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Bilingualism across the lifespan: Neuroanatomical correlates

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Dedicada a mis hijos por robarles tiempo de mi tiempo para ellos

y a mi padre porque inspiró mi camino en la ciencia.

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Abstract

Bilingualism across the lifespan: Neuroanatomical correlates

Lorna García Pentón

Recently, an increasing number of studies addressing the neuroanatomical bases of bilingualism have appeared (García-Pentón et al., 2016). However, the results are variable and in some cases conflicting, and consequently it is still a matter of debate how brain structure changes due to bilingual experience. The present study will try to shed some light on the field by adding fresh new evidence invetigating children and elderly high proficient Spanish-Basque bilinguals. The proposed work will use large-scale brain-mapping techniques to explore the relationship between structure and function, as a more holistic and realistic approach to understanding comprehensively the neural bases of bilingualism. This integrational perspective will also promote convergent evidence about the specialization and integration of the neural network in bilingualism. As such, this work will study the organisation of brain networks, either due to slow changes in brain areas and their wiring (namely, the structural plasticity), or due to fast modulation of their interactions (namely, functional plasticity) in bilingualism. Importantly, this thesis will employ Functional Magnetic Resonance Imaging (fMRI) during resting-state in combination with Diffusion-Weighted Magnetic Resonance Imaging (DW-MRI) to determine functional and structural connectivity, respectively. Both techniques will make it possible to model the large-scale structural/functional connectivity maps by means of a high-dimensional parcellation (90 grey matter regions) of the brain instead of limiting the analysis to specific regions of interest, as done in previous studies. A 3D high-resolution whole-head anatomical scan will be used in order to generate the grey matter parcellation employed in the connectivity analysis, but also to identify regional grey matter differential structural patterns associated with bilingualism using voxel-based and surface-based analyses. Network-based statistics (Zalesky et al., 2010) and graph theoretical approaches (Latora & Marchiori, 2001; Rubinov and Sporns, 2010) will be employed to investigate differences between groups in connectivity patterns, by isolating sets of regions interconnected differently between groups, and in topological properties of the networks, by measuring global/local graph-efficiency. The main findings of this research

on bilingualism across different groups of age (childhood and elderly) suggested that structural brain plasticity related to bilingualism was so small, unstable, subtle and transient that it was very difficult to detect even in lifelong bilinguals. A fact that is consistent with the current ambiguous picture in bilingualism studies (García-Pentón et al., 2016; see also others, Baum & Titone, 2014; Costa, & Sebastián-Gallés, 2014; Li, Legault, & Litcofsky, 2014; Paap et al., 2015; de Bruin et al., 2015a). However, this study suggested that even when the brain did not display focal brain differences related to bilingualism (i.e. did not show any specialization) it could still show differences at the global level. Specifically, the evidence draws attention that lifelong bilingualism could pinpoint a gain toward a better neural reserve in aging due to the whole network graph-efficiency observed in the elderly lifelong bilinguals.

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Preface

In this day and age, bilingualism is a common reality for millions of people in the world: in the context of globalization, members of all societies are exposed to languages other than their own, with estimates that more than half of the world's population uses two or more languages (Grosjean, 2010) and two thirds of the world's children grow up in a bilingual environment (Crystal, 1997). The familial, social, economical and theoretical repercussions of this topic mean that studies that investigate the biological basis of a second language are particularly desirable (Kennedy & Norman, 2005).

Although critical brain areas related to language have been extensively studied and described (Price, 2010), less is known about the impact of bilingualism in brain structure. Whether we need different or extra brain language regions or sub-networks to support more than one language still remains controversial. Importantly, contemporary neuroimaging techniques and methods provide the tools to investigate bilingualism and brain plasticity.

The Basque Country is a bilingual region in Spain where two languages coexist: Euskara and Spanish. Approximately half of the population is bilingual and individuals learn two languages from birth with extensive contextual presence of both. This situation must potentially enhance any brain structural plasticity due to bilingualism. Thus, it is undoubtedly one of the best places to carry out research on bilingualism. In this context, therefore, this thesis will investigate brain structural and connectivity differences between monolinguals and bilinguals linked to lifelong experience of bilingualism.

Part I: Introduction

Chapter 1: General Introduction

1.1 DEFINING BILINGUALISM

The term *bilingualism* has been not uniformly defined in the specialized literature. There are restrictive definitions that demand native-like mastery of two languages (e.g., Bloomfield, 1935). There are others more flexible that consider a bilingual to be one who alternates use of two languages, irrespective of proficiency (e.g., Weinreich, 1953; Mackey, 1968). In the latter category is the definition provided by Grosjean (2010), defining bilinguals as individuals who use two or more language in their everyday lives. Obviously this definition does not distinguish the different degrees of competency or proficiency of bilinguals giving rise to different degrees of bilingualism that can be classified based on multiple factors:

- *Language proficiency*. The use of two languages involves a complex set of skills that are difficult to measure exactly. However, it can be operationalized as the use of two languages, in which the degree of proficiency varies from '*low*' (not mastered) to '*high*'-proficiency as measured by tests (such as verbal fluency, flexibility, vocabulary size, lexical decision, etc.) and self-reports as indicators of linguistic or communicative competence in both languages.

- *Language competence.* It is possible to distinguish between '*balanced*' bilinguals that are those who have exactly the same degree of linguistic and social competence in both languages, performing in each language as a monolingual (equivalent to the restrictive definition), and '*non-balanced*' bilinguals or '*second language learners*' that are those who can speak two languages but not with equal facility.

- *Age of acquisition* (AoA) or the time of initial exposure to both languages. Bilinguals could be defined then as '*early*' or '*late*' bilinguals. There is no well-defined cut-off point for these sub-categories, so that the boundary between early and late bilingualism in general has been variously set out at various ages such as at 3, 5, 7, even 12 years old (Birdsong and Molis, 2001; Birdsong, 2005). Importantly, the AoA is usually understood as the most influential factor in the degree of competency in the languages.

- *Order in which languages are acquired*. According to this factor there are '*simultaneous*' bilinguals who learn two languages from birth and '*sequential*' bilinguals who acquire languages sequentially, having learned the first language before the second begins to be acquired (Baetens Beardsmore, 1982).
- *Context of acquisition of languages*. '*Natural*' or '*primary*' bilinguals are bilinguals who acquire languages in a natural language-learning context. On another hand, '*acquired*' or '*secondary*' bilinguals are those who have acquired the second language in the context of formal and instructed learning (Pap, 1982).
- *Regular use.* '*Active*' bilinguals are those who use both languages regularly. '*Passive*' bilinguals are those who are exposed to two languages but produce only one.

Measurement of bilingual competences has always been very controversial and problematic. This is complicated, on the one hand, by the heterogeneity of the bilingual population and on the other hand by the lack of standardized instruments and valid measurements. It could be expected that studies devoted to finding neural signatures of bilingualism would help to delineate better the concept of bilingualism, finding specific patterns of brain structure and functioning linked to different type of bilinguals. While plausible, however, this idea of identifying biological markers of bilingualism would seem difficult to implement without being affected by the same problems as other measurements.

1.2 BILINGUALISM: ADVANTAGE OR DISADVANTAGE?

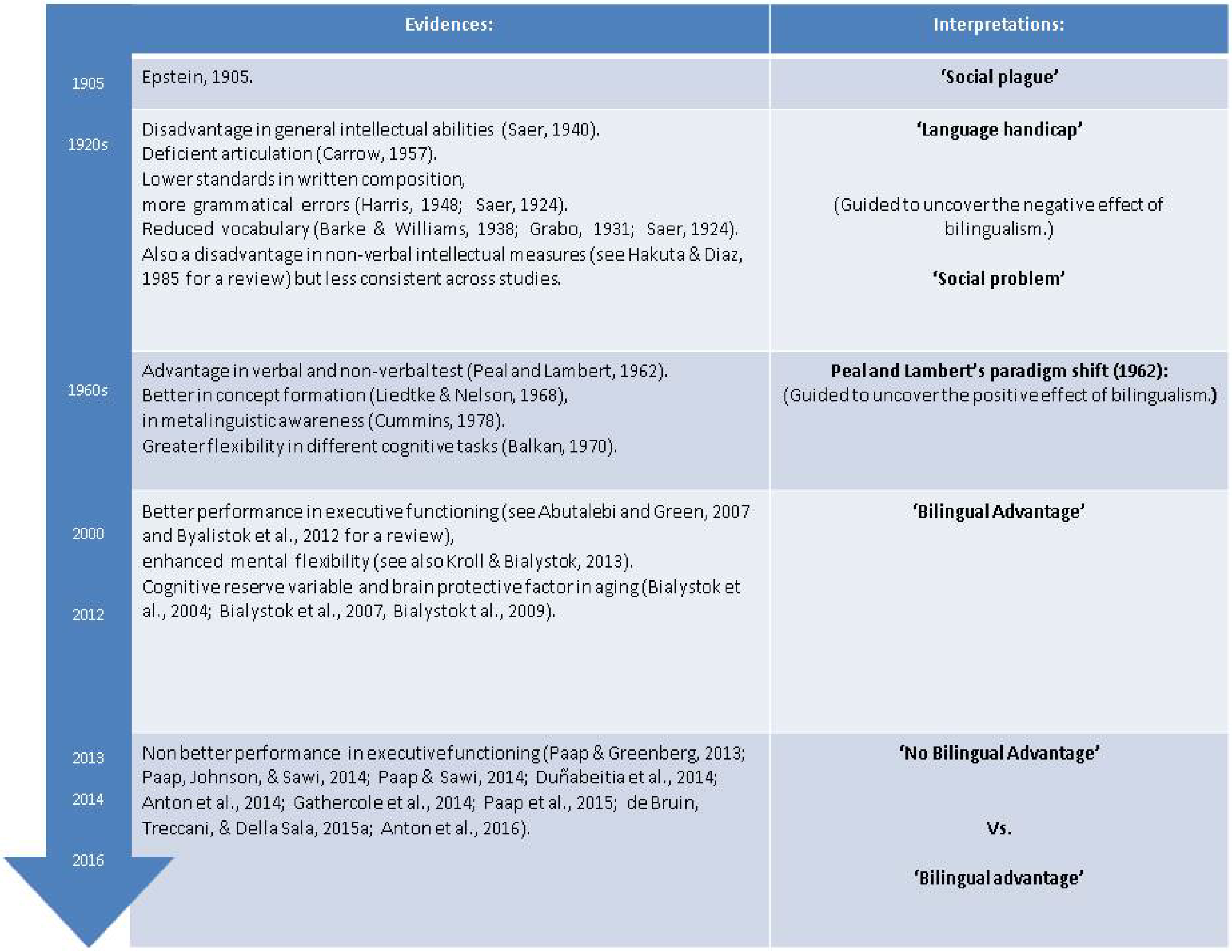
How bilingualism interacts with other cognitive abilities has changed in the specialized literature from the early years of the 20th century until nowadays. The findings of the pioneer literature pointed to the existence of a so-called 'language handicap' in bilinguals. In these studies, bilinguals showed 'linguistic disadvantages' such as deficits in articulation, written compositions and vocabulary (see Darcy, 1953; for a review). The interpretation was that bilingualism resulted in disadvantages for the intellectual abilities of children. The conception of bilingualism as negatively impacting academic performance was deeply rooted at that time and was exacerbated by the growing popularity of psychometric tests (in the 1920s) that measured intelligence in a manner very dependent on verbal skills (Lezak, 2004). Furthermore, those studies suffered from a wide range of methodological problems. For instance, they did not control for demographic variables (such as socioeconomic level, age and sex) or adequately test proficiency and fluency in both languages of the supposed 'bilingual' participants. Thus, such findings are currently considered unreliable by the scientific community (Cummins, 1976).

A paradigm shift about bilingualism began with the seminal study by Peal and Lambert in 1962. This was the first time a study reported that bilinguals outperformed monolinguals on verbal intelligence and nonverbal tests. It contrasted with almost all the prior literature showing the opposite. Notably, the study controlled the sample selection in terms of the linguistic profiles of the participants. This was the first time that a study made a distinction between truly *balanced bilinguals*, who were proficient in both languages from an early age and used both languages for communication, and *pseudo-bilinguals* who knew one language much better that the other and did not use the second language in communication. Additionally, it was also the first time that demographic variables were controlled. Although Peal and Lambert´s study has other important limitations related to the measure they used to evaluate language proficiency when selecting the bilingual/monolingual samples, their work had a profound impact on the field. The conception of bilingualism as a *linguistic handicap* and a *social problem* started to change (Hakuta & Diaz, 1985) and the way bilingualism was studied also changed.

Since the 1960s, the number of research reports making claims for a generalized 'advantage' of bilinguals over monolinguals, instead of the old conception of a 'disadvantage', has grown. The tasks used in these studies mainly tap into attentional resources, memory skills and executive control mechanisms (see Adesope et. al, 2010 for review and meta-analysis). More recent years have witnessed an exponential increase in studies addressing the so-called ‘bilingual advantage’. Evidence has been accumulated demonstrating that bilinguals outperform monolinguals mainly in executive control tasks (see Abutalebi & Green, 2007; Bialystok, Craik, & Luk, 2012 for a review). This new perspective continues to expand and is radically opposed to the old conception of bilingualism. It has been posited that speaking several languages can lead to benefits that go beyond the realm of language. With evidence showing that bilingualism even delays the onset of dementia in old age (see Bialystok et al., 2016 for a review), bilingualism has been shown to impact on brain structure and improve global cognitive functioning (Bialystok et al., 2012).

At present, the conception of bilingualism is changing again. The topic has recently given rise to much more controversy and heated discussions than before. A very recent and provocative article entitled “There is no coherent evidence for a bilingual advantage in executive functioning” (Paap and Greenberg, 2013) has been the detonator for this new viewpoint. In this and successive papers (Paap, Johnson, & Sawi, 2014; Paap & Sawi, 2014), Paap calls into question the seemingly monolithic idea of a ‘bilingual advantage’ with two main observations. The first accounts for the existence of a publication bias that gives preference to results favouring the ‘bilingual advantage’ hypothesis. While remaining a controversial issue (see the recent debate between Bialystok et al., 2015, and de Bruin, Treccani, & Della Sala, 2015b), this observation highlights the fact of the inconsistency of results across tasks and groups of participants (i.e. many do not support the advantage). The second observation is methodological. It refers to statistical concerns about small sample size, the high variability among language profiles of bilingual individuals and (again) how the bilingual and monolingual groups were matched on socio-demographic factors. See Figure 1 for a time line showing critical studies investigating bilingualism.

It seems that progress has been made, since in a 1952 textbook on child psychology you could read: “There can be no doubt that the child reared in a bilingual environment is handicapped in his language growth” (Thompson, 1952) while in 2012 in the New York Times journal it was published: “Being bilingual, it turns out, makes you smarter” (Bhattacharjee, 2012). However, the fact is that the current evidence is still incomplete and does not give consistent answers regarding whether there is or there is not a real ‘bilingual advantage’.



	Evidences:	Interpretations:
1905	Epstein, 1905.	**'Social plague'**
1920s	Disadvantage in general intellectual abilities (Saer, 1940). Deficient articulation (Carrow, 1957). Lower standards in written composition, more grammatical errors (Harris, 1948; Saer, 1924). Reduced vocabulary (Barke & Williams, 1938; Grabo, 1931; Saer, 1924). Also a disadvantage in non-verbal intellectual measures (see Hakuta & Diaz, 1985 for a review) but less consistent across studies.	**'Language handicap'** (Guided to uncover the negative effect of bilingualism.) **'Social problem'**
1960s	Advantage in verbal and non-verbal test (Peal and Lambert, 1962). Better in concept formation (Liedtke & Nelson, 1968), in metalinguistic awareness (Cummins, 1978). Greater flexibility in different cognitive tasks (Balkan, 1970).	**Peal and Lambert's paradigm shift (1962):** (Guided to uncover the positive effect of bilingualism.)
2000 2012	Better performance in executive functioning (see Abutalebi and Green, 2007 and Byalistok et al., 2012 for a review), enhanced mental flexibility (see also Kroll & Bialystok, 2013). Cognitive reserve variable and brain protective factor in aging (Bialystok et al., 2004; Bialystok et al., 2007, Bialystok t al., 2009).	**'Bilingual Advantage'**
2013 2014 2016	Non better performance in executive functioning (Paap & Greenberg, 2013; Paap, Johnson, & Sawi, 2014; Paap & Sawi, 2014; Duñabeitia et al., 2014; Anton et al., 2014; Gathercole et al., 2014; Paap et al., 2015; de Bruin, Treccani, & Della Sala, 2015a; Anton et al., 2016).	**'No Bilingual Advantage'** **Vs.** **'Bilingual advantage'**

Figure 1. *The time line of research on bilingualism. Key studies and interpretations.*

1.3 THE DEBATE ON THE BILINGUAL ADVANTAGE IN EXECUTIVE FUNCTIONS

How the cognitive processes involved in the performance of complex actions, behaviours, thoughts and cognition are controlled and coordinated? Executive functions (EFs) are: "general-purpose control mechanisms that modulate the operation of various cognitive sub-processes and thereby regulate the dynamics of human cognition" (Miyake et al., 2000; Miyake et al., 2012). In other words, they are essential components in the self-control ability which make people differ regulating complex verbal and non-verbal behaviours. Miyake et al. (2000) demonstrated the unity and diversity of these functions, showing that EFs are separable but moderately correlated (i.e. that they share some basic commonalities). However, they demonstrated three relatively independent EFs: information ‘*updating*’ (constant monitoring and rapid addition/deletion of working-memory contents or representations); ‘*shifting*’ of mental sets (switching flexibly between tasks or mental sets); and ‘*inhibition*’ of prepotent responses (deliberate overriding of dominant or prepotent responses). Although, the EFs could be split into more fine-grain level sub-processes, it is difficult to find tasks that can capture individual differences in such subtle sub-process. Thus, these three EFs have been the most frequently postulated in subsequent studies that aimed to understand the nature and organization of different EF systems.

Bilingualism is essentially a linguistic (verbal) and social experience that might primarily impact linguistic skills, but that may also extend to non-linguistic abilities. Managing two languages is a demanding task that could require enhanced executive control mechanisms. The proposal that bilingualism enhances the EF system for verbal and non-verbal processing was postulated by Bialystok (2001). This hypothesis suggests that speaking two languages every day has consequences for the higher cognitive functions, resulting more efficiently developed, such as inhibition and attention (see Barac et al., 2014). There is much evidence demonstrating that bilinguals outperform monolinguals in executive control tasks (see Abutalebi & Green, 2007; Bialystok, Craik, & Luk, 2012 for a review; see also Bialystok & Barac, 2012; Costa, Hernández, Costa-Faidella, & Sebastián-Gallés, 2009; Costa, Hernández, & Sebastián-Gallés, 2008). At a broad level, the underlying hypothesis for the so-called ‘bilingual advantage’ in EFs is that bilinguals are constantly dealing with different languages and for preventing mutual interference between languages they select the target language while inhibiting the non-target language(s). This practice provides bilinguals with a somewhat enhanced mental flexibility, which results in augmented or improved skills related to the management of conflicting information, as compared to monolinguals (see Kroll & Bialystok, 2013, for a review). In other words, speaking several languages can lead to benefits that go beyond the realm of language, impacting on global cognitive functioning (Bialystok et al., 2012) and, more specifically, on the mechanisms responsible for selecting one language while managing interference from the other(s).

But as indicated above, the matter of the bilingual advantage[1] in the behavioural domain is not without its complications. Some recent behavioural studies investigating the supposed bilingual advantage tested very large numbers of simultaneous and early bilinguals and reported no difference between bilinguals and monolingual peers in tasks such as the verbal Stroop, number size congruency (a non-verbal version of the Stroop) (Duñabeitia et al., 2014), attentional network test (Antón et al., 2014), card sorting test, Simon test and metalinguistic judgments test (Gathercole et al., 2014). The recent review by Paap, Johnson and Sawi (2015) points out that the incongruity and inconsistency of the behavioural findings from tasks related to EFs extend to different paradigms, age ranges (i.e., from childhood to the elderly), and types of bilinguals (e.g., early vs. late bilinguals).

One could try to account for this discrepancy by refining the theory in various ways. For instance, the Adaptive Control Hypothesis (Green & Abutalebi, 2013) takes into account the different interactive contexts that give rise to varying degrees and types of language switching behaviour, and thus to different specific cognitive and linguistic demands. The way in which a second (or subsequent) language is acquired varies significantly across and within societies, ranging from individuals learning two languages with extensive contextual presence of both languages from birth (the case of simultaneous bilinguals in settings such as the Basque Country, Wales or Catalonia, among many others), to late learners of a second language (L2, hereafter) with restricted or low contextual presence in the environment (e.g., learning a second language through classroom instruction without L2 natural immersion). These varying conditions for the acquisition of more than one language clearly impose sociolinguistic differences between bilingual individuals.

From this perspective, the specific nature of the bilingual samples from the studies just cited above could explain the lack of differences between bilinguals and monolinguals: these studies examine bilingual communities in which dense code-switching between highly interchangeable languages prevails, so these bilinguals do not need to exercise mechanisms of control between their languages in the same way that other bilinguals have to. This refined

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[1] For the sake of simplicity, henceforth we will use the term ‘bilingual advantage’ exclusively to refer to the differences sometimes reported for bilinguals outperforming monolinguals in tasks tapping into EFs.

version of the bilingual advantage hypothesis continues to assume that certain dual-language contexts (e.g. without dense code-switching) give rise to enhanced executive control, and there is evidence to support this hypothesis from both children and adults of different ages. However, it should be noted that an increasing number of studies testing bilingual samples from similar dual-language contexts (in which code-switching occurs to a lesser degree) have failed to find differences between bilinguals and controls (Paap & Greenberg, 2013; Paap, Johnson, & Sawi, 2014; Paap & Sawi, 2014). Thus, it remains to be seen whether some other factor(s) can explain the lack of a bilingual advantage in these cases.

One of the main problems for these divergent results could be the scant attention paid to the high variability among language profiles of bilingual individuals (Paap & Greenberg, 2013), which can in turn increase the variability in their ability to control for the interference caused by the non-target language(s) (e.g. Green & Abutalebi, 2013). Another limitation of existing behavioural studies in this regard is the non-systematic use of different tasks that involve very different weights of the components of the executive control system (e.g. monitoring, inhibition, shifting) (Miyake et al., 2000; Friedman et al., 2008; Miyake & Friedman, 2012). Furthermore, as shown by Paap & Greenberg (2013), tasks typically used to explore some of these components of executive control (e.g. inhibition) do not correlate with each other, pointing to the multidimensional nature of the measures obtained (see also Kroll & Bialystok, 2013). A refined unity/diversity framework of EFs have been proposed more recently (Miyake et al., 2012), were each EF is actually a combination of what is common for all EFs (unity) and what is specific for each EF (diversity). Under this refined approach, the *inhibition*-specific factor is not longer supported because once the investigators accounted for what is unique or shared between these EFs in the factorial analyses nothing specific remained for the inhibition component as an individual EF (see also Friedman et al., 2011).

Hence, despite the large number of studies on this topic, no convergence has been reached on whether bilinguals exhibit better executive functioning than monolinguals at the behavioural level, or in the contexts in which this difference could be observed. The difficulty in reaching a unified theoretical account given the presence of both null and significant differences between bilinguals and monolinguals in tasks related to different aspects of executive control is undeniable.

1.4 TACKLING THE NEUROANATOMY OF BILINGUALISM

The fact that language learning happens so readily –whether it be a child picking up languages effortlessly or an adult, albeit with more effort, learning a foreign language late in life in a natural environment or under classroom instruction– points in the direction of neuroplasticity. Therefore the search for brain changes is almost certainly a necessary preliminary step in this complex task of understanding the specific biological processes underlying bilingualism. Investigating the brain mechanisms underlying these cognitive processes may help to gain a better understanding of the putative bilingual advantage, particularly for identifying which conditions give rise to this cognitive advantage. Thus, an important contribution to the debate over the behavioural data on bilingualism could come from studies that investigate these issues using neuroimaging methods.

It is well established that the brain constantly changes structurally and functionally under many challenging situations, and this neuroplasticity plays an important role in learning and memory. Bilingualism, like many other fields of expertise (Carreiras et al., 2009; Draganski et al., 2004; Gaser & Schlaug, 2003; Lee et al., 2007; Maguire et al., 2000), involves structural and functional consequences for the brain. In that sense, the biological underpinnings of bilingualism have been approached from both functional and structural perspectives. The guiding hypothesis underlying this approach is clear-cut: if it is the case that bilingualism leads to enhanced language-related as well as domain-general executive control processes, then structural/functional differences may be found in the neural regions that underlie these processes. These regions are assumed to be "most likely found in the executive control system that is largely based on a network of processes in the frontal cortex" (see Kroll & Bialystok, 2013, p. 498), but there are other regions involved (see below for a detailed description of the brain network for language control). Thus, findings suggesting that language control and cognitive control recruit similar neural mechanisms have been taken to support this hypothesis (see Abutalebi & Green, 2007; Luk, Green, Abutalebi & Grady 2012 for review). However, demonstrating that both language and cognitive control mechanisms overlap in a distributed fronto-parietal network (De Baene, Duyck, Brass & Carreiras, 2015) does not necessarily imply a bilingual advantage.

Abutalebi & Green (2007) proposed an overall network of regions responsible for cognitive control and bilingual language production (see Green & Abutalebi, 2013, for an updated and better-defined version). This network is made up of the anterior cingulate cortex (ACC), the left prefrontal cortex (including mainly inferior frontal cortex), the left basal ganglia and the inferior parietal/supramarginal gyrus. They suggested that a single language network mediates the representation of both languages for a bilingual and that the executive control network modulates activation of this language network on an adaptive basis depending on the specific characteristics of the language context and the code-switching demands (cf. Green & Abutalebi, 2013).

Some studies have explored whether the bilingual experience alters the structure and function of these regions (see Bialystok et al., 2012; Costa & Sebastián-Gallés, 2014; Li et al., 2014; García-Pentón et al., 2016 for reviews). Leaving aside task-related functional neuroimaging techniques (see Abutalebi & Green, 2007; Hernandez, 2009; Luk et al., 2012, for a review), which could be influenced by task-related factors boosting multifaceted assessment of the interface between language control and executive control (see Paap & Greenberg, 2013), structural measurements seem well-suited to exploring task-independent differences between bilinguals and monolinguals in the structure of regions involved in language and general executive control mechanisms. Structural neuroimaging studies usually examine the anatomical substrate of the grey and white matter. For the study of grey matter (GM), researchers have typically used high-resolution T1-weighted magnetic resonance imaging (T1-MRI) to obtain measures such as GM volume or density (see Appendix 1 for a description of each measure). Measuring volume/density of the GM typically involves voxel-based morphometry (VBM) and region of interest (ROI) analysis. The thickness of the cerebral cortex is also another measure that can be automatically extracted from the T1-weighted MRI, which allows cross-subject statistical comparisons to be performed in order to detect focal changes in the brain (Fischl & Dale, 2000). For the study of white matter (WM), almost all the studies have employed diffusion-weighted magnetic resonance imaging (DW-MRI) and tract-based spatial statistics (TBSS) (Smith et al., 2006), implemented in the

FMRIB software library (FSL) (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012), to compare images using different diffusion measures: mean diffusivity (MD), axial diffusivity (AD), radial diffusivity (RD), and fractional anisotropy (FA). Although, some studies have employed tract of interest (TOI) approach to compared DW-MRI measures (i.e. similar to ROI-based approach). See Appendix 1 for descriptions of these measures and techniques.

While functional magnetic resonance imaging (fMRI) usually analyses how different brain areas respond to a stimulus or series of stimuli embedded in a given cognitive task (Shen, 2015), fMRI studies during resting-state also show spontaneous fluctuations that could reflect inter-regional neuronal activity that is coherent, synchronised and correlated between remote brain regions that operate together (Yan and Zang, 2010). This suggests that one would be able to map the brain without the need to design any specific task. This is crucial in order to study both healthy and abnormal brains, particularly in children and elderly populations unable to complete difficult cognitive tasks (Shen, 2015). In recent years, this approach, based on the analysis of the brain networks at rest as an approximation for understanding the functional architecture of the human neural circuitry, has become common in cognitive neuroscience. Importantly, contrary to task-based imaging, which typically emphasizes a single brain network associated with a particular task, resting-state fMRI (rfMRI) allows researchers to investigate the large-scale (at the whole brain level) human connectome. rfMRI shares this characteristic with T1-MRI and DW-MRI techniques described above, which are very useful to associate abnormal patterns to pathologies and can also be used to study many human conditions and abilities (e.g., bilingualism). In combination with DW-MRI, rfMRI can be used to investigate how brain structure determines and constrains functional networks.

In recent years, a growing body of evidence addressing structural and functional neuroplasticity in bilingualism has begun to emerge (see Li et al., 2014; García-Pentón et al., 2016, Pliatsikas and Luk, 2016 for a review). However, taken together, the results of these studies demonstrating how brain changes due to bilingual experience are heterogeneous and sometimes conflicting. While some studies have found a variety of neural regions that differ between bilinguals and monolinguals with a degree of consistency, others have failed to show any bilingual-specific effects or have reported localized differences in inconsistent brain areas. In contrast to the findings from studies exploring forms of expertise not related to language (Maguire et al., 2000; Maguire, Woollett & Spiers, 2006; Gaser & Schlaug, 2003), the hazy picture obtained from neuroimaging studies of bilingual-specific effects indicate that it is still unclear where precisely the neural differences between monolingual and bilingual populations lie.

One factor that could be limiting the reproducibility and scope of already existent studies is the sample age selection. The majority of the studies on bilingualism structural brain plasticity have been carried out with young adults (20-40 years old). This is an age when general cognitive abilities are presumed reach a peak of development (Hartshorne & Germine, 2015). Following this idea, young adults probably have a ceiling effect on some cognitive functions (such as executive functions) that make differences between bilinguals and monolinguals more difficult to detect (Bialystok et al., 2012; Bialystok et al., 2016). In fact, Bialystok et al. (2005) found that the bilingual advantage was present in children, middle-aged adults and older adults but absent in the young adults. Other behavioural studies have also reported that the bilingual advantage is more evident in the elderly than in young adults (Bialystok, Craik, & Luk, 2008; Bialystok, Craik, Klein, & Viswanathan, 2004; Hernandez Costa, Fuentes, Vivas, & Sebastián-Gallés, 2010; Gold, Kim et al., 2013). Consequently, it is possible that neural plastic changes linked to bilingualism may be stronger in children and the elderly than in young adults. This is one of the key hypotheses guiding the present investigation (see further in the Motivation and Aims section below).

Additionally, in the case of the elderly, there is plenty evidence about positive brain modifiers (such as exercise, occupation, education, intelligence, socioeconomic status and music) and also negative modifiers (hypertension, stress, hormonal depletion, etc.) that alter the normal aging trajectory of the brain (Raz & Rodríguez, 2006). The positive modifiers protect the brain against normal decline and the negative accelerate brain aging. This indicates that the aging brain is highly vulnerable to changes. In fact, elderly bilinguals do not show ceiling effects and are not at the peak of their cognitive function, they are rather declining. Under this condition the brain is susceptible to neural compensation and neural reserve mechanisms (Stern, 2009). Thus, any potential difference should be more clearly observable in this group. There is also the idea that greater benefits are expected with more years of active bilingualism (Bialystok et al., 2016). Older adult early bilinguals with longer life time experience of active bilingualism (i.e. higher number of years continually selecting between two languages in any social context) might be more trained experts in language selection mechanisms and, by extrapolation, in general cognitive executive functions, which must boost greater brain plasticity (e.g. increased grey and white matter volume) in brain regions related to EFs or language processing. This might result in the neural reserve (and/or compensation) that produces the protective benefit in the brain against decline. In other words, bilingualism might stimulate these brain areas (or the whole brain) which become more resistant to brain atrophy and pathology. The belief in bilingualism as neural reserve is based mainly on retrospective studies in which monolingual and bilingual patients diagnosed with dementia are compared on age of symptom onset; showing that bilinguals display symptoms of dementia significantly (4-5 years) later than monolinguals (Alladi et al., 2013; Bialystok et al., 2007; Gollan et al., 2011; Woumans et al., 2015). In general, there is a strong claim pointing to bilingualism as a cognitive reserve variable and brain-protective factor in aging (see Experiment II in Chapter 6 for details), although there is not a direct demonstration of that claim.

In the case of children, it is well known that brain regions and networks have an exponential growth during childhood (see Weiss-Croft & Baldeweg, 2015 for a review). The brain maturation curve has been described as having an asymptotic growth form; showing an exponential growth from birth until approximately 22 years old, with most rapid maturation between birth and 7 years old (Dosenbach et al., 2010). It has extensively suggested that during the first year of live the brain is extremely sensitive to neuroplasticity and is when occuring the faster pace of learning, during this time the brain is optimally predisposed to learn and store information about the world and is specially sensitive for language (Pierce et al., 2015). There is evidence that the infant’s brain during this period is fine-tuned to specific sounds of their native language and neural representations for that language start to establish (Kuhl et al., 2005; Werker & Hensch, 2015), which shapes the brain for more complex language acquisitions later, such as vocabulary and grammar (Werker & Tees, 2005; Pierce et al., 2014; Pierce et al., 2015). It is well known that the learning of an L2 is undeniably affected by the age at which learning begins (Hernandez and Li, 2007), affecting directly the level of competence in the languages acquired. Observation of the effects of AoA on language skills have given rise to the hypothesis of sensitive periods, where the outcomes of the learning are optimal during a time window and after this period the ability to learn optimally decreases (Knudsen, 2004). L2 AoA effects clearly indicate brain maturation developmental constraints (see for example ‘The sensorimotor hypothesis’, Hernandez & Li, 2007). Furthermore, there is evidence that birth exposure to a birth language still influences the brain later on development when that language is not present anymore (this is the case where the birth language is discontinued) (see Pierce et al., 2015). Other neuroimaging studies suggest that the neural circuitry that supports language processing is different in bilingual than in monolingual children (Garcia-Sierra et al. 2011), and that it involves different networks particularly with a greater connectivity to prefrontal areas for the former group (Petitto et al. 2012). Considering also that the development of executive functioning occurs critically during early childhood (see Garon et al., 2008) and that bilingualism could improve this (Kovacs and Mehler, 2009), it is highly plausible that the early acquisition of an L2 triggers greater brain plasticity in bilingual than monolingual children, especially in regions related to language processing and executive cognitive abilities. The idea is that extensive training in linguistic (and non-linguistic) skills would have a deeper impact on the brain that is more susceptible to changes due to developing brain maturation. Thus, if there are bilingual effects in the structure and connectivity of the brain they should be captured in children.

In general, there seems to be a strong case for exploring bilingualism effects in children and the elderly, as potential structural/functional brain differences between bilinguals and monolinguals will be more clearly observable when cognitive functions are still developing (in childhood) or already declining (in the elderly).

Next section (Chapter 2) will bring together in detail all the structural and connectivity reports about bilingualism and will show that these results are scarce and inconsistent across studies and exhibit a somewhat erratic pattern of differential effects in the expected regions and differential effects in other regions outside the proposed network as well. This section discusses cross-sectional and longitudinal structural studies in three different groups: children, young and old adults (sections 2.1, 2.2 and 2.3, respectively). Each of these subsections is further divided into grey matter (GM) volume/density, cortical thickness (CT) and white matter (WM) studies, given that these different measures can vary independently (see Li et al., 2014). Section 2.4 is dedicated to functional and structural brain connectivity studies conducted in this field, and it provides a brief insight into how functional/structural connectivity may contribute to the current debate. After dealing with the group comparisons for both GM and WM, there is also a separate subsection (2.5) looking specifically at the evidence from correlation analyses showing different effects of AoA and L2 proficiency on the brain. Such correlational studies offer valuable information on the factors that may drive brain changes in the context of bilingualism and they provide suggestive evidence on how these changes evolve across time. Nevertheless, the results described in sections 2.1, 2.2, 2.3 and 2.4 based on group comparisons are more robust and provide direct evidence that shows how the brain changes in bilingualism. Importantly, in order to make the conclusions as clear as possible, this dissertation only takes into consideration results that have been corrected for multiple comparisons, since uncorrected results just show a tendency and cannot be generalized. The final section (section 2.6) summarizes the most specific brain changes in bilinguals described in a review of the literature, and it discusses the main methodological differences among the studies that may be responsible for the inconsistencies.

Chapter 2: Brain Changes in Bilingualism

2.1 STRUCTURAL STUDIES IN CHILDREN

To our knowledge there are no structural studies investigating GM difference between bilinguals and monolinguals children. There are only two recently diffusion tensor imaging (DTI) studies investigating WM changes related to bilingualism in children: Mohades et al. (2012) used a TOI approach to compare FA maps. Their method involved first reconstructing the fiber tracts for the whole brain using a tractography algorithm. Then manually defined ROIs were used to separate four left language-related pathways and one bundle as a control tract: the Inferior Fronto-Occipital Fasciculus (IFOF), Superior Longitudinal Fasciculus (SLF), the bundle from the anterior part of the corpus callosum (CC) to orbitofrontal lobe, the fiber from anterior-midbody CC to motor cortices and right IFOF (as the control pathway not related to language). Finally, the FA values from the voxels included in these tracts provided a mean FA value for each tract and each individual. The samples consisted of 15 simultaneous bilingual children who started to learn the L2 before the age of three (mean age, 9.3) and 15 sequential bilinguals who started to learn the L2 after the age of three (mean age, 9.7). The native language of all bilingual children was Dutch and the L2 was variable between participants. The control group was 10 Dutch monolinguals (mean age, 9.6). The authors obtained higher mean FA values for the bilinguals as compared to the monolinguals in the IFOF, and lower mean FA values in the tracts going from the anterior part of the CC to the orbitofrontal lobe (see Table 2). Mohades et al. (2015) carried out a follow up study on the same 40 children previously tested. In the first study (time 1) the children had a mean age of 9 years old and in this second assessment (time 2) they were two years older. The authors used the same TOI approach described above, limiting the investigation to those tracks. Their results revealed that simultaneous bilinguals had the highest mean FA value in the left IFOF as compared to sequential bilinguals and monolinguals at time 2. The lower mean FA value they observed at time 1 in bilinguals in the anterior part of the CC was no longer evident at the later observation. More studies in children are needed to replicate these interesting results and so to provide greater insights into the structural evolution that accompanies second language learning.

2.2 STRUCTURAL STUDIES IN YOUNG ADULTS

2.2.1 Grey matter volume/density studies

2.2.1.1 Using whole-brain approach

Anatomical changes for bilinguals as compared to monolinguals were reported for the first time by Mechelli et al., (2004). They compared 25 early English-Italian bilinguals (who started to learn their L2 before the age of 5), 33 late bilinguals (who started to learn the L2 between 10 and 15 years old), and 25 English monolinguals. All groups were comparable in age and educational level. VBM analysis of the GM density, using the statistic parametric mapping (SPM) software package (http://www.fil.ion.ucl.ac.uk/spm/), revealed significant GM increases for the bilinguals in the left inferior parietal lobule (IPL) corrected for the family-wise error (FWE, see Appendix 1 for details) at voxel-level (see Figure 2, red and Table 1).

More recent studies have also obtained significant differences between bilinguals and monolinguals using different methods. Pliatsikas, Johnstone & Marinis (2014) compared 17 Greek-English bilinguals (mean age, 27.5; mean L2 AoA, 7.7; mastery proficiency in the L2) with 22 English monolinguals (mean age, 24.5). They performed a whole brain comparison using the threshold free cluster enhancement (TFCE, see Appendix 1 for details) technique (Smith & Nichols, 2009) implemented in the FSL software (Smith et al., 2004) to correct the FWE. They showed a large increment of GM volume for bilinguals in the cerebellum (Pliatsikas et al., 2014) (see Figure 2, dark-blue and Table 1). However, this result in the cerebellum must be taken with caution. On the one hand, it is important to take into account the location of the effect. The cerebellum may lie close to the edges of the field of view (FOV) in the images where the FOV is not large enough, so more artifacts and typical image deformations around the border can be expected (Morelli et al., 2011). Also, some limitations in this area related to poorer segmentation have to be considered (Ashburner & Friston, 2000).

Olalude et al. (2016), compared 15 young adults simultaneous English/American Sign Language (ASL) bimodal bilinguals (CODAs) (mean age, 26.4) and 16 early Spanish-English unimodal bilinguals (mean age, 22.3 years, AoA L2 < 6 years old) with 15 English monolinguals (mean age, 25.9). They performed a VBM at the whole-brain correcting the FWE at cluster-level, using SPM. The unimodal bilinguals had greater GM volume than monolinguals in a cluster including the right precentral covering as well the postcentral gyrus, the inferior frontal gyrus (IFG), frontal operculum and IPL. A second cluster was located in the right middle, medial and superior frontal gyri, and a third one in the superior and middle temporal gyrus (MTG). In the left hemisphere they obtained the largest cluster located in the middle and IFG, but also another one in the middle, inferior and superior occipital lobes, extending into the cuneus and into the MTG (see Figure 2, purple and Table 1). They also obtained a third cluster in the left precentral gyrus, but very small. Contrary to Pliatsikas et al. (2015) study, they obtained greater GM volume bilaterally in cerebellum, but for monolinguals as compared to unimodal bilinguals. Finally, they obtained greater GM volume in the right precentral and postcentral gyri for monolinguals as compared to bimodal bilinguals.

Burgaleta et al. (2016) compared 42 young simultaneous Catalan-Spanish bilinguals (mean age, 21.64) and 46 Spanish monolinguals (mean age, 21.85). They performed a whole brain comparison using the VBM-FSL protocol and TFCE technique to correct the FWE. They showed a large increment of GM volume for bilinguals in the right inferior temporal gyrus, left Heschl, right IPL and bilaterally in the IFG pars orbitalis and cerebellum (see Figure 2, light-blue and Table 1). They also performed a more subtle analysis restricted to the subcortical structures using the FIRST protocol implemented in the FSL and showed a bilateral expansion of putamen and thalamus for bilinguals with respect to monolinguals, as well as of left globus pallidus and right caudate.

Despite these results, other studies also performing VBM analysis failed to find significant differences (see Table 1) between young adult bilinguals and monolinguals correcting for multiple comparisons across the whole brain (Grogan et al., 2012; Ressel et al., 2012). Grogan et al. (2012) studied 31 young multilingual adults who learned English as L2 (the native and other languages varied between participants); the mean age of the group was

30.9 years old. They compared the multilingual group with 30 young non-native English bilingual adults (who also had different native languages); the mean age of the group was 30.6 years old. Although the L2 AoA was variable in both the multilingual and bilingual groups, it was balanced between them. Notice that the sample profiles are very different between these two studies and also different from the studies described above. In contrast, the study by Ressel et al. (2012) used a more similar sample profile to the Mechelli et al. (2004) study. Even so, this study also failed to find any significant differences at voxel-level. They compared 22 young Catalan-Spanish bilinguals who started to learn the L2 before the age of seven (mean age, 23.1) and 22 Spanish monolinguals (mean age, 21.5).

The above studies investigating structural brain changes related to bilingualism are cross-sectional studies that have focused on bilinguals who have already learned and experienced the L2 for long periods of time. Nevertheless, there is a study looking at how the brain changes during the process of learning an L2. Hosoda et al. (2013) studied Japanese students of English, 24 of whom received 4 months of laboratory training on vocabulary and 20 of whom did not. The mean age for both groups was 20 years old. The results of VBM on GM segmentations showed training by group interaction effect in the pars opercularis (IFGOp) of the inferior frontal gyrus (i.e. increased GM volume for learners as compared to controls after training).

In summary, the studies in young adults explicitly looked at whether or not the bilingual brain differs from that of the monolingual, correcting the FWE across the whole brain. On the one hand, differences appear mostly in three regions: the ***left/right IPL*** (Mechelli et al., 2004; Burgaleta et a., 2016, Olulade et al., 2016), the ***cerebellum*** (Pliatsikas et al., 2014; Olalude et al., 2016; Burgaleta et al., 2016), the ***left IFG*** (Hosoda et al., 2013; Olalude et al., 2016; Burgaleta et al., 2016) (Figure 2, Table 1). However, the studies used different FWE controlling methods (i.e. random field theory (RFT) or TFCE and permutations) and different levels of inferences (i.e. voxel-level or cluster-level), which means different levels of sensitivity: cluster-level inferences are more powerful than voxel-level inferences but also imply less localizationist power. Importantly, with respect to the effect in the cerebellum, while some studies found increased GM volume for bilinguals (Pliatsikas et al., 2014; Burgaleta et al., 2016) others found decreased volume (Olalude et al., 2016). Consequently, more studies are needed to confirm this result. Conversely, there are two cross-sectional studies that consistently showed negative results: no differences between bilinguals and monolinguals (Grogan et al., 2012; Ressel et al., 2012) (Table 1). Nevertheless, as the next section will show, when these studies limited their analysis to a region or volume of interest, effects start to appear in expected regions. In any case, these negative results provide interesting findings and help researchers in the field to form new hypotheses. So far, three studies have found the bilingualism effect in expected regions: the left IPL, cerebellum and IFG, but only the left IFG have been seen more consistently across studies.

2.2.1.2 Using ROI-based approach

Some of the studies described in the previous section also used a ROI approach to compare groups. For example, Ressel et al. (2012) manually extracted the mean volume from the right and left Heschl gyri to compare between bilinguals and monolinguals, and obtained significantly larger volumes in bilinguals, bilaterally. Although ROI analysis increases statistical power with respect to whole-brain analysis, the use of ROIs can limit the fine-grain spatial resolution of the effect of interest. Additionally, this type of analysis can miss true differences as a result of the averaging if the variation in the entire ROI is not uniform because parts with no significant difference and parts with a significant difference may be averaged over in the same ROI. The result is that a significant effect is blurred over an entire region. Or, conversely, such averaging may highlight differences if there is a fairly uniform difference that is not very great, rendering a significant effect that would not be deemed significant in a voxel-based analysis after correction for multiple comparisons.

Alternatively, some studies performing VBM used small volume corrections (SVC) as a way of limiting the analysis to specific regions without suffering from the problems of ROI averaging. This can be helpful as a middle point between ROI and whole-brain approaches. Two of the previously mentioned studies used this approach after failing to find differences when correcting across the whole brain. Grogan et al. (2012) found an effect not on the left but in the right IPL using Mechelli et al.’s coordinates for the SVC. However, the comparison was between multilinguals vs. bilinguals instead of bilinguals vs. monolinguals (see Figure 3, green and Table 1). In their VBM analysis, Ressel at al. (2012) additionally performed SVC on the Heschl gyri and differences appeared in just the left hemisphere (Figure 3, red and Table 1).

Two other studies have used SVC implemented in the SPM software but without reporting whole-brain results. Abutalebi et al. (2013) studied 14 German-Italian-English multilinguals who started to learn the L2 before the age of five and the third language (L3) after the age of ten, comparing them with 14 Italian monolinguals. The mean age for both groups was 23.5 years old. After SVC they obtained higher GM volume in the left putamen for multilinguals as compared to monolinguals (Figure 3, purple and Table 1). However, this was done using false discovery rate (FDR), which is a different correction to the FWE. In addition, Zou et al. (2012) studied 14 bimodal Chinese/Chinese Sign Language (CSL) adult bilinguals, (mean age, 49; mean L2 AoA, 19; 29 years of experience with CSL). They compared the bimodal Chinese-CSL bilinguals with 13 Chinese monolinguals (mean age, 48) and found an increased volume after SVC for bilinguals in the left caudate (Figure 3, blue and Table 1).

Additionally, Mårtensson et al. (2012) performed a longitudinal study comparing 14 native Swedish interpreter students (mean age, 20) who took a 3-month intensive language course focusing on vocabulary for different languages (4 Arabic, 8 Dari and 2 Russian) and 17 native Swedish non-learners (mean age, 21) as a control group. Volume measures from left and right hippocampus (the volumetric study was restricted to these regions) revealed larger volume on the right side for learners as compared to non-learners.

In summary, when some of the studies performing VBM in young adults limited their analysis to the scope of certain regions of interest, effects started to appear in the ***Heschl gyri*** (Ressel et al., 2012), the ***right IPL*** (Grogan et al., 2012), the ***left putamen*** (Abutalebi et al., 2013) and the ***left caudate*** (Zou et al., 2012). But again, these are isolated results. Unfortunately, there is no uniformity in the samples compared across these studies: for example, bilinguals vs. monolinguals (Ressel et al., 2012), multilinguals vs. bilinguals (Grogan et al., 2012), multilinguals vs. monolinguals (Abutalebi et al., 2013), bimodal bilinguals vs. unimodal monolinguals (Zou et al., 2012). Therefore, the origin of these effects is variable and may not represent a clear effect of bilingualism. In addition, it is striking that any studies investigated the same region (namely, the IPL), making it extremely difficult to arrive at any solid conclusions from these ROI analyses. Again, differences between bilinguals/multilinguals and monolinguals have been found in different regions in different studies. Equally important, the quality/noise of the data is variable across these studies since some of them used 1.5T and others 3.0T MR scanners (see table 1).

2.2.2 Cortical thickness studies

Klein et al. (2014) performed a cortical thickness study on 12 simultaneous bilinguals (mean age, 23; AoA, below 3 years old), 25 early bilinguals (mean age, 26; L2 AoA, after 4 years old and before 7 years old; mean L2 AoA, 5 years old), and 29 late bilinguals (mean age, 28; L2 AoA, after 8 years old and before 13 years old; mean L2 AoA, 10 years old), all French-English bilinguals. They compared the bilingual groups with a control group of 22 English monolinguals (mean age, 25). The results showed greater cortical thickness for early and late bilinguals as compared to monolinguals in the pars triangularis (IFGTr) and pars orbitalis (IFGOr) of the left IFG (inferior frontal gyrus) and less cortical thickness in the right IFGOr for late bilinguals as compared to monolinguals. Mårtensson et al. (2012) also performed a vertex-wise CT analysis in their longitudinal study (see previous section for details of the study). The learner group showed increased cortical thickness in left dorsal middle frontal gyrus (MFG), IFG and superior temporal gyrus (STG). To date these are the only studies investigating cortical thickness in young adults. Overall, these two studies show as a target region for plastic changes the ***IFG***. Interestingly, they replicate the results on GM volume.

2.2.3 White matter studies

Recently, there have been an increasing number of studies investigating WM changes related to bilingualism. The first study looking for WM differences between bilinguals and monolinguals was Mechelli et al. (2004), who, in addition to the GM analysis, used a VBM analysis to look for differences in WM segmentations but failed to detect any differences in the whole brain (see Table 2). Ressel et al. (2012) also looked for WM differences using the same approach and found no differences either (see Table 2). However, most studies looking at WM changes have employed diffusion tensor imaging (DTI) (see Appendix 1) instead of

T1-weighted MRI. Cummine & Boliek (2013) studied young adult Chinese-English bilinguals (mean age, 24.2; L2 AoA before the age of five) and 11 English monolinguals (mean age, 28.5). They obtained significant decreases of the FA for bilinguals as compared to monolinguals in the right IFOF (see Figure 4, red and Table 2). They also obtained decreased FA in the anterior thalamic radiation, especially in the right superior portion and bilaterally in the inferior portion. More recently, Pliatsikas, Moschopoulou & Saddy (2015) studied 20 sequential bilinguals (mean age, 31.85) who had highly proficient English as L2 (mean AoA, 10.15; mean immersion, 91 months; L1 varied across participants) and were highly proficient in English. The bilinguals were compared with 25 English monolinguals (mean age, 28.16). The authors performed a TBSS analysis, revealing higher FA values for these sequential bilinguals bilaterally in the whole CC (genu, body and splenium), the IFOF, the uncinate and the SLF (see Figure 4 and Table 2).

Schlegel et al. (2012) investigated WM changes during 9 months of intensive Chinese learning without immersion. They studied a training group of 11 English monolingual learners and a control group of 16 English monolingual non-learners. The mean age for both groups was 20 years old. They obtained the most significant FA increase in the genu of the CC, corrected across the whole brain. They also found increased FA and decreased RD in left frontal language-related regions and in the counterpart regions on the right hemisphere. For the whole-brain analysis, they used a non-parametric permutation test and TFCE to achieve significant cluster effects. Interestingly, since they acquired (nine) monthly MR images from participants, they were able to show that the global mean FA (extracted from all the voxels that showed significantly increased FA in the prior whole-brain analysis) described a significant linear increase over the nine time points for learners. They also showed that the amount of increased FA correlated positively with the amount of language learned across these time points. Additionally, they extracted mean FA and RD values from 111 TOIs that showed higher connectivity between language regions and found increased FA and decreased RD mean values for learners as compared to non-learners in 16 of these TOIs: 5 of them terminated in the caudate nucleus and 10 of them connected together different frontal regions of the left hemisphere or frontal regions between hemispheres (these connections passed through the genu of CC). Even though the sample is small, this is a very germane experimental design since it allows for variability between brains to be eliminated. If changes related to bilingualism are small (and this is a very plausible scenario), this strategy of analysis is more beneficial than the cross-sectional studies described above.

Hosoda et al. (2013) longitudinal study on Japanese students of English (see section 2.2.1), also performed TBSS analysis on FA maps. They showed training by group interaction effect in the IFGOp (i.e. increased FA values for learners as compared to controls after training). They also traced 8 pathways known to be related to language: IFGOp-to-Caudate, IFGOp-to-STG (dorsal language pathway), IFGTr of the IFG-to-MTG (ventral language pathway) and the ILF in each hemisphere. The results showed increased connectivity for the right IFGOp-to-caudate and IFGOp-to-STG pathways.

So far, the ***IFOF*** and ***CC*** seem to be the focus of neuroplasticity in bilingualism in young adults. However, while one study found increased FA values in the ***IFOF*** (Pliatsikas et al., 2015) and ***CC*** (Pliatsikas et al., 2015; Schlegel et al., 2012) other found decreased FA values in the ***IFOF*** (Cummine & Boliek, 2013).

2.3 STRUCTURAL STUDIES IN ELDERLY

2.3.1 Grey matter volume/density studies

2.3.1.1 Using whole-brain approach

Abutalebi et al. (2014) performed a VBM study using SPM, comparing 23 older adult bilinguals (12 Cantonese-English and 11 Cantonese-Mandarin; mean age, 62.2; mean L2 AoA, 18.87) with 23 Italian monolinguals (mean age, 61.9). This study obtained a significant volume increase for bilinguals in the left anterior inferior temporal gyrus (aITG) (see Figure 2, yellow and Table 1) using cluster-level correction of the FWE in SPM, a different method of inference from previous studies. Abutalebi, Guidi, Borsa, Canini, Della Rosa, Parris & Weekes (2015) performed a VBM study using SPM, comparing 19 older adult bilinguals (11 Cantonese-English and 8 Cantonese-Mandarin; mean age, 61.68; mean L2 AoA, 12.68) with 19 Italian monolinguals (mean age, 60.93). The results showed a significant volume increase for bilinguals in the left/right ACC (see Figure 2, green and Table 1) using FWE cluster-level correction. However, Gold, Johnson, & Powell (2013) study also performing VBM analysis failed to find significant differences (see Table 1) between bilinguals and monolinguals correcting for multiple comparisons across the whole brain. Gold et al. (2013) studied 20 older adult English native bilinguals who started to learn the L2 before the age of 5; the L2 was variable between participants and the mean age of the group was 63.9 years old. The study compared the bilingual group with 20 English monolinguals with a mean age of 64.4 years old.

In summary, these studies explicitly looked at whether or not the bilingual brain differs from that of the monolingual in old adults, correcting the FWE across the whole brain. Differences appear in the ***left aITG*** (Abutalebi et al., 2014) and the ***ACC*** (Abutalebi, Guidi, et al., 2015) (Figure 2, Table 1). Then again, there is one more study in elderly that showed negative results: no differences between bilinguals and monolinguals (Gold et al., 2013) (see Table 1).

2.3.1.2 Using a ROI-based approach

Using the automatic anatomical labelling atlas (AAL, Tzourio-Mazoyer et al., 2002), in the same study, Abutalebi et al. (2014) extracted the mean volume for the right/left temporal pole (TmP) and right/left orbito-frontal cortex (OFC), and found greater mean volume for bilinguals as compared to monolinguals in both regions and hemispheres. In a more recent study, Abutalebi, Canini, Della Rosa, Green & Weekes (2015) extracted GM volume from ROIs in the left/right IPL using the coordinates reported by Mechelli et al. (2004). This time, they studied 30 older bilinguals (mean age, 63.2; 16 Cantonese-English bilinguals and 14 Cantonese-Mandarin bilinguals) who started to learn the L2 at a mean age of 18.27 years, and compared them to 30 older Italian monolinguals (mean age, 61.85). They found that the volume in the left/right IPL was significantly greater for the bilingual group.

Olsen et al. (2015) extracted and averaged the volume of the GM for the frontal, temporal, parietal and occipital lobes for both right and left hemispheres. They investigated structural differences in the brain of 14 older bilingual adults (mean age, 70.4) who reported regular use of both English and another alphabetic language since before the age of 11. They compared the bilinguals with 14 English monolinguals (mean age, 70.6) and did not obtain any significant group effects in their GM analysis.

In summary, when some of the studies performing VBM limited their analysis to the scope of certain regions of interest, effects started to appear in the ***right/left TmP*** and ***OFC*** (Abutalebi et al., 2014), and the ***right IPL*** (Abutalebi, Canini et al., 2015). With the exception of the right IPL, these are isolated results. Besides ROI studies per se are limited in the scope of their hypothesis, if moreover investigate different regions, then it is impossible to reach conclusions from these analyses. Again, differences between bilinguals and monolinguals have been found in different regions for the different studies. And there is one more study obtaining no differences between bilinguals and monolinguals (Olsen et al., 2015).

2.3.2 Cortical thickness studies

There is only one study about CT in elderly. In their study, Olsen et al. (2015) also performed a CT analysis on the bilingual and monolingual samples described above. They did not find any group differences. However, their analysis was limited to the entorhinal cortex and temporal pole. What they observed was a significant negative correlation between the cortical thickness of the temporal pole and age in the monolinguals but not for bilinguals. They suggested that bilingualism preserves this region from a structural decline in aging.

2.3.3 White matter studies

Luk et al. (2011) studied 14 older adult native English bilinguals who started to learn an L2 after the age of 11 (the L2 was variable across participants) and compared them with 14 English monolinguals. The mean age between groups was 70.5 years old. They performed a TBSS analysis and found significantly increased FA values for bilinguals as compared to monolinguals in parts of the CC (Figure 4, green) that extended bilaterally into SLF (Figure 4, blue) and into to the right IFOF (Figure 4, red) and Uncinate. They also obtained significantly decreased radial diffusivity (RD) values for bilinguals in the body of the CC, overlapping with some of the areas of increased FA (Luk et al., 2011) (see Table 2). Gold et al. (2013) obtained a different result using the same approach to study 20 English bilinguals (mean age, 63.9) who started to learn the L2 after the age of five (the L2 varied across participants). They matched the bilinguals with 20 English monolinguals (mean age, 64.4). The authors obtained a significant decrease in the FA values for bilinguals as compared to monolinguals in many portions of the CC (Figure 2, green) and bilaterally in the ILF, IFOF (Figure 4, red) and fornix. They also obtained significantly increased RD values in regions of reduced FA, particularly in IFOF and CC but also in smaller parietal and occipital tracts. These results do not support those of Luk et al. (2011). This difference may be due to the fact that the samples in each study were slightly different (see Table 2). In Olsen et al. (2015) study, they also extracted the volume of the WM for the frontal, temporal, parietal and occipital lobes in both hemispheres of their sample (described above). They found that the WM volume in the frontal lobe was significantly higher for their older adult bilingual sample as compared to monolinguals (see Table 2).

Overall, two fasciculi seem to be the focus of neuroplasticity in bilingualism across the different groups of age, the ***CC*** and ***IFOF***. However, while some studies found increased FA values in the CC for older and younger adult bilinguals (Luk et al., 2011; Pliatsikas et al., 2015; Schlegel et al., 2012) others found decreased FA values for older adult bilinguals (Gold et al., 2013) and children (Mohades et al., 2012). And while some studies found increased FA in the IFOF for older adult bilinguals (Luk et al., 2011), younger adults (Pliatsikas et al., 2015) and children (Mohades et al., 2012), others found decreased FA values for older (Gold et al., 2013) and younger (Cummine & Boliek, 2013) adult bilinguals. Since almost all of the WM studies use the same methodological approach (i.e. TBSS) in the analysis of the diffusion-derived measures, this makes them more comparable to each other than the GM studies, and yet they show many inconsistencies and sometimes the results are contradictory. However, there are several confounding factors among the samples, such as the chronological mean age and the AoA of the L2. Consequently, it is possible that these inconsistencies are due to a combination of maturation/degeneration processes and second language acquisition processes. Previous studies have demonstrated that WM declines linearly with age in some local areas, such as CC, internal capsule and prefrontal regions, while other areas remain relatively preserved, such as temporal and posterior regions (Good et al., 2001; Salat et al., 2005). As such, the two focal tracts for bilingualism (CC and IFOF) seem to be particularly vulnerable to age effects. Thus, greater interaction with work on changes in WM during development and aging is required to progress in this area.

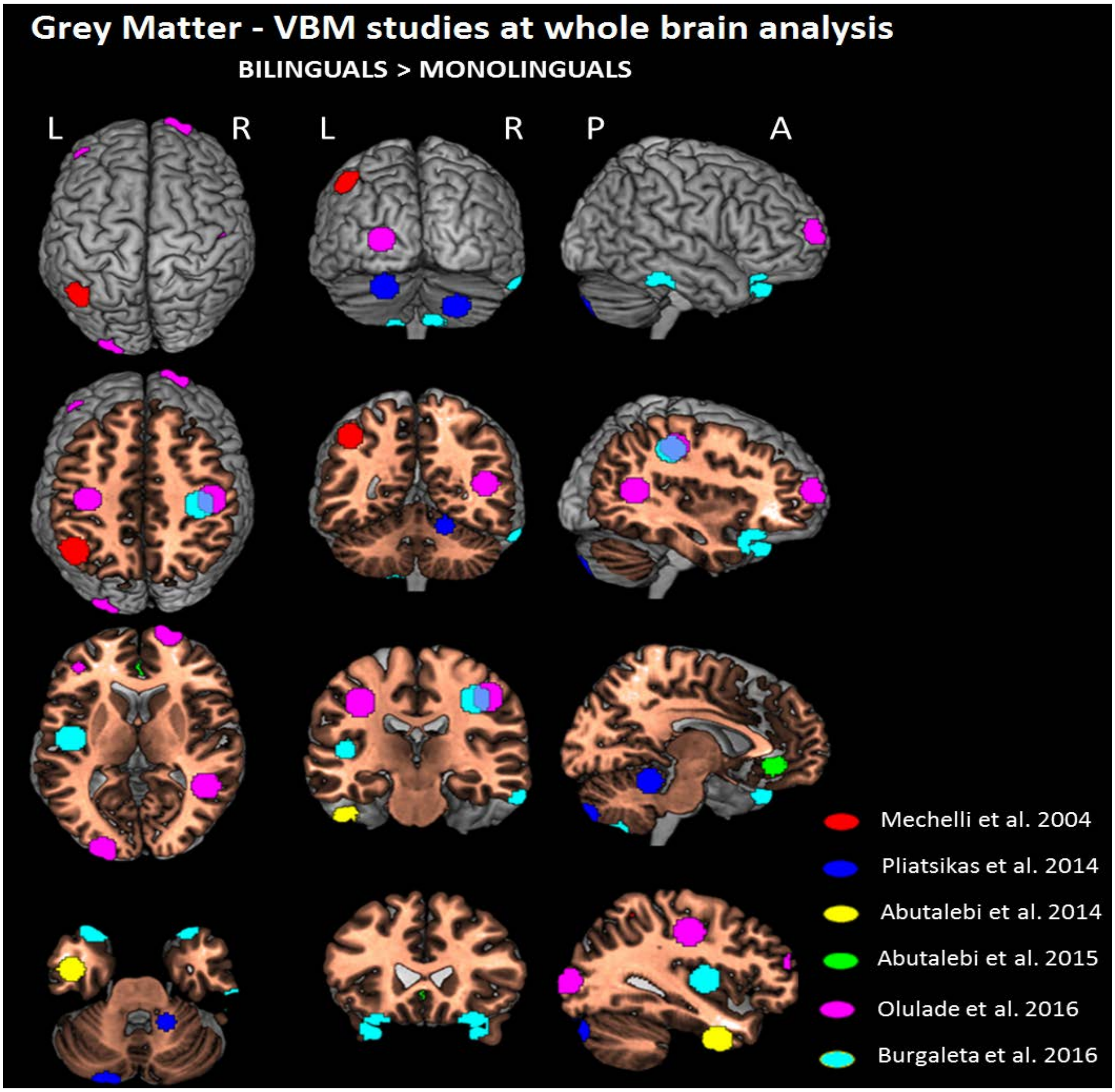

Figure 2. *Cross-sectional studies showing significant differences in GM volume/density in bilinguals as compared to monolinguals. Results from VBM studies at whole-brain analysis. The colours represent the relative location in the brain of the results from different studies.* ***Red****: Mechelli et al. (2004), showing significant GM density increase in left inferior parietal lobule [IPL].* ***Dark-blue****: Pliatsikas et al. (2014), showing increased GM volume in the cerebellum.* ***Yellow****: Abutalebi et al. (2014), showing increased GM volume in left anterior inferior temporal gyrus [aITG].* ***Green****: Abutalebi, Guidi, et al. (2015) showing increased GM volume in right/left anterior cingulate cortex [ACC].* ***Purple****: Olulade et al. (2016) showing increased GM volume in bilateral frontal, temporal, parietal and occipital regions.* ***Light-blue****: Burgaleta et al. (2016) showing increased GM volume in the right inferior temporal gyrus, left Heschl, right IPL and bilaterally in the IFG pars orbitalis and cerebellum. Abbreviations: R (right); L (Left); A (anterior); P (posterior).*

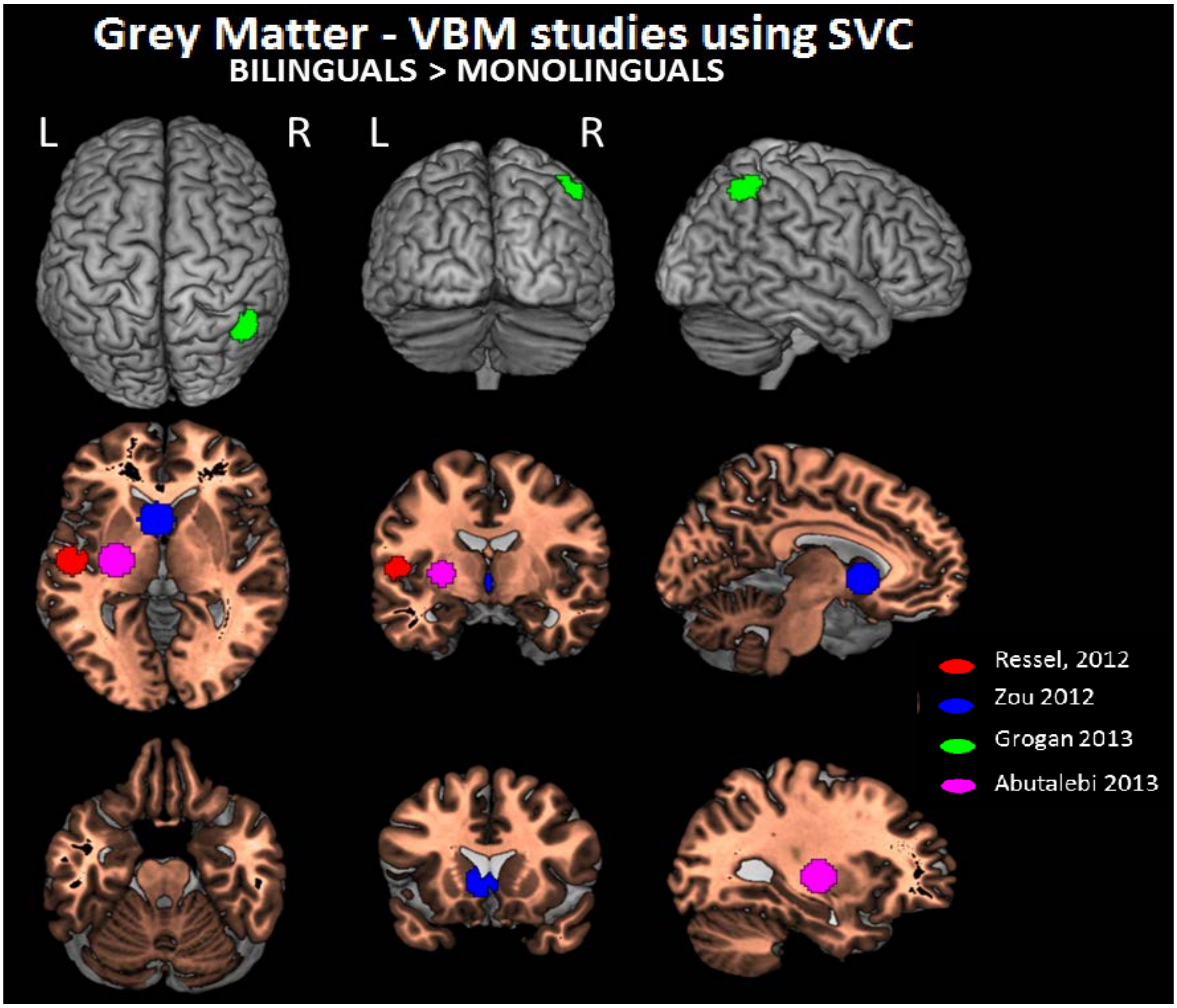

Figure 3. *Cross-sectional studies showing significant differences in GM volume/density in bilinguals as compared to monolinguals. Results from VBM studies using SVC, these studies also showed higher GM volume/density in bilinguals as compared to monolinguals –with the exception of Abutalebi et al. (2013), which compared multilinguals vs. monolinguals, and Zou et al. (2012), which compared bimodal bilinguals vs. monolinguals.* ***Red****: Ressel et al. (2012), showing increased GM volume in left Heschl gyrus.* ***Blue****: Zou et al., (2012) showing increased volume in the left caudate.* ***Green****: Grogan et al. (2012), showing increased GM volume in right IPL, using Mechelli et al.'s (2004) coordinates for the SVC.* ***Purple****: Abutalebi et al. (2013), showing increased GM volume in left putamen. Abbreviations: VBM (voxel-based morphometry); SVC (small volume correction); R (right); L (Left); A (anterior); P (posterior).*

Table 1: *Cross-sectional GM studies related to bilingualism (ages given in years)*

Authors	Sample	Methods	Comparison	Main results
(Mechelli et al., 2004)	25 English-Italian early bilinguals (L2 AoA < 5) 33 English-Italian late bilinguals (10 < L2 AoA < 15) 25 English monolinguals.	VBM analysis (density) Low-resolution method for registration (in SPM versions older than SPM5)	Early bilinguals vs. monolinguals Late bilinguals vs. monolinguals	**Left IPG**: all bilinguals > monolinguals (FWE correction at voxel-level)
(Ressel et al., 2012)	22 Catalan-Spanish bilinguals (L2 AoA < 7; mean age, 23.1). 22 Spanish monolinguals (mean age, 21.5). Matched for gender.	1.5T scanner, voxel size:1 mm^3 VBM & ROI analysis, Modulated images (volume), Standard Unified segmentation (in SPM8), DARTEL for own template creation, High-resolution registration, 8 mm (FWHM), WM+GM as covariate.	Early bilinguals vs. monolinguals	**No significant differences** (FWE correction at voxel-level) **Left Heschl**: bilinguals > monolinguals (SVC of the FWE) **Left/right Heschl**: bilinguals > monolinguals (ROI approach)
(Zou et al., 2012)	14 Chinese-CSL bimodal bilinguals (mean L2 AoA, 19; mean age, 49) 13 Chinese monolinguals (mean age, 48).	3T scanner, voxel size:1.3x1.0x1.3 mm^3 VBM analysis, Modulated images (volume), Optimized VBM5 protocol (SPM5), Low-resolution registration.	bimodal bilinguals vs. monolinguals	**Left Caudate**: bimodal bilinguals > monolinguals (SVC of the FWE)
(Gold et al., 2013)	20 English-variable_L2 bilinguals (L2 AoA < 10; mean age, 63.9) 20 English monolinguals (mean age, 64.4) Matched for gender	3T scanner, voxel size:1 mm^3, VBM analysis, Modulated images (volume), Standard unified segmentation (SPM8), Own template creation, High-resolution registration, 8 mm (FWHM), TIV as covariate.	bilinguals vs. monolinguals	**No significant differences** (FWE correction at voxel-level)
(Grogan et al., 2012)	31 multilinguals (variable_L1, English as L2, variable_L3; mean age, 26.7) 30 bilinguals (variable_L1, English as L2; mean age, 26.7) L2 AoA balanced between groups.	1.5T scanner, voxel size:1 mm^3, VBM analysis, Modulated /unmodulated images (volume/density), Standard unified segmentation (SPM5), Low-resolution registration, 8 mm (FWHM), Age as covariate.	multilinguals vs. bilinguals	**No significant differences** (FWE correction at voxel-level) **Right IPL**: multilinguals > bilinguals (SVC of the FWE) (just in density images)

Table 1. (continued)

Authors	Sample	Methods	Comparison	Main results
(Abutalebi et al., 2013)	14 German-Italian-English multilinguals (L2 AoA < 5; L3 AoA > 10 years) 14 Italian monolinguals Groups matched in age (mean age, 23.5), all females.	3T scanner, voxel size:1 mm^3, VBM analysis, Modulated images (volume), Optimized VBM5 protocol, Low-resolution registration, 4 mm (FWHM), TIV as covariate.	multilinguals vs. monolinguals	**Left putamen**: multilinguals > monolinguals (SVC of the FWE).
(Pliatsikas et al., 2014)	17 Greek-English bilinguals (L2 AoA > 6; mean L2 AoA, 7.7; mean age, 27.5). 22 English monolinguals (mean age, 24.5).	3T scanner, voxel size:1 mm^3, VBM analysis, Modulated images (volume), FSL-VBM protocol, Own template creation, Medium-resolution registration, 3 mm (sigma), Age and gender as covariates.	Late bilinguals vs. monolinguals	**Right/left cerebellum**: bilinguals > monolinguals (TFCE correction).
(Abutalebi et al., 2014)	12 Cantonese-English bilinguals 11 Cantonese-Mandarin bilinguals (mean AoA, 18.87; mean age, 62.17) 23 Italian monolinguals (mean age, 61.9).	3T scanner, voxel size:1 mm^3, VBM & ROI analysis, Modulated images (volume), Optimized VBM8 protocol, Low-resolution registration, East Asian brain ICBM template for bilinguals, European brain ICBM template for monolinguals, DARTEL for registration, High-resolution registration, 8 mm (FWHM) Sex/TIV/education-level/age as covariates.	Late bilinguals vs. monolinguals	**Left aITG**: bilinguals > monolinguals (FDR correction at cluster-level). **Left/right OFC, TmP**: bilinguals > monolinguals (ROI approach).
(Klein et al., 2014)	12 simultaneous bilinguals (L2 AoA < 3; mean age, 23), 25 early bilinguals (4 < L2 AoA < 7; mean age, 26), 29 late bilinguals (8 < L2 AoA < 13; mean age, 28), All French-English bilinguals 22 English monolinguals.	1.5T scanner, voxel size: 1 mm^3, Cortical thickness analysis, Vertex-based approach, CIVET processing pipeline.	Simultaneous bilinguals vs. monolinguals Early bilinguals vs. monolinguals Late bilinguals vs. monolinguals	**Left IFGTr, left IFGOr**: early, late bilinguals > monolinguals **Right IFGOr**: early bilinguals < monolinguals. late bilinguals < monolinguals, simultaneous & early bilinguals. (FDR correction at whole-brain).

Table 1. (continued)

Authors	Sample	Methods	Comparison	Main results
(Abutalebi, Guidi, et al., 2015)	11 Cantonese-English bilinguals 8 Cantonese-Mandarin bilinguals (mean AoA, 12.68; mean age, 61.68) 19 Italian monolinguals (mean age, 60.93).	3T scanner, voxel size:1 mm^3, VBM analysis, Modulated images (volume), Optimized VBM8 protocol, Low-resolution registration, East Asian brain ICBM template for bilinguals, European brain ICBM template for monolinguals, DARTEL for registration, High-resolution registration, 8 mm (FWHM).	Late bilinguals vs. monolinguals	**Left/Right ACC**: bilinguals > monolinguals (FDR correction at cluster-level).
(Abutalebi, Canini, et al., 2015)	16 Cantonese-English bilinguals 14 Cantonese-Mandarin bilinguals (mean AoA, 18.27; mean age, 63.2) 30 Italian monolinguals (mean age, 61.85).	3T scanner, voxel size:1 mm^3, ROI analysis, Modulated images (volume), Optimized VBM8 protocol, Low-resolution registration, East Asian brain ICBM template for bilinguals, European brain ICBM template for monolinguals, DARTEL for registration, High-resolution registration.	Late bilinguals vs. monolinguals	**Left/Right IPL**: bilinguals > monolinguals (ROI approach).
(Olsen et al., 2015)	14 English-variable_L2 bilinguals (L2 AoA < 11) 14 English monolinguals (mean age, 70.6).	3T scanner, voxel size:1 mm^3, ROI analysis, (volume) linear and nonlinear registration (ANT algorithm), Cortical thickness analysis, ROI approach, Freesurfer processing pipeline.	Late bilinguals vs. monolinguals	**No significant differences** (Explore GM volume from temporal, parietal, frontal and occipital lobe). **No significant differences** (Explore CT from entorhinal cortex, hippocampus and temporal pole).

Table 1. (continued)

Authors	Sample	Methods	Comparison	Main results
Olalude et al., 2016)	15 Spanish-English unimodal bilinguals (mean age, 22.3, L2 AoA > 6) 16 SL-English bimodal bilinguals (mean age, 26.4, CODAs: simultaneous bimodal bilinguals) 15 English monolinguals (mean age, 25.9).	3T scanner, voxel size:1 mm^3, VBM analysis, Modulated images (volume), New Segment, DARTEL for registration and group-template creation, High-resolution registration, 10 mm (FWHM), TIV as covariate.	unimodal bilinguals vs. monolinguals, bimodal bilinguals vs. monolinguals	Extensive clusters in **bilateral frontal and parietal regions** unimodal bilinguals > monolinguals **bilaterally cerebellum** monolinguals > unimodal bilinguals **No significant differences** bimodal bilinguals > monolinguals **right precentral and postcentral** monolinguals > bimodal bilinguals (FWE correction at cluster-level).
Burgaleta et al., 2016)	42 simultaneous Catalan-Spanish bilinguals (mean age, 21.6) 46 Spanish monolinguals (mean age, 21.8).	3T scanner, voxel size:1 mm^3, VBM analysis Modulated images (volume), FSL-VBM & FIRST protocol, Own template creation, Medium-resolution registration, 3 mm (sigma), TIV as covariate.	bilinguals vs. monolinguals	**bilaterally IFGOr, cerebellum, left Heschl, right IPL and ITG and basal ganglia** bilinguals > monolinguals monolinguals > unimodal bilinguals **No significant differences** (FWE correction at whole-brain).

Abbreviations: aITG = anterior inferior temporal gyrus; AoA = age of acquisition; FDR = false discovery rate; FWE = family wise error; FWHM = full-width at half-maximum; GM = grey matter; IFGOr = pars orbitalis, inferior frontal gyrus; IFGTr = pars triangularis, inferior frontal gyrus; IPL = inferior parietal lobule; OFC = orbito-frontal cortex; ROI = region of interest; **;** *STG = superior temporal gyrus; SVC = small volume correction; TFCE = threshold free cluster enhancement; TIV = total intracranial volume; TmP = temporal pole; VBM = voxel-based morphometry; WM = white matter; ACC = anterior cingulate cortex; CT = cortical thickness.*

Table 2: *Cross-sectional WM studies related to bilingualism (ages given in years)*

Authors	Sample	Methods	Comparison	Main results
(Mechelli et al., 2004)	25 English-Italian early bilinguals (L2 AoA < 5) 33 English-Italian late bilinguals (10 < L2 AoA < 15) 25 English monolinguals.	VBM analysis Unmodulated images (density).	Early bilinguals vs. monolinguals Late bilinguals vs. monolinguals	**No significant differences** (FWE correction at voxel-level).
(Ressel et al., 2012)	22 Catalan-Spanish bilinguals (L2 AoA < 7, mean age, 23.1). 22 Spanish monolinguals (mean age, 21.5). Matched for gender.	1.5T scanner, voxel size:1 mm^3 VBM & ROI analysis, Modulated images (volume), Standard Unified segmentation (in SPM8), DARTEL for own template creation, High-resolution registration, 8 mm (FWHM) WM+GM as covariate.	Early bilinguals vs. Monolinguals	**No significant differences** (FWE correction at voxel-level).
(Luk et al., 2011)	14 English-variable_L2 bilinguals (L2 AoA < 11yo) 14 English monolinguals (mean age, 70.5).	3T, 30 directions, 5 mm slice thickness, TBSS protocol FA, RD, AD. Sample-specific target image for registration, Medium-resolution registration.	Bilinguals vs. Monolinguals	**FA in CC, SLF, Right IFOF and uncinate:** Bilinguals > Monolinguals **RD in CC:** Monolinguals > Bilinguals.
(Mohades et al., 2012)	15 Dutch-variable_L2 simultaneous bilinguals (L2 AoA < 3, mean age, 9.3) 15 Dutch-variable_L2 sequential bilinguals (L2 AoA > 3, mean age, 9.7) 10 Dutch monolinguals (mean age, 9.6).	3T scanner, 15 directions, voxel resolution: 1.75x1.75x2 mm^3, TOI analysis, FA mean values.	Simultaneous bilinguals vs. Monolinguals Simultaneous vs. Sequential bilinguals Sequential bilinguals vs. Monolinguals	**Mean FA in both IFOF:** Simultaneous > Sequential bilinguals > Monolinguals **Mean FA in anterior CC to orbitofrontal lobe tracts:** Monolinguals > Simultaneous bilinguals (Bonferroni correction).
(Gold et al., 2013)	20 English-variable_L2 (L2 AoA < 10; mean age, 63.9) 20 English monolinguals (mean age, 64.4) Matched for gender.	3T scanner, 36 directions, voxel resolution: 1.75x1.5x3 mm^3, TBSS protocol FA, RD, AD, MD 5000 permutations.	Bilinguals vs. Monolinguals	**FA in both ILF/IFOF, fornix, CC** Monolinguals > Bilinguals **RD in IFOF, CC:** Bilinguals > Monolinguals (TFCE correction).

Table 2. (continued)

Authors	Sample	Methods	Comparison	Main results
(Cummine & Boliek, 2013)	13 Chinese-English bilinguals (L2 AoA > 5, mean age, 24.2) 11 English monolinguals (mean age, 28.5).	1.5T, 12 directions, 4 mm slice thickness, TBSS protocol FA, MD.	Bilinguals vs. Monolinguals	**Right IFOF & Anterior Thalamic Radiation *(Right superior portion & inferior portion bilaterally):*** Monolinguals > Bilinguals.
(Pliatsikas et al., 2015)	20 variable_L1-English (L2 AoA < 10.15; mean age, 31.85; mean immersion, 91 months) 20 English monolinguals (mean age, 28.16).	3T, 30 directions, 2 mm slice thickness, TBSS protocol FA.	Sequential late learners vs. monolinguals	**Bilaterally CC, IFOF, Uncinate and SLF:** Bilinguals > monolinguals.
(Olsen et al., 2015)	14 English-variable_L2 bilinguals (L2 AoA < 11) 14 English monolinguals (mean age, 70.6) (Same sample as Luk et al., 2011).	3T scanner, voxel size:1 mm^3, ROI analysis, (volume) linear and nonlinear registration (ANT algorithm).	Late bilinguals vs. monolinguals	**Mean volume in frontal lobe**: bilinguals > monolinguals (ROI approach).
(Mohades et al., 2015)	14 Dutch- variable_L2 simultaneous bilinguals (L2 AoA < 3, mean age, 11.4) 16 Dutch-variable L2 sequential bilinguals (L2 AoA > 3, mean age, 11.33) 10 Dutch monolinguals (mean age, 11.8) (Same sample as Mohades et al., 2011, but 2 years later).	3T scanner, 15 directions, voxel resolution: 1.75x1.75x2 mm^3, TOI analysis, FA mean values.	Simultaneous bilinguals vs. Monolinguals Simultaneous vs. Sequential bilinguals Sequential bilinguals vs. Monolinguals	**Mean FA in left IFOF:** Simultaneous > Sequential bilinguals > Monolinguals (TOI approach, Bonferroni correction).

Abbreviations: AD = axial diffusivity; AoA = age of acquisition; CC = corpus callosum; FA = fractional anisotropy; FWE = family wise error; FWHM = full-width at half-maximum; GM = grey matter; IFOF = inferior frontal-occipital fasciculus; ILF = inferior longitudinal fascicule; MD = mean diffusivity; RD = radial diffusivity; ROI = region of interest; SLF = superior longitudinal fasciculi; TBSS = tract-based spatial statistic; TFCE = threshold free cluster enhancement; TOI = tract of interest; VBM = voxel-based morphometry; WM = white matter.

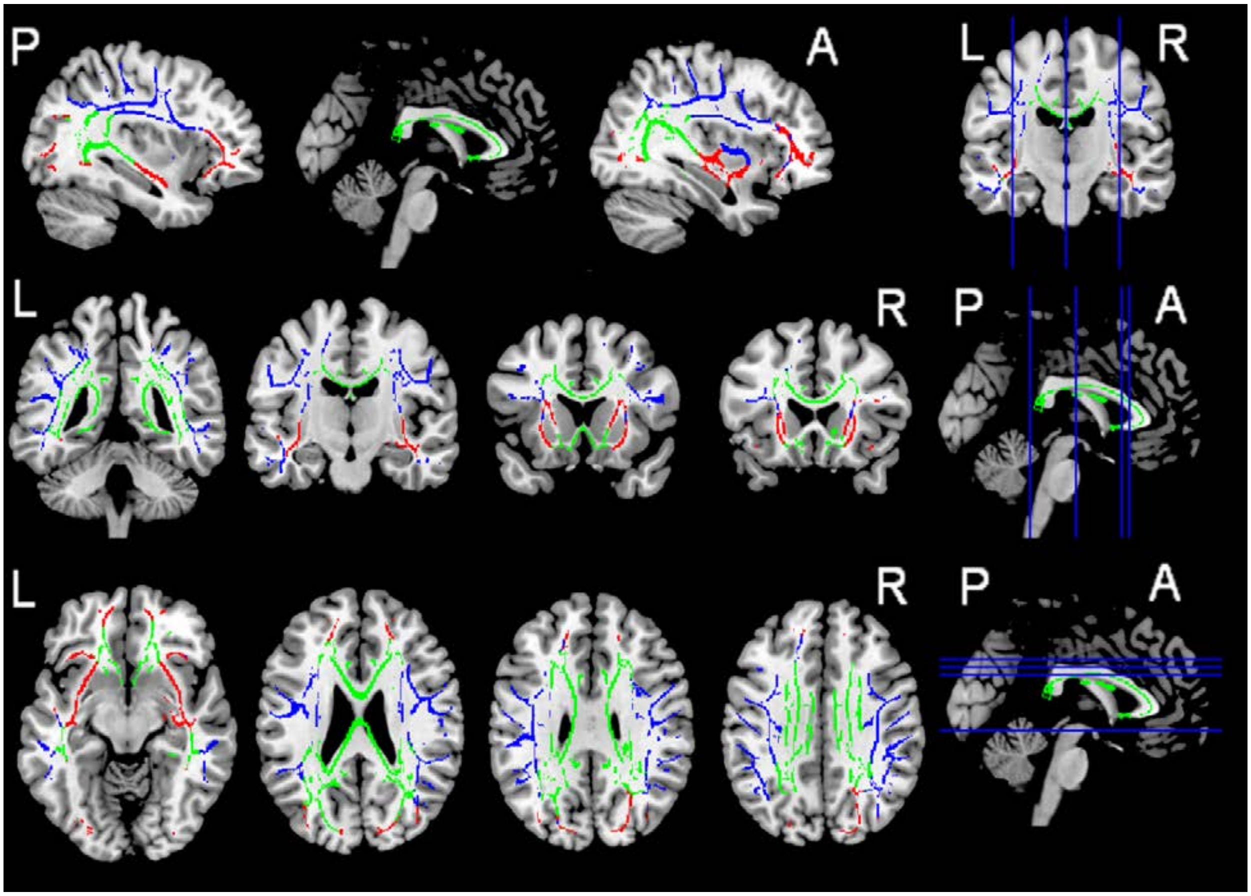

***Figure 4**. Regions showing significant differences in FA values between bilinguals and monolinguals from TBSS studies. **Green**: corpus callosum [CC] (Luk et al., 2011; Gold et al., 2013; Pliatsikas et al., 2015). **Red:** inferior frontal-occipital fasciculus [IFOF] (Luk et al., 2011; Cummine & Boliek, 2013; Gold et al., 2013; Pliatsikas et al., 2015). **Blue**: superior longitudinal fasciculi [SLF] (Luk et al., 2011; Pliatsikas et al., 2015). Abbreviations: R (right); L (Left); A (anterior); P (posterior).*

2.4 BRAIN NETWORK STUDIES IN BILINGUALISM

Studies of brain networks examine the relationship and interaction between brain regions to provide a more complete information about the organization and configuration of these regions and the brain as a whole. Potentially, this might offer a better understanding of the possible mechanisms underlying the cognitive processes associated with learning and using a second language. Although it is important to understand brain architecture and functional integration per se, the search for neural underpinnings of any aspect of basic neuroscience must be done in the wider context of its applicability. In this sense brain mapping techniques that capture the structure-function relationships of complex distributed cognitive processes can have important implications, mostly in clinical settings, education and healthy aging, which are crucial social concerns. Importantly, studying brain connectivity could reveal important individual variations (Sporns, 2011). For example, could shed light on why two patients with the same lesion could have such different symptoms and others with different injuries have the same symptoms. Regions do not operate in isolation, hence connectivity is an important aspect of brain functioning. Only recently has interest been directed towards a distributed and integrated approach in bilingualism (see Li & Grant, 2016 for a review). The next two sections describe the few studies that have investigated structural and functional relationships between GM regions in order to provide a network perspective instead of separated regions in bilingualism.

2.4.1 Structural connectivity

Modifications in the axonal connectivity of the whole brain associated with bilingualism are not well understood. In particular, the relationship between bilingualism and the topological properties of the brain anatomical (structural) network are received less attention. There are many studies modelling the human brain as a complex network (Li et al., 2009; Basset et al., 2009; Iturria-Medina et al., 2010; Zalesky et al., 2011). From this perspective the brain is modelled as a graph whose nodes (structural/functional brain regions) are interconnected by edges (structural/functional connections) (Bullmore and Bassett, 2011). Therefore, measures of network integration that characterize the ability to rapidly combine specialized information from distributed regions by estimating the ease with which the regions communicate can be obtained. They are commonly based on the concept of path, which is the sequence of distinct nodes and links, representing the network's basic organization. One common measure of integration are the 'global efficiency' of the graph brain network. But also it is possible to obtain measures of specialization that describe the clustering hierarchy properties of all regions in the networks, identifying hub regions or cluster of regions particularly highly connected to other parts of the networks. 'Local efficiency' is one of the measures that characterize clustering properties of the graph brain network. The topological properties of the brain graph are important for its performance and, conversely, functional performance can impact these topological properties (Sporns et al., 2000). The neural basis of bilingualism provides a unique window into the ability of our brains to both integrate and segregate information dynamically within common neural structures.

With the aid of complex network analysis and network-based statistics, García-Pentón et al. (2014) examined the impact of bilingualism on the large-scale structural brain network. This study investigated WM structural brain connectivity differences between 13 early Basque–Spanish bilinguals (mean age, 24.08; L2 AoA before 3 years old) and 13 Spanish monolinguals (mean age, 29.07). They performed an anatomical connectivity and complex network analyses based on DW-MRI. The connection density between 90x90 pairs of GM regions was estimated from a tractography algorithm. A network-based statistic (Zalesky, Fornito, & Bullmore, 2010a) and graph-theory approaches were employed to identify differences in connectivity patterns and properties of the networks between both groups. The authors identified two different anatomical subnetworks that were more strongly connected and graph-efficient in early bilinguals as compared to monolinguals. Sub-network I contained left frontal and parieto-temporal brain regions, most of them previously described in the literature as language-relevant: Insula—STG—IFGTr—SMG—IFGOp—Medial Superior Frontal Gyrus. Sub-network II also included some brain regions that have also been extensively related to language processing (i.e. left angular gyrus (AnG) and left superior TmP) while the others have been implicated in other cognitive processes related to language: left Superior Occipital gyrus—right Superior Frontal Gyrus—left SPL—left superior TmP—left AnG. This investigation revealed that early bilingualism modifies the structural configuration of the WM in relevant brain subnetworks. Specifically, bilingualism facilitates the emergence of efficient, highly interconnected neural subnetworks in response to the burden of processing two languages. Importantly though, this outcome also decreases the efficiency of the larger brain network as a whole. Thus, the optimization of circumscribed subnetworks impacts negatively the general configuration of the overall network hosting these specific subnetworks. From an information processing perspective these findings provide a striking example of coping with increasing computational and representational demands under the pressure of limited neural resources.

Until now, this is the only study investigating structural connectivity in bilingualism. However, most bilingualism neuroimaging studies toward brain networks perspective have been focused on functional connectivity, as describe the next section.

2.4.2 Functional connectivity

Task-related functional connectivity studies particularly under the brain networks perspective investigating neural changes related to training on non-native sounds (Sheppard et al, 2012; Veroude et al., 2010; Ventura-Campos et al., 2013) and artificial grammars (Yang & Li, 2012) have growing fast during the last years. An extensive review of this literature is beyond the scope of this thesis introduction, but see Li & Grant (2016) for details. In general, those studies are suggesting that functional brain networks could predict successful L2 learning (see also Yang et al., 2015 for a naturalistic language training). However, functional connectivity studies are still scarce on bilingualism research. Particularly, large-scale functional network have not been investigated. Contrary to task-based fMRI, which typically emphasizes a single brain network associated with a particular task, resting state fMRI allows researchers to investigate connectivity at the whole brain level, which allows us to associate any possible spatially distributed connectivity pattern to bilingualism.

The seminal study about resting state activity by Biswal et al. (1995) showed how different regions of the sensorimotor system fluctuated rhythmically and in a synchronized manner during rest. Later, was described for the first time a brain network that displayed increased activity during rest and decreased activity when the subject is involved in a cognitive task. This network was suggested to play an important role as a baseline of the brain, thus being called: the default mode network (DMN, Raichle et al., 2001). In addition to the sensorimotor and DMN, many other brain networks that are involved in vision, hearing, and memory have been also observed at rest (Damoiseaux et al., 2006). These networks that are not externally driven by any stimulus or task are also highly dynamic and interactive, and can be seen modified under many conditions.

The first study about resting-state fMRI in bilingualism was performed by Luk et al. (2011). They combined WM results from the TBSS analysis (see section 2.3.4) with resting-state functional connectivity measures. They performed a resting-state functional connectivity analysis taking as seeds the regions of GM adjacent to the cluster showing higher FA values for bilinguals in the prior TBSS analysis and which they considered important for language switching (i.e. right/left IFG). This analysis showed stronger functional connectivity between left IFG and posterior brain regions (i.e. with MTG, right IPL, precuneus, middle occipital gyri and left caudate) for bilinguals as compared to monolinguals. In contrast, monolinguals showed a different connectivity pattern, showing higher connectivity between left IFG and other frontal regions.

Grady et al. (2015), performed a resting-state and task-based fMRI connectivity study in 14 old monolinguals English speaker (mean age, 70.6) and 14 lifelong bilinguals (mean age, 70.3), who speak English and another alphabetic language since before 11 years old. They investigated functional connectivity in two brain networks that are typically engaged in EF: the fronto parietal control (FPC) network and the salience network (SLN), but also study the DMN. They found stronger intrinsic functional connectivity in the FPC network and DMN in bilinguals than in monolinguals. Although they did not find group differences in activations for the tasks-related fMRI paradigm, bilinguals showed stronger correlations than monolinguals between the intrinsic connectivity in the FPC network and the task-related increases of activity in prefrontal and parietal regions. Consequently, they also suggested greater anterior-posterior functional connectivity in bilinguals.

Li et al. (2015) computed the resting state functional connectivity between two language control regions: dorsal ACC and left caudal nucleus and crucial language processing regions: precentral gyrus, STG, Rolandic operculum and MTG, bilaterally. In this study, 14 mandarin-CSL bimodal bilinguals (mean age, 49.5) were compared with 15 monolinguals (mean age, 43.54). They found decreased functional connectivity between the dorsal ACC and the left STG and left rolandic operculum for bimodal bilinguals.

Finally, Berken et al. (2016) studied the resting-state fMRI signal comparing 16 French-English simultaneous (mean age, 23.3) and 18 sequential bilinguals (mean age 25.7, L2 AoA > 5 years). Similarly, they focused on the IFG and found greater functional connectivity between the left and right IFG and between IFG and dorsolateral prefrontal cortex, IPL and cerebellum. They also found that the earlier the L2 was acquired, the stronger the functional connectivity between the left IFG and the right IFG and IPL. In addition, they found that the increased resting-state functional connectivity was correlated with decreased neuronal activity in the left IFG during a speech production fMRI paradigm.

Very recently, it was suggested that resting state functional connectivity could also predict the acquisition of the L2 in adults (Chai et al. 2016). Chai et al. collected rfMRI data from 15 English speakers immediately before 12 weeks of intensive French immersion training course and performed a brain connectivity analysis focused on two seed regions related to language processing: the left frontal operculum (FO) and the visual word form area (VWFA), obtaining maps of functional connectivity between these regions and all the voxels in the brain (seed-voxel connectivity maps). Then, a correlation analysis was performed between the functional connectivity maps and behavioural scores obtained from two language tasks performed to evaluate the L2 learning: lexical retrieval and reading speed scores. This study showed that the connectivity between left-FO-and-left-posterior-STG and between left-FO-and-ACC positively correlated with lexical retrieval scores and connectivity between VWFA-and-left-mid-STG correlated with the reading speed scores.

In general, both functional and structural connectivity studies consistently identified or focus in the ***IFG***, a region related to cognitive/language control, and showed how this region is differently related to a more extended set of regions in bilinguals and monolinguals. However, the brain network approach is particularly useful for studying large-scale structural and functional connectivity plasticity associated with any cognitive functions (Guye, Bartolomei & Ranjeva, 2008), such as language and executive functioning, a topic that still largely unexplored on bilingualism. Until today, there is only one study investigating brain networks as a whole in bilingualism (García-Pentón et al., 2014). The search for differences between bilinguals and monolinguals cannot be limited to locating differences in specific structures and/or in the patterns of connectivity of these structures. However, these new studies put us on the right way: if there are bilingualism effects, they may be evident not just as a change in the volume of one or more regions, but also as a change in the connections between the different regions of a circuit.

2.5 EFFECTS OF AGE OF ACQUISITION AND PROFICIENCY IN L2

Although this is not the main focus of this dissertation it is important to take into account how structural changes related to bilingualism evolve across time. For that reason it is also necessary to discuss correlational studies between AoA and L2 proficiency with brain structural measures. Firstly, considering the brain as a non-linear dynamic system and (individual) bilingualism as a dynamic process (Hernandez, 2013), it is relevant to consider both the point at which bilingualism begins to influence the system (i.e. AoA) and how the effect of bilingualism cumulatively interacts with the system (i.e. L2 proficiency and AoA). Secondly, adaptive models of the neural underpinnings of bilingualism (e.g. Green & Abutalebi, 2013; Abutalebi & Green, 2007) take into account the dynamic development of the relevant brain networks over time. Therefore, this section looks at the relationship between second language experience and brain structure.

Some of the studies mentioned above have also investigated the effect of AoA and L2 proficiency on the brain. The study by Mechelli et al. (2004) used SVC around the region where they obtained the group effect – the left IPL – and obtained a negative correlation between GM density and L2 AoA, which means that GM density increased as the L2 AoA decreased. Additionally, they obtained a positive correlation between the GM density and L2 proficiency, which means that GM density increased as proficiency increased. Grogan et al. (2012) also showed a positive correlation between GM volume and L2 proficiency in the pars opercularis (IFGOp) of the left IFG.

Similarly, Hosoda et al. (2013) found the same positive correlation in the IFGOp and also in FA values of WM tracts beneath the right IFGOp and inside the right ILF and Arcuate fasciculus, two tracts that typically connect language regions. They studied 137 Japanese-English bilinguals who started to learn the L2 after the age of 7 (mean age, 11). In addition, they performed a tractography analysis and also found a positive correlation between L2 proficiency and connectivity in the right pathways connecting IFGOp to caudate, and IFGOp to superior temporal gyrus (STG)/supramarginal gyrus (SMG).

Using a ROI analysis, Abutalebi et al. (2013) showed that the GM volume in the left putamen increased as proficiency in the third language increased, and they reported this effect solely for the third language because no correlation effects were obtained for either the native language or the L2. In a later study also using a ROI approach, Abutalebi et al. (2014) found a positive correlation between GM volume in the left TmP and proficiency in L2 in a group of multilingual subjects. Additionally, the Abutalebi, Canini et al. (2015) study found no significant correlation between the L2 AoA and the IPL GM volume, but did reveal a positive correlation between the L2 naming performance and the GM volume in the left IPL, and between the L2 exposure time and the GM volume in the right IPL.

On the other hand, Klein et al. (2014) showed that CT correlates positively with L2 AoA in the left IFG and the left superior parietal lobule (SPL) for bilinguals, and negatively in the right IFG. It is important to note that CT is a different measure and has a different interpretation to that of GM volume. Differences in local GM volume can arise from differences in CT and variation in surface area due to the folding pattern (Kanai & Rees, 2011). However, GM volume is more correlated with surface area and much less correlated with CT (Winkler et al., 2010). There are studies (Chung, Dalton, Shen, Evans, & Davidson, 2007) showing a negative correlation between CT and GM density. Thus, although CT results are difficult to interpret, it seems that Klein et al.'s correlation results (Klein et al., 2014) between CT and L2 AoA are in line with correlation results in GM volume/density and ultimately are also consistent with what would be expected for the IFG and parietal regions (Kanai & Rees, 2011; Winkler et al., 2010). In addition, Mårtensson et al. (2012) longitudinal study showed that the CT in left STG and volume in right hippocampus correlated positively with proficiency in the L2.

A longitudinal study by Stein et al. (2012) described GM changes based on a correlation analysis with behavioural measures. Specifically, they studied GM density changes in a group of 10 English monolinguals after a brief immersion in an L2 (5 months of learning German in Switzerland), showing a positive correlation between the increase of L2 proficiency and the increase of the density in the left IFG and also in the anterior temporal lobe (aTL), using cluster-level correction of the FWE. Notice that this is the only VBM longitudinal study of L2 learning within an immersion context.

In conclusion, the most consistent effect regarding AoA and L2 proficiency in the brain is in the GM of the ***IFG***, and the WM connecting the ***IFG*** with other GM regions, such as caudate, STG and SMG/IPL. These results suggest that mastery of the L2 (i.e. increased

L2 proficiency and L2 experience) is associated with higher GM volume, higher WM connections and less CT in regions related to EFs and language control, specifically the IFG. Abutalebi and Green's model (Abutalebi & Green, 2007; Green & Abutalebi, 2013) predicts that the degree of involvement or activation of these regions changes as a function of L2 proficiency. Bringing together the predictions of the model and the findings for structural changes in the brain described here requires a link between function and structure, particularly of how 'involvement' and 'activation' spell out in terms of structure. Very tentatively, if (as Abutelebi and Green predict) greater L2 proficiency involves more automatic processing of the language, greater ability in control and thus less activation of the associated regions in the control network, these results suggest that these changes are associated with greater GM volumes and WM connectivity in these regions. However, this is just speculation and only further testing can shed more light on the issue.

2.6 SUMMARY AND METHODOLOGICAL CONCERNS IN BILINGUALISM STUDIES

To summarize, a total of nine cross-sectional studies performing VBM looked for GM differences between bilinguals and monolinguals across the whole brain. Three of them (3/9) were cross-sectional studies that reported no significant brain differences (Gold et al., 2013; Grogan et al., 2012; Ressel et al., 2012). In contrast, three other studies using different techniques/measures and experimental designs consistently reported GM changes in the IFG: Klein et al. (2014) obtained increased cortical thickness for bilinguals in the left IFG but decreased cortical thickness in the right; Mårtensson et al. (2012) also found increased CT in the left IFG, and Hosoda et al. (2013) obtained increased GM volume in the right IFG. These last two studies looked at intensive L2 learning experiences longitudinally. Interestingly, there are two very recent studies (Olulade et al., 2016; Burgaleta et al., 2016) showing also increased GM volume in the IFG. Finally, five cross-sectional studies (6/9) performing VBM at the whole brain level found increased density/volume in different regions: the left or right IPL (Mechelli et al., 2004; Burgaleta et al., 2016; Olulade et al., 2016), cerebellum (Pliatsikas et al., 2014; Burgaleta et al., 2016; Olulade et al., 2016), left aITG (Abutalebi et al., 2014) and ACC (Abutalebi, Guidi et al., 2015). Each of the studies used different methods for the pre-processing and analysis of the data (see table 1). Importantly, with respect to the effect in the cerebellum, while some studies found increased GM volume for bilinguals (Pliatsikas et al., 2014; Burgaleta et al., 2016) other found decreased volume (Olalude et al., 2016).

Eight studies performed ROI analysis, extracting mean volumes from the regions or reducing the analysis to the scope of a volume of interest. Two studies showed increased GM in the right IPL (Grogan et al., 2012; Abutalebi et al., 2015), and the rest showed isolated results.

Considering WM, four cross-sectional studies looked for differences between bilinguals and monolinguals across the whole brain using TBSS. The most consistent WM changes were observed in CC and IFOF. However, while two studies found increased FA values in CC for bilinguals (Luk et al., 2011; Pliatsikas et al., 2015), another found decreased FA values (Gold et al., 2013). And some studies found increased FA values in IFOF for bilinguals (Luk et al., 2011; Pliatsikas et al., 2015); others found decreased FA values (Cummine & Boliek, 2013; Gold et al., 2013). On the other hand, two other studies performing VBM reported no significant differences in WM volume (Mechelli et al., 2004; Ressel et al., 2012). However, these last two studies used a completely different measurement and methodology to those of the former studies. There is one more cross-sectional study showing decreased and increased FA values in the CC and IFOF, respectively, for bilinguals but using TOI approach (Mohades et al., 2012). In line with results on cross-sectional studies, there are also three longitudinal studies: one showing increased FA in CC for learners vs. non-learners (Schlegel et al., 2012), another one showing increased FA of the white matter inside the right IFG (Hosoda et al., 2013) and one more showing increased FA in the IFOF for bilingual (Mohades et al., 2015, the same children investigated in Mohades et al., 2012).

Regarding brain network approach, there are three functional (Luk et al., 2011; Grady et al., 2015; Li et al., 2015) and one structural (García-Pentón et al., 2014) connectivity studies that compared bilinguals and monolinguals. Luk et al. (2011) and Grady et al. (2015) showed greater anterior-posterior functional connectivity in bilinguals, especially between the IFG and posterior brain regions in the temporal, parietal and occipital gyri. García-Pentón et al. (2014) revealed that early bilinguals showed a different axonal structural configuration of the brain, developing more highly interconnected and efficient subnetworks to achieve the processing of the two languages, and that these changes seem to be at the expense of decreased graph-efficiency for the whole brain network. This is in line with previous accounts broadly showing that bilinguals are less accurate and slower than monolinguals of each language in linguistic tasks (e.g. picture naming, word recognition, lexical decision) (Gollan et al., 2011; Martin et al., 2012), under the supposition that the over-developed structural subnetworks allow bilinguals to deal with two languages but do not improve linguistic skills per se in each language. Furthermore, Luk et al. (2011) showed stronger functional connectivity between the left IFG and other frontal regions in monolinguals and this pattern could be important in supporting better performance in linguistic tasks as compared to bilinguals. On the other hand, the fact that regions and subnetworks important in executive control mechanisms (i.e. IFG, DNM and FPC network) are involved (García-Pentón et al., 2014; Luk et al., 2011; Grady et al., 2015) is in line with Abutalebi and Green's model. Be that as it may, large-scale functional/structural connectivity studies are still needed to complement previous findings.

Assessing these findings in the light of Abutalebi and Green's Adaptive Control Hypothesis (Abutalebi & Green, 2007; Green & Abutalebi, 2013), there is just one region predicted by the model that consistently shows up across studies as a structural difference due to bilingualism: the left/right ***IFG*** (Hosoda et al, 2013; Klein et al., 2014; Grogan et al, 2012; Luk et al., 2011; García-Pentón et al., 2014; Olalude et al., 2016; Burgaleta et al., 2016). Some of the studies used alternatives to the traditional methods of VBM and ROI-based analysis and analysed the whole brain to reveal effects of bilingualism in the IFG (Klein et al., 2014) and in the connections between the IFG and other regions (Luk et al., 2011; García-Pentón et al., 2014; see also Grady et al., 2015; Berken et al., 2016). Furthermore, others studies support these results showing that this region is also sensitive to L2 AoA and proficiency (Grogan et al., 2012; Hosoda et al., 2013; Klein et al., 2014). Additionally, several WM structural studies have also confirmed differences between bilinguals and monolinguals in the tracts connecting IFG with many other regions in the frontal lobe (including the contralateral side) and the temporal, parietal and occipital regions in the back of the brain, specifically the CC (Luk et al., 2011; Pliatsikas et al., 2015; Gold et al., 2013; Mohades et al., 2012) and the IFOF (Luk et al., 2011; Pliatsikas et al., 2015;

Mohades et al., 2012, 2015; Gold et al., 2013; Cummine & Boliek, 2013). Nevertheless, although these results identified the same WM regions, they are contradictory because some show increases while others show decreases FA values of the WM. As stated above, chronological age and age of acquisition of the second language are clearly confounding factors between these studies.

In the same vein, some other regions predicted by the model have also appeared. This is the case of the IPL, which was initially demonstrated by Mechelli et al. (2004) and then replicated by Grogan et al. (2012) and Abutalebi, Canini et al. (2015) but using a different methodology (namely, ROI-based rather than a whole-brain approach). However, more recently have been replicated by Burgaleta et al. (2016) and Olulade et al. (2016) using whole-brain approach. It is also the case of the ACC (Abutalebi, Guidi et al., 2015) but replication to support this finding is lacking. Additionally, some regions have emerged that are not predicted by the model: the aITG (Abutalebi et al., 2014) and cerebellum (Pliatsikas et al., 2014; Burgaleta et al., 2016; Olulade et al., 2016). Although the Adaptive Control Hypothesis could possibly account for the cerebellum effects in the context of dense code-switching, the sample in the Pliatsikas et al. (2014) study did not come from this sort of environment: the bilinguals in the study were in a relatively monolingual immersion setting, and more critically, their mean L2 AoA was 7.7 years old.

Although current neuronal models of bilingualism, such as Abutalebi and Green's Adaptive Control Hypothesis, are logical and consistent with task-based fMRI data, the current structural and connectivity evidence does not provide complete support for the models' postulates. While the results offer limited support for some aspects of the Adaptive Control Hypothesis, taken as a whole they suggest that the model is incomplete and requires adjusting for those regions that cannot be accounted for or are altogether unexpected under its present formulation.

Critically, the current experimental evidence for plasticity changes in the brain due to bilingual experience is relatively weak. Neuroimaging studies in this field are still very small in number and far from being consistent enough. With the evidence currently available, it is possible to be confident about consistent and reproducible structural changes related to bilingualism in only a few regions, such as IFG and its connections with other areas. The remaining findings provide an unclear picture that makes it difficult to arrive at generalizations or to confirm or refute current models. Therefore, the debate over the bilingual advantage does not seem to have become clearer with current neuroimaging data. To a great extent much of this lack of conclusive evidence is due to methodological differences among the studies and these inconsistencies will be identified below. Against this backdrop, the Adaptive Control Hypothesis is a good candidate for a working model in which the integration of structural, functional and behavioural evidence would allow us to confirm and/or fine-tune how the brain is shaped by bilingualism. For that to happen, new studies are required in order to accumulate more stable data.

Various methodological issues have already been touched upon in the previous sections. One of the major concerns is about the different approaches used for the pre-processing and analysis of data, which can give rise to different results (Ashburner & Friston, 2011) and thus could contribute toward explaining the inconsistencies in the field of bilingualism. Tables 1-2 summarize the pre-processing and analysis of the studies included in this introduction. Notice that the greatest variation exists in GM studies, particularly in segmentation and registration procedures. Some of the studies used the unified segmentation approach (Ashburner & Friston, 2005), others used the improved unified segmentation approach implemented in the New Segment toolbox for SPM8 or even older segmentation algorithms (Ashburner & Friston, 2000), and yet others used the VBM5 protocol that does not use prior tissue information for the segmentation step (Good et al., 2001). There is also other studies using the segmentation approach implemented in the FSL software (Zhang, Brady, & Smith, 2001), which relies on different algorithms. The registration step also depends on the software used for the processing of the images (see Table 1): old versions of SPM use a low spatial resolution method for the non-linear registration; FSL uses a medium spatial resolution method; and SPM8 uses a high spatial resolution registration method. Each of these methods can produce different results (Radua, Canales-Rodriguez, Pomarol-Clotet, & Salvador, 2014). The size of the filter used to smooth the images also affects results and can be an important source of variability (Jones, Symms, Cercignani, & Howard, 2005; Salmond et al., 2002). In this case, no variability was observed across studies, with the single exception of the Abutalebi et al. (2013) study, which used an isotropic Gaussian kernel of 4 mm of FWHM, compared to the other studies, which used 8 mm (FWHM) or sigma of 3 mm (approximately equivalent to 8 mm FWHM). Additionally, although almost all of these studies used volume (modulated images) as the GM measure, a few studies used density (unmodulated images) as the GM measure (see Appendix 1). These different choices can give rise to different results and require different interpretations (Radua et al., 2014). Also, although some studies corrected images for brain size using the total intracranial volume (TIV), WM+GM raw volumes or age, others followed different statistical procedures or did not correct for brain size at all. Additionally, some studies used their own group template for the registration to the standard space, using DARTEL (Ashburner, 2007), FSL or other methods, while the rest of the studies used standard templates for registration. The former usually improves the registration in the group. These methodological choices need to be considered when interpreting the results and accounting for their variability.

In contrast, there is no such heterogeneity of methods in WM studies because – with the exception of only two studies that used a TOI approach, which suffers from the same shortcomings as the ROI approach (Furutani, Harada, Minato, Morita, & Nishitani, 2005; Kanaan et al., 2006; Snook, Plewes, & Beaulieu, 2007; Tapp et al., 2006) – the remaining studies follow a standardized method implemented in FSL to perform the TBSS approach on diffusion-derived measures. However, it is important take into account that the use of data acquired from different scanners (1.5T or 3.0T) and using different parameters for the acquisition of the images (see tables 1 and 2 for details of the different studies reviewed here) can produce important differences in the quality/noise of the images across studies and also influence variability in the results.

Finally, the most important difference between studies is the approach used for the statistical analysis of the data: ROI vs. VBM analysis, each of which answers different questions. The former looks for differences between groups at ROI-level and the latter looks for differences across the whole brain (i.e. voxel-level, peak-level, cluster-level inferences). Advantages and disadvantages of both procedures have been mentioned throughout this review, and there has been much debate about the use of voxel-based or ROI-based approaches (Good et al., 2001). Various studies have compared both methods (Furutani et al., 2005; Giuliani, Calhoun, Pearlson, Francis, & Buchanan, 2005; Kanaan et al., 2006; Kubicki et al., 2002; Snook et al., 2007; Suzuki et al., 2005; Tapp et al., 2006; Testa et al., 2004), finding, on the whole, similar results for both methods but some advantages for voxel-based over ROI-based analysis. Although VBM can overlook small differences (Saxe, Moran, Scholz, & Gabrieli, 2006), the ROI approach limits the chances of coming up with new, unexpected findings (Friston & Henson, 2006) making it difficult to expand and generalize the body of knowledge. Ultimately, the two techniques are complementary and cannot be used separately (Snook et al., 2007). This is important because even when there is a prior hypothesis about particular regions, it is useful to obtain information about the whole brain, since regions are unlikely to be working in isolation, making it crucial to perform a whole brain analysis in the first place. Additionally, even though the ROI-based analysis increases the sensitivity of the test with respect to the whole-brain analysis thanks to a reduction in the amount of testing and consequently in the problem of multiple comparisons, ROIs face other problems due to the effect of averaging discussed in section 2.2.1.

The VBM approach also has its weaknesses. The fact that different ways of performing VBM analysis can lead to disparate results is a huge problem for the integration of different studies. Different pre-processing steps applied to the images, such as the choice of segmentation or registration algorithms, or even the decision to modulate images (or not) after registration, can also lead to different results (Ridgway et al., 2008). However, ROI analysis holds no advantage over VBM in this respect because the definition of the regions by hand also introduces errors into the process. Usually, good segmentations are produced by well-trained and highly experienced research staff, and the difficulty lies in finding individuals with this kind of expertise. Furthermore, almost all the ROI studies reviewed here suffer from the same problem of image pre-processing as the VBM studies because they performed automatic extraction of the ROIs or they also normalized the images before the delineation of the ROIs by hand.

As far as data analysis is concerned, in order to make studies replicable and more generalizable, certain standards are needed (see Borgwardt, Radua, Mechelli, & Fusar-Poli, 2012; Ridgway et al., 2008, for comprehensive guidelines on good practice when reporting VBM studies). Firstly, the various techniques of correcting for multiple comparisons require special attention and clarification: within the different methods of controlling the FWE rate or the FDR (described in Appendix 2), various options exist which impact on the interpretation of the results, and full details of the correction process should be provided. Secondly, although reporting uncorrected results should be avoided, doing so requires providing even more information to make the results meaningful to interpretation (see Ridgway et al., 2008 for details of these recommendations). Thirdly, even if there is some justification for performing ROI analysis or SVC, a prior exploratory whole-brain analysis is needed to complement the ROI approach and even negative results must be reported (Borgwardt et al., 2012). This is crucial to have the full picture before reaching any conclusions.

In the same way that a correct and thoughtful methodological approach to data acquisition and analysis is important, so is the need for an adequate characterization of the bilingual sample being tested. Unfortunately, almost all the studies described here have small samples and some of them provide minimal information regarding the type of bilinguals tested, thus making it difficult to draw conclusions that could generalize to other bilingual samples. (This may be another source of some of the above-mentioned discrepancies among existing studies.) The concept of bilingualism is broad enough to cover a finite but wide range of language combinations, and it might be the case that languages of different typologies shape the human brain and its functions in different ways (Carreiras, Duñabeitia, Vergara, de la Cruz-Pavia, & Laka, 2010; Zhu, Nie, Chang, Gao, & Niu, 2014). Hence, it should be no surprise that different language combinations (pairs) give rise to the differing development of the neural substrates that support language use and control. Similarly, even if the results obtained from studies exploring the influence of L2 proficiency and L2 AoA partially converge, there is convincing evidence that these two factors independently contribute to language processing in bilinguals' comprehension and production behaviour (Dimitropoulou, Duñabeitia, & Carreiras, 2011; Dowens, Vergara, Barber, & Carreiras, 2010; Duñabeitia, Dimitropoulou, Uribe-Etxebarria, Laka, & Carreiras, 2010; Duñabeitia, Perea, & Carreiras, 2010; Perea, Duñabeitia, & Carreiras, 2008). Thus, a thorough description of the knowledge and use of each of the languages is essential for a precise characterization of the samples being tested to make possible the replication and discussion of findings in the context of the specific linguistic background of the participants (see Tables 1 and 2 for an illustration of the variability between studies). Finally, but equally important, a precise definition of the manner in which the second language has been acquired and of the context in which each language is being used is critical, given numerous demonstrations of the differential effects derived from naturalistic vs. classroom-based learning (Muñoz, 2008; Pliatsikas & Marinis, 2013; see Stein, Winkler, Kaiser, & Dierks, 2014, for an overview), as well as of dominance-switch effects (Basnight-Brown & Altarriba, 2007), and of the role of immersion in language processing, and therefore, in the neural assemblies supporting bilingualism (Baus, Costa, & Carreiras, 2013). Since bilingualism is in and of itself a heterogeneous phenomenon, a wide range of studies taking in this variability could reduce this methodological problem and provide better answers. Unfortunately, to date there are insufficient studies covering and replicating this variability.

Chapter 3: Motivation, Aims, Predictions and General Scheme

The first motivation for this dissertation is that even though there are so many controversies about the bilingual advantage, studies about brain plasticity in bilingualism are still scarce. Additionally, the little existing evidence has not been replicated and very often is conflicting. The present study will try to shed some light on the field by adding fresh new evidence testing children and elderly high proficient early Spanish-Basque bilinguals, two very different languages from a linguistic typology point of view. This thesis will embrace an interdisciplinary approach to address an ambitious but clearly outlined goal: *the neural modelling of bilingualism*. As such, the proposed work has the potential to illuminate a number of core issues in the field and to use brain-mapping techniques that comprehensively capture the structure-function relationships of systems of highly complex distributed cognitive processing (e.g., language and executive control).

Measure the differences between bilinguals and monolinguals is difficult, mainly due to the high degree of variability between bilingual individual linguistic profiles and also due to the variability in language control and processing abilities from one individual to another, from one population to another and one age group to another. However, if a pattern appears among so much variability, presumably this will be able to be generalized. This thesis will try to overcome some of these problems investigating age groups at either end of the lifespan: children and the elderly. Children's brains are in the process of development and in a period where the brain is more sensitive to changes and in particularly to changes related to language acquisition and executive functioning. In the elderly, on the other hand, there is typical structural and functional decline of the brain. There is evidence showing that the brain at this stage is sensitive to many cognitive reserve variables such as intelligence, social status, fitness (Stern, 2009) and likely bilingualism (Bialystok et al., 2016). Therefore, in this study, *it is expected that the potential brain differences between bilinguals and monolinguals* *are most salient in both children and elderly. It is also expected that if these differences appear, they should be more salient in seniors than in children, given the lifelong experience of bilingualism of the elderly as compared to the children.*

Additionally, the bilingual population proposed for this study (Basque-Spanish bilinguals), is mostly simultaneous and early bilingual that is constantly exposed to both languages and switches between their languages very often in everyday life, and indeed they often interleave both languages in a single utterance within a conversation. According to Green and Abutalebi's bilingualism model (2013) this represents a dense code-switching language interactional context. Moreover, both languages, Spanish and Basque, are very distant lexically (Duñabeitia et al., 2010), orthographically (Casaponsa et al., 2015) and syntactically (Carreiras et al., 2010). It is expected, therefore, that the kind of bilingual population and context proposed here and the typological distance between languages will also result in more brain plasticity, as it is well-known that AoA and language proficiency are determinant factors in the neural underpinnings of bilingualism (see Laka, 2012 and Caffarra, Molinaro, Davidson and Carreiras, 2015 for a review), and that when the difference between properties of the two target languages differ more widely, the neural systems involved in the processing of both languages are more differentiated (i.e. may have distinct underlying neural correlates) (Kovelman et al., 2008; Li & Shu, 2010; Zhao & Li, 2010; Lucas et al., 2004; Zhu et al., 2014), which could increase the possibility of finding brain differences between bilinguals and monolinguals.

The research questions of interest in this study will be addressed by combining different neuroimaging modalities (i.e. T1-MRI, DW-MRI and rfMRI). As each neuroimaging modality in isolation is limited by the nature and extent of information it can provide, this fusion enables complementary data of value in understanding brain plasticity. In this sense, different measurements can be obtained, such as GM volume, cortical thickness, DTI-derived measures and large-scale structural/functional connectivity maps that could provide compelling evidence for neural changes related to the acquisition of more than one language. With such complementary imaging results, commonalities can be arrived at, helping to overcome previous methodological problems in the field.

Specifically, *it is expected that bilinguals will show regional increased GM volume and decreased cortical thickness in regions important for bilinguals living in a dense code-switching language interactional context (i.e. cerebellum, left IFG, caudate/putamen, insula, premotor and motor cortex) and/or in regions that have previously shown increased grey matter volume/density, and/or different functional demands in bilinguals. Local modifications of WM structure are also expected in comparison to monolinguals; showing increased/decreased FA values in WM tracts connecting these regions. But also functional/structural specialized subnetworks are expected, by showing higher axonal connection density and/or higher functional correlation between these GM regions.*

Brain regions may have undergone a different configuration to increase their processing capacity in order to fulfill the increased language demands. The relationship between bilingualism and the topological properties of the brain functional/structural networks will also be investigated here by modelling the human brain as a complex network (Basset et al., 2009; Iturria-Medina et al., 2011; Li et al., 2009; Zalesky et al., 2010b). Here, the brain is modelled as a graph whose nodes (structural/functional brain regions) are interconnected by edges (structural/functional connections) (Bullmore and Bassett, 2011). Global graph-efficiency (a measure of integration) and local graph-efficiency (a measure of clustering) of the brain structural/functional networks will be estimated. It have been demonstrated that these complex network topological properties are important for the performance of the brain graph. On the other hand, functional performance of the brain graph can impact these topological properties (Sporns et al., 2000). Thus, *changes in topological properties of the brain structural/functional networks in bilinguals are also expected*. Taken into account previous findings on bilingualism (García-Pentón et al., 2014), *it is expected that the bilingual brain will show higher efficient specialized subnetworks related to language as compared to monolinguals.*

Overall, the **main objective** of this dissertation is:

- To better understand the neuroplasticity in bilingualism studying two different age groups: children and elderly, and using a multimodal neuroimaging approach.

The central **research questions** of this dissertation are: ***a)*** *What are the effects of bilingualism on the brain functional/structural configuration* ***b)*** *Whether we need different or extra brain regions/sub-networks to deal with more than one language* ***c)*** *Whether the network's capacity for management and integration of information differs from monolinguals*. This dissertation will focus on age groups at either end of the lifespan and will include multiple neuroimaging techniques to exhaustively investigate these questions. The specific research questions asked are:

- *How does the bilingual experience change the structure of the brain in childhood?*
- *How does lifelong bilingualism alter the structure of the brain in the elderly?*
- *Are these changes more evident in the elderly than in children?*
- *How are the large-scale functional/structural brain networks (i.e. at the whole-brain level) modified by bilingualism?*
- *What commonalities due to bilingualism can be detected in the brains of these two bilingual groups?*

Accordingly, in order to reach a unified neural modelling of bilingualism, we will investigate differences between bilinguals and monolinguals using structural and functional measures and their interrelationships. For that purpose, this dissertation will tackle these **specific objectives**:

(i) *To evaluate how the bilingual experience changes the GM structure in childhood and old age by measuring and comparing the GM volume and CT between bilinguals and monolinguals, at the whole brain level.*

(ii) *To explore how the WM is modified in children and elderly bilinguals as compared to monolinguals, by measuring and comparing FA maps at the whole brain level.*

(iii) *To map and compare the underlying structural/functional connectivity in the bilingual brain using a large-scale connectivity approach in children and elderly bilinguals and monolinguals.*

(iv) *To perform a fully comprehensive study integrating both populations (children and elderly) in order to determine the most outstanding differences between monolinguals and bilinguals.*

Summarizing, this study utilises a combination of neuroimaging techniques (T1-MRI, DW-MRI and rfMRI) to promote convergent evidence about the specialization and integration of the neural networks in bilingualism. As such, this work expects to uncover the neural mechanism for bilingualism by mapping the structural/functional networks in bilinguals as compared to monolinguals. In other words, this dissertation will study the organisation of brain networks, either due to slow changes in brain areas and their wiring (namely, the structural plasticity), or due to fast modulation of their interactions (namely, functional plasticity). T1-MRI (i.e. 3D high resolution whole-head anatomical scan) will be used to identify regional grey matter differential structural patterns associated with bilingualism using voxel-based morphometry (VBM; Ashburner and Friston, 2000) implement in FSL (Smith et al., 2004) and surface-based analyses implement in FreeSurfer (Dale et al., 1999; Fischl et al., 1999). Importantly, this work will bring evidence of how brain structure constrains and determines functional connectivity. DW-MRI and rfMRI data will be used to determine structural and functional connectivity, respectively. Both techniques will make it possible to model the large-scale structural/functional connectivity maps by means of a high-dimensional parcellation of the brain instead of limiting the analysis to specific regions of interest, as done in previous studies. For that purpose the T1-MRI will be also used to generate the grey matter parcellation employed in the connectivity analysis. It is crucial to use small parcellations (e.g. 90 GM regions) in order to obtain a better high-dimensional structural individual atlas that can then be more readily matched to the functional regions. DW-MRI data will be pre-processed using FSL and rfMRI data will be pre-processed using the Data Processing Assistant for Resting-State fMRI (DPARSF, Yan and Zang 2010; Yan et al. 2016, available at http://rfmri.org/DPARSF), which is based on SPM (http://www.fil.ion.ucl.ac.uk/spm). For the data analysis, network-based statistics (Zalesky et al., 2010) and graph theoretical approaches (Latora & Marchiori, 2001; Rubinov and Sporns, 2010) will be employed to investigate differences between groups in connectivity patterns, by isolating sets of regions interconnected differently between groups, and in topological properties of the networks, by measuring global/local graph-efficiency. This network analysis represents a fine-grained spatial analysis of the specialisation and the integration of the brain. In other words, the connectivity of each region separately along with how regions interact with each other and form communities/subnetworks. This approach provides information about whole-brain structural/functional organization. This methodology has been widely used before in healthy subjects and patients looking for normal and abnormal intrinsic connectivity patterns (e.g. Zalesky et al., 2010b; Bai et al., 2012; Verstraete et al., 201), and it has also been used in healthy populations to study bilingualism (García-Pentón et al., 2014). The direct comparison between the monolingual and bilingual groups will allow the extraction of subnetworks and regions that are critical for our understanding of the neural bases of bilingualism. As a result, *the detection of different structural/functional connectivity patterns (subnetworks) in bilinguals as compared to monolinguals is expected. On the basis of previous studies I hypothesize that the subnetwork(s) related to bilingualism will be made up of a distributed network of regions including the cerebellum, left IFG, caudate/putamen, insula, premotor and motor cortex, but also other brain regions related to language processing and control that have been previously suggested to be influenced by bilingualism. I also hypothesize that the IFG will be a hub region in bilinguals identified by the topological analysis*.

The most innovative aspect of the research agenda is the approach to understanding the mind in terms of nested and distributed systems that establish dynamical interactions in order to realise a given cognitive process. The mind cannot be explained simply as a collection of segregated brain modules that are in charge of specific behaviours or processes (Spivey, 2007). In this context, mapping the human brain is a necessary step to uncovering the principles of the brain’s functional and structural organization. However, little has been unambiguously mapped to date. The distributed integrational connectivity perspective of the brain outlined highlights the difficulty in explaining the distributed neural mechanisms underlying cognitive capacities without a multimodal neuroimaging approach. Recently, such an approach has been followed to provide a distributed and integrated view of bilingualism in young adulthood (see García-Pentón et al., 2014). Within this new integrational perspective, it becomes necessary to explore the relationship between structure and function, as a more holistic and realistic approach to understanding the comprehensive nature of bilingualism. Additionally, using different measures to complement brain information extracted from different healthy populations will ensure that the results obtained will lead to solid conclusions, which ultimately will contribute to refining current models of bilingualism. The fact that the issues dealt with by this dissertation have not yet been widely explored means that many interesting questions may be uncovered by this work.

Having outlined the background of this dissertation, Part II (the experimental section) first reports and discusses the findings from 2 experiments carried out separately to examine the effects of bilingualism on the brain in the two populations under study (chapter 4 describes Experiment I with bilingual and monolingual children and chapter 5 describes Experiment II with elderly bilinguals and monolinguals). Finally reports and discusses the results of a third analysis that integrates both age groups in a more exhaustive statistical model (Chapter 6).

Part III includes Chapter 7, with the summary of the effects and general discussion of the dissertation. This chapter summarizes the results of the experiments and discusses these results in the light of current models of bilingualism in section 7.1. Finally, section 7.2 concludes by identifying issues that should be taken into account so that studies in this field are more comparable and provide evidence that can be collected, processed and integrated more easily. This chapter closes with methodological recommendations to follow in future studies (section 7.3) with the aim of providing a methodological framework that will enable the field to progress.

Part II: Experimental Section

Chapter 4: Experiment I. Structural Brain Changes in Bilingual Children

4.1 INTRODUCTION

Language exposure during the first years of life sculpts the brain even before infants can speak, and toddlers develop an astonishing capacity to discriminate between their native language's sounds and those that are not meaningful from early stages of the development (Kuhl, 2010). While monolingual infants lose the ability of non-native phonetic discrimination by approximately 10 months old, bilingual infants can discriminate between their two languages (Byers-Heinlein, et al., 2010) and even can distinguish languages different to their own for longer periods (Weikum et al., 2007; Sebastián-Gallés et al., 2012). However, and in spite of the evidence gathered regarding the manner in which infants discriminate among the languages of their environment, our understanding of how the exposure to two languages from infancy shapes the brain remains far from complete.

Recent neuroimaging studies suggest that the neural circuitry that supports language processing is different in bilingual than in monolingual children (Garcia-Sierra et al. 2011), and that it involves different networks particularly with a greater connectivity to prefrontal areas for the former group (Petitto et al. 2012). Furthermore, it has been suggested that a relatively brief exposure period to a seemingly bilingual linguistic setting is sufficient to alter the ulterior brain activation pattern and to influence cognitive abilities related to language processing (e.g., Pierce et al., 2015).

In general terms, it is assumed that children acquire languages with greater ease than adults (Flege, Munro & Mackay, 1995; Weber-Fox & Neville, 1996). It is well known that the proficiency in a second language is modulated by the age at which that language was acquired (Hernandez and Li, 2007), given the sensitive time periods related to the different linguistic levels of processing. Due to the sensitivity of the infant's brain to neuroplasticity during the first years of life, it has been proposed that this period is the best suited for acquiring a language (Pierce et al., 2015). Hence, being infancy the best setting for language acquisition, and considering that the toddlers' brains bear critical developmental changes that shape their future structural and functional architecture during this period, one may wonder whether acquiring two languages in childhood may yield a neural configuration that is inherently distinct from that of the children who acquire a single language. The current study aims at shedding light on this issue by comparing the anatomical and functional brain organization of monolingual and bilingual children.

Considering the course of development of many cognitive functions that are necessary to efficiently use and control the language(s) spoken by a person, it has been suggested that childhood represents a perfect test scenario to investigate the existence of differences between bilinguals and monolinguals at multiple levels of cognitive processing. In fact, it has been suggested that childhood sets the best test case for the so-called bilingual advantage (e.g., Bialystok et al., 2012). According to this hypothesis, bilingual children may develop enhanced executive functions as compared to their monolingual peers as a consequence of their constant need to monitor the environment and to inhibit the inappropriate language to effectively communicate (e.g., Bialystok et al., 2016). While this conclusion has been refuted in recent years from data coming from young and older adults (see Antón et al. 2016; Paap & Greenberg, 2013), and even from large-scale studies testing bilingual and monolingual children (see Duñabeitia et al., 2014; Antón et al., 2014), it remains to be fully explored whether bilingualism can shape the infant brain at a level that is not seen in merely behavioural tasks.

In the current study the resting-state fMRI technique, in combination with DW-MRI and T1-MRI, was used to study structural/functional brain plasticity related to bilingualism in children. This neuroanatomical approach was followed in order to explore task-independent differences between the bilingual and monolingual brain during childhood; leaving aside the discrepancies at the behavioural and functional level on the existence of a bilingual advantage (e.g., Paap & Greenberg, 2013; Kroll & Bialystok, 2013; Paap et al., 2015; de Bruin et al., 2015a; see also García-Pentón et al., 2016; Abutalebi & Green, 2007; Hernandez, 2009; Luk et al., 2012, for a review). This combination of methods has also the advantage of allowing large-scale network analysis, by mapping functional/structural brain networks at the whole-brain level. Considering also that the development of executive functioning follows a trajectory whose most critical moments occur during early childhood (see Garon et al., 2008) and that some authors have suggested that bilingualism could modulate this course of development (e.g., Kovacs & Mehler, 2009), it could be tentatively suggested that the simultaneous acquisition of more than one native language could trigger greater structural/functional brain plasticity than the acquisition of a single language, and therefore that this effect can be salient in children's brains. Moreover, these changes should be expected especially in regions related to language processing and executive cognitive abilities.

The evidence on the structural/functional brain changes associated with bilingual experience is scarce and inconsistent, and it mainly comes from studies testing young and older adulthood (see Costa & Sebastián-Gallés, 2015; García-Pentón et al., 2016; Li et al., 2014, for reviews). Across the studies, just one region shows up in a relative consistent manner showing differences between bilinguals and monolinguals: the left/right IFG (e.g., Hosoda et al, 2013; Klein et al., 2014; Grogan et al, 2012; Luk et al., 2011; García-Pentón et al., 2014, Olalude et al., 2016; Burgaleta et al., 2016). This difference is correctly predicted by current neuro-functional models (e.g., Abutalebi & Green, 2007; Green & Abutalebi, 2013), but these same accounts also predict differences in other regions which are not so stable across studies (such as, cerebellum, caudate/putamen, insula, premotor and motor cortex). Nonetheless, as said, these pieces of evidence mainly come from studies testing young and older adults.

So far, there are only two structural neuroimaging studies exploring the differences between bilingual and monolingual children's brain (Mohades et al., 2012; 2015) and they just investigate the white matter (WM) plasticity. In a first study, Mohades et al. (2012) examined WM changes in some tracts of interest cross-sectionally by comparing groups of simultaneous bilingual, sequential bilingual and monolingual children. These same groups were followed in a longitudinal study, as recently reported in Mohades et al. (2015). They extracted the mean FA values from four major tracks that are well-know to connect language regions (i.e. the left arcuate fasciculus/SLF, the left IFOF and the bundles arising from the anterior and middle part of the CC). In the cross-sectional study (Mohades et al., 2012), the authors found a group effect showing higher mean FA values in the left IFOF for bilinguals than for monolinguals, and a decreased mean FA values in the anterior part of the CC. In the longitudinal study (Mohades et al., 2015), the results revealed that simultaneous bilinguals had the highest mean FA value in the left IFOF as compared to sequential bilinguals and monolinguals. Interestingly, the lower mean FA value they observed at the first tested moment for bilinguals as compared to monolinguals in the CC was no longer evident when these same participants were retested in the longitudinal study.

While the two studies by Mohades et al. (2012, 2015) are of clear-cut relevance for our understanding of the manner in which bilingualism shapes the neural circuitry in childhood, the evidence in this regard is too limited to grant generalization. To our knowledge, there are no studies in children evaluating the influence of bilingualism on the structure of the grey matter. In addition, modifications in the structural/functional connectivity of the whole brain associated to bilingualism in childhood are still unknown. Particularly, the relationship between bilingualism and topological properties of the brain network has never been investigated in children. Besides, the previous WM studies focused on specific tracts of interest, and did not follow a whole-brain quantitative neuroimaging approach which could result appropriated 1) to uncover differences in tracts other than the targeted ones, and 2) to statistically circumscribe the analysis (see García-Pentón et al., 2016, for a discussion). These limitations were overcome in the current study by combining different cutting-edge neuroimaging analysis techniques that allowed us to obtain different structural/functional measurements (i.e., GM volume, cortical thickness, FA values, structural/functional connectivity measures and complex network parameters) at the whole-brain level.

Summarizing, this study is the first known attempt to identify local GM differential structural patterns associated with bilingualism by investigating a group of simultaneous Basque-Spanish bilingual children and a group of well-matched Spanish monolingual controls and critically, using voxel-based and surface-based analyses. This study will also identify WM structural differences by means of DTI-derived measurements using TBSS approach (Smith 2006). Besides, in order to determine differences in structural/functional connectivity patterns between both groups, a network-based statistic (NBS) approach will be follow to isolate sets of highly interconnected regions, providing information about whole-brain structural and functional organization (Zalesky et al., 2010a; see García-Pentón et al., 2014, for a similar approach with young adult bilinguals). Finally, to assess for the configurational properties of the whole-brain networks and possible differential sub-networks, a graph theoretical approach is used (e.g., Latora & Marchiori 2001; Watts & Strogatz 1998). This is done in order to explore the differences in the topological parameters associated with the structural/functional networks of bilingual and monolingual children with a special focus on global/local graph network efficiency, which is a measure of the intrinsic capability of the network to guarantee high information exchange between nodes or regions.

4.2 PARTICIPANTS

14 native Spanish monolinguals (6 females, age range from 6 to 14 years, mean age, 10.98 years, 2.45 std) and 14 early Spanish–Basque bilinguals (6 females, age range from 6 to 14 years, mean age, 10.95 years, 2.48 std) took part in the experiment. The groups were paired in age and sex (see table 3). All bilingual children had acquired both languages before preschool and used both languages every day. A language questionnaire was applied to their parents and the children were rated as very highly proficient in both languages (mean rates: 9.57, ±0.53 std for Spanish and 8.20, ±1.39 std for Basque) on a scale from 1 (very poor level) to 10 (perfectly fluent). The ratings were based on reading, writing, listening and speaking skills. The monolinguals children used only Spanish for daily life and had no knowledge of any other language (see Table 4). All participants were healthy people with no reported history of neurological/mental illness and/or treatment with psychotropic medication. Participants gave verbal and written informed consent prior to involvement, in accordance with the Declaration of Helsinki, and the research protocol was approved by the Basque Center on Cognition, Brain and Language (BCBL) Ethics Committee. IQ scores were measured with the Spanish version of the Kaufman Brief Intelligence Test (K-BIT) and Wechsler Intelligence Scale for Children (WISC), and was controlled as a nuisance covariate. Handedness scores averages assessed by the Edinburgh Handedness Inventory (Oldfield R.C. 1971) were not statistical significantly different between groups (p-value = 0.2157). Notice that for the white matter study only 13 participants per group took part in the

study because one of them had to leave the resonance before the acquisition of the DW-MRI sequence.

Table 3. *Age in years, gender of participants and IQ.*

Monolingual group (N = 14)			Bilingual group (N = 14)		
Age	**Gender**	**IQ Composite (standard score)**	**Age**	**Gender**	**IQ Composite (standard score)**
6.36	F	141	6.42	F	103
8.32	M	100	8.54	M	109
8.30	F	102	8.35	F	102
9.20	M	121	8.73	M	114
10.57	M	110	10.45	M	106
10.98	F	100	10.71	F	108
11.05	F	124	10.74	F	106
12.10	M	101	11.86	M	105
12.78	M	100	12.79	M	104
13.10	M	115	12.95	M	102
13.16	M	122	13.35	M	111
13.25	F	116	13.51	F	109
13.61	M	119	13.78	M	90
14.14	F	117	14.22	F	109

Note: N, sample size. IQ, intelligence quotient. F, female. M, male.

Table 4. *Language profile of monolingual and bilingual groups.*

Variables	**Mean (±SD)**	
	Monolinguals (N = 14, 6 female)	**Bilinguals (N = 14, 6 female)**
AoA of Spanish	0	0.23 (±0.83)
AoA of Basque		0.91 (±1.5)
% daily exposure to Spanish	100	47.69(±20.17)
% daily exposure to Basque	-	43.69 (±19.25)
% daily exposure to other language	-	8.23 (±3.52)
Spanish proficiency	10	9.57 (±0.53)
Basque proficiency	0	8.20 (±1.39)
Other language proficiency	-	1.35 (±3.16)

Note: N, sample size. AoA, age of acquisition (the age at which participants started to learn these languages). SD, Standard deviation.

4.3 METHODS

4.3.1 Magnetic resonance imaging (MRI) data acquisition

All images were acquired on a 3-T Magnetom Trio Tim scanner (Siemens AG, Erlangen, Germany) located at the BCBL in Donostia-San Sebastián. For each participant, a high-resolution T1 weighted scan was acquired with a 3D ultrafast gradient echo (MPRAGE) pulse sequence. Acquisition parameters used were: matrix size 256 × 256; 176 contiguous axial slices; voxel resolution 1 × 1 × 1 mm^3; TE/TR/TI = 2.97 ms/2530 ms/1100 ms, respectively; and flip angle 7°. The scan lasted 6 min.

DW-MRI data were recorded using a single-shot spin echo-planar imaging (EPI) sequence, along 64 gradient directions at b-value = 1500s/ mm^2 and 1 unweighted (b = 0) image. Acquisition parameters used were: eco time (ET) = 99ms, repetition time (RT) = 9300ms, FOV = 1840 × 1840 mm^2, matrix size 1024 × 1024, 58 contiguous slices and an isotropic voxel resolution = 1.79 × 1.79 × 1.79 mm^3). The total scan time for the DW-MRI protocol was approximately 10 min.

Blood oxygen level dependent (BOLD) fMRI during resting-state was acquired using a whole-brain, single-shot, multi-slice, gradient-echo echo planar imaging (EPI) sequence of 180 volumes with the following parameters: TR/TE = 2000/30 ms, flip angle = 78°, FOV = 192 × 192 mm, matrix = 64 × 64, 33 slices, slice thickness = 3 mm, slice spacing = 3.59 mm, descending acquisition. The resulting nominal voxel size was 3.0 × 3.0 × 3.59 mm. A fixation cross was displayed as images were acquired. Subjects were instructed to stay awake, keep their eyes open, fixate on the displayed crosshair, and remain still. The total scan time for the resting-state fMRI protocol was approximately 12 min.

4.3.2 Data pre-processing and analysis

4.3.2.1 Voxel-based morphometry (VBM)

In this study, structural data was analysed with FSL-VBM (Douaud et al., 2007, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM), an optimized VBM protocol (Good et al., 2001) carried out with FSL tools (Smith et al., 2004). First, structural images were brain-extracted and grey matter-segmented before being registered to the MNI 152 standard space using non-linear registration (Andersson et al., 2007). The resulting images were averaged and flipped along the x-axis to create a left-right symmetric, study-specific grey matter template. Second, all native grey matter images were non-linearly registered to this study-specific template and 'modulated' to correct for local expansion (or contraction) due to the non-linear component of the spatial transformation. The modulated grey matter images were then smoothed with an isotropic Gaussian kernel with a sigma of 3 mm (see figure 5). See Appendix 1 for more information about VBM technique.

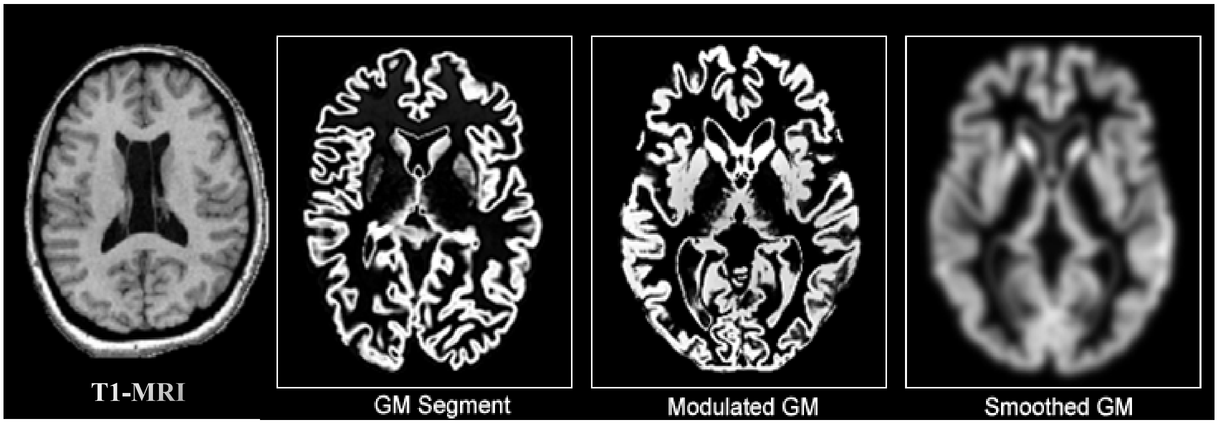

Figure 5. *Different VBM preprocessing steps over the T1-MRI structural images.*

Group comparison between bilinguals and monolinguals was carried out using a voxel-wise general linear model (GLM) and permutation-based non-parametric testing (Winkler et al., 2014). The number of permutations was set to 10000 and the IQ was included as continuous nuisance regressor. Two contrasts were examined: Bilingual > Monolingual and Monolingual > Bilingual. Regional differences were reported as significant at $p < 0.05$, fully corrected for multiple comparisons across space using threshold free cluster enhancement (TFCE) (Smith and Nichols, 2009) where cluster-like structures are enhanced but the image remains fundamentally voxel-wise. In addition, an extent threshold of 100 voxels was also set. Anatomical locations of significant regions were determined by reference to the MNI structural atlas (Mazziotta et al., 2001) integrated into FSL atlas tool and AAL atlas (Automated Anatomical Labeling, Tzourio-Mazoyer et al., 2002) implemented in MRIcron software.

Because the interpretation of the obtained results with such voxel-wise analysis can be interpreted either as a difference in GM folding or thickness, a cortical thickness analysis as described in the next section has been also performed.

4.3.2.2 Surface-based morphometry analysis

To measure the cortical thickness FreeSurfer (version 5.1) image analysis suite (http://surfer.nmr.mgh.harvard.edu/) was used. Cortical reconstruction and volumetric segmentation is performed as follow: briefly, this processing includes motion correction, removal of non-brain tissue, automated Talairach transformation, segmentation of the subcortical WM and deep GM volumetric structures, tessellation of the GM and WM boundaries, automated topology correction, and surface deformation following intensity gradients to optimally place the GM/WM and GM/CSF borders at the location where the greatest shift in intensity defines the transition to the other tissue class (Dale et al.,1999; Fischl and Dale, 2000; Segonneet al., 2004). Once the cortical models are complete, a number of deformation procedures were performed in the data analysis pipeline, including surface inflation and a high-dimensional nonlinear registration to a spherical atlas. A cortical surface-based atlas has been defined based on average folding patterns mapped to a sphere. The registration is based on aligning the cortical folding patterns and so directly aligns the anatomy instead of image intensities. The spherical atlas naturally forms a coordinate system in which point-to-point correspondence between subjects can be achieved. This coordinate system can then be used to create group maps (similar to how MNI space is used for volumetric measurements). This method uses both intensity and continuity information from the entire three-dimensional T1-MR images in the segmentation and deformation algorithms to produce representations of cortical thickness, calculated as the closest distance from the GM/WM boundary to the GM/CSF boundary at each vertex on the tessellated surface (see Figure 6). These maps are not restricted to the voxel resolution of the original data and are thus capable of detecting sub-millimetre differences between groups. Prior to the statistical analysis, the individual cortical thickness was smoothed in cortex using a Gaussian filter with (FWHM) of 10 mm.

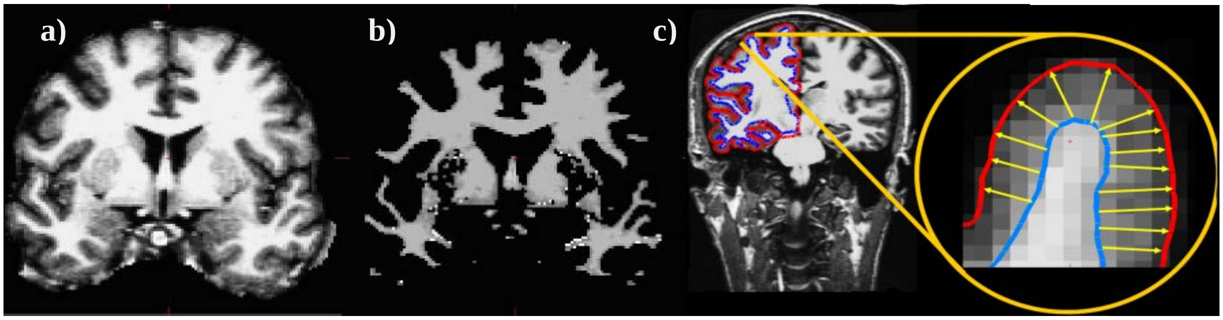

Figure 6. *Freesurfer's preprocessing steps over the T1-MRI.* ***a)*** *Skull stripped image.* ***b)*** *Subcortical WM segmentation.* ***c)*** *Lines over the T1-MRI represent the cortical surfaces. Blue line: surface between WM and GM. Red line: surface between GM and pia mater. Yellow lines represent cortical thickness measurement.*

Finally, a vertex-wise GLM was applied. Statistical inference was carried out with FreeSurfer tools based on non-parametric Monte Carlo testing (10000 iterations), using a cluster-wise correction method for multiple comparisons with initial vertex-wise threshold for cluster-formation of $t = 2$ ($p < 0.01$). In this analysis, only those clusters with a corrected value of $p < 0.05$ using Bonferroni correction were considered as significant. GLM analysis on the surface enables us to test group differences by two contrasts: Bilingual > Monolingual and Bilingual < Monolingual.

4.3.2.3 Diffusion-weighted magnetic resonance imaging (DW-MRI) pre-processing

For this study the DW-MRI dataset was pre-processed using FMRIB’s Diffusion Toolbox (FDT) as part of FSL 5.0.2 software package (available at http://www.fmrib.ox.ac.uk/fsl/). Eddy currents correction was applied to correct for the distortions induced by the application of the diffusion encoding gradients and for simple head motion, using affine registration to the $b = 0$ image (first volume in the dataset). After correction, individual diffusion parameters were estimated in each voxel (Figure 7a) by fitting a tensor model to the raw diffusion data using FDT (Smith et al., 2004). From this step the FA images were obtained.

4.3.2.4 Tract-based spatial statistic (TBSS) analysis

The voxel-wise statistical analysis of the FA data was carried out using TBSS (Smith 2006), part of FSL (Smith 2004). The FA images created were brain-extracted using BET

(Smith 2002). All subjects' FA data were then aligned into a common space using the nonlinear registration tool FNIRT (Andersson 2007), which uses a b-spline representation of the registration warp field (Rueckert, 1999). Next, the mean FA image was created and thinned to create a mean FA skeleton that represents the centers of all tracts common to the group (see Figure 7). Each subject's aligned FA data was then projected onto this skeleton and the resulting data fed into voxel-wise cross-subject statistics.

Group comparison between bilinguals and monolinguals was carried out using a voxel-wise GLM and permutation-based non-parametric testing (Winkler et al., 2014). The number of permutations was set to $k = 10000$ and the IQ was included as continuous nuisance regressor. Two contrasts were examined: Bilingual > Monolingual and Monolingual > Bilingual. Regional differences were reported as significant at $p < 0.05$, fully corrected for multiple comparisons across space using TFCE (Smith and Nichols, 2009) with an extent threshold of 50 voxels. Anatomical locations of significant regions were determined by reference to the Johns Hopkins University (JHU) white-matter tractography atlas (Mori et al., 2005) integrated into FSL atlas tool.

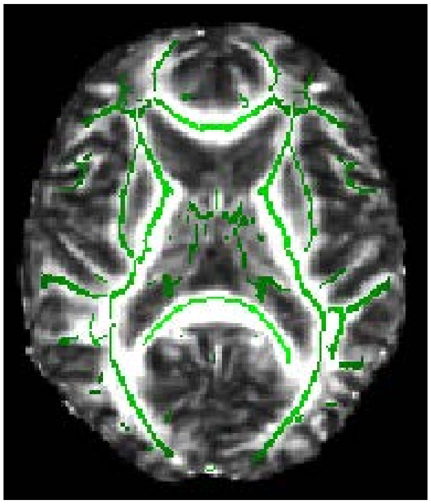

Figure 7. *Mean FA skeleton template used in the TBSS analysis*.

4.3.2.5 White matter connectivity analysis

For this analysis T1-weighted images of each participant were first co-registered to the participant’s b0 and then segmented in 3 tissue probability maps: GM, WM and cerebrospinal fluid (CSF) using SPM8 software package (available at http://www.fil.ion.ucl.ac.uk/spm/). The three tissue classes in native space and the matrix transformation to MNI space obtained from segmentation were then used to automatically parcellate the cerebral cortex of each participant into 90 GM regions taken from AAL atlas (Tzourio-Mazoyer et al., 2002), using IBASPM toolbox (available at http://www.fil.ion.ucl.ac.uk/spm/ext/#IBASPM) (Alemán-Gómez et al., 2006). The individual atlases in T1 native space (see an individual atlas example in Figure 8) were then resliced to DTI space using a nearest-neighbour interpolation in SPM8 to create seed points mask for tracking.

For each participant, axonal connectivity values between each brain voxel and the surface of each of the 90 GM regions considered (voxel-region connectivity) were estimated using the probabilistic fiber tractography algorithm implemented in the FSL software (Behrens et al., 2003). For each brain voxel, an index of connectivity, representing the number of generated paths that passed through it from the seed region, was assigned (Figure 8b). Tracking parameters used were 5000 as a number of generated paths from each seed point (defaults in the FSL software package as optimum to reach the convergence of the algorithm), 0.5 mm as step length, 500 mm maximum trace length, and a curvature threshold of ±80º.

4.3.2.5.1 Network construction

For each participant, the whole-brain undirected weighted network was created as follows: 1) A node was defined to represent each anatomic region considered (i.e. 90 grey matter regions of the AAL atlas template), 2) An undirected arc aij between nodes *i* and *j* was established if a nonzero connectivity value was found to exist between the boundary voxels of regions *i* and *j*, and 3) Arc weight w(a*ij*) was defined as the connection measurement (the output of the tractography algorithm) between regions *i* and *j* (Iturria-Medina et al., 2011), estimated by counting the 'effective' number of voxels over the surface of both regions and weighting each voxel by its voxel-region connectivity value with the opposite zone, relative to the total number of considered superficial voxels (Figure 8c).

4.3.2.6 Resting-state functional magnetic resonance imaging (rfMRI) analysis

The analysis was performed using statistical parametric mapping (SPM12) software (Wellcome Trust Centre for Neuroimaging) and the Data Processing Assistant for Resting State fMRI (DPARSF Advanced Edition, version 4.1) toolbox (Yan and Zang, 2010; Yan et al., 2016). Three methods were used to reduce motion effects in the rfMRI signal: global signal regression, 24-motion parameter regression and the scrubbing of high motion time points. These methods have been shown to significantly reduce the effects of motion (Power et al., 2014; Power et al., 2015). Using DPARSF the first 10 volumes were rejected in order to avoid magnetic saturation effects, and SPM were used for slice timing correction, realignment, and coregistration to the individual's T1-MRI. Additionally, nuisance covariates were regressed out and the images were normalized using DARTEL (Ashburner, 2007) and smoothed with a 6 mm full width half maximum (FWHM) Gaussian kernel. The results were filtered at 0.01–0.1 Hz, as recommended by Satterthwaite et al. (2013). Nuisance covariates including 24 motion parameters have been shown to be better than the six parameters alone at decreasing motion effects, especially on those individuals with a higher motion like children (Yan et al., 2013; Power et al., 2014; Power et al., 2015). A scrubbing regressor method was used with each bad time point included as a covariate. White matter, CSF, and global tissue signal were also used as covariates.

4.3.2.6.1 Network construction

The average time courses from 90 GM regions taken from AAL atlas (as in WM connectivity analysis) were extracted. Connectivity was calculated on residual time series following confound regression and band-pass filtering using Pearson's correlation with DPARSF. Functional correlation between each of the 90 regions (ROI-wise connectivity correlation) was calculated using Pearson correlation. Thus, a 90 × 90 connectivity matrix was constructed for each subject; a Fisher's r-to-z transformation was applied to improve normality (Rosner, 2006).

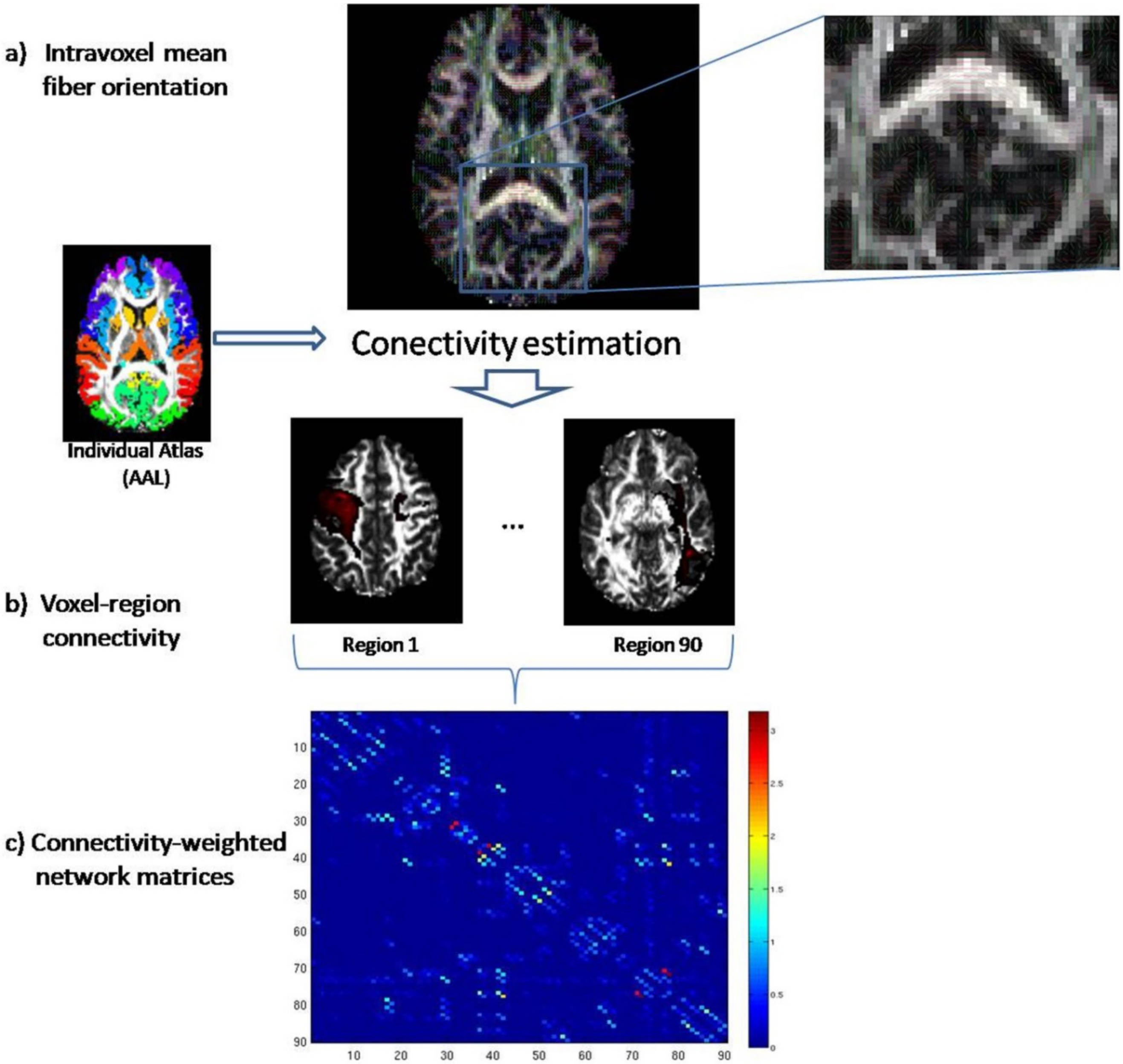

Figure 8. *A schematic representation of the connectivity estimation and network matrix construction in one participant as an example. a) Axial map representing intra-voxel mean fiber orientation (dyadic vectors) overlaid on the FA image; the inset figure provides details of the high fiber orientation coherence within the splenium of the corpus callosum. b) Axial voxel-region connectivity maps corresponding to region 1 (precentral gyrus) and region 90 (inferior temporal gyrus), overlaid on the FA image; voxels are color-coded according to whether the connectivity of each voxel is high (red) or low (black). c) Whole-brain structural network matrix derived from the tracking algorithm, as described in Methods.*

4.3.2.7 Network-based Statistic (NBS) analysis

An NBS approach (Zalesky et al., 2010a) was used to isolate the components of the 90 × 90 undirected connectivity matrices that differ significantly between the bilingual and monolingual groups. A component is a set of interconnected edges (i.e. sub-networks) in the connectivity matrix. The NBS analysis first performed a two-sample T-test at each edge independently to test the null hypothesis (H_0) that the values of connectivity between the two populations come from distributions with equal means. After that, a preselected T-value (T-threshold = 3) is used to threshold the statistical value calculated at each edge of the connectivity matrix to identify the set of supra-threshold edges. All interconnected components present in the set of supra-threshold edges are identified and their size (number of edges that the components comprise) is stored. Thus, a component is formed by interconnected supra-threshold edges at which the H_0 was rejected. To estimate the significance of each component, the NBS performed a nonparametric permutation test (K = 10000 permutations). A total of K random permutations are generated independently. For each permutation, the group to which each subject belongs is randomly exchanged, and then the statistical test is recalculated in each permutation. After that, the same threshold is applied to create the set of supra-threshold links for each K permutation. Then, the size of the largest component in the set of supra-threshold links derived from each K permutation is stored, thus providing an empirical estimation of the null distribution of the maximal component size. Finally, the p-value of each observed connected component was corrected, calculating the proportion of the 10000 permutations for which the largest component size was greater than the observed connected component size and then normalized by K. This allowed control of the FWE associated to each connected component, based on its size.

In other words, the NBS tries to identify components (structural/functional connected structures) that are formed by a set of links (structural/functional connections) between regions that exceed an appropriately chosen supra-threshold link. The topological extent of these connected structures is then used to determine their significance. The first step is creating the set of supra-threshold links computed for each pair-wise association. Then any connected components present in the set of supra-threshold links are identified and the number of links that they are comprised of (their size: m) is stored. A nonparametric permutation test is used to assign a p-value controlled for the FWE to each connected component, based on its size. The p-value of each connected component of size *m* is estimated, looking for the proportion of permutations for which the maximal component size is greater than *m,* and then is normalized by K (For more details see Zalesky et al., 2010a. For applications and examples of the NBS approach see Bai et al., 2012; García-Pentón et al., 2014; Verstraete et al., 2011; Zalesky et al., 2010b).

Schematic representations of the NBS results will be depicted using BrainNet Viewer version 1.1 (available at: http://www.nitrc.org/projects/bnv/). The anatomical name by which each node is labelled is taken directly from the AAL atlas (Tzourio-Mazoyer et al., 2002).

4.3.2.8 Complex network analysis

Global efficiency

In terms of the information flow, the global efficiency (E_{glob}) of a network *G* reflects how efficiently information can be exchanged over *G*, considering a parallel system in which each node sends information concurrently along the network. It is defined as (Latora and Marchiori, 2001):

$$E_{glob} = \frac{1}{n(n-1)} \sum_{\substack{i,j \in G \\ i \neq j}} \frac{1}{d_{ij}},$$

where *n* is the number of nodes, and d_{ij} is the geodesic length over all pairs of nodes. In the unweighted network context, the shortest path length d_{ij} is defined as the number of arcs along the shortest path connecting nodes *i* and *j*. In the case of weighted networks, the path with the minimum number of nodes is not necessarily the optimal d_{ij} and it is necessary to define a physical length associated to each arc (this should be a function of the characteristics of the hypothetical link among any nodes *i* and *j*). In this study, it is assumed that the physical length of an arc connecting nodes *i* and *j* is inversely proportional to the strength of the analysed connection (Iturria-Medina et al., 2008), i.e., $l_{ij} = 1/w_{ij}$. Thus, the shortest path length d_{ij} is finally computed as the smallest sum of the arc lengths throughout all the possible paths from node *i* to node *j*. Note that for the particular case of unweighted graphs, $l_{ij} = 1$ for all arcs and the geodesic lengths d_{ij} reduces to the minimum number of arcs traversed to get from i to j.

Local efficiency

The local efficiency (E_{loc}) of G is defined as the average efficiency of the local sub-graphs (Latora and Marchiori 2001):

$$E_{loc} = \frac{1}{n} \sum_{i \in G} E_{glob}\left(G_i\right),$$

where G_i is the sub-graph of the first neighbours of node i. This measure has been used to reveal how much a system is fault tolerant, showing how efficient the communication is among the first neighbours of i when i is removed.

In a physiological sense, the global efficiency of a structural brain network reflects the potential parallel exchange of neural information between the anatomical regions involved (a high global efficiency value, i.e., $E_{glob} \approx 1$, may indicate highly parallel information transfer in the brain system, in which each element node could efficiently send information concurrently along the network). The local efficiency of a structural brain network reflects its potential tendency to present communities or clusters of anatomically and physiologically different regions that deal with common neural information (where regions connected to a same region tend also to link to each other). In addition, concurrent higher values of global and local efficiencies indicate a system with a high balance between local necessities (fault tolerance) and wide-scope interactions.

Statistical analysis of topological measures

A Wilcoxon rank-sum test was used to investigate differences between both groups of participants. This is a nonparametric method to test the null hypothesis (H_0) that medians for each graph network measure are equal. In order to minimize false positive findings (proportion of incorrectly rejected H_0) arising from the high number of tests performed in the topological analysis, FDR correction (at $q = 0.05$) were used to set a critic p-value (Benjamini & Hochberg, 1995; Groppe et al., 2011). All p-values less than or equal to the critic p were significant; suggesting that there is a group difference (reject H_0).

4.4 GREY MATTER RESULTS

4.4.1 Voxel-based morphometry

A between-group voxel-based comparison of the GM volume at whole brain was performed, using the optimized FSL-VBM protocol (Douaud et al., 2007). A two-sample T-test adjusted for covariates was performed to evaluate whether the GM volume means of the two groups differ between them across the whole brain. IQ was included in the statistical model in order to verify that group differences cannot be explained by this nuisance covariate. The group comparison revealed a significant increased GM volume for the bilingual group as compared to monolingual at $p < 0.05$ TFCE corrected for multiple comparisons in a large cluster comprising parts of the right lingual, posterior cingulate (PC) and precuneus gyri. The significant cluster appears in Figure 9 overlaid in a standard brain template and in Table 5.

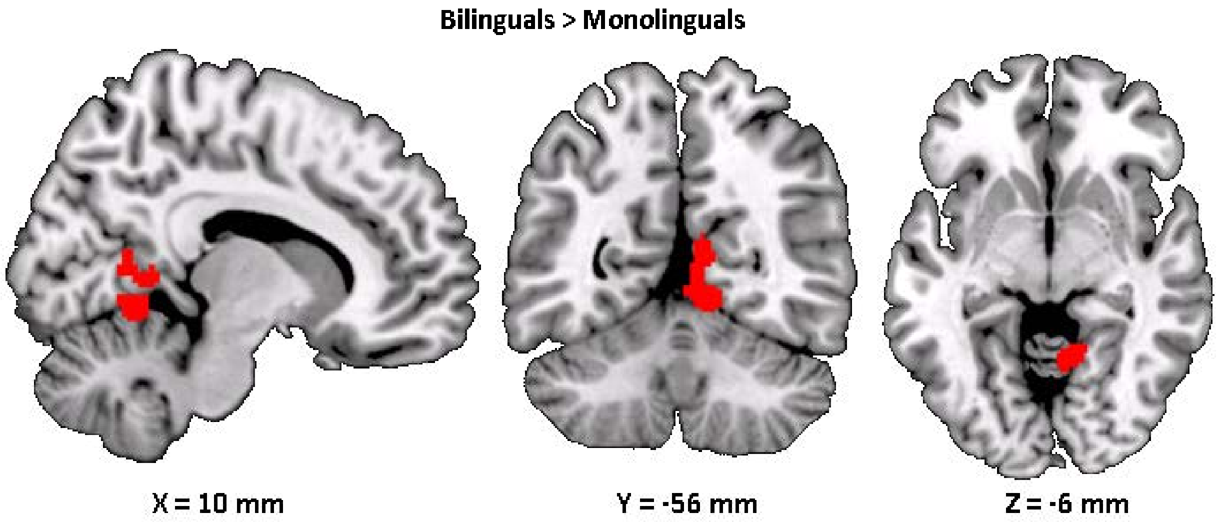

Figure 9. *Brain regions in the right hemisphere showing significant increased GM in bilinguals as compared to monolinguals. P-maps showing significant cluster effect for group comparison of bilinguals > monolinguals at $p < 0.05$ Threshold free cluster enhancement (TFCE) corrected for multiple comparisons. The background image is the MNI (Montreal Neurological Institute) brain template. Slices are taken from the X, Y, Z MNI standard coordinates displaying the value of maximum intensity within the cluster and are showing (from left to right): the sagittal, coronal and axial plane, respectively. The sagittal view represents the right hemisphere and in the coronal and axial views the right hemisphere is on the right side. IQ was used as covariate for the analysis, $K = 10000$ permutations. Bilinguals showed greater GM volume in the right precuneus, posterior cingulate and lingual gyri.*

Table 5. *Maximum peak coordinates within significant cluster effect for contrast Bilingual > Monolingual showing significant increased GM volume at p < 0.05 threshold free cluster enhancement (TFCE) corrected for multiple comparisons.*

Cluster/Regions	Nº Voxels	p-value	X (mm)	Y (mm)	Z (mm)	T-value
R. Lingual/PC/Precuneus	246	0.027	10	-56	-6	4.52

Note: PC, posterior cingulate; X, Y, Z coordinates in Montreal Neurological Institute (MNI) space. K = 10000 permutations, IQ as covariates.

4.4.2 Surface-based morphometry

A between-groups vertex-based comparison of the CT was performed using FreeSurfer. A two-sample T-test adjusted for IQ and non-parametric Monte Carlo testing (10000 iterations) was performed to evaluate whether the CT means of the two groups differ between them at the whole brain. At a $p < 0.05$ cluster-based corrected using Bonferroni, there were no areas where bilinguals had significantly thinner or thicker cortex than monolingual controls.

4.5 WHITE MATTER RESULTS

4.5.1 Tract-based spatial statistic

To investigate the effects of bilingualism in WM structure, a voxel-wise statistical analysis of the FA skeletonized data was carried out using TBSS. A two-sample T-test was performed using permutation non-parametric testing for the two-group comparison. IQ was included in the statistical model as nuisance covariate. The results were fully corrected for multiple comparisons across space using TFCE. The comparison between groups produced no significant effect at $p < 0.05$ TFCE corrected.

4.5.2 Structural Connectivity

The NBS (T-threshold = 3, K = 10000 permutations) was used to detect any connected structural sub-networks that were significantly different between groups. NBS analysis did not show any set (component) of GM regions more highly connected by means of WM tracts between groups at $p < 0.05$ FWE corrected. Neither at a lower initial cluster formation threshold (T-threshold = 2) a significant component was obtained at $p < 0.05$.

Additionally, a pair-wise analysis using FDR correction neither show any isolated pair of regions more connected between groups.

4.5.3 Complex network analysis

This analysis assessed for differences between bilingual and monolingual groups in the measure of integration (E_{glob}) and clustering (E_{loc}). It was conducted to understand how the higher language demands impact on the development of the brain network, for example, in terms of additional axonal cost. For the E_{glob} one single measure was obtained and for E_{loc} one single measure was obtained for each of the 90 GM regions per participant. No statistically significant differences between groups emerged from the topological parameters at a $p < 0.05$. This indicates that both groups have similar patterns in terms of graph-efficiency at both local (a measure of clustering) and global (a measure of integration) scales in the structural network.

4.6 RESTING-STATE FUNCTIONAL RESULTS

4.6.1 Functional Connectivity

The NBS (T-threshold = 3, K = 10000 permutations) was used to detect any connected functional sub-networks that were significantly different between groups. A single sub-network was found to be significantly more connected in bilinguals than in monolinguals at a level of $p < 0.05$ FWE corrected. This subnetwork connected the right Heschl's gyrus with the left amygdala [p(FWE) = 0.048, t-value = 4.52]. Due to the subnetwork only comprise a pair of regions, in order to obtain a stronger focal control for multiple comparisons a pair-wise analysis was performed using FDR correction. FDR is more sensitive to focal effects involving isolated connections, whereas the NBS analysis improves power to detect distributed networks comprising multiple connections. In other words, FDR have the capacity to perform inference at the level of individual connections and the NBS cannot (NBS just allows to control the FWE at the network level). The same pair of regions showed up significantly higher connected in bilinguals as compared to monolinguals [p(FDR) = 0.05, t-value = 4.21]. The significance of the connection between both regions was preserved when subject IQ was included as nuisance covariate.

Heschl’s gyrus is where the primary auditory cortex is located, being a crucial region for auditory processing and language. As will be discussed later, some modifications in Heschl's gyrus have been implicated in bilingualism (Ressel et al., 2012), with expert phoneticians (Golestani et al., 2011) and with learning/producing non-native speech sounds (Golestani et al., 2007; see also Golestani and Pallier, 2007). For its part, the amygdala has been suggested that could play some role in language acquisition since its size have been found that correlate with language skills in healthy (Ortiz-Mantilla et al., 2010) and autistic (Mosconi et al., 2009) children.

4.6.2 Complex network analysis

This analysis assessed for differences between bilingual and monolingual groups in E_{glob} and E_{loc} of the resting-state functional network. Again, for the E_{glob} one measure was obtained per participant and for the E_{loc} one measure was obtained for each of the 90 GM regions per participant. No statistically significant differences between the groups emerged. This indicates that both groups have similar patterns, in terms of graph-efficiency in both local and global scale in the functional network.

4.7 DISCUSSION

The present experiment investigated the neural correlates of bilingualism in GM and WM of early bilingual children who have been exposed to both languages before 3 years old. Crucially, the bilingual group was matched in age and sex with the monolingual control group. At a $p < 0.05$ corrected for multiple comparisons across the whole brain, significantly increased GM volume was found in the right lingual, PC and precuneus gyri in bilinguals. In contrast, no significant increases or reductions in FA values of the WM were found for bilinguals at $p < 0.05$ corrected. The NBS did not identify any sub-networks of nodes/regions differently interconnected by anatomical tracts between groups. But, a pair of region comprised by the right Heschl’s gyrus and the left amygdala was found showing greater functional connectivity at resting state. Finally, no differences between groups were obtained in the topological measures for the whole brain structural or functional networks.

So far this is the first study investigating GM structural plasticity linked to bilingualism in children. This experiment obtained compelling evidence for increased GM volume in regions of the right hemisphere including the lingual, PC and precuneus gyri for bilinguals as compared to monolinguals. These neural correlates indicate brain plasticity and reveal structural features that are linked to bilingualism, but are not compatible with very different proposals from other bilingualism studies (see García-Pentón et al., 2016 for a critical review). However, there are other studies demonstrating that precisely the precuneus is one of the central regions showing higher brain maturation in the growth curve (Dosenbach, 2011) and the regions most highly structurally (Hagman et al., 2008) and functionally (Tomasi and Volkow, 2010) connected in the brain. The precuneus and surrounding posteromedial regions such as the PC cortex are also essential regions in the functional default mode network (Eichele et al., 2008; Leech & Sharp, 2014; Pearson et al., 2011). Some studies have shown that this network undergoes prolonged development during childhood (Fair et al., 2008; Supekar et al., 2010; Thomason et al., 2008; Gao et al., 2015; Gao et al., 2009). Interestingly, precuneus and PC have also shown more deactivation with age increase, suggesting that greater maturation in these regions results in less involvement in cognitive processing (Weiss-Croft & Baldeweg, 2015), which means as well that they could be important regions in development. These are essential association areas (i.e. for the integration of information) especially involved in the regulation of attention that seems to be fundamental in general cognitive functioning (Weiss-Croft & Baldeweg, 2015). Particularly, the precuneus has been implicated in higher-order processes such as voluntary attention shift and more abstract mental imagery tasks, as well as episodic memory retrieval and self-processing tasks (see Cavanna & Trimble, 2006 for a review). Moreover, the precuneus, PC and lingual cortex have shown increased activation in dyslexic children immediately after training in oral language skills, perhaps as a compensatory mechanism (Temple et al., 2003), pointing the involvement of these regions in learning.

Contrary to what could be expected by this finding on the GM, no differences were obtained between bilinguals and monolinguals either in the NBS, or in the topological analysis that would have highlighted these regions as hub regions of the brain networks in bilingual children. Nonetheless, this is not the first time that one study on bilingualism reported the precuneus as targets of neuronal plasticity in children. Pierce et al. (2015) found that Chinese–French bilinguals activated left cingulate gyrus and right precuneus more strongly than the French monolinguals in a phonological working memory task. Thus, in general, these results could indicate that bilingualism might accelerate the maturation into these regions that are crucial for development and learning. However, this is just a neural correlate and does not imply causation (i.e. two events occurring together can not be taken as a cause-effect relationship), thus further complex studies are needed to explain the causes of the relationship between bilingualism and these regions.

In contrast to the GM volume results, there were no significant differences in WM between bilinguals and monolinguals children. In this analysis, no voxel survived the correction for multiple comparisons. However, the studies from Mohades et al. showed difference between bilinguals and monolinguals in the IFOF (Mohades et al., 2012; Mohades et al., 2015 longitudinal study with the same population) and CC (Mohades et al., 2012). The authors used a TOI approach. And in our study, a voxel-based approach was taken, that has the advantage of investigating brain differences at the whole brain level without limiting the search to specific brain tracts or regions, but has the disadvantage of a decrease in sensibility. There has been much debate about the use of voxel-based or ROI-based approaches (Good et al., 2001) and the relevant issues were discussed throughout the first part of this dissertation (Chapter 2). However, at a lower uncorrected threshold of significance ($p < 0.001$) decreased local FA values were found in bilinguals covering a small area (only 5 voxels) in the left IFOF. This effect was neglected because it was found to be non-significant across the whole brain and because a minimum of 25 voxels did not surpass the statistical threshold. However, this trend in the IFOF are favouring a decrease rather than an increased in bilinguals, which is not in line with the prior results in children (Mohades et al., 2012; Mohades et al., 2015). On the other hand, although the mean age of the samples of both studies (Mohades et al. and the current study) was not so different, notice that for this current experiment were used a carefully matched bilingual and monolingual children groups in age and sex, which is not the case in Mohades et al.. When participants are properly matched, small size effects tend to disappear (Paap & Greenberg, 2013), especially in small sample studies like these. Thus, given that these studies are not comparable in many aspects, we believe that more studies are needed to confirm the early effects of bilingualism on the IFOF.

The NBS analysis showed no differences in the structural connectivity patterns between bilinguals and monolinguals. However, NBS identified a pair of regions: the right

Heschl's gyrus (primary auditory cortex) and the left amygdala higher functionally connected in bilingual children as compared to monolinguals. This result suggests that early bilingualism might produce higher correlated activation between both regions perhaps as a compensatory mechanism of learning two languages. Interesting, Ressel et al. (2012) manually extracted the mean volume from the right and left Heschl's gyri to compare bilinguals and monolinguals, and they obtained significantly larger volumes in bilinguals. Additionally, there are other prior studies showing higher WM density in the left Heschl's gyrus (Golestani et al., 2007) for faster learners as compared to slower learners of non-native speech sounds, suggesting that WM differences across individuals could predict behavioural differences in some aspects of language learning. Later on, they investigated the Heschl morphology on an expert phonetician population (Golestani et al., 2011) and also found more WM in the right Heschl's gyrus for experts than non-experts individuals. However, they found that Heschl morphology did not correlate with the years of expert training. Thus, they suggested that experience during adulthood cannot modify Heschl, but rather that differences are established *intra utero* and can predispose individuals for the selection and acquisition of the expertise later in life. The role of the amygdala in language and specifically in bilingualism is unspecified and has yet to be elucidated. In the context of language processing the amygdala has been considered a region for processing the emotional aspects of word and phrases (Binder and Desai 2011), but in general is one of the regions most commonly linked to the processing of emotional significance of any type of stimuli (Olson, 2007). Thus, it is well-known that is strongly implicated in emotional learning and memory (Phelps and LeDoux, 2005). Additionally, there are studies showing a functional lateralization of the amygdala in language. The left amygdala have been shown more activated than the right amygdala when processing positive and negative emotional words as compared to neutral words (Hamann and Mao, 2002). Importantly, a relationship has been found between its size and language skills in adults and children with autism (from 3 to 4 and 6 years old): larger left amygdala correlated with better language skills and larger right amygdala and also smaller left amygdala correlated with poorer languages, communicative and social skills (Haznedar et al., 2000; Munson et al., 2006). Additionally, in healthy children larger left amygdala (measured at 6 months of age) also correlated with higher language abilities (Ortiz-Mantilla et al., 2007) and larger right amygdala correlated with lower language scores (measured at 2, 3 and 4 years old) (Ortiz-Mantilla et al., 2010). All these suggested that, specially, left amygdala it could play some role in the acquisition of language. Importantly, Hernandez (2009) observed higher bilateral fMRI activation of the amygdala in bilingual adults when they had to name pictures in their native language as compared to naming in their L2. And an increased activity of the right amygdala have been also found in bilingual children (11 year old) preforming a word-reading task in their native language as compared to their L2 (Owens et al., 2009). Our results therefore suggest that the increased correlated activity in the right Heschl's gyrus and left amygdala in the bilingual children as compared to monolinguals might be related to the extra demands in the acquisition of both languages. Notably, although functional connectivity changes were obtained, no plastic changes in the structural network were observed, suggesting that functional plasticity corresponds to dynamic and phasic changes that occur even before (slow) structural changes can be detected.

In summary, the present study suggests that early bilingualism may modify the brain during development, producing changes in GM volume of some regions (i.e. precuneus, posterior cingulate, lingual), supporting the important role of these regions in learning and development. Bilingualism maybe accelerates the normal maturation of these regions and uses them as compensatory mechanisms due to the intense use of both languages. In addition, early bilingualism may also yield higher functional connectivity between regions that are important for auditory and language processing (Heschl's gyrus and amygdala), probably to compensate the difficulty of learning and integrating two languages.

Chapter 5: Experiment II. Structural Brain Changes in Elderly Bilinguals

5.1 INTRODUCTION

Cognitive decline in the elderly may be due to multiple neurobiological factors: atrophy of the grey matter of different brain regions, lack of integrity of the white matter that causes disruption of connections between the regions of grey matter, reduction of vascular integrity and depletion of neurotransmitter systems (Raz & Kennedy, 2009; Brookmeyer et al., 2007). All these neurodegenerative changes may result in significant cognitive impairment. However, a significant number of people with brain deterioration still have normal cognitive functioning (Christensen et al., 1999; Kemper et al., 1994; Duarte et al., 2006; Lindenberger & Ghisletta, 2009). In fact, the theory of cognitive reserve holds that some variables improve the brain's ability to deal with damage, mitigating its effects on cognitive functioning (Stern, 2002; Stern, 2009). Variables that seem to enhance the quality of life of older people include, for example, education, occupation, intelligence, socioeconomic status, aerobic fitness and music (Albert et al., 1995; Christensen, 2001; Hillman et al., 2008; Hanna-Pladdy and MacKay, 2011; Steffener et al, 2012; Stern, 2009).

It has been proposed that cognitive reserve is supported by two neural mechanisms: neural reserve and neural compensation (see Stern, 2009). Neural reserve refers to brain network capacity that allows people to cope with increased environmental demands; this brain capacity can differ in its expression as a function of its efficiency and capability. Any condition that affects brain functions can affect the capacity and efficiency of the brain network, including normal aging (Achard and Bullmore, 2007). On the other hand, neural compensation is based on the existence of alternative brain networks (or set of brain regions) that would be recruited to compensate for the loss of cognitive efficiency and/or brain structures vulnerable to brain atrophy in aging or neurodegeneration in diseases.

The hypothesis that bilingualism enhances cognitive reserve, protecting the brain against age decline in normal and pathological aging has recently emerged (Bialystok et al., 2007; Bialystok, 2012). The belief in bilingualism as brain protective factor is based mainly on retrospective studies in which monolingual and bilingual patients diagnosed with dementia are compared on age of symptom onset; showing that bilinguals display symptoms of dementia significantly (4-5 years) later than monolinguals (Alladi et al., 2013; Bialystok et al., 2007; Bialystok et al., 2014 Chertkow et al., 2010; Craik et al., 2010; Gollan et al., 2011; Woumans et al., 2015) and mild cognitive impairment (MCI; Ossher et al., 2013; Bialystok et al., 2014). Also bilingual patients who have suffered brain stroke have shown significantly better cognitive recovery following stroke as compared to monolinguals (Alladi et al., 2016).

The first study suggesting that bilingualism improved cognitive reserve was Bialystok et al. (2007). They investigated 184 patients diagnosed with Alzheimer’s disease and found that bilinguals (93 of the patients) showed the symptoms four years later than monolingual patients (Bialystok et al., 2007). Craik et al. (2010) studying 211 patients replicated the delay in the diagnosis of Alzheimer’s disease for bilingual as compared to monolingual. Chertkow et al. (2010) studied 632 patients showing that in non-immigrant bilinguals the effect was not significant, but for immigrant multilingual they found approximately 5 years of delay of Alzheimer’s diagnosis as compared to monolinguals, which suggested that the immigrant status of the patients in previous studies could interact with the bilingual condition. However, another large-scale data sample study with 648 participants, demonstrated that simultaneous bilingual patients developed dementia 4.3 years later than monolinguals (Alladi et al., 2013) and when only illiterate participants were considered, the delay of symptoms increased to 6 years for bilinguals. In the same way, Gollan et al. (2011)’s study found that higher degrees of bilingualism were associated with a delay in the diagnosis only in bilinguals with low educational level. In general, greater benefits were found for less-educated groups than well-educated (Alladi et al., 2013; Chertkowet al., 2010; Gollan et al., 2011). See also other results reporting benefits in cognitive measures (Bak et al., 2014).

It is important to note that, the samples under study included immigrants as well as non-immigrants, educated as well as illiterate individuals, with high and low socio-economic background. Additionally, the samples include early as well as late bilinguals and the patterns of language use varied across these studies, from context predominantly monolingual to highly multilingual characterized by frequent language switching and mixing. Importantly, when some of these secondary factors are taken into account several studies failed to replicate the delays in bilinguals the symptoms of dementia (Chertkow et al., 2010, Crane et al., 2009; Gollan et al., 2011; Sanders et al., 2012; Clare et al., 2014; Zahodne et al., 2014; Kowoll et al., 2015; Lawton et al., 2015). Thus, the results of these studies are extremely controversial and required caution about the assumption of the neuro-protective effects of bilingualism (Calvo et al., 2016; Costa & Sebastián-Gallés, 2014; Paap, Johnson & Sawi, 2015a; 2015b).

The hypothesis of bilingualism as cognitive reserve variable predicts that greater benefits are expected with more years of active bilingualism (Bialystok et al., 2016). Given that older adult early bilinguals have longer lifetime experience of active bilingualism (i.e. higher number of years continually selecting between two languages at any social context) it might be expected they are more trained experts in language selection mechanisms and, by extrapolation, in general cognitive executive functions, boosting brain plasticity (e.g. increasing grey and/or increased/decreased white matter volume) in brain regions related to EFs or language processing. There is the idea that bilingualism might stimulate in some way these brain areas, which become more resistant to brain atrophy or pathology. However, the neural correlates of this neuroprotective effect of bilingualism in aging are still unclear.

Few neuroimaging studies have been trying to search into this matter. The study of Schweizer et al. (2012) tries to provide evidence in support of the protective effect of bilingualism on the brain in pathological aging populations. They matched one group of bilinguals with one group of monolinguals on cognitive level and clinical severity of Alzheimer’s disease (also matched in demographic variables). They reported comparable cognitive performance in memory tests between bilingual and monolingual Alzheimer’s patients, despite significant greater GM atrophy shown in medial temporal lobe for the former, using linear measurements derived from computerized tomography scans. In other words, despite having greater medial temporal lobe atrophy, the bilingual individuals with Alzheimer performed in the same manner on neuropsychological testing as their monolinguals peers with less brain atrophy, hence, compensating for the greater atrophy.

This study, however, is controversial, because it is possible that the observed group differences in brain atrophy, irrespective of the neuropsychological performance of both groups, could be attributable to other factors than bilingualism. For example, in this study the bilingual group had more immigrants than the monolingual group, a condition that also could interact with bilingualism. Additionally, the groups were not matched in some confounding variables (i.e. monolingual group had significant higher occupational and educational level than the bilinguals). As mentioned above, these are also important cognitive reserve variables (Stern, 2009) that could explain the less atrophy observed in the monolingual group, which interferes with the hypothesis of the study about the brain protective effect of bilingualism. Furthermore, Bilinguals under this study had been used two languages regularly since childhood, with a variety of languages being included in the group. So, doubts remain about which factors apart from bilingualism produced these group's brain differences.

Another structural study (Gold et al., 2013) using DTI measures compared healthy older bilingual adults with matched monolingual controls and the former showed lower FA values in posterior parts of the IFOF and the CC (in areas of these tracts that presumably connect regions related to memory processing). These authors reported that bilinguals performed equally in different memory tests to monolingual controls, despite showing lower FA values, something that they interpret as less integrity of the WM. However, we are far from understanding how increased/decreased WM or GM is related with a function or behaviour. Specifically, in FA studies, decreasing axonal density, increasing axonal caliber or decreasing the degree of myelination should all reduce the FA values in the white matter. But despite the extensive use of this measure in many fields of neuroscience, any differences in this value should not always be associated with or interpreted in terms of WM tissue 'integrity'. Different fiber configurations and variations in these configurations can produce different modifications in this measure (Jone et al., 2013). In any case, these two aforementioned studies failed to demonstrate that bilingualism is a neuroprotective factor of the brain.

There are three more neuroimaging studies suggesting the neuroprotective effect of bilingualism. Luk et al. (2011) found a different pattern than Gold et al. (2013) (an increase rather than a decrease) but in different parts of the same tracts. Luk et al. (2011), unlike Gold et al., found that bilingual elderly had increased FA in anterior parts of IFOF and CC (those parts of the tracts that might connect frontal regions). Additionally, Abutalebi et al., comparing Chinese older adult bilinguals with Italian monolinguals obtained significant volume increases for bilinguals in the aITG (Abutalebi et al., 2014) and in the left/right ACC (Abutalebi, Guidi et al., 2015) (see section 2.3 for details about these studies). However, images of bilinguals and monolinguals in Abutalebi et al. studies were acquired with different scanners, which could explain some difference obtained between the groups. In general, these three studies interpreted these focal increased in WM and GM as a protective effect of bilingualism in the brain.

In sum, the structural findings so far described are scarce, inconsistent and more importantly, not determined what neural mechanisms engage these neuroprotective effects of bilingualism. The aim of this experiment is to investigate the brain structural bases of bilingualism in aging, especially, to determine the neural mechanism by which bilingualism confers its advantage as a neuroprotective factor (i.e. by neural reserve and/or neural compensation). The idea is that bilingualism will make the cognitive system more efficient in the use of their cerebral resources and this fact should be easily captured in the brain configuration and the topological parameters of the brain network, perhaps developing higher efficient specialized subnetworks that will help to compensate the normal brain deterioration in aging. Thus, this study will try to identify differences in the organization and capability of the whole structural brain networks in elderly lifelong bilinguals as compared to monolinguals. To assess this spatial configuration and properties of the brain, structural networks based on DW-MRI will be created (Gong et al., 2009; Hagmann et al., 2008; Iturria-Medina et al., 2007, 2008). To explore the differences in the configuration and topological parameters of the networks, NBS (Zalesky et al., 2010) and a complex network analysis (Latora & Marchiori 2001; Watts & Strogatz 1998) will be used, respectively.

In fact, there are already some studies using complex network analysis based on graph theory approach, to measure differences between Alzheimer's disease patient and control groups. These studies have reported some alteration in parameters of brain networks derived from different neuroimaging modalities (such as fMRI, EEG and T1-MRI). In general, these studies found that the small-world characteristic of the whole brain network is reduced in Alzheimer's disease (see Bullmore and Sporns, 2009 for a review). The small-world property is a tradeoff between shorter path length among nodes (which means higher global graph-efficiency) and a higher clustering (which means higher local graph-efficiency) of the network. In detail, one study showed that clustering was significantly reduced in the entire network for Alzheimer's patients (Supekar et al., 2008), while other showed that path length was increased (Stam et al., 2007) and two of them showed abnormal topological configuration for nodes/regions considered important hubs in the brain (He et al., 2008, Stam et al., 2008), which also affect the small-world attribute of the brain graph-network.

There are other studies with healthy older populations showing that older people had reduced global/local graph-efficiency of the brain functional networks than younger people, which means that small-world attributes of the brain functional network (small-world attributes have been found in almost any biological network), are affected by normal aging (see Archad and Bullmore, 2007). Based on these previous studies and following the notion of bilingualism as brain protective factor, could be also expected that the whole structural/functional brain network in elderly lifelong bilinguals will show increased global/local graph efficiency than in monolinguals.

In the current study rfMI technique in combination with DW-MRI and T1-MRI, will be used to study structural/functional brain plasticity related to lifelong bilingualism. This neuroanatomical approach is followed in order to explore task-independent differences between bilinguals and monolinguals. This combination of methods has also the advantage of allowing large-scale network analysis, by mapping functional/structural brain networks at the whole-brain level. There are only two resting-state functional studies comparing elderly bilinguals and monolinguals (Luk et al., 2011 and Grady et al., 2015), but these studies did not investigate the large-scale functional brain network. Thus, modifications in the structural/functional connectivity of the whole brain associated with bilingualism and topological properties of the brain network have not been yet investigated in elderly. Luk at al. (2011), performed a resting-state functional connectivity analysis taking as seeds the regions of GM adjacent to the cluster showing higher FA values for bilinguals in the prior TBSS analysis (see section 2.3) and which they considered important for language switching (i.e. right/left IFG). This analysis showed stronger functional connectivity between left IFG and posterior brain regions (i.e. with MTG, right IPL, precuneus, middle occipital gyri and left caudate) for bilinguals as compared to monolinguals. In contrast, monolinguals showed a different connectivity pattern, showing higher connectivity between left IFG and other frontal regions. For its part, Grady et al. (2015) investigated functional intrinsic connectivity during resting-state and also during a task-related paradigm. They focused in two brain subnetworks that are typically engaged in EF: the fronto parietal control (FPC) network and the salience network (SLN), but also study the default mode network (DMN) (see section 2.4 for details about this study). They found stronger intrinsic functional connectivity in the FPC network and DMN in bilinguals than in monolinguals. Bilinguals also showed stronger correlations than monolinguals between intrinsic connectivity in the FPC network and the task-related increases of activity in prefrontal and parietal regions; suggesting greater anterior-posterior functional connectivity in bilinguals as Luk et al. study.

In sum, the current study attempt to identify local GM differential structural patterns associated with bilingualism by investigating a group of elderly lifelong Basque-Spanish bilinguals and a group of well-matched Spanish monolingual controls using voxel-based and surface-based morphometry. Additionally, local WM structural differences will be investigated by means of DTI-derived measurements using TBSS approach and structural/functional connectivity measures will be calculated to obtain large-scale brain network connectivity maps. In order to determine differences in connectivity patterns between both groups, a NBS approach was followed by isolating sets (subnetworks) of highly interconnected regions, providing information about whole-brain structural and functional organization (Zalesky et al., 2010a; see also García-Pentón et al., 2014, for a similar approach with young adults). Finally, a brain complex network analysis will be used to assess the topological properties of the networks in elderly bilinguals and monolinguals.

5.2 PARTICIPANTS

Thirty-four seniors in total who lived in the Basque Country were selected for this experiment (age range from 64-78, mean age, 69.35 years, 4.01 std). Participants were healthy people with no reported history of neurological/mental illness and/or treatment with psychotropic medication and all of them had normal or corrected-to-normal vision. All participants gave verbal and written informed consent prior to involvement, in accordance with the Declaration of Helsinki, and the research protocol was approved by the BCBL Ethics Committee.

The first group comprised 17 Basque-Spanish bilinguals (10 females, age range from 64 to 78 years, mean age, 69.41 years, 4.08 std). The second group comprised 17 Spanish monolinguals (10 females, age range from 64 to 78 years, mean age, 69.29 years, 4.07 std). They were carefully paired in age and sex (see Table 6). The bilingual group used both languages every day and rated themselves as mid-to-high proficient in both languages (mean rates: 8.83, 1.02 std for Spanish and 7.70, 1.48 std for Basque) on a scale from 1 (very poor level) to 10 (perfectly fluent). Information about language profile was obtained by means of a questionnaire before the experiment. The ratings were based on reading, writing, listening and speaking skills. The AoA of the L2 (Spanish) ranged from 0 to 11 years old. All bilinguals have a lifelong bilingualism index (LBI)[2] above 91.48% (i.e. they have been bilingual 91.84% of their lives) (see Table 7 for details about all these measures). The monolinguals used only Spanish for daily life and had no/little knowledge of Basque (proficiency mean rate: 9.47, 0.87 std for Spanish and 2, 1.41 std for Basque) or any other language (see Table 7 for participants' language profile). Only 1 monolingual had been in contact with Basque but received little exposure and rated themselves as very poorly proficient in Basque (AoA = 15 years old; proficiency rate = 3).

The groups were not significantly different in mean years of study and Mini-Mental State Examination Test (MMSE) scores (see table 8). The study contained only right-handed old adults. IQ scores were measured with the Spanish version of the Kaufman Brief Intelligence Test (K-BIT) and were controlled as nuisance covariate.

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[2] This index represents an estimation of the amount of active exposure to both languages as a function of the age and is calculated from the formula: LBI = 100–(AoA L2*100/Age). In this way, both lately acquired bilingualism and short periods of active use of the two languages are represented by lower values, while early bilingualism and extensive use of the two languages get higher scores.

Table 6. *Age in years, gender* and *IQ of participants (F for feminine, M for masculine).*

Bilingual group (N = 17)				**Monolingual group (N = 17)**			
Age	**Gender**	**IQ Composite (standard scores)**	**MMSE**	**Age**	**Gender**	**IQ Composite (standard scores)**	**MMSE**
66	F	123	29	66	F	123	29
69	M	119	29	69	M	117	27
68	F	107	30	68	F	120	28
76	M	131	30	76	M	87	28
70	F	117	29	69	F	102	29
75	F	90	28	75	F	85	28
67	F	118	30	67	F	87	29
78	M	98	27	78	M	99	30
71	F	85	30	71	F	86	30
73	M	103	29	73	M	110	30
70	M	125	26	69	M	110	29
68	M	92	29	68	M	84	28
65	F	123	30	65	F	95	28
64	F	122	30	64	F	89	30
69	F	120	28	69	F	113	26
66	F	125	28	66	F	83	28
65	M	133	29	65	M	105	30

Note: N, sample size. F, female. M, male. IQ, intelligence quotient. MMSE, Mini-Mental State Examination scores.

Table 7. *Language profile of monolingual and bilingual groups.*

	Mean (±SD)	
Variables	**Monolinguals** **N = 17 (10 female)**	**Bilinguals** **N = 17 (10 female)**
AoA of Spanish	0.0(±0.0)	5.94(±2.92) (L2)
AoA of Basque	-	0.0 (±0.0) (L1)
LBI (%)	4.58(±18.90)	91.48(±3.97)
Spanish proficiency	9.47(±0.87))	8.83 (±1.02)
Basque proficiency	2(±1.41)	7.70 (±1.48)
Other language proficiency	-	-

Note: N, sample size. AoA, age of acquisition (the age at which participants started to learn these languages). LBI, lifelong bilingual index. SD, Standard deviation. Proficiency was measured on a scale from 1 (very poor level) to 10 (perfectly fluent).

Table 8. *Group Means and SDs for Demographic & Neuropsychological Measures.*

	Monolinguals **N = 17 (10 female)**		**Bilinguals** **N = 17 (10 female)**	
	Mean	**SD**	**Mean**	**SD**
Age at completion of studies	16.706	4.1798	18.88	5.7758
MMSE (raw score)	28.65	1.17	28.88	1.17
General IQ (percentile)	99.70	13.84	113.58	14.84

Note: N, sample size. MMSE, Mini-Mental State Examination score. SD, Standard deviation. IQ, intelligence quotient.

5.3 GREY MATTER RESULTS

5.3.1 Voxel-based morphometry

A between-group voxel-based comparison of the GM volume at whole brain level was performed, using the optimized FSL-VBM protocol (Douaud et al., 2007), as described in section 4.3.2.1. A two-sample T-test adjusted for covariates was performed to evaluate whether the GM volume differ between the two groups across the whole brain. IQ was included in the statistical model in order to verify that group differences cannot be explained by this nuisance covariate. Two contrasts were performed: Bilinguals > Monolinguals and Bilinguals < Monolinguals. The group comparison revealed no significant difference between bilinguals and monolinguals at $p < 0.05$ TFCE corrected for multiple comparisons. These finding suggested that lifelong bilingualism did not produce any effect in the GM for the elderly group.

5.3.2 Surface-based morphometry

A between-group vertex-based comparison of the CT was performed using FreeSurfer (see section 4.3.2.2). A two-sample T-test adjusted for IQ was performed to evaluate whether the CT means of the two groups differ between them across the whole brain. At a $p < 0.05$ corrected for multiple comparisons, there were no areas where bilinguals had significantly thinner or thicker cortex than monolingual peers.

5.4 WHITE MATTER RESULTS

5.4.1 Tract-based spatial statistic

To investigate the effects of bilingualism in WM structure for the senior's group, a voxel-wise statistical analysis of the FA skeletonized data was also carried out (see the procedure described in section 4.3.2.4). A two-sample T-test was performed using permutation non-parametric testing for the group comparison. Two contrasts were performed: Bilinguals > Monolinguals and Monolinguals > Bilinguals. IQ was included in the statistical model as nuisance covariate. The results were fully corrected for multiple comparisons across space using TFCE. The comparison between groups produced no significant effects. At $p < 0.05$ TFCE corrected, no significant increases or reductions in local FA values were found in lifelong bilinguals.

5.4.2 Structural Connectivity

The NBS (T-threshold = 3, K = 10000 permutations) was used to detect any connected sub-networks that were significantly different between groups. Groups did not show any set of GM regions (subnetwork or component) more highly interconnected (at $p < 0.05$) by means of WM tracts. Even at a lower initial cluster formation threshold (T-threshold = 2) any significant component was obtained. A pair-wise analysis using FDR correction neither show any isolated pair of regions more connected between groups at $p < 0.05$.

5.4.3 Complex network analysis

This analysis assessed for differences between bilingual and monolingual groups in the measure of integration (E_{glob}) and clustering (E_{loc}) of the structural network. A significantly higher E_{glob} of the whole network ($p < 0.05$) was obtained for bilinguals as compared to monolinguals (see Table 9). This indicates that in the bilingual group the information flows more efficiently than in monolinguals. E_{glob} represents the efficiency of a parallel system, which means how efficiency the system exchanges concurrently information (Latora and Marchiori, 2001). This result is perhaps a positive consequence of the greater language demands during their lifetime. For E_{loc} measure no statistically significant differences between groups survived after FDR correction for multiple comparisons (at q = 0.05).

Table 9. *Global graph-efficiency (E_{glob}) comparison results between bilingual (N = 17) and monolingual (N = 17).*

Groups	**E_{glob} Mean (± SD)**
Bilingual	0.0802 (±0.01406)
Monolingual	0.0637 (±0.0162)
Z-value	2.57
P-value	0.01

Note: SD, standard deviation. The P-values correspond to the null hypothesis (H_0) that medians are equal. $P \leq 0.005$ suggests there is a group difference (reject H_0).

5.5 RESTING-STATE FUNCTIONAL RESULTS

5.5.1 Functional Connectivity

NBS (T-threshold = 3, K = 10000 permutations) was used to detect any functional sub-networks that were significantly different connected between groups. Groups did not show any set of GM regions (subnetwork/component) more highly interconnected at $p < 0.05$. Even at a lower initial cluster formation threshold (T-threshold = 2) no significant component was obtained. Additionally, a pair-wise analysis using FDR correction did not show any isolated pair of regions significantly different connected between the groups at $p < 0.05$.

5.5.2 Complex network analysis

This analysis assessed for differences between elderly bilingual and monolingual groups in E_{glob} and E_{loc} of the resting-state functional networks. No statistically significant differences between the groups emerged at $p < 0.05$. This indicates that both groups have similar patterns in terms of graph-efficiency at both global and local scales in the functional network.

5.6 DISCUSSION

A well-matched elderly Basque-Spanish bilingual group, comprised by lifelong bilinguals who have been bilinguals for more than 91% of their lives, were compared with Spanish monolinguals counterparts. These participants were presumably in a declining process due to normal aging, but their cognitive abilities were at normal levels according to the scores obtained in the MMSE (see Folstein, Folstein & McHugh, 1975). Participant's scores were above 26 (mean = 28.76; std = 1.16) and did not differ between groups. Results showed no focal structural brain differences between bilingual seniors and monolingual peers, either in GM or in WM. However, the graph theoretical analysis conducted to examine global and local graph network efficiencies showed that the graph-efficiency of the whole structural network increased for bilinguals as compared to that for monolinguals. These results suggest more efficient parallel information transfer between all nodes in the whole brain, which could mean a more optimized general configuration of the whole network. In functional terms, the more optimized network could benefit higher cognitive processes (for example, executive functioning) that might require greater efficiency in transferring parallel information across all regions in the brain (Bassett et al., 2009; Bullmore and Bassett, 2011; Li et al., 2009).

It was expected that the potential differences between bilinguals and monolinguals would be salient in the elderly, given their lifelong bilingualism. Contrary to what was expected, however, the elderly groups did not show focal differences in GM/WM structural measures even when the bilinguals under study were early bilinguals who have been immersed in a dense-code switching interactive bilingual context for almost their whole life and the languages in this bilingual context (Basque and Spanish) are extremely typological distant, which might imply that the cost of dealing with these two languages might affect the brain more. Rather, these results indicate that brain regional structural differences related to lifelong bilingualism, if exist, are small and difficult to detect.

In this sense, the complex network analysis was very helpful. This analysis suggested that even when no local/focal differences were observed in the brain, the consequence of lifelong bilingualism could bring a global benefit to the brain, given the increased global graph-efficiency gained in the entire structural network of bilinguals as compared to monolinguals. Taking together this topological result of the brain in lifelong healthy bilinguals and the results from preceding studies demonstrating differences in the brain network in Alzheimer's disease (AD) as compared to controls, some interesting links could be suggested. AD seems to imply a reduced *small-world* property of the brain network (Bullmore, 2009). The reduced small-word (economy) attribute of the network is related to the increased *path length* and decreased *clustering* (Supekar et al., 2008; Stam et al., 2007; He et al., 2008; Stam et al., 2008). These two measures are directly related to the loss in the *global/local graph-efficiency* of the network, respectively, since increased path length yields less global efficiency and decreased clustering yields less local efficiency. Thus, the less small-world characteristic of a network can also be defined as having less global and local graph-efficiency (Achard and Bullmore, 2007). Interestingly, complex network analyses in healthy older populations showed that small-world attributes of the functional brain network are affected by normal aging (i.e. older people had reduced global/local graph-efficiency of the brain functional network than younger people) (see Archad and Bullmore, 2007). Accordingly, the increased global graph-efficiency of the structural network in the bilingual group tested here could be compatible with the idea of a lesser degree of cognitive decline in bilingual population and a better prognostic of AD in bilinguals (see Bialystok et al., 2016 for a review). However, there is poor neural evidence supporting this claim (see García-Petón et al., 2016), and although the current study provides some potential explanation for how bilingualism might act as a brain protective factor, caution is required due to the small sample size tested here and given that the global network graph-efficiency is the only significant difference. No other differences on structural or functional measures were found. Thus, this result needs to be replicated.

Summarizing, the analysis of the brain did not show local or regional grey/white matter structural differences associated to lifelong bilingualism as was expected. But lifelong elderly bilinguals showed increased global graph-efficiency of the whole brain structural network as compared to their monolingual peers; providing new evidence that might explain how bilingualism could protect the brain against cognitive decline in normal aging.

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Chapter 6: A Comprehensive Study of Bilingualism Across Children and Seniors

6.1 INTRODUCTION

A more detailed analysis was performed with both age groups together in a fully comprehensive statistical model in order to increase power and see what differences survive across groups. In all the subsequent analyses, a 2 × 2 between-subject factors analysis of covariance (ANCOVA) was performed. The ANCOVA included two factors: Language-Profile (levels: bilinguals and monolinguals) and Age-Group (levels: children and elderly), adjusted for a covariate (IQ).

6.2 PARTICIPANTS

The same participants from Experiments I and II were included.

6.3 METHODS

The procedure followed for the analyses was the same that the followed in the Experiments I and II.

6.4 GREY MATTER RESULTS

6.4.1 Voxel-based morphometry

A mass univariate GLM was used, corresponding here to a two-way (2 × 2) between-factor ANCOVA adjusted for IQ as nuisance covariate, and found a significant Language-Profile by Age-Group interaction effect at $p < 0.005$ corrected for multiple comparisons using TFCE. One large cluster comprised an extended region in the right hemisphere including the Lingual/PC/Precuneus (see Table 10 and Figure 10). The post-hoc comparisons revealed that interaction effect in the right lingual/PC/precuneus was driven by the group of children. The children group showed a significantly increased GM volume for bilingual as compared to their monolinguals peers (see Table 10 and Figure 10).

Table 10. *Significant simple effect of Language-Profile in children showing increased GM volume in bilinguals as compared to monolinguals. The table is showing as well the interaction effect.*

Cluster Region	Voxels	P value	X, Y, Z (mm)	T value	***Interaction*** *Voxels*	*P value*	*X, Y, Z (mm)*	*T value*
R.Lingual/PC/ Precuneus	1058	0.001	12, -58, -8	4.71	*1235*	*0.007*	*2, -36, -8*	*4.9*

Note: PC, posterior cingulate; R, right; L, left; Voxels = number of voxels in the cluster; P≤0.005 threshold free cluster enhancement (TFCE) corrected. X, Y, Z = coordinates in Montreal Neurological Institute (MNI) space of local maxima. K = 10000 permutations. IQ as nuisance covariate.

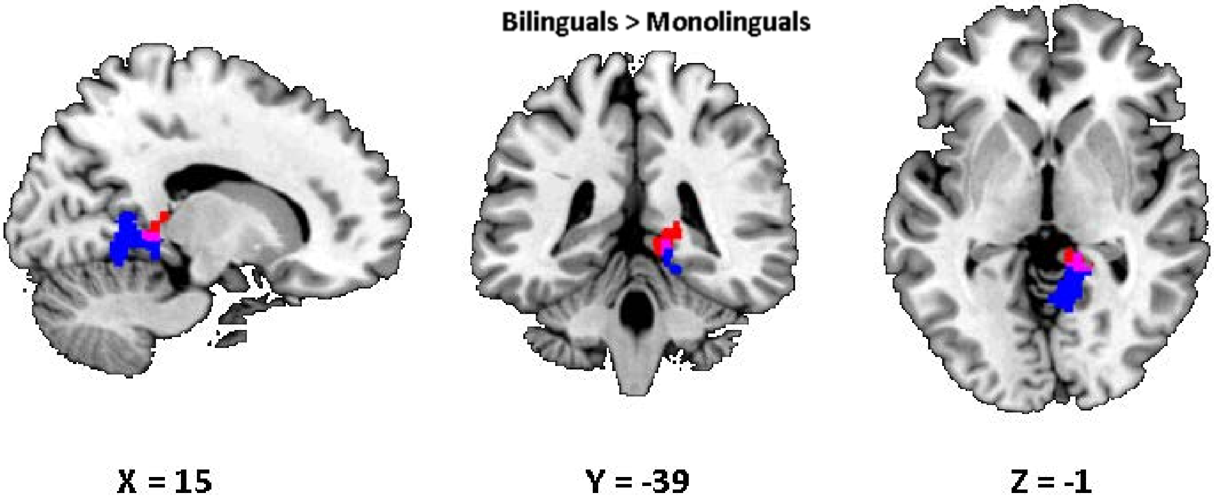

Figure 10. *Brain regions in the right hemisphere showing significant increased GM in bilinguals as compared to monolinguals. Significant cluster effects at p < 0.01 threshold free cluster enhancement (TFCE) corrected. Red: Language-Profile by Age-Group interaction effect. Blue: Simple effect Bilinguals > Monolinguals for children. Purple: Overlay between the interaction and the simple effect. Background image is the MNI (Montreal Neurological Institute) brain template. Slices are taken from the X, Y, Z MNI standard coordinates (in mm) displaying the maximal overlay between the interaction and the simple effect, and are showing (from left to right): the sagittal, coronal and axial plane. Sagittal view represents the right hemisphere. In the coronal and axial views the right hemisphere is on the right side. IQ as covariate, K = 10000 permutations. Bilinguals showed greater GM volume in the right precuneus, posterior cingulate and lingual gyri.*

6.4.2 Surface-based morphometry

In the vertex-based analysis of the CT, the 2 × 2 between-subject factor ANCOVA showed two significant interactions between factors (Language-Profile by Age-Group) effects at $p < 0.05$ corrected for multiple comparisons using Bonferroni correction. These interactions were observed in the right Precuneus extending into the lingual and PC (peak in MNI space [4.8 -58.1 22.1], $p = 0.013$, cluster size = 1298 vertices) and right Postcentral gyrus (peak in MNI space [20.1 -32.2 59.1], $p = 0.031$, cluster size = 1307 vertices). The post-hoc comparisons revealed that the interaction effects was driving by the children group, showing a significant thinner CT in bilinguals as compared to monolinguals peers in the precuneus (peak in MNI space [8.4 -60.7 39.8], $p = 0.006$, cluster size = 3764 vertices) and a significant thinner CT in monolinguals as compared to bilinguals in the postcentral (peak in MNI space [17.8 -32.5 59.0], $p = 0.020$, cluster size = 1440). See results in Figure 11.

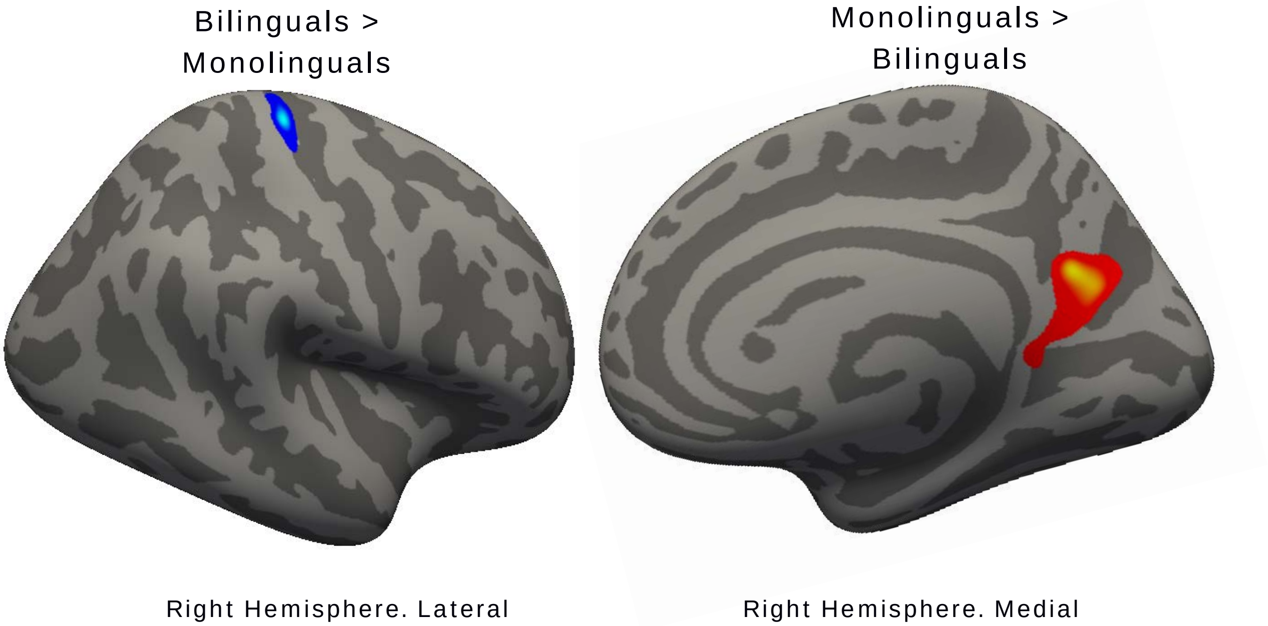

Figure 11. *Brain region on the right hemisphere showing significant increased/decreased cortical thickness (CT) in bilinguals as compared to monolinguals. ANCOVA results showing significant Language-Profile by Age-Group interaction effects at $p < 0.05$ Bonferroni corrected. Blue: Bilinguals > Monolinguals interaction driven by the group of children in the right postcentral. Red: Monolinguals > Bilinguals interaction driven by the group of children in the right Precuneuos. The first and second background brain images are the lateral and medial view, respectively, of the right hemisphere inflated template. IQ as a covariate. Right postcentral CT is increased in bilinguals relative to monolinguals and right precuneus CT is decreased in bilinguals relative to monolinguals. K = 10000 iterations.*

6.5 WHITE MATTER RESULTS

6.5.1 Tract-based spatial statistic

The TBSS analysis of the FA revealed a significant overall main effect of Language-Profile in the left IFOF mostly in the posterior part of the tract at $p < 0.05$ TFCE corrected (see Table 11 and Figure 12). The FA values were overall decreased in these WM tracts for bilinguals as compared to their monolinguals peers.

Table 11. *TBSS analysis of the FA showing significant main effect of Language-Profile.*

Main Effect	Cluster Region	Voxel	P-value	X (mm)	Y (mm)	Z (mm)	T-value
Monolinguals > Bilinguals	Left IFOF/ ILF	268	0.039	-32	-53	3	4.32

Note: p < 0.05 threshold free cluster enhancement (TFCE) corrected. X, Y, Z coordinates in Montreal Neurological Institute (MNI) space. K = 10000 permutations. IQ as covariate. L, left; IFOF, inferior frontal occipital fascicule; ILF, inferior longitudinal fascicule.

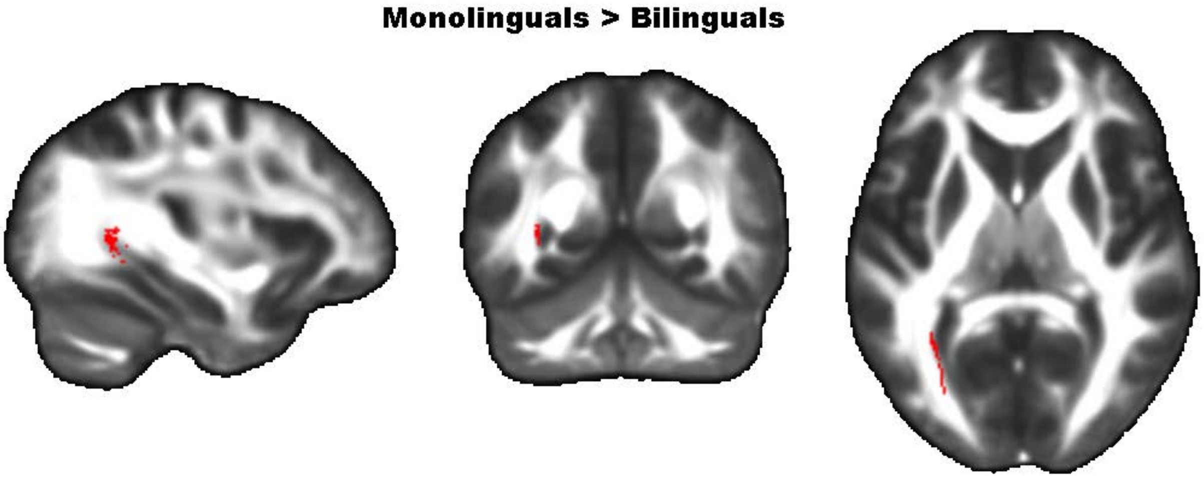

Figure 12: *Brain regions showing significant increased fractional anisotropy (FA) in monolinguals as compared to bilinguals in the left inferior frontal occipital fascicule and inferior longitudinal fascicule. Significant cluster of overall main effect Monolinguals > bilinguals at p < 0.05 threshold free cluster enhancement (TFCE) corrected. The background image is the FA brain template in MNI (Montreal Neurological Institute) space. The slices are showing (from left to right): the sagittal, coronal and axial plane. Sagittal view represents the left hemisphere. In the coronal and axial views the left hemisphere is on the left side. IQ as covariate, K = 10000 permutations.*

6.5.2 Structural Connectivity

NBS (T-threshold = 3, K = 10000 permutations) was used to detect any connected sub-networks that were significantly different between bilinguals and monolinguals. This analysis did not identify any significant main effects of Language-Profile, or interaction between Language-Profile and Age-Group at $p < 0.05$.

6.5.3 Complex network analysis

ANCOVA analysis showed a significant main effect of Language-Profile, F(1,56) = 6.29, p = 0.016 (see Figure 13) for E_{glob}. Looking at the main effect graphically it could be notice that the lines are not parallel. That is because it was also found a significant interaction between Language-Profile and Age-Group factors, F(1,56) = 4.17, p = 0.047 for E_{glob}. Post-hoc comparisons using Bonferroni indicated significantly higher E_{glob} for bilinguals as compared to monolinguals (p = 0.018) in the elderly group but not in the children at $p < 0.05$ (see Table 12). This result suggests that the intrinsic capability of the whole brain system to transmit parallel information between its nodes/regions, which is the capacity of each node to send concurrent information along the network, is tend to be higher (or more efficient) in bilinguals than in monolingual. But this efficiency became only significantly increased in the elderly bilinguals (i.e. after many years of languages exposure).

Table 12. *Global graph-efficiency comparison results between monolinguals and bilinguals.*

	Global Graph Efficiency Means (± SD)	
Groups	Children (N = 13/13)	Elderly (N = 17/17)
Bilinguals	0.0585 (±0.0162)	0.0802 (±0.0162)
Monolinguals	0.0576 (±0.0092)	0.0637 (±0.0148)
Mean differences	0.0009	0.0166
P-values (Bonferroni-corrected)	1.000	0.018

Note: SD, standard deviation. The P-values correspond to the null hypothesis (H_0) that means are equal. A small P-value ($p < 0.005$) suggests that there is a group difference (reject H_o).

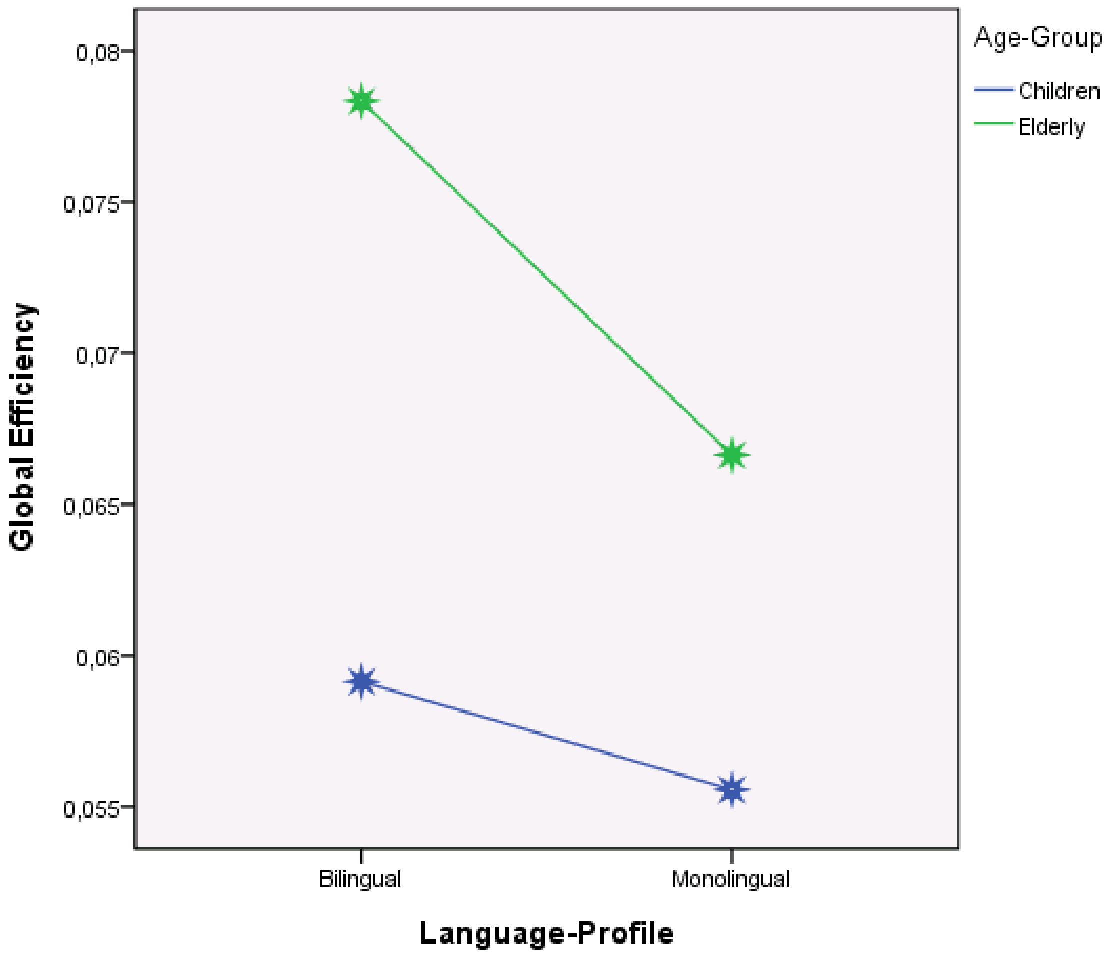

Figure 13: *Graphical representation of the main effect of Language-Profile (x-axis) and the interaction between Language-Profile by Age-Group (separated lines, blue, children; green, elderly) on global graph-efficiency measure of the whole structural network (y-axis). Asterisks represent* E_{glob} *mean values for each group.*

6.6 RESTING-STATE FUNCTIONAL RESULTS

6.6.1 Functional Connectivity

The NBS obtained a significant interaction effect between Language-Profile and Age-Group (T-threshold = 3, K = 10000 permutations, $p < 0.05$ corrected) identifying one functional subnetwork comprised by only two regions. Consequently, the pair-wise analysis was used and also obtained a significant interaction effect for this pair of regions [p(FDR-corrected) = 0.05, t-value = 4.21]. As in the independent analysis carried out on the children sample (Chapter 4), we found that the right Heschl's gyrus and left amygdala were significantly higher connected in bilingual than in monolingual children.

6.6.2 Complex network analysis

This analysis assessed for differences between bilingual and monolingual groups in E_{glob} and E_{loc} of the resting-state functional network. No statistically significant main effects or interaction between Language-Profile and Age-Group emerged at $p < 0.05$. This indicates that both groups (bilinguals and monolinguals) have similar patterns in terms of graph-efficiency at both local (a measure of clustering) and global (a measure of integration) scales in the functional network.

6.7 INTERIM DISCUSSION

In this analysis, a fully comprehensive statistical analysis of bilingualism was performed across children and seniors. A 2-way ANCOVA was run including two factors, Language-Profile (levels: bilinguals and monolinguals) and Age-Group (levels: children and elderly), adjusted for IQ. The ANCOVA analysis found a significant interaction between factors (Language-Profile by Age-Group) in the GM volume of the right *Lingual/PC/Precuneus* cortex. A significant interaction was also found between these factors in the CT for the right *precuneus* and *postcentral* gyrus. The post-hoc comparisons revealed that interactions were driven by the children group. Bilingual children showed a significant increased GM volume and a significant decreased CT in the right *Lingual/PC/Precuneus* as compared to their monolinguals peers and showed an increased CT in the *postcentral* gyrus.

The TBSS analysis of the FA revealed a significant overall main effect of Language-Profile: bilinguals showed a significant decreased FA of the WM across groups in the posterior part of the left *IFOF/IFL*. Previous studies using the standardized TBSS protocol have been shown consistently bilingualism effects in these tracts (Luk et al., 2011; Gold et al., 2013; Pliatsikas et al., 2015; Cummine & Boliek 2013). However, Gold et al. (2013) was the only study showing decreased FA in the posterior part of the IFOF, similar to the current result. Other studies obtained the effects in the anterior part of the tract (Luk et al., 2011; Pliatsikas et al., 2015; Cummine & Boliek 2013) showing an increased rather than a decreased. Mohades et al. (2012; 2015) although using a different approach (TOI approach) also obtained increase mean FA in the IFOF for bilinguals as compared to monolinguals.

The result related to the complex network analysis revealed that whole brain structural network was significantly higher global graph-efficient in bilinguals as compared to monolinguals but only in the elderly, suggesting that elderly lifelong bilingual brain networks have a better capability of transferring information between nodes/regions across the whole network than their monolingual peers. In terms of functional principles, these gain in global graph-efficiency seem to support the idea that demanding, highly specific processing in higher-level cognitive functions, such as language and/or executive functions, during lifelong might imply structural changes in the brain, as they could require sustained greater efficiency in transferring parallel information across all regions in the brain (Bassett et al., 2009).

Why is it the case that higher global network graph-efficiency is associated with reduced FA in the bilingual group? An increase in global graph-efficiency suggests that certain connections are stronger, which would lead us to expect greater density and higher FA values. However, this increased density may be masked by the existence of regions of crossing fibers. If a given tract strengthens its connections and becomes dense but intersects another tract that does not change, the FA values will be lower on the voxels of crossing fibers. This would explain why lower FA is related to increased global efficiency of the brain graph network. Nonetheless, these are different measurements that rely on different types of analysis. In fact, the higher global graph-efficiency could be related to more extended changes that do not reach significance at a local level.

Part III: Summary and General Discussion

Chapter 7: General Discussion

In the current study, a multimodal neuroimaging approach (each structural technique complements the other in understanding neural correlates) was used to study brain plasticity in early Basque-Spanish bilingual children and lifelong Basque-Spanish bilingual elderly who have been actively bilingual for many decades. Both bilingual groups were carefully-matched in age and sex with monolingual control groups. The neural effects of bilingualism were analysed by measuring GM volume, CT, FA values, structural/functional connectivity and topological brain network measures. This study was carried out on samples of children and the elderly, with the aim of better understanding whether or not bilingualism yields effects in the brain and especially to resolve inconsistencies in previous results. Age groups were selected at either end of the lifespan under the assumption that any effects that bilingualism may produce in the brain would be more salient in these groups because they are not at the peak of their cognitive abilities (children are under development and elderly are on decline). The bilingual population of this study is immersed in a Basque-Spanish bilingual context. In this context they have to switch between their two languages very often on a daily basis and even in the same conversation (i.e. a dense-code switching interactive bilingual context). An overview of the results can be found in Table 13.

In Experiment I, 14 carefully-matched Basque-Spanish bilinguals with 14 Spanish monolinguals children were compared. The most important result from this experiment was the increased GM volume in the bilingual children as compared to the monolingual peers, in a large area covering the *Lingual/PC/Precuneus* cortices. Although no change was observed in WM connectivity, bilingual children showed higher functional connectivity between the right Heschl's gyrus (right primary auditory cortex) and the left amygdala.

In Experiment II, the elderly participants (17 carefully-matched Basque-Spanish bilinguals with 17 Spanish monolinguals) showed normal MMSE scores and did not differ between groups in other controlled measures. The structural results showed no regional brain differences between Basque-Spanish bilingual seniors and their Spanish monolingual counterparts, either in GM or in WM, neither in the functional connectivity patterns. However, a higher graph-efficient of the structural network was observed in elderly bilinguals as compared to monolinguals.

Table 13: *Overview of the main results from studies reported in this dissertation.*

Study	**Grey Matter**	**White matter**	**Structural/ Functional Connectivity**	**Topological analysis**
Experiment I (Children)	***Bilinguals > Monolinguals:*** Increased volume in **R. Lingual/PC/ Precuneus.**	n.s	***Bilinguals > Monolinguals:*** Pair of regions showing higher functional connectivity: **R. Heschl** **L. Amygdala**	n.s
Experiment II (Elderly)	n.s.	n.s	n.s	***Bilinguals > Monolinguals:*** Increased E_{glob}.
ANCOVA (elderly and children)	*Language-Profile by Age-group Interaction* (**driven by children**): ***Bilinguals > Monolinguals:*** Increased volume in **R. Lingual/PC/ Precuneus**. Increased CT in **R. Postcentral** ***Bilinguals < Monolinguals:*** Decreased CT in **R. Precuneus**.	*Main effect of Language-Profile:* ***Bilinguals < Monolinguals:*** Overall decreased FA in **L. IFOF/ILF**	*Language-Profile by Age-group Interaction* (**driven by children**): ***Bilinguals > Monolinguals:*** Pair of regions showing higher functional connectivity: **R. Heschl** **L. Amygdala**	*Main effect of Language-Profile* and *Language-Profile by Age-group Interaction* (**driven by Elderly**): ***Bilinguals > Monolinguals:*** Increased E_{glob}.

Note: n.s. indicates not significant results; L, left. R, right; PC, posterior cingulate; IFOF, inferior frontal occipital fascicule; ILF, inferior longitudinal fascicule; CC, corpus callosum; E_{glob}, global efficiency.

Finally, the ANCOVA found a significant interaction between factors (Language-Profile by Age-Group) for the GM volume in the right *Lingual/PC/Precuneus* cortex and for the CT in the right *precuneus* and *postcentral* gyri. The post-hoc comparisons revealed that the interactions were driven by the children group. They showed a significantly increased GM volume and a significantly decreased CT in the *Lingual/PC/Precuneus* for bilinguals as compared to their monolingual peers. And for the *postcentral* gyrus bilinguals showed an increased CT as compared to monolinguals. The TBSS analysis of the FA revealed a significant overall main effect of Language-Profile in the *IFOF/IFL* across Age-Groups. The FA values were globally decreased in these WM tracts for bilinguals as compared to monolingual peers. The NBS analysis showed an interaction effect of language-Profile by Age-Group identifying a small functional subnetwork more highly interconnected in bilingual children as compared to monolinguals that comprised only two regions: the right Heschl's gyrus and the left amygdala. It was also found a significant main effect (bilingual > monolingual) for the global graph-efficiency (E_{glob}) of the structural network, but also a significant interaction of Language-Profile by Age-Group, which means that the whole brain structural network was more graph-efficient overall in bilinguals as compared to monolinguals but only significant in the elderly group.

Any potential brain effects of bilingualism were expected to be clearer in children and elderly bilinguals. Furthermore, it was expected that these effects would be stronger in elderly bilinguals than in children, given the lifelong bilingualism of the seniors as compared to the children. Clear predictions were also made for differences in specific brain regions that might be important for bilinguals living in a dense code-switching interactional language context (regions such as left IFG, caudate/putamen, cerebellum). It was also hoped to increase the possibility of convergent results by combining different neuroimaging modalities and measures.

Contrary to what was expected, however, the elderly group did not show local differences between bilinguals and monolinguals even when the bilinguals under study were essentially early bilinguals who have been immersed in a dense-code switching interactive bilingual context for almost their whole life and the languages in this bilingual context (i.e. Basque and Spanish) were extremely typologically distant, which might imply that the cost of dealing with these two language affects the brain more. Importantly, this result was in line with behavioural findings recently reported in our laboratory with the same elderly group

(Anton et al., 2016). 24 bilinguals and 24 carefully matched monolinguals (including the participants of this dissertation) were compared on verbal and numerical Stroop tasks, but no behavioural differences in these inhibitory measures were detected between them.

Furthermore, children showed more focal brain changes related to bilingualism than seniors. This could suggest that bilingualism might produce compensatory transient plastic changes in the brain when the languages are still been acquired, but later on when they are completely established these changes regress back to the previous state or diminish after complete acquisition of the languages skills. This was the most remarkable result of this dissertation, the extensive increased GM volume and decreased CT obtained for the bilingual children in the *right Lingual/PC/Precuneus*. These patterns of results seem plausible, as there has been demonstrations that the GM volume and the CT are inversely related (Chung, Dalton, Shen, Evans, & Davidson, 2007). These neural correlates indicate brain plasticity related to bilingualism, but are not compatible with other structural bilingualism studies in adults (see Costa & Sebastián-Gallés, 2015; García-Pentón et al., 2016; Li et al., 2014 for a review). However, the precuneus and as well the PC are essential regions in the 'default mode network' (Leech et al., 2016; Utevsky et al., 2014) that typically undergoes a prolonged development during childhood (Fair et al., 2008), being the precuneus a region showing one of the highest index of maturation during childhood (Dosenbach et al., 2010) and one of the regions most highly structurally (Hagman et al., 2008) and functionally (Tomasi and Volkow, 2010) connected in the brain. Importantly, some studies have shown the involvement of the lingual, PC and precuneus after oral language training in children with dyslexia (Temple et al., 2003), perhaps as a compensatory mechanism. Additionally, Pierce et al. (2015) found that Chinese–French bilinguals activated left cingulate gyrus and right precuneus more strongly than the French monolinguals in a phonological working memory task. In general, these current results suggest that bilingualism might accelerate the maturation into these regions that are crucial for development and learning. But we acknowledge that the result reported here is just a neural correlate and it does not imply any causation effect at the behavioural level. Future investigations need to combine behavioural with structural/functional measures in order to ascertain the relationship between these neuroanatomical correlates and the advantages or disadvantages at the behavioural level in bilingualism. In the absence of any further replication, we can only suggest that additional research is required in order to explain the relationship between bilingualism and these effects in the precuneus, the lingual and PC gyri.

In this sense, extreme caution is needed when associating a structural change with a given function or behaviour in development. In this regard, some brain differences that have been linked to the bilingual advantage are sometimes accompanied by disadvantages or null differences in behaviour or could be interpreted as a bilingual disadvantage (see Abutalebi & Green, 2007; Bialystok et al., 2012; García-Pentón et al., 2014). As such, it is not clear why some brain differences are labelled as a bilingual advantage. Whatever structural brain differences are found, they should not automatically be taken to support this hypothesis. In the context of the current disjunctive more studies are still needed to provide a direct demonstration of correlation between anatomical, functional and/or behavioural data. Otherwise we are just moving the same debate about possible advantages of bilingualism from the realm of cognition to the brain (Duñabeitia & Carreiras, 2015).

The second result obtained in this study concerning the GM was the increased CT in the right postcentral gyrus for bilinguals as compared to monolinguals. In a recent study, Olalude et al. (2016) found increased GM volume for Spanish-English bilinguals young adults as compared to English monolinguals in the right precentral gyrus covering in part the postcentral. The opposite pattern was found between simultaneous bimodal (ASL-English) bilinguals as compared to monolinguals (i.e. decrease in the right precentral that extends into the postcentral for the bimodal bilinguals). Another study of Chinese learners of English (Cao et al., 2014) found greater connectivity between the left postcentral gyrus and the right middle occipital gyrus in a pseudo word rhyming task. These previous and the current result could be suggesting the role of the somatosensory cortex for learning languages (Cao et al., 2014).

Concerning the analysis of the FA, only with the gain in power of the ANCOVA was it possible to detect a decrease across all participants in FA values for bilinguals as compared to monolinguals in the left *IFOF/IFL* (i.e. overall both levels of factor Age-group: children and seniors). WM differences between bilinguals and monolinguals have been observed consistently in the IFOF in the literature, but either an increase or a decrease in FA was detected in these studies for bilinguals as compared to monolinguals (Cummine & Boliek

2013; Gold et al., 2013; Luk et al., 2011; Mohades et al., 2012; 2015; Pliatsikas et al., 2015). While two studies found increased FA values for bilingual older adults (Luk et al., 2011) and young adults (Pliatsikas et al., 2015), another one found decreased FA values for bilingual older adults (Gold et al., 2013) and young adults (Cummine & Boliek, 2013). Similarly, Mohades et al., (2012; 2015) using a different approach (i.e. a TOI approach) also found an increase in the mean FA of this tract in children. Taken together all these previous WM studies, the results are difficult to interpret, as the effect may be an increase in young, children and older adult bilinguals or a decrease in young and older adult bilinguals. It has been argued previously in the introduction that it may be precisely the combination of the maturation/degeneration related to the chronological age and the L2 AoA effects (clearly confounding factors between prior studies) that bring about these disparate patterns, especially as the regions identified by these studies seem to be more sensitive to chronological age effects (Good et al., 2001; Salat et al., 2005). However, here we obtain an overall main effect across both group of age: children and elderly. Particularly, the overall main effect obtained here is in line with results showing effects into the same WM tract, but favouring a decrease rather than an increased in bilinguals. In any case, it should be pointed out that it is difficult to perform a fair comparison with these previous studies because in many cases the full local maximum coordinates of the cluster of the effect could not be obtained. As a result, only a general visual inspection of where these effects fell along the IFOF could be carried out. Nonetheless, these results are in line with one study (Gold et al., 2013), as their extensive clusters of the effect in the IFOF were more broadly extended into the posterior part of the brain.

The NBS analysis of the structural connectivity did not show any set of regions with increased interconnectivity for bilinguals as compared to monolinguals. However, NBS identified a small functional component more highly interconnected in bilingual children as compared to monolinguals that only includes a pair of regions: the right Heschl’s gyrus and the left amygdala. Interestingly, Ressel et al. (2012) obtained significantly larger volumes of the Heschl’s gyri in bilinguals as compared to monolinguals. Additionally, Golestani et al. (2007) found higher WM density in the left Heschl’s gyrus for faster learners as compared to slower learners of non-native speech sounds and more WM in the right Heschl’s gyrus for expert’s phonetician than non-experts (Golestani et al., 2011). They also showed that Heschl morphology did not correlate with the years of expertise, suggesting that experience during adulthood cannot modify Heschl, but rather that Heschl's gyri are established before birth (*intra utero*). In general, they suggested that WM differences across individuals in the Heschl gyri could be a neuroanatomical marker of skill that predicts behavioural differences in some aspects of language learning, and can even predispose individuals for the selection and acquisition of an expertise. Regarding the amygdala, it is a critical region for the processing of emotional significance of any type of stimuli (Olson, 2007). And it is well-known that is important for emotional learning and memory (Phelps and LeDoux, 2005). Notably, there are studies reporting the left amygdala more activated than the right amygdala when participants were processing positive and negative emotional words as compared to neutral words (Hamann and Mao, 2002). Especially, a relationship was found between its size and language skills in adults and children (from 3 to 4 and 6 years old) with autism (Haznedar et al., 2000; Munson et al., 2006): larger and smaller left amygdala correlated with better and worse language skills, respectively, and also larger right amygdala correlated with poorer languages abilities. Similarly, in healthy 6 month's children larger left amygdala correlated with higher language abilities (Ortiz-Mantilla et al., 2007) and larger right amygdala (measured at 6 month of age) correlated with lower language scores measured at 2, 3 and 4 years old (Ortiz-Mantilla et al., 2010), which suggested that amygdala could play some role in the acquisition of language, specially left amygdala. In addition, Hernandez (2009) observed bilateral fMRI activation of the amygdala in bilingual adults when they had to name pictures in their native language as compared to naming in their second language. And an increased activity of the right amygdala was also found in bilingual children preforming a word-reading task in their native language as compared to their second language (Owens et al., 2009). Our results therefore suggest that the increased correlated activity in the right Heschl's gyrus and left amygdala for bilingual children as compared to monolinguals could reflect early functional plasticity associated to the extra demands in the acquisition of both languages, perhaps as a compensatory mechanism for learning and memory.

On the other hand, the complex network analysis revealed that the structural brain network was more graph-efficient in elderly lifelong bilinguals than in monolinguals. This is an important finding insofar as it provides some plausible explanation on how bilingualism could serve as a protective brain factor. The increased global graph-efficiency of the structural network could be interpreted as an indication that the early acquisition of two languages and the lifelong use of those two languages could have a positive effect on the brain, allowing a more efficient flow of information across the structural brain network, which would, in turn, increase the brain's ability to cope with focal deterioration in normal and pathological cognitive decline. Interestingly, a preceding study in young adults showed that early bilingualism modified the structural configuration of the white matter, developing higher interconnected and efficient sub-networks to deal with the processing of the two languages (García-Pentón et al., 2014). But this change was associated with an observed decrease of the global graph-efficiency of the entire brain network. These results suggest that once the brain is more specialized and clustered (i.e. forming specialized subnetworks to manage with two languages) the global efficiency of the whole brain network tends to decrease. Together with these preceding results, the current finding suggests that after the complete acquisition of these abilities in the elderly group, the brain specialisation showed by young bilinguals tend to disappear and the brain becomes a more optimized or well-organized network, which could explain the increase global graph-efficiency. Tentatively, this can suggest that intensive practice with the two languages could act as a neural reserve mechanism, enabling the cognitive system to become more efficient at using cerebral resources. This might imply less specialization of the brain but better capability in transferring information across all brain network, which could protect the brain against a normal and pathological decline in aging. But, although the current study provides some tentative explanation for how bilingualism might act as a brain protector factor, caution is required due to the small sample size tested here and given that the global graph network efficiency was the only significant difference observed. No other differences on structural or functional measures were found in elderly. Thus, this finding needs to be replicated, and future investigations need to combine behavioural with structural and functional measures in order to ascertain the relationship between this structural finding and the advantages or disadvantages of bilingualism at the behavioural level. In the absence of any behavioural advantage (see Anton et al., 2016) and any further replication, this result is not definite.

These results also suggest that the structural and functional connectivity in the brain network undergo dynamic changes that could vary across the age range. However, the degree to which structural/functional connectivity could be modified, particularly in adult brains, as well as the timescale (fast, slow) in which these changes can occur is still a matter of current debate. Particularly, this study suggests that after many years of bilingual experience (in elderly lifelong bilinguals), the topological parameters of the structural network are affected. Conversely, in children, a higher functionally correlated subnetwork was identified for bilinguals, but this change did not affect the stability of the structural network. This suggests that functional plasticity can occur faster but structural changes could take more time to take place. Importantly, although functional connectivity is determined by underlying structural networks (Honey et al., 2007; Greicius et al., 2009; Johnston et al., 2008), differences at the level of the functional connectivity can occur without the explicit need for structural modifications (i.e. without differences in the wiring between the brain areas).

Summarising, this research on bilingualism across different groups of age (childhood and elderly) suggested that structural brain plasticity related to bilingualism is small, unstable, subtle, transient and it is very difficult to detect even in lifelong bilinguals. Perhaps because bilingualism is a complex and unpredictable variable hard to separate from linguistic, cognitive, psychological, social and individual factors. A fact that is consistent with the current ambiguous picture in bilingualism studies (García-Pentón et al., 2016; see also others, Baum & Titone, 2014; Costa, & Sebastián-Gallés, 2014; Li, Legault, & Litcofsky, 2014; Paap et al., 2015; de Bruin et al., 2015a). In sum, the evidence is weak, scarce and inconsistent, but draw attention to that lifelong bilingualism could point out gain toward a higher brain network global-graph-efficiency in aging.

7.1 THE BILINGUAL PUZZLE: SOME PIECES DO NOT FIT TOGETHER

There is no doubt that bilingualism is a multifaceted phenomenon and as such it will not result in simple effects or modify a single region. As a consequence, understanding such a complex phenomenon will require more brain network studies. It is generally agreed that structural and functional data, especially combined with behavioural data, can provide additional evidence and contribute to understanding the causal basis of the adaptive changes in bilingualism. Although in recent years we have made substantial progress in the field, we are still a long way from being able to make meaningful generalizations, and this will require running preferably more longitudinal studies with well-characterized samples, using standardized and sophisticated procedures. Furthermore, to avoid coming to loggerheads over apparently contradictory findings in the literature, studies should be conducted in different places to test hypotheses in different bilingual populations. In that sense, this is the first study to investigate brain plasticity in Basque-Spanish bilinguals, a very different bilingual context, thus contributing with new fresh results to the pool of evidence in this area. This increases the diversity of research in the field, which has tended to be the production of a limited number of research groups replicating results. Studies carried out in different locations enable access to populations that vary significantly in linguistic profile, possibly making findings more difficult to reproduce across labs but also more generalizable when replication does occur.

There is also a need for larger sample sizes in studies and for samples to be matched in essential variables. We are aware that the sample size per group under study does not appear very large to detect differences. At the same time, samples that are small but form part of well-controlled cross-sectional studies with carefully characterized language profiles – particularly with special populations– could offer good statistical power. Large sample sizes are desirable in neuroimaging studies, although this goal may be very difficult to achieve with special populations (such as the elderly and children) as participants may be difficult to find due to compatibility with the technique. In this context, the use of standard methods and statistical procedures to report significant results, as well as more sophisticated analysis, becomes even more important. These investigation studied carefully-matched groups of bilingual and monolingual participants in age and sex. Other important confounding variables were controlled for, such as language proficiency, daily and years of bilingualism exposure, IQ and MMSE. In addition, all bilinguals in this study were early bilinguals who start to acquire the second language before 3 years old in the children group and before a mean of 6 years old in the elderly. Moreover, a refined neuroimaging protocol was used that combines results from different structural and functional techniques in order to arrive coincidence from multiple methods.

Related to the issue of characterizing the samples adequately, it is important to bear in mind fundamental differences that may exist between different types of bilingual populations. Although acquiring a new skill could alter the brain and behaviour at any age, results at the neural and behavioural level seem to indicate that when the skill is acquired from birth, such as two simultaneous languages, it may be harder to find modifications. In this sense, simultaneous bilingualism is not exactly a typical case of expertise, such as driving a taxi, video gaming, doing sports, or playing musical instruments, and so the impact of the lifelong bilingual experience may be substantially different to what has been found for other types of expertise acquired later in life (Duñabeitia & Carreiras, 2015).

In this study, clear focal structural changes in GM were only obtained in bilingual children but not in lifelong bilingual seniors, suggesting that focal GM brain plasticity related to the early acquisition of a simultaneous or second language could be happening in childhood, as this is needed for the acquisition of both languages but not for the later use once languages have been completely acquired, thus being provisional changes that consequently diminish. Therefore, this evidence does not talk about a bilingual advantage rather talk about the development of skills for later use. Additionally, functional connectivity changes that represent short-term plasticity, that is: more phasic and transient changes were observed in children but not in the elderly group. Contrary, changes on the topological parameters of the structural brain network, which represent long-term plasticity (i.e. slow and more permanent changes), were observed in the elderly but not in the children group.

Importantly, the contribution of null effects reported here to the debate is, however, extremely important. It is critical that negative results be taken into consideration and not be dismissed. ‘Negative result’ here refers to instances where the difference between bilinguals and monolinguals is in the opposite direction to that expected (i.e. the mean measure of monolinguals is greater than the bilingual measure), or both groups perform equally and therefore no mean difference appears –see behavioural studies comparing very large samples of participants (Antón et al., 2014; Duñabeitia et al., 2014)–. These kinds of results fail to provide evidence in line with the hypothesis of the bilingual advantage, and in that sense they are ‘negative’ results for the proponents of such an advantage.

In addition to being problematic for the bilingual advantage hypothesis, to what extent do these results provide support for an alternative account? Within the framework of classical inference it is not possible to accept the null hypothesis (no difference between groups). We can only either reject the null hypothesis (by finding a difference between groups) or fail to reject the null hypothesis (by finding no difference). This means that we cannot really affirm that there is no difference between groups, because we are unable to distinguish whether the null hypothesis is true or whether we do not have enough sensitivity and/or power in the experiment to detect differences. In other words, the absence of evidence is not equivalent to evidence of absence. Particularly, in neuroimaging studies the absence of a statistically significant effect in a particular region does not prove that the region is not affected. Mainly, due to normalization (Crum et al., 2003) or smoothness (Ashburner and Friston, 2000) are likely to result in statistical variability. However, being aware of these possible sources of error and bias who can be introduced at different stages of a study –which can be amplified here due to the nature of the populations under study (elderly and children)–, a conservative approach based on solid findings was followed (Ioannidis, 2005). Thus, based on the null effects on the brain obtained here in the elderly bilinguals, as well as the inconsistent (unexpected) structural neural correlates obtained for children, and based on the null behavioural result in the same elderly participants (Anton et al., 2016), but also in similar bilingual children samples (see Anton et al., 2014; Duñabeitia et al., 2014 for null result in several EF tasks in Basque-Spanish bilingual children), the claim about the bilingual advantage hypothesis cannot be supported by the current study.

A theoretical model of bilingualism must account for the fact that, firstly, there are bilinguals who do not show an advantage over monolinguals in several control mechanisms (Anton et al., 2016; Anton et al., 2014; Duñabeitia et al., 2014; Paap et al., 2015b), and, secondly, there are bilinguals who do not show differences in brain regions as compared to monolinguals (see García-Pentón et al., 2016 for a critical review). Currently, those studies not supporting the hypothesis for the bilingual advantage tend to be overlooked and ignored. It would do well to remember that the scientific method advances through the falsifiability of theories: any result that does not conform to a given theory's predictions brings that theory into doubt and raises the need for a redefinition and generation of new questions and hypotheses (de Bruin et al., 2015a; David et al., 2013; Jennings & Van Horn, 2012). The intention of this thesis in considering negative/null results is to avoid the risk of the neuroanatomical debate falling into the same stalemate as the discussion at the behavioural level about the bilingual executive cognitive control advantage.

Finally, it should be pointed out that across previous studies, despite the variability of the samples, of linguistic profiles and dissimilar methods, if a pattern of consistent results had emerged, this would have represented significant and robust effects related to bilingualism. Unfortunately, this has not been the case. Instead, many of these studies looked for local differences using a ROI-based approach or SVC; some of them did this after reporting no significant differences at whole brain level but most went directly to look for differences with ROIs without providing information about the whole brain level. However, this strategy can be dangerous if the interpretation is not cautious. The fact that one particular region is affected or modulated by one condition in a ROI analysis does not mean that it is more relevant with respect to other brain regions. One region could be necessary but not sufficient for a process. This is why it is essential to look first at the whole brain level and then go further in a ROI based analysis to better understand the role of the region. Restricting analysis to a small brain volume or ROI is different from the whole brain approach and caution is needed when interpreting such findings in terms of the relevance of one specific region in relation to other regions in the brain.

In sum, although it has been suggested that the diverse findings in the field represent different pieces of a complex puzzle that need to be fitted together, our results from different bilingual age groups at either end of the lifespan (children and elderly) and from different neuroimaging techniques investigating at the whole brain level, indicate that bilingualism effects are transient and unstable. Although we are still in a poor state of affairs for solving the puzzle, being aware of these problems will hopefully put us on the right road. In the final sections, the results of this thesis are concluded and suggestions made about what else needs to be done in the light of the current findings.

7.2 CONCLUDING REMARKS

There are important conclusions from this dissertation. First, lifelong bilinguals did not show regional GM or WM structural differences. Contrary to what was expected, bilinguals as compared to monolingual showed more GM differences in children than seniors who have been active bilinguals during almost their whole lives. Second, bilingualism might accelerate the maturation in precuneus, PC and lingual gyri during childhood, regions that are crucial for development and learning. Third, even when the brain does not display regional differences (i.e. did not show any specialization) in lifelong bilinguals it can still show global differences in the brain as a whole. Specifically, a higher global structural network graph-efficiency was observed in lifelong bilinguals, which could point out a gain toward a better neural reserve in aging. Fourth, functional connectivity changes related to bilingualism were detected in children but not in elderly. Importantly, this functional changes can occur without differences in the wiring (structural changes) between the brain areas involved.

This dissertation refutes the suggestion that ceiling effects in young adults could be responsible for the lack of differences obtained between bilinguals and monolinguals at the neural level. If that would be the case, the bilingual effects on the brain should be clearer in elderly bilinguals because they do not show ceiling effects and are not at the peak of their cognitive function development, but are rather declining. Under this condition the brain is susceptible to neural compensation and neural reserve mechanisms. Thus, any potential difference between bilinguals and monolinguals should be more clearly observable in this group. The current results are an indication that something else than ceiling effects is responsible for the lack of differences in the expected regions. On the other hand, if greater brain changes are expected due to lifelong active bilingualism, any bilingualism effects should be even stronger in early lifelong bilinguals than in bilingual children. The present results show the opposite pattern, thus the hypothesis that increased active bilingual experience leads to greater brain changes in regions related to language processing and control is refuted. The inconsistent results in children and the elderly suggest that bilingualism is a variable phenomenon. Thus, the present study adds a cautionary note to the exaggerated optimism when reporting/interpreting brain findings related to bilingualism and recasts the existing debate on the bilingual advantage hypothesis from the perspective of brain structure.

Additionally, studies combining behavioural and functional/structural brain network approaches to confirm and shed more light on the neural correlates obtained in the bilingual children and elderly are needed. Thus, neuroimaging studies of bilingualism need to take greater care in data analysis, to use more sophisticated methods of analysis and approaches in order to reveal more subtle effects that could exist in any form of bilingualism. These are crucial points for future studies in order to add more evidence before arriving at conclusions favouring or not the hypothesis of the bilingual advantage (or any other hypothesis, for that matter). Not everything has been said yet in the field of bilingualism. More studies with higher numbers of participants, with well described samples and methodology are needed to accumulate an important body of evidence to illuminate whether and how bilingualism modulates brain structure and function and to obtain more stable results across studies. For future studies we need to combine behavioural and brain measures to understand among other things (1) how potential brain changes in specific areas/circuits (which should be replicated by several studies) are related to cognitive processes and behaviour, (2) how and whether these brain changes are modulated by AoA, proficiency and language combinations, and (3) whether bilingual advantages at the behavioural level are accompanied by observable changes in the brain, with the aim of understanding when these bilingual advantages do or do not appear and why.

Various methodological considerations deserve mention in the light of the new evidence. A position shared by some authors (Bialystok, 2015; Green & Abutalebi, 2015; Kroll & Chiarello, 2015; Luk & Pliatsikas, 2015) holds that instead of a hazy view, what the growing body of research is showing us are isolated pieces of the very complex puzzle of bilingual neuroplasticity. Admittedly, this is possible. However, another possibility is that what is driving the findings is that the current panorama in bilingualism studies is skewed by methodological issues and factors not contemplated by the hypothesis. Parallels can be drawn with the debate in the field at the behavioural level, where it is now recognized that methodological shortcomings (such as small sample sizes, ignoring null results or failing to control for nuisance covariates) have been made that have unnecessarily complicated the field of bilingualism and have led to a lack of replicability (Bialystok et al., 2015; de Bruin, Trecanni, & Della Sala, 2015a, 2015b; Paap et al., 2015a). Therefore, studies should try to replicate previous work using the same methodology and subsequently use more novel and sophisticated (but standardized) methods to complement or refine their analysis. It is essential that there be at least some standards if the puzzle of the effects of bilingualism is to be solved.

7.3 FUTURE DIRECTIONS

To solve the puzzle of the neural effects of bilingualism, firstly we need to make sure the pieces are not incommensurable: we need to perform studies with samples of participants sufficiently large to overcome the problems of statistical power, that are carefully matched to control for demographic factors and that have well-described linguistic profiles, and use alternative and multiple approaches for the analysis. These are key factors in improving the field.

Secondly, we need to fit all the pieces together: studies showing negative/null results should have the same opportunities for publication as studies showing positive results. This would make it possible to understand when, how and why the bilingual advantage appears. Thirdly, we need to make sure the pieces are well made so that they can fit together: data analysis procedures need to comply with basic standards and there must be transparency in the reporting of data so that we know what the findings represent. Additionally, studies of the whole brain network that exploit more methods and techniques would be a welcome development.

In addition to these methodological concerns, it is important to stress the need for more exhaustive studies that integrate functional, behavioural and structural data to get a full picture of bilingualism. Since there are so many factors that could affect the brain, and since their consequences could be functional and/or structural, the impossibility of finding differences between groups or the absence of common patterns across studies does not mean that there are no differences or commonalities in many other directions.

On the other hand, here it is relevant to advocate a type of meta-analysis that would be particularly useful. With a view to bringing together evidence from different studies, voxel-based meta-analyses are the best quantitative tool to identify where differences in the brain really are, especially when the sample size of individual studies is a limitation (Borgwardt et al., 2012). This technique is even better than a standard qualitative review, because it makes it possible to obtain new p-values from many VBM studies. The problem here is that the small number of studies makes it almost impossible to perform this kind of analysis yet. Thus, more neuroimaging studies that include behavioural measures are needed. However, in the meantime, a database with full statistical maps (not just the reported selections) of all the studies published so far could help to perform a meta-analysis that would clear up many issues and allow progress in the field.

In sum, in light of the present findings and given the variability in results related to bilingualism, the conclusion is that effects of bilingualism on the brain are transient and unstable. However, the methodological shortcomings and the sample variability in the literature are acute enough to cause an unacceptable amount of noise in the data. It is not a question of ‘oversimplifying’ the matter, but these issues require attention if the field is to advance. The current alternative model appears to be to ‘overcomplicate’ the matter by constructing a model that accommodates all these questionable findings. No denying that data only make sense in the context of a strong theoretical framework, there are, however minimum requirements about the data themselves and it will not do to have theories driven by specious or dubious findings. More research is needed that is well-designed, theoretically motivated and correctly executed and analysed before any final conclusions can be arrived at

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Appendix

APPENDIX 1. TECHNIQUES AND MEASURES USED IN THE ANALYSIS OF BRAIN STRUCTURE.

Voxel-based morphometry (VBM) is a whole-brain technique that allows the investigation of local differences in the brain using statistical parametric mapping. VBM requires T1-weighted MRI images to be registered to a template and classified into three different brain tissue classes: grey matter (GM), white matter (WM) and cerebral spinal fluid (CSF). The segmentation into different brain tissue classes can be done in one step by combining tissue classifications using image voxel intensities, bias field correction and the prior probability derived from registration to a set of a priori tissue probability maps (e.g. GM, WM, and CSF). After spatial registration, images are typically scaled to compensate for any contraction during registration (known as modulation), thus conserving the total amount of GM/WM/CSF as in the original images. By this means, volumetric differences can be tested for, which means that not only mesoscopic (i.e. between microscopic and macroscopic) regional changes in the brain, such as cortical thinning, can be detected, but also macroscopic regional changes, such as cortical folding (Radua et al., 2014). If the images are not corrected (unmodulated) concentration or density differences alone can be tested for, making it possible to detect only mesoscopic differences (Radua et al., 2014). Subsequently, images must be spatially smoothed to permit the comparison of the volume/density images across brains for each individual voxel, using the general linear model (Ashburner & Friston, 2000). Because this model involves the use of univariate statistics at each voxel of the brain, many statistical tests are conducted. Therefore, the statistical significance of the inferences must be adjusted to correct for the problem of multiple comparisons.

Cortical thickness (CT) measurement involves registration to the standard space, tessellation of the GM and WM boundaries, automated topology correction and surface deformation following intensity gradients to optimally place the GM/WM and GM/CSF borders at the location where the greatest shift in intensity defines the transition between the different classes of tissue. Deformation procedures include surface inflation and registration to a spherical atlas. The method uses both intensity and continuity information from the entire three-dimensional T1-weighted MRI in the segmentation and deformation procedures to produce representations of the cortical thickness, calculated as the closest distance from the GM/WM boundary to the GM/CSF boundary at each vertex on the tessellated surface. The resulting maps are not restricted to the voxel resolution of the original data, and thus can detect sub-millimetre differences between groups. Before performing statistical analysis, the individual cortical thickness maps must be smoothed and finally a vertex-wise general linear model can be applied or a ROI approach can be used.

Region of interest (ROI) approaches restrict the statistical analysis to a specific region or regions, which may be defined by manually drawing the limits of the individual native space or by automatic parcellation, which involves segmentation and registration pre-processes and then individual atlas labelling using a standardized atlas to demarcate the different anatomical structures or regions. The desired measure (e.g. GM volume or density) is extracted from the images and averaged to obtain a global measure for each region under consideration.

Diffusion tensor imaging (DTI) is estimated from the DW-MRI (Basser, Mattiello, & LeBihan, 1994), which measures the motion of the water molecules across the axon, providing information about the fiber orientation and organization. Then, scalar measures associated to each diffusion tensor are used to obtain invariant indices like the mean diffusivity (MD), which characterizes the overall water diffusion in each voxel of the brain (for example, MD is higher in ventricles, lower in bones and tends to decrease with increases of myelination). Other scalar measures are the axial/radial diffusivity (AD/RD) that describe, respectively, water mobility along the axis of the main fiber orientation and water mobility perpendicular to this axis (Jones, Knosche, & Turner, 2013). Perhaps the most widely used tensor-derived measure is Fractional anisotropy (FA), which is calculated as the relationship between AD and RD measures and provides information about the degree of anisotropy of the water diffusion in the voxel. The anisotropy is higher (close to 1) inside the axon since the water is impeded from moving across the axon membrane (but can move more freely along the axon), and is lower (close to 0) in regions where the water can move freely in any direction, such as ventricles. Importantly, increasing axonal density, reducing axonal caliber or increasing the degree of myelination should all reduce RD and therefore elevate FA. Despite the extensive use of these measures in many fields of neuroscience, any differences in values should not always be associated with or interpreted in terms of WM tissue ‘integrity’. Different fiber configurations and variations in these configurations can produce different modifications in these measures (Jones et al., 2013).

Tract-based spatial statistics (TBSS) uses an improved nonlinear registration procedure and a mean FA skeleton (which represents the center of all common tracts) to project each subject’s FA maps. This avoids data smoothing and increases the sensitivity of the voxel-wise cross-subject statistics. Another advantage of this technique is that it only examines areas where the fibers run parallel (i.e. the voxels inside the skeleton). These provide a better interpretation of the results, since in areas of crossing fibers the FA changes are more difficult to interpret in terms of WM volume or integrity.

Corrections for multiple comparisons: The most commonly used method to correct for multiple comparisons is to control the **family wise error** rate (FWE) using random field theory and resampling-based approaches (Nichols, 2012; Nichols & Hayasaka, 2003) and this can be applied at the voxel-level or cluster-level of inference. In general, voxel-level FWE controlling procedures have good spatial specificity but poor sensitivity, and cluster-level FWE controlling procedures have better sensitivity but poor spatial specificity. More recently, **false discovery rate** (FDR) has been used to correct for the multiple comparisons problem at voxel-level. Which method is more appropriate and accurate depends on whether the data fulfil the assumptions of Gaussian distribution underlying each technique.

Threshold free cluster enhancement (TFCE): Many of the thresholding techniques make use of the spatial neighbourhood information to account for the real extended area of the signal. The most common approach in this regard in neuroimaging is **cluster-based** thresholding, which often is more sensitive to the search for the true signal that **the voxel-**

based thresholding. However, a limitation of this approach is the need to define the initial cluster formation threshold. This threshold is arbitrary and the choice can have a big impact on the results, especially with lower thresholds cluster formation (e.g. t, $z < 4$) used frequently. In addition, the amount of space pre-smoothing is also arbitrary (since the extension of the expected signal is rarely known in advance). **TFCE** is a method that tries to maintain the benefits of sensitivity threshold based on cluster (and indeed the general concept of ‘cluster’ signal), while at the same time avoiding (or minimizing) these problems. The method takes a raw statistic image and produces an output image in which the voxel-wise values represent the amount of cluster-like local spatial support. Overall, TFCE gives better sensitivity than cluster-based and voxel-based thresholding, but also a richer and interpretable results than the threshold based on cluster (see Smith & Nichols, 2009 for a technical report).

Small volume corrections (SVC) of the FWE limit the analyses to the scope of certain sub-volume but without the averaging inherent to ROI approach. SVC makes it possible to correct for multiple comparisons based just on the number of voxels in the sub-volume, which is a more liberal correction.

APPENDIX 2. GLOSSARY OF ABBREVIATIONS USED IN THE DISSERTATION.

Note: Acronyms for neuroanatomical terms comply as far as possible to those used in NeuroNames available at http://braininfo.org (Bowden, Song, Kosheleva, & Dubach, 2012). Deviations can occur in the capitalization of certain acronyms (e.g. 'CC' rather than 'cc' for corpus callosum) to aid text legibility.

AAL	Automated Anatomical Labeling
ACC	Anterior cingulate cortex
AD	Axial diffusivity
aITG	Anterior inferior temporal gyrus
AnG	Angular gyrus
AoA	Age of acquisition
ASL	American Sign Language
CC	Corpus callosum
CSF	Cerebral spinal fluid
CSL	Chinese Sign Language
CT	Cortical thickness
DMN	Default mode network
DTI	Diffusion tensor imaging
DW-MRI	Diffusion-weighted magnetic functional imaging
EF	Executive functions
FA	Fractional anisotropy
FDR	False discovery rate
fMRI	Functional magnetic resonance imaging
FOV	Field of view

FPC	Fronto parietal control network
FSL	FRMIB Software Library
FWE	Family wise error
FWHM	Full-width at half-maximum
GM	Grey matter
IFG	Inferior frontal gyrus
IFGOp	IFG pars opercularis
IFGOr	IFG pars orbitalis
IFGTr	IFG pars triangularis
IFOF	Inferior fronto-occipital fasciculus
ILF	Inferior longitudinal fasciculus
IPL	Inferior parietal lobule
IQ	Intelligence quotient
ITG	Inferior temporal gyrus
L2	Second language
MD	Mean diffusivity
MFG	Middle frontal gyrus
MNI	Montreal Neurological Institute space
MRI	Magnetic resonance imaging
MTG	Middle temporal gyrus
NBS	Network-based statistics
OFC	Orbito-frontal cortex
PC	Posterior cingulate
RD	Radial diffusivity
RFT	Random field theory

ROI	Region of interest
rfMRI	Resting-state functional magnetic resonance imaging
SLF	Superior longitudinal fasciculus
SLN	Salience network
SMG	Supramarginal gyrus
SPL	Superior parietal lobule
SPM	Statistical parameter mapping
STG	Superior temporal gyrus
SVC	Small volume correction
T1-MRI	T1- weighted magnetic functional imaging
TBSS	Tract-based spatial statistics
TFCE	Threshold free cluster enhancement
TIV	Total intracranial volume
TmP	Temporal pole
TOI	Tract of interest
VBM	Voxel-based morphometry
WM	White matter