Research Report

DTI reveals structural differences in white matter tracts between bilingual and monolingual children

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ABSTRACT

The impact of bilingualism on the microstructure of the white matter pathways related to language processing is assessed in elementary school children by magnetic resonance diffusion tensor imaging (MR-DTI). Forty children, 8–11 years old, subdivided into 3 different groups (15 simultaneous bilinguals, 15 sequential bilinguals and 10 monolinguals), were scanned. The hypothesis was that the starting age and the manner of second language acquisition would affect the characteristics of language circuitry.

In each subject the mean fractional anisotropy (FA) was obtained for four major white matter pathways: 1 — the left arcuate fasciculus/superior longitudinal fasciculus (lAF/lSLF) that connects Broca’s area in the opercular and triangular regions of the left inferior frontal gyrus to the posterior language zone, 2 — the left inferior occipitofrontal fasciculus (lIFOF), connecting anterior regions in the frontal lobe with posterior regions in the temporal occipital lobes, 3 — the bundle arising from the anterior part of the corpus callosum projecting to the orbital lobe (AC-OL) and 4 — the fibers emerging from the anterior midbody (AMB) of the corpus callosum that associate with the premotor and supplementary motor cortices (AMB-PMC). The three groups did not show significant differences in mean FA over the lAF/lSLF or AMB-PMC tracts. In simultaneous bilingual subjects the lIFOF tracts had higher mean FA value compared to monolinguals and also sequential bilinguals, whereas the comparison for the AC-OL fibers yielded a significantly lower mean FA value in simultaneous bilingual subjects compared to monolinguals. In both cases the FA value for sequential bilinguals was intermediate to that of the other two groups. To our knowledge, this study provides the first evidence of bilingualism related adaptation of white matter microstructure in the human brain.

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Keywords: Diffusion tensor, Fiber tracking, Language processing, Bilingualism, Children

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Abbreviations: MRI, magnetic resonance imaging; DTI, diffusion tensor imaging; FA, fractional anisotropy; BA, Broca’s area; WA, Wernicke’s area; AF, arcuate fasciculus; SLF, superior longitudinal fasciculus; IFOF, inferior occipitofrontal fasciculus; CC, corpus callosum; AMB, anterior mid-body; PMC, Premotor and supplementary motor cortices

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1. Introduction and background

Exposure to two or more languages has an impact on the functional ability and microstructure of the brain (Kovelman et al., 2008a, 2008b). Over the last twenty years numerous fMRI and MEG studies have shown significant differences in bilingual and monolingual brain functioning during tasks involving linguistic and non-linguistic processing (Bialystok et al., 2005; Costa et al., 2009; Crinion et al., 2006; Emmorey et al., 2008; Kim et al., 1997; Klein et al., 1995; Kovelman et al., 2008a). It has been established that bilinguals recruit both common and dedicated cortical areas for their two languages (Giussani et al., 2007; Marian et al., 2003). Typical aspects that were investigated in bilingualism studies include the handling of more than one language, language interference and the switching between languages (Crinion et al., 2006; Marian et al., 2003; Mondt et al., 2009; Rodriguez-Fornells et al., 2002). In 2004, Mechelli et al. introduced the idea of testing the structural plasticity of bilingual brains by looking for differences in the density of gray and white matter between bilinguals and monolinguals. They found evidence for an increase in the gray matter density in the left inferior partial cortex in bilingual brains compared to monolingual brains (Mechelli et al., 2004). However, they did not demonstrate white matter differences. MR diffusion tensor imaging (DTI) has opened the opportunity of in vivo neuroimaging, tracking the nerve fibers non-invasively using the information of directional diffusion of water in white matter to characterize structural connectivity (Conturo et al., 1999; Glasser and Rilling, 2008; Morgan et al., 2009).

There have been various investigations on language and the brain from different aspects. The language pathways in the human brain have been thoroughly investigated using post-mortem dissections and in vivo studies (Geschwind, 1972; Martino et al., 2010). These in vivo studies, using various linguistic and imaging techniques, have provided evidence for left hemispheric dominance of speech and language functions in right handed normal subjects (Purves et al., 2001; Vikingstad et al., 2000).

This proven left lateralization has been taken into account for the design of our own study. Broca’s area (BA) and Wernicke’s area (WA) have been identified as the main cortical regions associated with language. BA is located in the opercular and triangular parts of the left inferior frontal gyrus (Brodmann area 44 and 45, respectively) and is associated with language output (Drönkers et al., 2007). WA is a left posterior temporal area devoted to language input (comprehension). These gray matter subdivisions are connected by white matter tracts (Catani et al., 2005; Friederici, 2009), providing a dorsal link.

The dorsal white matter connections between the temporal and inferior frontal language cortices follow the arcuate fasciculus (AF) and the superior longitudinal fasciculus (SLF) (Catani and Mesulam, 2008; Catani et al., 2002, 2005; Crosby et al., 1962; Nieuwenhuys et al., 1988). Since the reliability of distinction between these two pathways is poor due to the spatial resolution limitations of current DTI methods (Friederici, 2009), they are traditionally treated as a single dorsal language pathway (notation: AF/SLF).

The ventral connection between the inferior frontal cortex and the posterior temporal regions is provided by the inferior occipitofrontal fasciculus (IFOF) (Duffau et al., 2002, 2005; Parker et al., 2005). The left IFOF (IFOF) is a large bundle that connects the inferolateral and dorsolateral frontal cortex with the posterior temporal and occipital lobe, extending below the insula and along the inferolateral edge of the claustrum (Catani et al., 2002; Jellison et al., 2004). It is known to play a major role in language semantic processing (Duffau et al., 2005; Leclercq et al., 2010; Mandonnet et al., 2007; Rodrigo et al., 2008).

In addition to these two bundles, language research has indicated some other tracts, passing through the corpus callosum (CC), to be of interest. The observed variations of the CC associated with various linguistic traits (e.g. verbal fluency, learning to read and write and dyslexia) support the hypothesis of probable differences in these bundles between bilinguals and monolinguals (Beaton, 1997; Castro-Caldas et al., 1999; Gazzaniga, 2000; Hines et al., 1992; Hynd et al., 1995; Nosarti et al., 2004a).

Differences of lateralization of language functions between monolinguals and bilinguals (Hull and Vaid, 2006), and the relationship between the lateralization of language functions in the brain and the size of the CC was established earlier (Abotiz et al., 1992; Josse et al., 2008; Witelson, 1989). The first bundle of interest, emerging from the anterior third of corpus callosum (AC-OL), was selected on the basis of the work of Putnam, who specifically showed in a DTI study the relationship between the size of anterior CC and lateralization of brain function (Putnam et al., 2008), and also the proven role of the orbital frontal lobe in perspective-taking and understanding another person’s mental state (Hynes et al., 2006). Another reason to choose this bundle was the affirmed contribution of orbitofrontal lobe to auditory stimuli (Frey et al., 2004) and also the fact that the orbitofrontal cortex receives input from all sensory modalities including auditory which plays a distinct role in absorbing linguistic information (Kringelbach and Rolls, 2004).

The second one, passing through the anterior midbody (AMB) of the corpus callosum, was selected on the basis of the work of Coggins, who used MRI to demonstrate adaptation of the size of the AMB to multilingualism (Coggins et al., 2004).

Recent DTI studies have covered Broca’s area (Drönkers et al., 2007), Wernicke’s area (Blank et al., 2002; Morgan et al., 2009), the AF (Catani and Mesulam, 2008; Glasser and Rilling, 2008; Saur et al., 2008) and other fiber tracts concerned with speech (Hickok and Poeppel, 2000; Morgan et al., 2009; Parker et al., 2005). However, these studies were limited to pure tracking applications and there has been no quantitative study of the effects of multilingualism on the characteristics (microstructure) of white matter in the brain. Yet, DTI provides the basis for doing quantitative investigations, for instance by calculating fractional anisotropy (FA), a quantity that measures the directional diffusion of water. It relates to the tissue axonal microstructure and can be used as a biomarker for white matter anisotropy and fiber organization. FA is known to depend on the number of axons, the axon density, the size of axons and the degree of myelination (Basser et al., 2000; Schmithorst et al., 2005). The aim of this study was therefore to use FA in order to compare the white matter microstructure of the four language related pathways introduced above (Fig. 1) in the brain of simultaneous bilingual,
sequential bilingual and monolingual children. Sequential bilinguals were defined as having acquired their second language after the age of 3 (at school), while simultaneous bilinguals were exposed to both languages from birth. The reason to include both sequential and simultaneous bilinguals was to test the effect of starting age for learning the second language (whether from birth or at a later stage of life). The hypothesis was that the brain plasticity will have had stronger expression in the group that was subjected to the second language at an earlier stage. To our knowledge, this is the first report quantifying the influence of speaking more than one language on the neuroanatomical structure of the white matter.

2. Results

The results of the fiber tracking procedure for the four bundles of interest are illustrated in Fig. 1. The bundles could be traced for all subjects, with one exception: in 4 subjects the lAF/lSLF could not be drawn consistently. Table 2 lists for each group the values of mean FA for the four bundles and their standard deviations. The distribution of data is illustrated in error-bar plots for each bundle of fibers (see Fig. 3). The results of the Shapiro–Wilk test showed that the FA data were normally distributed within the groups. The ANOVA indicated the existence of significant differences between the three groups for two of the bundles under consideration: the relevant p-values were 0.98, 0.004, 0.039 and 0.43 for lAF/lSLF, lIFOF, AC-OL and AMB-PMC respectively. The post hoc results for the two bundles with a significant difference are summarized in Table 3. In the lIFOF, FA differed significantly between simultaneous bilinguals and sequential and monolingual subjects, while in the AC-OL tract only the difference between simultaneous bilinguals and monolinguals remained significant.

<table>
<thead>
<tr>
<th>Table 1 – Group information.</th>
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<tr>
<td>Group</td>
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<tr>
<td>Simultaneous bilinguals</td>
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<td>Sequential bilinguals</td>
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<td>Monolinguals</td>
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</table>

Table 2 – Mean FA values for the four bundles of reconstructed fibers (1 — lAF/lSLF; 2 — lIFOF; 3 — AC-OL; 4 — AMB-PMC).

<table>
<thead>
<tr>
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<th>Simultaneous bilinguals</th>
<th>Sequential bilinguals</th>
<th>Monolinguals</th>
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</thead>
<tbody>
<tr>
<td>lAF/lSLF</td>
<td>0.525±0.029</td>
<td>0.523±0.045</td>
<td>0.522±0.036</td>
</tr>
<tr>
<td>lIFOF</td>
<td>0.548±0.019</td>
<td>0.526±0.025</td>
<td>0.516±0.025</td>
</tr>
<tr>
<td>AC-OL</td>
<td>0.589±0.024</td>
<td>0.595±0.025</td>
<td>0.613±0.022</td>
</tr>
<tr>
<td>AMB-PMC</td>
<td>0.577±0.020</td>
<td>0.582±0.018</td>
<td>0.571±0.018</td>
</tr>
</tbody>
</table>

3. Discussion

Previous research has shown that differences in mean FA values could, in principle, correspond to variations in the number of axons, axon density, size of axons and degree of myelination, and that FA values correlate with information transmission properties (auditory reaction time (Böhr et al., 2007), cognitive processing speed (Turken et al., 2008), information processing speed (Penke et al., 2010; Segura et al., 2009, 2010)).

Based on existing studies and the specificities of language processing, we aimed our investigation of FA at four bundles of fibers among several bundles associating with language processing and communication. In two of these, we found differences in mean FA between the groups under study.

The lAF/lSLF couldn’t be reconstructed in some of the cases. A possible explanation for this could be that, as AF has been reported to continue to myelinate until the later stages of life (Friederici, 2009; Rauschecker et al., 2009), the myelination in these children could be very partial, making it difficult to track the whole bundle. In a recent study Brauer et al. have reported a significantly lower anisotropy in children’s AF/SLF compared to adults, supporting the immature status of this pathway in children (Brauer et al., 2011).

Tracking the lIFOF led to our most interesting finding, which is the higher mean FA value in simultaneous bilinguals compared to monolinguals and also compared to sequential bilinguals. Higher anisotropy of white matter over the lIFOF in simultaneous bilinguals supports the idea that the semantic processing and transmission of semantic information in simultaneous bilinguals might be faster than in monolinguals.

The AC-OL tract was shown to have a significantly lower mean FA value in simultaneous bilinguals compared to monolinguals. Left hemispheric dominance of language in monolinguals and late bilinguals and bilateral cerebral language patterns in early bilinguals ( Hull and Vaid, 2006) may lead to differences in the size of CC between these two groups (Josse et al., 2008) and can suggest an explanation for significantly lower FA value over AC-OL in simultaneous bilinguals compared to monolinguals. However the exact relevance is to be found with more investigations. Although the role of these
fibers in language processing is not completely clear, our finding may indicate that the information is transferred more efficiently between BA and its homologue in the right hemisphere in monolinguals compared to bilinguals.

Finally, in the AMB-PMC tract, contrary to our hypothesis no differences between the three groups could be found. On the basis of this, we could conjecture that bilingualism (which was reported to lead to a larger area of the AMB) may not affect their diffusion characteristic fibers passing through this part of the CC.

In Fig. 3, the mean FA values over the two bundles with significant differences between bilinguals and monolinguals (i.e. lIFOF and AC-OL) were compared. The fact that the mean FA in sequential bilinguals is intermediate to that of the two other groups and also the significant difference in the mean FA value over the lIFOF between simultaneous and sequential bilinguals suggest that the age and manner of being exposed to the second language may have an effect on the anisotropy of the white matter in these two bundles of fibers.

Due to the proven fact of left hemispheric dominance of language in more than 95% of right-handed people (Bethmann et al., 2007; Vikingstad et al., 2000), we limited our investigations to the lIFOF and IAF/ISIF. This can be extended in the future to find inter-hemispheric differences between bilinguals and monolinguals.

These results support our hypothesis that there are differences between the brains of simultaneous language learners and sequential learners. Simultaneous bilinguals by definition acquired their second language from birth (age) at home (manner), while sequential bilinguals acquired the second language after the age of 3 (age) at school (manner). An impact of the age of acquisition could be expected, as the main process of myelination of fibers in the human brain occurs in early childhood, before the second year of life (Lenroot and Giedd, 2006). An additional effect of the manner of acquisition (at school or at home) is not clear at this moment. Further research could try to find out its impact.

Although limiting the age of our population to the range 8–11 restricted the number of subjects, this had the advantage of immature brain structure, which facilitates further research on developmental changes in the same group. Restricting the subjects to a limited age range puts all of them in a more or less same state of brain maturation and the starting age of 8 provides a sufficiently long period for sequential bilinguals to reach an acceptable level of proficiency in their second language.

It remains unclear what the lower FA value of the AC-OL bundle in bilinguals compared to monolinguals really implies as a consequence of bilingualism, and if this results in the slower transmission of linguistic information between the...
two hemispheres. Further studies need to be done to clarify this question and to understand the role played by this bundle of fibers in bilingualism.

The four bundles in our study are certainly not the only ones associated with language. For instance the relevance of the left uncinate fasciculus to the language has been studied by Duffau et al. (2009). There are also some additional sub-regions in the CC that are thought to play a role in language processing, such as the posterior CC which is assumed to be important for speech and language functions (Nosarti et al., 2004b) and the posterior portion has been reported to be involved in speech processing (Sammler et al., 2010). Comparing these sets of fibers and other possible bundles between bilinguals and monolinguals can be of interest for further studies. These investigations are open to be extended to a detailed quantitative comparison of the left and right hemispheres between these groups.

4. Experimental procedures

4.1. Subjects

Forty right-handed healthy male and female children, aged 96–141 months (mean: 114, SD: 11) and subdivided into 3 different groups (15 simultaneous bilinguals, 15 sequential bilinguals and 10 monolinguals) were scanned. The study had been approved by the Ethics Committee of the University Hospital of Brussels (UZ Brussels, Belgium) and informed consent was obtained from all parents. All subjects spoke French or

<table>
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<tr>
<th>FA</th>
<th>Simultaneous vs. sequential</th>
<th>Simultaneous vs. monolingual</th>
<th>Sequential vs. monolingual</th>
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<tbody>
<tr>
<td></td>
<td>IIFOF</td>
<td>AC-OL</td>
<td>IIFOF</td>
</tr>
<tr>
<td>P (ANOVA-Post hoc)</td>
<td>0.043 *</td>
<td>0.823</td>
<td>0.004 *</td>
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</table>

* p<0.05 shows significant difference. Significant differences are shown in bold.

Fig. 3 – Mean FA value over the reconstructed fibers for 3 groups (i.e. group 1: simultaneous bilinguals; group 2: sequential bilinguals; group 3: monolinguals); A: The values over the lAF/SLF fibers don’t differ much between 3 groups; B: The error-bar plots reveal a significantly lower FA value over lIFOF in monolinguals compared to simultaneous bilinguals (*p=0.004), with intermediate result of sequential bilinguals; C: A significantly higher mean FA value over AC-OL is found in monolinguals compared to simultaneous bilinguals (**p=0.039); D: There is no considerable difference in FA value for the AMB-PMC fibers among the 3 groups.
All scans were done with a Philips Achieva 3 T MR system (software release 2.5), with an 8 channel SENSE head coil. Diffusion weighted images were acquired using a single-shot, echoplanar Stejskal-Tanner sequence with 15 non-collinear diffusion gradient directions and b=700 s/mm². Other imaging parameters were: TR/TE=6484 ms/60 ms, FOV=224×224×120 mm³ covering 60 oblique axial with no gap and 1.75×1.75×2 mm³ resolution, total scan duration=454 s. The FOV was tilted in order to minimize the number of slices needed to cover the whole brain and thus shorten the acquisition time. Noise was reduced by averaging 4 acquisitions per scan. For registration purposes, a T1-weighted 3D anatomical image using a turbo field echo sequence was also acquired (TR/TE=12 ms/3.75 ms, FOV=200×200×200 mm³, 100 axial slices, 1×1×2 mm³ resolution, total scan duration=383 s).

### 4.2. Image acquisition

In order to correct for subject movement during the diffusion weighted scans, registration of the resulting images to the first image was done using the software available on the scanner.

DTI post processing used DTI Studio, a program written in visual C++ at the Johns Hopkins University (Jiang et al., 2006). The diffusion weighted images were imported in DTI Studio in PAR/REC format and the corresponding gradient table was calculated using the DTI gradient table creator developed by Farrell et al. (Farrell et al., 2007). For each voxel, the apparent diffusion coefficients (ADC), the diffusion tensor eigenvectors and eigenvalues ($\lambda_0$, $\lambda_1$, $\lambda_2$) and fractional anisotropy (FA) were calculated. FA was defined by

$$\text{FA} = \frac{3}{2} \sqrt{\frac{(\lambda_0-\lambda_1)^2 + (\lambda_1-\lambda_2)^2 + (\lambda_2-\lambda_0)^2}{\lambda_0^2 + \lambda_1^2 + \lambda_2^2}}$$

where $\lambda = \frac{\lambda_0 + \lambda_1 + \lambda_2}{3}$.

Color maps combining the values of FA (brightness) and the directional information (color coding: left-right in red; anterior-posterior in green and head-foot in blue) for the principal eigenvector for each voxel were derived. The FACT-algorithm (Fiber Assignment by Continuous Tracking) available in DTI Studio was used to reconstruct threedimensional fiber tracts. This algorithm was reported to be a reliable tool for reconstructing the neuroanatomical fiber tracts (Jiang et al., 2006; Mori and van Zijl, 2002; Mori et al., 1999). In this approach, the starting point for fiber tracking is the center of a voxel that has an FA value exceeding a user-defined threshold (0.25 in our study). The tracking proceeds along the direction of the principal eigenvector of the voxel. At voxel boundaries, the tracking direction is changed to the direction of the principal eigenvector of the neighboring voxel. The end point of the tract is the first voxel that has an FA value lower than a user-defined threshold (chosen to be 0.20, in order to track the fibers into the gray matter), or at which the tract deviates by more than a given angle (40° in our study). The next step was to interactively select the fibers passing through the predefined ROIs for each of the four bundles of interest. These ROIs were manually drawn for every individual on the FA weighted color maps. Fig. 2 illustrates the ROIs used to track four bundles. The IAF/ISLFL was derived by a two-ROI approach using the AND function of the fiber tracking package. The two ROIs were situated: 1) at the level of the rolandic operculum in coronal view (Fig. 2A, and plane 4 in 2I); 2) laterally to the ventricular trigone on an axial view (Fig. 2B, and plane 5 in 2I). Fibers that pass through both regions defined the IAF/ISLFL. To track the IFOF, an occipital ROI was drawn in a coronal slice, behind the splenium of the CC (Fig. 2D, and plane 3 in 2I) and a second frontal ROI was drawn on a coronal slice located half-way between the genu of the CC and the anterior margin of the insula (Fig. 2E, and plane 2 in 2I) (Rodrigo et al., 2007). This approach allowed separation of this tract from the IAF/ISLFL. The AC-OL fibers were traced by choosing the orbital cortex in the coronal view, locating the first ROI between the anterior edge of the CC and the anterior end of cerebral hemisphere (Fig. 2F, and plane 6 in 2I) and a second ROI placed on the anterior part of the CC in the midsagittal slice (Fig. 2G, and plane 1 in 2I). (Huang et al., 2005).

The AMB-PMC bundle was found by drawing a single ROI in the anterior mid-body of the CC (Fig. 2C, 2I plane 1) (Coggins et al., 2004). The “NOT” operation was sometimes necessarily used to remove a subset of unwanted projections from a reconstruction result (Wakana et al., 2007). All excessive fibers, connecting to regions other than those of interest were removed in all four tracts.
4.4. Quantitative and statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS 17.0). For each of the four bundles, the mean and standard deviation of the FA values of all the voxels within the bundle were calculated. To assess the significance of group differences in mean FA between simultaneous bilinguals, sequential bilinguals and monolinguals, a one-way ANOVA analysis with post hoc testing using Bonferroni adjustment for multiple comparisons was undertaken. Differences were considered significant for p-values less than 0.05 (Jiang et al., 2006; Mori et al., 1999). The normality of the data sets within the groups was assessed by the Shapiro-Wilk test.

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