Reduced Perfusion in Broca's Area in Developmental Stuttering

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Abstract: *Objective:* To study resting cerebral blood flow in children and adults with developmental stuttering. *Methods:* We acquired pulsed arterial spin labeling magnetic resonance imaging data in 26 participants with stuttering and 36 healthy, fluent controls. While covarying for age, sex, and IQ, we compared perfusion values voxel-wise across diagnostic groups and assessed correlations of perfusion with stuttering severity within the stuttering group and with measures of motor speed in both groups. *Results:* We detected lower regional Cerebral Blood Flow (rCBF) at rest in the stuttering group compared with healthy controls in Broca's area bilaterally and the superior frontal gyrus. rCBF values in Broca's area bilaterally correlated inversely with the severity of stuttering and extended posteriorly into other portions of the language loop. We also found increased rCBF in cerebellar nuclei and parietal cortex in the stuttering group compared with healthy controls. Findings were unchanged in child-only analyses and when excluding participants with comorbid illnesses or those taking medication. *Conclusions:* rCBF is reduced in Broca's region in persons who stutter. More severe stuttering is associated with even greater reductions in rCBF to Broca's region, additive to the underlying putative trait reduction in rCBF relative to control values. Moreover, a greater abnormality in rCBF in the posterior language loop is associated with more severe symptoms, suggesting that a common pathophysiology throughout the language loop likely contributes to stuttering severity. *Hum Brain Mapp 00:000–000, 2016*.

Key words: stuttering; developmental stuttering; pulsed arterial spin labeling; Broca; perfusion

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INTRODUCTION

Stuttering is a disorder of speech fluency. Manifestations include repetition of a sound, syllable, or one-syllable word, silent or audible prolongation, or both [Wingate, 1964]. The lifespan incidence of stuttering is approximately 5%, though recent evidence suggests that it may be higher. The average lifespan prevalence is about 1%, though it may be lower per recent data [Yairi and Ambrose, 2013]. Onset is typically in early childhood, with gradual attenuation in the frequency and severity of stuttering ultimately leading to recovery years later. Adult persistence is noted in less than 25% of those affected [Yairi and Ambrose, 1999]. Anatomical Magnetic Resonance Imaging (MRI), task-based functional MRI (fMRI), and resting state fMRI investigations of stuttering have noted abnormalities in widely distributed brain regions [Chang et al., 2009; Choo et al., 2011; Kell et al., 2009; Liu et al., 2014; Lu et al., 2012].

Task-based fMRI studies of stuttering have several limitations. First, the only brain regions interrogated are those that subserve the task. Second, the scientific utility of the activation map depends on selection of a pathogenically relevant activation paradigm. Third, participants of differing ages in task-based fMRI studies often use different strategies to perform the task, or they differ systematically in their cognitive and emotional responses to variations in task performance, creating systematic age-related differences in activation that have little or no relevance to the task itself [Horga et al., 2014]. The effects of differences in task performance therefore are confounded with age effects on task-based fMRI activation.

In contrast, measures of regional cerebral blood flow (rCBF) are a convenient surrogate measure of brain activity because blood flow is typically tightly coupled with neural activity. Consequently, rCBF measures can be acquired at rest, independent of any activation paradigm, making them potentially more useful than task-based fMRI for developmental studies; moreover, they are available from almost all brain regions simultaneously, at least when using modern measurement techniques. To the best of our knowledge, no prior studies have reported perfusion-based measures of rCBF in developmental stuttering.

Based on theoretical models and findings in prior brain imaging studies of stuttering [Beal et al., 2013, 2015; Bode et al., 2011; Cai et al., 2014; Chang et al., 2015; Cykowski et al., 2010; Lu et al., 2010; Salmelin et al., 2000; Sommer et al., 2002; Wu et al., 1995], we had strong *a priori* hypotheses about the primacy of Broca's region and, secondarily, of related regions in the language circuit in the pathogenesis of developmental stuttering. We hypothesized that we would detect reduced perfusion in brain regions involved in various stages of speech production, including sensory feedback, phonological encoding, and motor planning and programming.

METHODS

Participants

We recruited 26 participants with developmental stuttering via advertisements posted on the internet and at local clinics, hospitals, and stuttering support groups. A licensed speech-language pathologist diagnosed all participants with stuttering prior to enrollment in the study. We administered Kiddie-Schedule for Affective Disorders and Schizophrenia, Present and Lifetime Version to diagnose comorbid disorders in stuttering participants under age 18 [Kaufman et al., 1997]. We administered the Structured Clinical Interview for DSM-IV-TR Axis I Disorders to diagnose comorbid disorders in stuttering participants over 18 [First et al., 2003]. We allowed inclusion of participants with comorbidities because these are highly prevalent in stuttering speakers in general, and excluding those with comorbid illnesses would have impaired the generalizability of our findings. We evaluated stuttering severity on the day of MRI scan using the Assessment of the Child's Experience of Stuttering (ACES) for children [Yaruss et al. 2006], and the Overall Assessment of the Speaker's Experience of Stuttering (OASES) for adults [Yaruss and Quesal, 2006]. Stuttering severity ranged from mild to severe, with an average severity of 46.8, in the moderate range. We recruited 36 fluent controls randomly from a telemarketing list of 10,000 names in the local community, excluding those with lifetime Axis I disorders or any language disorders, and group-matching to the stutterers on age and sex.

In all participants we estimated IQ using the Wechsler Abbreviated Scale of Intelligence [Wechsler, 1981] the Edinburgh Inventory to measure the laterality of handedness [Oldfield, 1971], and Hollingshead's four-factor index to measure socioeconomic status [Hollingshead, 1975]. Participants, both stutterers and fluent controls, performed several other neuropsychological tasks, including three that are thought to index one or more aspects of cerebral lateralization (desirable because language is thought to be lateralized): (1) A finger-tapping task, which involved pressing a key on a personal computer (PC) with the index finger as rapidly as possible in a 10-second period; each participant underwent a total of 10 trials using the dominant and nondominant hands alternately [Reitan and Wolfson, 1985]. (2) A line bisection task using the dominant and nondominant hands separately, and a motor-free assessment using visual inspection, as previously described [Jewell and McCourt, 2000]. (3) The Purdue pegboard test [Tiffin and Asher, 1948]. Additional exclusionary criteria for all participants were: a history of premature birth, prior head trauma with loss of consciousness, past seizures, mental retardation, pervasive developmental disorders, or chronic medical illness. The Institutional Review Board of the New York State Psychiatric Institute and Children's Hospital Los Angeles approved the study procedures. We obtained written informed consent from all adult participants and the

parents of child participants. Children also provided written assent.

MRI Scanning

Images were acquired on a GE Signa 3T (Tesla) HDx system with an 8-channel, receive-only head coil. We optimized our Pulsed Arterial Spin Labeling (PASL) perfusion sequence for parallel imaging at 3T using a PICORE (Proximal Inversion with Control for Off-Resonance Effects) QUIPSS II (Quantitative Imaging of Perfusion using a Single Subtraction) pulse sequence [Wong et al., 1998]. We placed a 9-cm tagging slab 16-mm below the proximal edge of the imaging volume. To control for the off-resonance effects of the inversion pulses used for acquiring the labeled images, we acquired the control images by applying off-resonance adiabatic hyperbolic secant RF pulse with the same frequency offset as that for the labeled images without the slice-selective gradient. We used a single-shot gradient-echo EPI (echo planar imaging) sequence for image acquisition, with time to QUIPSS saturation TI1 = 600 ms, and inversion time of the first slice TI2 = 1,300 ms. Acquisition parameters were: FOV 24 cm, 64 × 64 matrix, TE(echo time)/TR (repetition time)- = 24/2,300 ms, flip angle 90°, slice thickness 6 mm, inter-slice spacing 0.5 mm, for a nominal spatial resolution of 3.75 × 3.75 × 6.5 mm. We acquired 18 slices from inferior to superior in sequential order. Each ASL scan with 151 acquisitions plus 5 dummies required 5 min 59 sec. We used gradient-echo EPI with TR = 15 sec at the same resolution and slice positions as the ASL scan to quantify signal from white matter (M_{0_WM}) [Wong et al., 1998].

Image Preprocessing

In native imaging space we aligned the PASL brain images and the M_{0_WM} image to the first PASL image for each participant to correct for head motion. We subsequently smoothed the coregistered PASL images using a Gaussian kernel of 6-mm Full Width at Half Maximum (FWHM) to remove spatial noise and enhance the signal-to-noise ratio in the images. The choice of a moderate 6-mm FWHM spatial filter was based on our desire to avoid loss of spatial precision in locating our effects of interest. We generated a brain mask for each participant based on the mean PASL image.

Image Analysis

Maps of rCBF

For each participant we constructed a voxel-wise map of rCBF from the PASL time series and M_{0_WM} image using in-house software, as follows: (1) We pair-wise subtracted the control images from the labeled images; (2) From the average of the subtracted images, we calculated rCBF at each voxel using the following equation: $\mathrm{rCBF} = \frac{\Delta I}{2 * M_{0\_B} * TI_1 * e^{-TI_2/T_{1B}} * \alpha}$ where ΔI is the image difference obtained in step 1; α is the tagging efficiency, for which we used the default value of 1.0; and T_{1B} is the T_1 of blood, for which we used the default value of 1,664 ms [Lu et al., 2004]; M_{0_B} is the MR signal from a voxel filled with arterial blood, which was estimated from the M_{0_WM} map according to $M_{0\_B} = rM_{0\_WM}\, e^{(1/T_{2WM} - 1/T_{2B})TE}$ where r is the proton density ratio of blood, for which we used the default value of 1.06; and where the default values for T_{2WM} and T_{2B} were 80 and 200 ms, respectively [Alsop and Detre, 1996; Jarnum et al., 2010; Wong et al., 1998].

Statistical analysis of rCBF maps

We used SPM-8 (Statistical Parametric Mapping) to coregister the anatomical brain images to the corresponding mean rCBF images for each participant using a 6 degrees-of-freedom linear transformation. We then coregistered the rCBF images for each participant to the canonical avg152T1_brain template, resampled with a voxel size of 3 × 3 × 3 mm^3 in SPM, using each participant's anatomical brain image as an intermediary source. The canonical avg152T1_brain is an average template derived from MRIs of 152 healthy persons (18.5–43.5 years of age, average 24.5 years) in the International Consortium for Brain Mapping database [Fonov et al., 2011; Mazziotta et al., 1995].

First-Level Maps of Normalized rCBF

For each participant, we averaged the spatially normalized rCBF maps across time, which produced a mean rCBF map. We converted this mean rCBF map into a Z-score map by subtracting from the rCBF value at each voxel the global mean across all voxels of the mean rCBF map and then dividing by the standard deviation across all voxels of the mean rCBF map. The purpose of transforming the raw mean rCBF map into a Z-score rCBF map was to provide rCBF maps that have the same mean and standard deviation across individuals, which emphasizes the location of individual rCBF values across the brain and improves our statistical power to detect effects of interest within and between diagnostic groups. It also obviated the need to include global CBF measures as covariates in statistical analyses.

Second-Level Maps of Group Differences

All group-level statistical analyses employed a general linear model (GLM), implemented in SPM8, at each voxel of the spatially normalized, individual Z-score rCBF maps, while covarying for age and sex. Our primary *a priori* analysis compared rCBF across diagnostic groups. To correct for multiple comparisons across voxels, we used Monte Carlo simulations (10,000 iterations) implemented in customized, MATLAB-based software to determine the cluster extent threshold (55 voxels in resampled space, assuming an individual voxel type I error of $P = 0.05$) that yielded a

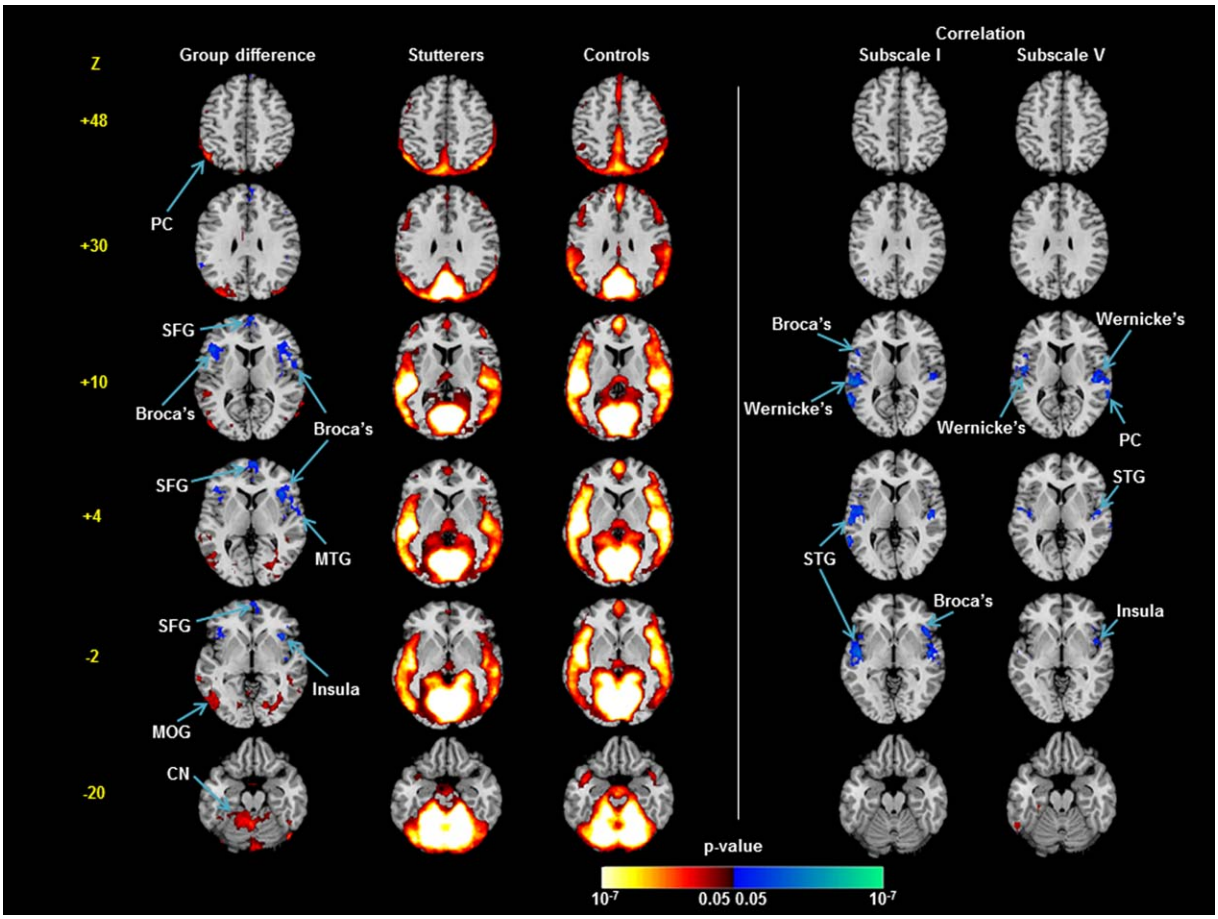

Figure 1.

Group differences and severity correlations. The right sides of the images correspond to the right side of the brain. Left Panel: The first column represents the statistically significant differences in rCBF Z-score perfusion values between the stuttering group and the fluent controls while covarying for age and sex of the participants, displayed at a threshold of $P < 0.05$ after correction for multiple comparisons. Voxels in blue indicate reduced perfusion values in the stuttering group, and voxels in red indicate increased perfusion values, relative to controls. Perfusion values at rest in the stuttering group were lower bilaterally in Broca's area and the superior frontal gyrus, and higher in cerebellar nuclei and the parietal cortex, compared with fluent controls. The second and third columns show the color-coded maps of P-value significance levels comparing individual-level rCBF Z-score perfusion values against a value of zero in a general linear model applied within the stuttering and control groups separately, thresholded at $Z = 1.65$, corresponding to $P = 0.05$ after correction for multiple comparisons. The vertically aligned z-values at the left side of the figure represent slice level (in millimeters) in the Montreal Neurological Institute coordinate system. Right Panel: Voxels in blue represent inverse correlations, and voxels in red represent positive correlations, between rCBF Z-score perfusion values and stuttering severity scores, displayed at a threshold of $P < 0.05$ after correction for multiple comparisons. Perfusion values in Broca's area correlated inversely with the severity of stuttering. Stuttering severity scale I incorporates information from stuttering persons about the severity of their symptoms as well as their general knowledge of stuttering; Scale V is a total severity score, the sum of scales I–IV. Abbreviations: PC, parietal cortex; SFG, superior frontal gyrus; MTG, middle temporal gyrus; MOG, middle occipital gyrus; CN, cerebellar nuclei. [Color figure can be viewed at wileyonlinelibrary.com]

corrected *a priori* significance threshold of $P < 0.05$ in all of our Z-score rCBF maps [Forman et al., 1995]. This between-group statistical map is shown under the column heading of "Group difference" in Figure 1. As an aid for understanding the group difference maps, we also created maps comparing Z-score rCBF values within each diagnostic group against a value of zero, indicating where in the brain the normalized Z-score rCBF values were significantly nonzero. These within-group statistical maps are shown under the column headings of "Stutterers" and "Controls"

TABLE I. Participant characteristics

	Participants		Group Comparison	
	Stutterers ($n = 26$)	Control ($n = 36$)	Test statistic	P-value
Age range, years	5–51	6–50		
Age, years (mean ± SD)	17.21 ± 11.73	21.76 ± 11.47	$t = 1.52$, $df = 60$	0.13
Age [n (%) children][a]	20 (77%)	19 (53%)	$x^2 = 3.77$, $df = 2$	0.15
WASI IQ (mean ± SD)	105.19 ± 17.36	116.47 ± 14.48	$t = 2.78$, $df = 60$	0.01
Sex [n (%) male]	15 (58%)	20 (56%)	$x^2 = 0.03$, $df = 2$	0.99
Ethnicity [n (%) Caucasian]	18 (69%)	27 (75%)	$x^2 = 0.25$, $df = 2$	0.88
Handedness [n (%) right]	18 (69%)	30 (83%)	$x^2 = 1.72$, $df = 2$	0.42
Socioeconomic status (mean ± SD)[b]	41.38 ± 15.07	47.75 ± 14.64	$t = 1.67$, $df = 60$	0.10
Stuttering severity (range; mean ± SD)[c]	25.62–72.12; 46.77 ± 10.95			
Taking medications, n (%)[d]	2 (8%)			

Abbreviations: WASI, Wechsler Abbreviated Scale of Intelligence.
[a]Child = Less than 20 years of age, Adult = 20 years or older.
[b]Socioeconomic status data were not available for 6 stutterers and 8 controls.
[c]Severity was based on the Impact Rating (total score) from the Assessment of the Child's Experience of Stuttering for child stutterers, and the Overall Assessment of the Speaker's Experience of Stuttering for adult stutterers. Severity ratings were missing in 4 children and in 2 adult stutterers.
[d]One stuttering speaker on antidepressants and 2 on psychotropic medications at the time of the scan. Medications were not mutually exclusive.

in Figure 1. They were used to indicate visually whether the statistically significant differences in the between-group comparison maps (the first column in Fig. 1) derived from larger or smaller overall Z-score rCBF values in one group or the other (the second and third columns in Fig. 1).

RESULTS

Sample Characteristics

Our groups were generally well matched on demographic characteristics, including age, sex, socioeconomic status, ethnicity, and handedness (Table I). However, controls had slightly higher, but statistically significant, full scale IQ scores than did the stutterers. Five stuttering participants had one comorbid disorder each, including attention deficit hyperactivity disorder combined type ($N = 2$), social anxiety disorder ($N = 1$), chronic motor tics ($N = 1$), and mild Tourette syndrome ($N = 1$).

Group Comparison of Perfusion Values

rCBF values at rest were lower in Broca's area ($P < 0.0043$) and the superior frontal gyrus ($P < 0.0015$) in the stuttering group compared with fluent controls ($P < 0.0001$) (Table II). Values were higher in cerebellar nuclei ($P < 0.0002$) and the parietal cortex in the stuttering group ($P < 0.0001$). Our findings remained consistent when including only children (age < 20) in the analyses (Supporting Information Fig. e-1 and Supporting Information Table e-1). rCBF values in the basal ganglia did not differ significantly between the two groups, although resting rCBF values in the basal ganglia were low in both groups, limiting our ability to detect group differences.

Within-Group Correlations

rCBF values in Broca's area correlated inversely with the severity of stuttering measured using subscales I and V (total severity) of the ACES and OASES ($r = 0.67$; $df = 25$; $P < 0.0001$) (Fig. 1) and were consistent with correlations for the other subscales as well (not shown). The findings remained unchanged in analyses of children alone. Inverse correlations of perfusion with severity of stuttering extended posteriorly into the arcuate fasciculus within the superior temporal and inferior parietal cortex, and into Wernicke's area.

Potential Confounds

Our findings did not change upon statistical covariation for IQ. Additionally, our findings remained consistent when excluding the 5 stuttering participants who had comorbid illnesses or when excluding 2 stuttering participants who were taking medications.

Neuropsychological Measures

Thirty-eight participants (22 stutterers and 16 fluent controls) from the 62 total participants underwent neuropsychological testing. Post-hoc analyses for these 38 participants revealed positive correlations of rCBF with finger tapping speed for the dominant hand in contralateral sensory and motor language areas (left inferior temporal, left insula, left middle temporal), while covarying for age, sex, and diagnostic

TABLE II. Locations of statistically significant group differences identified in Figure 1

Brain region	Brodmann's Area	Hemisphere	Cluster size	MNI coordinates of local maxima			T-statistic	P-value (df = 60)
				x	y	z		
Broca's	BA-44	Right	490	48	32	4	3	0.0019*
				54	11	13	2.8	0.0034
				51	14	10	2.75	0.0039
		Left	252	−45	23	10	3.46	0.0005*
				−48	17	19	3.37	0.0006
				−51	20	16	3.22	0.001
Parietal cortex	BA-39	Left	1046	−48	−61	49	4.76	0.0001*
Middle occipital gyrus	BA-19	Left	1046	−42	−85	16	3.68	0.0002*
				−33	−88	28	3.66	0.0002
				−48	−82	7	3.53	0.0004
Superior frontal gyrus	BA-10	Right	311	3	65	7	3.66	0.0002*
		Left	311	0	53	40	2.71	0.0043*
Insula	BA-45	Right	490	39	23	4	3.86	0.0001*
				36	20	1	3.63	0.0002
				36	26	7	2.79	0.0035
Middle temporal gyrus	BA-21	Right	194	51	−43	−5	2.96	0.0022*
				60	−58	−2	2.44	0.0088
Cerebellar nuclei	NA	Left	2289	−21	−76	−44	4.76	0.0001*
				−21	−64	−29	3.4	0.0006

Only clusters in which *P*-values survive correction for multiple comparisons at $P < 0.05$ are shown. The *P*-values listed are uncorrected. Cluster size is number of voxels in resampled space. The local maxima represent the peak values within the corresponding brain regions. The *P*-values with an asterisk represent the regional maxima.
NA, Not applicable.

group ($P < 0.01$) (Fig. 2). We also detected significant inverse correlations of rCBF with finger tapping speed for the dominant hand in bilateral sensorimotor areas cephalad to the language regions (right operculum, left rolandic, right parietal cortex) ($P < 0.01$) (Fig. 2). We did not detect significant correlations for finger tapping speed in the nondominant hand or for scores on the Purdue pegboard or line bisection tasks.

DISCUSSION

This study is, to our knowledge, the first to use perfusion MRI to investigate differences in brain activity in persons who stutter. We found reduced rCBF at rest in Broca's area bilaterally and the superior frontal gyrus in the stuttering group compared with healthy controls. rCBF values in Broca's area correlated inversely with the severity of stuttering and extended posteriorly into other portions of the language loop. We also found increased rCBF in the stuttering group compared with healthy controls in cerebellar nuclei and the parietal cortex. All findings were unchanged in children-only analyses. The consistency of findings across ages suggests that reduced rCBF in Broca's region may represent a stable trait vulnerability, without which stuttering may not manifest. The inverse correlations of perfusion with stuttering severity suggest that more severe stuttering is associated with even greater reductions in rCBF to Broca's region and posterior portions of the language loop, additive to the underlying putative trait reduction in rCBF relative to control values.

Broca's area is a key component of the neural network for speech production. Cortical stimulation studies in monkeys and humans point to a critical role for Broca's in the planning of fine movements necessary for speech production [Luders et al., 1995]. Additionally, intracranial recordings have documented its participation in phonological and syntactic processing, and in lexical retrieval [Sahin et al., 2009]. Reduced rCBF in Broca's region could therefore disrupt motor planning, phonological and syntactic processing, and lexical retrieval, leading to speech dysfluency. Severity correlations extended posteriorly into adjacent tissue of the arcuate fasciculus, suggesting that when tissue disturbances present in Broca's region extend posteriorly into the arcuate fasciculus, function of the language circuit worsens to produce more severe symptoms.

Post-hoc analysis correlating rCBF with measures of motor performance in all participants supported interpretations of our primary findings. Positive correlations of finger tapping speed using the dominant hand with rCBF in contralateral sensory and motor language areas suggests that better motor performance accompanies greater rCBF in language regions, and that reduced rCBF in language regions in stutterers represents relative impairment in

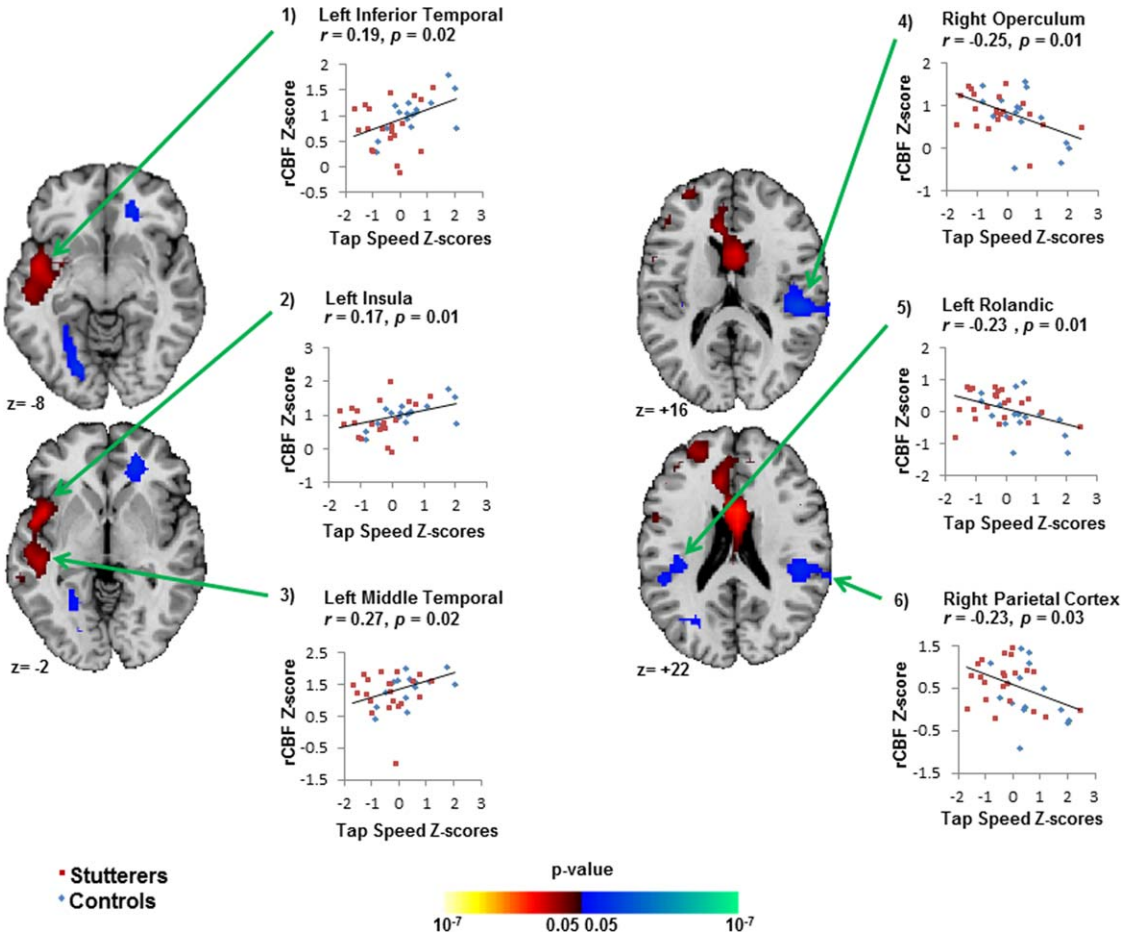

Figure 2.

Correlations of perfusion values with dominant hand finger tapping speed. These correlation analyses included 38 participants (22 stutterers and 16 fluent controls, those who had finger tapping scores from the 62 total participants). The maps reveal positive correlations of rCBF Z-score values with Z-scores for finger tapping speed in the dominant hand that were located in contralateral sensory and motor language areas (Fig.2). The correlations covaried for age, sex, and diagnostic group, and are displayed at a threshold of $P < 0.05$ after correction for multiple comparisons. We also detected significant inverse correlations of perfusion with finger tapping speed for the dominant hand in bilateral sensorimotor areas cephalad to the language regions. Scatterplots for correlations at selected voxels are displayed to demonstrate that the correlations are not driven by outliers. Shown above each scatterplot are the Pearson's correlation coefficient (r) and associated P-value. The rCBF Z-score values in the scatterplots are adjusted for age, sex, and diagnostic group. Abbreviation: rCBF, regional cerebral blood flow. [Color figure can be viewed at wileyonlinelibrary.com]

planning and execution of not only speech actions, but also simple sensorimotor processes more generally. Inverse correlations of rCBF with finger tapping speed for the dominant hand in bilateral sensorimotor areas cephalad to the language regions suggests that speed on this simple motor task is greater in persons who require relatively less rCBF in sensorimotor cortices to generate comparable levels of motor performance and who therefore are more efficient in neural processing within their sensorimotor cortices.

Our findings are generally consistent with other neuroimaging studies that have reported abnormalities in Broca's region in stuttering, including altered gray and white matter composition [Beal et al., 2013, 2015; Lu et al., 2010], reduced fractional anisotropy on diffusion tensor imaging (DTI) [Cai et al., 2014; Chang et al., 2015; Cykowski et al., 2010; Sommer et al., 2002], decreased connectivity on fMRI [Bode et al., 2011], decreased glucose metabolism on fluorodeoxyglucose-positron emission tomography (FDG-PET) [Wu et al., 1995], and aberrant activation on magnetoencephalography (MEG) [Salmelin et al., 2000]. Several of these prior studies have reported predominantly lateralized findings [De Nil et al., 2000, 2003; Fox et al., 2000; Ingham et al., 2004]. A seminal PET study, for example, reported increased activation in the

right hemisphere in stuttering adult men [Fox et al., 1996], consistent with the longstanding theory that abnormal hemispheric lateralization is the cause of developmental stuttering [Kushner, 2012]. Several imaging studies have subsequently suggested, however, that lateralized activation could represent a compensatory brain response to the presence of stuttering [Braun et al., 1997; Preibisch et al., 2003; Sowman et al., 2014]. We suspect that we detected significant reductions in rCBF to Broca's region bilaterally, rather than unilaterally, because our findings represent a trait-like vulnerability to stuttering, rather than a compensatory response to its presence. This interpretation is consistent with findings from a prior anatomical imaging study that reported bilateral trait-like abnormalities in the language system in both symptomatic and recovered children with developmental stuttering [Chang et al., 2008].

Animal studies have provided indirect evidence of the key role of speech motor areas in learning and production of stereotyped song, with dysfunction in these regions leading to song dysfluency. Song speed slows if the premotor nucleus of the High Vocal Center (HVC, the songbird equivalent to Broca's area) is cooled [Long and Fee, 2008]. Bilateral ablation of the medial HVC induces a positive disruption of song (an increase in atypical sequences of syllables), whereas bilateral ablation of the lateral HVC induces a negative disruption (omission of one or more syllables) [Basista et al., 2014]. Bilateral lesions of the thalamic nucleus Uvaeformis (Uva) in songbirds prevent proprioceptive feedback from reaching the HVC and affects the timing and sequencing of note delivery but not the production of sounds, reinforcing belief that the learned patterns for these syllables reside in the HVC and RA (robustus archipallium, the equivalent of face-motor cortex) [Nottebohm and Liu, 2010]. The HVC contains inhibitory interneurons and two classes of excitatory neurons that project to the RA and Area X (a basal ganglia equivalent) [Mooney and Prather, 2005]. Excitatory projection neurons during song production fire in phasic bursts that are tightly and precisely locked to song motifs, while the interneurons are active throughout the song [Kozhevnikov and Fee, 2007]. Lower rCBF in Broca's area in our stuttering participants, when interpreted most parsimoniously in light of these animal models of song dysfluency, likely represents reduced firing of excitatory neurons that produces speech dysfluency.

Intraoperative subcortical mapping with electrical stimulation has recently identified the frontal aslant tract, a deep frontal pathway that connects Broca's area to the superior frontal gyrus, which is responsible for speech initiation and spontaneity. Lesions of this tract produce speech slowing and arrest [Fujii et al., 2015]. Our findings of decreased rCBF in the region of superior frontal gyrus may reflect tissue disturbances in the aslant tract. Neuroimaging and lesion studies suggest that the cerebellar nuclei contribute to speech motor control [Marien et al., 2014]. Increased cerebellar rCBF in our stuttering group could represent a compensatory response for the presence of motor-based dysfluency based within Broca's region and related language circuits. Similarly, increased rCBF in parietal cortex may represent the use of higher-order, multisensory integration processes to compensate for dysfluencies in our stuttering group, given that functional MRI studies have shown extensive connections of parietal cortex with the primary sensorimotor areas involved in speech production [Fuertinger et al., 2015]. Abnormal rCBF in areas beyond the sensorimotor language systems suggests the involvement of networks beyond the key anatomical areas responsible for normal speech production.

Our study has several limitations. First, the age range of stuttering participants was wide. We do not believe that the wide age range compromised our findings because our statistical analyses covaried for age, and our results were unchanged when including only children in the analyses. Moreover, we included both children and adults by design, so as to aid identification of trait-like abnormalities in rCBF, which should be age-independent. Second, we studied these language-based systems at rest, not when taxing them with a relevant task. Again, however, this was by design, because resting measures are more appropriate for developmental studies, as they do not conflate measures of task performance with age or with trait abnormalities. Third, the pulsed ASL sequence used for image acquisition employed gradient recalled Echo Planar Imaging (ge-EPI). This sequence produces signal drop-out in several key areas, including orbitofrontal cortex and inferior basal ganglia. Future ASL studies in stuttering populations should employ pulse sequences that do not suffer from this localized signal drop-out. Fourth, we did not register the images of our children and adult participants to separate, age-appropriate templates [Fonov et al., 2011], as doing so would have precluded analysis of our data across all participants in our study. Moreover, the template we used is an average from 152 mostly young adult participants; the averaging introduces smoothing that should render inconsequential the distinction of this template from a pediatric template. Finally, we assessed the severity of stuttering based on the ACES and OASES, subjective measures of sustained dysfluency over time, rather than on potentially more transient measures of dysfluency made from short-duration speech samples. Nevertheless, future studies of stuttering should include objective measures of dysfluency.

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CONTRIBUTIONS

Conceived and designed the experiments: BSP. Performed the experiments: RV, BSP. Analyzed the data: JD, YH, ZW, RB, BSP. Contributed reagents/materials/analysis tools: SCRW, DL, FOZ, BSP. Wrote the first draft: JD, BSP. Edited the article: YH, ZW, RB, SCRW, DL, FOZ.

DISCLOSURE OF FINANCIAL RELATIONSHIPS AND CONFLICTS OF INTERESTS

JD is on the editorial board of Journal of Child Neurology and Pediatric Neurology, and has received grants from Epilepsy Foundation of Greater Los Angeles.

YH has nothing to disclose.

ZW has nothing to disclose.

FOZ is a co-investigator on research projects funded by the UK Medical Research Council, the UK Biotechnology and Biological Sciences Research Council, Diabetes UK, the British Heart Foundation and Unilever UK.

SCRW has nothing to disclose.

RB has nothing to disclose.

DL has acted as a consultant for Ixico PLC.

BSP is a joint editor of the Journal of Child Psychology and Psychiatry; holds 3 patents (application numbers 61/601,772, 8,143,890B2, 61/424,172); and has received support from NIDA (1R01DA027100), NIMH (2T32MH16434, 1P50MH090966, 5R01MH36197, 1R01MH093677, 1R21MH101564), NIEHS (4R00ES020364).

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