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RESEARCH ARTICLE

Functional connectivity changes in the language network during stroke recovery

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Abstract

Objective: Several neuroimaging studies have examined language reorganization in stroke patients with aphasia. However, few studies have examined language reorganization in stroke patients without aphasia. Here, we investigated functional connectivity (FC) changes after stroke in the language network using resting-state fMRI and performance on a verbal fluency (VF) task in patients without clinically documented language deficits. **Methods**: Early-stage ischemic stroke patients ($N = 26$) (average 5 days from onset), 14 of whom were tested at a later stage (average 4.5 months from onset), 26 age-matched healthy control subjects (HCs), and 12 patients with cerebrovascular risk factors (patients at risk, PR) participated in this study. We examined FC of the language network with 23 seed regions based on a previous study. We evaluated patients' behavioral performance on a VF task and correlation between brain resting-state FC (rsFC) and behavior. **Results**: Compared to HCs, early stroke patients showed significantly decreased rsFC in the language network but no difference with respect to PR. Early stroke patients showed significant differences in performance on the VF task compared to HCs but not PR. Late-stage patients compared to HCs and PR showed no differences in brain rsFC in the language network and significantly stronger connections compared to early-stage patients. Behavioral differences persisted in the late stage compared to HCs. Change in specific connection strengths correlated with changes in behavior from early to late stage. **Conclusions**: These results show decreased rsFC in the language network and verbal fluency deficits in early stroke patients without clinically documented language deficits.

Introduction

Resting-state functional connectivity MRI (rs-fcMRI) is a useful way to study network level changes following stroke given that there are no exogenous task demands on the patient and individual differences in task strategies are obviated.[1,2] Several neuroimaging studies have examined brain reorganization changes following stroke in patients with aphasia.[3] Few studies, however, have investigated alterations in the language network and its effect on behavioral performance in stroke patients who have no documented language deficits in the clinical setting. Language is a high-level cognitive process that involves semantic and phonological processes, and requires access to memory representations during task performance. It is therefore likely that a stroke-induced lesion could cause disruptions in the network of brain regions involved in language processing and this may manifest as subtle behavioral deficits that are not easily discernible on clinical examination. In contrast, tasks such as the verbal (or phonemic) fluency task, involving both language and other high-level cognitive processes that subserve language, may capture these deficits.

Our goal in this study was to examine rsFC in the language network in the early and late stages in stroke patients without clinically documented language deficits, compared to healthy controls (HCs) and patients with cerebrovascular risk factors for stroke (patients at risk, PR). In order to examine the behavioral differences, we chose the verbal fluency (VF) task which requires patients to generate multiple responses within a limited time period based on phonemic criteria. Additionally, we investigated brain–behavior correlations at each time-point and change over time.

Methods

Participants

The research described in this study is part of a longitudinal project investigating brain reorganization changes following stroke. *Inclusion criteria* for the stroke group were patients aged 18 years or older with ischemic stroke and ability to provide written consent. We also recruited two types of control subjects in the study: (1) Healthy normal controls – HCs (subjects 18 years or older) were recruited from the campus community by means of flyers and campus-wide recruitment email. (2) In addition to HCs, patients with (Transient Ischemic Attack defined as resolution of symptoms within 24 h and stroke excluded by anatomical MRI), or with other risk factors for stroke, were chosen to serve as a second group of controls – PR. These patients were chosen because of similar vascular risk factors, medications, stressors of medical care comparable to those of the stroke patients as well as similar changes in terms of vasculopathic brain changes such as white matter disease burden, old lacunar infarcts, and other chronic changes. It is necessary to enroll these controls, to compare the deficits observed in stroke patients with a better matched control which takes into account normal aging as well as other chronic changes associated with patients with vascular disease or risk factors and also are on similar medications. A neuroradiologist (V. P.) examined all brain MRI scans of normals and patients to verify that they meet inclusion criteria and lesion location in stroke patients.

Exclusion criteria for all groups were history of psychiatric illness, confounding neurological disorders, drug abuse, and contraindications to MRI. Here, we report results based on the data from 26 early-stage stroke patients, without clinically recorded language deficits as determined by NIHSS and clinical history (mean $\pm$ SD age = 62.5 $\pm$ 7.96, 16M, see Table 1 for clinical characteristics including raw scores on VF task, Table 2 for demographics), 12 PR (mean $\pm$ SD age = 61.42 $\pm$ 16, 7M), and 26 HCs age matched to the acute stroke patients (mean $\pm$ SD age = 60.19 $\pm$ 7.38 years, 14M). Details regarding patient deficits were recorded from the clinical notes of the attending stroke neurologist (J. S., M. C., or M. J., Table 1). Fourteen of the stroke patients were also tested in late stage (average 4.5 months from stroke) and the results compared with a subset of 14 age-matched HC. The study was conducted in accordance with protocol approved by the local Health Sciences Institutional Review Board. All subjects provided written informed consent.

Data acquisition and preprocessing

Behavioral

VF outside the scanner was assessed by the forms of the Controlled Oral Word Association Test (COWAT),[4] which requires subjects to produce words beginning with the letters, "F," "A," "S" in three respective 1-min trials. Responses to each letter were recorded and letter fluency scores were based on the total number of correct responses produced by the participants across the three letter conditions. Analyses were done using both raw scores and normed scores (corrected for age and education). However, because both analyses yielded similar patterns, results are reported using the raw scores.

MRI scanning

MRI data were obtained on 3T GE 750 scanners (GE Healthcare, Waukesha, WI) equipped with an eight-channel head coil. Eyes closed, 10-min resting scans were

Table 1. Clinical characteristics of stroke patients.

Patient	Age at test	Time since stroke (days)		NIH stroke[1] score (NIHSS)	Attending stroke neurologist	Lesion location	VF raw score	
		Early stage	Late stage				Early	Late
1[1]	76	4	137	10	MC	R. corona radiata	23	11
2	74	7		1	JS	R. temporo-occipital	53	
3[1]	67	5	165	4	MC	R. perirolandic/occipital	34	35
4[1]	65	4	163	0	JS	R. thalamus	17	22
5	62	3		0	MJ	B. temporal	48	
6	67	5		0	JS	L. medulla	39	
7[1]	69	4	74	0	MC	L. occipital, cerebellar	38	50
8[1]	57	6	180	1	MC	L. cerebellum	66	56
9[1]	58	5	75	0	MC	L. cerebellum	31	30
10[1]	63	3	71	0	MC	R. fronto-parietal-temporal	44	37
11[1]	59	7	124	2	MJ	R. MCA	27	18
12	59	4		2	JS	L. frontal	54	
13[1]	58	7	178	1	JS	L. frontal	39	50
14	70	2		2	MC	L. parietal	42	
15	63	2		2	[1]	L. putamen	13	
16[1]	62	9	128	0	MJ	L. parietal	27	19
17[1]	75	6	145	1	JS	L. occipital	41	39
18	51	7		13	MC	R. internal capsule	41	
19	64	1		0	MC	R. occipital	40	
20[1]	61	6	220	1	MC/MJ	R. cerebellum	10	12
21	46	3		0	MJ	R. occipital	53	
22	44	5		7	JS/MC	L. insula, frontal	27	
23	63	5		0	JS	R. paramedian pontine	23	
24[1]	59	2	150	2	JS	L. insula, angular gyrus	21	28
25	62	5		2	JS	L. frontal	31	
26[1]	73	4	106	0	MC	R. frontal operculum, insula	47	40
Tested in late stage[1]		Average = 4.65 days	Average = 133.7 days		No data[1]	R = right, L = left, B = bilateral, MCA = middle cerebral artery		

VF, verbal fluency.

[1]NIHSS Language subscore was 0 in all of these patients; no language deficits were noted on clinical exam by the attending Stroke Neurologist and the clinical stroke team.

obtained using single-shot echo-planar T2*-weighted imaging: TR = 2.6 sec, 231 time-points, TE = 22 msec, FOV = 22.4 cm, flip angle = 60°, voxel dimensions $3.5 \times 3.5 \times 3.5$ mm^3, 40 slices. T1-weighted axial anatomical scans were acquired at the beginning of each scan using FSPGR BRAVO sequence (TR = 8.132 msec, TE = 3.18 msec, TI = 450 msec) over a 256×256 matrix and 156 slices (flip angle = 12°, FOV = 25.6 cm, slice thickness = 1 mm).

fMRI data preprocessing

fMRI data were processed using AFNI package.[5] Images were despiked, slice time corrected, motion corrected, aligned with the anatomical scan, normalized to MNI space, resampled to 3.5 mm^3, and spatially smoothed with a 4-mm FWHM Gaussian kernel. Motion censoring (per TR motion >1 mm or 1°), nuisance regression, and bandpass filtering (0.009–0.08 Hz) were performed simultaneously in one regression model.[6] Nuisance signals regressed out included six motion estimates and their temporal derivatives, and the voxel-wise locally averaged white matter signal.

Functional connectivity

Seed regions

Seed regions in the language network were based on a recent study of resting data from 970 healthy subjects (age range 15–85) from the 1000 Functional Connectomes

Table 2. Demographic characteristics of participants in the study.

Healthy normal controls	Age at test	M/F	Hand	Early stroke	Age at test	M/F	Hand	Patients with risk factors	Age at test	M/F	Hand
1	77	M	R	1	74	M	L	1	80	F	R
2	62	M	R	2	76	F	R	2	65	M	R
3	63	M	R	3	67	F	R	3	47	M	A
4	68	M	R	4	65	M	A	4	69	M	R
5	71	F	R	5	62	M	R	5	49	M	R
6	67	M	R	6	67	M	L	6	80	F	R
7	74	F	R	7	69	M	R	7	58	M	R
8	61	F	R	8	57	F	R	8	61	F	R
9	55	M	R	9	58	M	R	9	76	M	R
10	60	F	R	10	63	M	R	10	22	M	R
11	60	M	R	11	59	F	R	11	62	F	R
12	51	M	R	12	59	M	R	12	68	F	R
13	55	F	R	13	58	F	R				
14	59	F	R	14	70	F	R				
15	63	F	R	15	63	M	L				
16	61	F	R	16	62	M	R				
17	56	M	R	17	75	M	R				
18	58	F	R	18	51	M	R				
19	61	M	R	19	64	F	R				
20	53	M	R	20	61	M	R				
21	62	F	R	21	46	M	L				
22	61	M	R	22	44	F	R				
23	58	M	L	23	63	M	R				
24	57	F	R	24	59	F	R				
25	44	M	R	25	62	F	R				
26	48	F	R	26	73	M	R				
	Avg 60.2 years (44–77 years)	14M	25R		Avg 62.5 years (44–76 years)	16M	22R		Avg 61.4 (22–80 years)	7M	11R

Project http://www.nitrc.org/projects/fcon_1000/.[7] The areas identified in this study encompass a broad language network including phonological and lexical-semantic functions. Regions of this network include the inferior frontal network, middle frontal gyrus, inferior temporal, and temporo-parietal areas as well as subcortical regions that included the bilateral caudate and left putamen/globus pallidus, ventral thalamic, and subthalamic nuclei. Several studies have reported the involvement of these regions in language processing including speech comprehension and production.[8–11] The MNI coordinates for 23 seed regions of interest (ROIs) reported in this study were used to create 8-mm spherical seed ROIs for this study. For each subject, time series from the 23 seed ROIs were extracted from the spatially standardized residuals of the resting EPI data. The language mask constituting these 23 regions was then used to extract the time series for each region and a 23 × 23 ROI correlation matrix computed (total of 23 $(23-1)/2 = 253$ connections) for each individual. Correlation coefficients were transformed to z-scores and subsequently used in the NBS toolbox.

Connectivity matrix and application of network-based statistic

A connectivity matrix of size 23 × 23 was computed for each subject. A 23 × 23 connectivity matrix stores the estimates of a total of $23\times (23-1)/2 = 253$ unique pairwise connections. Each row/column of the connectivity matrix corresponds to a distinct seed region of interest (ROI) or a node, such that position (i, j) represents the correlation between the ith and jth regions. To identify impaired connections, we used the network-based statistic, proposed by Zalesky et al.,[12] that controls for family-wise error (FWE), and allows one to make inferences about the subnetworks of connected ROIs, similar to cluster-based voxel thresholding in task fMRI studies. NBS first identifies supra-threshold connections that survive an initial t statistic threshold and then applies permutation testing to identify subnetworks that are differentially significant among groups or over time.[12,13] In the case of FC analyses, by combining a connection-level threshold with network-based statistics, it is possible to make infer-

ences about the extent of specific subnetworks of connected ROIs. The NBS assigns a corrected *P*-value to each subnetwork (consisting of nodes or seed ROIs and edges or connections among these nodes) that is significantly different between the groups. This kind of permutation testing is similar to that used in task fMRI studies with conventional cluster-based thresholding of statistical parametric maps. A primary *t*-statistic threshold of 2.0 corresponding to a $P < 0.05$ and number of iterations = 5000 were used for the NBS. Results of NBS were viewed using the BrainNet viewer toolbox.[14]

Statistical tests

Fisher's exact tests were run to examine group differences on variables of gender and handedness. Two-sample *t*-tests were used to examine the differences in age, education, and raw VF scores. Within the NBS, for FC, because we had an a priori hypothesis that early stroke patients would be more impaired than HCs and PR, we performed two-sample *t*-tests to test for group differences and paired *t*-test to assess differences from the early to late stages of stroke. Correlations among brain connectivity measures (*z*-scores) and raw behavioral scores (corrected for age and education) were computed using Pearson's correlation coefficients. Both false discovery rate (fdr)-corrected and uncorrected *P* values are reported for the correlations. Corrected *P* values were computed using the "mafdr" function in Matlab,[15] which corrects for multiple comparisons by controlling the false discovery rate.

Results

Nearly all patients were discharged to home and received no specific speech- or language-related therapy. Two of the 14 patients tested in the late stage (#1 and #20 in Table 1) were discharged to in-patient rehabilitation; speech therapy was recommended for one (#1, Table 1) of the two patients.

Demographic factors

There were no significant differences in age, handedness, and gender among the three groups (Table 3). There was a significant difference in education between late versus HCs on years of education.

Behavioral

VF task

There were significant deficits in performance when early-stage ($P < 0.001$), late-stage stroke patients ($P = 0.003$), and PR ($P = 0.02$) were compared to HC (Fig. 1, Table 4). There was no significant difference between early stroke patients, late stroke patients when compared to PR or between early-stage and late-stage stroke patients. Analysis of normed scores[16] which takes into account education and age also showed a similar pattern as raw scores.

FC

Early-stage stroke and HC

Compared to HC, early-stage stroke patients showed impaired connectivity in a language subnetwork of 24 connections. Table 5 and Figure 2A show connections that form this subnetwork. There were significant differences in interhemispheric and intrahemispheric connection strengths between early stroke and controls (Fig. 3).

Early-stage and late-stage stroke

Compared to early stage, patients in the late stage showed stronger connectivity in a language subnetwork comprising of 31 connections. Table 5 and Figure 2B show connections that form this subnetwork. There were significant differences in interhemispheric and intrahemispheric connection strengths from early to late stage (Fig. 3).

No subnetwork was identified as being significantly different between early stage and PR, PR and HC, late stage and HC, or late and PR.

Brain–behavior relationship

For stroke patients in the early stage, Pearson *r* correlations between VF performance and FC trended toward significance for connections between right caudate and right pars orbitalis ($r = 0.37$, $P = 0.06$, $P_{corr} = 0.13$), and left superior temporal and right superior temporal regions ($r = 0.33$, $P = 0.09$, $P_{corr} = 0.06$). HCs showed significant correlations or trend toward significance for several connections (Table 6).

Change in connection strength from early to late stage ([score at late minus score at early stage]/score at early stage) correlated significantly with change in behavioral performance for some connections, specifically for connections from left putamen to right superior parietal ($r = -0.80$, $P = 0.001$, $P_{corr} = 0.016$), and a trend toward significance from left superior frontal to left ventral thalamus ($r = 0.46$, $P = 0.08$, not significant when corrected for multiple comparisons). Table 7 gives a summary of the significant results by *P* values for the raw and normed behavioral and brain FC analysis.

Table 3. *P* values for group differences on different factors.

Factor	Early (N = 26) vs. HN (N = 26)	Late (N = 14) vs HN (N = 14)	PN (N = 12) vs. HN (N = 26)	Early (N = 26) vs. PN (N = 12)	Late (N = 14) vs. PN (N = 12)
Age	0.27	0.92	0.75	0.77	0.48
Sex/gender	0.77	1.00	1.00	1.00	1.00
Education	0.15	0.03*	0.56	0.55	0.17
Handedness	0.19	1.00	0.54	0.64	1.00

*$P \leq 0.05$ was considered significant.

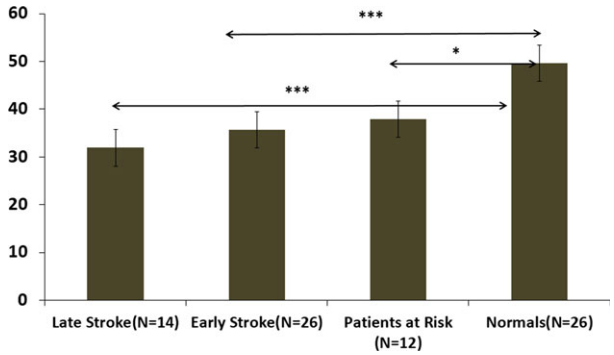

Figure 1. Average letter fluency scores for each group. ***$P < 0.001$,**$P < 0.005$, *$P < 0.05$.

Sample size and power considerations

Stroke patients, in general, are a heterogeneous group with varying age, size and location of infarct, and large variability in comorbidities, among other things. However, focusing only on a small highly selected subgroup also has the disadvantage that the results may not be easily generalizable. In order to address the power issue, we also provide below a post-hoc power analysis using the G*Power software package.

$$\text{Effect size for group differences in overall language network connectivity} = \frac{(\text{Mean connectivity for patients} - \text{Mean connectivity for controls})}{\text{standard deviation for controls}}$$

The computed effect size = 1.76. Power corresponding to this effect size is close to 1 using the G*Power software. This indicates that for the type of results reported here with $N = 26$ in each group, our study is sufficiently powered. For effect sizes estimated based on group differences, values between 1.15 and 2.7 are considered a moderate to strong effect.[17] Additionally, in our study, all the stroke patients had no overt clinical language deficits, a majority of them were discharged to home, and were all able to participate in a 10-min scanning session, and in that sense this makes it a relatively homogenous group.

Discussion

Our results demonstrate that stroke patients without clinically significant language deficits in the early stages show impaired rsFC in the language network and impairments on behavioral language task compared to HC. Stroke patients in the late stage did not show differences in brain FC compared to HC, although behavioral differences persisted. Relationship between rsFC and behavioral performance was significant for specific language connections in HC and in early stroke, and change in FC in specific connections from early to late stage correlated with change in language performance.

Early-stage stroke shows alterations in rsFC in the language network

Several of the impaired connections in the early-stage stroke patients involved Wernicke's area and other regions such as the left superior/middle frontal, right pars opercularis (right homologue of Broca's area), superior temporal, posterior parietal, and bilateral caudate regions. Several of these regions have been impli-

Table 4. Verbal fluency scores by group.

	HN	PN	Early stroke	Late stroke
Verbal (average raw)	49.6 (N = 26) 51.07 (N = 14)	37.9* (N = 12)	35.7** (N = 26) 33.2** (N = 14)	31.9 (N = 14)**

**$P < 0.002$, *$P < 0.05$ significantly different from HN. The comparisons between early stroke and PN ($P = 0.63$), late stroke and PN ($P = 0.25$), early and late stroke ($P = 0.55$) were not significant.

Table 5. Connections forming part of the significant subnetwork in each comparison.

Connections that significantly decreased in early strokes when compared to HC. Connection between	*t*-stat	Connections that significantly increased in late stroke when compared to early stroke. Connection between	*t*-stat
R Caudate – R pars Orbitalis‡	2.01	Cingulate – L Caudate	2.04
L inferior Temporal – R Striate	2.01	R inferior Parietal – R Posterior Parietal	2.06
L superior Temporal – R pars Triangularis	2.02	Broca – Wernicke	2.09
R Caudate – R pars Opercularis*	2.04	L superior Frontal – R superior Parietal	2.11
L Caudate – R pars Orbitalis	2.11	R Cerebellum – R Extra Striate	2.14
L pars Orbitalis – R superior Parietal*	2.13	Cingulate – R inferior Parietal	2.14
L pars Orbitalis – R Striate	2.14	Cingulate – Broca	2.18
L superior Temporal – R superior Temporal‡	2.17	R Pars Opercularis – R pars Orbitalis	2.23
L superior Frontal – R ExtraStriate*	2.2	L Putamen/globus pallidus – R pars Orbitalis	2.25
L inferior Temporal – L Middle Frontal	2.26	L Putamen/globus pallidus – R superior Parietal	2.25
R posterior Parietal – R Striate	2.28	L Caudate – L superior Temporal	2.26
L superior Frontal – R inferior Parietal*	2.29	L superior Temporal – R inferior Parietal	2.26
L Caudate – R pars Opercularis	2.33	Cingulate – Wernicke	2.32
R inferior Parietal – R pars Opercularis	2.34	L Putamen/globus pallidus – R superior Temporal	2.35
L pars Orbitalis – R superior Temporal	2.35	L superior Frontal – L ventral Thalamus†	2.4
L superior Frontal – Wernicke*	2.36	Wernicke – R pars Triangularis	2.49
Wernicke – R inferior Parietal*	2.38	R posterior Parietal – R superior Parietal	2.52
L superior Frontal – L superior Temporal	2.41	Broca – R Posterior Parietal	2.58
L Middle Frontal – Wernicke	2.53	L Caudate – R Extra Striate	2.6
Cingulate – L pars Orbitalis	2.57	L superior Frontal – Wernicke	2.66
L superior Frontal – R superior Temporal	2.57	R inferior Parietal – R pars Triangularis	2.66
Cingulate – R superior Temporal	2.66	Broca – L inferior Temporal	2.72
R ExtraStriate – R posterior Parietal	2.75	Wernicke – R superior Temporal	2.73
Wernicke – R pars Opercularis*	3.11	R Cerebellum – R Striate	3
		L Caudate – R superior Parietal	3.18
		L superior Temporal – Wernicke	3.23
		L Putamen/globus pallidus – R inferior Parietal	3.57
		Wernicke – R Posterior Parietal	3.64
		L superior Frontal – R Extra Striate*	3.83
		L ventral Thalamus – R pars Orbitalis	4.04
		L ventral Thalamus – R pars Triangularis**	4.18

L, left; R, right; table lists all connections that form part of the subnetwork identified as significant by NBS for each comparison arranged by the *t*-statistic for each connection. Changes in connection strength from early stage to late stage ([score at late stage minus score at early stage]/score at early stage) correlated significantly with change in behavioral performance, specifically for connections from left ventral thalamus to the right pars triangularis region ($r = -0.62$, $P = 0.017$)**, and a trend toward significance from left superior frontal to left ventral thalamus ($r = 0.46$, $P = 0.09$)†. *Common connections between Tables 5 and Table 6 (connections that correlated with VF for HC). ‡For patients in the early stage, Pearson *r* correlations between VF performance and FC trended toward significance for connections between right caudate and right pars orbitalis ($r = 0.37$, $P = 0.06$, Pcorr = 0.13), and left superior temporal and right superior temporal regions ($r = 0.33$, $P = 0.09$, Pcorr = 0.06).

cated in language processing including tasks of phonemic and semantic fluency.[8,18] Neuroimaging studies have identified language pathways that connect the temporal regions to parietal, and parietal to frontal regions.[19] Subcortical structures such as the caudate are also involved in language production specifically in monitoring and controlling lexical and language alternatives.[9] Weakening of connections among these regions following stroke may have led to subtle deficits in language task performance.

Decreased FC in language regions seen in this study was accompanied by impaired performance on the VF task. Few of the connections that decreased were positively correlated with performance on the VF task in early stroke. However, our results also showed that majority of connections in HC that were maladaptive or negatively correlated with behavior, significantly decreased in strength in early strokes (Tables 5 and 6). This also provides evidence for adaptive reorganization even during early stages of stroke.

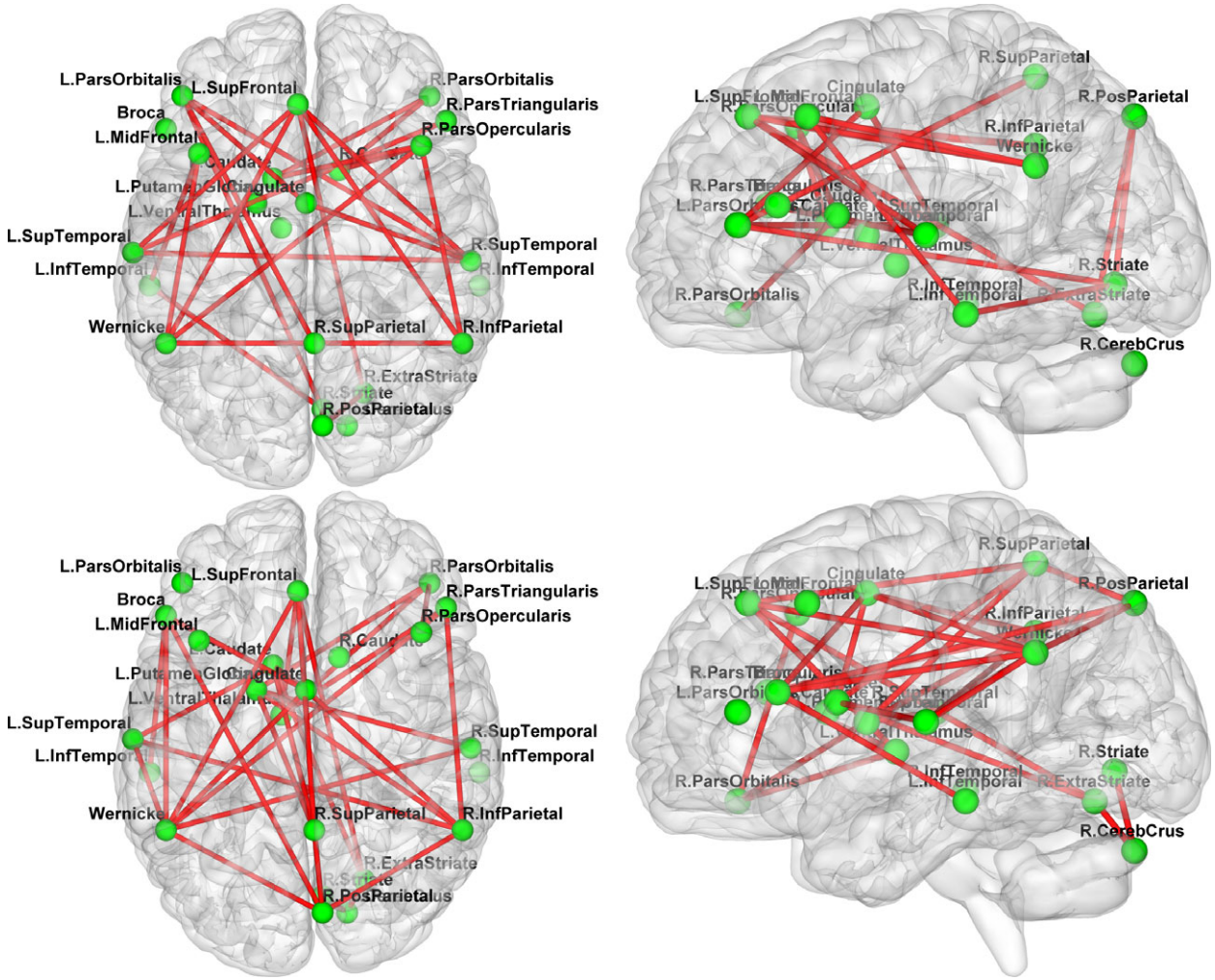

Figure 2. FC maps in the axial (left) and sagittal (right) sections. Top panel (A) shows subnetwork of connections more impaired in early stroke compared to HC. Bottom panel (B) shows subnetwork of connections that strengthened in late stroke when compared to early stroke patients. Node/Node labels that are further away appear indistinct. Left hemisphere is on left in axial view. FC, functional connectivity.

Late-stage strokes show rsFC patterns similar to HCs

Patients in our study were examined approximately at 4.5 months in the subacute phase during which time spontaneous recovery is expected to occur and activation patterns tend to normalize. A language subnetwork involving cortico-cortico and cortico-striatal connections was significantly stronger in late-stage compared to early-stage stroke patients. A few of these stronger connections involved seed ROIs in traditional left hemisphere language areas, suggesting that connections to and from these regions had recovered over time in these patients. Interestingly, nearly all of the subnetwork connections were different than the connections that showed significant decrease in strength in early stroke (when compared to HCs). One possibility is that there may be redundant connections which are easier to strengthen rather than connections that were initially affected by stroke. Two mechanisms of recovery have been suggested – recovery from *diaschisis* and concomitant reorganization.[20] According to the now well-accepted concept of *diaschisis,* originally proposed by von Monakow (as cited by Feeney and Baron[21]), preserved cortical areas adjacent to or distant, but functionally connected to the lesion, show impaired functioning likely due to hypometabolism in an area downstream of the injured location. Recovery from *diaschisis* may occur when there is additional input from other intact regions of the brain leading to recovery of function.[20] Reorganization of function by which perilesional areas or homologous regions in the undamaged hemisphere take over the specific function occurs in the

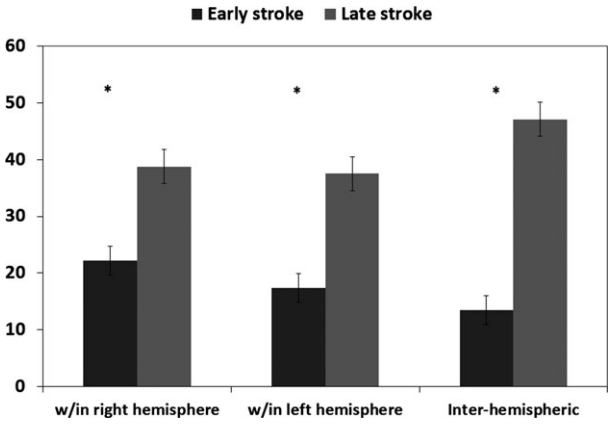

Figure 3. Within hemisphere and interhemispheric total connectivity scores in the subnetwork significantly different between early and late stroke. $P < 0.0002$.

Table 7. *P* values for raw and normed verbal scores and rsFC.

	PR	Early stroke	Late stroke
Verbal (average raw)			
HCs	0.02*	0.001**	0.001**
PR		0.63	0.25
Early stroke			0.55
VERBAL (normed score)			
HCs	0.03*	0.002**	0.002**
PR		0.70	0.28
Early stroke			0.41
Brain functional connectivity			
HCs	0.39	0.03*	0.38
PR		0.98	0.94
Early stroke			0.02*

HCs, healthy control subjects; PR, patients at risk; rsFC, resting-state functional connectivity.
$^{**}P \leq 0.01$, $^{*}P \leq 0.05$.

months following stroke which could explain the return to the normal-like brain FC pattern in the late stage. Although brain FC returned to normal-like pattern, late stroke patients continued to show reduced scores on the VF task. Notably, increase in FC strength correlated with both positive and negative changes in behavioral scores for some connections, suggesting that significant improvement in FC in specific connections in the late stage may be adaptive as well as maladaptive, respectively (Table 5).

PR versus stroke patients

Patients with risk factors for stroke were worse than HC but performed slightly better on the verbal task than early stroke patients, although there were no differences in brain FC in the language network. It is possible that the PR group was underpowered to detect any significant differences with stroke patients. Given that a significant percentage of patients with TIAs and other risk factors go on to have a stroke, it is important to carefully characterize network level changes in this group and identify predictors of cognitive function so that evidence-based models can be developed to increase awareness about treatable vascular conditions, develop focused treatment plans, and reduce the incidence of subclinical behavioral deficits.

We observed no significant differences in connectivity strengths between PR and HC in the language network. This is in contrast to a recent study that reported alterations in TIA in several resting functional networks such as default mode, dorsal attention, sensorimotor, visual, and auditory.[22] However, this latter study examined connectivity in 21 TIA-only patients, whereas our PR group had a mixture of patients with TIAs and other risk factors. Given additional evidence for changes in connectivity with aging[23] and in other groups at risk for stroke,[24] it is important to investigate network level changes in this group with a larger sample size.

Table 6. Correlation between connection strengths and behavioral scores for HCs.

Connection between	Pearson *r*	Significance *P*	*P* corrected (fdr)
L. Superior Frontal and Wernicke	−0.42	0.03*	0.11
R. Caudate and R. pars Opercularis	−0.51	0.007**	0.03*
L. Superior Frontal and R. Extra Striate	−0.41	0.04*	0.08†
L. Superior Frontal and R. Inferior Parietal	−0.43	0.03*	0.14
Wernicke and R. Inferior Parietal	−0.47	0.02*	0.06†
Wernicke and R. Pars Opercularis	−0.63	0.001**	0.005**
L. ParsOrbitalis and R. Superior Parietal	0.36	0.07†	0.06†

$^{**}P \leq 0.01$, $^{*}P \leq 0.05$; †$0.05 < P < 10$.

Stroke versus PR versus HCs

Alterations in both connectivity patterns and behavior were found in these different groups (Table 7). There was a decrease in connectivity strength in early-stage stroke compared to HC, but no significant differences in connectivity between early stroke and PR and between PR and HC. A similar pattern was seen in behavioral difference with early-stage stroke showing a significant deficit compared to HC, but no significant difference between

early stroke and PR, and a small but significant deficit in performance in PR when compared to HC. This suggests that these groups may lie on a continuum as to the level of impairment in brain and behavioral measures (i.e., early strokes more impaired than PR who are more impaired than HC). There seems to be gradual improvement in late-stage stroke with increase in strength compared to early-stage stroke with no significant difference in connectivity when compared to HC and PR, and no difference in behavior with PR but with residual behavioral deficit compared to normals. Changes in vasculature with healthy aging as well as vasculopathic changes (risk factors for stroke) may accelerate structural and functional changes that serve as a catalyst for cerebrovascular disease and eventually cognitive impairments.[25] Both stroke patients and PR are characterized by a background of vasculopathic changes as well as are on similar medications that may affect functional tissue and subsequently lead to macro- and microstructural changes in the brain.[24] Our study suggests that it is important to take into account these vasculopathic changes in stroke studies to isolate the disruption from acute stroke on brain and behavior, given that the background of chronic vasculopathic changes can also contribute to disruption of brain and behavior.[26]

Methodological considerations

A potential confound of age difference between the groups was eliminated by matching late strokes with controls on age, thus controlling for any differences in the groups that could be ascribed to age-related changes in vasculature and functional network connectivity. Study limitations include the heterogeneity in stroke location that could have differing impacts on language function. Given the small sample size for the PR, our study could be underpowered to detect a significant difference when compared to HC (although see recent stroke studies that reported recruitment of similar small control groups[27–30]). We also used only one task for assessing behavioral performance, given the inherent challenges of testing stroke patients in the early acute stage. Despite these limitations, our study has several strengths – there were no significant differences among the groups on sex, age, and handedness. By including patients with risk factors as controls, we also controlled for other sources of variation such as vasculopathic changes with age- and health-related factors. Although the lack of homogeneity in the PR group preclude any definitive conclusions regarding effect of actual lesion in the stroke patients vis-à-vis the PR, these results could be considered exploratory and future studies should study brain and behavioral changes employing a battery of language tests in a larger and more homogenous sample of stroke patients as well as PR for stroke.

Summary

Our results suggest that language network connectivity and function may be altered in stroke patients even with no overt clinically discernible language deficit and is important to investigate during early and late stages of recovery following stroke. Additionally, network-level connectivity pattern may be both adaptive and maladaptive to behavior suggesting that brain–behavior relationships need to be taken into account when evaluating and rehabilitating stroke patients.

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Author Contributions

V. N. was involved in study design, data collection, analysis, and writing. C. L., B. Y., J. S., and S. V. were involved in data collection and manuscript editing. S. V., P. R., T. N., and N. A. were involved in data analysis. J. L. S. was involved in patient recruitment and test administration. K. M., M. J., and M. C. were involved in patient referrals, and clinical documentation. J. S. was involved in patient referrals, clinical documentation, and manuscript editing. V. P. is the lead PI and was involved in study conception and design, manuscript editing, and supervised all aspects of the study.

Conflict of Interest

None declared.

References

1. Carter AR, Shulman GL, Corbetta M. Why use a connectivity-based approach to study stroke and recovery of function? NeuroImage 2012;62:2271–2280.
2. Park C, Chang WH, Ohn SH, et al. Longitudinal changes of resting-state functional connectivity during motor recovery after stroke. Stroke 2011;42:1357–1362.
3. Saur D, Lange R, Baumgaertner A, et al. Dynamics of language reorganization after stroke. Brain 2006;129:1371–1384.
4. Benton A, Hamsher K, Sivan A. Multilingual aphasia examination. Iowa City, IA: AJA; 1976.

5. Cox RW. AFNI: Software for analysis and visualization of functional magnetic resonance neuroimages. Comput Biomed Res 1996;29:162–173.
6. Jo HJ, Gotts SJ, Reynolds RC, et al. Effective preprocessing procedures virtually eliminate distance-dependent motion artifacts in resting state FMRI. J Appl Math 2013;21:2013.
7. Tomasi D, Volkow ND. Resting functional connectivity of language networks: characterization and reproducibility. Mol Psychiatry 2012;17:841–854.
8. Vigneau M, Beaucousin V, Herve PY, et al. Meta-analyzing left hemisphere language areas: phonology, semantics, and sentence processing. NeuroImage 2006;30:1414–1432.
9. Crinion J, Turner R, Grogan A, et al. Language control in the bilingual brain. Science 2006;312:1537–1540.
10. Price CJ. The anatomy of language: a review of 100 fMRI studies published in 2009. Ann N Y Acad Sci 2010;1191:62–88.
11. Whelan BM, Murdoch BE, Theodoros D, et al. Re-appraising contemporary theories of subcortical participation in language: proposing an interhemispheric regulatory function for the subthalamic nucleus (STN) in the mediation of high-level linguistic processes. Neurocase 2004;10:345–352.
12. Zalesky A, Fornito A, Bullmore ET. Network-based statistic: identifying differences in brain networks. NeuroImage 2010;53:1197–1207.
13. Zalesky A, Fornito A, Seal ML, et al. Disrupted axonal fiber connectivity in schizophrenia. Biol Psychiatry 2011;69:80–89.
14. Xia M, Wang J, He Y. BrainNet Viewer: a network visualization tool for human brain connectomics. PLoS One 2013;8:e68910.
15. Storey JD. A direct approach to false discovery rates. J Roy Stat Soc 2002;64:479–498.
16. Spreen OSE. A compendium of neuropsychological tests : administration, norms, and commentary. New York: Oxford University Press; 1998.
17. Ferguson CJ. An effect size primer: a guide for clinicians and researchers. Prof Psychol: Res Pract 2009;40:532–538.
18. Shirer WR, Ryali S, Rykhlevskaia E, et al. Decoding subject-driven cognitive states with whole-brain connectivity patterns. Cereb Cortex 2012;22:158–165.
19. Catani M, Jones DK, ffytche DH. Perisylvian language networks of the human brain. Ann Neurol 2005;57:8–16.
20. Marsh EB, Hillis AE. Recovery from aphasia following brain injury: the role of reorganization. Prog Brain Res 2006;157:143–156.
21. Feeney DM, Baron JC. Diaschisis. Stroke 1986;17:817–830.
22. Li R, Wang S, Zhu L, et al. Aberrant functional connectivity of resting state networks in transient ischemic attack. PLoS One 2013;8:e71009.
23. Antonenko D, Brauer J, Meinzer M, et al. Functional and structural syntax networks in aging. NeuroImage 2013 Dec;83:513–523.
24. Cheng H-L, Lin C-J, Soong B-W, et al. Impairments in cognitive function and brain connectivity in severe asymptomatic carotid stenosis. Stroke 2012;43:2567–2573.
25. Austin BP, Nair VA, Meier TB, et al. Effects of hypoperfusion in Alzheimer's disease. J Alzheimer's Dis 2011;26(suppl 3):123–133.
26. Mitchell GF, van Buchem MA, Sigurdsson S, et al. Arterial stiffness, pressure and flow pulsatility and brain structure and function: the Age, Gene/Environment Susceptibility–Reykjavik study. Brain 2011;134(Pt 11):3398–3407.
27. Fan F, Zhu C, Chen H, et al. Dynamic brain structural changes after left hemisphere subcortical stroke. Hum Brain Mapp 2013;34:1872–1881.
28. Wang LE, Tittgemeyer M, Imperati D, et al. Degeneration of corpus callosum and recovery of motor function after stroke: a multimodal magnetic resonance imaging study. Hum Brain Mapp 2012;33:2941–2956.
29. Warren JE, Crinion JT, Lambon Ralph MA, Wise RJ. Anterior temporal lobe connectivity correlates with functional outcome after aphasic stroke. Brain 2009; 132(Pt 12):3428–3442.
30. Zhu D, Chang J, Freeman S, et al. Changes of functional connectivity in the left frontoparietal network following aphasic stroke. Front Behav Neurosci 2014;8:167.

