Reorganization of Brain Function after a Short-Term Behavioral Intervention for Stuttering

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Abstract

This study investigated changes in brain function that occurred over a 7-day behavioral intervention for adults who stutter (AWS). Thirteen AWS received the intervention (AWS+), and 13 AWS did not receive the intervention (AWS-). There were 13 fluent controls (FC-). All participants were scanned before and after the intervention. Whole-brain analysis pre-intervention showed significant differences in task-related brain activation between AWS and FC- in the right inferior frontal cortex (IFC) and left middle temporal cortex, but there were no differences between the two AWS groups. Across the 7-day period of the intervention, AWS+ alone showed a significant increase of brain activation in the left ventral IFC/insula. There were no changes in brain function for the other two groups. Further analysis revealed that the change did not correlate with resting-state functional connectivity (RSFC) that AWS showed in the cerebellum (Lu, et al., 2012). However, both changes in task-related brain function and RSFC correlated with changes in speech fluency level. Together, these findings suggest that functional reorganization in a brain region close to the left IFC that shows anomalous function in AWS, occurs after a short-term behavioral intervention for stuttering.

Key words: Stuttering; Intervention; Reorganization; Inferior frontal cortex; Cerebellum

1. Introduction

Whilst most children acquire language effortlessly, about 5% of them have problems that lead them to begin to stutter (Craig & Tran, 2005). Roughly four out of five of the cases where stuttering starts in childhood recover spontaneusely. Stuttering usually persists into adulthood in the remaining cases, resulting in about 1% of the adult population being affected (Howell, 2011; Yairi & Ambrose, 2005). At present, the

neurophysiology behind childhood stuttering, how the brain compensates for stuttering, and what brain reorganization occurs when an intervention is given are not completely understood. Some general consensus has arisen concerning the first two of these issues over the past two decades. Adults who stutter (AWS) show overactivations in regions of the right hemisphere such as the inferior frontal cortex (IFC) and anterior insula, but lower activations in regions of the left hemisphere such as the left IFC and temporal cortex, compared to fluent controls in several speech production and auditory perception tasks (Brown, Ingham, Ingham, Laird, & Fox, 2005; De Nil et al., 2008; Jiang, Lu, Peng, Zhu, & Howell, 2012; Lu et al., 2016). AWS also show altered connnectivity between the basal ganglia/cerebellum and cortical brain regions compared to fluent controls in speech production tasks (Chang, Horwitz, Ostuni, Reynolds, & Ludlow, 2011; Howell, Jiang, Peng, & Lu, 2012b; Jiang et al., 2012; Lu, Chen, et al., 2010; Lu et al., 2009; Lu, Peng, et al., 2010). Moreover, the abnormal brain activation in the left hemisphere and altered connectivity in the circuits between basal ganglia and cererbral cortex have been confirmed in children who stutter in auditory speech processing tasks or in the resting-state condition, but the right hemispheric abnormalities have not (Chang & Zhu, 2013; Sato et al., 2011). With respect to the third issue, less is known about the neurophysiological changes that occur when a behavioral intervention for stuttering is given, particularly those that happen when the period of intervention is short-term.

Compared to fluency-enhancing techniques such as choral speech or altered auditory feedback, behavioral interventions have the advantage that they can supress stuttering to a degree for a relatively long period of time. Several previous studies have used positron emission tomography or functional magnetic resonance imaging (fMRI) methods to examine changes in brain function over the course of behavioral

interventions. For instance, after a three-week behavioral intervention, the speech-related brain activation in AWS re-lateralizes to the left hemisphere (De Nil, Kroll, Lafaille, & Houle, 2003; Neumann et al., 2003). In particular, activation in the left ventral IFC (vIFC) adjacent to the functionally anomalous region increases in speech production tasks (Neumann et al., 2005). Another study showed that after practice to pace speech along with a metronome for eight weeks, the abnormal activation in the basal ganglia was eliminated, and the activation in the cerebellar vermis decreased (Toyomura, Fujii, & Kuriki, 2015). However, further evidence showed that one to two years later, the overactivations returned to the right hemisphere albeit to a lesser extent (De Nil et al., 2003; Neumann et al., 2003).

This previous evidence suggests that different patterns of changes in brain function occur at different times after behavioral interventions have been delivered to AWS. Evidence from animals also indicates that experience-induced changes in brain structures in the first two weeks differed from those seen after two weeks (Comeau, McDonald, & Kolb, 2010). Thus, it is plausible that the changes in brain function differ between short-term (within two weeks) and long-term (beyond two weeks) interventions for AWS. Whilst the changes in the neural systems over the course of long-term interventions have been investigated in vocal or auditory task conditions previously (De Nil et al., 2003; Kell et al., 2009; Neumann et al., 2003; Neumann et al., 2005), little is known about what changes happen in neural systems after a short-term behavioral intervention (i.e., within 2 weeks).

In fact, only one study has investigated changes in brain function and structure in AWS over the course of a short-term behavioral intervention. In this work they looked at changes of brain function in the resting-state condition (Lu et al., 2012). Lu et al.'s (2012) intervention was based on recent dual-route models of stuttering. Alm's (2004,

2006) dual premotor model of stuttering suggested that the basal ganglia-supplementary motor area complex is impaired in AWS, and that the cerebellum-premotor area (PMA) is employed to bypass the impaired circuit. The EXPLAN model complements this by focusing on the coordination or 'interlocking' of linguistic planning and execution stages at the language–speech interface and proposes that the cerebellum organizes motor plans for output (Howell, 2004, 2007; Howell, Au-Yeung, & Sackin, 2000; Howell & Dworzynski, 2005). However, EXPLAN lacked imaging evidence that directly supported the model. Consequently, a dual-route neural model was developed and tested empirically in classic speech production tasks (i.e., overt and covert picture naming tasks) (Lu, Chen, et al., 2010; Lu et al., 2009; Lu, Peng, et al., 2010). This dual-route model assumed that two neural circuits were impaired in AWS: 1) The connectivity in the basal ganglia-IFC circuit was altered and this was closely associated with atypical linguistic planning; 2) the connectivity in the cerebellum-PMA circuit was affected and this was associated with atypical speech motor execution (Howell et al., 2012b; Jiang et al., 2012; Lu, Chen, et al., 2010; Lu et al., 2009; Lu, Peng, et al., 2010). The dual-route model also hypothesized that improvement in linguistic planning (particularly phonological processing) and articulatory motor execution and repair of both the basal ganglia-IFC and cerebellum-PMA circuits are probably essential for full recovery from stuttering in adulthood. Thus, training on phonological processing and articulatory motor execution should change the function of brain regions in the basal ganglia-IFC circuit and/or cerebellum-PMA circuit, and reduce the severity of stuttering.

Lu et al. (2012) showed that a behavioral intervention administered to AWS for a 7-day period significantly enhanced speech fluency in AWS. The intervention also eliminated the stronger resting-state functional connectivity (RSFC) between the

midline of the cerebellum and the whole language network in AWS compared to fluent controls. According to the dual-route neural model of stuttering (Lu, Chen, et al., 2010; Lu et al., 2009; Lu, Peng, et al., 2010), the basal ganglia-IFC and cerebellum-PMA circuits should show functional changes when an intervention targeting phonological processing and articulatory motor execution is given to AWS. However, Lu et al. (2012) did not detect any functional changes in the left vIFC that have been reported in other studies that employed speech tasks rather than a resting-state paradigm (Kell et al., 2009; Neumann et al., 2005). The left IFC is involved in various aspects of speech production such as phonological processing (Costafreda et al., 2006) and phonetic encoding (Papoutsi et al., 2009). Its functional and structural anomalies have also been implicated in stuttering (Cykowski, Fox, Ingham, Ingham, & Robin, 2010; Jiang et al., 2012; Kell et al., 2009; Lu, Chen, et al., 2010; Lu et al., 2012; Lu et al., 2009; Salmelin, Schnitzler, Schmitz, & Freund, 2000). Moreover, it appears that an increase of activation in the left vIFC relates to full recovery from stuttering (Kell et al., 2009). Hence, it remains necessary to determine whether the left vIFC shows changes in brain function in a speech task conducted before and after a short-term behavioral intervention for AWS.

The cerebellum-PMA circuit was involved in the atypical motor execution in AWS in the dual-route neural model of stuttering (Lu, Chen, et al., 2010; Lu et al., 2009; Lu, Peng, et al., 2010). Thus, the cerebellum was also expected to show functional changes after a short-term intervention, which was confirmed (Lu et al., 2012). Previous evidence has shown that individual variability in resting-state neural activity can predict individual differences in task performance such as perceptual learning and memory (Baldassarre et al., 2012; Hampson, Driesen, Skudlarski, Gore, & Constable, 2006; Tambini & Davachi, 2013; Tambini, Ketz, & Davachi, 2010; Wang et al., 2010). This raises the possibility that similar influences may apply to speech

tasks, too. Thus, if the task-related changes of brain function in the vIFC were identified, it would also be interesting to know whether the two types of changes in brain function, i.e., the task-independent changes in the resting-state condition and task-related changes in the speech tasks, are related to one another or not.

The present study examined task-related changes in brain function after a short-term behavioral intervention for stuttering. Based on the dual-route neural model of stuttering (Lu, Chen, et al., 2010; Lu et al., 2009; Lu, Peng, et al., 2010), brain regions in the basal ganglia-IFC and/or cerebellum-PMA circuits may show functional changes after a short-term behavioral intervention that targets phonological processing and articulatory motor execution. The results were compared to previous findings that identified differences between AWS and controls in the resting-state condition (Lu et al., 2012) in order to provide a comprehensive picture of changes in brain function after a short-term behavioral intervention. This comparison should elucidate the relationship between task-related and task-independent changes in brain function after the short-term intervention.

2. Materials and methods

2.1. Participants

Twenty-eight AWS were recruited who had participated in the study of Lu et al. (2012). They were randomly assigned to groups who received the intervention (AWS+) or who did not receive the intervention (AWS-). Any AWS assigned to AWS+ who reported that they could not adhere to the intervention schedule during the test period was moved to AWS-, and another AWS from AWS- was selected at random and re-assigned to AWS+. Two AWS+ did not complete the task-related fMRI experiment and were excluded from this study, resulting in 13 AWS+ in the data reported. Thus, 13 AWS+ received a short-term behavioral intervention (mean age = 23 ± 2.25 years), and 13

AWS- did not (mean age = 29 ± 6.06 years). Thirteen fluent controls were also recruited (FC-, mean age = 24 ± 1.45 years). All participants were male native Mandarin speakers. The participants reported that they had no personal or family history of psychiatric, neurological or other disorders (other than stuttering in AWS). There were no statistically significant differences between the two groups for handedness, years in education, percent of stuttered syllables (%SS), Stuttering Severity Instrument III (SSI-3) scores, or Overall Assessment of the Speaker's Experience of Stuttering (OASES) (Table 1). The only significant difference between participants was age, which was partialled out as a covariate in all the analyses below.

The fluency assessments were performed by two native Mandarin researchers who had 2- and 9-years' clinical experience respectively. A spontaneous speech sample and a reading of a standard text were recorded from all participants (Riley, 1994). The spontaneous speech sample was recorded during a conversation between the experimenter and the participant. The length of the sample was determined by the number of syllables required for SSI-3 analysis: This was fixed at 300-syllable as specified by Riley (1994) and was consistent across all participants. Riley's (1994) standard reading text was also 300-syllables in length and took 3-4 minutes to elicit. Time to read the text varied depending on the reading speed (of all participants) and stuttering severity of AWS. Any physical concomitants were noted independently by two research assistants while the recordings were made as described by Riley (1994). Inter-judge consistency was indexed by the intra-class correlation (ICC) coefficients. ICC coefficients had values of 0.972 (pre) and 0.985 (post) that showed that reliability of assessments was high (Koch, 1982). Participant's spontaneous speech samples were divided into two equal parts for repeat measurement reliability. The two judges estimated SSI-3 on each part on separate occasions and ICC was 0.76.

Table 1 about here.

The study was approved by the ethics committee of the State Key Laboratory of Cognitive Neuroscience and Learning, Beijing Normal University. Written informed consent was obtained from each participant.

2.2. Intervention given to AWS+

The behavioral intervention for AWS+ was developed at Beijing Normal University and was conducted by QY (a qualified speech therapist). The intervention focused on three levels of speech processing (phonemic, syllabic, and sentential). The timetable for intervention was the same for all AWS+ participants: It took place over 7 consecutive days, with 3 sessions per day and 9 blocks per session. The stimuli were spoken by a man who scored at the highest level at the National Putonghua Proficiency Test (National Linguistics Work Committee, the Ministry of Education of P.R. China). In the first block of each session, the participant heard two-syllable words. The repetition task allowed AWS+ to build the auditory-motor representations of the voicing pattern of each phoneme for the words. The next two blocks required AWS+ to read aloud visually-presented two-syllable words that were written in Pinyin. Pilot work showed that Pinyin reading can facilitate phonological awareness in AWS. All of the words were bi-syllabic and each syllable was represented by a consonant-vowel character selected from a standard database of frequently used words in the National Putonghua Proficiency Test Handbook (Ma, 2007). There was no time limit in either the repetition or reading task. This sequence (from block 1 to block 3) was repeated two times in the subsequent six blocks but with different speech materials. For each sequence (three blocks), all materials had either the same vowel with varying consonants or had the

same consonant with varying vowels. Thus, this intervention targeted both phoneme and syllable processing. At the end of each day's intervention AWS+ listened to an audio-recording of their intervention session. The therapist discussed the performance individually with each AWS+. AWS+ were also required to practice the newly learnt speaking pattern in their daily life, i.e. when producing sentences AWS+ were required to produce the syllables in the new way. At the beginning this slowed their speaking rate, but their speaking rate gradually recovered to normal. This procedure helped AWS+ to transfer the speaking pattern established on two-syllable words to sentence production in naturalistic environments. AWS- and FC- did not perform any language or speech improvement exercises whilst the AWS+ received the 7-day intervention. Changes in severity of stuttering were assessed by the SSI-3 and changes in experiences of stuttering were assessed by OASES. The day before intervention was used for pre-clinical assessments and imaging, then AWS+ received the 7-day intervention, and the day after the intervention was used for post-clinical assessments and imaging. This timetable was fixed for all AWS+ participants.

2.3. fMRI task and materials

An overt single-word reading task was employed while participants were scanned; this task was not part of the intervention for AWS+. Specifically, 240 Chinese characters were selected from a standard character database (Institute of Linguistics, 1986). All characters were monosyllables pronounced with a falling tone in order to keep the tone feature stable across conditions (Howell, Jiang, Peng, & Lu, 2012a; Howell et al., 2012b). The frequencies of usage of the characters were less than fifty per million. The characters were divided into two sets so that half were used at each scanning session (i.e., scanning session 1 before the intervention and scanning session 2 after the

intervention). The assignment of the sets to the scanning sessions was counterbalanced across the participants.

Each of the scanning sessions had 12 blocks with the task and baseline period alternated (10 characters per block). The 120 characters for each scanning session were allocated to these blocks at random without replacement. Each block had a 15-sec baseline period and a 30-sec task period. A fixation cross was viewed during the baseline period, and the participants were required to fix their gaze on the cross. An additional 6-sec resting period occurred at the beginning of the scanning session in order to stabilize the scanner's and the participant's performances. Images that were collected during this period were excluded from the below data analyses. Individual trials during the task period lasted for 3 sec (delay = 1.5 sec). This is a modified version of the sparse sampling method (Perrachione & Ghosh, 2013). That is, the first 1.5 sec involved stimulus presentation and collection of participants' response, whilst the last 1.5 sec (i.e., delayed period) collected images (Fig. 1). During the first 1.5 sec, a character appeared on the screen for 1 sec for each trial, followed by a 0.5-sec blank screen. The participant read the character aloud as quickly and accurately as possible when it appeared on the screen. The spoken responses were recorded digitally through an MRI-compatible headphone system, and quality was sufficient to allow voice-onset-time (VOT) to be calculated. A row of three asterisks was displayed for 1.5 sec after the screen went blank and brain images were collected during this period. The speech of the participants was fluent in both scanning sessions as the task was simple. Fig. 1A and 1B summarize the design.

E-prime software (www.pstnet.com) was used to display the stimuli. The stimuli were back-projected from a liquid crystal display onto a screen at the foot of the MR

scanner. Participants viewed the stimuli via a mirror attached to the head coil above their eyes.

2.4. Imaging data acquisition

Imaging data were acquired on a Siemens TRIO 3T scanner at the MRI Center of Beijing Normal University. Participants lay supine within the MR scanner with their head secured with foam padding. The MRI-compatible headphone system was used to reduce the scanner noise. The resting-state scan was always performed at the start, and the task and structural scans followed.

2.4.1. Resting-state scan

Participants were instructed to close their eyes, relax, and remain stationary. The axial gradient-recalled echo-planar images (EPI) were acquired in an 8-minute resting-state scan. The parameters were as follows: Repetition time (TR) = 2,000 msec; echo time (TE) = 30 msec; flip angle = 90°; slice thickness = 4 mm; in-plane resolution = 3.1 * 3.1mm^2; number of interleaved slices = 33.

2.4.2. Task scan

EPI were acquired using the following parameters: TR = 3000 ms (delay = 1500 ms); TE = 30 ms; flip angle = 90°; slice thickness = 5 mm; in-plane resolution = 3.1 × 3.1 mm^2; number of interleaved slices = 25.

2.4.3. Structural scan

A high-resolution T1-weighted MP-RAGE sequence was employed: TR = 2,530 msec; TE = 3.30 msec; flip angle = 7°; slice thickness = 1.3 mm; in-plane resolution = 1.3 * 1.0 mm^2; number of interleaved sagittal slices = 128.

2.5. VOT analysis of the speech responses

VOTs were measured and compared between participant groups. This was done both pre- and post-intervention in order to test whether the speech production performance differed across participant groups (all pairs of groups were compared).

2.6. Task fMRI data analysis

2.6.1. Preprocessing and individual-level statistics

Data preprocessing and statistical analyses were conducted using the Analysis of Functional NeuroImages package (AFNI, http://afni.nimh.nih.gov/afni) (Cox, 1996, 2011). The first two volumes of each participant's functional images were discarded prior to data analysis. During preprocessing, the functional images were slice-time corrected and realigned. During spatial normalization, the functional images were co-registered to high-resolution T1 images at individual participant level. The images were then spatially normalized to the Montreal Neurological Institute (MNI) template (spatial resolution = 2 mm × 2 mm × 2 mm) using the corresponding T1 images (Ashburner & Friston, 2005). Finally, the functional images were smoothed using a 6-mm full-width at half-maximum Gaussian filter.

Brain activity associated with laryngeal motor control in the current speech task was examined to validate the sparse sampling scanning procedure. To do this, the time courses of the preprocessed BOLD fMRI data were extracted from the laryngeal motor cortex (X, Y, Z = -48, -14, 40) that is known to be associated with laryngeal motor control (Brown, Ngan, & Liotti, 2008; Howell et al., 2012a). These time courses were plotted together with the box-shape time course of the experimental design (see Fig. 1C). The dark black line and grey line with triangle markers represent the time courses of the group-average across all fluent controls and a randomly selected fluent control (#13) respectively. The grey line with circle markers represents the box-shape time course of the experimental design. As the figure shows, both the group-averaged and

the individual-participant's BOLD time courses fit well to the time course of the experimental design. The goodness of fit was estimated by general linear models (GLM) (group-average: $t = 5.028$, $P < 0.001$; individual participant: $t = 6.92$, $P < 0.001$). The estimated motion parameters were included in the GLM to exclude potential confounding of head movement artefacts on the estimation of goodness of fit. The fit was good, confirming the validity of the sparse sampling procedure used in this study, as was expected based on previous evidence (Perrachione & Ghosh, 2013).

The estimated motion parameters [i.e., motion correction parameters for translation (X, Y and Z) and rotation (pitch X, roll Y and yaw Z)] were also examined. First, a one-way ANCOVA was conducted to examine potential differences across groups pre-intervention. Age was included as a covariate. No significant differences were found pre-intervention in any parameters ($P > 0.05$) except for rotation Y ($F_{(3,35)} = 4.443$, $P = 0.01$). *Post-hoc* analysis showed a significant difference between FC- and AWS- ($P = 0.046$), but no significant differences were found between FC- and AWS+ ($P = 0.97$) nor between AWS+ and AWS- ($P = 0.109$). Second, paired two-sample *t*-tests were conducted between pre- and post-intervention data for each group. No significant differences were found for FC-, AWS+, and AWS- after Šidák correction for multiple comparisons ($P > 0.05$) (Abdi, 2007).

Fig. 1 about here

The preprocessed data were subjected to GLM analysis to obtain individual level statistics. The brain activation for each individual participant was calculated as the contrast (β) between task and baseline. Each β value was then converted into % signal

change. The estimated motion parameters were included in the GLM to exclude potential confounding of head movement artefacts with brain activations.

2.6.2. Group level statistics 1: Comparison between FC-, AWS-, and AWS+ pre-intervention

A one-way ANCOVA was performed to: 1) establish the initial consistency of brain activation between AWS+ and AWS- as required when examining whether any changes in brain function in AWS+ were due to the intervention they received; 2) determine whether FC-, AWS+, and AWS- showed different patterns of brain activation during the task. The threshold for significance was $P < 0.05$ (corrected by Monte Carlo simulation at the cluster level, cluster volume > 668 mm^3, individual voxel $P < 0.005$) (Forman et al., 1995; Xiong, Gao, Lancaster, & Fox, 1995).

The % signal change for each brain region that survived the ANCOVA analysis was extracted for each participant. Then, the extracted % signal change was examined for group difference patterns based on a *post-hoc* test with Šidák correction [the cluster-based ANCOVA were not conducted here in order to avoid double-dipping (Kriegeskorte, Simmons, Bellgowan, & Baker, 2009)].

2.6.3. Group-level statistics 2: Comparison between brain activations across the two test sessions (post-intervention minus pre-intervention)

Paired two-sample *t*-tests were conducted to examine changes in brain function between the two scanning sessions. This analysis was performed separately for the three participant groups ($P < 0.05$, corrected in the same way as described under 2.6.2 *Group-level statistics 1*). Then, the % signal change for any brain regions that survived the *t*-test was extracted for each participant, and examined for group difference patterns post-intervention based on a cluster-based ANCOVA and *post-hoc* test with Šidák correction.

2.7. Resting-state fMRI data analysis

The resting-state data analyses were reported in Lu et al. (2012). The analysis procedures are described briefly below as they help evaluate the present study.

2.7.1. Preprocessing and individual-level statistics

Imaging data were pre-processed using AFNI. The first four volumes of the EPI images were discarded to allow the magnetic field to stabilize. Slice-time correction, image registration, motion correction, and spatial smoothing (full width at half maximum = 6 mm) were performed next. After the global linear trend was removed, the imaging data were band-pass filtered using a Fourier transform procedure (0.01-0.08 Hz) to extract the low frequency fluctuations (LFFs). These LFFs were then scaled and converted into % signal change.

The preprocessed data were then subjected to independent component analysis (ICA) using the FMRIB Software Library's Melodic software (http://www.fmrib.ox.ac.uk/fsl/melodic/index.html). ICA separates a set of signals into independent maximally non-Gaussian components (ICs) (Beckmann & Smith, 2004). , The optimal number of ICs for each participant was determined using the Laplace approximation to the Bayesian evidence for a probabilistic principal components analysis model during IC decomposition (Beckmann, DeLuca, Devlin, & Smith, 2005; Beckmann & Smith, 2004). Each IC has a time course pattern. The voxels whose time series correlated significantly with the IC's time course comprise a spatial pattern. The voxel's value represents the degree of correlation between the time series of that voxel and the time course of the IC. Sample maps associated with each IC were then transformed to *z*-scores. The *z*-scores reflected the degree to which a given voxel's time series was correlated with the specific IC's time course. All data sets were then normalized into the standard MNI space.

Finally, following previous research (Greicius, Srivastava, Reiss, & Menon, 2004), a modified quantitative procedure was used to select the ICs for each participant that matched most closely the spatial map of the speech-language network.

2.7.2. Intervention-induced RSFC reorganization

In order to examine any potential reorganization of the RSFC arising from intervention in AWS+, the ICs of these participants were selected and compared across the intervention (pre- vs. post-intervention) in a second-level random-effect analysis (paired two-sample *t*-test). The same procedure was applied to AWS- and FC- to confirm the stability of RSFC when there was no intervention ($P < 0.05$, corrected).

2.8. Analyses to test whether changes in RSFC and task-related brain function were related to one another

Lu et al. (2012) showed that AWS+ have decreased RSFC between the midline of the cerebellum (X, Y, Z = -5, -70, -15, $z = 4.327$, cluster volume = 363 mm^3, declive of vermis) and the whole language network compared to fluent controls after the intervention. Thus, the relationship between the RSFC changes in the midline of the cerebellum and task-related changes in brain function were examined using a *Pearson* correlation method.

2.9. Analyses to test whether changes in brain function were related to changes in behavioral performance

Estimates of individual variations in the impact of the intervention on speaking behaviors (changes of SSI-3 score, %SS, and OASES score) were obtained and correlated with changes in brain function to further establish the relationship between changes in brain function and the impact of the intervention on behaviors.

3. Results

3.1. Group difference in the VOT

None of the comparisons between any pairs of groups were significant either pre- or post-intervention ($P > 0.05$).

3.2. Group difference in task-related brain function pre-intervention

As previously reported (Lu et al., 2012), %SS, SSI-3, and OASES in AWS+ showed significant changes after the intervention for both overt stuttering behavior [%SS, $t(12) = 8.015$, $P < 0.0001$; SSI-3, $t(12) = 5.82$, $P < 0.001$] and covert stuttering experiences [OASES, $t(12) = 5.26$, $P < 0.001$]. As expected, no such changes were found in AWS-

.

Whole-brain activation results for each group pre- and post-intervention are presented in Fig. S1 ($P < 0.001$, cluster volume > 800 mm^3). The results were not corrected for multiple comparisons in order to show general activation patterns.

Whole-brain ANCOVA revealed two brain regions that showed significant group differences in task-related brain activation among AWS+, AWS- and FC- pre-intervention (Fig. 2A and 2C). These were the left middle temporal cortex (MTC, BA39, X, Y, Z = -42, -80, 10; $F_{(2,36)} = 10.161$, cluster volume = 2056 mm^3) and the right IFC/PMA (BA44, X, Y, Z = 44, 8, 18; $F_{(2,36)} = 8.545$, cluster volume = 1528 mm^3).

A cluster-based *post-hoc* analysis was conducted on the MTC and IFC/PMA to clarify the pattern of group differences. The results showed that, compared to the FC-group, both groups of AWS had significantly higher activation in the right IFC/PMA (AWS+: $P = 0.019$; AWS-: $P = 0.005$), but lower activation in the left MTC (AWS+: $P < 0.029$; AWS-: $P = 0.002$). There were no significant differences between the two AWS groups (IFC/PMA: $P = 0.951$; MTC: $P = 0.686$) (Fig. 2B and 2D).

3.3. Inter-session difference in task-related brain function (post-intervention minus pre-intervention)

AWS+ showed a significant increase in activation from pre- to post-intervention in the left vIFC/insula (BA47/13, X, Y, Z = -28, 14, -16; $t(12) = 5.02$, cluster volume = 4224 mm^3) (Fig. 2E). As expected, the AWS- and FC- groups did not show any significant changes in brain function between the two test sessions. These findings confirmed that the changes in brain function found in AWS+ were induced by the intervention.

Further analysis of this region based on cluster-based ANCOVA showed significant group differences post-intervention ($F_{(2,36)} = 7.574$, $P = 0.002$). *Post-hoc* analysis (again with Šidák correction) showed that post-intervention the activation of this region was significantly higher for AWS+ than for AWS- ($P = 0.014$) and FC- ($P = 0.003$). There were no significant group differences in activation among the three groups pre-intervention ($F_{(2,36)} = 0.848$, $P = 0.437$) (Fig. 2F).

In addition, a correlation analysis between the change in brain function in the left vIFC and age in AWS+ was conducted to exclude the potential relationship between age and intervention. The correlation was not significant ($r = 0.429$, $P = 0.143$). Thus, the effect of age on the results, if any, was minimal in this study.

Fig. 2 about here

3.4. Relationship between changes in RSFC and that in task-related brain function

Changes in task-related brain function of the left vIFC did not correlate significantly with changes in RSFC of the cerebellum ($r = 0.037$, $P = 0.905$).

3.5. Are changes in brain function related to behavioral changes?

The increase of brain activation in the left vIFC correlated significantly with the decrease of stuttering severity as measured by the SSI-3 score ($r = -0.714$, $P = 0.006$).

The correlation with SSI-3 score was still significant (partial correlation: r_p = -0.679, P = 0.022) when the changes in the duration of stuttering events, physical concomitants, and OASES were partialled out (Fig. 3A). However, when one participant with outliers was removed, the correlation was then only marginal significant (partial correlation: r_p = -0.542, P = 0.085). In addition, the changes in brain function also partially correlated with %SS when the changes in OASES were partialled out (r_p = -0.589, P = 0.044) (Fig. 3B). However, the change in brain function did not correlate significantly with the change in OASES score irrespective of whether (partial correlation: r_p = -0.246, P = 0.44) (Fig. 3C) or not (correlation: r = 0.5, P = 0.87) the changes in stuttering severity were partialled out. Together, these results indicated that individuals for whom the intervention was more effective in enhancing their fluency level showed a larger change in the left vIFC than those for whom the intervention was less effective.

The resting-state data for the participants who provided task data were re-computed and correlations with changes in behavioral performance were examined. There was a significant correlation between the decrease of RSFC in the cerebellum and reduction in stuttering severity when the effects of duration of stuttering events, physical concomitants, and OASES were partialled out (partial correlation: r_p = 0.605, P = 0.048) (Fig. 3D). However, the correlation between the change in brain function and change in %SS was not significant even when OASES scores were partialled out (r_p = 0.008, P = 0.98) (Fig. 3E). Also, no significant correlation was found between the change in brain function and change in OASES scores irrespective of whether (partial correlation: r_p = 0.061, P = 0.851) (Fig. 3F) or not (correlation: r = 0.06, P = 0.846) the changes in stuttering severity were partialled out.

Fig. 3 about here

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4. Discussion

This study examined how the brain responds to a short-term behavioral intervention for stuttering. The results revealed increased activation in the left vIFC/Insula after the successful 7-day intervention on AWS+. No functional changes were detected in fluent controls and AWS- who were not given the intervention. This result suggested that the changes in brain function in this region were induced by the behavioral intervention. Convergent evidence has shown that the degree of activation of the left IFC in normal speakers and damage to the left IFC in aphasic speakers are associated with performance level in speech production tasks (Schnur et al., 2009). Specifically, this region plays a role in lexical selection (Schnur et al., 2009), phonological processing (Costafreda et al., 2006), phonetic encoding (Papoutsi et al., 2009), and integration of information (Hagoort, 2005). Studies on AWS have reported both functional anomalies (Lu et al., 2012; Salmelin et al., 2000) and structural disconnections (based on diffusion tensor imaging) in this region (Cykowski et al., 2010; Kell et al., 2009). Other studies also revealed functional anomalies and structural disconnections in regions that surround, or are connected with, the left IFC (Sommer, Koch, Paulus, Weiller, & Buchel, 2002; Watkins, Smith, Davis, & Howell, 2008). Most importantly, after a 3-week intervention, a region adjacent to the anomalous part of the left IFC showed increased activation compared to that before intervention (Neumann et al., 2005). Moreover, people who have recovered from stuttering without intervention also showed stronger activation in the adjacent region (vIFC, BA47) compared to AWS who did not recover (Kell et al., 2009). Thus, it is likely that the increase of activation in the left vIFC is related to stuttering that recovers fully (Kell et al., 2009). The present findings

suggest that the changes in brain function that are related to full recovery from stuttering can be induced by a short-term behavioral intervention.

This finding also supports the speculation that different patterns of changes in brain function may occur at different times after behavioral interventions have been delivered to AWS. In previous studies, brain functions in AWS have been found to dynamically change from pre-intervention, immediately post-intervention, to one- or two-years later (De Nil et al., 2003; Neumann et al., 2003; Neumann et al., 2005). Some of these changes in brain function are related to full recovery of stuttering (Kell et al., 2009). These changes can be induced at the start by a short-term intervention and are retained at full recovery (e.g., the changes of brain function in the left IFC) (Kell et al., 2009). Other changes are likely to be temporary and are not directly related to the optimal recovery of stuttering (De Nil et al., 2003; Neumann et al., 2003; Neumann et al., 2005).

Ideally there should have been a placebo condition in which AWS- and fluent controls receive a "sham" intervention. However, it is difficult to create a "sham" intervention that appears to AWS to be an "intervention" that would have no effects on fluency at all. Because a sham condition is lacking, although the changes in brain function may be induced by the intervention in the current study, they might not be directly attributable to the current intervention procedure. On the other hand, although all individuals in the AWS+ group received the same intervention, some individuals showed more change than others. Thus, the individual differences in the pre-post changes were examined by estimating individual variation in the impact of the intervention on behavioral performance. The individual variations were then correlated with changes in brain function. When this was done, the results showed that individuals for whom the intervention was more effective had a larger change in the left vIFC.

However, there was no significant correlation between the changes in brain function and the changes in stuttering experiences as measured by OASES. These correlations suggest that the functional changes in the left vIFC was closely associated with improvement of speech fluency level, but independent of the general changes in stuttering experiences.

Previous evidence suggested a close relationship between the pars opercularis (BA44) of the left IFC and phonological and articulatory control during speech production (Costafreda et al., 2006). Studies on stuttering have also found functional differences in this region between AWS and fluent controls in a resting-state paradigm. For example, Lu et al. (2012) conducted an IC analysis that compared difference in RSFC between AWS and controls. A weaker RSFC between the pars opercularis of the left IFC and the whole language network was identified in AWS compared to fluent controls. Moreover, this neural difference remained stable after a successful short-term behavioral intervention. When a seed-based correlation analysis was performed, Xuan et al. (2012) found decreased connectivity between the pars opercularis of the left IFC and right inferior parietal lobule, but increased RSFC between the same region of the left IFC and left PMA. Interestingly, Chang and Zhu (2013) examined children who stutter, and also found weaker RSFC between the pars opercularis of the left IFC and the left posterior superior temporal cortex (in both male and female AWS) and between the same region of the left IFC and the left PMA (only in male AWS). However, the current study did not detect any functional differences between AWS and controls during task performance in this region. Similarly, previous fMRI studies using various speech tasks also failed to find functional activation differences between AWS and controls in this region (Brown et al., 2005; Chang, Kenney, Loucks, & Ludlow, 2009; De Nil et al., 2008; Howell et al., 2012b; Kell et al., 2009; Watkins et al., 2008).

Although some studies (e.g., Watkins et al., 2008) identified lower brain activation in a nearby region in the ventral PMA, the ventral PMA might play a different role to that performed by the pars opercularis of the left IFC. Thus, although there are still inconsistencies in the specific patterns of group differences in the RSFC (i.e., stronger or weaker), this evidence suggests that the abnormal functionality of the pars opercularis of the left IFC can be identified in the resting-state condition alone. Another possibility is that the abnormal functionality of the left IFC occurred in specific conditions. For instance, a recent study (Lu et al., 2016) showed that the left IFC had significantly stronger activity in multi-syllabic word production than in single-syllabic word production in controls, but not in AWS, indicating a reduction in the function of this area during speech production. Another study further showed lower BOLD signal amplitude in the left IFC when AWS producing more typical stuttering symptoms (Jiang et al., 2012). Thus, it is also possible that the abnormal functionality of the left IFC can be identified only when the more typical symptoms of stuttering are specifically tapped.

Consistent with previous studies, overactivation in the right IFC but less activation in the left MTC were found. Overactivation in the right frontal cortex/anterior insula and less activation in the left temporal cortex have been considered to be two neural signatures of stuttering (Brown et al., 2005). A recent review further distinguished state-related (by comparison of AWS and controls when speech was fluent in both groups) and trait-related (comparison of stuttered and fluent speech in AWS) functional difference (Belyk, Kraft, & Brown, 2015). This study showed overactivation in regions in the right hemisphere including the right IFC, and lower activation in the left hemisphere including the temporal cortex, both of which were associated with trait stuttering. More diverse group difference patterns were associated with state stuttering.

It seems that the abnormal functional activations in the right IFC and left MTC found in the current study reflected trait features of AWS rather than temporary stuttering phenomenon.

There was no significant correlation between changes in RSFC of the cerebellum and those in task-related brain function of the left vIFC. Previous dual-route models of stuttering have postulated different roles for the basal ganglia-related and cerebellum-related neural circuits in stuttering (Alm, 2004, 2006; Howell, 2004, 2007; Howell et al., 2000; Howell & Dworzynski, 2005; Howell et al., 2012b; Jiang et al., 2012; Lu, Chen, et al., 2010; Lu et al., 2009; Lu, Peng, et al., 2010). Most importantly, in a speech task, the left vIFC did not show any activation before the intervention, but did after the intervention. In contrast, in the resting-state condition, higher-level RSFC in the cerebellum was found in AWS+ before the intervention, but was eliminated after the intervention. Different patterns of changes in brain function in the left IFC and cerebellum suggest that they might have different roles in stuttering. However, the *Pearson* correlations that showed no correlation provide weak evidence to support distinct roles of the vIFC-related and cerebellum-related neural circuits. A more rigorous approach would have been to look at task-related networks and examine whether there were differences in connectivity pre- and post- intervention.

The present findings have some general implications for the mechanism of brain plasticity in the speech motor system. First, although it is well known that training can lead to an enhancement of brain function (Kerr, Cheng, & Jones, 2011; Kleim, 2011; Kleim & Jones, 2008; Ludlow et al., 2008), it is not clear how this happens in the speech motor system (Ludlow et al., 2008). On the one hand, in aphasic cases in which speech functions eventually recovered, the neural control returned to the original form seen in controls that involved the left IFC and motor cortex (Saur et al., 2006). On the other

hand, spontaneous recovery from cortical dysarthria post-stroke showed a contralateral transfer of the neural control (Riecker, Wildgruber, Grodd, & Ackermann, 2002). After a short-term behavioral intervention for stuttering in the current study, both plastic changes, i.e., contralateral and peri-anomalous plasticity, occurred but in different ways (losing an anomalous function vs. acquiring a new function). Thus, the present findings provide new insight into the mechanism behind brain plasticity in the speech motor system.

Second, during the treatment of speech motor disorders, several recovery stages may occur (Howell & Lu, in press). For example, as shown by the current results, after a short-term intervention, the peri-anomalous regions were recruited. It is predicted that at later stages after long-term intervention, whole-brain functional lateralization will shift dynamically between the left and right hemisphere (De Nil, Kroll, & Houle, 2001; Neumann et al., 2003; Neumann et al., 2005). Thus, a general mechanism of dynamic brain plasticity in both acquired speech production disorders with brain injuries (Saur et al., 2006) and developmental speech production disorders that do not show clear-cut brain damage is required. In future studies it is important to report at what stage post-treatment the plasticity is found.

There are several limitations in this study. First, the design of this study could not dissociate the factor of intervention length and that of intensity. However, previous studies on stuttering interventions usually employ an intervention with similar or even higher-level intensity (e.g., the Kassel Stuttering Therapy, KST) (Euler & Wolff von Gudenberg, 2002). Interestingly, recruitment of an area around the left speech motor cortex was reported around 6-12 weeks after the intensive KST intervention (Neumann et al., 2003; Neumann et al., 2005). Thus, it seems that the current findings are not related to intensity of intervention. However, more research is needed to clarify this

issue. Second, although a sparse sampling method helps to avoid potential artifacts due to articulator movement on the BOLD signal, it also resulted in poor spatial resolution of the imaging data in the current study (i.e., 25 slices with 5 mm slice thickness). Specifically, we only have large voxels that average across many subpopulations of neurons, thus losing spatial and functional resolution. Finally, examinations of the relationship between brain plasticity in different speaking tasks or different treatment methods are also required.

5. Conclusions

This study reported that the postulated optimal brain repair appeared after a short-term behavioral intervention for AWS+. This finding is consistent with previous propositions of the dual-route model and EXPLAN concerning the behavior and neurophysiology of stuttering. This suggests that repair of the basal ganglia-IFC circuit and cerebellum-PMA circuit is the key to the recovery from stuttering in adulthood. Future studies are needed to test whether direct brain stimulation on brain regions in the two circuits can enhance speech fluency, and whether stimulation to different brain regions can induce different effects on behavior.

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References

Abdi, H. (2007). The Bonferonni and Šidák corrections for multiple comparisons. *Encyclopedia of measurement and statistics*, 3, 103-107.

Alm, P. A. (2004). Stuttering and the basal ganglia circuits: a critical review of possible relations. *J Commun Disord*, 37(4), 325-369.

Alm, P. A. (2006). *A new framework for understanding stuttering: The dual premotor model*. Paper presented at the International Fleuncy Association. Section 3. Theory and Prevalence of Stuttering, Dublin.

Ashburner, J., & Friston, K. J. (2005). Unified segmentation. *Neuroimage*, 26(3), 839-851.

Baldassarre, A., Lewis, C. M., Committeri, G., Snyder, A. Z., Romani, G. L., & Corbetta, M. (2012). Individual variability in functional connectivity predicts performance of a perceptual task. *Proc Natl Acad Sci U S A*, 109(9), 3516-3521.

Beckmann, C. F., DeLuca, M., Devlin, J. T., & Smith, S. M. (2005). Investigations into resting-state connectivity using independent component analysis. *Philos Trans R Soc Lond B Biol Sci*, 360(1457), 1001-1013.

Beckmann, C. F., & Smith, S. M. (2004). Probabilistic independent component analysis for functional magnetic resonance imaging. *IEEE Trans Med Imaging*, 23(2), 137-152.

Belyk, M., Kraft, S. J., & Brown, S. (2015). Stuttering as a trait or state - an ALE meta-analysis of neuroimaging studies. *Eur J Neurosci*, 41(2), 275-284.

Brown, S., Ingham, R. J., Ingham, J. C., Laird, A. R., & Fox, P. T. (2005). Stuttered and fluent speech production: an ALE meta-analysis of functional neuroimaging studies. *Hum Brain Mapp*, 25(1), 105-117.

Brown, S., Ngan, E., & Liotti, M. (2008). A larynx area in the human motor cortex. *Cerebral Cortex*, 18(4), 837-845.

Chang, S. E., Horwitz, B., Ostuni, J., Reynolds, R., & Ludlow, C. L. (2011). Evidence of left inferior frontal-premotor structural and functional connectivity deficits in adults who stutter. *Cereb Cortex*, 21(11), 2507-2518.

Chang, S. E., Kenney, M. K., Loucks, T. M., & Ludlow, C. L. (2009). Brain activation abnormalities during speech and non-speech in stuttering speakers. *Neuroimage*, 46(1), 201-212.

Chang, S. E., & Zhu, D. C. (2013). Neural network connectivity differences in children who stutter. *Brain,* 136(Pt 12), 3709-3726.

Comeau, W. L., McDonald, R. J., & Kolb, B. E. (2010). Learning-induced alterations in prefrontal cortical dendritic morphology. *Behavioural Brain Research,* 214(1), 91-101.

Costafreda, S. G., Fu, C. H., Lee, L., Everitt, B., Brammer, M. J., & David, A. S. (2006). A systematic review and quantitative appraisal of fMRI studies of verbal fluency: role of the left inferior frontal gyrus. *Hum Brain Mapp,* 27(10), 799-810.

Cox, R. W. (1996). AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Computers and Biomedical Research,* 29(3), 162-173.

Cox, R. W. (2011). AFNI: What a long strange trip it's been. *NeuroImage*.

Craig, A., & Tran, Y. (2005). The epidemiology of stuttering: The need for reliable estimates of prevalence and anxiety levels over the lifespan. *International Journal of Speech-Language Pathology,* 7(1), 41-46.

Cykowski, M. D., Fox, P. T., Ingham, R. J., Ingham, J. C., & Robin, D. A. (2010). A study of the reproducibility and etiology of diffusion anisotropy differences in developmental stuttering: a potential role for impaired myelination. *Neuroimage,* 52(4), 1495-1504.

De Nil, L. F., Beal, D. S., Lafaille, S. J., Kroll, R. M., Crawley, A. P., & Gracco, V. L. (2008). The effects of simulated stuttering and prolonged speech on the neural activation patterns of stuttering and nonstuttering adults. *Brain Lang,* 107(2), 114-123.

De Nil, L. F., Kroll, R. M., & Houle, S. (2001). Functional neuroimaging of cerebellar activation during single word reading and verb generation in stuttering and nonstuttering adults. *Neurosci Lett,* 302(2-3), 77-80.

De Nil, L. F., Kroll, R. M., Lafaille, S. J., & Houle, S. (2003). A positron emission tomography study of short- and long-term treatment effects on functional brain activation in adults who stutter. *Journal of Fluency Disorders,* 28(4), 357-380.

Euler, H., & Wolff von Gudenberg, A. (2002). The Kassel Stuttering Therapy: Do follow-up compliance incentives help maintain fluency shaping treatment effects. In

M. Gross & E. Kruse (Eds.), *Aktuelle phoniatrisch-pädaudiologische Aspekte 2001/2002* (pp. 107-110). Heidelberg: Median-Verlag von Killisch-Horn.

Forman, S. D., Cohen, J. D., Fitzgerald, M., Eddy, W. F., Mintun, M. A., & Noll, D. C. (1995). Improved assessment of significant activation in functional magnetic resonance imaging (fMRI): use of a cluster-size threshold. *Magn Reson Med,* 33(5), 636-647.

Greicius, M. D., Srivastava, G., Reiss, A. L., & Menon, V. (2004). Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. *Proceedings of the National Academy of Sciences of the United States of America,* 101(13), 4637-4642.

Hagoort, P. (2005). On Broca, brain, and binding: a new framework. *Trends Cogn Sci,* 9(9), 416-423.

Hampson, M., Driesen, N. R., Skudlarski, P., Gore, J. C., & Constable, R. T. (2006). Brain connectivity related to working memory performance. *J Neurosci,* 26(51), 13338-13343.

Howell, P. (2004). Assessment of Some Contemporary Theories of Stuttering That Apply to Spontaneous Speech. *Contemp Issues Commun Sci Disord,* 31, 122-139.

Howell, P. (2007). A model of serial order problems in fluent, stuttered and agrammatic speech. *Hum Mov Sci*, 26(5), 728-741.

Howell, P. (2011). *Recovery from Stuttering*: Psychology Press/Taylor & Francis Group.

Howell, P., Au-Yeung, J., & Sackin, S. (2000). Internal Structure of Content Words Leading to Lifespan Differences in Phonological Difficulty in Stuttering. *J Fluency Disord*, 25(1), 1-20.

Howell, P., & Dworzynski, K. (2005). Planning and execution processes in speech control by fluent speakers and speakers who stutter. *J Fluency Disord*, 30(4), 343-354.

Howell, P., Jiang, J., Peng, D., & Lu, C. (2012a). Neural control of fundamental frequency rise and fall in Mandarin tones. *Brain Lang*, 121(1), 35-46.

Howell, P., Jiang, J., Peng, D., & Lu, C. (2012b). Neural control of rising and falling tones in Mandarin speakers who stutter. *Brain Lang*, 123(3), 211-221.

Howell, P., & Lu, C. (in press). Facts and theories about stuttering. In P. v. Lieshout, B. Maassen & H. Terband (Eds.), *Speech motor control in normal and disordered*

speech: Future developments in theory and methodology (pp. 147-172). Rockville, MD: ASHA Press.

Institute of Linguistics, B. L. C. (1986). A Freqency Dictionary for Modern Chinese (pp. 1491). Beijing: Beijing Language College Press.

Jiang, J., Lu, C., Peng, D., Zhu, C., & Howell, P. (2012). Classification of types of stuttering symptoms based on brain activity. *PLoS One,* 7(6), e39747.

Kell, C. A., Neumann, K., von Kriegstein, K., Posenenske, C., von Gudenberg, A. W., Euler, H., et al. (2009). How the brain repairs stuttering. *Brain,* 132(Pt 10), 2747-2760.

Kerr, A. L., Cheng, S.-Y., & Jones, T. A. (2011). Experience-dependent neural plasticity in the adult damaged brain. *Journal of Communication Disorders,* 44(5), 538-548.

Kleim, J. A. (2011). Neural plasticity and neurorehabilitation: teaching the new brain old tricks. *Journal of Communication Disorders,* 44(5), 521-528.

Kleim, J. A., & Jones, T. A. (2008). Principles of experience-dependent neural plasticity: implications for rehabilitation after brain damage. *Journal of Speech, Language, and Hearing Research,* 51(1), S225-239.

Koch, G. G. (1982). Intraclass correlation coefficient. In S. Kotz & N. L. Johnson (Eds.), *Encyclopedia of Statistical Sciences* (pp. 213–217). New York: John Wiley & Sons.

Kriegeskorte, N., Simmons, W. K., Bellgowan, P. S., & Baker, C. I. (2009). Circular analysis in systems neuroscience: the dangers of double dipping. *Nature neuroscience*, 12(5), 535-540.

Lu, C., Chen, C., Ning, N., Ding, G., Guo, T., Peng, D., et al. (2010). The neural substrates for atypical planning and execution of word production in stuttering. *Exp Neurol*, 221(1), 146-156.

Lu, C., Chen, C., Peng, D., You, W., Zhang, X., Ding, G., et al. (2012). Neural anomaly and reorganization in speakers who stutter: a short-term intervention study. *Neurology*, 79(7), 625-632.

Lu, C., Long, Y., Zheng, L., Shi, G., Liu, L., Ding, G., et al. (2016). Relationship between Speech Production and Perception in People Who Stutter. *Front Hum Neurosci*, 10, 224.

Lu, C., Ning, N., Peng, D., Ding, G., Li, K., Yang, Y., et al. (2009). The role of large-scale neural interactions for developmental stuttering. *Neuroscience,* 161(4), 1008-1026.

Lu, C., Peng, D., Chen, C., Ning, N., Ding, G., Li, K., et al. (2010). Altered effective connectivity and anomalous anatomy in the basal ganglia-thalamocortical circuit of stuttering speakers. *Cortex,* 46(1), 49-67.

Ludlow, C. L., Hoit, J., Kent, R., Ramig, L. O., Shrivastav, R., Strand, E., et al. (2008). Translating principles of neural plasticity into research on speech motor control recovery and rehabilitation. *Journal of Speech, Language, and Hearing Research,* 51(1), S240-258.

Ma, X. B. (2007). *Handbook of National Putonghua Proficiency Test* (1 ed.): Jinan University Press.

Neumann, K., Euler, H. A., Gudenberg, A. W. v., Giraud, A. L., Lanfermann, H., Gall, V., et al. (2003). The nature and treatment of stuttering as revealed by fMRI. *Journal of Fluency Disorders,* 28(4), 381-410.

Neumann, K., Preibisch, C., Euler, H. A., von Gudenberg, A. W., Lanfermann, H., Gall, V., et al. (2005). Cortical plasticity associated with stuttering therapy. *J Fluency Disord,* 30(1), 23-39.

Papoutsi, M., de Zwart, J. A., Jansma, J. M., Pickering, M. J., Bednar, J. A., & Horwitz, B. (2009). From phonemes to articulatory codes: an fMRI study of the role of Broca's area in speech production. *Cereb Cortex,* 19(9), 2156-2165.

Perrachione, T. K., & Ghosh, S. S. (2013). Optimized design and analysis of sparse-sampling FMRI experiments. *Front Neurosci,* 7, 55.

Riecker, A., Wildgruber, D., Grodd, W., & Ackermann, H. (2002). Reorganization of speech production at the motor cortex and cerebellum following capsular infarction: a follow-up functional magnetic resonance imaging study. *Neurocase,* 8(6), 417-423.

Riley, G. (1994). *Stuttering severity instrument for children and adults* (3rd ed.). Austin, TX.: Pro-Ed.

Salmelin, R., Schnitzler, A., Schmitz, F., & Freund, H. J. (2000). Single word reading in developmental stutterers and fluent speakers. *Brain,* 123 (Pt 6), 1184-1202.

Sato, Y., Mori, K., Koizumi, T., Minagawa-Kawai, Y., Tanaka, A., Ozawa, E., et al. (2011). Functional lateralization of speech processing in adults and children who stutter. *Front Psychol*, 2, 70.

Saur, D., Lange, R., Baumgaertner, A., Schraknepper, V., Willmes, K., Rijntjes, M., et al. (2006). Dynamics of language reorganization after stroke. *Brain*, 129(Pt 6), 1371-1384.

Schnur, T. T., Schwartz, M. F., Kimberg, D. Y., Hirshorn, E., Coslett, H. B., & Thompson-Schill, S. L. (2009). Localizing interference during naming: convergent neuroimaging and neuropsychological evidence for the function of Broca's area. *Proc Natl Acad Sci U S A*, 106(1), 322-327.

Sommer, M., Koch, M. A., Paulus, W., Weiller, C., & Buchel, C. (2002). Disconnection of speech-relevant brain areas in persistent developmental stuttering. *Lancet*, 360(9330), 380-383.

Tambini, A., & Davachi, L. (2013). Persistence of hippocampal multivoxel patterns into postencoding rest is related to memory. *Proc Natl Acad Sci U S A*, 110(48), 19591-19596.

Tambini, A., Ketz, N., & Davachi, L. (2010). Enhanced brain correlations during rest are related to memory for recent experiences. *Neuron,* 65(2), 280-290.

Toyomura, A., Fujii, T., & Kuriki, S. (2015). Effect of an 8-week practice of externally triggered speech on basal ganglia activity of stuttering and fluent speakers. *Neuroimage,* 109, 458-468.

Wang, L., Laviolette, P., O'Keefe, K., Putcha, D., Bakkour, A., Van Dijk, K. R., et al. (2010). Intrinsic connectivity between the hippocampus and posteromedial cortex predicts memory performance in cognitively intact older individuals. *Neuroimage,* 51(2), 910-917.

Watkins, K. E., Smith, S. M., Davis, S., & Howell, P. (2008). Structural and functional abnormalities of the motor system in developmental stuttering. *Brain,* 131(Pt 1), 50-59.

Xiong, J. H., Gao, J. H., Lancaster, J. L., & Fox, P. T. (1995). Clustered pixels analysis for functional MRI activation studies of the human brain. *Human Brain Mapping,* 3(4), 287-301.

Xuan, Y., Meng, C., Yang, Y., Zhu, C., Wang, L., Yan, Q., et al. (2012). Resting-state brain activity in adult males who stutter. *PLoS One,* 7(1), e30570.

Yairi, E., & Ambrose, N. (2005). *Early Childhood Stuttering*. Austin, TX: PRO-ED, Inc.

Figure legends

Fig. 1 Summary of the experimental procedure. (A) The overall arrangement of the experiment. (B) Experimental procedure. (C) Preprocessed BOLD time courses in the laryngeal motor cortex. Solid black line, the experimental design; solid grey line with circle, the time course for one randomly selected fluent participant; solid grey line with triangle, the group-averaged time course in fluent controls. The x-axis represents trial number. The baseline lasted 15 sec, which is equal to a period of 5 trials (3 sec per trial).

Fig. 2 (A) and (C) show ANCOVA results of functional activation differences among the three groups pre-intervention. (B) and (D) show the pattern of % signal change that was extracted from each cluster of (A) and (C). (E) shows changes in brain function between pre- and post-intervention, whereas (F) shows the pattern of % signal change that was extracted from the cluster of (E). The data points in (B), (D), and (F) show the results of each participants.

Fig. 3 Partial regression plots showing results of the partial correlations between residuals of changes in task-related brain activation of the left vIFC and residuals of changes in SSI-3 score (A, Speaking behaviors and OASES are partialled out), %SS (B, OASES is partialled out), and OASES score (C, SSI-3 score is partialled out).

Results of similar correlation analyses using RSFC in the cerebellum are presented in (D), (E), and (F).

Fig. S1 Patterns of brain activation for each of the three groups at the two time points. (A), (C), and (E) show patterns of brain activation for FC-, AWS-, and AWS+ pre-intervention; (B), (D), and (F) show the patterns post-intervention. $P < 0.001$, cluster volume > 800 mm^3, uncorrected.

Table 1 Speech fluency information in all participants.

		AWS+	AWS-	FC-	ANCOVA/t-test	*post-hoc* test for ANCOVA			
						AWS+ vs. FC-		AWS- vs. FC-	AWS+ vs. AWS-
Age		23(2.25)	29(6.06)	24(1.45)	$F_{(2,36)} = 8.126$, $P = 0.001$	$P = 0.839$		$P = 0.002$	$P = 0.001$
EY		17(0.48)	15(2.73)	17(0.76)	$F_{(2,36)} = 2.921$, $P = 0.067$				
HS		70(23.88)	79(22.37)	76(15.77)	$F_{(2,36)} = 0.571$, $P = 0.57$				
Pre-intervention	**%SS**	12(2.06)	12(2.08)	0(0.01)	$F_{(2,36)} = 208.647$, $P < 0.001$	$P < 0.001$		$P < 0.001$	$P = 0.73$
	SSI-3	31(4.68)	29(5.91)		$t(24) = 0.957$, $P = 0.348$				
	OASES	61(6.67)	57(11.56)		$t(24) = 1.039$, $P = 0.309$				
Post-intervention	**%SS**	7(3.13)	12(2.15)		$t(24) = -5.041$, $P < 0.001$				
	SSI-3	15(4.96)	29(5.84)		$t(24) = -6.441$, $P < 0.001$				
	OASES	52(6.35)	56(12.97)		$t(24) = -1.018$, $P = 0.319$				

Note: EY, years of education; HS, handedness scores. Standard deviations are inside the brackets. Stuttering assessment was not applicable to FC-. In addition, there are also no *post-hoc* test when ANCOVA results did not reach significance (the last column) and when *t*-test were conducted. Thus, the corresponding places were left with blank (column 5 and the last column).

Highlights

1. Changes in the left vIFC's function were identified after a short-term behavioural intervention for stuttering.

2. Distinct roles of task-related changes in the vIFC and task-independent RSFC changes in the cerebellum were clarified.

Significance

This study demonstrated that a brain area in the left ventral inferior frontal cortex (vIFC) showed intervention-induced increase of activation in a speech production task. This area was close to an area of the left IFC where both functional and structural anomalies have been identified in adults who stutter. Thus, it is likely that the recruitment of this nearby brain area to compensate for the anomalies is an important process in recovery. This study shows that recovery can be induced even after a short-term behavioral intervention for stuttering.

Figure 1

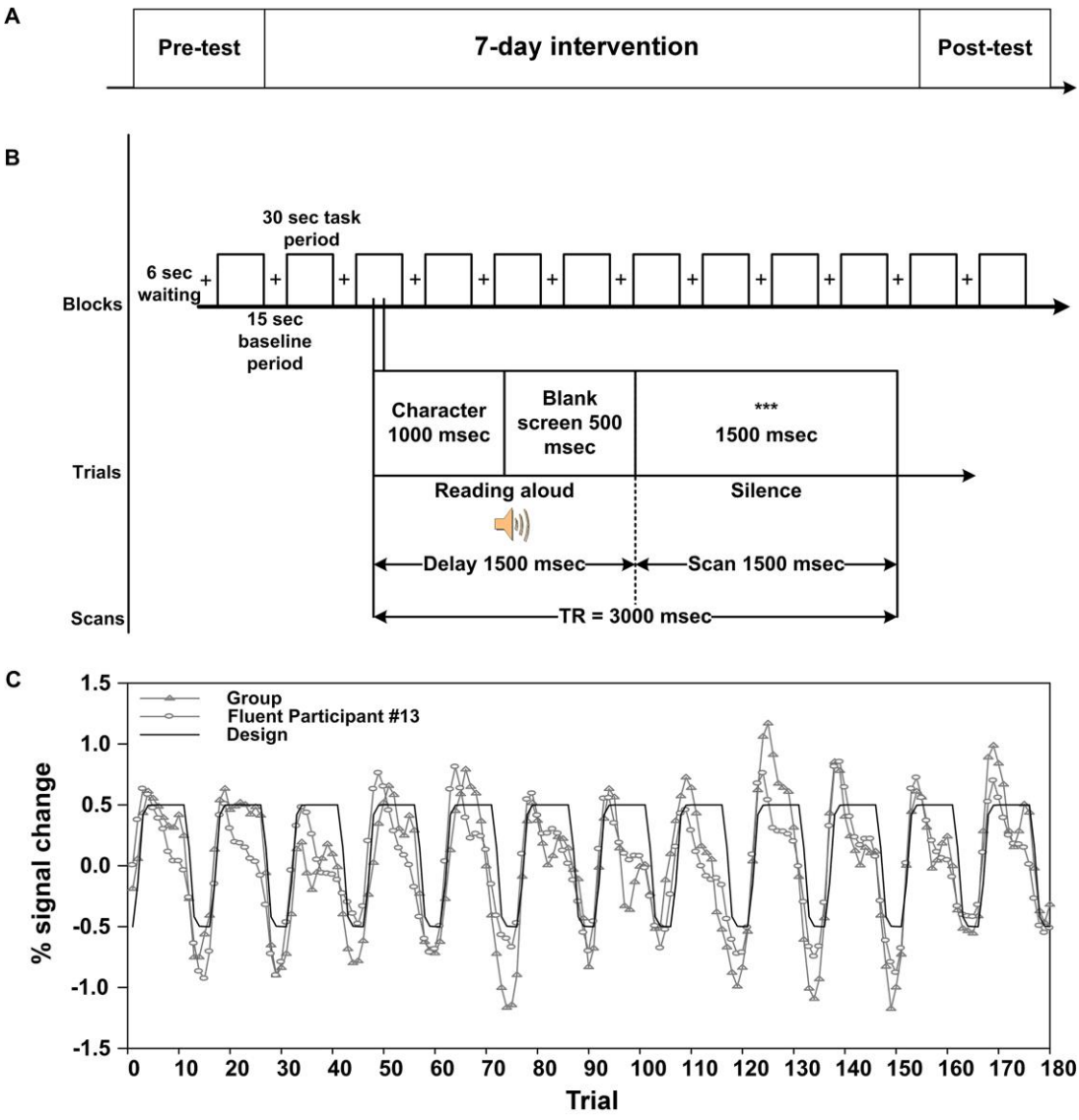
A
Pre-test
7-day intervention
Post-test
B
Blocks
30 sec task period
6 sec waiting
15 sec baseline period
Trials
Character 1000 msec
Blank screen 500 msec
***
1500 msec
Reading aloud
Silence
Delay 1500 msec
Scan 1500 msec
Scans
TR = 3000 msec
C
Group
Fluent Participant #13
Design
% signal change
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180
Trial

Figure 2

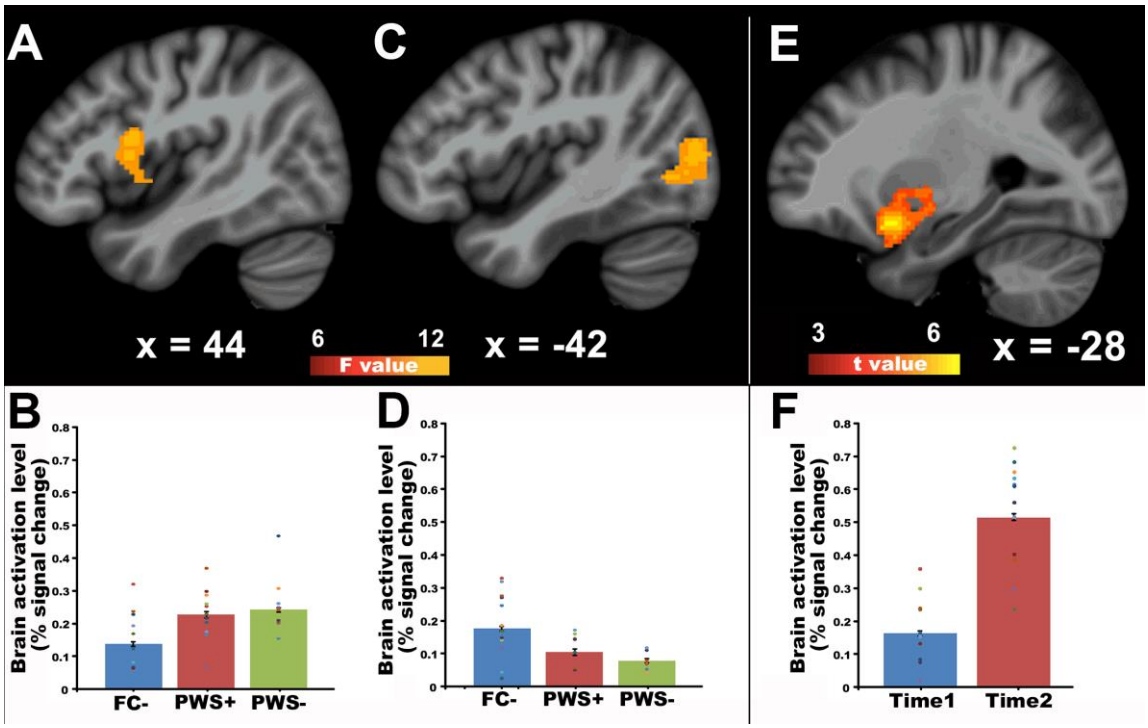
A
C
E
x = 44
6
12
F value
x = -42
3
6
t value
x = -28
B
D
F
Brain activation level (% signal change)
FC-
PWS+
PWS-
Time1
Time2

Figure 3

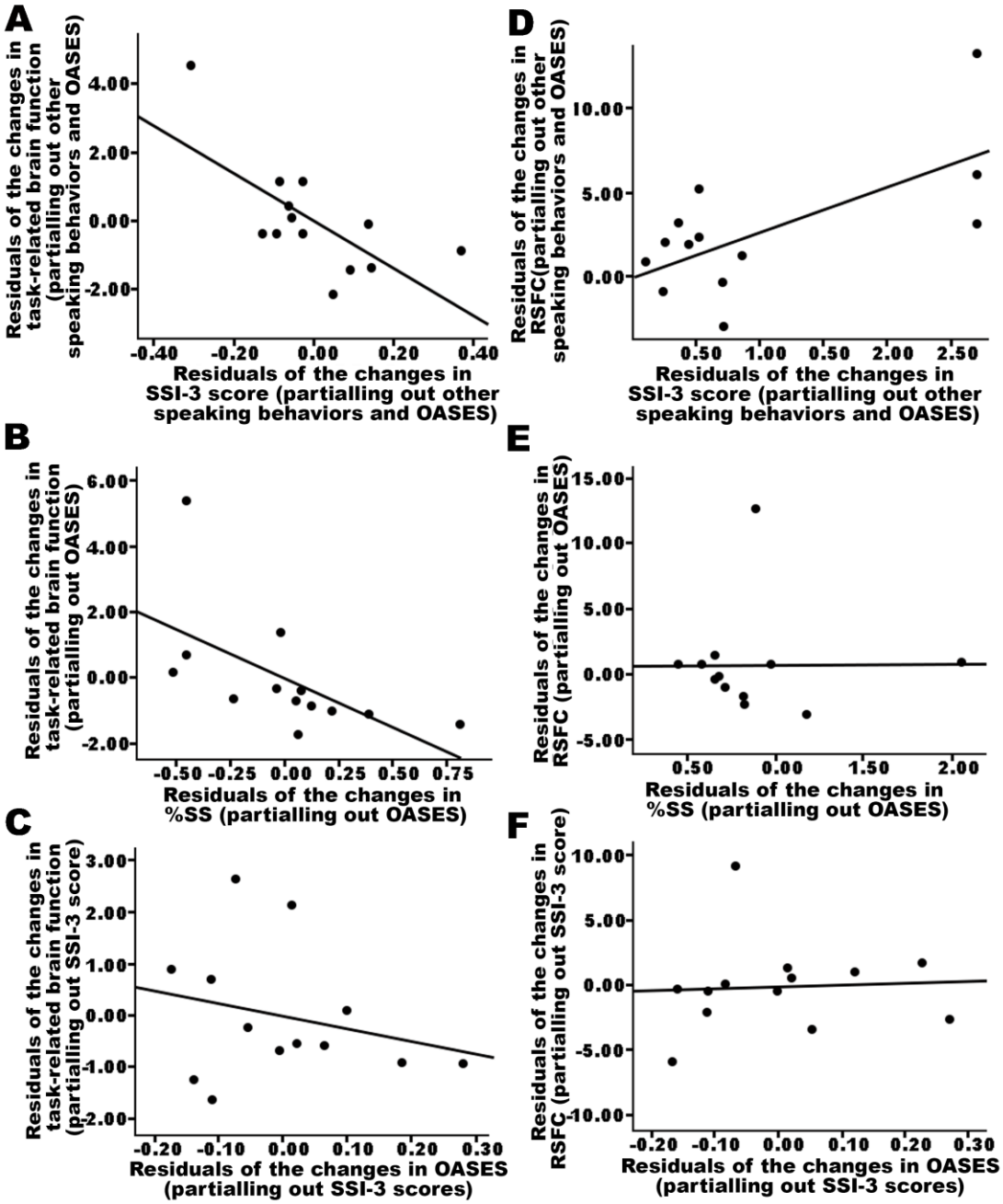
A
Residuals of the changes in task-related brain function (partialling out other speaking behaviors and OASES)
4.00
2.00
0.00
-2.00
-0.40 -0.20 0.00 0.20 0.40
Residuals of the changes in SSI-3 score (partialling out other speaking behaviors and OASES)
D
Residuals of the changes in RSFC(partialling out other speaking behaviors and OASES)
10.00
5.00
0.00
0.50 1.00 0.50 2.00 2.50
Residuals of the changes in SSI-3 score (partialling out other speaking behaviors and OASES)
B
Residuals of the changes in task-related brain function (partialling out OASES)
6.00
4.00
2.00
0.00
-2.00
-0.50 -0.25 0.00 0.25 0.50 0.75
Residuals of the changes in %SS (partialling out OASES)
E
Residuals of the changes in RSFC (partialling out OASES)
15.00
10.00
5.00
0.00
-5.00
0.50 0.00 1.50 2.00
Residuals of the changes in %SS (partialling out OASES)
C
Residuals of the changes in task-related brain function (partialling out SSI-3 score)
3.00
2.00
1.00
0.00
-1.00
-2.00
-0.20 -0.10 0.00 0.10 0.20 0.30
Residuals of the changes in OASES (partialling out SSI-3 scores)
F
Residuals of the changes in RSFC (partialling out SSI-3 score)
10.00
5.00
0.00
-5.00
-10.00
-0.20 -0.10 0.00 0.10 0.20 0.30
Residuals of the changes in OASES (partialling out SSI-3 scores)

Figure S.1