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RESEARCH PAPER

Second language use rather than second language knowledge relates to changes in white matter microstructure

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Abstract Learning and learning to regulate more than one language is shown to have an impact on the structural connectivity of the brain in networks related to language processing and executive control. The available evidence remains however variable in terms of the occurrence, localization and extent of these effects. Variability likely depends on the fact that grouping heterogeneous linguistic profiles under a dichotomous condition (bilingualism vs. monolingualism) may obscure critical aspects of language experience underlying white matter changes. Here, we treated the main quantifiable features in which bilingual experience can be partitioned—that is, age of acquisition, proficiency and use of a second language—as continuous variables, and tested their effects on a sample of young adult participants. Findings indicate that the time spent using a second language, rather than the age of acquisition or knowledge of that language, significantly modulates white matter microstructure in a bilateral cingulo-frontal cluster encompassing structures primarily related to language control. Taken together, these data point to a usage-dependent remodeling of cingulo-frontal connections, and substantiate the conceptualization of bilingualism as a complex and dynamic experience.

Keywords Bilingualism · Second language use · Structural connectivity · White matter microstructure

Introduction

There is extensive evidence from both humans and animal models that skill learning is associated with dynamic changes in the brain's white matter (WM), neural tissue mainly composed of myelinated axons which enable efficient information transfer between different parts of the brain. Longitudinal MRI studies in humans have shown that learning/training activities such as piano practicing (Bengtsson et al. 2005), spatial learning (Hofstetter et al. 2013) or working memory training (Takeuchi et al. 2010) impact WM microstructure in pathways that are functionally relevant to the learned or improved skill. Activity-dependent myelination—either via new myelin formation or via myelin remodelling of already myelinated tracts—has been recently indicated as a putative mechanism by which the structural properties of brain connections may be shaped by experience-related factors, ultimately resulting in behavioural adaptation (Fields 2015; Fields and Dutta 2019). Converging evidence has been provided that WM plastic changes are also associated with the learning and use of a

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second language (L2), which entails not only the acquisition of additional linguistic knowledge, but also the skills necessary to select and control two languages according to the given communicative circumstances (e.g. Elmer et al. 2011; Singh et al. 2018; Schlegel et al. 2012). Irrespective of whether individuals are just learning an L2 or highly proficient bilinguals, speaking more than one language is associated with the cognitive effort of handling competing information from multiple language systems which are jointly activated—both in comprehension and production—at all levels of language processing (phonology: Goldrick et al. 2014; lexicon: Malt et al. 2015; grammar: Hartsuiker et al. 2004). Extending beyond language to domain-general cognition, the orchestration of a dual language system is known to engage executive control functions underpinned by a bilateral network of cingulo-fronto-parietal and subcortical structures (Abutalebi and Green 2016; Green and Abutalebi 2013). Two major approaches have been adopted to probe the structural consequences of L2 learning and use across the WM: (a) Longitudinal experimental investigations which assess the impact of L2 training programs by collecting data on multiple occasions from intervention and comparison groups; (b) Cross-sectional investigations which assess the impact of bilingualism by collecting data on a single occasion from bilingual and monolingual groups. Longitudinal studies are overall consistent in reporting that L2 learning leads to enhanced connectivity (i.e., increased axonal density or myelination) in several perisylvian and cortico-basal ganglia tracts related to language processing and control (e.g. Hofstetter et al. 2017; Hosoda et al. 2013; Mamiya et al. 2016; Schlegel et al. 2012). Evidence from cross-sectional investigations is instead more mixed, and possibly influenced by cohort effects. Pliatsikas et al. (2015), for example, compared young adult bilinguals who were highly immersed in a bilingual environment and active users of their L2 to monolingual individuals. Results indicated increased fractional anisotropy (FA) values (i.e., a proxy measure of increased axonal density or myelination) in the corpus callosum (CC), the inferior fronto-occipital fasciculus (IFOF), the uncinate fasciculus (UF), and the superior longitudinal fasciculus (SLF) bilaterally (see also Rahmani et al. 2017). Negative findings came, by contrast, from Cummine and Boliek (2013), who reported higher FA for young adult monolinguals versus age-matched bilinguals in the right inferior IFOF, which ventrally connects occipital, posterior temporal and orbito-frontal areas and is primarily associated with semantic processing (e.g. Almairac et al. 2015; Martino et al. 2010). Along similar lines, Kuhl et al. (2016) found higher FA for monolingual US citizens versus English–Spanish bilingual immigrants across virtually all the white matter regions studied, in both the cerebrum and the cerebellum. Of note, when splitting the bilingual sample for length of residence in the US, differences between bilingual and monolingual groups were significant only for the lower immersed bilingual subgroup. The patterns of effects from these studies have been interpreted as pointing to L2 exposure or immersion in a bilingual environment as key predictors of WM plasticity, implying that bilingualism-related differences may be driven by specific types of bilingual experience rather than bilingualism per se. It is in fact increasingly recognized that grouping heterogeneous linguistic profiles under a dichotomous condition (bilingualism vs. monolingualism) may obscure critical aspects of language experience underlying neural changes (see Del Maschio et al. 2018; Luk and Bialystok 2013). In addition, a more practical consideration hinders the operationalization of bilingualism as an all-or-none phenomenon: mainly due to the progressive transformation of English into a global language, testing participants who have no knowledge or exposure to an L2 is becoming less realistic, especially among younger people, and even in countries traditionally considered as largely monolingual. On these grounds, and in line with recent trends in the neuroscience of bilingualism (e.g. DeLuca et al. 2019; Kousaie et al. 2017), we treated the main quantifiable features in which bilingual experience can be partitioned as continuous variables, and tested their effects on a sample of young adult participants. Specifically, while controlling for other subject-related variables, we attempted to pinpoint the contribution of the age at which an L2 is acquired (L2 AoA), the amount of time spent using an L2 (L2 usage), and the level of attained L2 knowledge (L2 proficiency) on FA data across the whole brain. Growing evidence suggests that increased amounts of gray matter as well as a more efficient structural and metabolic connectivity are modulated by continuous bilingual practice and extensive immersion in bilingual environments (Kuhl et al. 2016; Luk et al. 2011; Perani et al. 2017;

Pliatsikas et al. 2017). Thus, we predict that second language use rather than second language onset or knowledge will be associated with higher FA values in language- and control-related WM tracts.

Materials and methods

Participants

Fifty (n = 50) young adult participants (Mean age = 25.78, SD = 4.8, 30 F, Range = 18–38) with no history of neurological or psychiatric disorder took part in the study. All participants were right-handed, as established by the Edinburgh Handedness Inventory scale (Oldfield 1971). The MacArthur Scale of Subjective Social Status (https://macses.ucsf.edu/research/psychosocial/subjective.php#measurement) was used to measure participants' socio-economic status (SES) and educational attainment. Verbal intelligence was assessed using the Test di Intelligenza Breve (TIB) (Colombo et al. 2002), the Italian equivalent of the National Adult Reading Test (NART; Nelson 1982). All participants were native Italian speakers who spoke English as an L2. The online Cambridge test for adult learners (https://www.cambridgeenglish.org/test-your-english/general-english/) was administered to all participants to assess L2 proficiency (see Sulpizio et al. 2019). The test is composed of 25-items that evaluate English grammatical and conversational knowledge. The score (0–25) provides an estimation of proficiency within the Common European Framework of Reference for Languages (CEFRL) (i.e., A1, A2, B1, B2, C1, C2). To evaluate L2 vocabulary knowledge, participants completed an additional proficiency assessment consisting of an L1-to-L2 90-items translation task (i.e., 30 high-frequency, 30 medium-frequency, and 30 low-frequency words) (see Abutalebi et al. 2012). The level of L2 usage was assessed with a detailed questionnaire in which participants were asked to estimate how many hours per day they used their native (Italian) and second language (English). The questionnaire covered the following areas: media (television, radio, social networks), family, friends and/or classmates, partner, study and/or job, reading and writing (extra-job), other activities (hobbies, sports). Details on demographic, cognitive, and linguistic measures are reported in Table 1.

The present study was conducted with ethical approval from the Human Research Ethics Committee of the Vita-Salute San Raffaele University (Milan, Italy). Written informed consent was obtained from all participants.

MRI acquisition

MRI scanning was performed using a 3 Tesla Philips Ingenia CX MR scanner (Philips Medical Systems, Best, Netherlands) with a 32 channels SENSE head coil at the C.E.R.M.A.C., San Raffaele Hospital/University, Milan (Italy). For each participant a high-resolution MPRAGE (Magnetization Prepared Rapid Gradient Echo) T1-weighted anatomical image was acquired with the following parameters: repetition time (TR) = 9.9 ms, echo time (TE) = 4.9 ms, flip angle = 8°, FOV = 260 mm, matrix size = 256 × 256, number of axial slices = 243, slice thickness = 1.4 mm, voxel size = 0.7 × 0.7 × 0.7 mm^3, Phase Encoding direction (PE) = A/P, SENSE factor = 2, whole brain coverage. Diffusion weighted images (DWI) were acquired with a multi-shell sequence (b values = 700, 1000, 2855 s/mm^2, 10 non-diffusion-weighted b0 distributed in the sequence) with the following parameters: 106 diffusion-encoding gradient directions, TR = 5900 ms, TE = 78 ms, flip angle = 90°, FOV = 240 mm, matrix size = 128 × 128, number of axial slices = 56, slice thickness = 2.3, voxel size = 1.875 × 1.875 × 2.3 mm, PE = A/P, SENSE factor = 2, whole brain coverage. Additionally, four b = 0 images were collected with reversed phase-encode blips (i.e. volumes with distortions going in opposite direction).

DWI preprocessing and TBSS analysis

Image processing and tensor calculation were carried out using FSL version 6.0.1. (Smith et al. 2004; Woolrich et al. 2009). A subset of images comprising only the b = 0 (non-diffusion-weighted) and b = 1000 volumes was selected for the analyses from the shelled DWI data. First, images were visually inspected to exclude major artefacts. Diffusion weighted volumes were then blip-up-blip-down corrected for susceptibility induced distortions by means of the b = 0 images acquired with opposite phase encoding direction using

Table 1 Descriptive statistics of socio-demographic, cognitive, and linguistic measures. Mean, standard deviation (SD) and range for each measure are reported. Raven's matrices raw scores are corrected according to participants' age and years of education; corrected scores range from 0 to 36 (cut-off = 18)

	Mean (SD)	Range
Age (years)	25.7 (4.8)	18–38
Education (years)	17.4 (1.8)	13–21
Annual family income (score)	3.6 (1)	1–5
Test Intelligenza Breve (TIB)	47.2 (2.1)	43–50
Raven's Matrices (corrected scores)	31.3 (2.8)	26–36
Age of Acquisition (AoA)	8.4 (5.3)	3–28
L2 Usage	5.5 (3.3)	0–17.5
Cambridge test (score)	19.2 (4)	10–25
Translation task (L1 > L2) (% of correct responses)	61 (14.3)	19–89

Topup (Andersson et al. 2003; Smith et al. 2004). Eddy current and head motion correction was performed with Eddy tool, with outlier drop-out slices replacement (Andersson and Sotiropoulos 2016; Andersson et al. 2016). Local fitting of diffusion tensors was performed by means of DTIFIT, using FMRIB's diffusion toolbox (FDT). Tract-Based Spatial Statistics (TBSS) (Smith et al. 2006, 2007) method was adopted in order to estimate FA changes driven by bilingual experience. Each participant's FA volume was spatially normalized to the Montréal Neurological Institute (MNI) space and projected onto a mean skeleton image representing the center of all major tracts. The mean skeleton was thresholded at FA > 0.20 and binarized.

Statistical analyses

Preliminary correlation analyses were run to investigate the occurrence of high intercorrelations among independent (linguistic) variables in our multiple regression model. Spearman's correlations were performed between L2 AoA, Usage, and Proficiency (i.e., Cambridge test and Translation task scores). In case two linguistic measures were highly correlated ($r \geq 0.50$; cfr., e.g., Taylor 1990), only one of them was entered in our model.

Data were entered into a General Linear Model (GLM) with the following regressors: Gender, SES, Education, TIB, AoA, Usage and Proficiency. *Randomise* was used to perform voxel-wise DTI analyses with nonparametric permutation inference (Winkler et al. 2014). Linear effects of AoA, Usage and Proficiency were investigated. 5000 permutations were tested at $p < 0.05$, with a threshold-free cluster-enhancement (TFCE) correction for multiple comparisons with a spatial threshold of $k \geq 100$ voxel (Smith and Nichols 2009).

Results

A high significant correlation was detected between the two proficiency measures, that is Cambridge test and translation task ($r_s = 0.69$, $p < 0.001$). We considered the Cambridge test to be a more ecological measure which also evaluates aspects related to the speaker's conversational ability. Hence, the Cambridge test was chosen as an index of participants' proficiency and entered in the model.

WM clusters with significant differences in FA values are reported in Table 2 and Fig. 1. AoA and proficiency did not modulate any significant change in WM FA. Usage-driven WM changes were found in a large cingulo-frontal cluster encompassing the left cingulum bundle (CB), the corpus callosum (CC) (genu, body), the anterior and superior arms of the corona radiata (CR) bilaterally, and the left SLF. A second significant cluster was found in the right SLF.

Discussion

The question of how to control for the multiple factors that characterize the variable nature of bilingualism

Table 2 Significant effects of second language use on WM FA. The John Hopkins University ICBM-DTI-81 atlas (Mori et al. 2005) was used for white matter tracts labelling

Cluster index	Voxels	p TFCE corr.	MAX X (mm)	MAX Y (mm)	MAX Z (mm)	COG X (mm)	COG Y (mm)	COG Z (mm)	WM tracts
1	4541	0.025	17	35	36	− 4.226	26.577	26.392	L Cingulum (cingulate gyrus) Genu of corpus callosum Body of corpus callosum R anterior corona radiata L anterior corona radiata R superior corona radiata L superior corona radiata L superior longitudinal fasciculus
2	196	0.045	37	− 20	28	37.683	− 22.175	30.744	R superior longitudinal fasciculus L Superior longitudinal fasciculus

p TFCE corr. p value corrected for multiple comparisons with Threshold Free Cluster Enhancement, *MAX (x, y, z)* peak value MNI coordinates, *COG (x, y, z)* center of gravity MNI coordinates, *WM* white matter

continues to pose theoretical and methodological challenges to present-day research. In this study, we treated L2 AoA, use, and proficiency as continuous variables, and tested their effects on the neural structural connectivity of a sample of young Italian participants who spoke English as a foreign language. Results indicated that both the age at which English was acquired and the level of attained English knowledge at testing did not relate to significant whole-brain changes in WM microstructure. On the other hand, the amount of daily hours of English use was associated with higher FA (i.e., a proxy measure of increased axonal density or myelination) in a large cingulo-frontal cluster encompassing the left cingulum bundle (CB), the genu and body of the corpus callosum (CC), the anterior and superior corona radiata (CR) bilaterally, and the bilateral superior longitudinal fasciculus (SLF). In the following, we will discuss the association of language use and specific brain structures in an itemized manner for each neural region.

Cingulum bundle

The CB is a C-shaped association tract which connects the temporal pole with the orbitofrontal cortex, arching around the CC (Mori et al. 2005). As a whole, the CB is suggested to serve a multimodal role in cognitive function. Accumulating evidence from both healthy and clinical populations implicates the anterior dorsal CB in attention, working memory, and executive processes, whereas the retrosplenial and parahippocampal subdivisions of the tract have been associated with visuo-spatial and episodic memory functions (see, for review, Bubb et al. 2018). Interestingly, whilst especially the dorsal anterior cingulate cortex/pre-supplementary motor area complex (dACC/pre-SMA) is well known to play a role in bilingual language control (Abutalebi and Green 2016), the involvement of the anterior dorsal CB in relation to bilingual experience represents a relatively novel finding. To our knowledge, the only study which previously reported the cingulum as related to

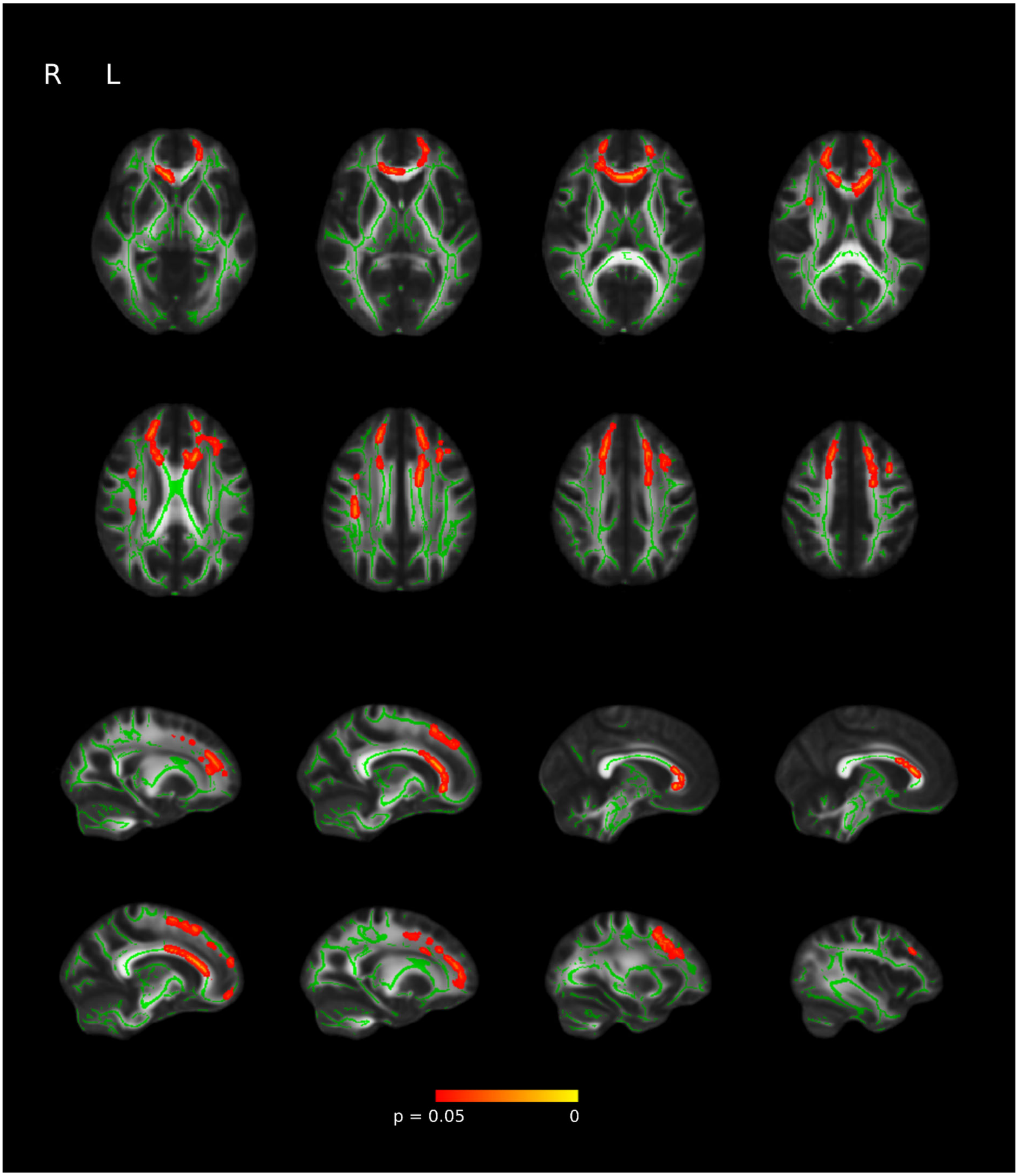

Fig. 1 Significant changes in Fractional Anisotropy (FA) driven by second language use. Clusters thresholded at $p < 0.05$ are overlayed on the mean FA skeleton mask and displayed over the mean FA template. Results are shown in radiological display convention

bilingualism is Rahmani et al. (2017), who performed diffusion MRI connectometry analyses on existing data from Pliatsikas et al. (2015) and found higher quantitative anisotropy (QA) of the CB in late bilingual adults compared to monolinguals. However, Rahmani and colleagues also reported no correlation between QA of CB and duration of immersion period in their bilingual group, which seems to be at odds with the usage-driven changes here documented in the same tract. These idiosyncratic patterns of effects may be due to methodological differences in diffusion MRI analysis (TBSS vs. dMRI connectometry) and different measures employed to quantify second language use (daily hours of L2 usage vs. length of residence in the foreign country). Overall, we report that higher levels of L2 usage are associated with enhanced connectivity in the dorsal anterior division of the cingulum, which encompasses vast reciprocal connections of ACC to dorsolateral prefrontal and orbitofrontal cortices.

Corpus callosum

Immediately ventral to the CB, the CC stretches across the brain's midline and connects homologous areas in the two hemispheres, playing a pivotal role in interhemispheric communication (Gazzaniga et al. 1962; Gazzaniga 2005). Coggins et al. (2004), who were the first to report bilingualism-mediated neuroplasticity across the WM, were also the first to show larger volume in the anterior midbody of the CC for bilinguals versus monolingual controls. This finding, and more in general the structural modification of the genu and body of the CC associated with bilingual experience, have been largely replicated (e.g. Felton et al. 2017; Luk et al. 2011; Schlegel et al. 2012; Singh et al. 2018) even in sequential bilinguals (Mohades et al. 2012; Pliatsikas et al. 2015). Nichols and Joanisse (2016), for instance, reported a positive relationship between FA values in the left CC and AoA in Chinese-English speakers (see also DeLuca et al. 2019), suggesting that older learners rely more heavily on this structure to compensate for a late L2 onset. A positive association between FA values in the CC and second language use has been reported, among others, by Pliatsikas et al. (2015). It has been proposed that the neuroplastic changes in the CC may result from the greater cognitive effort associated with multiple language processing, which would require stronger interhemispheric communication of cortical regions bridged by the CC. A stronger reliance on a bilateral recruitment of the CC network in bilinguals as compared to monolinguals has been recently proposed as a putative explanation for the differential effect of the ACC sulcal variability on executive control performance in these groups (Del Maschio et al. 2019).

Corona radiata

It has long been suggested that normal language processing is supported not only by cortico-cortical WM pathways between language regions, but also fiber tracts connecting cortical language centers and deep brain structures which participate in language processing and control (Crosson 1992; Wallesch and Papagno 1988). In bilinguals, a supplementary recruitment of subcortical structures and cortico-subcortical networks has been attributed to the need for selectively attend to one language and switching from one language to another in conversation, but also to efficiently manage articulatory processes, especially in demanding language control conditions such as simultaneous interpreting (see, for a recent review, Calabria et al. 2018). The CR is a white matter sheet of projection fibers that ascend from the thalamus up to the cortex and descend from the frontal and parietal lobes to the basal ganglia and the brainstem, containing axonal bundles that carry nearly all of the neural traffic from and to the cortex (Mori et al. 2005). Clinical evidence suggests that the CR may play a role in lexical retrieval (Schnur et al. 2006) and speech control (Avila et al. 2004). The superior CR has been found to be part of a networked system activated during simultaneous interpreting in young multilingual participants (Hervais-Adelman et al. 2014). Rossi et al. (2017) reported higher FA values for late, relatively proficient L2 learners in the anterior and posterior CR, but failed to detect any correlation between FA and length of immersion in the L2 environment. The authors interpreted the absence of an effect in light of the small variability in length of immersion of their participants.

Superior longitudinal fasciculus

The SLF is a branched WM pathway dorsally connecting parietal and temporal lobes with ipsilateral frontal cortices (Makris et al. 2004). As this tract connects areas integral to the language network, not surprisingly it can be remodeled by L2 learning and use. Enhanced structural connectivity in the left SLF has been consistently reported both in young (Pliatsikas et al. 2015; Rossi et al. 2017) and older adult bilinguals (Anderson et al. 2018; Luk et al. 2011) versus age-matched monolinguals. Furthermore, suggesting a rapid time scale of WM plasticity, Hofstetter et al. (2017) showed that WM changes in the left parietal cortex are detectable after only 1 h of vocabulary training in L2, with a positive correlation between lexical learning rate and FA values in the left SLF. Evidence on the role of L2 exposure/immersion as a catalyst for changes in SLF microstructure has been provided by Mamiya et al. (2016), who reported a positive association between FA in the bilateral SLF and the amount of time L2 learners spent in a language immersion program. A similar finding came from Kuhl et al. (2016), who reported a positive correlation between FA values in the SLF and the amount of time spent by their bilingual group speaking English as a second language (although, as previously mentioned, higher FA was detected for monolingual versus bilingual participants across most of the white matter regions studied).

Overall, the findings here reported should be interpreted in light of a number of limitations, among which the efficacy of diffusion metrics such as FA. Arguably the most commonly used diffusivity measure, higher or increased FA is commonly treated as an indication for low water diffusivity caused by higher amounts of myelin, which, in turn, is supposed to be related with increased axonal density or myelination within a given tract. It is however impossible to pinpoint the cellular events underlying changes in the diffusion signal, which can be modulated by many different WM features, including myelin, axon diameter, and axonal density (Assaf and Pasternak 2008). A further limitation of the present study relates to its cross-sectional nature: we cannot rule out the possibility that pre-existing differences in WM indices inclined speakers to different patterns of use.

Conclusions

Bilingualism has been routinely operationalized as an all-or-none-phenomenon in past research, whereas it is a multi-componential experience with a number of variable factors underlying brain structural changes. By treating the main quantifiable features in which bilingual experience can be partitioned as continuous variables, the present study shows that the time spent using a foreign language, rather than the age of acquisition or knowledge of that language, significantly modulates WM microstructure in a large cingulo-frontal cluster encompassing networks primarily related to language control. Traditional research in bilingualism has mainly focused on knowledge rather than practice of languages, with only few studies examining the influence of the actual pattern of language use on neurocognitive functions. Our findings shed new light on the neuroanatomical adaptations resulting from bilingual language use, with special reference to changes in fiber tracts which have been rarely reported in previous literature, such as the cingulate bundle and the corona radiata. The data point to a usage-dependent remodeling of cingulo-frontal connections and substantiate the conceptualization of bilingualism as a complex and dynamic experience.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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