Neurological Soft Signs Are Associated With Altered Cerebellar-Cerebral Functional Connectivity in Schizophrenia

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Cerebellar dysfunction is associated with neurological soft signs (NSS), which is a promising endophenotype for schizophrenia spectrum disorders. However, the relationship between cerebellar-cerebral resting-state functional connectivity (rsFC) and NSS is largely unexplored. Moreover, both NSS and cerebellar-cerebral rsFC have been found to be correlated with negative symptoms of schizophrenia. Here, we investigated the correlations between NSS and cerebellar-cerebral rsFC, explored their relationship with negative symptoms in a main dataset, and validated the significant findings in a replication dataset. Both datasets comprised schizophrenia patients and healthy controls. In schizophrenia patients, we found positive correlations between NSS and rsFC of the cerebellum with the inferior frontal gyrus and the precuneus, and negative correlations between NSS and rsFC of the cerebellum with the inferior temporal gyrus. In healthy controls, NSS scores were positively correlated with rsFC of the cerebellum with the superior frontal gyrus and negatively correlated with rsFC between the cerebellum and the middle occipital gyrus. Cerebellar-prefrontal rsFC was also positively correlated with negative symptoms in schizophrenia patients. These findings were validated in the replication dataset. Our results suggest that the uncoupling of rsFC between the cerebellum and the cerebral cortex may underlie the expression of NSS in schizophrenia. NSS-related cerebellar-prefrontal rsFC may be a potential neural pathway for possible neural modulation to alleviate negative symptoms.

Key words: neurological soft signs/schizophrenia/cerebellar-cerebral connectivity/functional connectivity

Introduction

Neurological soft signs (NSS), originally defined as minor non-localizable neurological impairments, are characterized by deficits in sensory integration, motor coordination, and disinhibition.[1,2] Accumulating evidence[3–8] suggests that NSS may be one of the promising endophenotypes for schizophrenia spectrum disorders,[3,9] which can bridge the gap between heterogeneous phenotypes (clinical symptoms and behaviors) and complex genotypes.[3,10,11] With the advent of neuroimaging techniques, investigating the neural underpinnings of NSS may help to ascertain the etiology of schizophrenia.

Resting-state functional magnetic resonance imaging (rsfMRI) is an important tool to understand the intrinsic organization of spontaneous signal fluctuations or coupling among distributed brain networks, which can be measured by resting-state functional connectivity (rsFC).[12] Dysfunction of the cerebellum has been proposed to play a role in the pathophysiology of schizophrenia.[13] More recently, evidence suggests that the cerebellum is a critical node in multiple functional networks,[14,15] and changes in cerebellar-cerebral rsFC in patients with schizophrenia have also been reported.[16–18] Although previous studies have suggested that cerebellar-cerebral rsFC in schizophrenia is associated with NSS,[19,20]

few empirical studies have directly examined such relationship. To the best of our knowledge, only two previous studies have utilized rsfMRI to investigate the relationship between NSS and neural correlates in schizophrenia. Galindo et al[21] found that NSS were correlated with altered functional connectivity (FC) in the default mode network in patients with schizophrenia, while Hirjak et al[22] found that NSS were correlated with alterations of the frontocerebellar and frontoparietal networks in patients with schizophrenia. However, these two studies used the independent component analysis (ICA), which is a data-driven method and is different from the whole-brain seed-based FC approach. The latter rather than the former method could test a-priori hypothesis involving specific regions of interest (ROIs).

Since NSS are related to sensorimotor dysfunctions[1,8] and general cognitive impairment in schizophrenia,[23–26] we constructed cerebellar-cerebral rsFC by focusing on the sensorimotor and executive control networks, using cerebellar ROIs which has been found to be involved in the respective functional networks.[14,15,27,28] Specifically, substantial evidence suggests that the function of the cerebellum is beyond motor control and coordination[29] and is linked to cognitive function.[30,31] Moreover, rsFC studies have confirmed the functional heterogeneity of the cerebellum and found that cerebellar-cortical circuits within the anterior lobe and lobule VII are mapped onto the sensorimotor network and those in the posterior lobe are mapped onto the executive control network.[14,15,27,28] Utilizing cerebellar ROIs in the sensorimotor and executive control networks may provide opportunities to explore the various rsFC patterns in schizophrenia.

In addition, previous studies have reported that cerebellar-cerebral rsFC is correlated with negative symptoms in schizophrenia.[18,32] Importantly, NSS, especially the motor coordination signs, have been found to correlate with negative symptoms in schizophrenia.[23–34] Taken together, these previous findings suggest that the cerebellar-cerebral functional circuits may be the shared neural underpinnings between NSS and negative symptoms in schizophrenia.

In this study, we aimed to (1) investigate whether patients with schizophrenia had more NSS deficits compared with healthy controls; (2) examine the relationship between NSS and cerebellar-cerebral rsFC in patients with schizophrenia and healthy controls using a ROI-whole-brain voxel rsFC approach; (3) explore whether the NSS-related cerebellar-cerebral rsFC was correlated with negative symptoms in schizophrenia; and (4) validate the significant findings in an independent dataset. Based on previous findings, we hypothesized that patients with schizophrenia would exhibit more NSS.[2] Regarding the NSS-related rsFC, we hypothesized that rsFC between the cerebellum and the frontal cortex would be correlated with NSS in patients with schizophrenia,[22] and rsFC between the cerebellum and more generalized cerebral regions would be correlated with NSS in healthy controls.[35] We further hypothesized that negative symptoms would be correlated with motor coordination-related rsFC of the cerebellum with the frontal cortex in schizophrenia patients.[23,32] Lastly, we hypothesized that the independent dataset would replicate and validate the significant findings.

Methods

Participants

Two independent samples were included in this study: a main dataset of 51 patients with schizophrenia and 50 healthy controls as well as a replication dataset of 34 patients with schizophrenia and 34 healthy controls. The detailed information of recruiting participants can be found in the Supplementary Methods. The diagnosis of schizophrenia was ascertained by qualified psychiatrists using the Structure Clinical Interview for DSM-IV Axis I Disorder.[36] Their clinical symptoms were assessed by qualified psychiatrists using the Positive and Negative Syndrome Scale (PANSS)[37] and the Scale for Assessment of Negative Symptoms (SANS).[38] All patients were in clinical stabilization.

This study was conducted in accordance with the Helsinki Declaration and approved by the Ethics Committees of the Institute of Psychology, the Chinese Academy of Sciences (protocol number: H13014, H15027, H16015), the New Territories West Cluster Clinical and Research Ethics Committee (protocol number: NTWC/CREC/1293/14), and the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (UW14-325).

Measures

The abridged version of the Cambridge Neurological Inventory (CNI) was used to assess NSS.[8,39] It consists of 25 items divided into the motor coordination (eg, rapid finger tapping and fist-edge-palm), sensory integration (eg, extinction and stereognosis) and disinhibition (eg, go/no-go response and head movement) subscales. Each item was scored on a dichotomized scale with "0" for absence and "1" for presence of NSS by trained experimenters. Higher score indicates more NSS. The intraclass correlation coefficients for the subscales and the total scale were greater than 0.80.[39]

Participants' IQ was estimated using the short form (the information, arithmetic, similarities, and digit span subtests) of the Chinese version of the Wechsler Adult Intelligence Scale-Revised.[40]

MRI Data Acquisition and Preprocessing

The rsfMRI data and high-resolution T1-weighted structural imaging data were acquired using a 3-T MR scanner (see Supplementary Methods for scanning sequences). Details on imaging preprocessing can be found in the Supplementary Methods. In brief, rsfMRI data were preprocessed using the Statistical Parametrical Mapping 12 (SPM 12; https://www.fil.ion.ucl.ac.uk/spm/software/spm12) and the Conn Functional Connectivity Toolbox (CONN).[40] Standardized denoising steps were performed using the ART[41] and the anatomical component-based noise correction method (aCompCor)[42] in CONN (see Supplementary Methods for details). Global signal was not regressed out as it may lead to artefactual negative FC results.[43] The mean frame-wise displacement (FD) for each participant was taken as a covariate in the subsequent analysis to minimize the effects of head motion.[44]

FC Analysis

Previous studies[27,28] have found that the cerebellar seeds are able to identify cerebellar-cerebral executive control and sensorimotor networks. Thus, six ROIs for the executive control network[27] and four ROIs for the sensorimotor network[27,28] were defined with a radius of 6 mm in the bilateral cerebellum to construct cerebellar-cerebral rsFC in the CONN (Supplementary table 1). Second, mean time series of each seed were calculated and correlated with the rest of the whole-brain voxels. Then, the correlation maps for each participant across all ROIs were transformed to Z-maps through Fisher's r-to-z transformation.

Statistical Analysis

Demographic and Behavioral Data Analysis Demographic and behavioral data were compared between groups in each dataset using independent sample *t*-tests or chi-square tests in SPSS (Version 17).

Regression Analysis Between Cerebellar-Cerebral rsFC and NSS Before regression analysis, the spatial topography of cerebellar-cerebral rsFC in healthy controls and schizophrenia patients was verified by one-sample *t*-tests in each dataset (see figure 1, Supplementary Results, and Supplementary table 2). The threshold for significance was set at voxel-level family-wise error (FWE) corrected *P* < .05.

To establish the neural correctional patterns between cerebellar-cerebral rsFC and CNI total and subscale scores for each group in the main dataset, Fisher Z-maps were included into a linear regression model comprising CNI total score (or subscale scores) as a regressor for patients with schizophrenia or healthy controls separately in the CONN. Age, gender, and mean FD[44] were entered as covariates. Given the correlation between NSS and cognition,[26,45] we did not include Intelligence Quotient (IQ) as a covariate. Length of education, duration of illness,

average daily antipsychotic dosage, and gray matter volume of the cerebellum were included as covariates separately to control for the potential confounding effects (see Supplementary Material). The clusters were considered significant if they reached a threshold of voxel-level *P* < .001 with cluster-level FWE corrected *P* < .005 (in order to correct for multiple comparisons of 10 ROIs).

Correlation Analysis Between NSS-Related rsFC and Clinical Symptoms The rsFC values of significant clusters were extracted in CONN. Partial correlation analysis was conducted to investigate the relationship between the NSS-related rsFC values and scores on the SANS subscales as well as the composite score in schizophrenia patients in the main dataset using SPSS. Covariates for the partial correlation analysis in the two schizophrenia patient groups included age, gender, mean FD, and antipsychotic dosage. The significance level for the partial correlation analysis was set at *P* < .05.

Validation Analysis

The significant findings in the main dataset were validated in the replication dataset. In order to test whether the significant correlations between CNI scores and the corresponding cerebral rsFC of the cerebellar functional network in the main dataset were also found in the replication dataset, each CNI score which was significantly correlated with rsFC in the main dataset and the Fisher Z-map from the corresponding cerebellar functional network were adopted into a linear regression model in the replication dataset. A mask of the significant cerebral regions found in the main dataset was generated using the WFU PickAtlas software (http://www.nitrc.org/projects/wfu_pickatlas/) for small volume correction. The threshold was voxel-level *P* < .005 with cluster-level FWE corrected *P* < .05.

As for the significant correlations between NSS-related rsFC and clinical symptoms found in the main dataset, the correlation between the rsFC of the Crus I$_{exe1}$ with the inferior frontal gyrus and the SANS avolition subscale score was carried out in the replication dataset to verify its stability.

Results

Behavioral Results

The demographic, behavioral, and clinical characteristics of the participants are shown in table 1. Patients with schizophrenia had significantly higher CNI subscale and total scale scores than healthy controls.

Correlations Between Cerebellar-Cerebral rsFC and CNI Scores

Table 2 and figure 2 show the significant correlations between cerebellar-cerebral rsFC and CNI scores in patients
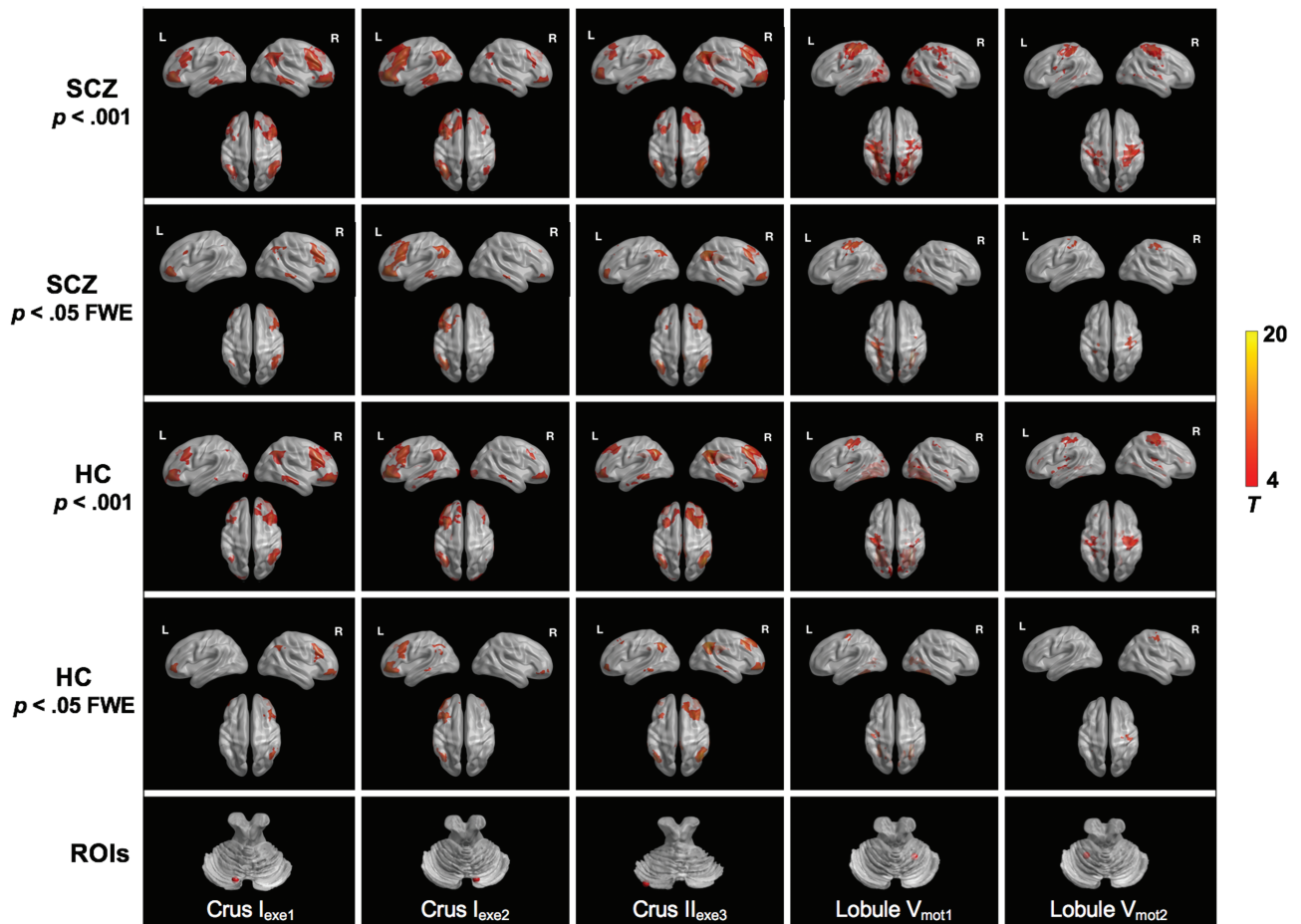
Fig. 1. Resting-state cerebellar-cerebral functional connectivity in SCZ and HC in the main dataset. The representative cerebellar seeds shown here include: Crus I$_{exe1}$, Crus I$_{exe2}$, Crus II$_{exe3}$, Lobule V$_{mot1}$, Lobule V$_{mot2}$. SCZ, patients with schizophrenia; HC, healthy controls; ROIs, regions of interest.

with schizophrenia in the main dataset. The rsFC between the Crus I$_{exe1}$ and the inferior frontal gyrus and the rsFC between the lobule VIII$_{mot4}$ and the precuneus were significantly and positively correlated with the motor coordination subscale score, indicating stronger connectivity with more severe motor coordination deficits. Moreover, the rsFC between the Crus II$_{exe3}$ and the inferior temporal gyrus was negatively correlated with the motor coordination subscale score, indicating weaker connectivity with more severe motor coordination deficits. CNI total score was positively correlated with the rsFC between the precuneus and the Lobule V$_{mot2}$ and negatively correlated with the rsFC between the inferior temporal gyrus and the Crus II$_{exe3}$ as well as the Crus II$_{exe4}$. There were no significant correlations between scores on the sensory integration and disinhibition subscales and rsFC.

Table 2 and figure 2 show the significant correlations between cerebellar-cerebral rsFC and CNI scores in healthy controls in the main dataset. Scores on the motor coordination subscale were positively correlated with the rsFC between the Crus I$_{exe1}$ and the middle occipital gyrus as well as the precuneus. Scores on the sensory integration

subscale were positively correlated with the rsFC of the fusiform gyrus with the Crus I$_{exe2}$ as well as the Crus II$_{exe3}$, and the rsFC of the superior frontal gyrus with the Lobule VIII$_{mot3}$. Moreover, there were significant positive correlations between CNI total score, motor coordination and disinhibition subscale scores and the rsFC of the occipital cortex with the Crus I$_{exe1}$, the Lobule VIII$_{mot4}$, and the Lobule VIII$_{mot3}$, respectively. Most rsFC results remained significant after including the covariates (see Supplementary Tables).

Cerebellar-Cerebral rsFC and Clinical Features of Schizophrenia

For schizophrenia patients in the main dataset, the rsFC between the Crus I$_{exe1}$ and the inferior frontal gyrus, which showed positive correlation with the CNI motor coordination subscale score in the FC analysis, was positively correlated with the SANS avolition subscale score ($r = 0.34$, $P = .02$; figure 3). Such correlation remained significant after including daily antipsychotic dosage, duration of untreated psychosis, illness duration, and

Table 1. Demographic, Clinical, and NSS Information of Main and Replication Datasets

	Main Dataset					Replication Dataset				
	HC ($n = 49$)	SZ ($n = 50$)	t/χ^2	P	Cohen's d	HC ($n = 34$)	SZ ($n = 34$)	t/χ^2	P	Cohen's d
Demographics										
Age (y)	27.45 (6.42)	27.68 (6.27)	$t_{97} = -0.18$	.857	0.036	43.18 (12.81)	43.94 (12.17)	$t_{66} = -0.25$	.804	0.061
Gender (male, %)	46.94%	52.00%	0.25	.689		35.29%	33.33%	0.03	1.000	
Education (y)	15.92 (2.85)	12.50 (3.34)	$t_{97} = 5.47^{**}$	<.001	1.102	12.15 (3.46)	11.48 (2.67)	$t_{66} = 0.88$	.384	0.217
Estimated IQ	128.84 (12.00)	99.26 (13.96)	$t_{97} = 11.29^{**}$	<.001	2.272	119.65 (11.91)	105.78 (16.63)	$t_{66} = 3.91^{**}$	<.001	0.959
CNI										
Motor coordination	1.33 (1.28)	3.04 (1.93)	$t_{97} = -5.20^{**}$	<.001	1.044	1.18 (1.19)	2.67 (1.88)	$t_{66} = -3.88^{**}$	<.001	0.947
Sensory integration	0.37 (0.70)	1.18 (1.38)	$t_{97} = -3.69^{**}$	<.001	0.740	1.21 (0.98)	1.79 (1.02)	$t_{66} = -2.38^{*}$	.020	0.580
Disinhibition	1.18 (0.69)	1.90 (1.27)	$t_{97} = -3.66^{**}$	<.001	0.704	0.44 (0.70)	1.09 (0.98)	$t_{66} = -3.12^{*}$	.003	0.763
CNI total	2.86 (1.93)	6.14 (3.30)	$t_{97} = -6.03^{**}$	<.001	1.213	2.82 (1.93)	5.55 (2.92)	$t_{66} = -4.52^{**}$	<.001	1.103
Clinical characteristics										
Course of illness (y)		1.18 (1.08)					19.89 (10.63)			
DUP (y)		0.82 (1.06)					NA			
PANSS_Positive		9.72 (3.09)					11.25 (5.88)			
PANSS_Negative		11.38 (5.96)					12.62 (5.34)			
PANSS_General		20.70 (5.09)					24.33 (8.32)			
SANS_Affective		5.06 (6.37)					2.68 (3.76)			
Flattening										
SANS_Alogia		1.78 (2.92)					1.50 (2.46)			
SANS_Avolition		1.58 (2.32)					2.32 (3.07)			
SANS_Anhedonia		2.72 (4.08)					2.44 (3.47)			
SANS_Inattention		2.38 (2.22)					0.76 (1.52)			
SANS_Composite total		3.44 (3.97)					8.94 (10.87)			
CPZ equivalent dose (mg)		340.83 (174.32)					224.92 (192.11)			

Note: HC, healthy controls; SZ, patients with schizophrenia; CNI, the abridged version of the Cambridge Neurological Inventory; NA, not applicable; PANSS: the Positive and Negative Syndrome Scale; CPZ: chlorpromazine; DUP, duration of untreated psychosis; SANS: the Scale for Assessment of Negative Symptoms; SANS_Composite total, the total score of composite items but excluding attention item.

$^{*}P < .05$, $^{**}P < .001$.

Table 2. Associations of CNI Subscale and Total Scores With Cerebellar-Cerebral Functional Connectivity in the Main Dataset

| Cerebellar Seeds	Direction of Association	Regions	P (FWE-Corr)	Cluster Size	MNI Coordinates			T
					x	y	z	
A. Patients with schizophrenia								
CNI MC								
Crus I$_{exe1}$	Positive	**Inferior frontal gyrus**	.001	123	56	8	18	5.54
Crus II$_{exe3}$	Negative	**Inferior temporal gyrus**	.001	126	−44	6	−36	4.47
Lobule VIII$_{mot4}$	Positive	**Precuneus**	<.001	195	−4	−54	34	5.64
CNI SI	No significant results							
CNI DIS	No significant results							
CNI total								
Crus II$_{exe3}$	Negative	**Inferior temporal gyrus**	.003	106	−46	8	−40	4.66
Crus II$_{exe4}$	Negative	**Inferior temporal gyrus**	.003	110	−46	10	−40	5.13
Lobule V$_{mot2}$	Positive	Precuneus	.001	121	−8	−50	38	5.21
B. Healthy controls								
CNI MC								
Crus I$_{exe1}$	Positive	Middle occipital gyrus	.001	130	−46	−78	28	5.4
	Positive	Precuneus	.001	128	−12	−64	30	4.51
	Negative	Superior temporal gyrus	<.001	339	−62	−2	−2	5.82
	Negative	Postcentral gyrus	.004	101	60	−10	24	5.33
Crus I$_{exe2}$	Negative	Superior temporal gyrus	.0046	98	−64	−8	8	4.7
Lobule VI$_{exe5}$	Negative	Medial superior frontal gyrus	.0046	95	10	54	40	5.3
Lobule VIII$_{mot4}$	Positive	Middle occipital gyrus	<.001	180	−22	−78	12	4.77
	Positive	Middle occipital gyrus	<.001	129	28	−68	18	4.47
CNI SI								
Crus I$_{exe2}$	Positive	Fusiform gyrus	.001	121	−26	−42	−12	5.15
Crus II$_{exe3}$	Positive	Precuneus	.004	100	24	−50	18	5.34
	Positive	Fusiform gyrus	.003	101	−26	−44	−12	5.05
	Positive	Fusiform gyrus	.001	125	30	−44	−14	4.83
Crus II$_{exe4}$	Positive	Inferior parietal lobule	<.001	141	30	−46	40	5.03
Lobule VI$_{exe5}$	Negative	Fusiform gyrus	<.001	299	−34	−38	−16	5.49
	Negative	Fusiform gyrus	<.001	139	30	−42	−20	4.98
Lobule VIII$_{mot3}$	Positive	**Superior frontal gyrus**	.002	100	30	60	−2	4.5
CNI DIS								
Lobule VIII$_{mot3}$	Positive	Calcarine sulcus	<.001	132	−6	−100	−8	5.84
CNI total								
Crus I$_{exe1}$	Positive	Cuneus	<.001	304	−20	−58	22	5.77
	Positive	Middle occipital gyrus	.001	118	−44	−74	24	5.25
	Negative	**Middle occipital gyrus**	.002	109	38	−86	8	5.11
	Negative	Superior temporal gyrus	<.001	170	−60	4	4	4.55

Note: CNI, the abridged version of the Cambridge Neurological Inventory; MC, motor coordination subscale; SI, sensory integration subscale; DIS, disinhibition subscale. The brain regions in bold were validated in the replication dataset. The threshold was voxel-level P < .001 and cluster-level FWE correction P < .005.

gray matter volume of the cerebellum as covariates (see Supplementary Results). However, correlational results did not survive multiple comparison correction (P < .002). Exploratory analysis did not find any other significant correlations between rsFC and clinical symptoms.

Validation Results in the Replication Dataset

The replication dataset confirmed that schizophrenia patients had higher CNI scores than healthy controls (table 1). Significant cerebellar-cerebral rsFCs in the executive control network and the sensorimotor network were also found (Supplementary table 3).

Confirming the patterns in the main dataset, patients with schizophrenia in the replication dataset exhibited the same correlations between scores on the motor co-ordination subscale and the rsFC of the corresponding cerebellar network and cerebral regions (Supplementary table 4 and figure 2). As for CNI total scores, patients with schizophrenia exhibited a negative correlation with the rsFC of the inferior temporal gyrus and the cerebellar executive control network. Healthy controls exhibited a significant positive correlation between the sensory integration subscale score and the rsFC between the superior frontal gyrus and the cerebellar sensorimotor network, and a significant negative correlation between the CNI
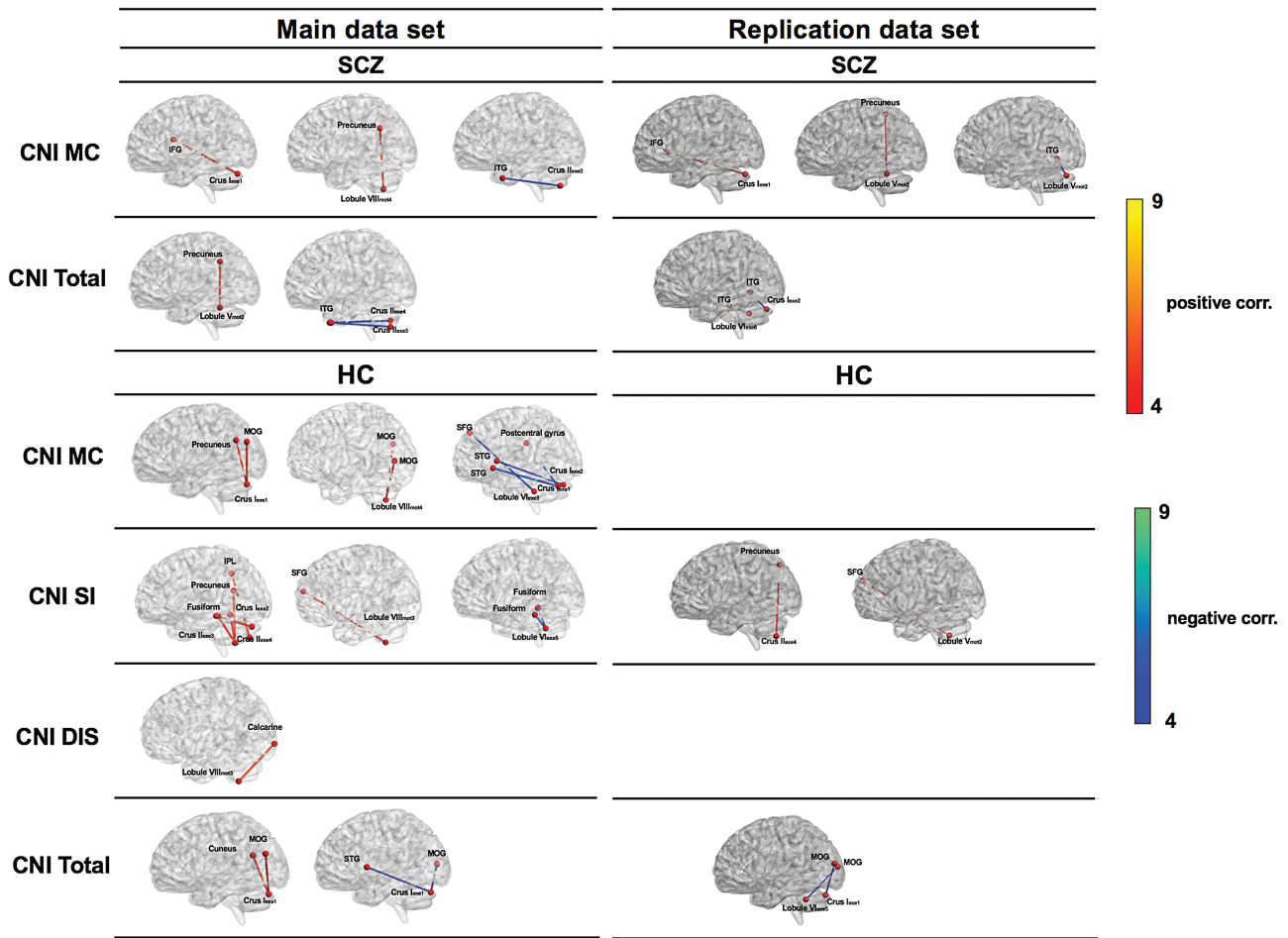
Fig. 2. Associations of CNI subscale and total scores with cerebellar-cerebral resting-state functional connectivity in the main ($P < .001$ with cluster-level FWE corrected $P < .005$) and the replication ($P < .005$ with cluster-level FWE corrected $P < .05$) datasets. CNI, the abridged version of the Cambridge Neurological Inventory; MC, motor coordination subscale; SI, sensory integration subscale; DIS, disinhibition subscale; IFG, inferior frontal gyrus; ITG, inferior temporal gyrus; MOG, middle occipital gyrus; SFG, superior frontal gyrus; STG, superior temporal gyrus; IPL, inferior parietal lobule.
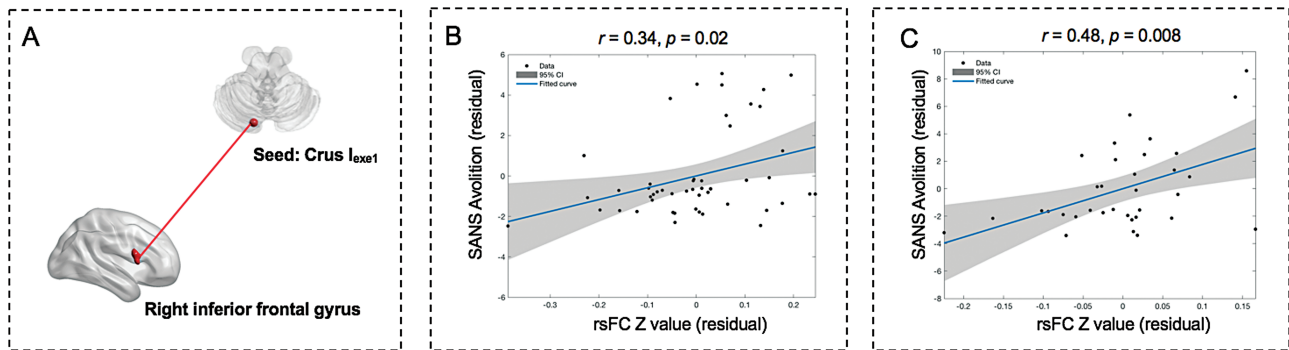
Fig. 3. Relationship between CNI-related cerebellar-prefrontal rsFC and negative symptoms. (A) rsFC between the inferior frontal gyrus and the Crus I$_{exe1}$. (B) Scatterplot of the significant correlation between two unstandardized residuals for reflecting relationship between SANS avolition subscale score and rsFC of the Crus I$_{exe1}$ with the inferior frontal gyrus in the main dataset. (C) Scatterplot of the significant correlation between two unstandardized residuals for reflecting relationship between SANS avolition subscale score and rsFC of the Crus I$_{exe1}$ with the inferior frontal gyrus in the replication dataset. CNI, the abridged version of the Cambridge Neurological Inventory; rsFC, resting-state functional connectivity; SANS, the Scale for Assessment of Negative Symptoms.

total score and the rsFC of the middle occipital gyrus with the cerebellar executive control network. Most of the results remained significant after including length of education, duration of illness, and gray matter volume of the cerebellum as covariates (see Supplementary Tables). However, only the rsFC between the inferior frontal gyrus and the Crus I$_{exe1}$ was found to be significant after including daily antipsychotic dosage as a covariate.

Lastly, the same significant positive correlation between scores on the SANS avolition subscale and rsFC ($r = 0.48$, $P = .008$; figure 3) was found. Such correlation remained significant after including daily antipsychotic dosage and gray matter volume of the cerebellum as covariates ($r = 0.51$, $P = .009$) and became marginally significant after including daily antipsychotic dosage and illness duration as covariates ($r = 0.49$, $P = .05$).

Discussion

The current study aimed at investigating cerebellar-cerebral rsFC underlying NSS in schizophrenia patients and healthy controls. Our study has two main findings: (1) cerebellar-cerebral rsFC involving the executive control and motor networks were correlated with NSS differently in patients with schizophrenia and healthy controls; and (2) cerebellar-prefrontal rsFC was significantly and positively correlated with motor coordination deficits and negative symptoms in schizophrenia patients.

The correlations between NSS and rsFC of the cerebellum with the inferior frontal gyrus, the inferior temporal gyrus, and the precuneus in schizophrenia patients are generally in line with previously morphological,[46,47] task-based fMRI,[48–50] and rsfMRI studies,[22] providing evidence to support the role of cerebello-thalamo-cortical circuit (CTCC) dysfunction[51,52] in the pathophysiology of schizophrenia. Specifically, our findings indicate that increased rsFC of the Crus I$_{exe1}$ with the inferior frontal gyrus (a part of the prefrontal cortex) and the lobule VIII$_{mot4}$ with the precuneus and the decreased rsFC of the Crus II$_{exe3}$ with the inferior temporal gyrus are correlated with more motor coordination deficits in schizophrenia patients. These findings extend previous structural and fMRI findings. Previous morphological studies have revealed that the cortical thickness of the prefrontal cortex, the inferior temporal gyrus, and the precuneus were negatively correlated with NSS score in patients with schizophrenia.[46,53] Moreover, a task-based fMRI study also found that patients with schizophrenia showed altered prefrontal cortex connectivity during a fist-edge-palm imaging task.[48]

On the other hand, the inferior frontal gyrus plays a critical role in movement observation, execution of visually guided movements and action monitoring.[54–56] Therefore, the uncoupling of brain activities between the inferior frontal gyrus and the cerebellum, in which the inferior frontal gyrus perceives movement information, exerts top-down control and sends signal to the cerebellum, could lead to a failure in visuomotor transformation. The precuneus is known for somatosensory control and movement coordination.[57,58] The aberrant cerebellar-cerebral rsFC between the sensorimotor network and the precuneus may lead to impaired processing of basic sensorimotor information and contribute to failure in movement control and coordination. The inferior temporal gyrus has been implicated in auditory processing[59] and visual recognition.[60] The decreased connectivity between the inferior temporal gyrus and the cerebellum may indicate insufficient communication between the cerebral and cerebellar regions, suggesting abnormal processing for basic auditory and visual stimuli.

Furthermore, we found that several cerebellar-cerebral rsFCs were also significantly correlated with NSS in healthy controls. A previous study using rsfMRI has reported that NSS were positively correlated with activity of the cerebellar lobule VI[61] in the healthy population. Our results are consistent with these previous findings and suggest that more cortical regions including the occipital, temporal, and frontal cortices are involved in the correlations between cerebellar-cerebral rsFC and NSS. For example, rsFC between the Crus I$_{exe1}$ and the postcentral gyrus (the primary somatosensory cortex, S1) is negatively correlated with motor coordination deficits. The postcentral gyrus is responsible for attention, proposing somatic sensations and spatial working memory.[62] Previous brain structural studies in the healthy population have also found that motor coordination deficits are correlated with morphological changes of the postcentral gyrus.[63,64]

Another important finding of this study is the significant positive correlation between negative symptoms and cerebellar-prefrontal rsFC, which was also positively correlated with motor coordination deficits in patients with schizophrenia (figure 3). Motor coordination deficits have been found to be correlated with negative symptoms of schizophrenia.[23,33] Moreover, a diffusion-tensor imaging study found that white matter volume of the inferior frontal gyrus was negatively correlated with negative symptoms in schizophrenia.[65] Another study has found that schizophrenia patients with persistent negative symptoms had smaller gray matter volumes of inferior frontal gyrus compared with patients with nonpersistent negative symptoms.[66] These structural MRI studies suggest that the inferior frontal gyrus may play an important role in negative symptoms of schizophrenia. Furthermore, modulation of the connectivity between the cerebellum and the prefrontal cortex using transcranial magnetic stimulation (TMS) has been proposed as a possible intervention to alleviate negative symptoms of schizophrenia.[32] Moreover, the application of transcranial direct current stimulation (tDCS) to the prefrontal and cerebellar cortex has been found to improve NSS in patients with

bipolar disorder.[67] Our finding suggests that cerebellar-prefrontal rsFC may be the common neural pathway of NSS and negative symptoms, and a potential therapeutic target for noninvasive brain stimulation such as TMS and tDCS to improve NSS and negative symptoms in schizophrenia.

The main strength of this study is that a replication dataset has been used to test the reproducibility and reliability of the results found in the main dataset. Furthermore, the majority of the findings remained significant after including covariates such as length of education, duration of illness, and gray matter volume of the cerebellum, although the change in results after including daily antipsychotic dosage suggests that antipsychotic medications may influence brain activity.

This study also has several limitations. First, our patients with schizophrenia were medicated and previous studies have demonstrated that exposure to antipsychotics may affect motor function and brain activity in schizophrenia.[68,69] Second, the demographics and clinical features of the two datasets were not matched, and our findings in the control groups of the two datasets differed. However, our main outcomes, such as motor coordination scores, SANS avolition subscale scores, and cerebellar-prefrontal rsFC were not significantly different between two patients' groups, suggesting that the different illness duration between the two datasets would unlikely affect our findings. Furthermore, a previous study of 738 schizophrenia patients showed that NSS deficits barely changed across the lifespan.[4] Third, we chose ROIs in the cerebellum to represent specific networks. Nevertheless, previous evidence suggest that other methods to construct rsFC network are also valid and useful.[35] For instance, the established cerebellar network parcellation[14] which covers comprehensive networks could be used to explore cerebellar-cerebral rsFC among other networks in schizophrenia in future studies. Finally, other sensorimotor abnormalities in schizophrenia such as catatonia or parkinsonism could also be examined in future research. Previous studies have shown that these abnormalities (NSS, catatonia, and parkinsonism) are related to negative symptoms,[70–72] cognition,[73,74] and outcome[70,71,75] in schizophrenia. Further studies on the neural mechanisms underlying these sensorimotor abnormalities can advance our insights on the psychopathology and development of new treatment for schizophrenia.

In conclusion, we demonstrated altered coupling between NSS and cerebellar-cerebral rsFC in patients with schizophrenia and healthy controls. Furthermore, we found that cerebellar-prefrontal rsFC was positively correlated with both motor coordination deficits and negative symptoms in schizophrenia. Our findings indicate that cerebellar-prefrontal rsFC may be the shared neural mechanism between NSS and negative symptoms in schizophrenia. Altered coupling of NSS-related cerebellar-cerebral functional networks may be potential biomarkers for schizophrenia spectrum disorders.

Supplementary Material

Supplementary material is available at *Schizophrenia Bulletin*.

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