Evidence of Left Inferior Frontal–Premotor Structural and Functional Connectivity Deficits in Adults Who Stutter

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The neurophysiological basis for stuttering may involve deficits that affect dynamic interactions among neural structures supporting fluent speech processing. Here, we examined functional and structural connectivity within corticocortical and thalamocortical loops in adults who stutter. For functional connectivity, we placed seeds in the left and right inferior frontal Brodmann area 44 (BA44) and in the ventral lateral nucleus (VLN) of the thalamus. Subject-specific seeds were based on peak activation voxels captured during speech and nonspeech tasks using functional magnetic resonance imaging. Psychophysiological interaction (PPI) was used to find brain regions with heightened functional connectivity with these cortical and subcortical seeds during speech and nonspeech tasks. Probabilistic tractography was used to track white matter tracts in each hemisphere using the same seeds. Both PPI and tractography supported connectivity deficits between the left BA44 and the left premotor regions, while connectivity among homologous right hemisphere structures was significantly increased in the stuttering group. No functional connectivity differences between BA44 and auditory regions were found between groups. The functional connectivity results derived from the VLN seeds were less definitive and were not supported by the tractography results. Our data provide strongest support for deficient left hemisphere inferior frontal to premotor connectivity as a neural correlate of stuttering.

Keywords: DTI tractography, functional connectivity, motor, psychophysiological interaction (PPI), speech perception, speech production, stuttering

Introduction

Developmental stuttering affects 5% of preschool children and occurs in 1% of adults (Bloodstein 1995). Stuttered speech is characterized by unintended occurrences of sound syllable repetitions, prolongations, and blocks, particularly on the initial part of words and sentences. Compared with stuttering, speech production is a deceptively easy skill for people who do not stutter, and learning to produce fluent speech is achieved without much conscious effort. For most, speech becomes an automatic and highly efficient skill by adolescence (Smith and Zelaznik 2004). The motor preparation and execution for fluent speech requires the development of well-integrated corticocortical and basal ganglia–thalamocortical speech motor and perceptual systems for proficient speech production ability (Hickok and Poeppel 2004; Riecker et al. 2005; Guenther 2006; Ghosh et al. 2008). Most studies that have examined the neural correlates of stuttering suggest that the underlying deficits may be those which affect dynamic interactions among cortical and subcortical systems that support speech motor planning, initiation, and execution (Ludlow 2000; Watkins et al. 2008; Lu, Chen, et al. 2010).

Several types of connectivity deficits have been suggested in stuttering. First, positron emission tomography and functional magnetic resonance imaging (fMRI) studies have shown that during stuttering, different patterns of brain activation occur in the left motor and auditory regions, with increased activation in the right hemisphere in comparison with fluent speakers (Fox et al. 1996; Braun et al. 1997). Studies in normal speakers have shown that when their auditory feedback is perturbed, compensatory motor adjustments occur along with the posterior superior temporal gyrus (pSTG) being activated bilaterally (Hashimoto and Sakai 2003). One hypothesis, then, might be that auditory feedback is abnormally integrated with the speech motor execution regions in stuttering speakers. Rather than feedback-based processing, however, a more efficient feedforward processing mechanism is proposed for fluent speech processing, which relies on inferior frontal to motor connectivity (Golfinopoulos et al. 2010). According to this model, left inferior frontal to motor connectivity may be affected in stuttering. Others have proposed that such a disruption may affect feedforward processing in stuttering, leading to overreliance on feedback-based speech production, in turn resulting in disfluency (Max et al. 2004; Civier et al. 2010). Using magnetoencephalography during single word reading, fluent speakers have activity changes first in the inferior frontal region, which are then followed by changes in the primary motor area. However, the reverse order in sequences of brain activity occurred in stuttering adults even when they were reading fluently (Salmelin et al. 2000). This may suggest that stuttering speakers have abnormal connectivity between the inferior frontal and the motor areas for speech production that persist even during fluent speech.

Diffusion tensor imaging (DTI) studies have suggested that anatomical deficits in the white matter tracts underlying the primary oral motor areas in the left hemisphere are present in both children and adults who stutter. Decreased fractional anisotropy in the left superior longitudinal fasciculus (SLF) underlying the inferior frontal and motor regions has been found in several studies (Sommer et al. 2002; Chang et al. 2008;
Watkins et al. 2008). On the other hand, a recent study found high functional connectivity between the thalamus and the posterior middle temporal gyrus (MTG) and the presupplementary motor area (preSMA) in stuttering subjects compared with controls (Lu, Ning, et al. 2009). The thalamus including the ventral lateral nucleus (VLN), receives projections from the dentate nucleus of the cerebellum and projects to the motor cortex (Strick 1976; Hoover and Strick 1999; Dum and Strick 2003). Different parts of the VLN also have connections to the posterior parietal (Schmahmann and Pandya 1990), prefrontal (Middleton and Strick 1994, 2001), and superior temporal cortices (Yeterian and Pandya 1991). Thalamocortical pathways are involved in motoric aspects of speech and language (Johnson and Ojemann 2000; Schmahmann and Pandya 2008). Because of connections with cortical and subcortical structures, the VLN of the thalamus likely plays an integral role in fluent speech processing and may be affected as many investigators have suggested a possible subcortical involvement in stuttering (Wu et al. 1997; Alm 2004; Smits-Bandstra and De Nil 2007; Giraud et al. 2008). Given past evidence that many individuals who stutter exhibit motor planning and execution deficits not limited to speech production (Max et al. 2000; Max and Yudman 2003; Smits-Bandstra, De Nil, Saint-Cyr 2006), there may be deficits in subcortical interrelationships with sensorimotor cortical activity in stuttering speakers.

In the current study, we examined both functional and structural connectivity using fMRI and DTI data, respectively, to investigate the corticocortical and thalamocortical connections in stuttering and control groups. Although others have examined effective connectivity using structural equation modeling (SEM) in stuttering and nonstuttering groups (Lu, Ning, et al. 2009), we have combined both functional and structural analyses in the same subjects to determine if anatomical connectivity differences underlie functional connectivity differences in 3 possible regions within the left hemisphere: between the inferior frontal gyrus (Brodmann area 44 [BA44]) and the pSTG affecting auditory feedback during speech, between BA44 and the premotor and primary motor areas affecting the integration between planning and motor execution, and between the VLN in the thalamus and the MTG and the preSMA affecting subcortical and cortical integration.

To measure functional connectivity, we used psychophysiological interaction (PPI) (Friston et al. 1997; Kim and Horwitz 2008) analysis to examine correlated activity with a priori defined seed regions during speech and nonspeech oral motor production tasks compared with a control condition, silent fixation. PPI measures the influence of activity in one region of the brain (seed) on the activity of spatially distant regions, which may change from one task to another.

For structural connectivity analysis, we employed probabilistic tractography (Behrens, Johansen-Berg, et al. 2003) using DTI data. To examine corticocortical sensorimotor connectivity, we focused on the tracts that interconnect inferior frontal region including the opercular part of the inferior frontal gyrus (BA44) with posterior structures. BA44 was chosen as a seed region for both PPI and DTI tractography based on previous findings reporting aberrant connectivities between this inferior frontal region with premotor and motor and sensory areas in stuttering speakers, supported by both structural (Sommer et al. 2002; Watkins et al. 2008; Cykowski et al. 2010) and functional studies (Fox et al. 1996; Braun et al. 1997). In children who stutter, gray matter volume in this region was found significantly decreased compared with age-matched controls, even in children who had naturally recovered from stuttering (Chang et al. 2008). To examine thalamocortical connectivity, we examined tracts passing through the VLN of the thalamus, which has anatomical interconnections with the basal ganglia, cerebellum, and the motor/premotor regions (Strick 1976; Hoover and Strick 1999; Dum and Strick 2003).

We tested 3 sets of hypotheses regarding cortical and subcortical network abnormalities in stuttering. Given previous evidence of aberrant structural and functional findings in the left perisylvian region, we predicted that stuttering speakers would have reduced functional and structural connectivity 1) between the left BA44 with the pSTG, 2) between BA44 and the premotor and primary oral motor cortex, and 3) between the VLN of the thalamus and cortical targets when compared with controls. We also examined whether any structural or functional connectivity differences were related to the severity of stuttering.

Materials and Methods

Participants

Twenty-three healthy normally fluent adults (11 females) (mean age = 33, standard deviation [SD] = 9.97) and 23 stuttering adults (10 females) (mean age = 35, SD = 8.65) participated in the PPI portion of the study which was a reanalysis of fMRI data gathered for Chang et al. (2009). In addition, 29 of the 46 subjects also participated in the DTI study; 14 fluent adults (7 females) and 15 stuttering adults (6 females) for the tractography portion of this study. FA analysis based on the DTI data from these subjects was reported in an earlier paper (Chang et al. 2010). All subjects were right handed on the Edinburgh handedness inventory (Oldfield 1971), and native North American English speakers. All subjects underwent testing with a battery of standardized speech, language, and cognitive tests, audiometric hearing screening, oral motor screening, and cognitive evaluations. The tests included the Peabody Picture Vocabulary Test (PPVT-3), Expressive Vocabulary Test (EVT-3), Test of Nonverbal Intelligence (TONI-3), Revised Token Test, Wechsler Digit Span Test, Goldman–Fristoe Test of Articulation, Kahn–Lewis Phonological Analysis, and Test of Auditory Comprehension of Language (TACL). For study inclusion, the participants had to score within 1 SD of the norm on all standardized testing.

Stuttering severity in the stuttering participants was assessed using the Stuttering Severity Instrument (SSI-3) (Riley 1972), while they engaged in conversation, monologue, and reading tasks in front of a small audience. The SSI scores varied from very mild to severe (mean = 23 [moderate], SD = 6.04). To determine measurement reliability of the SSI score ratings, an intraclass correlation (ICC) analysis was conducted on the ratings made by 2 experienced speech-language pathologists. The ICC for the overall SSI measurement was 0.98, 0.88 for stuttering frequency measurements during conversation/monologue, and 0.99 during the reading task.

All subjects were free of neurological or medical disorders based on self-report, passed audiometric screening, and had normal structural MRI scans as confirmed by a radiologist. None of the stuttering subjects was under active behavioral or pharmacological stuttering therapy at the time of study participation, although a majority reported having been treated during various points in their lifetime. All subjects signed an informed consent form approved by the Internal Review Board of the National Institutes of Neurological Disorders and Stroke and were paid for their participation.

Procedure

For functional imaging, auditory and visual stimuli were delivered using Eprime software (version 1.2, Psychology Software Tools, Inc.) running on a PC, which synchronized each trial with functional image acquisition. Sound was delivered binaurally through MRI-compatible
headphones (Silent Scan Audio Systems, Avotec Inc.). The auditory stimuli were set at a comfortable volume level for each subject before the experiment and remained constant throughout the experimental runs. Subjects’ productions were monitored and recorded using an MR-compatible microphone attached to the headphones (Silent Scan Audio Systems, Avotec Inc.). The task conditions have been described in detail in Chang et al. (2009). Briefly, each trial started with auditory presentation of a sequence of 2 nonsense speech syllables or 2 nonspeech sounds. Six different stimuli pairs were randomly presented for the speech and nonspeech conditions. The speech stimuli were pairs of meaningless vowel-consonant vowel-consonant speech syllables /bem/-/dauk/, /hik/-/λdfl/, /saip/-/kuf/, /lok/-/chim/, and /raig/-/sot/, devoid of lexicality but following the rules of English phonology. The nonspeech gestures included vocal tract gestures that involved sound targets but were devoid of phonological content: cough-sigh, sing (‘/x/’ on a tone)-raspberry, kiss-snorl, laugh-tongue click, and whistle-cry. All nonspeech targets were easily reproduced by each subject, yet involved complex oral motor sequencing. The nonspeech and speech stimuli were similar in duration (subject, yet involved complex oral motor sequencing. The nonspeech and speech stimuli were similar in duration (t speech = 820 ms (SD = 136), t nonspeech = 916 ms (SD = 142)) and amplitude on root-mean-square power (s speech = 0.15 (SD = 0.04), s nonspeech = 0.12 (SD = 0.07), with no statistically significant difference (P > 0.05). An auditory stimulus containing 2 targets was presented during a 4-s silent period, followed by 2.7 s of scanning. Next the participant was visually cued to either repeat the previously presented speech response or reverse the order of the 2 items. The responses then took place during the following transient silence of 4 s, which were then immediately followed by 2.7 s of scanning (Chang et al. 2009).

All subjects underwent a training session on the day of the experiment to familiarize them with the stimuli and tasks. Both groups of subjects were able to produce the speech sequences without difficulty. The majority of stuttering participants did not stutter on either the speech or nonspeech task. Hence, the number of stuttered trials was too few to justify a separate analyses on neural responses related to stuttering itself. All subjects’ speech was monitored online during the experiment and any unclear responses were reviewed from the tape-recorded responses offline after the experiment. Because our main interest was to examine brain activity differences that existed during perceptually fluent production, we identified and excluded the few trials containing stuttered utterances from the analysis. The time points associated with a stuttered or inaccurate production were then deleted from further analysis.

**Image Acquisition**

All images were obtained using a 3.0 T GE Signa scanner equipped with a standard head coil. Subjects’ head movements were minimized using padding and cushioning of the head inside the head coil. Gradient echo-planar pulse sequence was used for functional image acquisition (time echo [TE] = 30 ms, time repetition [TR] = 6.7 s, field of view [FOV] = 240 mm, 6-mm slice thickness, 23 contiguous sagittal slices). An event-related sparse sampling design (Amunts et al. 1999; Badecker et al. 1999; Eden et al. 1999; Hall et al. 1999; Eickhoff et al. 2005) was used. Scanning followed the presentation of auditory stimuli, and the production of sounds or syllables to minimize the effects of scanner noise and movement-related susceptibility artifacts. High-order shifting before echo-planar image acquisition optimized the homogeneity of the magnetic field across the brain and minimized distortions. A high-resolution T1-weighted anatomical image was also acquired for registration with the functional data, using a 3D inversion recovery prepared spoiled gradient-recalled sequence (3D IR-Prep SPGR; time to inversion = 450 ms, TE = 3.0 ms, flip angle = 12°, bandwidth = 31.25 mm, FOV = 240 x 240, matrix 256 x 256, 128 contiguous axial slices).

For DTI, single-shot spin-echo echo-planar imaging sequence with paired gradient pulses was acquired with whole-brain coverage. Imaging parameters for the diffusion-weighted sequences were: TE/TR = 73/4/2 13 000, FOV = 2.4 x 2.4 cm; matrix = 96 x 96 mm2; zero filled to 256 x 256 mm2; on 51 contiguous slices with a slice thickness of 2.6 mm. Diffusion was measured along 35 noncollinear directions with a b factor of 1000 s/mm2. Three reference images were acquired with no diffusion gradients applied (b0 scans). Three anatomical scans were additionally acquired in all participants and were sent to a staff radiologist for clinical evaluation, to rule out gross abnormalities.

**Data Preprocessing**

Image preprocessing for fMRI data used in the PPI analysis was carried out using the Analysis of Functional Neuroimages (AFNI) software (Cox 1996). The first 4 volumes were excluded from analysis to allow for initial stabilization of the fMRI signal. To correct for small head movements, each volume from the 3 functional runs was registered to the volume collected closest to the high-resolution anatomical scan using heptic polynomial interpolation. The percent signal change in each voxel was normalized and functional data from each run were concatenated into one 3D + time file and subsequently spatially smoothed using a 6-mm full-width at half-maximum Gaussian filter.

The amplitude coefficients for production (speech and nonspeech) for each subject were estimated using multiple linear regression. This created statistical parametric maps of the t statistic for the linear coefficients. Statistical images were thresholded at t > 3.1 at P < 0.01 (corrected). Correction for multiple comparisons was achieved using Monte Carlo simulations. We selected a voxel-wise false-positive P threshold of 0.001 for each voxel and a minimum cluster size of 4 contiguous voxels (345 mm3) to give a corrected P value of 0.01. These statistical maps were used to obtain subject-specific peak seed coordinates for the subsequent PPI analyses. Each individual’s statistical map was transformed into standardized space (MNI 27 T1-weighted MRIs from a single subject) by using a 12 parameter affine registration.

For preprocessing of DTI data, we used tools available through FMRIB’s Diffusion Toolbox (FDT), included in the FMRIB Software Library (FSL, http://www.fmrib.ox.ac.uk/fsl). The processing steps included correction for eddy current distortion, and modeling of diffusion parameters, which calculated distributions of diffusion parameters at each voxel that were later used to run probabilistic tractography. More details on the probabilistic tractography algorithm can be found elsewhere (Behrens, Johansen-Berg, et al. 2003; Behrens, Woolrich, et al. 2003).

**Analyses**

**Psychophysiological Interaction**

We used PPI to measure functional connectivity with seeds in the inferior frontal region (BA44) on the left and right hemispheres during speech or nonspeech production compared with silent fixation. The seed coordinates for the left and right BA44 were identified for each individual by taking the peak voxel extracted during speech and nonspeech production (separately) that fell within the anatomically defined maximum probability maps of left and right BA44 (Amunts et al. 1999; Eickhoff et al. 2005). The locations of all subjects’ seed coordinates were distributed within the 40% probability map of the cytoarchitectonically defined left and right BA44. Subjects in the 2 groups did not differ in the manner of distribution of seed coordinates (i.e., the coordinates of the 2 groups did not cluster into 2 separate groups nor did the variability differ between the 2 groups). The time course of each seed region was extracted as the average across an 8-mm radius sphere around the seed coordinate for both task conditions.

A general linear model was implemented to examine brain regions that showed significant increases in functional connectivity with seed activity (left BA44, right BA44) during task performance versus silent fixation. In contrast to usual fMRI analyses, our model included not only the experimental condition (task; psychological) as the predictor variable but also activity level in the seed region (physiological) and interaction between the task and seed activity variables (PPI). This interaction was of primary interest, which gave us information on which regions had increased functional connectivity with the seed region during the task of interest (e.g., speech production), relative to a control task (e.g., silent fixation). Because our fMRI paradigm originally included perception and planning trials in addition to production trials, we included the effects of each of those conditions into the model, in addition to the other main effects (e.g., speech production, LBA44 activity). This was done so that common effects were accounted for and we could identify the effects that were unique to the PPI for each
subject, the correlation coefficients of the interaction regressor were converted to Z-scores. To examine the first and second hypotheses, 3-way analyses of variance (ANOVA) were conducted to compare PPI results for the left BA44 and the right BA44 seeds between the 2 groups (group and region of interest [ROI] as fixed factors and subjects as random factor). The PPI maps for speech and nonspeech were thresholded at a $Z > 2.68$ and a cluster corrected significance threshold of $P = 0.01$. The same procedure was repeated for the left and right VLN (LVLN and RVLN) to examine the third hypothesis. The thalamus ROIs were also subject-specifically defined based on the fMRI data, and seed coordinates for each ROI were determined by taking the peak voxel that fell on each of the Talairach daemon-defined thalamic nuclei of interest (LVLN and RVLN). All later analyses were repeated as was done for the BA44 seeds as mentioned above.

Finally, because in a previous fMRI study, we found significant correlations between stuttering severity scores and activity in the primary motor regions (i.e., Chang et al. 2009), we sought to examine the correlation between stuttering severity and functional connectivity of BA44-4p in both hemispheres. This was done by calculating the correlation coefficient for the variables SSI scores and PPI values extracted from the 4p region in each individual’s PPI maps derived using the BA44 seed (one for each hemisphere).

**Probabilistic Tractography**

The probabilistic tractography algorithm used by Behrens, Johansen-Berg, et al. (2003) and Behrens, Woolrich, et al. (2003) was applied to the DTI data to examine white matter tracts interconnecting speech-relevant frontal-temporal-parietal regions as well as thalamocortical tracts. The algorithm involves calculating the probability density function (PDF) at each voxel, the width of which represents the uncertainty of the fiber direction due to crossing fibers, movement artifacts, and/or noise. The algorithm allows tracking of fibers that originate from a chosen seed voxel (or mask) by considering both the local PDF and the global connectivity patterns. We used the same subject-specific seeds as originating points for tractography as were used in the PPI analysis for speech and nonspeech, yielding 2 analyses. A 5-mm cube surrounding each individual’s speech or nonspeech-specific seed voxels (left and right BA44, VLN) were generated as masks and were entered into the tractography algorithm as seed regions for deriving tracts. As no studies have previously examined white matter tracts using tractography in stuttering speakers, we chose not to constrain the tracking by defining an endpoint, as this would have limited tracking to only one tract.

To quantify the tractography results for testing the first and second hypotheses, we conducted an ROI analysis using a priori defined ROIs along the SLF, in the premotor (BA6), motor (4p), and pSTG regions. The choice of these ROIs was based on findings in previous studies that suggested connectivity deficiencies involving the inferior frontal–premotor (Watkins et al. 2008), inferior frontal–motor (Salmenlin et al. 2000), and inferior frontal–temporal–parietal (Fox et al. 1996; Braun et al. 1997; Salmenlin et al. 1998) regions in stuttering speakers. The ROIs examined for the tracts generated with VLN seeds were the motor 4p and premotor BA6 regions to be consistent with anatomical tracing studies of the ventrolateral thalamus-cortical connectivity (Strick 1976; Schell and Strick 1984; Katayama et al. 1986; Anderson and DeVito 1987; Nakano et al. 2000). To test the third hypothesis, we calculated the number of voxels that passed through these ROIs within the tractography-generated tracts derived from either the BA44 or the VLN seeds. The 4p and BA6 masks were cytoarchitectonically defined (Zilles et al. 1995; Eickhoff et al. 2005), and the pSTG was based on the Talairach daemon STG map (Fox et al. 1985) that was modified to contain only the posterior part of the STG (posterior to the first Heschl’s sulcus). An ANOVA examined group effects (between subjects), and the repeated factors of side (left, right), and ROI (BA6, 4p, pSTG) on the number of voxels passing through each tract. For the tracts generated using the VLN thalamus seed, the endpoint ROIs were the motor 4p and BA6 regions on each side. Here, repeated measures ANOVA examined group effects (between subjects) and the repeated factors of side (left, right), ROI (4p, BA6). All endpoints were based on cytoarchitectonic maps that were registered to standardized templates.

We also examined the relationship between tract density in the 4p with stuttering severity by calculating the correlation coefficient between SSI scores and the density measures extracted from 4p from tracts derived using the BA44 seed on each side.

**Results**

**Functional and Structural Connectivity Differences Involving Left BA44 for Speech and Nonspeech Production**

**Functional Connectivity with the Left BA44**

Functional connectivity increases with the LBA44 during speech production relative to silent fixation, in controls, are shown in Figure 1 (see also Supplementary Table 1). These regions included the left premotor area (BA6), left MTG, and the left pSTG/inferior parietal regions. These increases were somewhat greater during speech compared with nonspeech production (Fig. 1A, Supplementary Tables 1 and 2). In stuttering speakers, no significant functional connectivity increases occurred between the LBA44 with the left motor or premotor areas, while increases occurred mostly with the cerebellum for both speech and nonspeech conditions (Fig. 1B, Supplementary Table 2).

**Figure 1.** Functional connections of the left BA44 shown with PPI analysis. (A) Conjunction map showing regions that significantly increased functional connectivity (PPI) with left BA44 in normally fluent controls during speech (red), nonspeech (blue), and for both speech and nonspeech (yellow). (B) Conjunction map showing regions that significantly increased functional connectivity with the left BA44 in stuttering speakers during speech (red), nonspeech (blue), and both speech and nonspeech (yellow). (C) Contrast of PPI results between stuttering and control groups during speech production. Warmer color blobs show regions, where the control group exhibited higher functional connectivity than the stuttering group and the blue blobs show, where stuttering speakers had more functional connectivity than the control group ($p < 0.01$, corrected).
Tables 1 and 2). A direct contrast between the 2 groups showed that the functional connectivity with LA44 was greater in bilateral premotor cortex (BA6), right MTG, STG, anterior temporal and supramarginal gyrus regions in the controls (Supplementary Table 3), while connectivity between LA44 and the right postcentral gyrus, left anterior cingulate cortex (ACC), left inferior temporal gyrus, MTG, and the cerebellum was greater in the stuttering group (Fig. 1C, Supplementary Table 3). The patterns of group differences in functional connectivity were similar for both speech and particularly for nonspeech production tasks (Supplementary Tables 1–4).

Structural Connectivity with the Left BA44
The tracts from LA44 to motor, inferior parietal, and posterior temporal regions tended to extend further posteriorly in the control group than in the stuttering group (Fig. 2). In fact, visual inspection of individual tracts showed at least 10 of 14 controls and 6 of 14 the stuttering group showed robust tracts of the SLF. Tract density in the left and right SLFs generated from the left and right BA44 seeds were assessed by measuring the number of voxels that passed through the premotor (BA6), motor (4p), and pSTG ROIs within the respective SLF on each side. There was a significant side × group interaction \( F = 6.485, P = 0.017 \) and a significant ROI × side × group interaction \( F = 3.256, P = 0.047 \). Hence, we conducted additional group comparisons separately for the left and right ROIs (right BA44 results presented in the next section). There was a significantly smaller number of voxels passing through the BA6 \( F = 5.623, P = 0.025 \) as well as the 4p \( F = 6.562, P = 0.017 \) in the left SLF in the stuttering group compared with controls (Fig. 3). The difference in the left pSTG was not significant \( F = 1.632, P = 0.213 \).

Because our tracking of fibers appeared to be less reliable in the posterior temporoparietal regions, we conducted an additional ROI analysis based on tracts that were generated using the posterior STG seeds. From these tracts, the number of voxels that passed through the 4p and BA44 ROIs was assessed for each individual. There was an overall significant difference between the groups \( F = 7.432, P = 0.014 \), a significant side × group interaction \( F = 5.954, P = 0.026 \), and a significant ROI × group interaction \( F = 11.159, P = 0.004 \). Hence, we conducted additional group comparisons separately for the left and right ROIs. The stuttering group had significantly smaller number of voxels relative to controls passing through the left BA44 ROI in tracts that were generated using the left pSTG seed \( F = 7.252, P = 0.015 \) (Fig. 4). The difference in the left 4p ROI was not significant \( F = 3.195, P = 0.092 \). There were no significant differences found for the right hemisphere tracts for either BA44 or 4p.

Functional and Structural Connectivity Differences Involving the Right BA44 for Speech and Nonspeech Production

Functional Connectivity with the Right BA44
In the control group, no significant increases in functional connectivity over silent fixation were found in the right hemisphere and small increases were observed in the left inferior parietal lobule (IPL) and the left pSTG (Fig. 5A). In addition, only in the controls, there were regions that exhibited negative PPI values (indicating decreased functional connectivity relative to fixation) in the left cerebellum, left MTG, left angular gyrus, left precentral gyrus (4p), left Rolandic operculum, right postcentral gyrus, and SMA, among others. In stuttering speakers, extensive regions in the bilateral inferior frontal gyrus (IFG), bilateral STG, bilateral angular gyrus, and cerebellum, had increased functional connectivity with the right BA44 (Fig. 5B). There were no areas with negative PPI in the stuttering group. These differences were somewhat more pronounced during the speech condition compared with nonspeech (Supplementary Tables 1 and 2). The group contrasts revealed that the differences were significant in left IFG, bilateral PreCGy, MTG, SMA, cingulate gyrus, as well as the cerebellum, with the stuttering group consistently having more connectivity with the right BA44 in these areas relative to controls (Supplementary Tables 3 and 4). Group differences in functional connectivity patterns were greater for speech than nonspeech production tasks; the stuttering speakers had more functional connectivity between the bilateral angular gyrus and the RBA44 during speech than during nonspeech.

Structural Connectivity with the Right BA44
The tract density in the right hemisphere did not differ in the 2 groups for the BA6 \( F = 2.087, P = 0.160 \), 4p \( F = 2.363, P = 0.136 \), or pSTG \( F = 0.570, P = 0.457 \) (Fig. 3).

Correlation with Stuttering Severity
Correlation coefficients were calculated to examine the relationship between stuttering severity as measured through the SSI with each of the functional and structural connectivity measures. Surprisingly, there was an absence of any significant correlation between SSI scores with each of the connectivity measures \( r = 0.202, P = 0.44 \) for SSI and degree of functional connectivity between left BA44–4p; \( r = 0.138, P = 0.60 \) for SSI and functional connectivity between right BA44–4p; \( r = 0.292, P = 0.31 \) for SSI and structural connectivity between left BA44–4p; and \( r = 0.08, P = 0.78 \) for SSI–structural connectivity between right BA44–4p.)
Functional Connectivity Differences Involving the Thalamocortical Loop for Speech and Nonspeech Production

Functional Connectivity with LVLN

Group specific maps showing regions with significant functional connectivity with the LVLN are shown in Figure 6.

When the 2 groups were contrasted, functional connectivity with the LVLN was significantly lower in the right thalamus, left precentral gyrus, left middle frontal gyrus, right cingulate gyrus, right IPL, and left cerebellum in controls relative to the stuttering group (Supplementary Table 5). In the stuttering group, functional connectivity with LVLN was significantly increased relative to controls in the left IFG (Fig. 6A, Supplementary Table 5).

During nonspeech, the stuttering group had negative PPI similar to controls (Fig. 6B). The stuttering group additionally showed decreased functional connectivity in the right middle frontal gyrus, left precentral gyrus, and bilateral cerebellum relative to controls during nonspeech production and increased connectivity in the left angular gyrus (Fig. 6B, Supplementary Table 5).
show, where stuttering speakers had more functional connectivity than the control group. warmer color blobs show regions, where the control group exhibited higher functional connectivity than the stuttering group, and the blue blobs show regions relative to controls in the left IPL (angular gyrus) (Fig. 7).

During speech production, the control group had significantly increased functional connectivity with the RVLN. The left STG, right thalamus, and right ACC showed significantly less functional connectivity differences in the stuttering group relative to controls. The functional and structural connectivity results from the corticocortical network are discussed first followed by the thalamocortical results in later sections.

Functional connectivity between the left BA44 and the premotor (BA6) region for both speech and nonspeech oral motor sound production tasks were greater on the left in normally fluent controls but were not seen in stuttering participants. Instead, significantly increased functional connectivity was found between the right BA44 and bilateral speech-relevant cortical regions in the stuttering subjects. These functional connectivity increases with the right BA44 were not seen in controls. The group differences were similar for both speech and nonspeech oral motor sound production.

Structural connectivity measures showed results consistent with the functional connectivity results in the left hemisphere. Major white matter tracts that passed through the same BA44 seed had significantly less tract density in the stuttering participants only in the left premotor and precentral motor regions relative to controls; no group differences in tract density were found on the right side. Hence, both structural and functional connectivity measures supported left-sided group differences, whereas only functional connectivity differences were present for the right hemisphere. These findings underscore previous suggestions of left-sided deficits in stuttering, particularly in the connections between the inferior frontal and the premotor/motor regions (Salmelin et al. 2000; Sommer et al. 2002; Neumann et al. 2003; Neumann et al. 2005; Chang et al. 2008).

A number of recent studies across different research groups have reported attenuated white matter coherence in the left SLF underlying the orolarynx representation of motor cortex for people who stutter both in adulthood and as children (Sommer et al. 2002; Chang et al. 2008; Watkins et al. 2008; Cykowski et al. 2010). Stuttering adults also differed in the cortical sequence of activity changes involving the left inferior frontal and motor areas during fluent picture naming (i.e., even when they were fluently speaking, the sequence of cortical activity in the left ventral premotor/motor areas were reversed compared with controls) (Salmelin et al. 2000). A recent study showed that successful remediation of stuttering involved regaining activity in the left inferior frontal region as opposed to engaging the right homologue or adopting other compensatory activity (Kell et al. 2009). Watkins et al. (2008) found attenuated white matter coherence as measured with fractional anisotropy and decreased functional activity in the left overall group effect was not significant ($F=0.372, P=0.546$). The side $\times$ group effect did not reach significance ($F=4.173, P=0.05$).

**Discussion**

This study examined 2 neural networks that have been postulated as affected in stuttering. One network involved corticocortical connections linking the articulatory regions within the frontal motor areas and with auditory-sensory areas in temporal-parietal cortex. The other involved the thalamocortical pathway, involving the VLN of the thalamus, which has anatomical connections with motor and premotor cortical regions. The results demonstrated both functional and structural connectivity differences between the stuttering and normally fluent groups in the left inferior frontal to premotor connections, but only functional connectivity differences in the thalamocortical pathway in the stuttering group relative to controls. The functional and structural connectivity results from the corticocortical network are discussed first followed by the thalamocortical results in later sections.

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ventral premotor/inferior frontal region in stuttering speakers (Watkins et al. 2008). Thus converging evidence point to abnormalities involving the connectivity between the left inferior frontal and the premotor/motor cortex, as areas particularly relevant to the neuropathophysiology of stuttering. The present data suggests, however, that stuttering speakers

Figure 6. Functional connections of the LVLN region shown with PPI analysis. Warmer color blobs show regions showing positive functional connectivity, whereas the blue blobs show regions with negative functional connectivity with the seed region. (A) PPI results for speech production. (B) PPI results for nonspeech production.

Figure 7. Functional connections of the RVLN region shown with PPI analysis. Warmer color blobs show regions showing positive functional connectivity, whereas the blue blobs show regions with negative functional connectivity with the seed region. (A) PPI results for speech production. (B) PPI results for nonspeech production.
may exhibit decreased connectivity in motor regions not limited to orolaryngeal representation of the motor cortex. It may be the case that stuttering speakers have less distinction between speech versus adjacent motor representations in the motor cortex. Support for this idea comes from several studies that have reported subtle motor deficits in stuttering speakers not limited to speech movements but also when performing hand and finger movement tasks (Max et al. 2003; Smits-Bandstra, De Nil, Rochon 2006).

There was reduced functional connectivity between the left BA44 and premotor (area 6) regions in stuttering speakers relative to controls, although other functional neuroimaging studies found that stuttering speakers exhibited hyperactivity in the bilateral motor cortices during speech tasks. Further, it was previously found that there were significant correlations between bilateral motor cortex (4p) activity with stuttering severity (Chang et al. 2009). When the degree of functional connectivity in 4p was examined in relation to BA44 activity during speech/nonspeech production, there was no significant correlation with stuttering severity. This may indicate that left-sided connectivity differences are present in stuttering speakers, regardless of stuttering severity. On the other hand, motor cortex hyperactivity may be present as a reaction to stuttering that appear to be heightened with increasing stuttering severity.

Based on our first hypothesis, evidence of both functional and structural disconnection was expected between BA44 and the left STG in the stuttering group. The lack of functional connectivity differences between BA44 and STG may have been due to the speech and nonspeech tasks that were used. After the subjects were told whether to imitate or reverse the order of the stimuli, they had ample planning (rehearsal time) before producing the speech/nonspeech production. Productions were very short, within a couple of seconds, possibly reducing the need for self-monitoring with auditory feedback during production. To address this issue more directly in the structural analyses, we also conducted a tractography analysis using pSTG seeds to track white matter and measured tract density in the BA44 and motor 4p regions in both hemispheres for these tracts. We found significant tract density differences between the groups only in the left hemisphere in the BA44 but not in motor 4p. This suggests that stuttering subjects may not differ from controls in their ability to send auditory feedback to the motor cortex (4p) for motor adjustment but may still have a disconnection along the tracts flowing between left BA44 and 4p.

When studying adults who have stuttered since childhood it is difficult to determine which differences are part of the initial speech disorder or the result of chronic lifelong attempts to produce speech despite the disorder. The present data offer little support for significant macroscopic structural connectivity differences in the right corticocortical structures between the 2 groups. On the other hand, enhanced functional connectivity in the right hemisphere was demonstrated here and increased right-sided activity has been reported by others (Braun et al. 1997; De Nil et al. 2000; Fox et al. 2000; Chang et al. 2009). Significant differences in functional connectivity without differences in structural connectivity might suggest that the right hemisphere differences may be the result of, rather than contributors to, the emergence of stuttering symptoms. Heightened right-sided activity during speech tasks (Fox et al. 1996), and attenuated leftward asymmetry in the planum temporale (Foundas et al. 2001, 2003), have been reported in stuttering adults. In light of the present findings, perhaps these right-sided increases in function may develop as a result of stuttering into adulthood. This is also consistent with the fact that in children who stutter, no right-sided increases and no differences in asymmetry patterns in perisylvian anatomy were found relative to fluent peers (Chang et al. 2008).

In regard to the thalamocortical loop, group differences in functional connectivity involving the LVN included those found in the cerebellum, thalamus, insula, and the STG, which were present regardless of speech or nonspeech tasks. While functional connectivity differences were found in the LVN in both hemispheres, structural connectivity data indicated that the groups did not differ significantly on either side.

The differences found in LVN functional connectivity between the 2 groups suggest that stuttering speakers may differ significantly from controls in how structures within the cerebellum-thalamocortical loop function as a network for volitional speech and nonspeech motor execution tasks. Recently, a similar finding was reported, where using SEM, negative path coefficients were found from the thalamus to the preSMA in controls but positive projections were found from the thalamus to preSMA in the stuttering group (Lu et al. 2010). In the present data, we did not find significant group differences in the preSMA or SMA but did find differences in regions inferior to these premotor areas, in the cingulate cortex, particularly for speech relative to nonspeech production.

In sum, the present data provide clarifications to previously held suggestions of the neural bases of stuttering that involved deficient corticocortical as well as thalamocortical networks. Of these, we found strongest support for a deficient left inferior frontal to premotor connection in stuttering, as this connectivity was significantly decreased when measured with both functional and structural connectivity analyses. The connections between the LVN thalamus and cortical regions including the premotor and motor areas differed significantly only according to the functional connectivity measures but not structural connectivity.

The functional and structural connectivity differences between stuttering and control groups were not limited to speech production. Nonspeech production that involved similar oral motor structures as used in speech also resulted in very similar patterns of connectivity differences in stuttering speakers relative to controls. Although these connectivity changes were similar for both tasks, some differences were noted. The heightened connectivities on the right side that might be attributed to compensatory connections in stuttering were greater during speech than during nonspeech. For instance, heightened functional connectivity with right BA44 was seen in the motor and cerebellar regions during speech compared with nonspeech in stuttering speakers. These findings underscore the fact that left connectivity differences, which were similar for both speech and nonspeech, may be a trait difference in stuttering, whereas right connectivity differences, found more during speech than in nonspeech, may represent state related differences in stuttering. The convergent finding of attenuated left-sided white matter integrity in stuttering (Sommer et al. 2002; Chang et al. 2008; Watkins et al. 2008) is consistent with a possible disconnection syndrome that may have a genetic basis (Buchel and Watkins 2010). The recent discovery of mutations of specific genes associated with lysosomal dysfunction which were found in stuttering families (Kang et al. 2010) might be a neurochemical basis for the white
matter deficits, however, much more research is needed to substantiate these claims.

This study has several limitations. We used subject-specific functionally defined seeds in each of the bilateral regions of interest (BA44, VLN) to conduct both PPI analyses and DTI tractography. This procedure, combined with the use of cytoarchitectonically defined maps in the BA44, may have provided a robust estimation of each subject's peak voxel for speech and nonspeech production for this ventral premotor ROI. However, because we did not have cytoarchitectonic maps of the thalamus, we only relied on each subject's functional peak and a less anatomically specific map (Talairach daemon) of the VLN seeds for each individual. This procedure may have led to less accurate anatomical estimation of the peak voxel for these thalamic nuclei. Future studies would benefit from more accurate anatomical localization of the specific thalamic nuclei of interest in each subject. These data are also limited to providing macroscopic differences between the stuttering and fluent speaker groups. There could be microscopic differences (e.g., at the level of lamina, columns, etc.) that we are not detecting due to the nature of the measures used here. Finally, we note that interpretation of functional and effective connectivity differences is tricky and requires caution, especially concerning effects that appear to be compensatory (Kim and Horwitz 2008). To address this limitation, it might be ideal to track functional and anatomical changes in connectivity measures starting near the onset of stuttering symptoms in children. This may provide a clearer picture of what relates to pathophysiology versus compensatory effects.

In conclusion, we found evidence for both functional and structural connectivity differences in stuttering speakers among the left inferior frontal and premotor cortices. The thalamocortical connectivity differed between the groups only in functional connectivity but not in structural connectivity. The connectivity differences were not only found during speech but also during nonspeech oral motor sound production tasks. Heightened functional connectivity within right hemisphere and interhemispheric cortical regions, as well as between the thalamus and the premotor/motor, cerebellum, and temporoparietal regions were also observed in stuttering speakers but were not corroborated by increased structural connectivity. We propose, based on these results, that the neuropathophysiology of stuttering may involve deficient connectivity among the cortical network of regions that normally allows left-sided engagement of the inferior frontal and premotor cortices for efficient planning and execution of sound production. We also suggest that many previously reported differences involving right hemisphere and subcortical activity in stuttering speakers likely reflect altered functional responses to deficient connectivity of the left premotor/motor areas, although this remains to be determined by studying young children close to symptom onset.

**Supplementary Material**

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

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**Notes**

Conflict of Interest: None declared.

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