Using Diffusion Tensor Imaging to examine brain structural plasticity and language experience

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Abstract

Recent advances in neuroimaging methods have led to a renewed interest in the brain correlates of language processing. Most intriguing is how experiences of language use relates to variation in brain structure and how brain structure predicts language acquisition. These two lines of inquiry have important implications on considering language use as an experience-dependent mechanism that induces brain plasticity. This paper focuses on the structural connectivity of the brain, as delivered by white matter, i.e. the collections of the axons of the brain neurons that provide connectivity between brain regions. Tract-Based Spatial Statistics (TBSS), a method commonly used in the field, will be presented in detail. Readers will be introduced to procedures for the extraction of indices of variation in WM structure such as fractional anisotropy. Furthermore, the role of individual differences in WM and changes in WM pertaining to bilingual experience and language processing will be used as examples to illustrate the applicability of this method.

Keywords: White matter, bilingualism, language, individual experiences, TBSS, DTI

1) Structural plasticity in the human brain

Recent scientific advances have enabled scientists to examine brain changes in relation to life experiences. These brain changes have been investigated in the framework of experience-expectant and experience-dependent mechanisms (Greenough, Black & Wallace, 1987; Galvan, 2010). These mechanisms describe biological readiness that is similar across members in the same specie (experience-expectant) as well as characteristics that are specific to individuals within the specie (experience-dependent). Language use is one such experience that has captured research attention because of human's capacity to acquire language and the individual differences in how language acquisition unfolds due to diversity in exposure and usage. Of particular interest is how human adapts to the demand of acquiring two (or more) languages (Bialystok, 2017). This research inquiry has been examined in the field of cognitive neuroscience using a variety of methods, such as functional and structural magnetic resonance imaging (MRI, e.g., Silva & Clitterio, 2017), diffusion tensor imaging (DTI, e.g., Rollans, Cheema, Georgiou, & Cummine, 2017), electroencephalography (EEG) and Event-Related Potentials (ERPs) (for a recent review, see Beres, 2017), functional near-infrared spectroscopy (fNIRS) (e.g., Wan, Hancock, Moon, & Gillam, 2018; for a general review, see Pinti, Tachtsidis I, Hamilton, Hirsch, Aichelburg, et al., 2018), and magnetoencephalography (MEG) (e.g., Brodbeck & Pylkkänen, 2017). As expected, each of these methods has its strengths and weaknesses. One common strength is that all the methods provide insights into how brain *functions* while processing language. However, only structural MRI and DTI allow researchers to examine brain *structure*, grey and white matter respectively, in relation to language acquisition. In this paper, we provide an overview of the methods associated with collecting white matter data and its application in psycholinguistics.

The human brain composed of grey matter, white matter, and cerebral spinal fluid. Grey matter is largely composed of neurons, while white matter forms pathways, including axons and glia cells, that connect neurons in different regions of the brain (Edgar & Griffiths, 2009; for an overview video on neuroanatomy, please see <https://www.jove.com/science-education/5204/an-introduction-to-neuroanatomy>). Functional MRI (or fMRI) allows researchers to capture the Blood-Oxygen-Level Dependent signal in the human brain, providing reasonably accurate spatial information of *where* the activity is recorded as well as on a temporal scale that can be mapped onto the hemodynamic responses (for a review, see Pike, 2012). However, fMRI does not allow researchers examine the white matter pathways. Another sequence, namely Diffusion Tensor Imaging (DTI), allows researchers to examine microscopic structure of white matter pathways by analysing water molecules. White matter pathways are wrapped with myelin sheath which is made up of fatty tissues that act as a barrier wrapping the axons. The high-resolution structural image allows the visual inspection of grey matter, white matter, and cerebral spinal fluid (see Figure 1). While fMRI measures hemodynamic responses and oxygen consumption, DTI extracts information about water diffusion within the white matter pathways. Both techniques are carried out in the MRI environment.

Figure 1. High resolution T1-weighted MR image of a human brain in the axial orientation.

Structural changes in the brain associated with language experience can be studied using two research designs: cross-sectional design that compares individuals with different levels of experiences or longitudinal designs that compares the same individuals before or after some language training. Each of these designs has strengths and challenges. A cross-sectional design requires researchers to ensure that the groups are comparable except for the language experience in question. However, given language contact and interactions are often confounded. Researchers may need to conduct large-scale screening and include matching participants, typically in terms of age, education level, gender, and other potentially confounding demographic variables. Given participant assignment is based on language characteristics instead of random assignment, the research design recruits non-equivalent group comparison (May, 2012). Although cross-sectional design does not allow random assignment, this design is prevalent because of the feasibility and lower costs relative to longitudinal studies. In a longitudinal design, white matter pathways are measured more than once for each participant. Unlike cross-sectional design, longitudinal design treats each participant is his or her own comparison group. The issue of non-equivalent comparison will be less apparent in this design. Both naturalistic language acquisition (across time) and foreign language training are possible to be examined using longitudinal design. One caution is to acknowledge that time is always a confounding factor. In the case of developmental studies involving children studying bilingualism or training study, it is important to include a control group that have different language acquisition and training experience that have comparable experience at pre-test (ideally with random assignment for a training study). Examples of studies using these designs are described in section 5 of this paper. In the next section, procedures involved in conducting a study examining the white matter pathways are described.

2. Data acquisition

a) Scanning and participant preparation

Like any study involving human subjects, conducting a neuroimaging study requires approvals from research ethics board at a local university or hospital (depends on where the MRI scanner is located). Ethical clearance is often regulated by federal or governmental agency, hence it is a requirement beyond the institutional level. MRI contraindications generally establish exclusion criteria of participants. Typically, participants with any type of implants and devices that containing ferromagnetic material are by default excluded, in order to avoid the risk of the magnetic field interacting with, and disrupting the function of, these implants. Similarly, participants reporting history of claustrophobia are typically excluded, as the MRI scanners are considerably confined spaces, with typical bore sizes between 55-70 cm. Tattoo and body piercing may pose scanning risk as well. For studies recruiting children or adolescents, orthodontic implementation may pose a risk as well as influencing the quality of data. A MR technologist is usually available for consultation about safety. Panych and Madore (2018) has compiled a recent review and explanation of the physics behind the safety rules. Screening participants to ensure that they are safe to be in the MR environment has the utmost importance.

b) Image acquisition

To ensure a high signal-to-noise ratio and data quality, we would recommend the use of at least a 3T scanner and a 32-channel head coil, as it is common practice in contemporary studies in the field. The following images should then be acquired:

- 1) A diffusion-weighted image (DWI), which is the main image for the analysis. This comprises several 3D acquisitions (or *volumes*) of the brain which will vary in terms of the *strength/duration* (defined by the *b* value) and the *direction* of magnetic gradient fields that are applied by the scanner, which are used to investigate water diffusivity in the brain. The first volume in this image will typically be the b0 volume, i.e. a baseline acquisition where no gradient fields have been applied. The scanning protocol will vary across different labs/scanners, but a recommended protocol would include 60 transversal slices, slice thickness=2mm, acquisition matrix 256 x 256 mm with in-plane resolution 128 x 128 (yielding 2x2x2 isotropic voxels), 2 or more averages, echo time (TE)= 70ms, repetition time (TR)= 1800ms, b=1000, and acquisition in 64 directions (see Soares, Marques, Alves, & Sousa, 2013, for more detailed recommendations).
- 2) A separate brief image containing 2-3 b0 volumes acquired in the opposite direction from the main DWI, but otherwise with the acquisition parameters remaining the same. This is used during preprocessing to correct for inhomogeneities in the field (see below). In some cases, an additional high-resolution 3D T1-weighted image is used to register the DWI and provide anatomical effects with precision, particularly for separate volumetric analyses of grey and white matter.

c) Software and hardware

There are several software packages available for the pre-processing and analysis of DTI data (see Soares et al., 2013, for a list). In this paper we refer to the tools included in the FSL package (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012). FSL is an open access software library for the analysis of most types of MRI data. It is free to install and use, it runs on a Linux environment, including most of the more common distributions, or on Windows via a virtual machine, and it requires basic knowledge of shell scripting. More details, including installation instructions, tutorials and sample data, can be found here: <https://fsl.fmrib.ox.ac.uk/fsl/>. It is recommended that the analysis be run on a computing cluster rather than an individual computer as the analysis are often computationally intensive.

3. Preprocessing

The scanner will normally output raw data in a scanner-specific format (e.g. DICOM for Siemens scanners), which will need to be converted into NIfTI images to be used with FSL. Raw diffusion-weighted images have to be pre-processed before any analysis can be run. The next section describes the appropriate pre-processing steps as run by the FDT toolbox (Behrens et al., 2003):

- a) FDT first corrects of image distortion. DWI are likely to be distorted by two main factors, the first being participant movement in the scanner: Although typically MRI labs take steps to immobilize participants heads' in the scanner (e.g. with cushions) it is possible that the head will move slightly throughout a typical 10-15 minute scan. Any movement will distort the alignment of the acquired volumes, meaning that the head will be scanned in slightly different positions for each volume. Moreover, DWI are affected by eddy currents produced by the scanners gradient coils, which might cause stretches and shears in the images which will vary for different gradient directions. These distortions are corrected with the *EDDY* and *topup* tools.
- b) After correcting for the distortion, the second step is to fit a *diffusion tensor model* on each voxel on the corrected data, which is carried out by the *DTIFIT* tool. This step takes into account water diffusion in all directions as measured by the data and estimates the *directionality* of diffusion within each voxel. Since a voxel is a 3D object, water diffusivity can be estimated in three directions and can be represented as a tensor. In other types of brain tissue (e.g. cerebrospinal fluid) the diffusivity of a voxel is expected to be *isotropic*, i.e. equal towards all directions, yielding a spherical tensor. In white matter however, the presence of myelin restricts water diffusivity, simply because it is harder for the water molecules to cross a myelinated axon than travel inside it, i.e. along the myelin sheath. This means that diffusivity in the white matter is *anisotropic*, i.e. greater along the axons than perpendicular to them, yielding an ellipsoid tensor. DTIFIT uses this information to output a new set of images, or *maps*, including the following commonly used ones:
 - a. λ_1 , which corresponds to Axial Diffusivity (AD), or diffusivity parallel to a white matter tract
 - b. λ_2 and
 - c. λ_3 . These two values give us Radial Diffusivity (RD) based on this calculation ($RD = (\lambda_2 + \lambda_3)/2$). This is diffusivity that is perpendicular to a white matter tract
 - d. Mean diffusivity (MD): Average diffusivity across all directions (so $MD = (\lambda_1 + \lambda_2 + \lambda_3)/3$)
 - e. Fractional anisotropy (FA): A measure using the indices above to provide an estimate of the asymmetry of diffusion between 0 (isotropic diffusion) to 1 (anisotropic diffusion).

Of these indices, the most commonly used are FA and MD. High FA and AD, and low RD and MD, are usually treated in the literature as reflecting low diffusivity caused by high amounts of myelin in a given voxel, which can be due to reasons such as increased myelination or axonal density. These images are subsequently used for standard DTI analyses (see next section). Figure 2a shows an image of the diffusion directions in each voxel in the corpus callosum. Corpus callosum is the white matter pathway that connects the left and right hemispheres. Since there are few crossing fibres in the corpus callosum, the lines reveal that there is a high

level of directional diffusion in this white matter pathway. In contrary, in Figure 2b, there are crossing fibres in the sagittal stratum which includes the left inferior fronto-occipital fasciculus and left inferior longitudinal fasciculus. When the two pathways cross, the FA values in this region decreases because of the different directions of the pathways. Therefore, interpretation of FA values should be combined with familiarity of anatomical structure of the white matter architecture. Additionally, partial volume fractions can be used to accommodate the crossing fibre issue and increase accuracy (Jbabdi, Behrens, & Smith, 2010).

Figure 2. Directional vectors in a) corpus callosum showing the left-right directions along the axial plane. b) sagittal stratum showing different directions in the white matter pathways.

a)

b)

4. Analysis

4.1 Available methods, and TBSS

There are several options available for the analysis of white matter. These range from more classical whole brain Voxel-Based Morphometry routines applied on the FA or MD maps (Mechelli, Price, Friston, & Ashburner, 2005; Rugg-Gunn, Eriksson, Symms, Barker, & Duncan, 2001), to Tractography (Behrens, Berg, Jbabdi, Rushworth, & Woolrich, 2007), where typically specific tracts are a priori targeted across which the above WM indices are extracted. In this paper we will focus on the state-of-the-art method that is commonly used in the literature, which is Tract-Based Spatial Statistics (TBSS) (Smith et al., 2006). A detailed overview of the benefits and drawbacks of the various analytical methods is beyond the scope of this paper; however, it is worth discussing the principles of TBSS.

4.2 Whole brain analysis on FA with TBSS

Analysis in TBSS is usually run across the entire brain, and it can be applied to all four FDT outputs. This section describes the recommended analysis on FA maps, i.e. the most commonly used index of diffusivity (see below for analysis on the other indices). The TBSS pipeline comprises four scripts, which are followed by the appropriate statistical analysis. The first script, *tbss_1_preproc*, performs a quick trimming of the FA maps to account for potential outliers, and organises them in a specific folder structure which will be used by the next steps. The next steps, *tbss_2_reg* and *tbss_3_postreg*, is where the transformation of the images happens. In this step, all raw images are *registered*, or aligned, to a template image, in order to ensure comparability between the participants of a group. This template image can be a standard space image provided by FSL, an option recommended for studies with typical adult populations, or a study-specific template created by the user, recommended for populations such as children or patients, where the standard space image will not

apply. At the end of these procedure new versions of the participant's data are produced, which are registered in standard space, and also concatenated into a single 4D image (with participant being the fourth dimension). Moreover, a mean FA image is created, averaging across all participants, as well as a *skeleton* FA image. The skeleton image is central to the TBSS analyses, and it represents the centre of all tracts that are common to all participants in the study. The next, and final, processing step (*tbss_4_prestats*) creates a thresholded FA skeleton, which is then binarized (1=skeleton voxel, 0=non-skeleton voxel) to create a mask which will restrict the subsequent analyses to the skeleton only, therefore eliminating the issue of aligning participants. Subsequently, the individual FA data from the 4D image are projected onto this binarized skeleton; this means that, for each participant, each skeleton voxel takes the FA value from the centre of nearest tract. This results in *skeletonised data* per participant which are concatenated into a new 4D image with FA values per subject for those voxels that comprise the skeleton only.

This 4D skeletonised image is what is then input into a statistical analysis. For example, in standard between-groups comparison, one would want to see whether FA values across the voxels that comprise skeleton differ between group A and group B (voxelwise analysis) (note that all participants, independently of their group, are included in the 4D image). In FSL the recommended way of running statistical comparisons is via *randomise* (), which normally requires from the research to input (a) the 4D data (b) the binarized skeleton mask as created by *tbss_4_prestats* (c) a design matrix and (d) an operator determining the appropriate correction for multiple comparisons. We have already covered (a) and (b). For (c), the design matrix, the researcher will normally need to use *Glm* command, which provides a handy GUI for building a design. In *Glm*, the researcher will have to specify the number of inputs or rows (equal to number of participants and the number of volumes in the 4D file), and then add columns determining the group each participant belongs to. Then, the number of contrasts, or statistical tests, will also need to be specified. In all, an experiment with x number of participants belonging into two groups, and testing for between groups differences in FA in both directions (group A > group B AND group B > group A) should be set up as shown in Figure 3.

Figure 3. Group and contrast design as illustrated in the two-group comparisons with x participants.

Group	Group A	Group B
1	1	0
1	1	0
1	1	0
1	1	0
1	0	1
...	0	1
x	0	1

Contrast	Title	EV2	EV1
1	A>B	1	-1
2	B>A	-1	1

Once *randomise* has finished running, it will produce statistically corrected images which will be the same number of contrasts set up in *Glm* (i.e. two in the example above: A > B and B > A). These are the reportable between groups differences, which provide a *p* value per voxel across the skeleton. To display these findings, it is recommended to use an appropriate viewer (e.g. fsleyes in FSL or MRICROGL) on which the following images should be loaded: (a) A high resolution image matching the dimensions of the data (usually with a 1x1x1 voxel size), (b) the binary white matter skeleton mask, as derived from the previous steps, and (c) the statically corrected image, thresholded at the appropriate significance level (e.g. $p < 0.05$, which requires thresholding the images where $1 - p > 0.95$).

4.3 Tract of interest analyses

The above pipeline described a whole brain analysis, i.e. statistical comparisons were run on the skeletonised data across the entire brain. In cases where the researcher is particularly interested in FA in one or more tracts of interest (e.g. the IFOF or the UF), it is possible to run a Tract of Interest (TOI) analysis. The process for this will be identical to the whole-brain analysis, but the skeleton mask in *randomise* should be replaced with a new binary mask with a skeletonised version of the tract of interest, which can be created with FSL. Alternatively, it is possible for the researcher to extract and quantify the FA values of a tract of interest (or even the entire skeleton) in order to use them in analyses outside FSL. This can be done with the *fsstats* command by inputting the skeletonised 4D image and a binary mask of the tract of interest, which can be created from the white matter atlases as provided by FSL. This script can provide all descriptive stats for the FA across the defined mask (e.g. mean, standard deviation, range, etc.). Note that for any TOI analysis the same procedure should be repeated for every tract separately.

4.4 Using continuous predictors

Beyond the typical between groups comparisons, it is possible that the researcher would want to use continuous variables and to see how they predict FA values in the brain, especially looking at tracts that are involved in language acquisition and processing. For example, these continuous variables could be participant demographics (e.g. proficiency in, or age of acquisition of, a second language), language test scores (e.g. scores in reading or vocabulary tests), or online performance in language tasks (e.g. reaction times in a naming or a lexical decision task). These correlations are possible to run in *randomise* by setting up the appropriate designs in *Glm* where a single column should now contain the value of this variable per participant, and it should also be modelled as a single contrast. If covariates of no interest are to be included in this model (typically age and sex), these should be demeaned and expressed in different columns, and they do not need to be modelled as separate contrasts. The same procedure should be used for covariates used in between groups designs, where typically each covariate should be demeaned separately per group broken down in as many columns as the groups- for example, in a between groups experiment comparing monolingual and bilingual children and controlling for age and sex, there should be four covariate columns, one for age and one for sex, separately for each group. Finally, it is worth reminding that the above pipelines can be used with all the FDT outputs, not just FA (e.g. with MD). This can be done by simply copying the original non-FA maps in a new subfolder under the one originally created by TBSS for the FA analysis, and then running the *tbss_non_FA* script.

5. Applications in psycholinguistics

Human brain plasticity in the white matter pathways have been studied in many different domains (for a review, please see Sampaio-Baptista & Johansen-Berg, 2017). With language experience being one of the most persistent and prevalent experience, researchers began researching white matter pathways that are crucial for language processing. One of the early studies on white matter pathways and language compared adults with normal or poor reading abilities (Klingberg, Hedehus, Temple, Salz, Gabrieli, et al., 2000). The results showed that poor readers had lower FA values in bilateral temporo-parietal areas than normal readers. Importantly, the FA values in the specific tracts that showed group difference on the left temporo-parietal areas significantly correlated with the adults' reading performance. This finding established a relationship between behaviour and diffusion anisotropy in white matter pathways. Subsequent studies with on language

development and learning has contributed our understanding of brain plasticity in humans. In this section, we provide an overview of empirical evidence connecting white matter plasticity and language development, language experience, and language training.

a) White matter pathways associated with language development

To appreciate the how the human brain changes along with language development, researchers examined the neural pathways responsive to human language in the earliest stage of life. Perani and colleagues (2011) reported that one to three days old infants showed functional connectivity between the bilateral temporal regions when responding to human speech, whereas adults showed more intrahemispheric connectivity between frontal and temporal regions in the left hemisphere. Using DWI tractography, Perani and colleagues further reported that white matter pathways connecting the inferior frontal gyrus and temporal region (part of the extreme capsule system) and connecting the temporal cortex to premotor cortex (part of the arcuate fasciculus and superior longitudinal fasciculus) exist in newborns. However, the critical pathway that connects the prefrontal cortex and temporal cortex has not reached maturity. This finding illustrates that there is biological readiness for newborns to be responsive to language, with white matter architecture supporting the functional regions responsible for language acquisition. A subsequent follow-up study further confirmed that the immature pathway in newborns is developing in school age children, which shows more developed pathways than newborns, but still immature relative to that observed in the adults (Brauer, Anwender, Perani & Friederici, 2013).

Skeide, Brauer, and Friederici (2016) continued to examine the relationship between white matter pathways development and functional neural network when children and adults were listening to syntactically simple and complex sentences. Using a cross-sectional design, children from the ages of three to ten and adults performed a sentence-picture matching task while undergoing fMRI scans. Results showed older children responded faster and more accurate than younger children, with overall performance level approaching adults. Importantly, the same pattern of results was observed in the magnitude of BOLD signal in the inferior frontal gyrus and the variation of fractional anisotropy in the two white matter pathways connecting the frontal and temporal regions of the brain, namely the arcuate fasciculus. Magnitude of BOLD signal change and variation of fractional anisotropy in white matter pathways were highest in adults followed by the older children and the young children.

Subsequent research on a large sample (N = 183) children from three months to four years also reported the volume fraction in myelination in major white matter pathways, such as bilateral corona radiata, pathways in the left inferior frontal regions, and anterior corpus callosum, are significantly correlated with age (O'Muircheartaigh, Dean III, Ginestet, Walker, Waskiewicz, et al., 2014). Strikingly, these variation in the volume fraction of these white matter pathways is also correlated with the children's expressive and receptive language. Notably, there are differential correlations between white matter pathways and receptive and expressive language respectively. In the corpus callosum, the volume fraction in the anterior portion of the pathway is significant correlated with both receptive and expressive language. However, the volume fractions in the slightly superior portion underneath the left and right premotor cortex correlate with receptive and expressive language respectively. The interpretation is that segments of the same pathway may manifest in different relationships with development. These findings demonstrated the crucial role of white matter pathways in language acquisition: While white matter pathways, particularly the arcuate fasciculus and corpus callosum, are ready to support language acquisition at birth, the

maturity of these white matter pathways also depends on environmental exposure to language, as well as the individual's interaction with the language environment. Research on the relationship between language development and white matter pathways has also been investigated in children with atypical development. Although it is beyond the scope of the present paper, interested readers can refer to Mayes, Reilly, & Morgan (2015) for a comprehensive systematic review.

b) Individual differences in language experience

In addition to typical and atypical language development, the investigation on white matter pathways has also been focusing on individual differences in language acquisition, such as bilingualism. Bilingualism refers to the use of two (or more) languages on a daily basis (Grosjean, 2013). For many bilinguals, using both languages daily is part of their life and the experience is persistent. This presents an opportunity for researchers to employ neuroimaging techniques to investigate brain functions and structures associated with natural everyday experience. Notably, like many life experiences, bilingualism is complex and dynamic. This complexity has been a challenge in research as researchers around the world are conducting work on bilingualism, the operational definitions of bilinguals (and sometimes monolinguals) may not be consistent (Surrain & Luk, 2017). This is understandable given bilingualism is an interactional experience between an individual and his/her environment. Notably, this complexity is especially relevant for neuroimaging researchers. Given the high cost of the research, many studies adopt group comparisons to achieve an efficient design. As such, it is crucial for researchers to report individuals' experience as a group as well as the sociolinguistic environment for readers to understand the different language interactions in the groups.

The investigation of brain plasticity associated with bilingualism has largely been on adult and the aging populations, with much limited research on developing samples. Luk, Bialystok, Craik, and Grady (2011) has shown that lifelong bilingual older adults had higher FA in corpus callosum extending to the right inferior frontal regions. This finding was also reported in young adults in another study (Pliatsikas, Moschopoulou, and Saddy, 2015). Findings in these two studies contradict other studies in which monolingual young adults (Cummine & Boliek, 2013; Kuhl, Stevenson, Corrigan, van den Bosch, Can & Richards, 2016) and older adults (Gold, Johnson, & Powell, 2013) showed no difference in FA or higher FA in selected regions respectively. Gold et al. (2013) has pointed out that the bilingual participants in the study reported higher incidence of preclinical Alzheimer's Disease whereas the participants in Luk et al.'s (2011) study did not have these symptoms. Recently, Anderson, Grundy, De Frutos, Barker, Grady & Bialystok (2018) revisited the paradoxical results and reported that when participants were matched using propensity scores in their groupings and statistically control for other covariates, bilingual older adults were reported to have higher axial diffusivity in the left superior longitudinal fasciculus. Axial diffusivity represents the rate of diffusion (Feldman, Yeatman, Lee, Barde, & Gaman-Bean, 2010) whereas fractional anisotropy is a summary measure of estimate between isotropy and anisotropy of diffusion. Collectively, results showed that there are noticeable differences in white matter architecture between monolinguals and bilinguals. However, the nature and magnitude of this difference based on groups worth further investigation. Furthermore, operationally defining the monolingual and bilingual groups is essential to understanding white matter differences.

In addition to comparing monolinguals to bilinguals, researchers have examined differences in white matter pathways in bilinguals with different experiences. Generally, three dimensions define bilinguals' experiences: onset age of second language (L2) acquisition, second language proficiency, and frequency or usage of two or more languages (Luk, de Sa, & Bialystok, 2011; Luk & Bialystok, 2013). If bilingualism is considered as an experience-dependent mechanism, then onset

age of L2 acquisition is an important variable because brain plasticity peaks during childhood and gradually declines (for a general review, see Power & Schlagger, 2018; for a review specifically related to bilingualism, see Berken, Gracco & Klein, 2016). Young adults who acquired L2 early in life (before 5) had qualitatively different white matter structures compared to young adults who acquired L2 later in life (around 9; Hämäläinen, Sairanen, Leminen, & Lehtonen, 2017), although the differences were observed in different tracts with different metrics: Early bilinguals had higher FA in the anterior region of the left arcuate fasciculus but higher MD in bilateral inferior fronto-occipital fasciculi. The nature of these findings suggests the timing of L2 acquisition is important to consider when investigating bilingualism as a contributing factor to brain structural plasticity.

In addition to studies using group design comparing bilinguals with different characteristics in one dimension of bilingualism, researchers have examined correlations between bilingual experience and white matter structures. While group comparisons are informative, group categorization is usually relative within a sample and therefore, may not be reproducible in samples with different quality of bilingualism. For example, the groups form in communities with a strong dominant language is not the same as the groups form in multilingual communities. Therefore, studies using a single sample of bilingual correlating dimensions of bilingualism and white matter pathways. Nichols and Joanisse (2016) reported that age of L2 acquisition (English in this case) and L2 proficiency differentially predicted variation in the white matter pathways in the left (corpus callosum, arcuate fasciculus, and inferior longitudinal fasciculus) and right (inferior longitudinal fasciculus, arcuate fasciculus, forceps minor) respectively. The participants were a group of Chinese students studying in Canada who have not been fully immersed in the English-speaking environment at the time of testing. Similarly, DeLuca, Rothman, Bialystok and Pliatsikas (2019) reported a positive correlation between L2 AoA and FA in the corpus callosum of bilinguals that were highly immersed in an L2-speaking environment, suggesting that the later the L2 AoA the higher the FA values. To further examine the role of immersion, Kuhl, Stevenson, Corrigan, van den Bosch, Can, and Richards (2016) reported that duration of residence was significantly correlated with distributed white matter pathways in bilingual Spanish-English speakers living in the U.S. such that the longer an individual stayed in an L2 immersive environment, the higher FA (and lower diffusivity) was observed in white matter pathways in both left and right hemispheres. While the duration of residence may not imply L2 acquisition, Rossi, Cheng, Kroll, Diaz, and Newman (2017) compared individuals who were dominant English speakers but learned a second language at different point in childhood, significant correlations between onset age of L2 acquisition and bilateral white matter pathways were reported. This finding echoed those reported in Kuhl et al. (2016) and highlights the relationship between L2 acquisition and changes in white matter pathways.

c) Longitudinal changes in white matter pathways after language learning

The strongest evidence supporting brain plasticity associated with language acquisition comes from longitudinal studies. However, due to the high cost and logistical challenges, there are few neuroimaging studies on white matter pathways changes following naturalistic language acquisition or structured language learning. Mohades and colleagues (2012, 2015) had reported in two studies following a group of children longitudinally. In their 2012 study, the research team compared simultaneous bilinguals, sequential bilinguals, and monolinguals who were between the ages of 8 and 11 on four white matter pathways: left arcuate fasciculus, left inferior occipitofrontal fasciculus, anterior corpus callosum projecting to orbital lobe, and the anterior midbody of corpus callosum projecting to premotor and supplementary motor area. Results showed that simultaneous bilinguals had higher FA than monolingual children in the left inferior occipitofrontal fasciculus while the monolinguals had higher FA in the anterior corpus callosum projecting to orbital lobe. Sequential

bilinguals had FA values in between the simultaneous bilinguals and the monolinguals, but the values were not statistically different from either group. Two years later, the children were being assessed again. Simultaneous bilinguals had the highest level of FA in the left inferior occipitofrontal fasciculus. However, in regard to the rate of change, sequential bilinguals demonstrated the largest level of growth compared to the other groups. These findings showed that onset age of second language acquisition is associated with white matter plasticity in developing samples. More recently, DeLuca, Rothman and Pliatsikas (2018) showed that bilingualism-induced neuroplastic changes continue to take place even in highly proficient L2 speakers residing in an immersive environment. Specifically, DeLuca and colleagues reported increases in MD in the left forceps minor after three years of immersion in an L2-speaking environment. Given that increased MD signifies reductions in the amount of myelin, these results suggested decreased reliance on those frontal pathways that are implicated in language control as an effect of increasing experience in language control in immersive environments (see also Pliatsikas, 2019).

6. Future research directions and conclusions

Human's ability of becoming bilingual affords an exciting research opportunity to examine brain plasticity and a persistent naturalistic life experience. One possible future research direction is to investigate how bilingualism and biliteracy independently relate to white matter plasticity. Although it is widely believed that bilingualism and biliteracy is related, many bilinguals who reported using a non-dominant language are only capable of reading and writing in the community dominant language or the language of instruction. Neuroimaging research has shown that bilingualism modulates the white matter pathways relevant to reading (Bakhtiari, Boliek, & Cummine, 2014) and that there are substantive differences in white matter pathways between typical and atypical readers during childhood (Christodoulou, Murtagh, Cyr, Perrachione, Chang, et al., 2017). However, it is still unclear how bilingualism and biliteracy separately modulates white matter pathways in development. Importantly, there is little understanding in how bilingualism interacts with developmental disorders, such as reading difficulty. This investigation will shed light on the qualitative differences among bilinguals as well as enriching behavioural comparisons on typical and atypical reading development.

Another future research direction is to examine the relationship between foreign language ability and variation in white matter pathways. Recent research has demonstrated robust laterality in white matter pathways and ability to imitate foreign speech sounds (Vaquero, Rodríguez-Fornells, & Reiterer, 2017), suggesting one source of the high variability in L2 speech production. Foreign language pronunciation has attracted growing interests in applied linguistics, particularly with the recent advancement in assessing foreign language pronunciation. Saito (2017) reported that measures for L2 pronunciation loaded on the same factor and this factor was only marginally correlated with sound-symbol correspondence, but not the other lexical measure in the LLAMA Meara, 2005). Identifying the underlying neural network supporting different aspects of L2 acquisition will enhance further understanding of the process of language acquisition and inform developing effective pedagogies for L2 learning. Following the investigation L2 acquisition, future research could benefit from longitudinal designs that followed participants over time to examine changes in white matter pathways before and after learning a new language or as language proficiency increases in developmental samples. Results from a longitudinal design illustrate the strongest evidence of brain structural plasticity as related to language usage experience.

Human brain's capacity to learn and adapt to her environment has providing exciting opportunities for researchers from multiple disciplines. Language use marks an important ability that is unique to human. With the recent advances of neuroimaging technique allowing researchers to investigate changes in the human brain, diffusion weighted imaging holds the promise to further our understanding on the human brain structure adapts to the linguistic environment. This paper serves as a primer for researchers who are interested in conducting neuroimaging research examining white matter pathways. The focus of this paper has been on analysis. It should note that many technicalities are involved in the data acquisition stage. Researchers interested in pursuing neuroimaging methods without prior experience are recommended to reach out to researchers or institutions that have existing infrastructures and personnel. Interdisciplinary collaboration enriches each participating field and collectively contributes to understanding language use and brain plasticity.

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