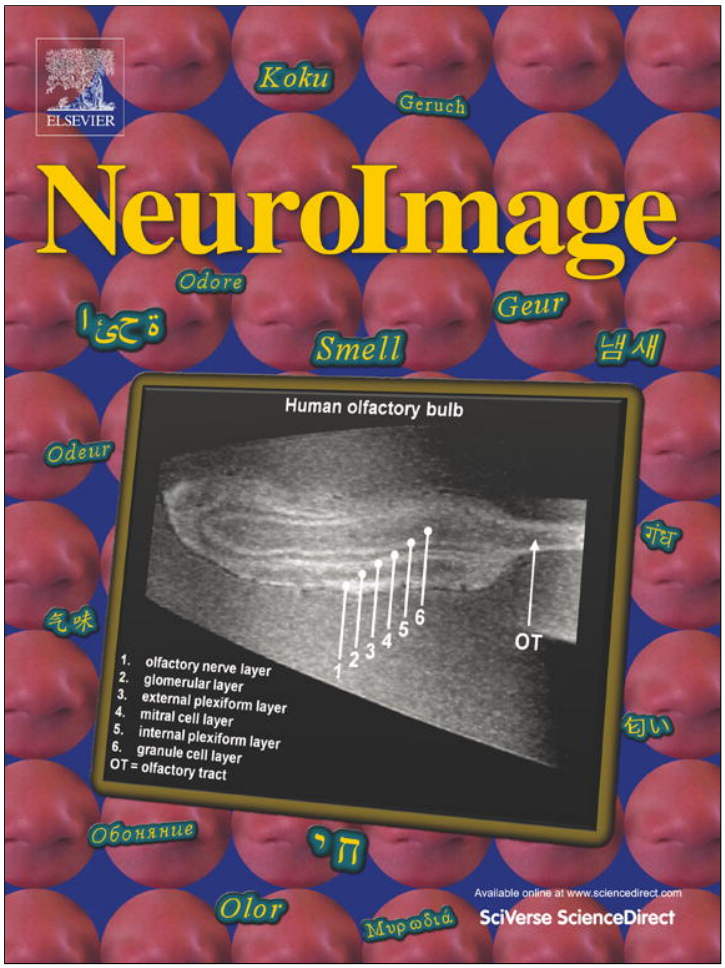

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Structural brain changes linked to delayed first language acquisition in congenitally deaf individuals

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ABSTRACT

Early language experience is essential for the development of a high level of linguistic proficiency in adulthood and in a recent functional Magnetic Resonance Imaging (fMRI) experiment, we showed that a delayed acquisition of a first language results in changes in the functional organization of the adult brain (Mayberry et al., 2011). The present study extends the question to explore if delayed acquisition of a first language also modulates the structural development of the brain. To this end, we carried out anatomical MRI in the same group of congenitally deaf individuals who varied in the age of acquisition of a first language, American Sign Language —ASL (Mayberry et al., 2011) and used a neuroanatomical technique, Voxel-Based Morphometry (VBM), to explore changes in gray and white matter concentrations across the brain related to the age of first language acquisition. The results show that delayed acquisition of a first language is associated with changes in tissue concentration in the occipital cortex close to the area that has been found to show functional recruitment during language processing in these deaf individuals with a late age of acquisition. These findings suggest that a lack of early language experience affects not only the functional but also the anatomical organization of the brain.

1. Introduction

Plasticity can be defined as the brain's ability to be shaped by experience at functional (Karni et al., 1998) and structural (Draganski et al., 2004; Maguire et al., 2000) levels. In this way, learning a new skill or acquiring a high level of proficiency at such varied tasks as juggling (Draganski et al., 2004), navigating a taxi through a city (Maguire et al., 2000), playing a musical instrument (Bermudez et al., 2009; Gaser and Schlaug, 2003; Hutchinson et al., 2003) or studying for an exam (Draganski et al., 2006) can be associated with structural changes in the adult brain.

Several aspects of language acquisition have also been associated with differences in brain structure (see Richardson and Price, 2009 for a review), be they present at birth or acquired through experience, such as differentiating or pronouncing novel phonemes (Golestani and Pallier, 2007; Golestani et al., 2002; Golestani et al., 2007), learning a tonal language (Wong et al., 2008), acquiring new vocabulary (Lee et al., 2007; Richardson and Price, 2009), learning Morse code (Schmidt-Wilcke et al., 2010) or acquiring a second language (Mechelli et al., 2004). While most studies have focused on structural changes following learning language in adulthood, to date no study has explored if a lack of language input early in life could alter brain development and result in lasting changes in the anatomical organization of the adult brain.

To address how early linguistic experience, and the lack of it, shapes the anatomical development of the brain, we studied a group of congenitally deaf individuals who varied in the age at which they acquired a first language, American Sign Language (ASL), and who were the same group of subjects who had shown functional Magnetic Resonance Imaging (fMRI) changes during language processing tasks related to the timing of language onset (Mayberry et al., 2011). In that fMRI study, Mayberry et al. (2011) observed a negative linear relationship between hemodynamic activity in the left frontal language regions and age of sign-language acquisition and a positive linear relationship between hemodynamic activity in the left visual cortex and age of sign-language acquisition. These activation patterns were found during both a phonemic hand-judgment and a grammatical-judgment task. Here, using Voxel-Based Morphometry (VBM), we asked if the same reciprocal relationship might be observed in the anatomical organization of the brain and whether we might observe local differences in

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white matter (WM) and gray matter (GM) concentration across the whole brain related to age of language acquisition in these individuals.

VBM is a fully automated technique that searches for local differences in gray-matter and white-matter concentrations across the whole brain between two groups (Ashburner and Friston, 2000). While VBM has been used to study the deaf population in the past, these studies have focused on isolating structural differences between deaf and hearing individuals related to auditory deprivation (Li et al., 2012; Penhune et al., 2003; Shibata, 2007; Smith et al., 2011). The results have showed, for the most part, that deaf individuals have less white matter than hearing individuals in an area underlying the planum temporale and Heschl's gyrus (Li et al., 2012; Shibata, 2007; Smith et al., 2011), an expected finding given the plasticity which could accompany auditory deprivation. Such findings are consistent with the findings of an earlier anatomical study using regions of interest (ROI) (Emmorey et al., 2003). However, the behavioral sequelae of these structural differences in auditory cortex are currently unknown (Leonard et al., 2012). Plasticity-related differences have also been observed in other brain regions in deaf individuals, for example, several studies have examined differences in visual cortex associated with deafness (e.g. Bavelier et al., 2001), and Penhune et al. (2003) observed increased gray-matter density localized in the left motor cortex that they interpreted as being related to increased fine-motor control of the dominant hand during signing. Structural differences in the posterior insular cortex have been documented in deaf signers compared to hearing signers that have been interpreted to be related to lip-reading requirements for deaf individuals as compared to hearing signers (Allen et al., 2008). Although there is an emerging body of literature focusing on the consequences of deafness associated with brain morphological changes, to date no studies have yet capitalized on the age range at which congenitally deaf individuals acquire their first language to explore how brain structure may be shaped by language experience during development.

We predicted that delayed first-language acquisition would result in the modulation of tissue concentration in the left frontal lobe and in the left occipital cortex as previously reported in the fMRI study with these same subjects (Mayberry et al., 2011).

2. Material and methods

2.1. Subjects

Anatomical magnetic resonance images (MRI) from 23 congenitally deaf individuals (12 males; 11 females, average age ± SD = 39.2 ± 12.3 years, range = 25–61 years; 23 right handed; Table 1) were acquired as part of a larger study that also involved functional magnetic resonance imaging (fMRI) (Mayberry et al., 2011). Eighteen of the recruited deaf subjects were profoundly deaf (>90 dB in both ears), 3 subjects had profound loss in one ear (>90 dB) and severe loss (>80 dB) in one ear, and 2 subjects who had severe loss in both ears (>80 dB) — see Table 2 for details). All subjects had used ASL as their primary means of communication on a daily basis for an extended period of time of at least 18 years (ASL experience: mean ± SD = 33.5 ± 11.5 years, range = 18–55 years; see Fig. 1 for illustration of ASL signs). Subjects were unable to understand spoken language sufficiently to engage in it for functional communication with others. The deaf subjects varied in their age of ASL acquisition; age of acquisition being defined as the chronological age at which each deaf subject gained daily access to fully perceivable linguistic input. The subjects chosen represented three developmental epochs (infancy, early childhood and late childhood) related to the age at which they first started acquiring ASL: (1) *Infant signers* were first exposed to ASL before age 3 by a parent who signed to them. (2) *Early signers* were first exposed to ASL between the ages of 4–7 years and; (3) *Late signers* were first exposed to ASL between the ages of 11–14 years. Early and late signers were born to hearing parents who did not use sign language as a means of communication with their children during childhood and were initially enrolled in schools where only speech was used; they were subsequently transferred to schools where sign language was used. All the deaf subjects were tested on two non-verbal subtests of the Wechsler Adult Intelligence Scale III (WAIS) consisting of Picture Completion and Picture Arrangement (Weschler, 1981) and scored within the normal range. To assess language deprivation apart from auditory deprivation, a separate set of anatomical scans using the same scanner and imaging parameters was also acquired from a control group of right-handed hearing subjects matched for age and gender who had never been exposed to sign language (n = 43, 21 males; 22 females, average age ± SD = 37.3 ± 11.5 years, range = 25–62 years); these subjects had been

Table 2
Range of hearing levels for deaf participants.

Subject	R-PTA	L-PTA
Native group		
N01	>95	>95
N02	>95	>95
N03	>95	>95
N04	>95	>95
N05	>95	>95
N06	>95	>95
N07	>95	>95
N08	>95	>95
N09	80	85
Early group		
E01	88	93
E02	>95	>95
E03	>95	>95
E04	93	93
E05	81	90
E06	>95	>95
E07	>95	>95
E09	85	85
Late group		
L01	93	93
L02	>95	>95
L03	>95	>95
L04	>95	>95
L05	88	>95
L06	>95	>95

Degree of hearing in dB HTL is taken at 500 Hz, 1000 Hz and 2000 Hz.
The average of these three frequencies is the Pure Tone Average or PTA. Severe Loss (71–90 dB) Profound Loss (>90 dB) is given for L (left) and R (right) ear. 95 dB represents the limits of portable audiometric testing.

Table 1
Demographic information of deaf subjects.

Developmental epochs	N	Age (Years)	Gender		Age of ASL Acquisition (Years)			ASL Experience (Years)
		Mean (SD)	M	F	Mean (SD)	Min	Max	Mean (SD)
Infancy	9	31.8 (8.6)	2	7	0.6 (1.0)	0	3	31.2 (8.6)
Early childhood	8	44.0 (12.1)	5	3	6.3 (1.0)	4	7	37.8 (11.7)
Late childhood	6	40.2 (11.9)	4	2	12.3 (1.0)	11	14	27.8 (11.4)
Total deaf	23	38.2 (11.6)	11	12	5.6 (4.9)	0	14	32.6 (10.8)

Fig. 1. Schematic showing photo of video frames of someone signing in ASL (the signs for WHY, DRIVING, and EAT respectively) shown here for illustrative purposes. For the VBM component of the study there was no task as this relates to the anatomical scan.

scanned previously at the Montreal Neurological Institute (MNI). This research was approved by and performed according to the guidelines of the Research Ethics Board of the Montreal Neurological Institute and written consent was obtained from each subject.

2.2. Scanning protocol

T1 weighted magnetic resonance (MR) images of the brain were acquired for each subject on a Siemens Sonata 1.5 Tesla scanner using 3D fast low-angle shot (FLASH) (TR = 22 ms, TE = 9.2 ms, flip angle = 30º, matrix size = 256 × 256, number of slices = 170, voxel size = 1 × 1 × 1 mm^3).

2.3. Data processing

Using an in-house pipeline developed at the MNI (http://wiki.bic.mni.mcgill.ca), the T1 weighted images were corrected for intensity non-uniformity using a non-parametric non-uniform intensity normalization (N3) method (Sled et al., 1998). This procedure aimed to minimize low-frequency variation of signal intensity that arises from inhomogeneity in the electromagnetic field and radiofrequency reception coil. Non-uniformity correction ensured a more accurate tissue classification (Sled et al., 1998). The corrected images were then linearly registered into a stereotaxic space based on the MNI 152 target, which itself was derived from the Talairach atlas (Talairach and Tournoux, 1988), to minimize variability due to differences in head position, brain size and brain shape across subjects, and allow for comparison of anatomical data between subjects (Collins et al., 1994). Using an automatic classification algorithm known as INSECT (Intensity Normalized Stereotaxic Environment for the Classification of Tissue), individual voxels in each image were classified as gray matter, white matter or cerebral brain fluid (Zijdenbos et al., 2002). The three-dimensional gray-matter and white-matter maps created by the classification algorithm were smoothed using an isotropic Gaussian kernel (Full Width Half Maximum (FWHM) = 10 mm) to compensate for imperfect registration (Ashburner and Friston, 2001), to normalize data distribution thereby indirectly increasing the validity of parametric statistics (Ashburner and Friston, 2001), and to make binary data into continuous data to allow correlational analyses with other continuous variables (Golestani et al., 2002).

2.4. Data analysis

2.4.1. Voxel-based correlation with age of sign-language acquisition

A whole-brain voxel-based correlation with age of ASL acquisition was carried out for gray matter and white matter for each deaf individual. Gray-matter and white-matter t-statistical maps were generated and voxels with *t*-values above the threshold value corresponding to $p < 0.05$ corrected for multiple comparisons using random field theory (Worsley et al., 1996) were deemed significant. The location of these peaks within the brain and their correspondence to specific functional brain areas were determined by using macroanatomical landmarks consisting of gyri and sulci as well as cytoarchitectonic maps when these were available (Amunts et al., 2007). Behavioral outcome measures were not used as covariates for the VBM correlational analysis as in the previous fMRI study with these individuals, because these variables were not correlated with age of ASL acquisition (Mayberry et al., 2011). All subjects had over 18 years of experience using ASL and there were no significant group differences in the duration of exposure to ASL among these subjects (see Table 1). It was not possible to do a correlational analysis related to the degree of deafness due to the limited range in degree of hearing loss among subjects as most had profound hearing loss and a few had severe hearing loss (see Table 2). To eliminate any possible contribution of chronological age and gender to the structural changes detected by our voxel-based correlational analysis (Good et al., 2001), these factors were included as covariates in the correlational analysis.

2.4.2. Comparison between deaf group as a whole and the hearing group

To ensure that modulation in tissue concentration related to age of ASL acquisition was independent of auditory deprivation, we conducted two types of analyses. First, we extracted the value of the tissue concentration in the brain areas modulated by age of ASL acquisition as determined by the VBM correlational analysis with the deaf group. Tissue concentrations in these areas shown to be modulated by age of ASL acquisition were then measured in a group of matched hearing controls. Tissue concentration for the whole deaf group was compared to those of the hearing using a Welch equality of means test (Welch, 1951), with separate analyses carried out for gray and white matter. Tissue concentration values corresponded to the voxels with the highest *t*-values yielded by the voxel-based correlational analysis.

In a second analysis, we carried out a VBM group comparison between the deaf group as a whole and the hearing control group to identify regional differences in tissue concentrations. Statistical thresholding methods were identical as the ones used for the other VBM analysis. In brain areas where *t*-values reached significance, gray- and white-matter concentrations at the peak voxel were regressed against the age of exposure to ASL in the deaf to examine if age of exposure to ASL modulated tissue concentration in the areas modulated by auditory deprivation.

2.4.3. Comparison between deaf subgroups and the hearing group in areas modulated by ASL age of acquisition in the deaf

To examine whether acquisition of ASL at a particular developmental stage results in a significant increase or decrease in tissue concentration compared to the hearing population, all of whom have early language acquisition, the deaf subjects were divided into three

subgroups according to the developmental epoch at which they were first exposed to sign language (infancy, early childhood and late childhood). A second Welch equality of means test, that takes into account unequal sample size when comparing multiple groups, was used to compare the tissue concentrations of brain areas modulated by age of ASL acquisition in deaf subgroups with the tissue concentration in the same areas in the hearing group. We then compared the mean gray-matter concentration at each peak for each deaf subgroup with the corresponding mean of the hearing control group using the Dunnett test (Dunnett, 1955). To avoid the problem of circularity, we compared each deaf subgroup with the hearing control group but did not compare deaf groups among themselves because these data were not fully independent of one another having been generated by the whole-brain voxel-based correlation with age of ASL acquisition, as detailed previously (Kriegeskorte et al., 2009).

2.4.4. Conjunction analysis comparing functional and anatomical data

A conjunction analysis was carried out to identify if structural brain areas modulated by age of language acquisition overlap with areas of functional activations previously reported in our fMRI study (Mayberry et al., 2011). As part of the conjunction analysis, the thresholded gray-matter t-statistic map ($t=-6.5$, $p<0.05$, corrected) was overlapped with the thresholded t-statistic map of blood oxygenation level dependent (BOLD) signal changes that were positively correlated with age of ASL acquisition ($t=3.1$, $p<0.05$, corrected) previously obtained in the same individuals during a grammatical judgment task during an fMRI experiment (Mayberry et al., 2011). This analysis was restricted to gray matter since blood oxygenation in the fMRI signal is generally attributed to the neuronal activity occurring in cell bodies (Raichle, 1998).

To further assess the relationship between structure and function, a regression analysis was carried out between the gray-matter concentration of the peak voxel yielded by the voxel-based analysis and changes in the BOLD signal at the peak voxel yielded by the fMRI analysis. Tissue concentration values and BOLD values were measured for each deaf subject and corresponded to the voxels with the highest *t*-values yielded by the voxel-based correlational analysis and the fMRI analysis. Using the same method, a regression analysis was also carried out between white-matter concentration and % BOLD signal change.

3. Results

3.1. Anatomical changes related to age of sign-language acquisition

The voxel-based whole brain correlational analysis considering gray matter detected two brain areas modulated by age of ASL acquisition. In both areas, later ASL acquisition was linked to a lower gray-matter concentration. The first peak spanned across the primary (V1) and secondary visual cortex (V2) ($x=-4$, $y=-79$, $z=1$, $t=-8.4$, $p<0.05$, corrected for multiple comparisons; Fig. 2A) as indicated by the spatial overlap of this peak with cytoarchitectonic probabilistic maps of these regions (Amunts et al., 2000). The second peak was located within the dorsal visual association cortex where the occipital and parietal lobes meet ($x=-23$, $y=-85$, $z=24$, $t=-7.2$, $p<0.05$, corrected for multiple comparisons; Fig. 2B). This peak was located in the vicinity of the regions delineated as V3A and V7 by an fMRI study mapping visual field maps within the occipital and parietal cortex (Swisher et al., 2007). To obtain a more precise visualization of the relationship between gray-matter concentration and age of ASL acquisition, gray-matter concentration at the most significant voxel was measured for each subject and was then plotted against age of ASL acquisition (Figs. 2C and D). Gray-matter concentration was negatively correlated with age of ASL acquisition in both V1/V2 ($r=-0.83$) as well as in V3A/V7 ($r=-0.81$).

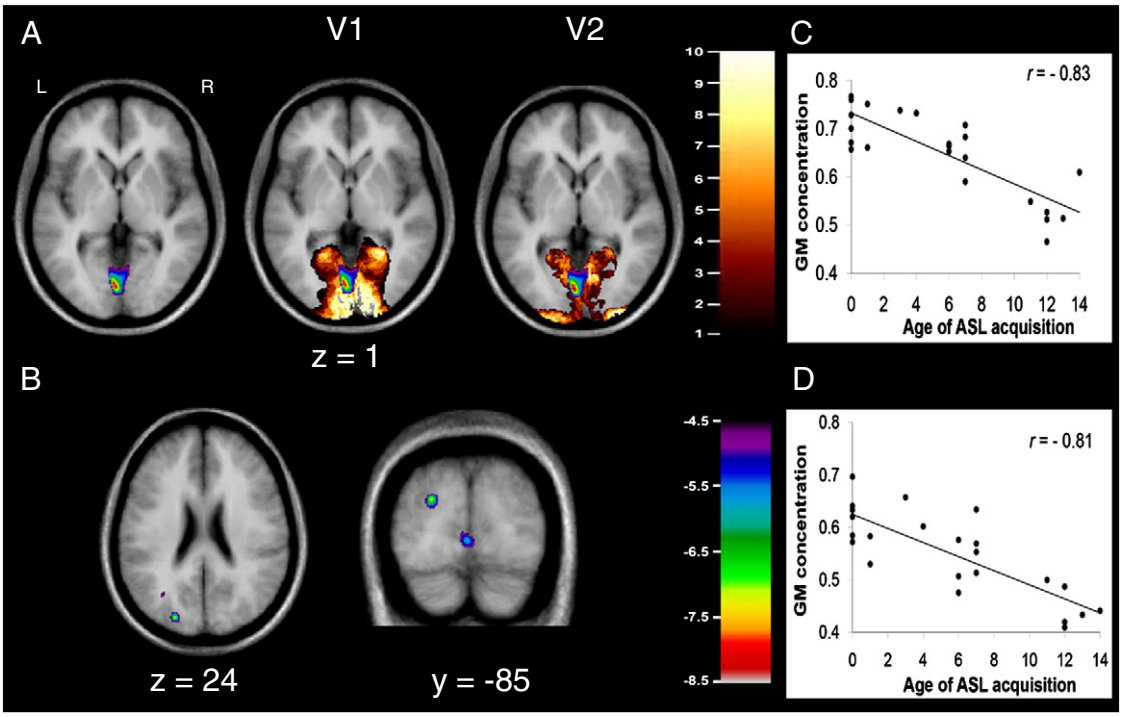

Fig. 2. Voxel-based correlational analysis of gray-matter concentration and age of ASL acquisition in the deaf group. Brain images show an average anatomical MRI image for deaf subjects (n=23) overlayed with a thresholded t-statistical map of brain areas where gray-matter concentration significantly decreases with age of ASL acquisition. Spectral color bar corresponds to *t*-values of peaks. (A) Peak located in the primary and secondary visual areas (left image; spectral) as shown by spatial overlap between the peaks and the cytoarchitectonic maps of V1 (center image) and V2 (right image). Hot metal color bar refers to cytoarchitectonic map of V1 or V2 (Amunts et al., 2000). (B) Peak located in dorsal visual association areas V3A/V7 as shown in horizontal and coronal slices (spectral). (C and D) Scatter-plots illustrate the presence of a negative correlation between gray-matter concentration and age of ASL acquisition (C) at the peak located in primary visual areas ($x=-4$, $y=-79$, $z=1$) and (D) at the peak located in the dorsal visual association area ($x=-24$, $y=-85$, $z=24$; D). In all anatomical images, the left side of the image corresponds to the left hemisphere and the right side corresponds to the right hemisphere. The *r*-value is provided as an indicator of effect size; *p*-values are not provided to avoid problems of circularity (Kriegeskorte et al., 2009).

The voxel-based whole-brain correlational analysis considering white matter revealed one peak which approached significance in the dorsal visual association cortex (area V3a/V7, $x=-24$, $y=-85$, $z=25$, $t=4.84$). In this area, later sign-language acquisition was associated with an increase in white-matter concentration. This peak was in close proximity to the gray-matter peak in area V3a/V7 ($x=-23$, $y=-85$, $z=24$) reported previously in Fig. 2B.

3.2. Comparison of tissue concentrations between deaf groups and the hearing control group

To rule out that the structural changes observed in areas modulated by age of sign-language acquisition were not influenced by auditory deprivation per se, tissue concentration in areas modulated by age of sign-language acquisition were compared to a baseline consisting of the average tissue concentration of the same brain areas in the group of right handed hearing controls matched for gender and age. The Welch test comparing gray-matter concentration between deaf and hearing groups detected no significant differences in distribution of gray-matter concentration between the two groups as a whole (V1/V2: $p=0.43$; V3a/V7: $p=0.82$). When the same analysis was carried in white matter in V3a/V7, no differences were detected between deaf and hearing groups as a whole ($p=0.47$).

To determine if sign-language acquisition at different developmental epochs resulted in a significant increase or decreased tissue concentration relative to the general hearing population, a second Welch test was implemented where the deaf group was divided according to the developmental epoch when these deaf individuals had first acquired ASL (infancy, early childhood and later childhood).

This test detected a significant difference in gray-matter concentration between hearing controls and all congenitally deaf signers in the peaks located in V1/V2: (F (3, 14.6) $=18.2$, $p<0.001$) and in V3a/V7: (F (3, 16.3) $=17.4$, $p<0.001$). The Dunnett test revealed that deaf individuals with sign-language acquisition during infancy had greater gray matter in these areas of the visual cortex than the hearing group and that individuals with late sign-language acquisition had a significantly lower gray-matter concentration than the hearing group, while individuals with ASL acquisition early in childhood did not differ significantly in the gray-matter concentration in these areas compared to the hearing controls (Fig. 3A, area V1/V2; Fig. 3B, area V3a/V7).

When the same test was carried out for white matter in V3a/V7, the Welch test detected a significant difference in white matter in this dorsal visual association cortex peak (V3a/V7, F (3, 15.8) $=9.90$, $p<0.001$). The Dunnett test revealed that late learners had significantly more white matter than hearing controls in this region (Fig. 3C, area V3a/V7).

In contrast, when a whole brain voxel-based comparison was carried out between the deaf group as a whole and the hearing control group no significant differences were found in the areas sensitive to age of sign-language acquisition. Rather, a decrease in white-matter concentration trending towards significance was found within the right superior temporal gyrus within the primary auditory cortex ($x=57$, $y=-3$, $z=2$, $t=-5.0$, $p<0.001$, uncorrected) as indicated by a probability map developed by Penhune et al. (1996) and a cytoarchitectonic map of the auditory cortex (area Te 1.2) (Morosan et al., 2001). Tissue concentrations in these regions did not show any correlation with age of language acquisition. No differences in gray-matter concentration were found between the two groups.

3.3. Relationship between structure and function modulated by age of sign-language acquisition

In the present study, we noted that areas of structural change in the occipital cortex (V3a/V7) associated with age of sign-language acquisition in the deaf subjects were closely located to areas of BOLD signal change previously observed in an fMRI study of age of language acquisition in the same individuals. A conjunction analysis was carried out to investigate if there was spatial overlap between the functional and structural peaks. Both the functional peak and gray matter change were located in close proximity to each other within the dorsal peak in the visual cortex (V3a/V7). The brain areas showing decreased gray-matter concentration related to delayed ASL acquisition ($x=-23$, $y=-85$, $z=24$) were just adjacent to brain areas showing increased BOLD signal change in relation to delayed ASL acquisition ($x=-20$, $y=-84$, $z=16$; Fig. 4A).

A regression analysis examining the relationship between gray-matter concentration of the peak voxel yielded by the voxel-based correlational analysis and the % BOLD signal change at the peak voxel yielded by the fMRI analysis for each subject revealed the presence of a negative relationship between BOLD signal change and gray matter in this region of dorsal visual association cortex in that a low gray-matter

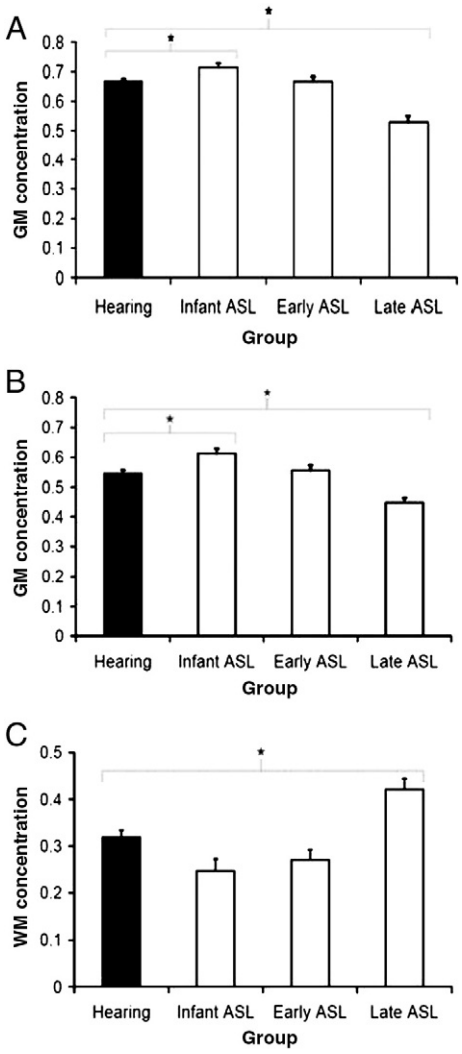

Fig. 3. Welch equality of means test comparing hearing group (black bar) and deaf individuals with sign language acquisition during infancy, early and late childhood (white bars). Gray-matter (GM) concentration was measured at the two peaks revealed by the voxel-based correlational analysis between gray-matter concentration and age of ASL acquisition. (A) Deaf individuals who acquired ASL during infancy had greater gray matter in than hearing individuals while deaf individuals with late ASL acquisition had a significantly lower gray-matter concentration at both peaks, respectively in primary visual areas ($x=-4$, $y=-79$, $z=1$) and (B) in the dorsal visual association area ($x=-23$, $y=-85$, $z=24$). (C) White-matter (WM) concentration was measured at one peak revealed by the voxel-based correlational analysis between white-matter concentration and age of ASL acquisition in the dorsal visual association area ($x=-24$, $y=-85$, $z=25$). Deaf individuals with late ASL acquisition had significantly more white matter in this area than hearing individuals.

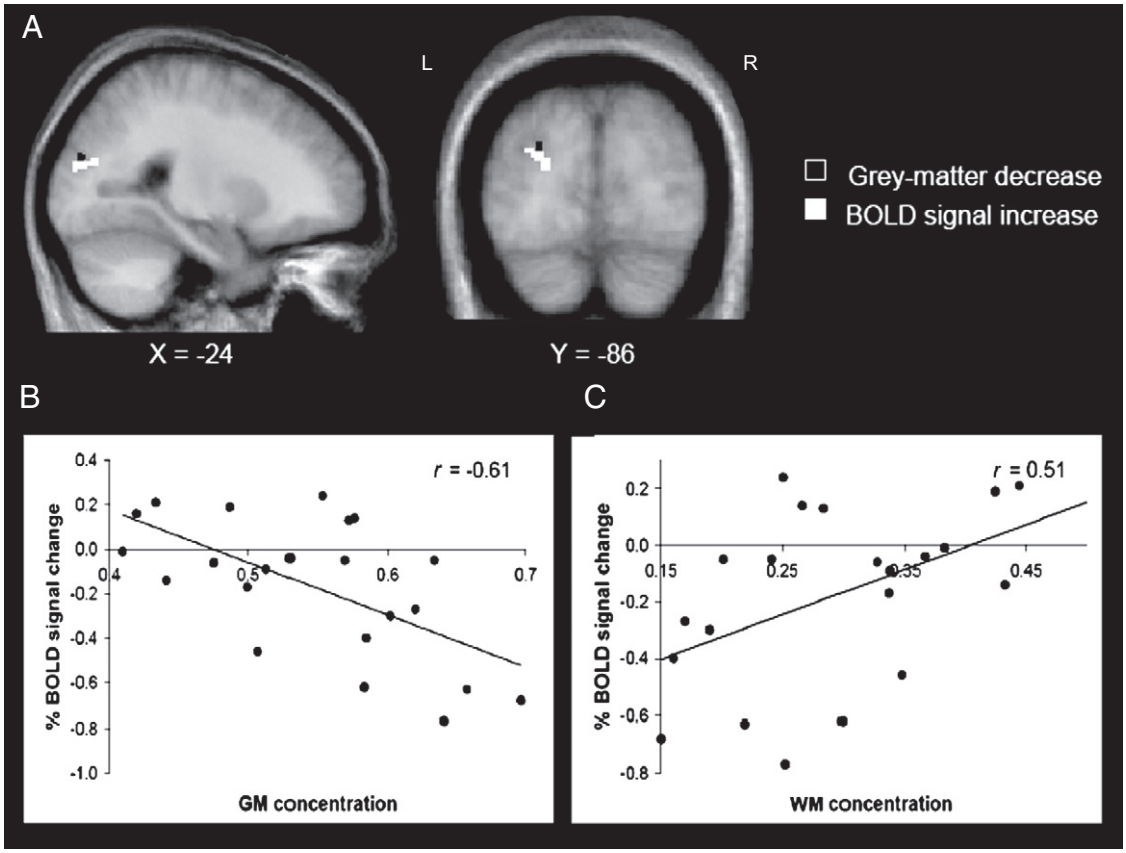

Fig. 4. Brain image showing the proximity of gray-matter structural and functional changes associated to late ASL acquisition. (A) Brain image of the peak yielded by the voxel-based correlational analysis showing an area of gray matter decrease in relation to age of ASL acquisition (black) and of the peak yielded by the fMRI analysis (Mayberry et al., 2011) showing an area of % BOLD signal change increase in relation to age of ASL acquisition (white). Peaks are superimposed onto an average of anatomical MR image of all deaf subjects (n = 23). (B) Scatter plot of regression analysis showing a negative relationship between gray-matter concentration and % BOLD signal change measured at the two peaks. (C) Scatter plot of regression analysis showing a positive relationship between white-matter concentration and % BOLD signal change in the same brain area.

concentration was associated with high BOLD signal change, while a high gray-matter concentration was associated with low BOLD signal change ($r = -0.61$, $p < 0.01$; Fig. 4B). A regression analysis carried out between white-matter concentration of the peak voxel yielded by the voxel-based correlational analysis and the % BOLD signal change at the peak voxel yielded by the fMRI analysis showed a positive relationship with higher white-matter concentration associated with higher BOLD signal change ($r = 0.51$, $p < 0.05$; Fig. 4C).

4. Discussion

Using VBM, we found that the timing of first language acquisition influences not only the functional (Mayberry et al., 2011) but also the neuroanatomical development of the brain. Based on previous functional imaging data (Mayberry et al., 2011), we predicted that individuals with late language acquisition would show anatomical differences in brain areas associated with functional change related to delayed age of ASL acquisition. It is of note that tissue concentration differences in the left frontal lobe and in the left temporal cortex were not observed. However, we did observe anatomical changes associated with age of ASL acquisition in the occipital cortex, in similar loci to the BOLD changes we previously found on an fMRI language processing task in these same individuals.

Our data show that gray-matter concentration in the V1/V2 and V3a/V7 areas of the occipital cortex is negatively correlated with age of language acquisition. We also find a trend towards a positive correlation between age of ASL acquisition and white matter in the V3a/V7 area. Gray- and white-matter concentrations in these areas do not significantly differ between deaf and hearing individuals overall, suggesting that the effect is independent of auditory deprivation and is driven in some way by age of ASL acquisition.

Moreover, although not the primary aim of the study, we collected anatomical images from hearing controls and carried out a VBM group comparison between deaf and hearing groups to identify regional differences in tissue concentrations in order to separate changes in brain anatomy associated with auditory deprivation from those associated with language deprivation. The group comparison of deaf versus hearing subjects showed a decrease in white-matter concentration within the right superior temporal gyrus within the primary auditory cortex, which is consistent with previous work relating the lack of auditory input at an early age to tissue loss in the auditory cortex (Emmorey et al., 2003; Shibata, 2007; Smith et al., 2011). Importantly, tissue concentrations in these regions did not show any correlation with age of sign-language acquisition indicating that the anatomical effects of early language deprivation and auditory deprivation are not the same.

In our study, late sign-language acquisition was associated with a decrease in the gray-matter concentration and a concomitant increase in white-matter concentration in the dorsal visual association cortex (V3a/V7). Although changes in the white matter only approached significance, the concomitant changes in gray and in white matter in opposite directions and in the same anatomical location are likely related to a partial volume effect, which is a characteristic of VBM analyses (Ashburner and Friston, 2000). This effect occurs in areas where both white matter and gray matter are present in close proximity and consequently these areas have voxels containing both white matter and gray matter. It is thus difficult to determine whether the tissue

concentrations observed are more related to a decrease in gray matter or to an increase in white matter. This issue is a well-known limitation of VBM. In general, there is not necessarily a straightforward relationship between VBM measures and underlying microanatomical features (for discussion, see Zatorre et al., 2012). Greater knowledge of the details of anatomical structure related to the phenomenon of interest will require complementary techniques, including for example cortical thickness measures, or diffusion imaging.

If tissue concentration changes found by VBM are interpreted as changes in gray matter, then early ASL acquisition is associated with an increase in gray matter in the visual cortex. In early learners, increased gray matter relative to hearing controls could correspond to greater computational power in the visual cortex leading to a more efficient visual–perceptual analysis of the sign language signal (Leonard et al., 2012) and thus more efficient sign processing for early compared to late learners. The second effect we observe in occipital cortex is that late ASL acquisition is associated with *less* gray matter compared to hearing controls. This finding suggests that early language acquisition, signed or spoken, gives rise to similar brain organization within the visual cortex. Early parent–infant interactions through language may focus on infant visual attention to salient elements in the environment. Such early linguistic exchanges may prompt the development of visual perceptual skills (Quittner et al., 1994). The finding that the native learners of ASL show more gray matter relative to the hearing controls suggests that early sign language exposure magnifies this effect in visual cortex.

If tissue concentration changes found by VBM are interpreted as changes in white matter, then late exposure to ASL is associated with greater white-matter concentration in the visual cortex. An increase in white matter in this area could point to the development of a greater connectivity between visual areas and other areas involved in language processing in the brain. A recent study shows that between childhood and adulthood dorsal language pathways connecting frontal and temporal regions seem to become more highly organized, and that alternate, ventral pathways may exist in children (Brauer et al., 2011). In a similar fashion, in the deaf signers with late language exposure, delayed language input could result in less maturation of the language network and hence increased connectivity between the visual and temporal cortex. This hypothesis is supported by a VBM study where deaf German sign language signers (DGS), some of whom had late age of acquisition, showed less white matter in the left posterior longitudinal and inferior uncinate fasciculi compared to hearing German speakers (Meyer et al., 2007).

Lesion studies have shown that the participation of the visual cortex in late signers may be essential for language processing, with late signers showing deficits in phonological processing following a lesion to the left occipital cortex (Hickok et al., 1995; Saito et al., 2007). Greater recruitment of the visual cortex may be an indication that more cognitive resources may be allocated to the initial stages of language processing and indicate that late signers engage in a shallower and less automatic form of language processing that relies to a greater degree on visual conceptual input (Mayberry et al., 2011). The present results suggest that increased neuronal activation in occipital cortex in late language learners may arise from inefficient neuronal organization and underlie the language processing difficulties previously reported in these individuals (Mayberry and Lock, 2003).

Increased gray matter in early learners of ASL and increased white matter in late learners are consistent with the idea that the timing of language acquisition in relation to post-natal brain growth may shape the language network to incorporate posterior brain regions in language processing, which ties in nicely with previous fMRI findings in this same group of individuals (Mayberry et al., 2011). In addition, the fact that the anatomical and functional changes are adjacent to each other (Fig. 4) suggests an important structural–functional link in this region for deaf signers, although to date it is still unresolved whether these functional changes precede the structural changes or vice versa. It is also not possible to say whether the functional effect is more related to changes in underlying white or gray matter, since both show similar correlations (Figs. 4B and C); future studies using additional imaging modalities will be required to disambiguate this finding. It is interesting to note that no such relationship between gray matter and white matter was found in the V1/V2 area, which suggests that different mechanisms may be at work in the structural reorganization of these two areas modulated by age of language acquisition in the visual cortex. In deaf individuals who acquired sign language in early childhood, the V1/V2 and the V3a/V7 areas are thought to be involved in motion processing just as is the case in hearing individuals (Bavelier et al., 2001).

It is of interest that we did not find changes in tissue concentration in frontal areas as had been observed in the fMRI study. One possibility is that late language acquisition does not lead to structural changes in this anterior frontal area, because at an early age, in the absence of language, this area may assume a role for other important cognitive functions, which may be sufficient to maintain the structural integrity of this region. Another possible explanation is that VBM has a differential sensitivity in different regions of the brain so that this technique was sensitive to changes in the visual cortex but may not have been sensitive enough to pick up changes in the left frontal area. In a recent tensor-based morphometric analysis, Leporé et al. (2010) observed greater volume in Broca's area in deaf native signers of LSQ (Quebec Sign Language) compared to hearing controls. The results of our study together with those of Leporé et al. (2010), show that both language input in early life and a lack of language input early in life affect the anatomical organization of the brain language system. Future studies with combinations of anatomical techniques and larger sample sizes may allow us to address this issue.

5. Conclusion

Age of sign-language acquisition leads to changes in the structural organization of the visual cortex that mirror changes in functional brain areas activated during a grammatical judgment task using fMRI (Mayberry et al., 2011). The finding of tissue concentration changes within the visual cortex of individuals in relation to age of ASL acquisition emphasizes the importance of undertaking structural analyses to understand the neural underpinnings of brain development and has allowed us to begin to shed light on the importance of language input early in life on the organization and the structural development of language in the brain.

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