

Handedness-related functional connectivity using low-frequency blood oxygenation level-dependent fluctuations

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Functional neuroimaging studies of handedness have traditionally focused on exploring regionally activated discrepancies, yet little is known regarding the effect of handedness on functional connections of brain regions. Here, we reported the relationship between cerebellar functional connectivity patterns and handedness by low-frequency blood oxygenation level-dependent fluctuations. Compared with the right-handers, the left-handers show higher cerebellar–prefrontal, cerebellar–parietal, and cerebellar–temporal functional connectivity, and lower cerebellar–limbic connectivity.

Keywords: blood oxygenation level-dependent, cerebellum, functional magnetic resonance imaging, functional connectivity, handedness, low-frequency fluctuations, resting state

Previous non-human primate studies have shown that these regions with known contributions to higher cognitive functions have afferent or efferent connections with the cerebellum. Our findings suggest that these observed low-frequency fluctuation correlations may reflect actually anatomic connections between the cerebellum and cerebral cortex, and the cerebellar involvement in higher function may be associated with handedness. *NeuroReport* 17:5–8 © 2006 Lippincott Williams & Wilkins.

Introduction

Approximately 90% of humans are right-handed, exhibiting a preference to use the right hand for skilled and unskilled activities, whereas the remaining 10% prefer to use the left hand [1]. Hand preference has been thought of as a clear example of behavioral lateralization in humans. A large body of evidence has indicated that behavioral lateralization shown in handedness is associated with the structural and functional lateralization of brain regions [2–4]. Most attempts to clarify functional lateralization have focused upon exploring activation pattern differences with functional neuroimaging in a given specific experimental task, such as the asymmetry in the activation of motor areas and cerebellum during movement [3]. Such studies for mapping only regional differences, however, may not be enough to understand the neurobiological basis for hand preference, because it is commonly agreed that brain function is a result of information integration among multiple brain regions, usually characterized in terms of functional connectivity [5]. Despite the interest in connectivity, little is known about whether functional connectivity of brain regions is related to handedness.

To address this issue, we used functional connectivity magnetic resonance imaging (fcMRI) analysis to examine the

relationship between cerebellar connectivity patterns and handedness. The fcMRI is an application of functional MRI by detecting inter-regional temporal correlations of low-frequency blood oxygenation level-dependent (BOLD) signal fluctuations during rest [6]. Regions with known anatomic connections usually show high correlated BOLD fluctuations. Unlike conventional task-specific functional connectivity, which may reflect coactivations resulting from the task itself, fcMRI may represent direct or indirect anatomical connections between brain regions. To date, fcMRI has been used to explore primary motor and sensory cortex [6–9], visual cortex [10], and subcortical regions, such as the thalamus and the hippocampus [11]. Recently, two fcMRI studies have examined functional connectivity patterns of anterior inferior cerebellum [12] and dentate nucleus [13]. Despite different cerebellar sites, the two studies have consistently revealed cerebellar–prefrontal, cerebellar–parietal and cerebellar–limbic functional connections, which provides support for previous findings from non-human primate studies showing anatomic connections between the cerebellum and cerebral cortex. In this report, we used fcMRI to examine the effect of handedness on cerebellar functional connections. The differences of bilateral cerebellar connectivity were also incidentally examined.

Methods

Volunteers

Fifteen healthy left-handed (six women; age range 21–32 years, mean 24.3 years) and 15 healthy right-handed (six women; age range 22–40 years, mean 25.9 years) volunteers participated in this study. All were Chinese. The right-handed participants (age and sex-matched) were chosen from a larger group for which we recently reported correlated low-frequency BOLD fluctuations [12]. All of the volunteers gave informed consent according to the procedure approved by the Institute of Automation, Chinese Academy of Sciences.

Handedness classification was completed according to a self-report questionnaire. Considering significant differences in language, living conditions, and habits between Chinese and other nationalities, both the direction and the degree of handedness for each volunteer in this study were assessed by Li's Handedness Inventory [14], which consists of a 10-item questionnaire (writing, using chopsticks, throwing, holding a toothbrush, using scissors, striking a match, threading a needle, holding a hammer, holding a racket, and towelng a face), each to be given an answer ('only right', 'only left' or 'right and left') for hand preference. A laterality quotient (LQ) was assigned to each volunteer using $LQ = (R - L) \times 100 / (R + L)$, in which R and L denote the sum of the scores of the questions answered in 'only right' and 'only left', respectively ('only right' or 'only left' for each question scores 10, but 'right and left' has a 0 score) [15]. The range of LQ was -100 for extreme left-handedness to $+100$ for extreme right-handedness. The absolute value of LQ was considered as a quantitative measure of the degree of handedness in both right-handed and left-handed volunteers.

Data acquisition

The functional images were scanned on a 1.5-T magnetic resonance scanner (GE Signa, Milwaukee, Wisconsin, USA: 1.5T Twinspeed). High-resolution T1 anatomical images were collected axially by using the following technical parameters: 1924/7.5/750 ms [repetition time (TR)/echo time (TE)/inversion time (TI)], 20 slices, 256×256 matrix, 24-cm field of view, 5-mm section thickness, and 1-mm gap. At the same slice locations as the anatomical images, functional images were acquired by using an echo-planar imaging (EPI) sequence with the following parameters: 2000/40 ms (TR/TE), 20 slices, 64×64 matrix, 90° flip angle, 24-cm field of view, 5-mm section thickness, and 1-mm gap. Participants were instructed to keep their eyes closed, relax their minds, and remain motionless as much as possible during EPI data acquisition. Foam pads were used to reduce head motion. The scan lasted for 400 s. A three-dimensional spoiled gradient-recalled (SPGR) whole-brain volume was also acquired in the axial plane with the following parameters: 11.3/4.2 ms (TR/TE), 15° flip angle, $0.94 \times 0.94 \times 2.4$ mm spatial resolution.

Data preprocessing

Imaging preprocessing [slice timing, realignment, spatial normalization, resampling to $3 \times 3 \times 3$ mm³ in size, spatial smoothing with a 4-mm full-width at half-maximum Gaussian kernel and low-pass temporal filtering ($f < 0.08$)] was performed by using the SPM2 package (www.fil.ion.ucl.ac.uk/spm). Prior to the process, the first 10 volumes were excluded

because of the instability of the initial MRI signal and the adaptation of the volunteers to the circumstances, leaving 190 volumes.

Regions of interest

The cerebellum regions of interest (ROIs) were obtained by using anatomical landmarks in the brain as follows: (i) An ROI template. First, on a representative volunteer's SPGR transformed to the space of Talairach coordinates [16], an approximately centered voxel [Talairach ordinates: $(-28, -61, -18)$] located in the left cerebellar hemisphere lobule VI was drawn, according to the anatomy proposed by Schmahmann *et al.* [17]. In order to automate our analysis and reduce the bias from a single voxel, the left cerebellum ROI, corresponding to low resolutions normalized functional images, was specified as a cluster made up of the approximately centered voxel and its six nearest neighbors. The right cerebellum ROI was then created by reflecting the left ROI symmetrically across the midline. Thus, a cerebellum ROI template was generated from a representative volunteer, as shown in Fig. 1A. (ii) Volunteer-specific ROIs. Taking into account the anatomical variance across volunteers, the ROI template was compared with each individual's Talairach-transformed SPGR, and the locations of an individual's ROIs were adjusted to ensure the precision of each individual's anatomy. Finally, 6/30 volunteers' ROIs were slightly adjusted. The resulting mean Talairach

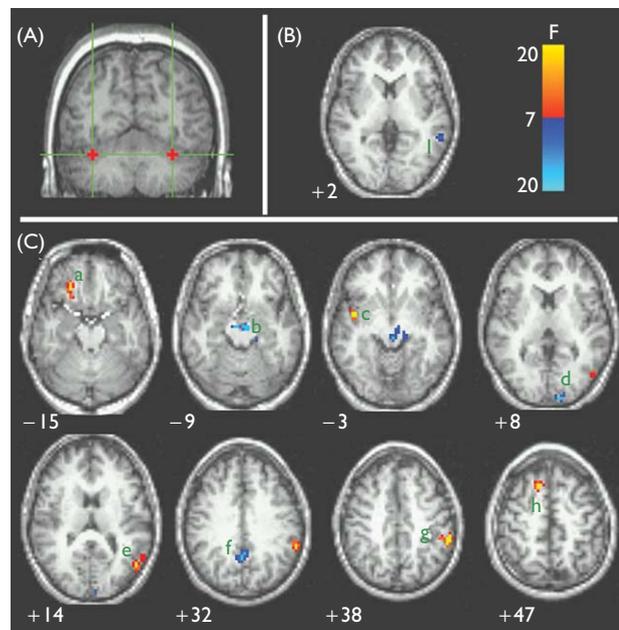


Fig. 1 The locations of the cerebellum in this study and results from a two-way analysis of variance. (A) A cerebellum region of interest template from a representative volunteer. (B) A main effect for side (left- and right-cerebellum) was observed in the left middle temporal gyrus (l). Cold color indicates left cerebellum < right cerebellum. (C) A main effect for handedness (left- and right-handed) was observed in the right inferior frontal gyrus (a), midbrain (b), right insula (c), left cuneus (d), left middle temporal gyrus (e), posterior cingulate cortex/precuneus (f), left inferior parietal gyrus (g), and right superior frontal gyrus (h). Hot color indicates left-handers > right-handers. Cold color indicates left-handers < right-handers. The statistical map was thresholded at $P < 0.001$ (corrected for multiple comparisons). The numbers beneath each axial image refer to the z-plane coordinates in the Talairach space [16]. The right side of the image corresponds to the left side of the brain.

coordinates of the ROIs centers across the volunteers were (−28.0, −61.0, −19.1) for the left and (28.0, −61.0, −19.1) for the right. According to the procedures, two volunteer-specific cerebellum ROIs for each volunteer were obtained, leading to further fcMRI analyses.

Functional connectivity magnetic resonance imaging analyses

Time series were first extracted for each volunteer-specific ROI by averaging the time series of all voxels in the ROI. Then, the resulting time series was used as a reference for cross-correlation analysis with that of all the voxels in a whole brain. Individual *r*-maps were further normalized to *z*-maps using Fisher’s *z*-transformation [18]. The obtained *z*-maps were subjected to a voxel-wise two-way analysis of variance (ANOVA) with factors of handedness (right-handed, left-handed) and side (left cerebellum, right cerebellum). Individual voxels’ *P*-value < 0.01 and cluster size > 540 mm³ were considered as significantly different. This combination yielded *P* < 0.001 after multiple comparisons correction by a Monte Carlo simulation method in Analysis of Functional NeuroImages (AFNI, <http://afni.nimh.nih.gov/afni/>) (AlphaSim, parameter: with mask, 10000 simulations, full-width at half-maximum=4 mm) [19].

Results

All of the right-handed volunteers in this study showed extreme right-handedness (LQ range 90–100, mean 99.3), whereas the left-handed volunteers showed significant interindividual variability in the degree of handedness (LQ range −80–0, mean −58.1).

Main effects from the two-way ANOVA are shown in Fig. 1B and C. Regions demonstrating a significant main effect for side were identified in the left middle temporal gyrus [Brodmann’s area (BA) 21] (Fig. 1B, left cerebellum < right cerebellum). Figure 1C shows brain regions demonstrating a main effect of handedness, including the right inferior frontal gyrus (BA 47), right superior frontal gyrus (BA 8), left inferior parietal lobe (BA 40), left middle temporal gyrus (BA 39), right insula (Fig. 1C, left-handers > right-handers), posterior cingulate cortex/precuneus (BA 31/7), left cuneus (BA 17), and midbrain (Fig. 1C, left-handers < right-handers). No voxels showing a significant handedness × side interaction, even in *P* < 0.05 (uncorrected), were observed, indicating that the observed differences between right- and left-handers occurred in both the right cerebellum and the left cerebellum. Further details about the above regions are presented in Table 1.

Discussion

In this report, we examined the relationship between cerebellar functional connectivity patterns and handedness. Compared with right-handers, left-handers showed higher cerebellar–prefrontal, cerebellar–parietal and cerebellar–temporal functional connectivity, and lower cerebellar–limbic connectivity. To our knowledge, this is the first report showing handedness-related functional connectivity in the cerebellum.

Previous functional neuroimaging studies of handedness have mainly focused on exploring regionally activated discrepancies. Studies from several research groups have indicated that activation patterns of brain regions are

Table 1 Brain regions showing significant statistical differences (*P* < 0.001, corrected) in cerebellar functional connectivity during rest

Brain regions	BA	x	y	z	Vol
Left cerebellum < right cerebellum					
Left MTG	21	−58	−40	2	702
Left-handers > right-handers					
Left IPL	40	−55	−37	38	1377
Left MTG	39	−49	−67	14	1053
Right IFG	47	22	31	−14	810
Right SFG	8	13	22	47	675
Right insula	N/A	40	−4	−3	540
Left-handers < right-handers					
Midbrain	N/A	−4	−16	−9	1863
PCC/PCu	7/31	−1	−49	32	1188
Left cuneus	17	−10	−97	8	567

BA, Brodmann’s area; x, y, z, coordinates of primary peak locations in the space of Talairach [16]; Vol, cluster volume in mm³; MTG, middle temporal gyrus; IPL, inferior parietal lobe; IFG, inferior frontal gyrus; SFG, superior frontal gyrus; PCC/PCu, posterior cingulate cortex/precuneus.

associated with the handedness [3,4]. Important for the present study, the cerebellum shows prominently lateralized activation in a movement task [3] and a silent verbal fluency task [4] in right- and left-handed volunteers. Little is known about whether functional connectivity pattern of brain regions is related to handedness, although functional connectivity has been a well-known technique for the characterization of brain function. Our exploration in handedness-related functional connectivity may provide an insight into the understanding of the neurobiological basis for hand preference.

We found that handedness-related functional connections focused on cerebellar–prefrontal, cerebellar–parietal, cerebellar–temporal, and cerebellar–limbic connectivity (Fig. 1C). Evidence has demonstrated that the cerebellum receives inputs from and sends outputs to the cerebral cortex, especially those regions with known contributions to higher cognitive functions, such as the prefrontal cortex, parietal cortex, and cingulate cortex [20]. A recent fcMRI study [13] in the dentate nucleus has indicated that such connections between cerebral and cerebellar cortices may reflect a major component of the anatomic substrate for the cerebellar involvement in higher function, such as cognition and emotion [21,22]. Our finding of handedness-associated connections suggests that cerebellar involvement in higher function may be related to handedness. Recent neuroimaging studies have indicated that cerebello-cortical functional connections can be modulated during different experimental tasks [9,23] or altered in specific brain diseases such as schizophrenia [24]. In this study, we highlighted the cerebellar functional connections differences in handedness. The discrepancies reflect that the left-handers, compared with the right-handers, may have different functional organization in cognitive and emotional processing involving the cerebellum [21,22]. This result, however, should be interpreted with caution. The direct evidence for hand preference associated with functional connectivity of brain regions is lacking now. Interestingly, recent diffusion tensor imaging studies have demonstrated that fractional anisotropy of white matter structures, such as cingulum [15] and corpus callosum [25], shows significant handedness relation. These findings of handedness-associated fiber connections may provide important implications for functional connectivity studies of

handedness. In this report, we did not display cerebellar connectivity patterns within the groups. Visual examination for the results within four groups indicated a large overlap in cerebellar–prefrontal, cerebellar–parietal, and cerebellar–limbic connectivity with two recent fMRI reports related to the cerebellum [12,13].

A final comment regarding the methodologies presented in the paper: First, we employed the low-frequency BOLD fluctuations to examine the handedness-related functional connectivity. Compared with task-specific functional connectivity, these low-frequency fluctuation correlations may reflect the anatomic connections more directly as it is task-free [6,12,13]. Nevertheless, the origin of the coherent fluctuations remains obscure to date, although some investigators have suggested that the spontaneous fluctuations may be related to the neuronal firing in the resting state [6]. The relationship between the low-frequency BOLD fluctuations and neuronal activity needs to be fully clarified in the future, using animal studies. Another issue in the present study is the use of a long TR during the EPI data acquisitions. In order to explore handedness-related connections across the entire brain, we used a low sampling rate (TR=2 s) for the multislice acquisitions. High-frequency noises like cardiac rhythm may not be completely excluded and thus may confound the low-frequency fluctuations analysis results [6,7]. A short TR (e.g. <500 ms) can be used to reduce these noise effects, but it usually limits the number of slices, which is obviously a disadvantage.

This report used low-frequency BOLD fluctuations to investigate a question of intrinsic brain organization (handedness) rather than a simple detection of networks. This extends previous fMRI applications. In future studies, hopefully, this technique can be used to explore cerebellar connectivity patterns in patients with known cerebellar abnormality (e.g. autism or schizophrenia).

Conclusion

The aim of this study was to examine the relationship between cerebellar functional connectivity and handedness by low-frequency BOLD fluctuations. Handedness-associated connectivity patterns were observed in cerebellar–prefrontal, cerebellar–parietal, cerebellar–temporal, and cerebellar–limbic connections. Previous non-human primate studies have shown that these regions with known contributions to higher cognitive functions have afferent or efferent connections with the cerebellum. Our findings suggest that these observed functional connections may offer a direct or indirect characteristic of anatomic connections between the cerebellum and cerebral cortex, and cerebellar involvement in higher function may be related to handedness.

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