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ARTICLE

Working memory processing deficit associated with a nonlinear response pattern of the anterior cingulate cortex in first-episode and drug-naïve schizophrenia

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Impaired working memory (WM) is a core neuropsychological dysfunction of schizophrenia, however complex interactions among the information storage, information processing and attentional aspects of WM tasks make it difficult to uncover the psychophysiological mechanisms of this deficit. Thirty-six first-episode and drug-naïve schizophrenia and 29 healthy controls (HCs) were enrolled in this study. Here, we modified a WM task to isolate components of WM storage and WM processing, while also varying the difficulty level (load) of the task to study regional differences in load-specific activation using mixed effects models, and its relationship to distributed gene expression. Comparing patients with HCs, we found both attentional deficits and WM deficits, with WM processing being more impaired than WM storage in patients. In patients, but not controls, a linear modulation of brain activation was observed mainly in the frontoparietal and dorsal attention networks. In controls, an inverted U-shaped response pattern was identified in the left anterior cingulate cortex. The vertex of this inverted U-shape was lower in patients than controls, and a left-shifting axis of symmetry was associated with better WM performance in patients. Both the above linear and U-shaped modulation effects were associated with the expressions of the genes enriched in the dopamine neurotransmitter system across all cortical brain regions. These findings indicate that a WM processing deficit is evident in schizophrenia from an early stage before antipsychotic treatment, and associated with a dopamine pathway related aberration in nonlinear response pattern at the cingulate cortex when processing WM load.

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INTRODUCTION

Working memory (WM) deficits are a core neuropsychological feature of schizophrenia [1], and often persist despite alleviation of acute psychotic symptoms with antipsychotic treatment [2]. The WM deficit explains a large proportion of variance in the disrupted activities of daily living in schizophrenia [3]; nevertheless, due to our limited understanding of its neural mechanisms, to date, no effective treatments have been devised to reverse this key deficit [4]. Uncovering the physiological basis of WM deficit may provide new treatment opportunities to improve rates of functional recovery in schizophrenia.

WM is characterized as a buffer for storage and processing of information [5], and its impairment in schizophrenia is particularly pronounced (Cohen's $d > 0.8$) when processing demands (i.e., load) are high [6]. Neuroimaging studies have associated WM with brain regions in both the fronto-striatal and fronto-parietal networks [7]. However, mixed results have been reported with both hyperactivation [8, 9] and hypoactivation [10] of the dorsolateral prefrontal cortex (DLPFC) in schizophrenia, while some studies failed to identify any significant aberrations in DLPFC activation [11]. Similarly inconsistent findings have also been reported in the literature for the anterior cingulate cortex (ACC) [8, 12].

These inconsistences may be due to the diversity of WM task paradigms, engaging various WM components to different extents [13]. The classic Sternberg Item Recognition Paradigm (SIRP) [14] engages only WM storage without processing demands, while n-back paradigms require an inseparable combination of the storage, updating, and attentional components of WM [15]. Here, we varied the difficulty levels for selective attention by applying a prospective cue with the presented stimuli, and the difficulty level peaked when targets and distractors were equally frequent.

The various levels of task difficulty (i.e., WM 'load') elicit different degrees of activation in the relevant brain areas. In HCs, both linear [16] and inverted-U shaped patterns of the DLPFC activation

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[8, 17] have been identified as WM load increases. In schizophrenia, not only the linear pattern [18] but also a flattened [8] or left-shifted inverted-U shaped [19, 20] pattern of DLPFC activation have been reported. Here, we used a modified parametric SIRP [21] to probe the load-sensitive pattern of brain activation while disentangling the storage and the processing components of WM.

The inverted-U pattern has been hypothesized to be associated with dopaminergic neurotransmission in the prefrontal cortex (PFC) based on the dopaminergic drug effects observed in both human and animal experiments [22]. A recent positron emission tomography (PET) study of the amphetamine-induced changes in dopamine in the DLPFC found that dopamine release was blunted in patients compared with controls and the D2/3 availability was associated with DLPFC activity during a WM task only in the pooled sample of patients and controls [23]. However, there is currently no direct evidence linking the distribution of inverted-U pattern of brain activity to dopaminergic neurotransmission in the human brain. We performed a transcriptomic analysis of brain tissues to test if the WM load modulations are indeed associated with the gene expressions for dopaminergic neurotransmission across different brain regions.

Another reason for inconsistent findings with regard to the neural underpinnings of WM deficit in schizophrenia are the confounding effects of long duration of psychosis and antipsychotic exposure in many studies; both of these can profoundly alter brain activations during WM performance [24]. During a WM task, greater activations in both the inferior and the middle frontal gyri have been observed in patients with 10–19 years of schizophrenia compared to patients with 3–9 years of this disorder [25]. Antipsychotics such as risperidone [26] and aripiprazole [27] can alter the activation patterns of several brain regions including the ACC during WM tasks. Therefore, we specifically recruited patients with first-episode and drug-naïve schizophrenia (FEDN).

Our major aim was to test the hypotheses that the load-dependent pattern of linear or inverted U-shaped brain activation during WM performance in healthy controls (HCs) is altered in the FEDN, and this load-dependent pattern is associated with dopamine system enriched gene expressions across different brain regions. We recruited 36 FEDN and 29 HCs matched for age, sex and years of education. Brain activations for the WM processing were isolated from the WM storage by our task design, while activation patterns that related to WM load were compared between patients and controls.

METHODS

Participants

In this study, 36 first-episode and drug-naïve patients with schizophrenia (SZ: FEDN) were recruited from the psychological counseling clinic at the Second Xiangya Hospital Affiliated to the Central South University, and 29 HCs matched with mean age, sex, years of education were recruited from the local community and the university. For patients, the clinical information was collected by an experienced psychiatrist at the hospital, including the Positive and Negative Syndrome Scale (PANSS) scores. All participants completed an WM task in the MRI scanner and another test for complex attention and executive function outside the scanner after the MRI experiment, namely the Trail Making Test (TMT)-A and B respectively [28]. The detailed inclusion and exclusion criteria are provided in Supplementary Method S1.

The study protocol was approved by the Second Xiangya Hospital Ethics Committee. All participants provided written informed consent after information on the research procedures had been provided by the study team.

Experimental paradigm

The experimental paradigm for working memory (WM) in this study was a modified version of a parameterized, event-driven SIRP paradigm [21], which consisted of 5 types of trials and each type had 8 trials (Fig. 1). The event-design of this paradigm enabled us to separately analyze correct trials only, thus minimizing any bias arising from the difference in task performances between patients and controls [21]. In the stimulus period, a black screen displayed four gray digits for 1500 ms. At the end of this period, 1, 2, 3 or 4 digits were illuminated for 500 ms in trials at load levels 1, 2, 3 and 4, respectively. During the subsequent 4000 ms delay period, participants were instructed to focus only on those illuminated digits (i.e., the cued set) and to memorize the digits which immediately followed them in the natural sequence of numbers (the manipulated set; e.g., for the illuminated digits 1,5,3 in Fig. 1, the correct responses would be 2,6,4). In the probe period, an illuminated digit was presented at the center of screen, and participants had to decide whether this digit was ('Yes') or was not ('No') part of the manipulated set using the left or right hand, respectively. The proportion of Yes or No answers was 1:1 and was counter-balanced by hand. While the WM 'load' (for both WM storage and WM processing) increased with the size of the manipulated set, the difficulty level of attentional processing peaked when the cued (i.e., illuminated) set and the distractor (i.e., non-illuminated) set had the same number of digits. On control trials, 4 gray digits appeared on the screen during the stimulus period and none of them was illuminated. Participants were instructed to memorize all 4 digits (i.e., the storage set) over the delay period and to determine whether the illuminated digit shown during the probe period was or was not part of the storage set. Trials were counter-balanced by their types.

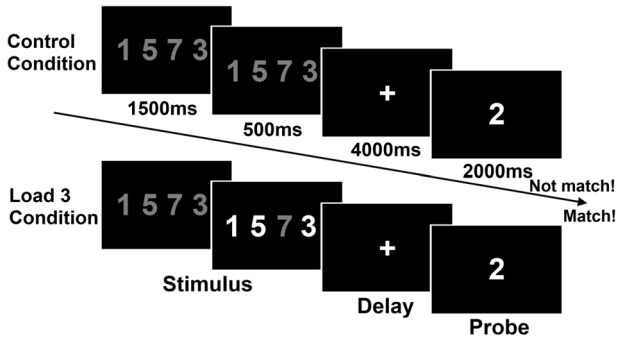

Fig. 1 Working memory task design. The upper row for a control trial: in the control trials displayed 4 gray digits and required participants to memorize these digits (i.e., the storage set). In the probe period, an illuminated digit was presented on the screen and the participants had to indicate whether this digit was or was not part of the storage set. The lower row for a trial at load level 3: In the stimulus period, 4 gray digits appeared on a black screen. In the trial at load level 3, 3 of the 4 gray digits were illuminated. During the subsequent delay period, participants were instructed to focus only on those digits which had been illuminated and to memorize the digits which immediately followed them in the natural sequence of numbers (i.e., the manipulated set; e.g., for the illuminated digits 1,5,3 the correct responses would be 2,6,4.). In the probe period, an illuminated digit was presented at the center of screen, and participants should decide whether this digit was ('Yes') or was not ('No') part of the manipulated set. The load levels varied among 1, 2, 3 and 4 in this task.

Data acquisition

Neuroimaging data were collected from all participants using a 3.0 T MRI system (Intera Achieva, Phillips, Netherland). The scan of T1-weighted images covered the whole brain with 36 slices on the axial position and aligned on the AC-PC were acquired with the following parameters: repetition time (TR) = 500 ms, echo time (TE) = 11 m, field of view (FOV) = 220 mm × 220 mm, matrix = 512 × 512; slice thickness = 4 mm, without gap, and flip angle (FA) = 8°. The functional images were acquired using the field echo-echo planar (EPI) imaging sequence during the execution of the tasks with the following parameters: TR = 2000ms, TE = 30 ms, FOV = 240 mm × 240 mm, matrix = 64 × 64, slice thickness = 4 mm without gap, in-plane resolution = 3.75 mm × 3.75 mm, FA = 90° and 36 axial slices.

Data preprocessing

The preprocessing of fMRI images was completed using the fMRIPrep 20.2.3 ([29] RRID:SCR_016216) based on Nipype 1.6.1 ([30, 31]; RRID:SCR_002502) with the first 6 fMRI scans removed. Processing procedure included skull-stripping, head-motion correction with six corresponding rotation and translation parameters were estimated, slice timing correction, co-registering to T1w with the boundary-based registration [32] cost-function,

Fieldmap-less susceptibility distortion correction and normalizing to standard spaces for generating a preprocessed BOLD run in MNI152NLin2009cAsym space (Supplementary Methods S2). Participants whose mean frame-wise displacement (FD) exceeded 0.5 mm were removed from the further analysis. The preprocessed images were resampled to $3 \times 3 \times 3$ mm^3 isotropic voxel and spatially smoothed with 6 mm full-width at half maximum Gaussian kernel. Head-motion parameters were used as covariates of no interest for further statistical analyses.

Quality control. (1) Participants whose accuracies in the control trials failed to achieve 50% were excluded from the following analyses. In total, 4 patients were excluded here. (2) Due to a software malfunctioning, 2 HCs were excluded for missing the records of the correct trials. 3) Two patients and 1 HC were further excluded due to excessive head movement (i.e., meanFD > 0.5 mm). Finally, 30 FEDN patients and 26 HC entered the following analyses in this study.

Statistical analysis

Demographic analysis. Both two-sample t-tests and variance analyses were used where appropriate for group comparisons. Covariates, including the age, sex and years of education, were controlled for when comparing task performances. The group comparisons were also verified by involving IQ as additional covariate. The false discovery rate (FDR; $q < 0.05$) was used for the correction of multiple comparisons.

Neuroimaging analysis

Brain activation: Brain activation was estimated by the general linear model with default settings in the SPM12 (http://www.fil.ion.ucl.ac.uk/spm). The WM contrast map was defined by comparing the delay stages between the trials at each load level and the control trials, for which only correct trials were included. Using the load level 4 contrast, we subtracted the contribution of WM storage (i.e., the control trials) from a combination of WM storage and processing (i.e., the trials at load level 4). Using the load level 1, 2 and 3 contrasts, the contribution of WM storage was subtracted from a combination of WM storage, processing and selective attention (i.e., the trials at load level 1, 2, 3). A linear regression model was used to identify changes in brain activation between FEDN and HC while controlling for the age, sex, years of education and mean FD. Following the literature [33], significant clusters were detected when both FDR < 0.05 and cluster size >10 voxels in this analysis and all the following analyses.

Linear modulation: A linear mixed-effect model was used to assess the significant modulation effect of the WM load on the brain activation across the four WM loads. The fixed effects included the WM load, age, sex, years of education and the mean FD, while the random effects included the subject ID. Mathematically,

$$Activation \sim C_{load} \times Load + Age + Sex + Education + meanFD + (1|subject).$$

where the linear modulation was estimated by the coefficient C_{load}.

U-shaped modulation: The significant U-shaped modulation of WM loads on brain activations was identified by a 2nd-order polynomial mixed-effect model for each voxel:

$$Activation \sim Load^2 + Age + Sex + Education + \mathrm{mean}FD + (1|SubjectID).$$

For each significant cluster, let O be the 4 activation values for the 4 WM load levels among participants, then the U-shape was characterized by

$$O \sim \mu \times (load - \alpha)^2 + \gamma.$$

where α and γ were the axis of symmetry and the vertex of the parabola, respectively.

Associations with behavior. Associations with neuroimaging-derived variables were assessed for both task performance (i.e., task accuracy in each WM load level and TMT response time) and symptom scores (i.e., PANSS) while controlling for age, sex, and education. Three groups of neuroimaging-derived variables were considered: 1) Brain activations of significant clusters as identified by the WM contrast map; 2) The linear modulation effect of WM loads (i.e., C_{load}) on brain activations for each significant cluster; 3) The U-shape parameters (i.e., α and γ) of the quadratic modulation effect of WM loads on brain activation for each significant cluster. Significance was confirmed by FDR correction.

Transcriptome and gene enrichment analysis. Gene expression data of the brain obtained from the Allen Human Brain Atlas (AHBA, http://www.brain-map.org) were applied in the transcriptomic analysis. Reannotation, data filtering, probe selection and normalization were included in the AHBA preprocessing procedures. Partial least square (PLS) regression was conducted. The significant PLS components were identified by 5000 permutations ($p < 0.05$) and were transformed to Z statistics using 5000 bootstraps. Gene enrichment analysis involving GO and Kyoto Encyclopedia of Genes and Genomes pathways was then conducted to identify the expressions of the genes that were correlated with the modulation effects of WM load on brain activations. More information can be found in Supplementary Method S3.

RESULTS

Demographics

Key demographic and clinical characteristics of the 30 FEDN patients (of whom 13 were females [43.33%] with a mean (SD) age of 20.43 (4.56) years) and 26 HC (of whom 14 were females [53.85%] with a mean (SD) age of 22.08 (2.64) years) are reported in Table 1. Lower IQ was observed in patients compared with controls ($t_{46} = -2.61$, $p = 0.0120$). Age, sex, years of education and mean FD were not different between patients and controls.

Working memory deficit in patients

Compared to control subjects, patients were impaired in both WM storage and WM processing, with deficits in processing being more pronounced than storage deficits, as indicated by lower accuracies during the control trials (Standardized Mean Difference, $SMD = -0.53, F_{1,46} = 4.55, p = 0.0386$;uncorrected) and the trials at load level 4 ($SMD = -1.05, F_{1,46} = 14.99, p = 0.0004$; FDR corrected; Table 1; Supplementary Table S9; Supplementary Fig. S2). When adjusted for control trials, the accuracy at load level 4 remained significantly lower in patients compared with controls ($F_{1,45} = 10.41, p = 0.0024$; FDR corrected). However, the significant association between the response time in TMT-A and the accuracy in the trials at load level 4 in HCs ($r = -0.76, p = 0.0006, n = 19$; FDR corrected; Supplementary Fig. S3) was disrupted in patients ($r = -0.24, p = 0.2395, n = 28; z = 2.35, p = 0.0188$). After considering IQ as an additional covariate in the group comparisons, the above findings of impaired WM performance (i.e., accuracy in the load level 4, $F_{1,40} = 6.34$, $p = 0.0161$) and the reduced WM-TMT-A association ($z = 2.97$, $p = 0.003$) in patients remained significant. Among patients, we found no significant association between symptoms burden and WM task accuracy, indicating that this deficit was not related to symptom severity.

Task-dependent brain activation

Among all participants, we found 3 activated clusters in the WM contrast map as defined in the Methods, including 2 left frontal clusters covering the supplementary motor area and precentral gyrus and a superior parietal cluster (Supplementary Table S10, Supplementary Fig. S4). We also found deactivations in 16 clusters located in the frontoparietal area, the temporal cortex and the occipital cortex (Supplementary Table S10; Supplementary Fig. S4). Among these significant clusters after FDR correction, the PANSS-positive score was associated with mean activations of two clusters in the left fusiform gyrus ($r = 0.63, p = 0.0006, n = 29$; FDR corrected; Supplementary Fig. S5a) and the left angular gyrus ($r = 0.63, p = 0.0005, n = 29$; FDR corrected; Supplementary Fig. S5b).

Among patients, we found 4 activation clusters, including 2 left frontal clusters covering the superior frontal cortex and precentral gyrus as well as 2 left parietal clusters covering the superior and inferior parietal cortices (Supplementary Table S10; Supplementary Fig. S6). We also detected 3 deactivated clusters located in the right middle temporal cortex, left middle occipital gyrus and fusiform gyrus (Supplementary Table S10; Supplementary Fig. S6) among patients. However, no

Table 1. The demographics of all participants.

	SZ patients (Mean ± SD)	HC (Mean ± SD)	T/χ^2/F	*P* value
Subjects (Female)	30(13)	26 (14)	0.617	0.4320
Age	20.43 ± 4.56	22.08 ± 2.64	−1.62	0.1117
Education (year)	11.63 ± 1.47	12.31 ± 1.09	−1.92	0.0598
Mean FD	0.16 ± 0.08	0.19 ± 0.11	−1.15	0.2539
IQ	113.62 ± 13.58	122.73 ± 9.87	−2.61	0.0120
PANSS				
Total	79.07 ± 10.64	/	/	/
Positive	21.86 ± 5.47	/	/	/
Negative	16.10 ± 6.69	/	/	/
General	41.10 ± 7.52	/	/	/
WM task performance[b]				
Load 0 accuracy	87.65 ± 12.80	93.25 ± 7.38	4.55	0.0386[*b]
Load 4 accuracy	88.70 ± 9.65	96.63 ± 3.85	14.99	0.0004[***a, b]
TMT task performance				
TMT-A time	36.41 ± 16.36	30.82 ± 11.09	1.53	0.2224[b]
TMT-B time	78.72 ± 35.47	57.62 ± 17.50	6.18	0.0166[*a, b]

FD frame-wise displacement, *PANSS* Positive and Negative Syndrome Scale, *WM* working memory.
*$p < 0.05$; ***$p < 0.001$.
[a]*p* value survived FDR correction.
[b]Comparison controlled for age, sex, and years of education.

Table 2. Significant clusters linearly modulated by the memory load during the working memory task in patients.

Role	Cluster ID	Brain region	BA	Cluster size	Peak (MNI)			
					X	Y	Z	Peak T
Activation >0	1	L_SPC, L_IPC	7	692	−27	−55	42	7.39
	2	L_Postcentral, L_Precentral	6	505	−51	−10	48	6.19
	3	L(R)_SMA	6	164	−3	3	63	4.88
	4	R_SPC	7	68	31	−70	48	4.80
	5	L_Lingual	18	16	−15	−64	−13	4.51
	6	L_ITC	37	20	−51	−52	−10	4.42
	7	R_Precentral	6	13	58	−4	48	4.20
	8	L_MFC	46	27	−27	39	18	4.04
	9	R_IPC, R_Postcentral	2	38	49	−34	57	3.83
	10	R_Postcentral	3	13	49	−25	42	3.78
Activation <0	11	R_MTP, R_MTC	21	43	55	9	−22	−4.99
	12	R_SupraMarginal	41	40	64	−40	36	−4.91
	13	L_MOG	18	47	−27	−91	3	−4.19
	14	R_IOC	18	28	31	−97	−4	−4.51
	15	L_MTC	14	39	58	−67	24	−3.93
	16	L_ STP	38	11	−39	15	−19	−3.9

SPC superior parietal cortex, *IPC* inferior parietal cortex, *SMA* Supplementary motor area, *ITC* inferior temporal cortex, *MFC* middle frontal cortex, *MTP* middle temporal pole, *MTC* middle temporal cortex, *MOG* middle occipital gyrus, *IOC* inferior occipital cortex, *STP* superior temporal pole.

significant deactivation cluster was detected among HC. There were no significant group differences in brain activation between patients and controls.

Linear modulation effect of WM load on brain activation observed in patients

We found significant modulation effects of the WM load on brain activations of 16 clusters located in the frontoparietal network, dorsal attention network, and the temporal cortex and the occipital cortex among patients only (Table 2; Supplementary Fig. S7). However, no significant associations between these linear modulation effects and either symptom scores or task performances were found after FDR correction. No significant group difference in the linear modulation was identified between HCs and patients.

Inverted U-shaped modulation of brain activation observed in controls

The lack of significant linear modulation effect of the WM load on brain activations in HC might be due to the well-known inverted

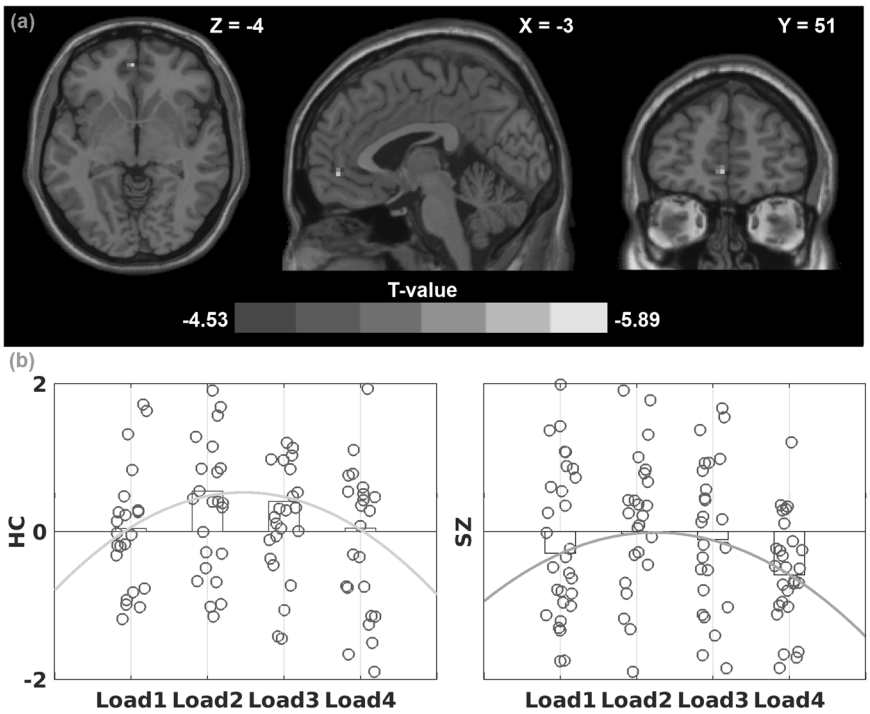

Fig. 2 Comparison of the inverted U-shaped modulation effect of working memory load on brain activation. a A significant cluster in the anterior cingulate cortex (ACC) modulated by the working memory load following an inverted U-shape in healthy controls (HC); **b** Inverted U-shapes fitted for the ACC activations against 4 loads in both HC (left) and patients (right) during the working memory task.

U-shaped modulation effect discussed earlier and reported elsewhere [8, 34]. Indeed, we found a significant cluster showing an inverted U-shaped modulation effect after FDR correction among HC, which was located in the left ACC (10 voxels; $T = -5.88$ at the peak voxel $[-3, 51, -4]$ Fig. 2).

Individual U-shaped function associated with WM performance in patients

Compared to the HCs, FEDN patients showed lowered vertex (i.e., γ; $F_{1,53} = 8.7, p = 0.0048$; FDR corrected), indicating an ineffective activation of this brain region in patients during the task. After including IQ as an additional covariate, the finding of the lowered vertex of the inverted U-shaped ACC activation in patients remained significant ($F_{1,44} = 7.04$, $p = 0.0112$). In patients, the greater axis of symmetry (i.e., α) was associated with worse load 4 accuracy performance ($r = -0.46, 95\%CI, -0.09 to -0.66, p = 0.0193, n = 28$; Fig. 3), indicating an earlier peak of engagement of this brain area at a lower level of task difficulty. No symptom association was significant for these U-shaped parameters.

Dopamine related biological processes associated with the modulation effects

For both the linear modulation effect in patients and U-shaped modulation effect in HCs, the first PLS components (PLS1) explained the maximum percentage of variance of the modulation effects (Supplementary Results S1). The robustness of the gene weights in PLS1 was confirmed by the leave-one-donor-out procedure. We found that a dopamine related GO term for biological process was enriched in the positive gene set associated with the linear modulation effect, i.e., the "dopamine receptor signaling pathway" (GO:0007212; Supplementary Tables S1–2). This GO term was also enriched in the positive gene set for the U-shaped modulation effect on the brain activations among HCs (Supplementary Tables S3–4). These enrichment findings remained significant when only the genes ranked in the top 1% were included in the positive gene sets (Supplementary Tables S5 and S7).

DISCUSSION

To our knowledge, this is one of the first studies of the nonlinear brain responses to a WM task in FEDN patients with schizophrenia. We found a significant disruption in the neural underpinnings of WM processing in the FEDN patients with a reduced prominence of the inverted U-shaped functional hemodynamic response pattern of the left ACC to various levels of the task difficulty. The use of FEDN patients precluded possible confounding effects of duration of illness and antipsychotic exposure. Among the FEDN patients, earlier engagement (i.e., the further left-shifted axis of symmetry in the U-shape activation) was associated with better accuracy in trials at load level 4 during the WM task. These findings in the FEDN sample highlight that the deficits in WM processing (i) occur early in the illness (FE), (ii) precede antipsychotic exposure (DN), (iii) occur regardless of symptom severity (lack of correlation with PANSS), and (iv) relate to insufficient or inefficient recruitment as necessitated by task demands (lowered vertex at median load of inverted U).

Our findings provide new evidence concerning the cognitive nature of WM deficits in FEDN patients with schizophrenia. Previous meta-analysis of cognitive performance in drug-naïve patients with schizophrenia found that the WM deficit was a large effect ($SMD = -0.97$) [35], comparable to that identified in first-episode patients receiving antipsychotic treatment ($SMD = -0.79$) [36]. In the current FEDN sample, we found a larger effect size for WM processing ($SMD = -1.05$) but a smaller effect size for WM storage ($SMD = -0.53$), with the processing deficit remaining significant even after controlling for the inter-individual variations in WM storage capacity. Compared with WM storage, WM processing is either likely to engage similar brain areas to a higher extent, or to activate more prefrontal cortical regions, or both of these [37]. Therefore, these findings in the FEDN sample indicate that the WM deficit in schizophrenia likely reflects the etiopathology of this disorder and establishing its early neural correlates may help to illuminate the mechanisms underpinning the psychotic symptoms as well as the cognitive deficits of schizophrenia.

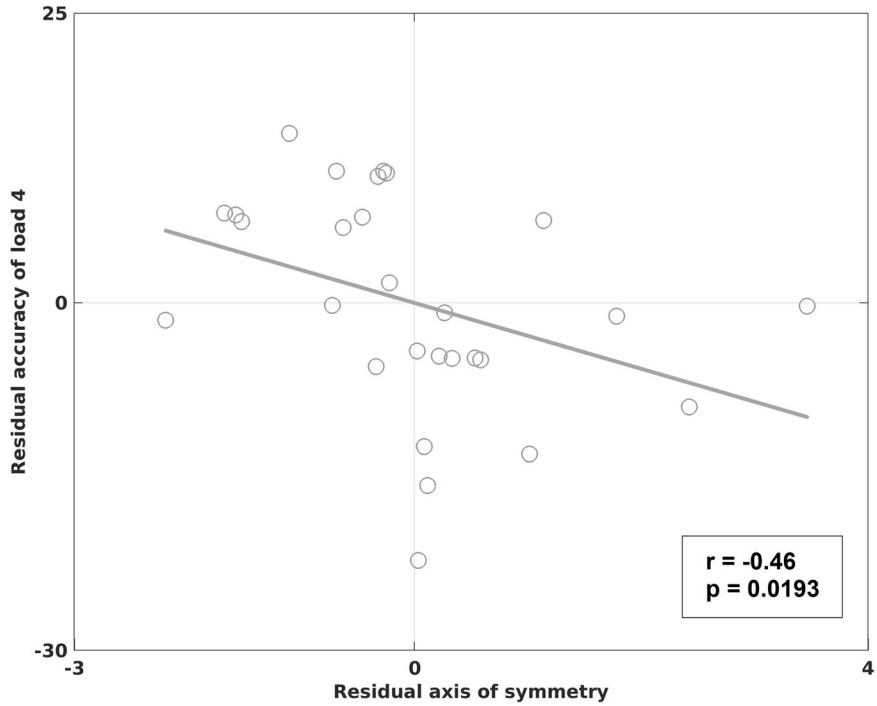

Fig. 3 Association between the inverted U-shape parameter and working memory performance. Performance was measured by accuracy in the trials at load level 4. U-shape was characterized by the axis of symmetry. These measures were first regressed against the covariates age, sex and education before plotting the residuals.

Notably, our finding of a linear modulation of the parietal cortex by WM load emphasizes executive attentional aspects of WM in schizophrenia. WM capacity is often considered to be limited [38], and it is essential to focus on certain aspects of the environment or certain neural representations in the memory buffer for superior performance in WM tasks [13, 37]. In the literature, WM performance has been correlated with the ability to control attention [39]. In the current sample, we found that WM performance (i.e., load-4 accuracy) was significantly correlated with attention (i.e., TMT-A) but not with executive function (i.e., TMT-B). Compared with HCs, this WM-attention correlation significantly decreased and became nonsignificant in patients. These results indicate a significant contribution of attentional impairment to the WM deficit in schizophrenia. Our neuroimaging findings of both the superior and the inferior parietal cortexes during WM in patients might provide neural correlates for this contribution. The involvement of both the dorsal and the ventral attention networks in WM, especially the parietal cortex for its selective attentional control during WM tasks, has long been reported in the literature [40–42]. However, antipsychotics have been reported to affect both WM [43] and selective attention [44]. Hence, the current finding provides new evidence emphasizing selective attention deficits associated with the parietal cortex in FEDN patients with schizophrenia during WM tasks.

Our WM task revealed a nonlinear response pattern within the rostral ACC (rACC) to varying difficulty levels of the attentional processing during the WM task. Structural connectivity analyses have shown that the rACC is a connection hub linking areas concerned with attention and motivation in both monkeys and humans, as it is anatomically located between the subgenual ACC and the dorsal ACC [45]. Here, our working memory task required selective attention to the illuminated digits with the gray digits as distractors. The inverted U-shaped activation of the rACC cluster suggested that selective attention requirements peaked when 2 digits were illuminated and 2 digits were gray. This nonlinear response may also reflect the well-known inverted-U shaped dopamine actions on the selective attention component of WM [22], given dopaminergic projections from the ventral tegmental area to the ACC [46]. Indeed, we found that the dopamine receptor signaling pathway was enriched in the genes with expressions correlated with WM load modulation effects across the cortical brain regions. Particularly, the Anaplastic Lymphoma Kinase gene (ALK, $Z = 12.55$) which enhances dopamine receptor D2 (DRD2) desensitization [47] was less expressed in the brain regions with stronger U-shaped modulations (e.g., rACC; $r = -0.14, p<0.001$; Supplementary Fig. S8) compared to brain regions with weaker U-shaped modulation in HCs (Supplementary Table S7). Taken together, these findings indicate that the dopaminergic neurotransmission system is associated with the pattern of load-dependent brain activation during the WM task, possibly linked with a putative role for cortical dopamine in cognitive effort [48]. Note in our experiment, the inverted U-shaped activation of the DLPFC was not significant; this may be due to the WM load in current task still being within the hypothesized limit of WM capacity (e.g., the magic number 4 in short-term memory [38]). It is notable that most demonstrations of the inverted-U shaped function in DLPFC have involved use of the n-back paradigm which requires active generation of items from working memory over time and with interpolated distraction, whereas the present SIRP task requires only 2-choice recognition. This delayed matching task requirement was also employed in another study apparently failing to show inverted-U shaped DLPFC activations [11]. Hence, the recruitment of DLPFC probably depends on precise working memory task requirements.

Disruptions of this inverted U-shaped activation of the rACC cluster in patients thus provide neural correlates of the WM deficit in schizophrenia. A previous n-back WM study in schizophrenia under stable antipsychotic medication had reported that the activity of the ACC increased from 0-back to 2-back and then decreased at 3-back [49]. Here, we found that in the FEDN patients, this inverted U-shape had a lowered vertex when compared with HCs. Possible interpretations of these findings

suggest a role for the anterior cingulate in exerting cognitive effort to resolve conflict resulting from ambiguity (e.g., in distraction conditions) [50]. Furthermore, those patients with left-shifted inverted U-shaped functions achieved better WM performance. These findings are supported in the literature from various imaging modalities with different analytic approaches. Changes in this cognitive control system including the ACC have been associated with schizophrenia in two recent meta-analyses in terms of gray matter volume and functional connectivity, respectively [51, 52]. The reduction of functional connectivity between the ACC in the ventral attention network and the anterior temporal lobe in the default mode network in schizophrenia is a disorder-specific dysconnectivity, since it was found to be intact in both bipolar disorder and depression [53]. Through effective connectivity analyses, the dysregulation of the attentional control aspect of WM in schizophrenia might be due to reduced communication between the default mode network and the fronto-parietal network when compared with HCs [54]. The reduced communication between these two networks might be disrupted during working memory tasks by aberrant salience signals from the attention network [55].

Several limitations of the current study should be mentioned. Sample size was limited, though we applied FDR correction to each analysis, with primary hypotheses tested with sufficient power. Some of our secondary analyses failed to reject the null hypotheses (e.g., activation/deactivation group differences, as well as symptom relationships with U-shape parameters), studies with a larger sample are needed to verify these findings. The current study excluded patients having a history of alcohol or drug abuse, but did not collect data on tobacco use. Given the possible effect of smoking on cognition [56], future studies are needed to test whether our findings would be affected by the tobacco use.

CONCLUSION

In a first-episode and drug-naïve cohort of patients with schizophrenia, deficits in WM processing (rather than WM storage capacity) are already present at early stages of this disorder and are linked partly to attentional impairment. Our WM task further discovered an inefficient engagement of an inverted U-shaped response pattern in the left ACC and its association with dopamine expression pathways and WM processing deficits in schizophrenia.

DATA AVAILABILITY

The data of this study are available within the limits set by ethically approval, upon reasonable request to the corresponding authors.

CODE AVAILABILITY

The Matlab and R codes of this study are available at https://github.com/Fengnanahub/task-fMR-processing-Code-for-manuscript.git.

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AUTHOR CONTRIBUTIONS

QL and XW made substantial contributions to the conception or design of the work; NF, LP, TR, LC, SF, XL, XW, and QL contributed substantially to the acquisition, analysis or interpretation of data for the work; NF, LP, TR, XW, QL contributed to the drafting the work or revising it critically for important intellectual content; All authors gave the final approval of the version to be published. All authors made agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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