Qiumei Zhang:** Resources, Investigation, Data curation. **Boqi Du:** Resources, Validation. **Xiaoxiang Deng:** Resources, Validation. **Feng Ji:** Resources, Validation. **Yu-Tao Xiang:** Resources, Validation. **Chuanyue Wang:** Resources, Validation. **Qi Dong:** Conceptualization, Supervision. **Chuansheng Chen:** Writing - review & editing, Validation, Visualization, Supervision. **Jun Li:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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We would like to thank all the volunteers. For the authors' contribution, Jun Li had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Jun Li and Qi Dong designed the study and wrote the protocol. Wan Zhao, Xiongying Chen, and Qiumei Zhang collected the data and managed the literature searches. Wan Zhao and Xiongying Chen completed the DNA extraction and genotyping. Boqi Du, Xiaoxiang Deng, Feng Ji, Chuanyue Wang and Yu-Tao Xiang selected the sample and evaluated them. Chuansheng Chen reviewed and polished the manuscript in language expression and logicality. Wan Zhao undertook the statistical analysis and wrote the first draft of the manuscript.

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Disclosure statement

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nicl.2020.102279.

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