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Functional and Anatomical Adaptations in Multilingual Language Users

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Functional and Anatomical Adaptations in Multilingual Language Users

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Abstract

Language is a quintessentially human trait. Many decades of neurolinguistic research provided evidence of neural structures which specialize in complex linguistic and cognitive processes supporting human communications. Because the world is multilingual, (Crystal, 2010; de Bot, 2019) a prominent question related to brain processes supporting language is whether the neural representation of language changes as a function of the number of languages one knows. This study attempts to depict a more comprehensive picture of brain plasticity in multilinguals, by integrating behavioral with functional, structural, and diffusion MRI data. The questions investigated stem from newer dual-stream models of language processing that frame brain architecture, supporting language function in terms of a language network (Friederici & Gierhan, 2013; Hickok & Poeppel, 2007). Based on this framework, language representation for multilinguals compared to monolinguals is investigated within brain regions specialized for language processing (a.k.a. core language nodes; Fedorenko & Thompson-schill, 2014), and regions of domain-generality, associated with language control. Three main findings surface from this investigation. First, monolinguals and highly proficient multilinguals similarly recruit core language brain regions during the processing of native and second languages. These same regions show similar restructuring patterns in grey matter structure and white matter connectivity. Second, compared to monolinguals, highly proficient multilingual speakers show stronger reliance on the cingulo-striatal subnetwork (Dosenbach et al., 2008; Wu et al., 2021) of the cognitive control system, during language comprehension. Decreases in grey matter thickness and volume, along with changes in white matter integrity within this subnetwork, accompany changes in the responsiveness of these regions during language tasks. Finally, contrary to predictions of recent models of bilingual language inhibition and control (Green, 1986, 1998), multilinguals show different patterns of language activation and inhibition. Additionally, these seem to be modulated by language dominance. The implications of these findings on current neurolinguistic theory and models of language processing in speakers of multiple languages (Abutalebi & Green, 2016; Green & Abutalebi, 2013; Grundy et al., 2017; Pliatsikas, 2020) are discussed.

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***

I dedicate this work to my loving parents, who have instilled in me a love for knowledge,
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Table of Contents

INTRODUCTION -- **1**

1. GENERAL INTRODUCTION -- **1**

1.1. NEURAL REPRESENTATION OF LANGUAGE -- 1
1.2. THE IMPORTANCE OF STUDYING MULTILINGUALISM. -- 2
1.2.1. The Bilingual Advantage --- *2*
1.2.2. Neuroprotective Effects Associated with Bi/multilingualism ------------------------------ *6*
1.2.3. Multilingualism and Aphasia -- *7*

2. FACTORS INFLUENCING LANGUAGE REPRESENTATION IN THE BRAIN ------------------- **9**

2.1. LANGUAGE PROPERTIES --- 10
2.2. INDIVIDUAL DIFFERENCES. --- 12
2.2.1. Age of acquisition. -- *12*
2.2.2. Proficiency --- *16*
2.2.3. Language Exposure and Language Use --- *19*

3. INTRODUCTION TO THE NEUROBIOLOGY OF LANGUAGE AND MULTILINGUAL LANGUAGE MODELS --- **21**

3.1. THE HISTORY OF NEUROBIOLOGY OF LANGUAGE --- 21
3.1.1. Neurolinguistic Models of Language -- *23*
3.2. THE HISTORY OF THE NEUROBIOLOGY OF MULTILINGUALISM -- 25
3.2.1. Neurolinguistic Models of Multilingualism. --- *26*
3.2.1.1. Adaptive Control Hypothesis --- 29
3.2.1.2. The Bilingual Anterior to Posterior and Subcortical Shift Hypothesis. ----------------- 31
3.2.1.3. The Dynamic Restructuring Model -- 32

4. THIS PROJECT'S AIMS -- **35**

4.1. INTRODUCTION TO METHODS AND ANALYSIS TECHNIQUES -- 38
4.1.1. Describing Proficiency Measures of Multilingualism -- *38*
4.1.2. Describing MRI Technique -- *38*
4.1.2.1. Describing the Structural MRI Technique -- 39
4.1.2.2. Describing the Functional MRI Technique --- 41
4.1.2.3. Describing the Diffusion Weighted Technique -- 44

5. SUMMARY AND GOALS OF EACH CHAPTER -- **47**

CHAPTER I -- **49**

1. INTRODUCTION -- **49**

2. THE IMPORTANCE OF USING DETAILED AND APPROPRIATE TESTING ----------------- **51**

2.1. CHOOSING THE APPROPRIATE SET OF ASSESSMENTS --- 52
2.1.1. Type of Assessments and Issues Related to Each -- *53*
2.1.1.1. Self-assessment Questionnaires -- 53
2.1.1.2. Standardized Tests -- 54
2.1.2. Proficiency Measures and Different Language Domains -------------------------------- *55*

3. THE PRESENT STUDY -- **56**

3.1. METHODS -- 57
3.1.1. The Self-Rating Questionnaire. --- *57*
3.1.2. The Development of the Adapted MiNT Test --- *57*
3.1.2.1. History of the Test -- 57
3.1.2.2. Adaptation our study -- 58
3.1.3. The Development of the Semantic and Vocabulary Knowledge Test. ------------------- *59*

        3.1.3.1. History of the Test ---- 59
        3.1.3.2. Adaptation our study ---- 59
    3.1.4. The Development of the GAT test ---- 60
        3.1.4.1. History of the Test ---- 60
        3.1.4.2. Adaptation for our study ---- 60
  3.2. Participants ---- 62
  3.3. Data Collection ---- 63
  3.4. Data Analysis ---- 63
  3.5. Results ---- 63
    3.5.1. Self-Rating Questionnaire Results. ---- 63
    3.5.2. Language Production and Vocabulary Assessment Results ---- 65
    3.5.3. Semantic and Vocabulary Knowledge Test Results ---- 65
    3.5.4. Grammatical Assessment Test Results ---- 67
    3.5.5. Average Proficiency Results ---- 68
    3.5.6. Correlation Analysis ---- 69

4. DISCUSSION AND LIMITATIONS ---- 72

  4.1. Self-Assessment and Proficiency Tests ---- 72
  4.2. Correlation Analysis ---- 74

5. **IMPLICATIONS AND FURTHER STEPS ---- 76**

CHAPTER II ---- 78

FUNCTIONAL ADAPTATIONS TO MULTILINGUAL EXPERIENCE WITHIN THE LANGUAGE NETWORK ---- 78

INTRODUCTION ---- 79

1. METHODS ---- 79

  1.1. Participants ---- 79
  1.2. fMRI Study Design and Materials ---- 79
  1.3. Data Acquisition ---- 83
  1.4. Data Preprocessing ---- 83
    1.4.1. Structural Data ---- 83
    1.4.2. Functional Data ---- 84
  1.5. Statistical analysis ---- 84
    1.5.1. Whole-Brain Analysis ---- 84
    1.5.2. ROI Analysis ---- 85
    1.5.3. Regression Analysis/Correlation Analysis. ---- 86

SECTION I ---- 86

1. THE LANGUAGE NETWORK ---- 87

  1.1. Core Language Brain Areas ---- 87
  1.2. Language Periphery Regions ---- 88

2. RESULTS ---- 90

  2.1. Whole Brain Analysis ---- 90
  2.2. ROI Analysis ---- 92
    2.2.1. Core Language Areas ---- 92
        2.2.1.1. Core Language Areas and AoA ---- 94
    2.2.2. Periphery Brain Areas ---- 95
  2.3. Discussion ---- 97

SECTION II ---- 101

1. RELEVANT BACKGROUND LITERATURE ---- 101

1.2 Language Competition and Inhibition ---101
1.3. Factors Influencing Brain Activation Patterns in Bilingual ------------------------------------103
1.4. The Adaptive Control Hypothesis ---104

2. PREDICTIONS --- 108

3. RESULTS: --- 110

3.1. Native L1 (Romanian) versus early L2 (Russian)--110
3.2. Late L3 (English) versus native L1 (Romanian) --111
3.3. Late L3 (English) versus Early L2 (Russian) ---111

4. DISCUSSION:--- 113

4.1. Cognitive Control Brain Regions ---113
4.2. Factors Influencing Language Control Mechanisms in Multilinguals ---------------------------117
4.3. Post-hoc Investigations: --119

CONCLUSION -- 120

CHAPTER III -- 122

BRAIN GREY MATTER RESTRUCTURING RELATED TO MULTILINGUAL PRACTICE----------- 122

1. INTRODUCTION -- 123

1.1. Background Information ---123
1.2. Evidence of Restructuring at Initial Stages of Language Learning ----------------------------125
1.3. Factors Associated with Brain Restructuring in Bilinguals ------------------------------------128
1.4. Evidence of Restructuring Beyond One Second Language --------------------------------------129
1.5. Evidence of Restructuring Beyond Initial Stages ---130

2. THIS STUDY --- 131

2.1. Material and Methods --133
2.1.1. Participants ---133
2.1.2. Data Acquisition ---133
2.1.3. Data Processing ---134
2.1.4. Data Analysis. --135
2.2. Results --138
2.3. Post-hoc Correlation Analysis ---142
2.4. Discussion ---144

3. CONCLUSION -- 151

4. LIMITATIONS AND FURTHER STEPS -- 151

CHAPTER IV-- 153

BRAIN WHITE MATTER RESTRUCTURING RELATED TO MULTILINGUAL PRACTICE --------- 153

1. INTRODUCTION -- 154

2. COMMON METHODS USED FOR QUANTIFYING WHITE MATTER IN THE BRAIN -------- 156

2.1. Issues Related to Traditional Models: ---158
2.1.1. Issues With Fitting the Data:---158
2.1.2. Issues With Biological Interpretability: --159

3. WHITE MATTER IN NEUROLINGUISTIC MODELS OF LANGUAGE PROCESSING--------- 162

3.1. White Matter Restructuring Patterns in Multilinguals ---165

4. PRESENT STUDY-- 168

4.1. Methods --170

4.1.1. Participants ---------- 170
4.1.2. Image Acquisition ---------- 170
4.1.3. Data Preprocessing ---------- 170
4.1.4. Statistical Analysis: ---------- 172
4.1.4.1. Whole Brain Analysis ---------- 172
4.1.4.2. Correlation analysis ---------- 173
4.2. RESULTS ---------- 173
4.2.1. Whole-Brain Analysis Results ---------- 173
4.2.1.1. White Matter Differences for a Comparison of Multilinguals > Monolinguals. ---------- 173
4.2.1.2. White Matter Differences for a Comparison of Multilinguals < Monolinguals ---------- 176
4.2.2. Correlation Analysis Results: ---------- 181

5. DISCUSSION ---------- 186

6. LIMITATIONS AND FURTHER DIRECTIONS ---------- 193

CHAPTER V ---------- 194

CONCLUSIONS ---------- 194

1. FRAMING THE RESULTS WITHIN CURRENT LITERATURE ---------- 199

2. LIMITATIONS AND FURTHER STEPS. ---------- 201

2.1. LIMITATIONS OF THE PROJECT ---------- 201
2.1. FURTHER STEPS ---------- 202

REFERENCES ---------- 205

APPENDIX A ---------- 224
APPENDIX B ---------- 225
APPENDIX C ---------- 228
APPENDIX D ---------- 229
APPENDIX E ---------- 230
APPENDIX F ---------- 231
APPENDIX G ---------- 232
APPENDIX H ---------- 233
APPENDIX I ---------- 235
APPENDIX J ---------- 236

COPYRIGHTED MATERIAL PERMISSIONS ---------- 237

List of Tables

TABLE 1. GRAMMATICAL ASSESSMENT TEST EXAMPLES ---------- 62
TABLE 2. SELF-RATING QUESTIONNAIRE ---------- 64
TABLE 3. PERCENTAGE ERROR FOR PROFICIENCY MEASURES ---------- 65
TABLE 4. PROFICIENCY MEASURES STATISTIC TESTING RESULTS ---------- 67
TABLE 5. P-VALUES OF THE CORRELATION TESTS FOR EARLY L2 ---------- 70
TABLE 6. P-VALUES OF THE CORRELATION TESTS FOR LATE L2 ---------- 71
TABLE 7. fMRI YES/NO TESTING SESSION RESULTS ---------- 82
TABLE 8. LIST OF CORE AND PERIPHERY LANGUAGE REGIONS ---------- 89
TABLE 9. STEREOTACTIC COORDINATES OF PEAK ACTIVATIONS FOR WHOLE-BRAIN ANALYSIS ---------- 92
TABLE 10. CORE LANGUAGE ROIs ---------- 93
TABLE 11. LANGUAGE CONTROL ROIs ---------- 96
TABLE 12. LIST OF CORE AND PERIPHERY LANGUAGE REGIONS AND RESULT COMPARISONS ---------- 98
TABLE 13. LIST OF COGNITIVE CONTROL LANGUAGE REGIONS ---------- 106
TABLE 14. PREDICTIONS BASED ON PREVIOUS LITERATURE ---------- 110
TABLE 15. REGIONS OF INTEREST WITH GREY MATTER CHANGES PREDICTED ---------- 132
TABLE 16. LIST OF ROI RESULTS FOR CORTICAL AND SUBCORTICAL GREY MATTER ---------- 141
TABLE 17. PREDICTED WHITE MATTER RESTRUCTURING PATTERNS ---------- 169
TABLE 18. FIXEL-BASED ANALYSIS RESULTS OF WHITE MATTER MORPHOMETRY ---------- 180
TABLE 19. COMBINED RESULTS OBTAINED FROM THE MRI DATA, ALL THREE STUDIES ---------- 198

List of Figures

Figure 1. Schematic Dual – Stream Model of Language -- 24
Figure 2. Simplified Language Control Network and Speech Production Regions --------------------------- 30
Figure 3. MINT Images with Increased Difficulty --- 58
Figure 4. Example of Sentence Construction for the GAT -- 61
Figure 5. Average Rating of Sentences of Ungrammaticality Scores -------------------------------------- 68
Figure 6. Correlation Matrix Proficiency and Language Use Early L2 ------------------------------------- 69
Figure 7. Correlation Matrix Proficiency and Language Use Late L2 -------------------------------------- 71
Figure 8. Sentence Tree Example for the fMRI Task -- 80
Figure 9. Task Design --- 80
Figure 10. Whole-Brain Analysis Results for Native Language Processing (all 30 subjects) -------------- 91
Figure 11. Average Beta-Values in A Priori Defined Core Language ROIs Mono vs Multi ------------------ 93
Figure 12. Average Beta-Values in A-Priori Defined Core Language ROIs, L2 vs L3 ----------------------- 94
Figure 13. ROI Analysis Results for Language Control Areas Across Groups ----------------------------- 97
Figure 14. Simplified Language Control Network and Speech Production Regions ------------------------105
Figure 15. ROI Analysis L1 (Romanian) vs L2 (Russian) ---111
Figure 16. ROI Analysis L3 (English) vs L2 (Russian) --112
Figure 17. Cerebellum Activation for the Multilinguals Across the Three Languages ---------------------113
Figure 18. Group Differences for Native Language Processing ---119
Figure 19. White Matter and Pial Surface Boundaries ---136
Figure 20. Subcortical ROIs Selected for the Structural Analysis --------------------------------------137
Figure 21. Cortical ROIs Selected for the Structural Analysis --138
Figure 22. Significant Mean Grey Matter Thickness Cortical ROIs ---------------------------------------139
Figure 23. Significant Mean Grey Matter Volume ---140
Figure 24. Correlation Analysis Between the AoA and the GM Thickness in the Right ACC ----------------143
Figure 25. Correlation Analysis Between the Amount of Immersion and Right ACC Thickness --------------144
Figure 26. The Dynamic Restructuring Model --156
Figure 27. A Dual-Stream Model of Language Processing ---164
Figure 28. White Matter Significant Fixels - Multilinguals > Monolinguals ----------------------------174
Figure 29. Tractograms of Significant White Matter Fixels – Multilinguals > Monolinguals ------------175
Figure 30. Reconstructed White Matter Pathways of Significant Fixels – Multi> Mono -------------------176
Figure 31. White Matter Significant Fixels - Monolinguals > Multilinguals ----------------------------177
Figure 32. Tractograms of Significant White Matter Fixels – Monolinguals > Multilinguals ------------178
Figure 33. Reconstructed White Matter Pathways of Significant Fixels – Mono > Multi ------------------179
Figure 34. Correlation Plots Between AoA and Fiber Density in the Inferior Cerebellar Peduncle -------182
Figure 35. Correlation Plots for Age and Fiber Density in the Inferior Cerebellar Peduncle -----------183
Figure 36. Correlation Plots Between Immersion and Fiber Density in the ICP --------------------------184
Figure 37. Correlation Plots Between Average Proficiency and Fiber Density in the ICP ----------------185

Introduction

1. General Introduction

1.1. Neural Representation of Language

A prominent question in the field of neurolinguistics is whether the neural representation of language changes as a function of the number of languages one knows. Language is often regarded as a quintessential property of the human species. Brain studies of language provide evidence for the expansion of neural structures and specialized cognitive processes that support linguistic communication (Bornkessel-Schlesewsky et al., 2015). However, much of the current work on the neurobiology of language has largely focused on the characterization of language in monolingual speakers. This is remarkable, as many have noted that most of the world's language users know two or even more languages (Grosjean, 2012). "The world is bilingual or multilingual" (Crystal, 2010; de Bot, 2019).

While burgeoning efforts are beginning to entertain models of language processing in bilingual speakers, the present study proposes to investigate language organization in fluent *multilingual* speakers, individuals who are versed in four or more languages. We have identified a local population of polyglots in the Sacramento area who have extensive expertise in at least four languages. This is the first multilingual study, to our knowledge, to test a subgroup of people that have Romanian as a first language. These individuals are also fluent in Russian, English, and a fourth language that differs from participant to participant (e.g. French, Spanish, Ukrainian, German, etc.). They will be compared to a group of English native speakers with no fluency in any other languages. I aim to understand whether extreme fluency in multiple languages leads to structural and functional changes in well-known language networks.

1.2. The Importance of Studying Multilingualism.

Understanding the neurobiology of language has important implications for both basic science and clinical practice. The proper characterization of the anatomical and functional networks that underlie human language processing is crucial for the understanding of properties that underlie the normal development and acquisition of language skills; and the development of therapies for individuals who have lost linguistic competencies as a function of acute brain injury.

Furthermore, multilingual research informs theoretical models of brain plasticity and provides testable hypotheses to establish biological and behavioral foundations of higher cognitive processes. Findings from this research can inform educational and public health policies (helping to identify best practices in language pedagogy), can help understand the cognitive and developmental advantages and/or disadvantages of multiple language experiences, can provide further evidence for considerations of language learning as a deterrent of cognitive decline, and also help develop appropriate therapy practices for bi/multilingual aphasia.

1.2.1. The Bilingual Advantage

Research on the brain organization in multilinguals helps characterize the normal development and acquisition of language skills in early infancy and examine their consequences into adulthood. Research suggests that the increased plasticity seen in early development is essential for multiple language learning (Petitto et al., 2012). Learning a second language simultaneously with a first language and may result in a longer period in which infants retain *universal categorical perception* and consequently more efficient learning of a new language(Bradley et al., 2013). Many researchers agree that the AoA of a language influences the cortical representation of the language network, with more overlap between the languages, acquired early in life versus the ones acquired later (Bloch et al., 2009; Cherodath & Singh, 2015). Monolingual babies are born with the ability to discriminate between the phonetic contrasts coming from all of the world's languages, referred to as universal categorical perception (Eimas et al., 1971; Kuhl, 2007). At the age of 10-12 months, infants lose this universal ability and become highly sensitive to phonetic contrasts from their native language. Petitto et al., 2012 looked at the neural networks and brain tissue that underlie the phonetic processing between monolingual and bilingual babies between 2 and 16 months old, using Near-Infrared Spectroscopy (fNIRS). The babies listened to three different stimuli: native language sounds, the non-native language sounds that were non - native for both bilinguals and monolinguals, and non-linguistic musical tones. Both groups of babies showed activation in the STG for all stimuli, with increased activation for the linguistic versus non-linguistic sounds. They have also noticed greater activity in the left IFC while processing linguistic sounds (both native and non-native) for all babies below the age of 12 months, while this increased activation was only observed for bilingual babies after the age of 12 months. The authors suggested that the bilingual input may prolong the plasticity period in which babies retain their universal categorical perception. These changes in the plasticity of the infant brain have been found to provide scaffolding for linguistic cognitive and reading benefits throughout life (Bialystok & Craik, 2010). Bradley et al., 2013 showed in an MRI study looking at differences in brain activation for monolinguals and bilingual adults during a lexico-semantic task, after being exposed to a novel language for only two hours. The monolinguals showed greater activation in the supplementary motor area (SMA), the anterior cingulate cortex (ACC), the dorsolateral prefrontal cortex (DLPFC), and the Caudate Nucleus (CN), while bilinguals recruited the DLPFC and regions of the basal ganglia (namely the putamen). The authors suggest that bilingual experience may result in developing a different learning strategy with the recruitment of different neural mechanisms of cognitive control, indicative of less effortful learning of new vocabulary items by the bilinguals compared to monolinguals.

One of the most discussed effects of multilingualism is the increase in executive function observed in bi/multilingual individuals (Bialystok et al., 2009; Dong & Li, 2015; Green &

Abutalebi, 2013). This has been long debated, with some authors suggesting that the practice of switching from language to language and constantly inhibiting the other languages (Marian and Spivey, 2003) leads to better cognitive control and flexibility (e.g. Bialystok et al., 2009; Green & Abutalebi, 2013; Luk et al., 2012; Pliatsikas & Luk, 2016; Poarch & Bialystok, 2015), while others argue that individuals that become bilingual are naturally better at switching (Festman et al., 2010). Nevertheless, studies show that bilinguals outperform monolinguals in multiple cognitive control and switching tasks requiring inhibiting, interference control/conflict resolution, switching, and control monitoring. For example, Poarch and Byalistok, 2015 examined 203 children, 8–11 years old that were either monolingual, partially bilingual, bilingual, and trilingual. The participants performed a flanker[1] task. The results showed that the bilinguals and the trilinguals outperformed the partial bilinguals and the monolinguals on the conflicting trials, suggesting that bilinguals have an advantage in recruiting the executive control function systems. Similar bilingual advantages were observed in a Stroop[2] task in Yang and Yang, 2017, that showed better performance on word retrieval for bilinguals versus multilinguals when higher cognitive control and working memory were required in the task. Bilinguals show slower reaction times in tasks requiring conflict resolution and interference control (e.g. Simon[3] task) as well. However, some studies fail to replicate these observations (e.g. Paap et al., 2015). One explanation for these inconsistencies may be related to the fact that these advantages seem to be modulated by a variety of factors,

---

[1] The flanker task was originally proposed by Eriksen & Eriksen, 1974. In a typical Flanker task, participants are presented with a series of arrows/chevrons on a screen and asked to indicate the direction of a target arrow occurring in the middle of the screen. In this study participants had had 4 trials: 1) baseline – only one chevron was at the middle of the screen; 2) neutral – middle chevron was flanked by two diamonds; 3) congruent – the chevrons were pointing in the same direction with the target; 4) incongruent – the chevrons and the target pointed in opposite directions.

[2] The Stroop task first presented by Stroop, 1935, is task in which participants are presented color words written in different colors and asked to name the color with which the word is written rather the meaning of the word. In this study the participants were asked to perform on tree different tasks:1) a baseline task -and *attention-span task* (Turner & Engle, 1989); 2) a low cognitive demand task - *Stroop-span task* (Hayes, Kelly & Smith, 2013); 3) a high cognitive demand task - *attention-impeded Stroop-span task* (Yang et al., 2005).

[3] In the Simon task introduced by(Carpenter & Simon, 1960), participants are asked to press a key on one of the sides of the screen based on the a specific picture (left side is red circle/right side if blue circle). The test usually has a congruent condition – the stimuli is presented on the same side of the screen as the correct key; an incongruent condition – the key and the stimuli are on opposite sides of the screen, and a neutral condition – stimuli is in the center of the screen.

including the age of participants, AoA, proficiency, task requirements, etc. (for a review see Dong & Li, 2015). Many questions as to whether these advantages are observed in highly multilingual populations are still unknown, and whether they are more strongly pronounced as an effect of multiple languages remains to be studied.

Experience with multiple languages may also lead to an increase in brain connectivity, which in turn allows for more efficient processing and control of language. Indeed, some authors have argued that these advantages are due to the connectivity between the language regions and the cognitive control regions. García-Pentón et al., 2014, analyzed the anatomical brain connectivity between a group of bilinguals and monolinguals. They identified two neural subnetworks of anatomically interconnected brain regions that were more connected in the bilinguals than monolinguals. The first was in the left hemisphere and comprised the insula, the superior temporal gyrus (STG), pars opercularis, and triungularis in the IFG, the supramarginal gyrus (SMG), and medial superior frontal gyrus (mSFG). The second network included the left superior occipital and parietal gyrus, superior temporal pole (STP), the angular gyrus (AG), and the superior frontal gyrus (SFG). The authors suggest that the demands imposed by processing multiple languages may lead to the development of more devoted specialized subnetworks, which in turn reduce the global efficiency of the network as a whole; affecting higher cognitive processes that rely on the whole network. It is still unknown whether these observations are also seen in multilinguals and whether brain connectivity and the efficiency of brain networks are further modulated (whether advantageously or disadvantageously) by the experience of using multiple languages. The studies presented in this dissertation assess the differences in brain connectivity between the monolinguals and the multilingual group, and the existence of such specialized functional and structural networks within multilinguals.

1.2.2. Neuroprotective Effects Associated with Bi/multilingualism

Lastly, multilingual experience seems to have important implications for brain organization in older adults. Although not consistently found across studies (Lawton et al., 2015), some results suggest that multilingualism has neuroprotective effects against cognitive decline (Alladi et al., 2013; Bialystok et al., 2007; Pliatsikas, Meteyard, et al., 2020). For example, a study looking at patients with dementia (648 patients, including 391 of them bilingual) have found that bilinguals showed significant later age of onset, regardless of the type of dementia (i.e. Alzheimer disease dementia, frontotemporal dementia, vascular dementia, etc.) and other individual factors were not shown to have an effect. This difference was also observed in illiterate patients; thus, the authors excluded the effect of education on the results (Alladi et al., 2013). Another study found greater white matter connectivity (as measured by fractional anisotropy (FA)for bilinguals in the corpus callosum (CC), bilateral superior longitudinal fasciculus (SLF), right inferior fronto-occipital fasciculus (IFOF), and the uncinate fasciculus (UF). The study has also investigated the connectivity of the frontal regions adjacent to these white matter tracts and found greater connectivity in the bilinguals compared to monolinguals. The authors suggest that bilinguals may benefit from preserved white matter integrity, which has been shown to decrease with age in adulthood (Luk et al., 2011). However, this is a highly controversial topic, and many others have failed to find similar bi/multilingual effects. One of these studies was a systematic review and meta-analysis by Mukadam et al., 2017. The authors observed that most retrospective studies report an average of 4.5 years of later onset of dementia in bilinguals compared to monolinguals, however, these effects may be due to other variables, that were poorly controlled in these, such as education socioeconomic status, and method of reporting initial symptoms.

Although most of the research in bilingual effects on brain structure and cognitive abilities concentrates on advantages, few studies identify some disadvantages associated with bilingualism. For example, Ivanova & Costa, 2008, measured picture naming performance in a group of monolinguals compared to highly proficient bilinguals with a dominant L1 language. The authors concluded that the lexical access in bilinguals seems to be slower than those in the monolinguals even in their L1. Other studies have looked at the vocabulary development in bilinguals compared to monolinguals and have noted that bilinguals have a smaller vocabulary in each language they speak but may have a higher total vocabulary. One study of 1738 children from 3 to 10 years compared the receptive vocabulary in bilinguals that were educated in English and spoke a second language at home. The overall vocabulary of the bilinguals was lower in English than those of the monolinguals of all ages. However, further analysis of the items in the vocabulary tests led the authors to conclude that the academic lexical items were comparable across groups, while the home life items showed a larger difference across groups, which was interpreted as a reasonable result given the language background of the bilinguals and was not interpreted as a disadvantage per se (Bialystok & Craik, 2010).

1.2.3. Multilingualism and Aphasia

Multilingual research has greatly contributed to the characterization of the deficits and impairments associated with aphasia. With an increase in the multilingual population worldwide, there is also an increase in the instances of multilingual aphasia (Cargnelutti et al., 2019a). Aphasia is a clinical condition that affects brain networks supporting language as a consequence of brain injury, brain trauma, or other neurological diseases. To date, rehabilitation of bilingual aphasia lacks optimal treatment practices that would suggest which languages (native or second languages, most or least impaired, acquired early or later in life, or most or least dominant languages) should be targeted for better recovery results and whether these treatments will generalize across languages is not yet known (Peñaloza & Kiran, 2019). Hence, one of the main reasons for which it is important to understand the language organization in the multilingual brain is to try to answer questions that help develop appropriate therapies for individuals that have lost their language abilities. Aphasia can manifest differently in the diverse languages known by the multilinguals (Cargnelutti et al., 2019b). Along with the multiple patterns of impairment (i.e. *parallel impairment* where both/all languages are impaired, *differential impairment* in which one language is more impaired than another, etc.) following aphasia, individuals may lose the ability to properly regulate and handle the languages they speak reflecting damage to the cognitive control systems that regulate the languages (for a review, see Paradis, 2001; also Fabbro, 2001a, 2001b). This inability may result in improper *switching* between language (e.g. switching to another language between sentences, even when the interlocutor does not speak the language in which the patient switches); or improper *mixing* of the languages within utterances (e.g. using words from a language that the interlocutor does not speak). Fabbro et al., 2000 in a case study of a bilingual reported involuntary switching from L1 to L2 and vice versa when the patient was asked to speak in only one language. Similarly, Kong et al., 2014 showed involuntary switching between the languages of a trilingual speaker with traumatic brain injury for the languages that were structurally more similar. Multilingual aphasia can be also manifested at the level of translation, especially from the less impaired to the more impaired languages (Adrover-Roig et al., 2011). By better understanding the brain networks supporting multilingual language processing and control, better therapy practices can be developed that target specific brain areas/ brain networks associated with the impairment observed (e.g. if the patient shows impaired switching pathologies, the proper characterization of the brain areas involved in switching between languages is of crucial importance).

In summary, multilingualism seems to affect brain organization from infancy and continues to have consequences into adulthood. Bi/multilingual infants show a prolonged universal categorical perception period that may promote more efficient language learning. Adult bi/multilinguals appear to have an advantage in tasks that require switching and higher cognitive control demands and may show later onsets of dementia compared to monolinguals. These advantages have been associated with greater connectivity between the brain regions, greater white matter integrity, and increased gray matter volume (Abutalebi et al., 2015). On the other hand, other authors have noted disadvantages related to juggling multiple languages. The effects of bilingualism on brain organization are still under debate. Whether multilingualism shows similar, differential, more pronounced, or lack thereof advantages or disadvantages is not known.

Interestingly, similar factors (i.e. language structure, individual differences, and language background) that influence language representation in the multilingual brain (I will discuss these in the next section) also modulate the patterns of language impairment observed in multilingual individuals (Cargnelutti et al., 2019b). It is crucial to characterize the influence of these factors in the restructuring of the multilingual brain, as these can be used to further understand language impairment in individuals with aphasia (Gray and Kiran 2013; Peñaloza & Kiran, 2019). Additionally, the levels of language at which all these factors have an effect may not only be used to develop treatment therapy but also predict rehabilitation outcomes (Paradis, 2001).

2. Factors Influencing Language Representation in the Brain

Decades of psycholinguistic and neurolinguistic studies of bilingual language processing and language representation in the brain have identified several factors that may contribute to anatomical and functional differences in the bi/multi-lingual brain. However, this rich literature provides a mixed picture of how these factors (and indeed the purported differences themselves) influence brain organization (de Bot, 2019; DeLuca et al., 2019; Marian, Blumfield, et al., 2007). Blackburn, 2019 differentiates between two types of factors that may influence language representation in the brain for multilinguals: language properties and individual differences.

2.1. Language Properties

One of the factors that may contribute to brain representation in multilinguals is the structure of the languages used. Most modern neuro/psycho cognitive theories and models of online language processing are built on the assumption of language universality, where the same neural and cognitive substrates reflect processing independent of language type. While a wide variety of typologically different languages have been shown to recruit roughly the same language network (e.g. Videsott et al., 2010), recent cross-linguistic studies point to a series of language-dependent processing differences resulting in respectively different brain activation patterns. Differences were observed at different levels of language processing. For example, (Tang et al., 2005) showed that processing Chinese characters, due to their visual-spatial complexity, requires the recruitment of additional brain areas, particularly in the right hemisphere, that are not recruited while processing English. In their meta-review, the authors identified the implication of slightly different brain regions for the processing of phonological features in Chinese versus English writing (e.g. dorsal lateral frontal regions versus posterior parts of the inferior frontal gyrus as well as stronger recruitment of the right occipitotemporal brain regions), that the authors attribute to the difference in mapping between orthography-to-phonology at the syllable level in Chinese and grapheme-to-phoneme mapping in English at the phonemic level.

At the level of morphosyntax, users of different languages seem to use the languages' specific cues (i.e. nominal case-marking, word order patterns, stress patterns, noun-verb agreement markers, clitic pronouns, verb voice markings, etc.) to assign hierarchical roles during sentence comprehension distinctly depending on the language (MacWhinney, 2008). Kovelman et al., 2008 showed that bilinguals demonstrate different brain activation patterns while processing OS (object-subject) "difficult" sentences versus SO (subject-object) "easy" sentences; with greater activation in the left IFG (particularly BA44/45) for the "difficult" sentences in English, and no difference in activation when processing SO versus OS sentences in Spanish. Additionally, (Bornkessel & Schlesewsky, 2006) propose a model that predicts morphological case informativity and word ordering preferences for languages with a different grammatical structure based on event-related potential (ERP) effects observed during processing sentences with certain grammatical violations. Namely, the authors suggest that for languages in which word order is the most informative cue for assigning semantic roles in the sentence, structures as (1), (documented by (Coulson et al., 1998)) will elicit a left anterior negativity (LAN) component at the position of “we”, as a result of a conflict elicited by the AGRT (agreement) mismatch once the pronoun is encountered.

E.g.

*The plane took we to paradise and back (1)

While, in languages such as German, in which morphological case is more informative for computing semantic roles, similar structures, as the one in (2), elicit an N400.

*Welcher Lehrer	besuchte	Der Priester	am	Sonntag (2)
[which teacher] NOM	visited	[the priest] NOM	on	Sunday

Similarly, Jeong et al., 2007, using functional MRI, compared brain activation related to syntactic processing of constructions in Chinese, Korean, Japanese, and English for multilingual speakers. They observed stronger activation in the left Broca’s area (namely, pars opercularis and pars triungularis) and right cerebellum, for comprehension tasks in English (L2) versus Korean (L2), for native Japanese (L1) and Chinese(L1) individuals. The authors attribute these differences to the fact that English (L2) typology, unlike Korean (L2), is more dissimilar to Japanese and Chinese. These findings suggest that various language properties may require different brain computations and may result in distinct brain patterns of activation.

In addition to language structure, there is evidence of differential recruitment of brain areas due to language modality. For example, Corina et al., 2013 - a review of neuroimaging and case studies of sign language use in deaf individuals - provide evidence that sign languages require additional somatosensory and visual-motor integration regions in the left hemisphere (i.e. left inferior parietal lobule (IPL), posterior parietal areas), as well as right hemisphere regions (e.g. posterior parietal region). The authors propose that the modality-driven differences in language representation may be due to the visual-spatial characteristics of syntactic properties of sign languages.

Together, these studies suggest that language typology, language-specific characteristics, and language modality may influence the organization of language representation in the brain of bilingual and multilingual speakers.

2.2. Individual Differences.

Although factors such as genetics, gender, age, and verbal intelligence have been proposed as potential contributors to language organization in bi/multilingual populations (e.g. Blackburn, 2019; similarly, Stocco et al., 2010 suggest that the neurotransmitter *dopamine* may play a prominent role in modulating the control networks, specifically the striatum, in multilingual individuals). The largest contributors to language representation in the brain are language proficiency (showing to have a greater impact on lexico-semantics), age of acquisition (AoA) (mostly impacting phonological and grammatical processes), and language exposure. Unfortunately, the last three are often confounded in studies of bilinguals, since generally AoA is negatively associated with proficiency, while higher exposure to a language leads to higher proficiency. Multilingual studies, as the one discussed here, allow for a dissociation between these by analyzing activation of two languages acquired at different times, with similar proficiency.

2.2.1. Age of acquisition.

One of the important factors influencing cortical representation of languages in studies of bi/multilinguals is AoA. The age at which a second language has been learned seems to lead to the development of different mechanisms related to lexico-semantic and grammatical processing. Important evidence of the effect of AoA on brain language organization comes from multilinguals studies, investigating participants that have learned their second languages at different ages. These data suggest that when a second language(s) is acquired at the same time as a native language, the same neural resources are used for both/all languages while learning an additional language later in life leads to stronger reliance on additional (task-dependent) brain areas.

Generally, multilinguals that have acquired their second language(s) earlier in life show more homogeneous patterns of cortical organization for all the languages (Cherodath & Singh, 2015) whereas late learners of L2s show a higher degree of inhomogeneity in brain activation patterns both in cortical domain-specific language areas (Bloch et al., 2009) and stronger recruitment of domain-general brain areas (especially in the PFC and the basal ganglia) while processing L2s. Liu & Cao, 2016, conducted a meta-analysis on the influence of the AoA on overlapping similarities between brain activation patterns while processing an L1 versus an early or late L2.

Their activation likelihood analysis revealed a few trending patterns: additional regions are recruited while reading a late L2 versus an early L2 compared to the L1; the AoA has an influence on L1 processing, namely the early L2 learners showed increase activation in the left fusiform gyrus during the processing of their L1. Although debated, there is also evidence suggesting that AoA affects more the cortical activity in brain areas involved in grammatical processing, and less, brain areas involved in lexico-semantic processing. Additionally, some authors (e.g.Golestani et al., 2006; Johanne et al., 2013; Ullman, 2001) suggest that AoA affects more the grammatical rather than lexico-semantic aspects of a second language since lexico-semantic processing is subserved by declarative memory systems (involving the left temporal lobe), and these do not differ regardless of how many languages are learned. While the grammar of an L1 is implicitly acquired - involving procedural memory subserved by a fronto-striatal network, a later L2 grammar is acquired explicitly and is dependent upon declarative memory. This view has been supported by the argument of observed increased activation in the basal ganglia as proficiency of the L2 increases, suggesting a shift from declarative to procedural memory (e.g. Golestani et al., 2006).

Other authors suggest that the same results may be interpreted as an increase in automaticity and need for cognitive control (Abutalebi, 2008; Perani & Abutalebi, 2005). These authors suggest that the same neural system subserves all learned languages, but later-learned languages require extensive cognitive control mechanisms (involving areas as the DLPFC, IPL, and the Basal Ganglia). For example, Waldron & Hernandez, 2013, observed greater activation for verb generation in the left middle frontal gyrus (dorsolateral prefrontal cortex), right superior frontal gyrus, angular gyrus extending into the left inferior parietal lobule), and left hippocampus for late bilinguals versus early bilinguals.

There are two general hypotheses in terms of brain representation of languages in the multilingual brain. One is that when a second language is acquired early in life, or simultaneously, the neural representation of the two languages will be shared, while a later learned language will recruit additional adjacent areas. This pattern of organization has been noted in studies using electrical stimulation mapping (ESM). For example, Lubrano et al., 2012, in a case study of a trilingual undergoing awake craniotomy, reported the results of language mapping between the patient’s L1 and L3. They observed overlapping as well as distinct brain regions across the two languages. The patient produced errors in both languages with stimulation in sites within BA 44, while BA 45 stimulation produced errors only in the patient’s L3 in the 2 sites mapped in the DLPFC, the patient produced switching errors in the L3, stimulation to frontal subcortical regions seemed to be shared by both languages (see also Bello et al., 2006)).

Another hypothesis concerning the neural representation of languages in the multilingual brain is that by learning two languages simultaneously, a bilingual language system develops that can accommodate both languages. Along with the development of the language system, general cognitive control systems are also developing. The concomitant development of both systems results in greater overlap, while a later acquired language will converge onto the already existing language and control systems. This process requires neurostructural changes and results in more non-overlapping brain regions. Bloch et al., 2009, in a verbal task, noted that trilingual individuals that learn an L2 early in life, show a homogeneous activation in Broca's and Wernicke's classical language network regions in all languages learned, even if the third language is learned later in life. While there is more variability in these areas for trilinguals that have learned their first L2 later in life. They have also noticed a gradual change related to the AoA of an L2 and brain activation overlap between the three languages spoken by the participants. The authors concluded that early exposure to a second language seems to give rise to a common language network that is accessed by all multilingual's languages. This network shows increasingly more variability in activation with increasing L2 AoA. Wattendorf et al., 2014 investigated the influence of AoA on cortical representation of language during silent language production. The study compared two groups of multilinguals, one group had learned an L2 by the age of 3, and the second group at the age of 9, both groups learned their L3 after 9 years old. A between-group comparison revealed higher activity in the bilateral inferior frontal areas (Broca's area), bilateral supplementary motor area (SMA), left anterior cingulate cortex (ACC), and left pre-central gyri for all three languages, for the early versus late multilinguals. Greater activity in the posterior superior temporal gyrus (pSTG) was observed for the late versus the early multilinguals in all three languages. The authors suggest that the AoA has an effect on how the cognitive control and the language network interact, with a reliance on the cortico-striatal sensorimotor loop that consolidates when languages are learned in parallel, while the late multilinguals have to rely on additional task-dependent brain regions. Additionally, the left striatum and the IFG (BA 47) were active for both groups in all languages except the L1 for late multilinguals. This pattern of results suggests that only languages that are learned in isolation, independent of other languages are subject to less interference and may require less control.

Lastly, in an overt picture naming paradigm of switch trials (the naming language of consecutive trials varies) versus non-switch trials (the naming language of consecutive trials was the same), for trilingual participants that had high proficiency in their early L2 and moderate proficiency for a late L3, authors showed that switching trials in the early L2 and late L3 compared to non-switching trials lead to greater activation in the rIFG and pre-SMA, while the same comparison in the L1 showed no difference in activation. Naming in both L2 languages versus naming in L1 elicited greater activation in the left IFG, pre-SMA, right SMA, left pre and postcentral gyrus, right Heschl's gyrus, right postcentral gyrus, right insula, bilateral middle cingulate cortex (BA6), left calcarine gyrus (BA17) and cerebellum, right inferior occipital gyrus (BA19), bilateral putamen and right caudate nucleus, and right cerebellum. However, the results did not show any significant differences between the early more proficient L2 and the late, less proficient L2, for either of the trial types (Bruin et al., 2014).

In summary, AoA affects the cortical representation of languages and leads to differences in the cortical overlap between multilingual's languages. Bi/multilingual studies suggest that an early learned language compared to a language that is learned later in life may potentially benefit from different mechanisms of lexico-semantic and grammar processing. Additionally, acquiring second languages earlier in life seems to develop different sensorimotor and control mechanisms that are then recruited for all languages, even if these are learned at a later age. Thus, AoA seems to be a major determinant of language representation in a multilingual brain.

2.2.2. Proficiency

Proficiency is one of the most studied factors influencing brain language representation in bilingual and multilingual individuals. Generally, with an increase in proficiency in a second language there is an increase in the overlap of the brain areas recruited (Abutalebi et al., 2001) AoA often interacts with proficiency. Abutalebi et al., 2001, suggest that while an early AoA results in greater neural representation overlap due to the concomitant development of the language and cognitive control systems. Higher proficiency leads to greater representation overlap due to higher convergence of the additional later learned language onto the existing systems. Contrary to AoA, proficiency seems to have a greater impact on the lexico-semantic system (e.g. Blackburn, 2019). Evidence supporting this view comes from studies looking at the temporal correlates of brain activity. A study by Leonard et al., 2011, combining magnetoencephalography (MEG) and magnetic resonance imaging (MRI) has looked at both the temporal correlates and activation maps during visual and auditory word processing in a group of bilinguals that have lost dominance in their L1 (native) and were more proficient in their L2 (learned around 6 years of age). They have noticed similar activity in the 'classical language areas' (i.e., IFG) but greater activity for the L2 versus L1 in the bilateral occipito-temporal areas, including the fusiform gyri, the ventral and lateral occipitotemporal regions, as well as the superior temporal sulcus (STS) and the anterior insula. These were observed from the earlier stages of encoding (170ms for visual words and 100ms for auditory words) and persisted up to ~400ms. Activity at around this time window is thought to occur at the level of lexico-semantic processing. Importantly, the effects seen at ~400ms were observed while participants processed "new" words (words that they did not encounter before in the task); suggesting that word familiarity affects the recruitment of these regions, while the right lateral occipitotemporal region showed this repetition effect only for the Spanish (the less proficient language), suggesting that proficiency modulated activity in this area.

Additionally, less proficient language processing seems to recruit more areas associated with general cognitive control, including the prefrontal control areas, left IFG (BA 47), the middle frontal gyrus (MFG), the bilateral cerebellum (Videsott et al., 2010), the basal ganglia (Abutalebi et al., 2009; Green & Abutalebi, 2013), and areas that are homologs to the 'left-lateralized language network' (Pang, 2012). There are multiple reasons for the increase in activity for the lower proficiency languages. One is that processing a less proficient language requires more effort resulting in an increase in the cognitive demand on the language network, and with an increase in proficiency, the efficiency of the network may become better. Another possible factor is that the increase in activity is driven by the process of interference suppression from the L1 or the more proficient languages. There is evidence showing a shift from the recruitment of cortical control areas, thought to be recruited when bilinguals require a conscious effort to process a less proficient language to a subcortical circuit (i.e. the basal ganglia), believed to be involved in more automated processes as a result of increased proficiency (Abutalebi & Green, 2016; J. A. E. Anderson et al., 2018). The basal ganglia have been shown to be involved in biasing cortical activity by prioritizing the cortical inputs to attain an intended goal and modify behavior (Stocco & Prat, 2014).

Videsott et al., 2010, compared the language activation patterns for a group of multilinguals across three languages. They found that high proficient languages elicited higher activity compared to lower proficiency languages in the right prefrontal areas, including the DLPFC (Ba46/BA10), the anterior frontal gyrus (BA11), the inferior frontal gyrus, and the right insula (BA 47). A conjunction analysis revealed the DLPFC to be a commonly activated area for both less proficient languages. An analysis of the opposite comparisons revealed higher activation in the left IFG (BA 44/46) and the cerebellum, with the cerebellum being a commonly recruited area. The authors suggest that the DLPFC is an area whose function is modulated by proficiency since it did not only show greater activation for both higher proficiency languages but was also functionally correlated with naming in the least proficient languages.

Another magnetoencephalography (MEG) study reported early and sustained (50 – 450 ms) activation in the left IFG for both L1 and L2 production, and homologous activation in the right IFG for L2 only, preceded by early and sustained activation in the right insula (BA 13) (Isel et al., 2010). Both languages activated the motor areas; however, for the L2, this activation was preceded by the anterior cingulate cortex (ACC, namely BA32). L1 recruited the left superior frontal gyrus (SFG), while the L2 activated the DLPFC. The authors suggest that the activation in the ACC is associated with the lower L2 proficiency, this area being involved in conflict-monitoring and error-detection. Meanwhile, the insula was proposed to be involved in activating an L2 and switching from L1 to L2 (Isel et al., 2010). Others relate its activity to L2 proficiency (Leonard et al., 2011).

More recently, the potential modulation of putamen activity by proficiency has been described in a multilingual study. The authors observed greater activity in the left putamen for the processing of the less proficient language. Additionally, the gray matter volume of the left putamen was associated with naming in the less proficient language. The authors propose that the left putamen is involved in articulatory processes, and it is more prominent when speakers articulate in non-native languages (Abutalebi et al., 2013).

Hence, proficiency is a contributing factor in multilingual language representation in the brain. Less proficient languages seem to be subserved by the same networks as the highly proficient languages. However, multilinguals show stronger activation in the classical language areas and additional recruitment of the cognitive control areas. These have been proposed to be due to the increased effort in controlling and suppressing interference from the more proficient languages while functioning in a less proficient language. As proficiency increases and processing becomes more automatic, there seems to be a shift between the recruitment of the cortical areas to the greater recruitment of the subcortical areas.

2.2.3. Language Exposure and Language Use

Multilingual language representation seems to also be affected by the amount of exposure to a second language and the amount of time a second language is used. The effect of exposure and use seems to have similar effects as proficiency. Diminished differences in cortical representation between the first and the second languages are associated with increased exposure and use. Language exposure affects the extent of brain activation while processing a second language. A study looking at the effect of AoA and exposure on brain activation during a verbal fluency task reported increased activity in the middle frontal gyrus (Ba 46/10) and the IPL (Ba 40) and control areas associated with less exposure (Perani et al., 2003). Abutalebi et al., 2007, showed that bilinguals with a less exposed L1, recruit the ACC and the subcortical areas (namely, the caudate) when switching into an L1 less exposed language. The authors suggest that this activation is due to the switching cost from a more dominant to a less dominant language modulated by exposure. Similarly, Tu et al., 2015, reported the effects of language exposure after only 30 days of differential language exposure. The participants were scanned two times, once after a period of exposure to both languages similarly, and a second time after a period in which participants used their L2 only 10% of the time. The second scan revealed greater recruitment of the dorsolateral prefrontal cortex (BA 46, Ba 9), the ACC, the IFG (namely, Ba 44), and marginally significant effects in the left caudate. Additionally, the activation in the left ACC was negatively correlated with the amount of exposure. The authors suggest that less exposure to an L2 may result in increased engagement of areas involved in monitoring and controlling languages.

To summarize, factors including both language properties and individual differences have been shown to contribute to multilingual brain representation patterns. At the level of phonological processing, studies have identified language-dependent activation patterns, as a function of whether the phonological mapping is at the syllable or phoneme level. Similarly, hierarchical role assignment as well as word order, during sentence comprehension seems to be computed distinctly depending on language typology and results in different brain patterns of activation. Finally, language modality is yet another contributing factor in brain recruitment during language processing. Along with language properties, individual differences pertaining to the multilinguals' background influence the results observed in neuroimaging studies of bi/multilinguals. Early age of acquisition seems to have an effect on the cortical overlap between the learned languages, while higher proficiency and exposure to a second language leads to higher convergence of the additionally later learned languages onto the neural network of the native language. Together, this evidence underly the importance of properly documenting and accounting for such factors in multilinguals studies in order to obtain a more comprehensive understanding of brain differences between multilinguals and bilinguals or monolinguals.

3. Introduction to the Neurobiology of Language and Multilingual Language Models

3.1. The history of Neurobiology of Language

Historically, there have been two major approaches to studying the neurobiology of language: neurophysiological approaches, studying language abilities in bilingual speakers with focal brain injuries (e.g. aphasia), and neuroimaging approaches (e.g. fMRI). Neurophysiological studies often report variable patterns of deficits and spared functions across bilingual aphasics (see Ijalba et al., 2013; Paradis, 1977, 2004, for reviews). The differential impairments are essentially a reflection of the complexity of the bilingual language experience, compounded by the indeterminacy inherent in lesion studies, which, on their own, can produce a variety of language comprehension and production profiles. Critical individual variables, such as language learning history, proficiency, and use, contribute to patterns of deficits and sparing, while variables of brain damage such as lesion size and extent of the functional damage result in complex interactions. Despite the allure of these fascinating case studies, the interaction of these factors makes generalizations difficult (Paradis, 2004). More recently, structural, and functional imaging techniques such as magnetic resonance imaging (MRI) and positron emission tomography (PET) have allowed researchers to rely less on investigations of aphasics and to study multilingualism in the intact brain, using more carefully targeted populations and more controlled language testing (Perani & Abutalebi, 2005). The variability between aphasic symptoms and patterns of recovery along with the evidence from neuroimaging data have led to the understanding that the classical language model, including Broca's and Wernicke's areas and the arcuate fasciculus, is inadequate in explaining how language works in the brain (Poeppel & Hickok, 2004). Advances in the cognitive neurosciences have led to refinements of the traditional Wernicke-Geschwind functional-anatomical model of language (Kolb & Wishaw, 2003). Consequently, the newer models of language processing propose to reframe the question of brain architecture, supporting language function in terms of a *language network*. Fedorenko & Thompson-Schill, 2014 propose a language network that has “core” component nodes (a.k.a. brain regions) that are specialized in language processing, and a set of “periphery” brain regions that may co-activate during language tasks but are also recruited during tasks from other domains. These periphery nodes are considered of a domain-general functionality, whereas the core regions are specialized for language processing only, thus considered domain-specific. The language-specific brain areas include, in large, areas that belong to the classical language model, comprising the inferior frontal gyrus (IFG), and the posterior part of the middle and superior temporal lobe (MTL, STL). This argument relies on the assumption that brain regions have a tendency to couple (i.e. co-activate) with other brain regions depending on their functional specialization. If a brain region supports computations in one specific task, it is more likely to co-activate with other regions supporting that same task, whereas brain regions supporting more general functions (i.e. cognitive control processes and working memory), necessary in different domains, will be recruited by multiple domains. Based on previous studies, the domain-general areas which have been consistently shown to be recruited during language processing (especially during L2 processing) are part of the cognitive control network and a fronto-basal ganglia network. These include the Anterior Cingulate cortex (ACC), the left Dorsolateral Pre-frontal cortex (lDLFPC), the pre-Supplementary Motor Area (pre-SMA), and subcortical regions, namely the left putamen, caudate nucleus, and thalamus of the basal ganglia (Abutalebi & Green, 2008, 2016). Together, the domain-specific and domain-general brain areas are engaged during language processing and create the language network.

Fedorenko and Thomson-Schill’s proposed language network provides an interesting framework for testable questions that can be addressed via MRI. These questions will be described in detail in the next sections and chapters.

3.1.1. Neurolinguistic Models of Language

The newer language processing models make reference to specialized processing pathways for associating speech sounds to their meaning and constructing auditory-motor models that guide speech production. Studies of bilingual speakers motivate the need for additional neural capacities for regulating the use of two (or more) languages. Neuroimaging and aphasia studies provide evidence for cortical and subcortical systems that mediate language control in bilinguals. Reviews of functional imaging studies of spoken language coupled with increasingly sophisticated assessments of auditory processing in nonhuman primates have led to a conception of acoustic language processing that includes parallel processing pathways, whose functions in the auditory realm mirror properties familiar from visual processing (Mishkin & Ungerleider, 1982).

In these dual-stream models (see Figure 1 for a schematic representation; Corina, 2015), a *dorsal pathway*, involved in language planning, auditory-to-motor mapping (Hickok & Poeppel, 2007; Saura et al., 2008), and complex syntactic processing (Friederici & Gierhan, 2013), and a *ventral pathway*, serving as a sound meaning interface and basic syntactic processing (Friederici & Gierhan, 2013), is proposed.

The *dorsal pathway* (more left-hemisphere dominant, i.e. (Hickok & Poeppel, 2007) includes two dorsal long-range fiber bundles supporting language functions: the arcuate fascicle (AF), connecting the pars opercularis (BA 44) with the middle and posterior superior temporal gyrus (MTG, STG) and parts of the superior longitudinal fascicle (SLF), connecting the middle temporal gyrus (MTG) and the superior temporal gyrus (STG) to the dorsal premotor cortex (dPMC), via the parietal cortex (PC), as well as frontal regions to the angular gyrus (AG) and supramarginal gyrus (SMG; (Friederici & Gierhan, 2013; Tremblay & Dick, 2016).

The *ventral pathway*, (believed to be bilaterally organized, e.g. Hickok and Poeppel 2007), connects regions that have been previously documented to be involved in semantic processing, namely the inferior frontal gyrus (IFG), the middle temporal gyrus (MTG), the angular gyrus (AG) and the posterior superior temporal gyrus (pSTG), connected through the Inferior Froto-Occipital Fascicle (IFOF; (Brauer et al., 2013; Friederici & Gierhan, 2013; Hickok & Poeppel, 2007). These pathways support communication between regions of the brain that have been documented through decades worth of research, to have language-specific functionality.

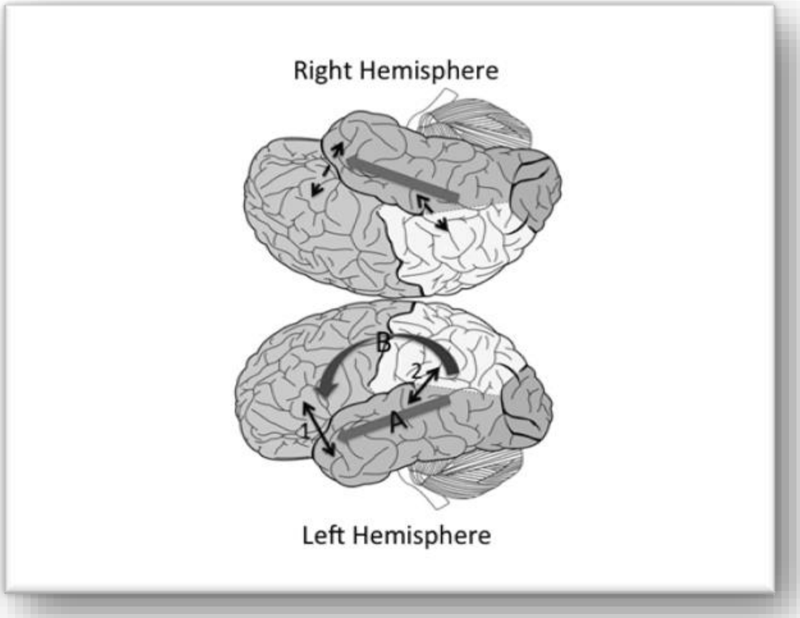


Figure 1. Schematic Dual – Stream Model of Language

Figure 1. Description: A: the ventral stream. B: the dorsal stream. Reprinted with permission from Corina, David P.: Neurolinguistic Studies of Sign Langugae Bilingualism, Marschark and Patricia Elizabeth Spencer, (ed), the Oxford Handbook ot Deaf Studies in Language, Oxford Library of Psychology, 276-287.

For example, Mashal et al., 2009, showed increased activation in the inferior frontal gyrus (IFG) (along with middle temporal gyrus (MTG) and middle frontal gyrus (MFG)) for a task that tested neural correlates of novel metaphorical sentence processing in which the participants were asked to read four-word sentences and to complete a semantic judgment task (rate if the sentence had a negative or positive meaning). The ventral part of pars triungularis (BA 45) has been reported to be involved in semantic processing. Wagner et al., 2000, shows activation in this area for a decision task that looked at the processing of non-semantic versus semantic priming pairs of words. Semantic processing has also been associated with activation in the pars orbitalis (BA 47, e.g. Vigneau et al., 2006) and the Angular Gyrus (i.e. semantically plausible versus implausible sentences; (Mashal et al., 2009). Figure 1. shows a schematic dual-stream model of language processing.

3.2. The History of the Neurobiology of Multilingualism

At the extreme highly proficient bilinguals show highly uniform language networks for L1 and L2, engaging familiar temporal - ventral systems observed in monolinguals. For proficient bilingual speakers, data indicate a shared L1/L2 lexicon rather than separate specialized and non-interactive lexicons (Dijkstra et al., 2013; Jones et al., 2012). In contrast to monolinguals, bilinguals are faced with additional needs, specifically neural resources necessary for the control of their multiple languages, as well as higher working memory capacity. Abutalebi & Green, 2007 mention the importance of a mechanism that is necessary for selective attention to only one of the languages spoken, monitoring interference from other languages while speaking in one of them, and the ability to switch from one language to the other. These are brain areas that are recruited by the language domain but are not specialized for language processing per se, rather shared with other domains (i.e. vision, emotion, etc.). These are often referred to as the domain-general brain areas.

Indeed, multilingual experience has been shown to be associated with neural adaptation in speakers of multiple languages. These adaptations have been observed at different levels of brain restructuring, for example, changes in structural resources or capacity (i.e. grey matter density or volume), changes in the tuning or changing of the responsiveness of neural populations (regional efficiency), or changes in the connectivity of the network (e.g. white matter connectivity). Research in the last decades identified the presence of distinct networks contributing to the executive control of language. One network, a fronto-basal-ganglia loop, is implicated in the inhibition of inappropriate languages during production. Alongside this network, another cortical fronto-parietal network sustaining more general switching mechanisms was proposed. These two systems, working in concert with language-specific brain areas, likely manage inhibitory control as well as language selection, both of which are necessary for the effective management of language in the bilingual brain (J. Crinion et al., 2006; Hervais-adelman et al., 2011). Generally, neuroimaging evidence shows that bi/multilinguals activate neural circuits that comprise the dorsolateral prefrontal cortex and the supplementary motor - suggesting a greater reliance on metalinguistic factors (Bialystok, 2001), the anterior cingulate – associated with control (Wattendorf et al., 2001), the left precuneus – associated with the allocation of attention (Luke et al., 2002) and the cerebellum. For example, Videsott et al., 2010, showed higher activation in the left inferior frontal gyrus, as well as the cerebellum (bilaterally), for Ladin (L1) compared to Italian (L3) in speakers of Ladin, Italian and English. Abutalebi et al., (2013), show larger activation in the left putamen for the less proficient L2 language versus L1. Similarly, Abutalebi et al., 2017 show increased activation in the caudate nucleus and anterior cingulate cortex, for a naming task in the less proficient language. The authors suggest that language control is performed by overlapping networks with domain-general executive control mechanisms.

3.2.1. Neurolinguistic Models of Multilingualism.

Most recent neuroimaging studies suggest that these control processes additionally needed by bi/multilinguals are managed by a cortico-subcortical network that is largely overlapping with executive control (domain-general) neural representations (Abutalebi & Green, 2016; Green & Abutalebi, 2013; Roelofs & Piai, 2011). As previously observed, this network includes brain regions such as the dorsal anterior cingulate cortex (dACC)/pre-supplementary motor areas (pre-SMA), the caudate (Cd) and the inferior parietal lobe (IPL) as well as the right pre-frontal cortex (pre-FC), the putamen, the thalamus (Thal), globus pallidus (GP) and the cerebellum (Cer) (Abutalebi & Green, 2007, 2016; Cools & D'Esposito, 2011; Green & Abutalebi, 2013; Luk, Green, Abutalebi, Grady, et al., 2012; Pliatsikas, 2020; Pliatsikas & Luk, 2016a).

Cognitive control is usually described as the part of working memory that comprises a set of mechanisms responsible for selecting the appropriate information for active maintenance in working memory, ensuring that information is maintained for a certain amount of time, controlling for interference so that the appropriate information can be maintained, updating the information when necessary, and using this information to influence other cognitive functions (Braver et al., 2007). Braver, 2012, proposes the dual mechanisms of control (DMC) framework, arguing that the cognitive control system operates in two dissociable and distinct modes, in terms of neural representation, computational properties, and temporal characteristics. These two modes are *proactive control* and *reactive control*. Proactive control refers to the sustained type of control that actively maintains a task goal (e.g. the maintenance of the goal of speaking in a target language). Reactive control is the transient type of control that resolves interference when it is detected, such as language switching. Braver suggest that the proactive control is a top-down process associated with anticipatory activation of the lateral PFC, reflecting the "early selection" of a task goal, and constantly biasing of the attention to help to maintain that goal; while the reactive control is a bottom-up process that reactivates the task goal as a result of encountered interference (e.g. through the engagement of conflict monitoring regions such as the anterior cingulate cortex (Van Veen et al., 2001). The distinction between the two types of control can be defined as a process of early selection and should be associated with sustained activity in the lateral PFC, versus the later correcting as needed, and should be associated with transitory activity in the PFC, the posterior cortical regions of the hippocampal-medial temporal lobe (MTL) complex, and the ACC, postulated to be involved in detecting the presence of conflict or uncertainty due to interference, the activation of an inappropriate response, or the likelihood of making an inappropriate response. In this framework, the PFC plays a central role in the active maintenance of a goal, and its function is supported by the hippocampus - medial temporal lobe subsystems, the dopaminergic system in the midbrain that actively gaits the entrance of information in the PFC, and the ACC – areas that show high interconnectivity with the PFC and have been suggested to play a dual role in both proactive and reactive control (De Pisapia & Braver, 2006).

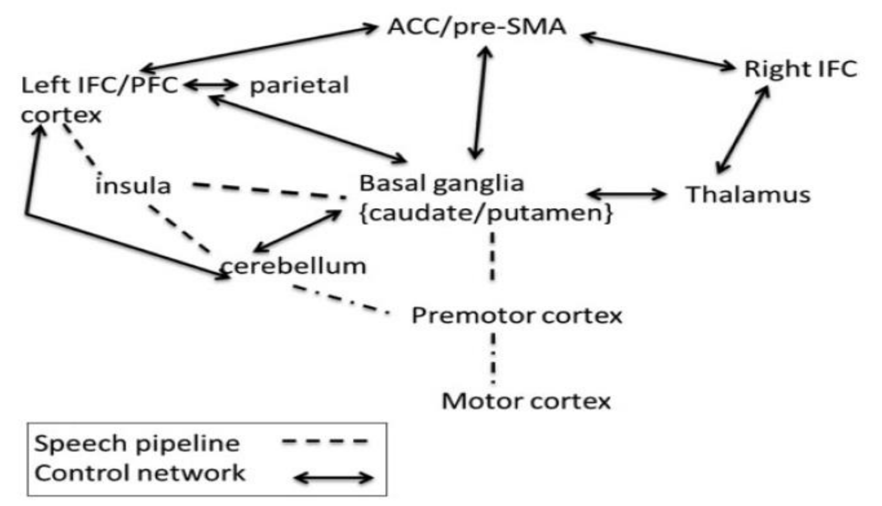
Despite the apparent congruence from the available evidence about neural changes observed in bi/multilinguals, contradictory evidence has also been reported. Consider for example measures of white matter tracts. While increased factional anisotropy (FA) and mean, radial, axial diffusivity (MD, RD, and AD) has been observed in the bi/multilinguals compared to monolinguals in some studies (Luk et al., 2011; Rossi et al., 2017), other studies have reported an opposite pattern (e.g. Cummine & Boliek, 2013) or partially different patterns (e.g. Kuhl et al., 2016, reported higher FA and lower RD and MD for bilinguals versus monolinguals). Similarly, while studies of grey matter differences between monolinguals and bilinguals report increases in grey matter density and volume in bilinguals (e.g. Grogan et al., 2012), the opposite patterns have also been reported (e.g. Elmer et al., 2014, reported reduced grey matter volume for bilinguals versus monolinguals). Finally, while some studies show increased recruitment by bilinguals compared to monolinguals of areas related to language control (e.g. Pliatsikas & Luk, 2016, report increased activation of the ACC for bilinguals compared to monolinguals). Luk, Green, Abutalebi, Grady, et al., 2012 in a study combining structural (VBM) and functional (fMRI), reported that bilinguals had larger ACC volumes than monolinguals, but recruited the ACC less than monolinguals, with better levels of behavioral performance (also, Rodríguez-Pujadas et al., 2014).

Three main proposals have tried to explain the variance, and to some extent conflicting evidence, in the bilingual literature. The *adaptive control hypothesis* (ACH) underlies the importance of the context in which a bilingual is situated when practicing their languages; the *bilingual anterior to posterior and subcortical shift* (BAPSS) hypothesis, which underlies the importance of proficiency (including the assumption that higher immersion leads to greater proficiency); the third and most recent - *dynamic restructuring model* (DRM) gives an account that combines the underlying observations noted in both previous models (i.e. the ACH and the BAPSS) and tries to account for these inconsistent observations by describing the multilingual experience effects in terms of trajectories reflecting the bilingual language experience. These three models will be described in the next sections.

3.2.1.1. Adaptive Control Hypothesis

Different forms of training (e.g. playing an instrument, developing a physical skill, learning a new language, etc.) shape how individuals perform non-verbal tasks (Bialystok et al., 2009). The bilingual advantages observed in non-linguistic cognitive tasks are believed to be due to the increased cognitive demands associated with the necessity to control multiple languages, which in turn leads to enhanced skills in cognitive control in general, and these skills are deployed for both language and non-linguistic tasks. Green and Abutalebi, 2013, proposes that the processes of cognitive control themselves adapt to the demand that is placed upon them. The authors propose the Adaptive Control Hypothesis (Abutalebi & Green, 2016; Green & Abutalebi, 2013) - a model of language control in bilingual language production, that describes the processes underlying the bilingual language control and the relationship between linguistic and non-linguistic components supporting such processes with their respective neural correlates (see Figure 2). In the context of the Adaptive Control Hypothesis, the language context (i.e. *single* language context in which bilinguals use the two languages in separate environments, *dual* language context, in which bilinguals use their languages with different speakers, where the bilinguals need to switch only when they change interlocutors, and *code-switching* language contexts in which bilinguals use both languages in the same utterance) may influence the adaptation of the frontal and subcortical structures involved in eight distinct control processes, namely goal maintenance and conflict monitoring (both part of the pro-active control mechanism), interference suppression, salient cue detection, selective response inhibition, task engagement, task disengagement, and opportunistic planning (instances of reactive control).

The authors propose a schematic representation of the demand on the cognitive control necessary for each of the control processes in bilinguals compared to monolinguals, depending on the interactional context. Similar to the predictions in Braver's DMC model, the ACH characterizes the pre-frontal cortex as the central neural hub responsible for controlling task-relevant information. This area is connected with the posterior brain areas (i.e. IPL), creating a circuit that manages selection across competing responses. Connected to the prefrontal cortex is another set of areas, namely the dACC/pre-SMA, the basal ganglia, and the cerebellum. The ACC has the prominent role of monitoring and detecting the presence of conflict (e.g. such as the conflict of choosing between translation equivalents in different languages) and sending information to the dorsolateral pre-frontal cortex (DLPFC) (Abutalebi & Green, 2008, 2016; Branzi et al., 2016; J. Crinion et al., 2006; Hernandez, 2009). The subcortical regions are related to task switching, sequence planning, and motor control (involved in language activation, inhibition, or both) and the cerebellum seems to be involved in the selection of the linguistic information most readily available and appropriate to achieve the linguistic goal.



Simplified language control network and speech production regions (see main text for explanation).

Figure 2. Simplified Language Control Network and Speech Production Regions

Figure 2. Description: Reprinted from Journal of Cognitive Psychology, Vol 25., David W. Green & Jubin Abutalebi, Language control in bilinguals: The adaptive control hypothesis., Pages 515-530., Copyright (2013)., Open Access.

3.2.1.2. The Bilingual Anterior to Posterior and Subcortical Shift Hypothesis.

Grundy et al., 2017, propose the *bilingual anterior to posterior and subcortical shift hypothesis* (BAPSS), based on the observation that the experiences managing multiple languages lead to domain-general cognitive changes observed both in the function and structure of the brain, stemming from observed effects of bilingualism on age-related cognitive decline, as well as the often reported advantage of bilinguals on executive function tasks requiring switching or fast-response times. The authors reviewed a set of magnetic resonance imaging (MRI) and electroencephalography (EEG) studies and converged on a few findings: 1) bilinguals show greater grey matter volumes in multiple areas of the brain, compared to monolinguals; 2) these increases are observed mostly in association with L2 proficiency increases; 3) bilinguals show less frontal activation than monolinguals in functional (MRI) studies; while children that start learning a language show the opposite effects (i.e. stronger recruitment of these areas); 4) functional connectivity between brain areas involved in language processing is stronger in bilinguals; 5) based on evidence from EEG studies, bilinguals show more reliance on earlier processes in control tasks. The authors suggest that due to these adaptations, bilinguals may be able to distribute effort across brain networks, while monolinguals rely more on the frontal regions for completing tasks of cognitive control (either verbal or non-verbal); hence, bilingualism leads to brain modifications that allow more efficient processing in cognitive tasks.

The authors propose that bilingualism is associated with a pattern of more efficient employment of the brain, particularly, greater recruitment of posterior/subcortical regions, and weaker reliance on the frontal regions by bilinguals compared to monolinguals in order to manage nonverbal executive tasks. While grey matter volume and language proficiency follow an inverted U shape; specifically, as bilinguals are in the process of gaining experience with a second language, the tissue volume in brain regions associated with language processing and control (especially the striatal regions) increase, however, when bilinguals gain a high level of expertise – associated with tissue specialization- these increases reduce and are no longer observed.

3.2.1.3. The Dynamic Restructuring Model

A third most recent model of the trajectorial neurostructural effects of multilingualism on the brain has been developed to account for the dynamic effects of language experience on the multilingual brain (Pliatsikas, 2020). The authors develop the Dynamic Restructuring Model (DRM), based on three main observations. First, simultaneous bilinguals showed similar patterns of neural adaptation with sequential bilinguals, only if they had high immersion in an L2 context (the adaptation in this context were mostly observed in the basal ganglia (namely structural adaptations of the putamen, globus pallidus, and caudate), the thalamus (Burgaleta et al., 2016; Pliatsikas et al., 2017), and bilateral cerebellum. Additional changes were observed in the white matter tracts (namely, the inferior fronto-occipital fasciculus (IFOF), anterior thalamic radiation (ATR), uncinate fasciculus (UF), inferior longitudinal fasciculus (ILF), superior longitudinal fasciculus (SLF), arcuate fasciculus (AF) and the corpus callosum (CC)) (García-Pentón et al., 2014; Mohades et al., 2012). Second, sequential bilinguals that did not have high immersion in an L2 context, showed restructuring in mainly the cortical areas, including the ACC, IPL, ATL, IFG, and MFG, the Heschl's gyrus, STL and SPL, while these changes seem to be negatively correlated with AoA. Hence, the reported changes in cortical grey matter were mostly observed in young sequential bilinguals with little immersion and lifelong older bilinguals. On the other hand, the subcortical changes were observed in the experienced bilinguals of all ages (e.g. Pliatsikas et al., 2015). Finally, the third observation came from studies of bilingual interpreters (which the authors suggest may be situated most frequently in a switching context) which showed reduced grey matter volume and white matter diffusivity compared to monolinguals and non-interpreter bilinguals.

These observations were additionally confirmed in training studies which suggest that the initial stages of structural adaptation in bilinguals are observed in cortical areas and are followed by changes in white matter and subcortical gray matter changes, while the initial cortical changes fade. Additionally, based on the very few available multilingual studies (Grogan et al., 2012; Hämäläinen et al., 2017, 2018, as cited in Pliatsikas, 2020), the authors observed that learning an additional language after a first sequentially learned L2 follows a similar pattern of adaptation as with a previously learned second language.

Pliatsikas, 2020, divides the bilingual brain adaptation trajectory into three stages:

1. The "*initially exposed stage"* – during which the multilingual is initially exposed to a second language. This period is associated with learning the vocabulary and concomitantly controlling for lexical alternatives, thus areas of the brain involved in such processes are responding to these environmental demands, resulting in increased grey matter volume (e.g. inferior and superior parietal lobes, anterior parietal lobe, and Heschel's Gyrus; as well as the inferior and middle frontal gyri and the ACC.
2. The "*consolidation stage*" – in which the bilingual shifts from learning lexical items to grammar and phonological acquisition. There is also an increase in control demands to control at all levels of language processing (i.e. phonology, lexical and grammatical). The brain adaptations associated with these processes are observed in the subcortical (namely the putamen and the left caudate) and cerebellar regions, associated with cognitive control (Abutalebi & Green, 2016). Alongside grey matter changes, adaptations are observed in the white matter diffusivity, namely the inferior and superior fasciculi (ILF, SLF), and the inferior fronto-occipital fasciculi (IFOF) and Corpus Callosum.
3. The "*peak efficiency stage"* – at this stage, the multilinguals become equally efficient in multiple languages (e.g. similar to simultaneous interpreters). At this stage, the changes described in the previous stages disappear (the grey matter and white matter differences), with a shift from the frontal adaptations in the white matter tracts to more posterior adaptations, within the same tracts (shift also predicted by the BAPSS).

In summary, contemporary "dual-route" neural models of spoken languages identify ventral and dorsal pathways that include bilateral temporal regions involved in registering the acoustic properties of speech. Pathways that manage language-specific and combinatorial properties of language are increasingly left-lateralized. Dynamic properties of language use, including cognitive processes of lexical selection in the production and maintenance of phonological codes, rely upon contributions of a strongly left-hemisphere dorsal parietal-frontal pathway. Data from bilinguals indicate that this same system is utilized in subsequently learned languages (i.e. L2, L3, etc.), especially with an early AoA, increased proficiency, or/and immersion. The neural control of multiple languages in bilinguals, including language switching and maintenance, results in additional neural circuitry that includes subcortical, cortical-striatal, thalamic processes, and a frontal-basal ganglia loop. Consistent with this characterization, bilingualism has been increasingly defined as a dynamic construct influenced by experience-based factors. These lead to differential brain adaptations observed in functional, structural, and connectivity measures of brain organization in bi/multilinguals. Consequently, models of bilingual language processing and control, take into account the bilingual language context, language history, language experience, and specific time points (level of proficiency and exposure) at which the bilinguals are studied, to describe the trajectories of brain adaptation resulting from these. A consistent pattern seems to surface, particularly, at an early stage of language learning, bilinguals seem to recruit more the cortical brain areas, and structural changes, as well as more involvement of these, should be observed in verbal and non-verbal tasks; with an increase in immersion and/or proficiency, a shift from the cortical to the subcortical and posterior regions is observed, along with structural and white matter diffusivity adaptations in these areas; at a peak efficiency stage, the changes observed in earlier stages start to fade due to greater efficiency, and with the newly learned language the same cycle of adaptation should be observed.

4. This Project's Aims

Effects of bi/multilingualism on the neural plasticity of the brain have been reported as differences in function, differences in grey and white matter volume and density, as well as brain connectivity and diffusivity. Most studies examine only one of these adaptations. This lack of consistency between measures and methodologies makes it hard to obtain a holistic effect of multilingualism on the brain and its trajectory. In this study, we propose to look at four different measures within the same population of multilinguals. We will obtain structural MR imaging data that will allow us to quantify grey and white matter volumes in the selected language and non-language regions of interest. We will obtain diffusion-weighted data, for which we will quantify fiber density (FD), fiber cross-section (FC), and a combination of the (FDC),_for principal white matter fasciculi shown to be modulated as a function of language learning: inferior fronto-occipital fasciculus (IFOF), superior longitudinal fasciculus (SLF) and uncinate fasciculus (UF). Additionally, we will investigate white matter differences in pathways involved in language control (e.g. corpus callosum (CC) and the cingulum). We will also obtain three fMRI measures of linguistic processing. Here, we will characterize BOLD activation during the processing of the subject's L1 (e.g. Romanian), early learned L2 (Russian) and a later learned L3 (English).

These different measures shed new light on the neuroanatomical adaptations resulting from different factors of language background. Altogether, they allow for a more holistic description of the multilingual effects on the brain and motivate the call for more consistent methodologies across multilingual studies.

There are multiple benefits of looking at populations of multilinguals instead of bilinguals. Some authors have argued that the languages spoken by a bilingual are active at all times (Marian et al., 2003); hence, speaking in two languages requires constant inhibition of the languages in non-use at the moment (a.k.a. joint activation; Kroll & Bialystok, 2013). The present study will help us test whether such assumptions hold for individuals that speak more than two languages (e.g. answering questions as "Are all languages in the multilingual brain, active at all times or are there certain mechanisms by which the brain is constantly controlling and regulating the activation of known languages, in order to only keep most active the languages used the most?"). Different factors have been shown to influence language brain organization in bilinguals (e.g. age of acquisition (AoA), proficiency, language experience, and use, sociocultural and socioeconomic background, etc.) - multilinguals allow for better control of such factors and the ability to disentangle the influence of each of these (i.e. not all languages might have been learned at the same time, spoken with the same fluency, or used to the same extend daily, thus the effect of each factor on language representation can be individually assessed).

Finally, multilingual studies further inform models of language processing in users of more than one language. For example, in the ACH, the most demanding environmental context is the dual-language context and thus requires the bilingual to use most of the executive control processes. The demands for each of these contexts may vary greatly for multilinguals compared to bilinguals. For example, in a dual-switching context where the bilingual speaker does not need to maintain a task goal, a multilingual, whose addressee may only speak a few but not all languages spoken by the multilingual, may still need to maintain a task goal. Similarly, the multilingual may not take advantage of the opportunistic planning to the same extent, needing to only select the appropriate information for the languages shared by the interlocutors (i.e. whatever comes most readily to hand may not be most appropriate). These executive brain areas may be differently recruited by the multilinguals due to the control demand differences for multilinguals versus bilinguals. Based on the rich linguistic background of our study's participants, we will be able to directly assess the effects of proficiency, immersion, and usage on brain restructuring.

There are a few specific hypotheses that we will test. First, based on the framework proposed by Fedorenko & Thompson-Schill, 2014, what can be expected is equal recruitment of language brain areas, across both groups (monolinguals and multilinguals). At the extreme, domain-specific language brain areas should be similarly recruited during language processing, independent of language typology or speaker background (i.e. age of acquisition and proficiency).

To the extent to which second languages modulate the recruitment of domain-general brain areas, these are expected to show stronger activation for the multilingual groups while processing the less proficient languages (namely Russian and English). It is worth noting that the present functional paradigm does not specifically target domain-general networks, however, domain-general regions will be specified based on previous studies through Region of Interest (ROI) statistical analysis. Furthermore, based on the ACH model, the multilinguals should show greater recruitment in the areas associated with cognitive control (more specifically the dorsolateral prefrontal cortex (DLPFC), the dACC/pre-SMA, the basal ganglia, and the cerebellum) while processing the L2 and L3 versus the native (L1).

Additionally, based on the predictions of the DRM, as long as the participant sample of our study is in the "*consolidation phase*" of their language learning, (as determined by the level of proficiency, immersion, and language background assessed by the background and language tasks) we will be able to assess specific region of interest (ROIs) and quantify grey matter and white matter for a comparison across the groups, as well as look at correlations of factors as AoA, proficiency, and immersion with the grey matter volume and density, as well as white matter diffusivity. We expect to observe differences in grey matter volume between the groups for areas predicted to show changes at this stage, namely the Putamen, Globus Pallidus, Thalamus, and Cerebellum. Similarly, we expect an increase in white matter diffusivity of white matter tracts associated with language control (namely, the IFOF, UF, SLF, AF, and CC).

4.1. Introduction to Methods and Analysis Techniques

4.1.1. Describing Proficiency Measures of Multilingualism

The language history and background, as well as the level of proficiency of the multilingual, have important implications for the appropriate characterization of the effects of multilingual experience on the brain. We constructed a set of background and linguistic experience tests that will allow us to describe and quantify measures of the age of acquisition (AoA), proficiency, immersion in an L2 context, and language use, for all languages known by the participants. The language tests will assess knowledge at different levels of language processing (i.e. production, lexical, semantic, and grammatical knowledge). This set of tests included a *Self-Rating Questionnaire*, a *Language Production and Vocabulary Assessment*, *a Semantic and Vocabulary Knowledge Test,* and *A Grammar Test*. These are described in detail in the first chapter.

4.1.2. Describing MRI Technique

The MRI is a non-invasive neuroimaging technique that uses strong magnetic fields, pulse sequences[4], and computer software, to produce detailed images of the human body. In the last two decades, MR scanners have become more powerful, while the application of the technique is increasingly covering a wide variety of complex topics (Huettel et al., 2004). MRI has become the most used method for the study of the human brain (Poldrack et al., 2011). There are multiple modalities[5] of imaging that the technique allows for; however, there are three modalities that are by far the most used in neuroimaging research: structural, functional, and diffusion imaging. Sometimes several different modalities will be used in a single study, either to use the images in one modality to correct for the limitations in another or to combine the modalities in order to provide clearer, more comprehensive interpretations

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[4] Pulse sequences – in MRI, a series of changing magnetic gradients and oscillating electromagnetic fields. These will be absorbed by hydrogen nuclei (prevalent in water molecules). The absorbed energy will be later emitted by the nuclei in an amount dependent on the number of nuclei.

[5] Modalities – different types of images can be acquired in an MR scanner, that capture different information about the brain.

of results. In our study, we have used all three modalities. These are concisely described in the next sections.

4.1.2.1. Describing the Structural MRI Technique

In recent years, there was an increase in studies reporting grey and white matter changes associated with bi/multilingualism. Structural changes in the brain are believed to be related to increased demand on a brain area due to the acquisition and use of a new skill (Maguire et al., 2000), hence it is an indirect measure of regional brain efficiency. Studying the structure of the brain informs our understanding of the function of the brain, thus, it has gained great interest in the last few decades in neurolinguistic research. Structural images provide information about the gross anatomy of the brain and are used to do morphometry[6] analysis. These analyses are based on MRI structural (a.k.a. T1 weighted) images. The basic analysis tool for obtaining such measures is segmentation which is a process that delineates the tissues and the structures in the brain. Once images are segmented different statistical analyses can be performed, such as cortical thickness, structural grey, and white matter density, as well as volume and shape measures (Jenkinson & Chappell, 2018).

It should be noted that the method poses certain limitations concerning the specificity to which results can describe microstructural changes. Though the results report overall changes in grey matter density or volume, the changes may be due to the increase of the cell bodies, increased number of dendrites, or increased number of neutrophils (Pliatsikas, 2019). The technique does not give enough detail to characterize which of these changes takes place.

Most MRI images require some degree of preparation in order to be ready for statistical analysis. The structural images are generally used to perform quantitative measures on the anatomy of the brain or to provide the lacking anatomical details in the functional images necessary for better normalization to a standard template. The preprocessing operations necessary for either of the uses, include correction for low-frequency artifacts (a.k.a. bias

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[6] Morphometry is the study of the shape and size of the brain.

fields) - images collected at high field strength (3-Tesla and above) have intensity variations across the images, and this step tries to correct for these non-uniformities; removal of non-brain tissue (called "*skull stripping*" in most software packages); and brain "*segmentation*" into different tissues types (i.e. white matter, grey matter, and cerebral spinal fluid) for use with functional data, and into both different tissue types as well as different structures (e.g. cortical regions, such as the pars triungularis of the IFG, and subcortical regions, as the Putamen, Thalamus, etc.) for structural quantitative analysis; and finally *spatial registration* (a.k.a. "*normalization*") to a common template space (the increasingly most common template is the MNI152, which is a is an average of 152 subject's structural images, from the Montreal Neurological Institute) or to the functional data (also called "*co-registration*" in some software packages; Poldrack et al., 2011). The most common methods of registration are "*volume-based*" methods, which involve changes to the three-dimensional volume, "*surface-based*" registration, which is a method that spatially transforms the data based on surface features (sulci and gyri), or a combination of both "*surface and volume-based*" registration (e.g. Postelnicu et al., 2008)

The most used method for quantifying gray and white matter in the brain is voxel-based Morphometry (VBM; Ashburner & Friston, 2000) which uses probabilistic mapping across the voxels of the volume. One of the issues that such methods suffer from is the "partial volume effect" (i.e. a voxel bridges two distinct anatomical regions, or functionally different regions). VBM methods do not allow to distinguish the individual contributions of each of those regions to the results. Increasingly, surface-based methods are used that compute probabilistic estimations of cytoarchitectonic boundaries based on the surface of the brain (e.g. Fischl et al., 2008). In contrast to the representation of the image in a three-dimensional surface as in volume-based analysis (i.e. voxels), the image is reconstructed into a two-dimensional image composed of vertices[7] and edged[8] and allow quantifying the volume, thickness, area, and curvature (i.e. brain geometry), rather than the voxel intensity (i.e. as in volume-based methods).

4.1.2.2. Describing the Functional MRI Technique

Functional Magnetic Resonance Imaging has become one of the central and most utilized techniques to study cognition and language (Paradis, 2019). It is an indirect measure of brain activity – one does not directly get images of neural activity (i.e. neuronal firing), rather physiological activity in the brain required to support neuronal activity via measures of blood flow (Huettel et al., 2004). There is no direct way to measure neuronal activity in the brain. The BOLD (Blood Oxygenated Level Dependent) effect measures blood deoxygenation in bulk neurons (i.e. in a typical 3x3x3 mm voxel there are approximately 10 million neurons), as an indirect measure, correlated to neural activity. The assumptions under the fMRI technique are that a region of the brain will require more oxygen and glucose when active compared to the basal state of activation in which the human brain is at rest. With increased metabolism in a certain region of the brain, there is an increase in blood flow and respectively an increase in blood oxygen in that specific region. Oxygen is carried to the brain by the hemoglobin molecule; when these molecules are "stripped off" of the oxygen they contain, the deoxygenated hemoglobin becomes paramagnetic (a.k.a. magnetically susceptible); these paramagnetic molecules increase the perturbation of the magnetic field in the brain when exposed to a strong magnet (i.e. the MRI machine). Since there is an increase in oxygen coming to the active region, the de-oxygenated hemoglobin is outstripped by oxygenated hemoglobin (which is diamagnetic, or magnetically unsusceptible). As a result, the active region is characterized by less perturbance in the magnetic field. Hence, the signal that is

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[7] In Graph Theory – a mathematical complex system (e.g. a network) defined in terms of the intersection of nodes (vertices) and the links between them called edges.

[8] The links between the nodes in a network like system.

acquired through the MRI scanner is due to the reduction of the level of deoxygenated hemoglobin in a specific brain area, relative to other brain areas.

One of the limitations of the measure is its temporal resolution as it is affected by the nature of the hemodynamic response (about 5 seconds). The technique does not measure neural activity but a "consequence" of neural activity, which takes time; thus, the temporal resolution is limited. Consequently, the fMRI measure may not be sensitive to processes that happen within a temporal resolution of fewer than 5 seconds. However, the measure provides relatively good spatial resolution (within one cubic millimeter of brain tissue). Another limitation of the measure is that the BOLD fMRI signal has no absolute, direct interpretation. Blood oxygenation in the brain can change for different reasons (i.e. respiration, increase heart rate, etc.) thus, the activation patterns observed in any brain state must be compared to other states (e.g. baseline measure of brain activity or other tasks).

Similar to structural data, functional MRI data requires a set of preprocessing steps in order to prepare the data for statistical analysis. Even though these steps vary across software packages, few standard methods are usually used. These include "*distortion correction"* the process that tries to correct the data for the inhomogeneity of the magnetic field; "*motion correction"* - as the term implies, it is an operation that corrects the images for head motion; "*slice timing correction"* – the process that corrects for the mismatch between the acquisition timing of different slices in the volume, "*spatial smoothing"* – the application of a filter to the data, that removes the high-frequency information, and finally "*spatial normalization"* – the process of spatially transforming the data into a common space, for further analysis and comparison across groups.

Although fMRI data can be modeled in many different ways, one of the most common approaches is conducting a general linear model (GLM) analysis on the whole brain. This is a lower level, univariate (voxel-by-voxel) analysis, looking at single regions of the brain. In this model the BOLD series plays the role of the dependent variable while the independent variable is the expected stimulus time courses, hence the model relies on the creation of regressors that model the BOLD signal convolved with the task time-series, and the other sources of variation and noise. The researcher specifies regressors of interest (generally one per condition), and regressors that model the noise coming from head motion (six-time courses of the rotation and translation parameters) as nuisance[9] regressors (Poldrack et al., 2011). The model estimation and statistical analysis are usually done in two steps, the first analysis is performed at the level of each subject and then the analyses are combined across subjects for group analysis. Once estimated the model returns beta values for every voxel in each regressor.

These values can be used in contrasts of interest (i.e. contrasting between two conditions) by either computing t-tests to reveal differences inactivation of different voxels (depending on the task condition) in the whole brain, or by extracting the betas values in the contrast for only specific regions of the brain (known as regions of interest (ROI) analysis), thus limiting the search for activation to that specific regions, and reduces the stringency of the correction for multiple comparison testing.

A second increasingly used method of neuroimaging data is multivariate analysis (a.k.a. complex network theory). This is a powerful higher-level modeling tool, describing the topological organization of the brain, in terms of its subcomponent regions (also called nodes) and their connectivity[10] (referred to as links). Since the brain is a complex system in which higher-level processes are performed by an interaction of a multitude of brain regions, it is important to look at the brain in terms of oscillatory patterns creating brain networks. In these models, brain networks are represented by adjacency matrices, in which each column and rows represent the nodes (anatomical brain regions) and the entries are the links between the brain regions. The networks can be constructed to denote structural or functional (dynamic or effective) connectivity, or a combination of both (Braun et al., 2015; Power et

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[9] Nuisance is a term used for the parameters included in the model in order to model-out sources of variance, but they are not parameters of interest, thus no inferences are performed on these.

[10] Connectivity – detecting brain regions that oscillate together at 0.1 Hz or less.

al., 2011; Rubinov & Sporns, 2010; Sizemore & Bassett, 2018). The multivariate analysis provides a means to quantify complex patterns of brain connectivity by using a branch of mathematics called Graph Theory[11]. After graphs are constructed, different measured of network characteristics are computed, including the degree – reflecting the importance of the nodes in the network, the clustering coefficient – a measure of functional segregation, path length – a measure of functional integration, and small worldliness – describing a mathematical graph design that enables distributive properties and regional specificity. These measures allow for two types of network properties interpretations, one is a comparison to a benchmark network (e.g. a random network), or a comparison to other real-world networks (e.g. another group of subjects).

4.1.2.3. Describing the Diffusion Weighted Technique

Language processing also requires communication between brain regions. The interactions between the brain areas are determined by their anatomical connection. These connections determine how the information is received by and conveyed to certain brain areas. The connection is conveyed by bundles of white matter that are called "*fascicles*". Fascicles can be three-dimensionally mapped by applying algorithms that use the attenuation of the MR signal, caused by the water molecule's diffusion[12] along the fiber tracks (Hubbard & Parker, 2014). The abundance of water in the human body makes it possible to perform diffusion-weighted MRI by applying controlled gradient magnetic fields that change the MR signal in order to quantify both the magnitude and the direction of diffusion of the water molecules in the brain. Due to the structure of the cellular environment, the water molecules display anisotropic diffusion[13], which allows for the creation of image contrasts based on the mobility

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[11] Graph Theory – the study mathematical structures for modeling pairwise relations between different variables or objects. These mathematical structures are called **graphs**, made up of vertices (also called nodes) which are connected to other vertices by edges (also called links).

[12] Diffusion is the term used to define the free movement of molecules (e.g. water molecules) over time, within a medium.

[13] *Anisotropic diffusion* – the unequal movement of the molecules within a medium. Due to the structure of the environment in which the molecules are found (e.g. the long narrow walls of axons), the diffusion is restricted and the molecules tend to diffuse along one axis, but not others. Compared to *Isotropic diffusion* – assuming equal diffusion along all directions within a medium (e.g. diffusion in a sphere).

of these molecules. These measures give us an indirect understanding of the effectiveness of the structural connectivity through the white matter fiber tracts. The *axial diffusivity* (AD) is a measure of water diffusion parallel to the WM tract; the *radial diffusivity* (RD) measures water diffusion perpendicular to the WM tract; *mean diffusivity* (MD) is the mean diffusion in all acquired directions within a given tract, and *fractional anisotropy* (FA) which is the most used metric, measures the directional asymmetry of water diffusion (i.e. it is scaled between 0 and 1, with an FA = 1 meaning fully anisotropic, and an FA = 0, fully isotropic). The main limitation of this technique is that the DTI model can only characterize one fiber tract in a voxel, this becomes problematic when the voxel encompasses multiple fiber tracts that are of different directionality. Different new techniques have been proposed that aim to resolve the orientation of crossing fibers (Seunarine & Alexander, 2014).

Voxel-based analyses have been used to testing FA images for vowel-vise differences between the groups (Johansen-Berg & Behrens, 2013). One of the disadvantages of this method is its reliance on registration accuracies. Park et al., 2004 discuss an alternative approach, which uses regions of interest (ROIs), usually drawn by hand for each subject. FA values are extracted from these ROIs and compared across subjects. More sophisticated approaches use tractography (fiber bundle[14] tracking) to identify the voxel from which the FA values are extracted and compared across subjects, in which standard space ROIs are used to constrain tractography in fiber bundles of interest and a mean FA value is obtained. The major problem with these approaches is that the FA values are averaged across a large number of voxels, which may bias the results toward the null hypothesis. A more recent method (Smith et al., 2006, 2007) tract-based spatial statistics (TBSS) allows for whole-brain analysis by estimating a "group mean FA skeleton". This mean image encompasses the centers of all the fiber bundles present in all the subjects in the study, and it allows for good registration across subjects. Tensor-based methods allow for different tensor-driven scalar

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[14] Fiber bundle – a collection of white matter axons with a similar anatomical path

quantities (e.g. FA, MD, AD, RD, etc.; Johansen-Berg & Behrens, 2013). There are a couple of issues with both voxel-based and tractography measures. They are heavily confounded by partial volume effects as well as bias in tract estimation due to crossing fibers (e.g. if a voxel contains crossing fibers, they will not be independently computed, but averaged). A recent study looking at the proportion of voxels affected by crossing fibers within a voxel estimated that approximately 90% of the white matter contains crossings fibers (Jeurissen et al., 2013). This makes the interpretation of measures obtained with such methods (i.e. FA, MD, AD, RD) challenging. Additionally, the effects of interest may be masked by the influence of these confounds.

In recent years newer methods have been proposed (Tournier et al., 2019) that try to account for fiber-specific characteristics within each voxel. This method, by looking at the volume fraction of different fiber populations and their orientation within each voxel (Jeurissen et al., 2014; Tournier et al., 2004) as well as fixel-based analysis[15] that gives a measure of apparent fiber density and fiber cross-section (Raffelt et al., 2012; 2015; 2017). Based on fixel analysis, we get measures of local capacity to transfer information, as measured by *fiber density* (FD) - a microstructural measure of the local level of axonal density within a fascicle (i.e. indicative of a change in density of the axons within a bundle while the size of the fascicle may be similar across groups); *fiber crossing-section* (FC) – a measure in size of the fiber bundle in each voxel (i.e. atrophy of a bundle, while the density within it may be the same across groups), as well as a combination of these two measures f*iber-density and cross-section (FDC).* Additionally, the newer models provide quantitative connectivity analysis (Christiaens et al., 2015; Smith et al., 2012, 2013, 2015).

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[15] Fixel-based analysis – analysis of population of Fibers in a specific voxel

5. Summary and Goals of Each Chapter

This project aimed to gain a better understanding of whether extreme fluency in multiple languages leads to structural and functional changes in previously documented language networks. I have proposed to achieve this goal using few measures of linguistic proficiency, and language history, as well as by assessing brain structure, function, and connectivity in a group of multilingual speakers, fluent in 4 or more languages. I will compare this group to a controlled monolingual group. Brain adaptation in response to learning a skill can happen in various ways. Adaptations can be observed in the structural resources or capacity of brain regions, changes in the responsiveness of certain regions (best measured by functional MRI), or in brain connectivity (better identified in studies investigating white matter). The presence or lack of activation in areas related to language processing does not necessarily suggest a lack of structural differences across the groups. Meanwhile, it is not yet understood whether the changes in structure (i.e. GM volume or thickness are a result of "learning" or a result of "activity" in the brain regions that are recruited. Thus, it is important to combine structural with functional data while using the MRI technique, in order to understand the nature of experience-related brain plasticity.

Language history and background, and proficiency have all been associated with differences in the brain organization of multilingual. The first study (Chapter I) gives a detailed description of a set of tests that were developed to assess all these factors in our monolingual and multilingual groups along with the statistical analysis and results of these behavioral metrics. These variables assessed in this chapter will be used in further MRI analysis to assess the influence of language experience and proficiency on brain function and structure in multilinguals.

As previously mentioned, effects of bi/multilingualism on the neural reorganization of the brain have been reported as differences in function, differences in grey and white matter volume and density, as well as brain connectivity and diffusivity. In the following chapters, I use different measures to assess each of these in our population. Chapter II describes a functional paradigm, in which I obtained three fMRI measures of linguistic processing and one acoustic control condition. Here, I characterize BOLD activation during the processing of the subject’s L1 (e.g. Romanian or English), early learned L2 (Russian) and a later learned L3 (English), and acoustic non-speech control. First, based on the framework proposed by Fedorenko & Thompson-Schill, 2014 I discuss activation patterns for native core versus periphery language brain regions. Second, I discuss differences in activation patterns observed for the native versus second languages in our multilingual participants in relation to recent models of language processing and control in bilinguals (i.e. the adaptive control hypothesis; ACH, Abutalebi & Green, 2016; Green & Abutalebi, 2013) Additionally, I discuss the effects of several multilingual background factors on brain activation patterns within the multilingual group.

Chapter III describes a structural MRI study in which I assess anatomical differences between the monolinguals and multilinguals participants. Here, I quantify brain grey matter thickness and volume across the groups. The results of the morphometry analysis are discussed with reference to recent models of brain restructuring patterns due to bilingual and multilingual experience, namely the dynamic restructuring model (DRM; Pliatsikas, 2020) and the bilinguals anterior to posterior and subcortical shift (BAPSS; Grundy et al., 2017).

Chapter IV describes a study that uses a novel method of estimating white matter morphometry (Raffelt et al., 2012; Raffelt et al., 2017) adaptations between the multilingual and the monolingual groups investigated in this project. Differences in fiber density (FD), fiber cross-section (FC), and fiber density and cross-section (FDC) are estimated across groups. Results are discussed concerning models of brain restructuring for highly proficient bi/multilinguals (DRM, BAPSS).

Finally, a concluding chapter describes the advantages of incorporating behavioral, functional, and structural MRI data, for studying brain plasticity in multilingual. An additional discussion of the limitations of the project and further steps are provided.

Chapter I

Abstract

The experience of practicing the native and the secondly learned languages for bi/multilinguals has important implications in recent models of bilingual language processing and control. In these models, factors from the multilinguals' linguistic background are described as predicting brain activation overlap and anatomical restructuring patterns. The present study discusses the three linguistic measures of proficiency at different levels of language processing. These tests are used to assess linguistic proficiency for the native language in a group of monolinguals (i.e. English) and Romanian (native, L1), Russian (an early acquired L2), and English (a later L3) for a group of multilingual participants. The results of the study show that self-rating proficiency does not always correlate with the results from standardized tests. Additionally, metrics assessing different levels of linguistic processing may reveal differences across abilities for different language domains. Finally, the specifics of the tests used, play an important role in assessing multilingual proficiency.

1. Introduction

The acquisition of additional languages leads to changes in the brain's structure and function. These changes are a consequence of adapting to the demands imposed by controlling and using multiple languages, instead of one. Neuroimaging studies of bi/multilinguals generally report overlapping patterns of brain representation for all languages known by a multilingual. For these individuals, language processing relies on a frontotemporal network, including the inferior frontal (Broca's) regions, middle and superior temporal gyri (MTG/STG), and other brain areas associated with motor planning, auditory and visual perception (Fedorenko & Thompson-schill, 2014). Additional control networks are recruited for the need to switch between languages, control for interference from other languages, and appropriate selection of the intended target language with its grammatical and lexical items. Importantly, the degree of overlap in the language and control network regions is modulated by interacting factors related to language properties and individual differences (Blackburn, 2019 de Bot, 2019).

As mentioned in the introductory chapter, the purpose of my dissertation is to look at the neuroanatomical adaptations observed in multilinguals, as a result of these different linguistic background variables. Understanding to what extent the multilingual's brain is different than that of a bilingual or a monolingual calls for a thorough understanding of the language history of the multilingual individual, for such factors as age at which a language was learned (age of acquisition (AoA)), the amount of time spent practicing one language (immersion and exposure), the modality used (spoken language versus signed language), the degree to which a language is known (language proficiency) or lost (language attrition), as well as language structure (Bornkessel & Schlesewsky, 2006), have all shown to have an impact on brain changes related to multilingual processing (de Bot, 2019; Blackburn, 2019; for a detailed discussion see introductory chapter). Furthermore, recent studies underline the importance of studying multilingualism as a dynamic process, involving transfer and interaction between languages (De Bot, 2019) and the need to move away from treating multilingualism as a categorical variable (Luk & Bialystok, 2013).

While the necessity of assessing each of these factors is agreed upon, there is still no standard method for measuring linguistic proficiency and creating a language profile for multilingual participants. The lack of availability of reliable and comprehensive assessment tools in the languages of the multilingual group investigated in my dissertation prompted the development and adaptation of the set of tests in three of the languages spoken by the participants (i.e. Romanian, Russian, and English).

In this chapter, I briefly discuss the importance of using detailed and appropriate testing in multilingual neuroimaging studies and issues related to the types of available tests. I then describe the process of developing a set of tests that will be used to obtain measures of language proficiency and linguistic background for a group of 15 monolingual English speakers and 15 multilinguals, fluent in four/five languages. The main goal of these assessments is to acquire comprehensive measurements of important factors influencing multilingual language representation and brain restructuring. These will not only give us a better understanding of the participant's background but also provide variables that will be used as factors predicting brain changes in further studies. The tests will assess the multilingual's proficiency in Romanian (native, L1), Russian (an early acquired L2), and English (a later L3) – languages for which we also investigated brain activation patterns in an fMRI study, described in the next chapter (see Chapter II). Finally, the results of the study will be discussed in relation to models of functional brain representation and restructuring (Green & Abutalebi, 2013; Pliatsikas, 2020), and further steps and limitations will be identified.

2. The Importance of Using Detailed and Appropriate Testing

The importance of using detailed and appropriate testing in neuroimaging studies of multilingualism has been underscored in recent models of bilingual language processing and control. In these models, the multilinguals' background peculiarities are identified as predicting factors in activation overlap and anatomical restructuring (i.e. changes in grey and white matter). For example, the Adaptive Control Hypothesis (ACH) predicts that the processes of control needed for bilingual practice adapt to the interactional context in which the bilingual is situated (Green & Abutalebi, 2013). The hypothesis predicts specific brain regional changes depending on whether the bi/multilingual is using their second languages in a single language[16] context, a dual-language[17] context, or a dense code-switching[18] context.

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[16] Single language context refers to an interactional context in which the bilingual uses each language they know in a single environment.

[17] A dual language context refers to an interactional context in which the bilingual uses both languages within a single context, but with different interlocutors.

[18] A dense code-switching context refers to an interactional context in which the bilingual uses both languages within the same context and with the same interlocutors.

Therefore, the multilingual experience and sociolinguistic background of the participants in this study (namely, the interactional context in which the multilinguals use their second languages), will be informative of the patterns of brain differences observed in the functional, structural, and connectivity analysis described in the following chapters, and therefore need to be appropriately assessed. A more recent model describing the neurostructural changes observed as a result of multilingual practice, namely the Dynamic Restructuring Model (DRM) predicts specific brain adaptation patterns depending on the dynamic effects of language experience and the linguistic profile of the multilingual (Pliatsikas, 2020). Based on the DRM, the age of acquisition, the level of language proficiency (i.e. whether individuals are at an initial stage or at more advanced stages of learning a language), and amount of language exposure will all result in specific brain change (for a detailed description of the stages and restructuring associated with these, see the introductory chapter, also Pliatsikas, 2020).

Inevitably, individual factors from the bi/multilinguals' background influence the brain activation and restructuring patterns observed in neuroimaging studies. Therefore, appropriate assessments need to be incorporated to obtain both measures of language experience and use in different international contexts, as well as proficiency tests associated with different stages of language learning. These can be used as predictors in statistical models or correlation tests used to describe brain changes associated with the multilingual experience. However, choosing appropriate assessments and finding a test that transfers across languages, is not an easy task. These issues will be briefly discussed in the following paragraphs.

2.1. Choosing the Appropriate Set of Assessments

What are the best types of assessment tests? Even though there are many different instruments to assess proficiency, researchers are often faced with at least three main challenges when choosing the appropriate set of tests. First, researchers struggle with the decision of using subjective tests (i.e. self-reported or parent-reported proficiency – which may be good at capturing the sociolinguistic contexts of the participants), objective tests (standardized/or “in-house” developed tests – which may be best suited for assessing proficiency), or a combination of both. Additionally, many of the tests available are not translated or adapted to the languages of interest. Finally, the majority of the existing tests focus on a specific aspect of the language - in most cases vocabulary knowledge (de Bot, 2019), that may not give a reliable assessment of the aspects of language knowledge overall.

2.1.1. Type of Assessments and Issues Related to Each

In a meta-review examining the description labels used for bilinguals in studies comparing monolinguals and different types of bilinguals, Surrain & Luk, 2019, reported that 29% of the studies used only subjective proficiency, 17% of the studies included both types of assessments, and 30% of the studies used objective assessment of proficiency only. The authors identified 186 peer-reviewed empirical studies, from the PsycINFO database, published between 2005 – 2015. In these studies, the tests used to assess bilinguals’ language proficiency, included in the vast majority, only vocabulary knowledge assessments (e.g. the Peabody Picture Vocabulary Test (PPVT); the Expressive One-Word Picture Vocabulary Test (EOWPVT), the Shipley Vocabulary Test, etc.) and 21% of these, reported language proficiency in only one of the languages of the bilingual, due to constraints of the multiple different first languages spoken by the bilinguals. More recently, Heron et al., 2020, in a meta-review of 57 neural imaging studies of bi-or multilingualism, published between 2010-2018, have noted that there seems to be a lack of consistency in the type, number, and way in which the language proficiency measures are used in neurolinguistic studies.

2.1.1.1. Self-assessment Questionnaires

There are generally two types of tests used to assess linguistic proficiency. These can be globally divided into objective discrete point tests (e.g. standardized tests), and self-evaluations. There are advantages and disadvantages related to the usage of each of the tests in these categories. One of the most convenient and most frequently used methods of assessing language proficiency in bilinguals is self-rating (Surrain & Luk, 2019). Although very often self-rating measures tend to be correlated with behavioral data (i.e. reading comprehension, reading fluency, grammaticality judgments, etc.; Gollan et al., 2012; Marian, Blumenfeld, et al., 2007), not everyone agrees with the accurateness of these ratings (i.e. Dunn & Tree, 2009). The correlation between self-assessment and standardized tests has been reported in a very wide range, from fairly low 0.25 (Weltens & Grendel, 1993) to fairly high 0.75 (Marian, Blumenfeld, et al., 2007). Generally, authors agree that rating of proficiency alone is not sufficient to determine the level of language knowledge of the bi/multilingual due to factors that are not in the control of the experimenter (i.e. mood, personality, external factors, etc.; Lemhöfer & Broersma, 2012). For example, in a study comparing the outcomes of self-evaluation depending on the participant's culture, Weltens & Grendel, 1993 observed that Dutch participants overestimated their proficiency in French (as a second language); while American participants showed closer results between self-evaluation ratings and other tests used (i.e. Common European Reference Format (CERF) tests). Also, assessing language proficiency becomes more difficult when participants need to assess their fluency on more than two languages. However, although not the optimal way of obtaining proficiency measures, the self-rating questionnaire is the most convenient ways to obtain information about language usage and experience.

2.1.1.2. Standardized Tests

On the other hand, various standardized tests have been proposed for language proficiency. For example, Lemhöfer & Broersma, 2012, proposed a short yes/no vocabulary test, called LexTale, a quick and valid Lexical Test for Advanced Learners of English. The test has been used to test proficiency in various studies and it is available in Dutch, Korean, English, and German. It has been shown to correlate with other translational tasks, especially for Dutch participants (0.78), however, lower correlations have been obtained when comparing tests of a Korean group (0.50). The test is mostly developed to test vocabulary knowledge in these four languages. Marian et al., 2007 developed a questionnaire of bilingual language status (the LEAP-Q). The goal of the questionnaire is to provide a reliable assessment of the bilinguals’ language profile; thus, it combines both questions of language proficiency and language experience. The test provides very detailed questions regarding the background experience factors contributing to the language profile of the bilinguals. Language proficiency questions are divided in their assessment of writing, listening, speaking, and reading proficiency, and the statistical analysis showed high validity and internal consistency of the LEAP-Q. Although, standardized tests are a better measure for linguistic proficiency, similar to self-proficiency assessments, these are subject to external factors that are not in the control of the experimenter.

Additionally, many of the available assessments are developed in only a few languages and may not coincide with the languages of interest. There are no neurolinguistic studies, to my knowledge, looking at the language proficiency of native Romanian speakers; thus, in order to use any of the available tests, they needed to be adapted to Romanian, in our study.

2.1.2. Proficiency Measures and Different Language Domains

Lastly, another important aspect of language that is rarely addressed in proficiency measures is the assessment of knowledge at different language domains (i.e. phonological, semantic, grammatical knowledge, etc.). Competence at different levels of language processing may not only be different within a second language (e.g. a second language learner may have good vocabulary knowledge but lower grammatical proficiency, it is also common for a second language learner to be able to understand a second language but be unable to speak it), but may also engage different brain regions depending on the language typology (e.g. Bornkessel & Schlesewsky, 2006; Kovelman et al., 2008). Different brain areas have been correlated with language ability in different linguistic domains. Xiang et al., 2012 showed that the lateralization of the white matter BA45 – temporal and BA6 – temporal pathways correlated with grammar inference tests. Both brain areas (BA 45 and BA6) have been reported in the literature to be involved in the processing of hierarchical structures in artificial grammar (e.g. Bahlmann et al., 2008). While fractional anisotropy (FA) measures in the pathway connecting BA47 and parietal regions were correlated with scores in a vocabulary learning task. Grey matter density increases in BA47 have been correlated with the number of vocabulary items learned in another brain morphometry study (Richardson & Price, 2009). Similarly, the processing of regular forms in monolinguals has been associated with the recruitment of the left fronto-temporal/basal ganglia circuit, while the processing of irregular forms relies on the superior and middle temporal gyri (Binder et al., 2009; Bozic & Marslen-wilson, 2010) with decreases in the engagement of the left inferior temporal gyrus (Tyler et al., 2005). Together, these studies underline the importance of assessing language proficiency at different levels of processing, particularly when looking at brain correlates of second language processing.

3. The Present Study

Based on these considerations, I created a set of tests and a background questionnaire reflecting a combination of all the types of tests described above. The decision to use both self-rated questionnaires, standardized and "in-house" tests were based on the goal of taking advantage of all the benefits of these measures. I have used a self-rating questionnaire to capture information about the age of acquisition, exposure, and amount of language use in different social environments and self-perceived proficiency. A previously validated production/vocabulary knowledge test (MINT; Ivanova et al., 2013) was also used, to assess production and vocabulary knowledge. A standardized test originally developed to assess semantic and vocabulary knowledge widely used in other studies, namely the Shipley Vocabulary Test (Shipley, 1940) was adapted/translated to Romanian and Russian. Additionally, a Grammar test was developed, based on another validated test, initially developed to assess grammatical knowledge in aphasic patients (Linebarger et al., 1983). Together, these allowed for a thorough characterization of the multilinguals' language background as well as language proficiency at different levels of linguistic processing in three languages (Romanian (L1), Russian (early L2), and English (late L2) spoken by the multilinguals and the one language (English) spoken by the monolinguals group. The set of tests used in this study included a *Self-Rating Questionnaire*, a *Language Production and Vocabulary Assessment (i.e. MINT)*, a *Semantic and Vocabulary Knowledge Test* (Shipley), and a *Grammatical Assessment Test (GAT) all adapted for the participants of this study*.

3.1. Methods

3.1.1. The Self-Rating Questionnaire.

Usually, measures of self-assessment rely upon the participant to report information about their linguistic background. The questionnaire used here asked participants to provide the age of acquisition, usage (including the environment in which they use a certain language, the amount of time spent speaking each language daily, etc.), and self-rated written, reading, and spoken proficiency of each of the languages they know. The questions included: *What was your first language(s)? / What is your preferred language(s)? / What language(s) do you understand? / What languages can you speak? / What language(s) do you speak at home? / What language(s) have you studied in school?* Etc.

The questionnaire also asked the participants to rate their fluency in all the languages on a continuum from 1 to 7, where 7 = native speaker; 6 = fluent; 5 = fluent conversational, 4 = conversational; 3 = almost conversational; 2 = still learning; 1 = beginner.

3.1.2. The Development of the Adapted MiNT Test

3.1.2.1. History of the Test

For the assessment of production proficiency and vocabulary knowledge, the participants were asked to complete the Multilingual Naming Test (MiNT; Ivanova et al., 2013). The original test consisted of 68 black-and-white line drawing images of different sources, an adaptation of a 32-image set first used by Ivanova, Salmon & Gollan 2012, was used for this study. The items in the images were selected to represent words of increased difficulty with presentation order. The participants were asked to name 32 black and white drawings of objects as the one in Figure 3.

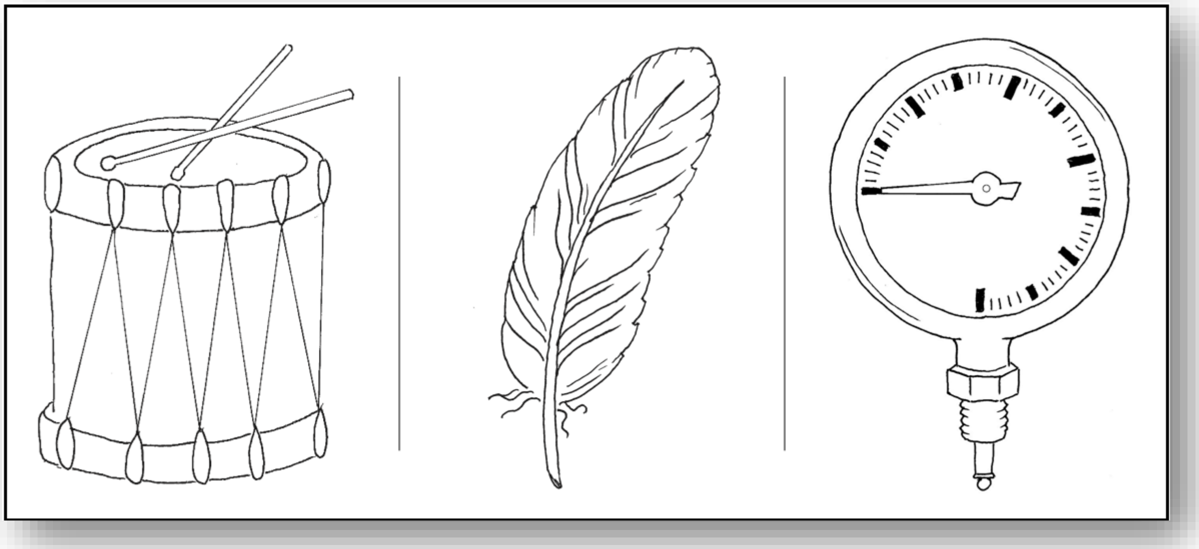

Figure 3. MINT Images with Increased Difficulty

Figure 3. Description: Images number 1 – easy (left), number 15 – medium difficulty (middle) and number 32 – difficult (right).

3.1.2.2. Adaptation our study

Multilingual participants were asked to name the images in Romanian, Russian and English, respectively. I excluded one of the images from all languages since they had no specific equivalent name in Romanian (i.e. the image showing a "plug"). Similarly, for few image items, more than one name was accepted as the correct answer (e.g. the equivalent for 'witch' in Romanian is both 'baba Iaga' or 'vrăjitoare').

3.1.3. The Development of the Semantic and Vocabulary Knowledge Test.

3.1.3.1. History of the Test

Additionally, the vocabulary section of the *Self-Administering Scale for Measuring Intellectual Impairment and Deterioration Test,* (also called Shipley Test in various studies) was used to assess the semantic and vocabulary knowledge of our participants. The original test (Shipley, 1940) was developed to assess vocabulary deterioration and abstract thinking and it has been widely used to assess intellectual ability and vocabulary of clinical as well as normal populations (e.g. Harnish et al., 1994; Martin et al., 1979). For this study, the vocabulary part of the original Shipley was used. This section included 40 items.

3.1.3.2. Adaptation our study

The English version of the *Self-Administering Scale for Measuring Intellectual Impairment and Deterioration* (a.k.a. Shipley Tests) was translated into Romanian and Russian. The vocabulary items were matched as closely as possible in meaning and difficulty. The normed frequency distributions of each of the items were computed in all three languages. First, words frequencies were computed from the Corpus of Contemporary American English (COCA, approximately 560,000,000 tokens) for each item in the original English test. Second, translation equivalents in Romanian and Russian (a few potential names for each item) were found. Normed frequencies were computed for each translated item in Romanian and Russian from two large corpora. Romanian item frequencies were extracted from the Balanced Corpus of Romanian Language, containing approximately 5,500,000 tokens (Ciochina et al., 2020), and in Russian, from Wikipedia text extracted from the Wiki dump, approximately 442,400,562 tokens. The final list of potential equivalent items was then created based on the frequency distributions obtained from each corpus using tools from the Natural Language Tool Kit, in Python (Bird et al., 2009). The selected words were then judged on a continuous scale from easy to difficult, by two university professors of the Russian language - native Russian speakers (one from Moldova and one from Russia) and two native language speakers of Romanian (both from Romania). This was done to ensure that the tests were matched across languages in terms of increasing difficulty with each succeeding item. Some items, though appearing with lower frequency in the corpora, were judged to be "easy" by the native speakers, whereas others, having high frequency were taught to be "harder", thus were replaced by appropriate words similar in meaning and frequency with the English version. Also, many words, especially towards the end of the test appeared with zero frequency in the Russian and Romanian corpora though were considered good matches for the English words. It is also worth mentioning that Romanian, as well as Russian, have a very rich system of declension and each of the lexical items was selected to have similar word forms (e.g. suffixes, case, number, etc.) across the options for each item in order to avoid potential identification of the correct answer. For a complete list of all items used in the *Semantic and Vocabulary Knowledge Test* in all three languages with their respective normed frequencies, see Appendix A.

3.1.4. The Development of the GAT test

3.1.4.1. History of the Test

The Grammatical Assessment Test (GAT) was constructed following a similar methodology as in Linebarger et al., 1983, originally developed to assess comprehension failures in agrammatic aphasics (Linebarger et al., 1983).

3.1.4.2. Adaptation for our study

A new set of sentences for each of the languages (including the English version) was created, specifically designed to assess grammatical judgment and sentence comprehension of Romanian, Russian, and English (namely, 72 sentences for each language). Half of the sentence set (36) were syntactically well-formed, and 36 were systematically ill-formed - representing 12 types of grammatical rule violations. Seven of the types were violations that translated in all three language structures (Romanian, Russian and English); whereas the other five were language-dependent.

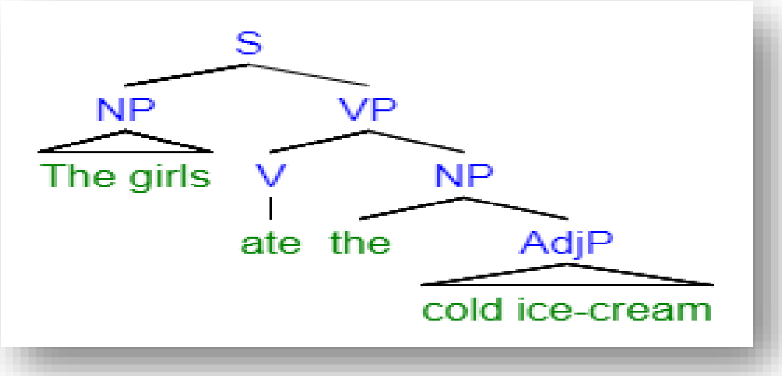

Figure 4. Example of Sentence Construction for the GAT

Figure 4. Description: Sentence tree example generated with the http://mshang.ca/syntree/. Note: S: sentence; NP: noun phrase; VP: verb phrase; AdjP: adjective phrase.

Table 1 below, describes the grammatical structure types, with examples of well-formed and ill-formed structures for each type. The syntactic structure of all sentences followed the same construction (see Figure 4), containing a noun phrase, verb phrase, and an adjunct or modifier phrase.

Six sentences for each type of sentence construction, three grammatical and three ungrammatical, were presented in the test in a randomized order from participant to participant, with no more than four consecutive sentences being grammatical or ungrammatical. Participants were asked to judge whether the sentence seemed grammatical or ungrammatical to them on a scale of 1 to 5. Below, is an example of a sentence in each language for one of the grammatical construction types used in the test. The ungrammaticality for this type of construction comes from the fact that the intransitive verb is followed by a direct object. Appendix B, lists examples of sentences in each language, for each grammatical error type.

Table 1. Grammatical Assessment Test Examples

*Elena	pleacă	trenul	de după masă.	Elena	pleacă	cu	trenul de după masă.
[*Elena	is leaving	train	afternoon.]	[Elena is leaving with the afternoon train.]			
*Он	вернулся		уроков.	Он	вернулся	с	уроков.
[*He	returned		the classes.]	[He	returned	from (his)	classes.]
* She sits the very comfortable chair.				She sits on the very comfortable chair.			

3.2. Participants

Thirty (15 monolingual and 15 multilingual) volunteers participated in this study, following written informed consent, approved by the Institutional Review Board at the University of California, Davis. All participants had no previous history of neurological or developmental disorders. All participants were between 19 and 33 years of age, (mean 24.8 years, SD 1.7 years for the monolingual group and mean 27.6 years, SD 3.2 years for the multilinguals) and were consistent right-handers, as measured by the *Edinburgh Handedness Inventory* (laterality index: mean of 85.8% for the monolinguals and a mean of 81.63% for the multilingual group, the scores ranging from 0 to +100 for all participants). All multilinguals were born and grew up in Moldova, a bilingual country with most of the population speaking Romanian and Russian fluently. They have acquired Romanian as their native language (L1), started learning Russian(L2) in elementary/middle school (mean age of 5.8 years), but also used it in everyday conversations, as well as watching Russian television. All participants learned an L3 (e. g. French, German or Spanish) starting in middle school. All participants moved to the United States at about 15 years of age (mean age of 15.2 years for the group), where they had started learning and using English 50% of the time on average (13 out of 15 participants went to high school or college in the U.S.). All monolingual participants have indicated to only have ever used English to communicate, and despite learning a foreign language in school (for six months on average), they are not able to communicate in any other language.

3.3. Data Collection

Monolingual participants completed the tests in writing, on one occasion, before or after the scanning session. The multilinguals completed the tests in two sessions. They have completed the language proficiency tests in the first session (the language tests were randomized across participants) and the background information and the self-rating questionnaire were completed in a second session before or after the scanning session. Two of the participants were excluded (and replaced) after the first session due to their low proficiency in English (compared to the other participants in the group) as assessed by the English tests.

3.4. Data Analysis

The behavioral data were manually digitalized for ease of processing. The test averages and statistical analysis were performed in R software (R Core Team, 2020; *www.R-project.org*). Other summaries and descriptive analyses were performed in Excel. Tables and figures were also created in Excel and R. I first present the results for the self-rating questionnaire, followed by the results for each of the proficiency tests used. Finally, results for correlation analysis between all variables in the behavioral data and self-rated proficiency for the second languages are shown.

3.5. Results

3.5.1. Self-Rating Questionnaire Results.

All monolingual participants rated their English proficiency at 100%; multilingual participants rated their L1 proficiency at 100%, L2 at 72% (SD = 0.94), and L3 at 78% (SD=0.91). Table 2 shows a summary of the background and self-rating assessments. In addition to the measures of AoA and self-rated fluency, I was able to obtain measures of language exposure and language usage. The multilingual participants have had several years of Russian exposure (with a mean of 11.3 years of using Russian every day) and English (with a mean of 12.5 years of English exposure).

Table 2. Self-Rating Questionnaire

Multilinguals Language AoA and Self-Rated Proficiency

Part. Num	*Age*		*Years of Ed.*		*Engl. AoA*		*SRP*	*AoA*	*SRP*	*AoA*	*SRP*	*Other Lang*	*AoA*
	Mono	*Multi*	*Mono*	*Multi*	*Mono*	*Multi*	*Engl*	*Rom*	*Rom*	*Russ*	*Russ*		
1	22	19	16	13	birth	14	6	birth	7	5	6	Span	15
2	28	29	21	19	birth	17	6	birth	7	6	4	Fr, It	8, 12
3	26	33	20	20	birth	10	6	birth	7	5	6	It, Ukr	6, 10
4	23	27	17	14	birth	21	4	birth	7	4	4	Ukr	4
5	23	28	18	14	birth	15	5	birth	7	5	5	Ukr, Fr	6, 13
6	23	29	17	13	birth	13	6	birth	7	2	5	Ukr	2
7	25	27	19	15	birth	17	4	birth	7	4	6	Ukr	9
8	26	30	18	13	birth	17	5	birth	7	6	6	Ukr, Fr	7
9	26	26	18	15	birth	20	4	birth	7	5	5	Ukr, Fr	11
10	23	26	17	15	birth	14	6	birth	7	11	4	Fr	10
11	24	31	17	13	birth	23	4	birth	7	0	5	Ukr	10
12	25	24	17	15	birth	13	6	birth	7	13	6	Span	16
13	26	27	18	18	birth	6	6	birth	7	12	4	Span, Ger	9, 12
14	24	29	18	17	birth	6	7	birth	7	1	6	Blr	1
15	27	29	16	20	birth	22	6	birth	7	9	3	It, Fr	9, 13

Table 2. Description: Part. Number: participant number; Years of Ed: years of education; Engl: English, Rom: Romanian, Russ: Russian; AoA: age of acquisition; SRP: Self-rated proficiency; Mono: monolingual; Multi: multilingual.

In terms of usage, the Russian was used from a mean of 5.5 years of age to a mean of approximately 15 years, for a total of 9.5 years. Importantly, after the age of 15 (group average), the participants started using only English daily (with a mean of 12.5 years), while Russian only on occasion. Thus, while English and Russian have similar years of exposure, they were used at different times in life. The languages that were used on an everyday basis at the time of the scanning were only Romanian (at home, with family and friends) and English (at school, work, church, store, etc.). Table 2 shows these data.

3.5.2. Language Production and Vocabulary Assessment Results

The mean for the percentage of error for the MINT was 6.9% (SD= 3.2%) for naming in native English for the monolingual group. The percentage error for the multilinguals was 5.4% (SD = 7.2 %) for L1, 25.4% (SD = 15.4%) for the L2, and 24.7% (SD = 6.9%) for the L3 (see Table 3). Two sample t-tests were carried out for comparing proficiency between the groups for the native language. The difference between the L1 monolinguals and L1 multilinguals ($t_{(30)}$ = 1.0 p <0.3; 95% CI, 1.85 to 0.65) was not significant. Paired sample t-tests were computed in order to assess the accuracy of the MINT, and further investigate differences in proficiency within the multilingual group between the languages.

Table 3. Percentage Error for Proficiency Measures

Percentage Error for the Proficiency Measures				
	MINT	SHIPLEY	GAT	Avg. Proficiency
	Perc. of Error	Perc. of Error	Perc. of Error	Perc. of Error
L1(mono)	6.9 (SD 3.2)	16.8 (SD 5.9)	13.9 (SD 8.7)	11.8 (SD 5.3)
L1(multi)	5.4 (SD 7.2)	12.8 (SD 7)	10.7 (SD 8.6)	10.0 (SD 5.3)
L2(multi)	25.4 (SD 15.4)	16.8 (SD 12.6)	18.2 (SD 7.5)	19.4 (8.5)
L3(multi)	24.7(SD 6.9)	24.5 (SD 18.5)	11.6 (SD 8.2)	18.4 (9.6)

Table 3. Description: Note: Mono: monolingual; Multi: multilingual; Perc. of Error: percentage of error; Avg. Proficiency: average proficiency

The differences between the L1 and L2 scores ($t_{(15)}$ = 7.2, p< 0.001; 95% CI, 4.45 to 8.21) and the L1 and L3 ($t_{(15)}$ = 4.04, p < 0.001; 95% CI, 2.87 to 9.38) were significant. There was no difference in scores between the L2 and L3 ($t_{(15)}$ = 0.1, p = 0.9; 95% CI, 3.3 to 2.9) (see Table 4).

3.5.3. Semantic and Vocabulary Knowledge Test Results

The mean for the percentage of error for the Semantic and Vocabulary Knowledge Test was 16.8% (SD= 5.9%) for the monolinguals group (see Table 3). The percentage errors for the multilingual group were 12.8% (SD = 7%) for the native L1, 16.8% (SD = 12.6%) for the L2, and 24.5 % (SD= 18.5 %) for the L3. A two sample t-test between the groups showed nonsignificant results ($t_{(30)}$ = 1.9, p= 0.6; 95% CI, 3.26 to 0.06). Paired sample t-tests showed statistically significant differences between L1 and L3 scores ($t_{(15)}$ = 3.6, p<0.01; 95% CI, 1.85 to 7.48), while the differences between L1 versus L2 ($t_{(15)}$ = 1.3, p=0.2; 95% CI, 0.95 to 4.15) and L2 versus L3($t_{(15)}$ = 1.7, p=0.1; 95% CI, -0.72 to 6.85) were not significant (see Table 4).

3.5.4. Grammatical Assessment Test Results

The mean percentage error produced in the GAT was 13.9 % (SD = 8.7 %) for the monolinguals. For the multilingual group, the percentage errors were10.7% (SD = 8.6%) for the test in L1, 18.2% (SD = 7.5%) for the L2, and 11.6 % (SD = 8.2%) for the L3 (see Table 3). A two sample t-test showed insignificant results between the two groups for the grammar test ($t_{(30)}$ = 0.9, p = 0.4; 95% CI, 5.7 to 2.24). Paired t-tests revealed differences between the scores in L1 versus L2 ($t_{(15)}$ = 3.2, p= 0.01; 95% CI, 1.78 to 9.14), and for L1 versus L3 ($t_{(15)}$ = 3.2; p = 0.01; 95% CI,7.9 to 1.6) and no differences between the scores for L2 versus L3 ($t_{(15)}$ = 0.5, p = 0.7; 95% CI, 2.44 to 3.78) (see Table 4).

Table 4. Proficiency Measures Statistic Testing Results

Proficiency Data Statistics

	MINT			***SHIPLEY***			***GAT***			***Avg. Proficiency***		
	p-value	*t stat.*	*CI (95%)*	*p-value*	*t stat*	*CI (95%)*	*p-value*	*t stat.*	*CI (95%)*	*p-value*	*t stat.*	*CI (95%)*
L1(mono) > L1(multi)	<0.4	-1	-1.85 to 0.65	< 0.6	-1.9	-3.26 to 0.06	< 0.4	0.9	5.7 to 2.24	<0.3	-1.05	-2.56 to 0.83
L1 (multi) > L2 (multi)	< 0.001*	7.2	4.45 to 8.21	< 0.2	1.3	-0.95 to 4.15	< 0.01*	3.2	1.78 to 9.14	<0.001*	4.4	2.3 to 6.63
L1 (multi) > L3 (multi)	< 0.001*	4.04	2.87 to 9.38	<0.01*	3.6	1.85 to 7.48	< 0.6	0.5	-2.4 to 3.7	<0.001*	4.2	1.87 to 5.76
L2 (multi) > L3 (multi)	< 0.9	-0.1	-3.3 to 2.9	< 0.1	1.7	-0.72 to 6.85	< 0.1	0.5	2.44 to 3.78	< 0.6	-0.5	3.3 to 2.01

Table 4. Description: P-values were FDR corrected for the number of tests. Note: Mono: monolingual; Multi: multilingual; GAT: Grammar test, MINT: Multilingual Naming Tests; Shipley: Self-Administering Scale for Measuring Intellectual Impairment and Deterioration, Lang. Use: language use

I have additionally looked at whether the sentences were considered grammatical or ungrammatical depending on whether the grammatical errors translated across languages or not. A comparison between the average rating for each sentence that had a cross-language versus a language-dependent error was computed. The paired two-sample t-tests were not significant for this comparison (see Figure 5). However, our sample had few data points (30 sentences per language), for a thorough comparison, and further analysis is needed for conclusive results.

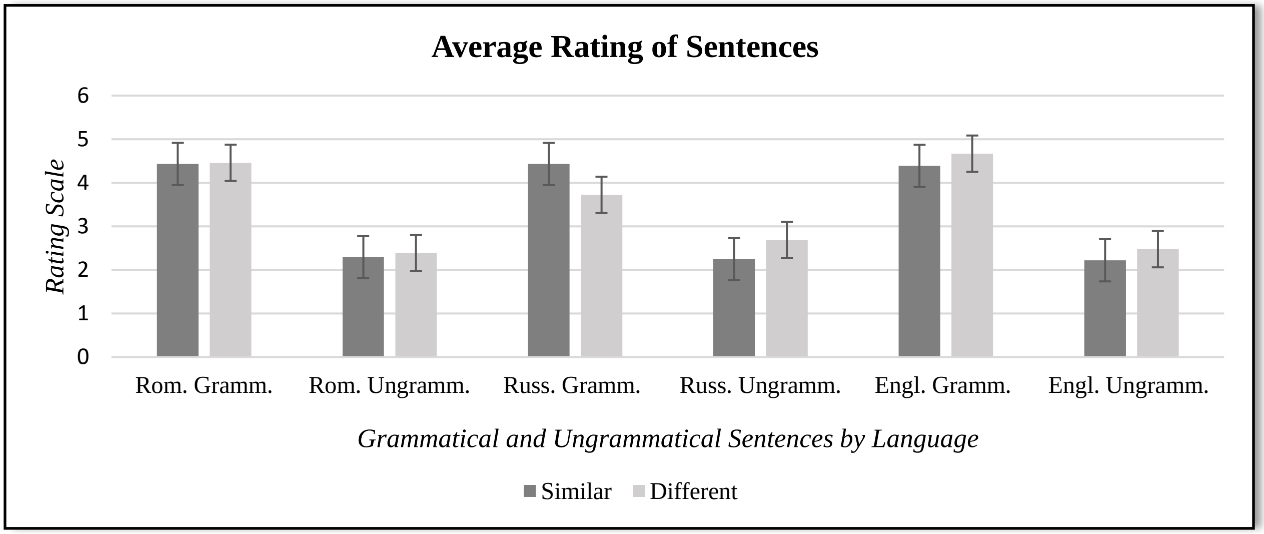

Figure 5. Average Rating of Sentences of Ungrammaticality Scores

Figure 5. Description: Similar – sentence construction with grammatical errors that translated across languages on a scale of 1(ungrammatical) to 5 (grammatical). Different sentence constructions with grammatical errors that did not translate across languages. Rom: Romanian; Russ: Russian; Engl: English. Gramm: Grammatical sentence types; Ungramm: Ungrammatical sentence types.

3.5.5. Average Proficiency Results

The overall percentage errors for the three tests were 11.84% (SD = 5.3%) for the monolingual group L1. For the multilingual group, the percentage error was 10.02% (SD = 5.3%) for L1, 19.39% (SD = 8.5%) for L2, and 18.04% (SD = 9.6%) for L3 respectively. A two-sample t-test was carried out for comparing proficiency between the groups for the native language. The difference between the L1 monolinguals and L1 multilinguals ($t_{(30)} = 1.05$, p

<0.3; 95% CI, 2.56 to 0.83) were not significant. Paired sample T-tests were computed in order to assess the accuracy of the proficiency test, and further investigate differences in proficiency within the multilingual group between the languages. The differences between L1 and L2 scores($t_{(15)} = 4.4$; $p < 0.001$; 95% CI, 2.3 to 6.63) and L1 and L3 naming ($t_{(15)} = 4.2$; $p< 0.001$; 95% CI, 1.87 to 5.76) were significant. There was not a statistical difference in scores between L2 and L3 ($t_{(15)} = 0.5$, $p = 0.6$; 95% CI, 3.3 to 2.01) (see Table 4).

3.5.6. Correlation Analysis

Finally, correlation analysis of self-proficiency and test measures were performed. Figures 6 and 7 below show the correlation matrices between the proficiency measures, self-rated proficiency, and other background variables for the multilingual group in their early L2 (Russian) and late L2 (English). Tables 5 and 6 show the p values calculated by Pearson's correlation tests between the proficiency test variables, the self-rated assessment, and other background factors as age of acquisition and language use.

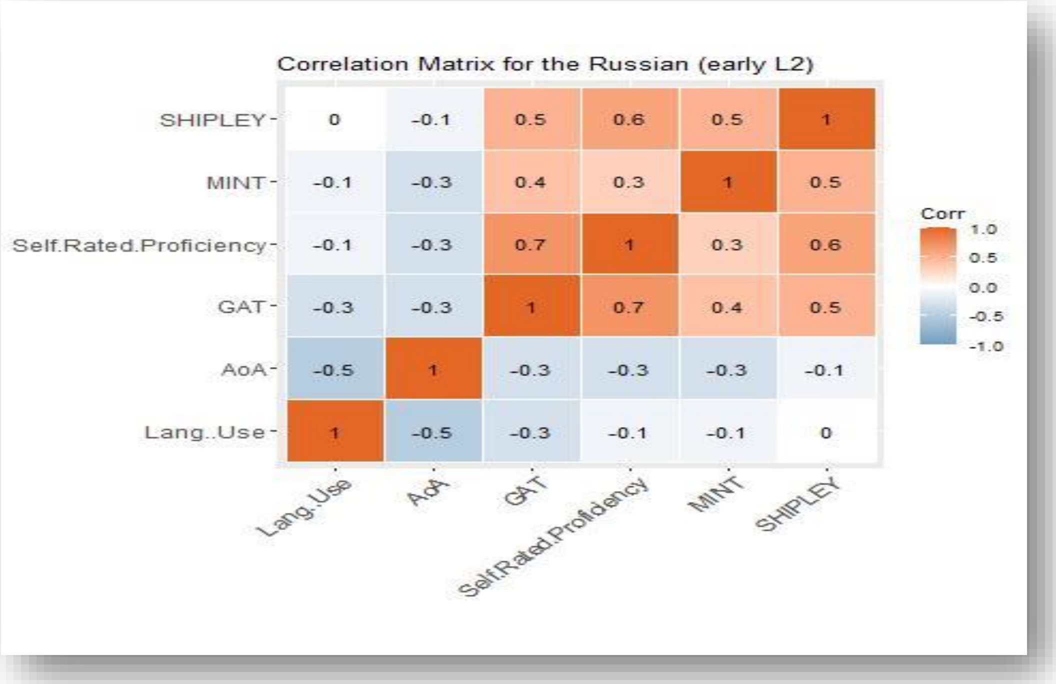

Figure 6. Correlation Matrix Proficiency and Language Use Early L2

Figure 6. Description: Correlation Matrix Across Variable of Proficiency and Language Use for the Russian (early L2) in Multilignuals. AoA: age of acquisition, GAT: Grammar test, MINT: Multilingual Naming Tests; Shipley: Self-Administering Scale for Measuring Intellectual Impairment and Deterioration, Lang. Use: language use.

Table 5. P-Values of the Correlation Tests for Early L2

P-values Correlation Matrix for the Russian (Early L2)

	MINT	*SHIPLEY*	*GAT*	*AoA*	*Lang.Use*	*Self.Rated. Proficiency*
MINT		0.05*	0.1	0.3	0.8	0.2
SHIPLEY	0.05*		0.06	0.6	0.9	0.008*
GAT	0.1	0.06		0.3	0.3	0.003*
AoA	0.3	0.5	0.2		0.08	0.3
Lang. Use	0.84	0.9	0.3	0.08		0.6
Self-Rated Proficiency	0.2	0.008*	0.003*	0.3	0.6	

Table 5. Description: P Values of the Correlation Tests for the Russian (Early L2), Multilingual Group. AoA: age of acquisition, GAT: Grammar test, MINT: Multilingual Naming Tests; Shipley: Self-Administering Scale for Measuring Intellectual Impairment and Deterioration, Lang. Use: language use.

The correlation analyses between the self-rated proficiency and the different tests were mixed. While there was a significant positive correlation between self-rated proficiency and the Shipley test ($r_{(15)} = .6$, $p < .01$), and self-rated proficiency and the GAT test ($r_{(15)} = .7$, $p < .01$), the correlation between the MINT and the self-rated proficiency were not significant in the early L2 (Russian).

Unexpectedly, age of acquisition did not correlate with either of the test measure scores. For the participants of this study, earlier acquisition of Russian did not lead to better proficiency. Compared to the Russian scores, the English self-rated assessment did not significantly correlate with the results of the test. Participants rated their self- proficiency higher if they started learning English at an earlier age, as shown by the negative correlation between the age of acquisition and the self-rated proficiency ($r_{(15)} = .8$, $p < .001$), however, the tests showed weak correlations between self-rated proficiency and test variables.

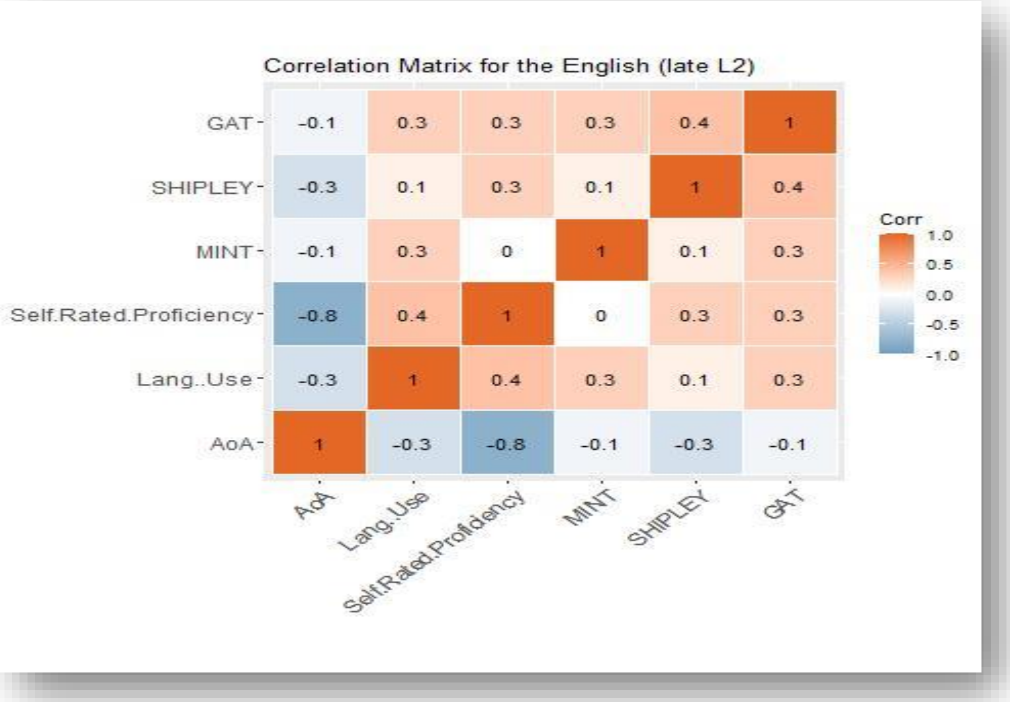

Figure 7. Correlation Matrix Proficiency and Language Use Late L2

Figure 7. Description: Correlation Matrix Across Variables of Proficiency and Language Use for the English (Late L2) for the Multilingual Group. AoA: age of acquisition, GAT: Grammar test, MINT: Multilingual Naming Tests; Shipley: Self-Administering Scale for Measuring Intellectual Impairment and Deterioration, Lang. Use: language use.

Table 6. P-Values of the Correlation Tests for Late L2

P-values Correlation Matrix for the English (Late L2)

	MINT	*SHIPLEY*	*GAT*	*AoA*	*Lang..Use*	*Self.Rated. Proficiency*
MINT		0.6	0.3	0.6	0.3	0.9
SHIPLEY	0.6		0.2	0.3	0.7	0.3
GAT	0.3	0.2		0.8	0.3	0.2
AoA	0.6	0.3	0.8		0.3	0.0006*
Lang. Use	0.4	0.7	0.3	0.3		0.1
Self-Rated Proficiency	0.9	0.3	0.2	0.0006*	0.1	

Table 6. Description: P-Values of the Correlation Tests for the English (Late L2), Multilingual Group. AoA: age of acquisition, GAT: Grammar test, MINT: Multilingual Naming Tests; Shipley: Self-Administering Scale for Measuring Intellectual Impairment and Deterioration, Lang. Use: language use

The correlation analysis between the self-rated proficiency and the different tests for English was not significant between the self-perceived proficiency and the test results. In contrast to Russian, for the English language, participants tended to rate their proficiency higher with more years spent practicing English, as shown by the statistically significant correlation between self-rater proficiency and AoA ($r_{(15)} = -.8$, $p < .0006$).

4. Discussion and Limitations

4.1. Self-Assessment and Proficiency Tests

In this study, three different measures of proficiency tests in English, Romanian and Russian, were presented. These metrics were developed to assess language proficiency at a phonological, lexico-semantic, and grammatical level. Through a background questionnaire, I was able to obtain the age of acquisition, educational background, language use, and experience measures for each participant. The three additional proficiency tests allowed for a detailed assessment of language knowledge for three of the languages spoken by the participants.

The results showed similar proficiency for both groups in the native languages; both groups showed excellent performance in all three tests, as well as 100% self-rated proficiency. For the multilingual group, the overall performance of the proficiency tests indicated that participants show good proficiency in all languages; however, statistically different results were obtained for native versus both non-native languages. The *Self-Rating Questionnaire* showed indicative lower fluency in the second languages, but no difference for different language domains. While the multilingual group showed overall lower proficiency for the early and late L2s compared to the native L1 in the *Language Production and Vocabulary Assessment* test (MINT), the *Semantic and Vocabulary Knowledge Test (SHIPLEY)* revealed no statistically significant differences between the early L2 and L1, while the GAT showed no significant differences between the L1 and the late (L2). Overall, the multilingual participants had equal proficiency of the L1 with the monolinguals, lower vocabulary proficiency in their late L2, and lower grammar knowledge in the early L2.

Inconsistent with the observation that AoA affects more the grammatical aspects of a second language (e.g. Golestani et al., 2006; for a detailed discussion see introductory chapter), the results revealed higher proficiency for vocabulary knowledge in the language learned earlier in life (Russian) and less proficiency for the grammatical aspects of the language compared to the L1. Conversely, higher proficiency was observed in the grammatical processing of late L2 (English) and lower for the vocabulary knowledge in this later acquired language in comparison to the native language. One possible reason for lower grammatical proficiency in the earlier learned language (Russian) may be due to the grammatical aspects of the language per se. The two second languages investigated in this study vary in the complexity of their morphological systems; English is an analytic language. Strongly analytic languages are made up of sequences of free morphemes, each word consists of a single morpheme with meaning. English has very few inflections, word order is the main cue used by the speaker to assign semantic and syntactic functions. Whereas Russian is a synthetic fusional language. Synthetic languages create meaning by attaching bound morphemes to other morphemes, thus a word may be made up of multiple meaningful elements. Word forms (particularly affixes) are an important cue in assigning semantic and syntactic roles in such languages. While processing languages with more complex morphological systems the speaker needs to keep in mind the case, number, gender, definiteness, etc., and computing the correct suffixes for those is necessary during sentence building and sentence comprehension. Comparative to English, Russian has very strong declension systems. There may not only be more online computation steps while processing sentences in Russian compared to English but also more complicated paradigms to store and access from memory at processing. Matthews, 1991, 2002, explains that different morphosyntactic categories (i.e. number, gender, case, etc.,) create sets of variables of different dimensions across languages.

For example, a noun in English is formed with a variable containing a single dimension:

Singular	***Plural***
book	books

Thus, the English noun can vary on a single dimension, and it is the only varying attribute to keep in mind for the speaker. Whereas the noun forms in Russian may vary on a three (masculine, feminine, and neuter) - by two (singular and plural) - by seven (nominative, accusative, genitive, dative, instrumental, prepositional) dimension. The appropriate selection among all these variants needs to be performed at each step of production or comprehension (e.g. Corbett, 1982) in Russian, thus resulting in lower grammatical proficiency, compared to English.

Another possibility for the unexpected results related to AoA may be the fact that most of the participants did not study Russian as a formal language of instruction (i.e. participants studied in Romanian – up to high school, and English – in college). Most participants acquired Russian in early childhood from watching TV, playing with and conversing with speakers of Russian, etc. It can be argued that many grammatical rules of sentence construction are formally taught in school and formal instruction manuals. These were not part of the linguistic background of the participants. However, it is important to note that there were no significant direct differences between the early and late L2 for the GAT, suggesting overall very similar proficiency between the second languages even within the grammar domain.

4.2. Correlation Analysis

The correlation analysis further confirmed the need for proficiency testing additional to the self-perceived proficiency. The self-perceived proficiency of the Russian was correlated with the performance on the GAT and the Shipley tests (the MINT did not correlate with self-proficiency for either of the languages). In contrast to Russian, none of the test results were correlated with self-perceived proficiency for English, however, the self-perceived proficiency showed a strong negative correlation with the AoA.

Interestingly, similar to the results for the Russian, MINT tests results did not correlate with the self-assessed proficiency and showed a weak correlation to the age of acquisition. One possible reason for which MINT showed these results might be due to the specifics of the tests, concerning both the type of test and the items selected. The MINT test comprised a collection of 32 images. Participants were asked to name the items in the images. Many of the participants reported "tip-of-the-tongue[19]" (TOT) phenomenon, most commonly reported in bi/multilingual speakers (Claussenius-Kalman & Hernandez, 2019). Gollan & Acenas, 2004, in a picture-naming task that induced TOT states in a group of Tagalog – English bilinguals, reported that bilinguals, compared to monolinguals showed more frequent TOT states, especially in cases in which the images to be named were not cognates. Because participants were asked to complete the test in one sitting, they might have not had the chance to remember the lexical items, even though they might have known them. Another factor that might have influenced these results is the fact that most of the items presented in the pictures were household items (e.g. hinge, blinds, axel, zipper, scarf, dustpan, etc.,). The participants in this study spoke Romanian as a native language and continue to speak Romanian at home. Since the items represented in the MINT were items encountered at home, most of these words were probably mostly used in Romanian, resulting in greater chances of TOT states or lack of lexical items in English and Russian for these items in the bilinguals' vocabulary. Research shows that although bilinguals hold an overall larger vocabulary, they have a smaller vocabulary in each of the languages spoken, as a consequence of spending less time in each language (Bialystok & Luk, 2012). These results come to underly the importance of appropriately choosing the type of tests when assessing proficiency in multilingual populations.

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[19] Tip-of-the-tongue states (TOTs) are word retrieval failures characterized by a feeling of imminent recall and by a greater than chance probability of accurately reporting partial information about the target word, e.g., the first phoneme (Gollan & Acenas, 2004)

Of course, language proficiency is a complex construct and cannot be completely assessed within one single study. However, this study underlines the importance of using detailed measures that may reveal different levels of proficiency in different language domains. Additionally, results show that the self-rating proficiency may not always correlate with and need to be combined with the results from other tests (i.e. such as standardized or in-house tests). Finally, the specifics of the tests used, play an important role in assessing proficiency, especially in multilinguals that may have acquired and continue to use different languages in the different socio-linguistic environments.

5. Implications and Further Steps

The motivation for constructing the measures presented in this study was to be able to test whether the participants in our groups vary in their linguistic background and language fluency at all levels of language. Based on the multilingual profiles obtained in this study, certain predictions can be made in terms of the brain adaptations that will be observed in the multilinguals. First, both groups showed great performance in their native languages, making the groups fit for between-group comparisons. Second, the multilingual group showed good proficiency in both second languages. However, the difference between the native versus non-native tests was significant, placing our participants at an intermediate (“consolidation stage”; see Pliatsikas, 2020) language learning stage. According to the DRM, in the consolidation stage, there is an increased demand for the control processes related to shifting from the learning of lexical items to grammar and phonological acquisition of the second language. The brain changes associated with these processes should be observed in subcortical and cerebellar regions, as well adaptations should be noticed in the white matter diffusivity, namely the inferior and superior fasciculi (ILF, SLF), and the inferior fronto-occipital fasciculi (IFOF) and corpus callosum.

Additionally, based on the self-rated questionnaire, the two-second languages were learned at different ages but were similarly acquired across the participants of the multilingual group. The participants used Russian for the first part of their life and moved to California, the U.S., where they started learning English and using mostly English (outside the home) and Romanian (at home and with family/friends), while Russian is only used on occasion (watching TV, or with Russian speaking friends). These unique similarities in the background related to language acquisition and use will allow a direct comparison between the languages within the multilingual group. Related to AoA, differences may be observed between the activation patterns of Russian (early L2) and English (late L2). We may observe more cortical overlap between the Russian and native Romanian, versus English and Romanian, due to the early age of acquisition of the latter.

Lastly, our results further underline the importance of considering multilinguals', rather than bilinguals' experience in the development of neurolinguistic models of multilingualism. The interactional context (see Green & Abutalebi, 2013) of the multilinguals compared to bilinguals is further complicated by the presence of additional second languages. For example, a multilingual may find themselves in a combination of interactional contexts (our participants reported using one language with one interlocutor only, in a context in which other two or three languages may be used with another interlocutor, etc.) in which they may experience single language, dual-language and dense code-switching language contexts, in different situations and with different interlocutors.

Chapter II

Functional Adaptations to Multilingual Experience within the Language Network

Abstract

Recent models of the neural underpinnings for language function in the brain, propose a language network, that has core component regions, specialized for language processing, and periphery nodes, overlapping with cognitive control networks that are domain-general. In this study, I use the localizer task paradigm, proposed by Scott et al., 2017, to investigate the neural correlates of language comprehension in a group of multilinguals compared to a group of monolinguals. The first section of this chapter describes an exploratory analysis looking at whole-brain activation patterns for language processing versus baseline, showing similar recruitment of brain regions identified by Scott et al., 2017. Following, and ROI analysis suggest similar activation in core language brain regions, and stronger recruitment of periphery brain regions, part of the cingulo-opercular subnetwork (including the anterior cingulate and insular cortex), for multilinguals compared to monolinguals. A second section of the chapter describes activation differences during language processing within three languages spoken by the multilingual group. The results of an ROI analysis suggest stronger recruitment of control brain regions associated with tonic activation related to maintaining alertness during task processing (i.e. the cingulo-opercular subnetwork; Dosenbach et al., 2008) for the more dominant languages (namely the native L1 and the later learned L3). Lower recruitment of control regions was observed during the processing of the less dominant early L2. These results are discussed in relation to recent models of bilingual language control (Abutalebi & Green, 2016; Green & Abutalebi, 2013).

Introduction

Language comprehension is a complex set of orchestrated processes that maps the speech signal onto meaningful concepts. These processes include the decoding of the articulatory phonetic aspects of the speech input, retrieval of the words, and their morphological and grammatical properties. The mapping of linguistic units results in a lawful transmission of intended informational concepts. For multilingual language users, these processes further entail the need to selects and control multiple linguistic mappings, for example, depending on the context, monitor and detect cues that may suggest a change in the target language used and guard against possible interference from the other languages spoken at any of the possible levels (e.g. the task schema, syntactic, lexical, morphological, etc.). Together, these processes are performed by the interaction of both linguistic and control mechanisms that are related to the executive control brain networks (Piai et al., 2013; Shao et al., 2012).

1. Methods

1.1. Participants

Thirty (15 monolinguals and 15 multilingual) volunteers participated in this study, following written informed consent approved by the Institutional Review Board at the University of California, Davis. A detailed description of participants' background and proficiency testing is provided in Chapter I.

1.2. fMRI Study Design and Materials

For the fMRI language stimuli, a set of 80 sentences were created for each language. E.g:

English		They put the diner plates on the table.
Romanian	*Mama le spune o poveste lungă.*	The mother tells them a long story.
Russian	*Волны вымыли длинный берег.*	The waves washed the longshore.

All sentences had a similar syntactic construction of a DP followed by a VP, containing a modifier.

i.e.

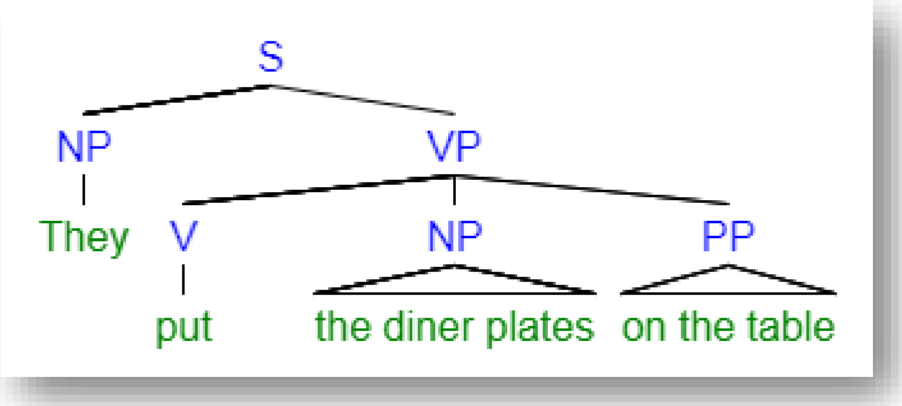

Figure 8. Sentence Tree Example for the fMRI Task

Figure 8. Description: Generated with the http://mshang.ca/syntree/

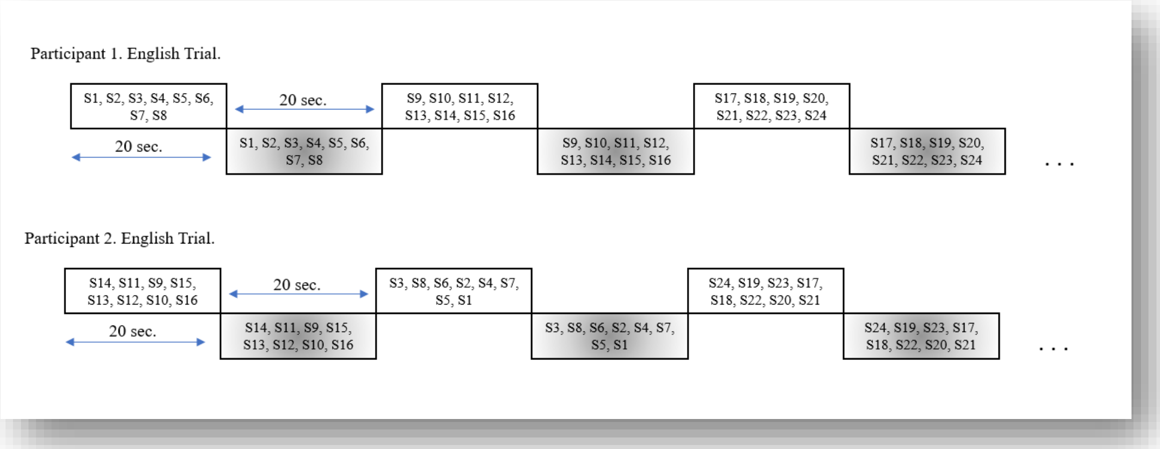

Figure 9. Task Design

Figure 9. Description: S-sentence; language block (box in white); baseline task (box in grey).

The sentences were audio recorded with the software Audacity (with a Rode NT1-A microphone, and Edirol UA-25 sound interface), in Romanian, Russian, and English by 3 female native speakers of each of the three languages. The individual sentences were then saved as individual .wav files, and pre-processed, compressed, and normalized in Matlab and Audacity tools. 8 blocks containing 7/8 sentences, with random pauses between sentences (ranging from 0.09s to 0.965s), for a total of 62 sentences per language, were constructed for each language trial. The sentences were presented in random order within each language block and were also randomized across trials (an illustration of the design is shown in Figure 9). The original audio files used in the language trials (for each language respectively) were modified according to Scott et al., 2017, to create the baseline condition. The modified speech .wav files were further reversed to suppress recognition of language identity by the multilingual individuals. Each trial consisted of 8 cycles of language interleaved with 8 cycles of modified reversed language (8 x 20sec. language + 8 x 20sec. baseline) for a total length of 5.20 minutes. Additionally, 5 audio files with individual sentences were randomly selected from the 62 sentences used in the language blocks, and 5 new individual sentences .wav files were used to construct the test sentence blocks.

During the scanning session, the participants were instructed to look at a fixation cross while attending to the auditory stimuli. They were also instructed to pay attention to the language sentences as they will be tested at the end of the trial and passively listen to the baseline condition. All participants listened to all three languages with approximately 1minute rest between language trials (for a total of approximately 18 minutes). The trials (i.e. language order) were pseudo-randomized across participants. Between language trials, instructions in printed English were presented on the screen containing information about the next language condition. Participants read the instructions and were free to ask clarification questions. The participants continued to start the trial when they were ready, with a button press.

After each language trial (English, Romanian, and Russian), the participants heard 10 sentences (5 new sentences and 5 they have heard during the trial) and were asked to answer with a button press (yes/no) whether they have heard the sentence before. This was done to insure attention to the stimuli. Monolingual speakers were not expected to be able to fully differentiate between previously heard words in the foreign language conditions, while we expected multilingual speakers to show performances that are similar to monolinguals processing their L1. Multilinguals responded to the yes/no test trial with 82% accuracy for the Romanian, 72% for Russian, and 74% for English. Monolinguals responded with 84% accuracy for the English, 62% for the unfamiliar Russian, and 64% for the unfamiliar Romanian. These results suggest that both groups were actively engaged in the task, while the multilinguals performed better, as expected since they were familiar with all three languages. Table 7 shows the results from the yes/no test task at the end of each language trial.

Table 7. fMRI Yes/No Testing Session Results

Yes/No Test Statistical Results				
Comparison	***t statistics***	***p value***	***95% CI***	***corr. p value***
Welch's t-test; $t_{(30)}$ both groups				
L1 (mono) > L1 (multi)	0.38	0.7	- 0.86 to 1.26	p < 0.8
paired t-test; $t_{(15)}$ monolingual group				
L1 (mono) > L1 (multi)	3.2	0.006	0.66 to 3.34	p < 0.04*
L1 (mono) > L2 (multi)	2.73	0.02	0.47 to 3.93	p < 0.05*
L1 (multi) > L2 (multi)	0.22	0.8	- 1.74 to 2.14	p < 0.8
paired t-test; $t_{(15)}$ multilingual group				
L1 (multi) > L2 (multi)	1	0.3	- 0.4 to 1.1	p < 0.4
L1 (multi) > L3 (multi)	1.67	0.1	- 0.2 to 1.8	p < 0.2
L2 (multi) > L3 (multi)	-1.07	0.3	- 1.4 to 0.5	p < 0.4

Table 7. Description: P-values were FDR corrected for multiple comparisons.

1.3. Data Acquisition

Participants were scanned with a Siemens 64-channel 3-Tesla "Skyra" MRI System (Siemens Healthcare, Erlangen, Germany) at the Imaging Research Center of the University of California, Davis. For the structural data, we used a T1-weighted imaging sequence using an MR-RAGE (TFL) sequence with a voxel size = 0.9x0.9x0.9 mm3, FOV = 243 × 243 mm, 208 sagittal slices, 7-degree flip angle, TR = 2500msec, TI = 1100 msec, and TE = 4.44 msec, Bandwith 160 Hz/Px; GRAPPA = 2, 32 reference lines. A multi-band two-fold slice acceleration scheme was used to acquire the functional data. GE-EPI (voxel resolution 3.5 × 3.5 × 3.5 mm3; FOV = 224mm; TE = 25 ms; flip angle = 90 deg.; bandwidth = 2232 Hz/Px; TA = 0.97 s; TR = 1 s; no in plane acceleration; 34 slices).

1.4. Data Preprocessing

FMRI data processing was carried out with several available tools. We used a Debian-based Ubuntu distribution to run the different command tools in bash (Bourne-Again Shell) command language interpreter. For each subject the first 10 volumes were discarded in each scan series, to ensure that the magnetization was at the equilibrium state in the volumes analyzed.

1.4.1. Structural Data

The raw MPRAGE images were converter to NIFTI format using the dcm2nii function obtained from www.nitrc.org. Non-brain removal using was carried out using Brain Extraction Tool (BET) (Smith, 2002), part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.ul/fsl). Bias Field Correction was performed with a B-spline nonparametric nonuniform intensity normalization approach, using the N4BiasFieldCorrection Tool (Tustison et al., 2010; N4ITK), part of ANTs (Advanced Normalization Tools), version 2.1 (http://stnava.github.io/ANTs/). A study template of all 30 participant's structural images was obtained with were then used to build the multi-variate template via ANTs' antsMultivariateTemplateConstruction2 (Avants et al., 2009).

1.4.2. Functional Data

The raw epi images were converter to NIFTI format using the dcm2nii function obtained from www.nitrc.org. Non-brain removal was carried out using the BET (Brain Extraction Tool)(S. M. Smith, 2002), and motion correction via MCFLIRT (Jenkinson et al., 2002); both, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.ul/fsl). The motion correction tool outputted a mean functional image and the movement parameters files that were later used as nuisance regressors in the 1st level analysis. Bias Field Correction was applied both on the man functional and the functional images, with the B-spline nonparametric nonuniform intensity normalization approach, via the N4BiasFieldCorrection Tool (N4ITK; Tustison et al., 2010), part of ANTs (http://stnava.github.io/ANTs/). Symmetric diffeomorphic registration of the functional data to the high-resolution structural images and standard MNI template space images was carried out using the AntsregistrationSyN tool (Avants et al., 2008), part of the ANTs (Advanced Normalization Tools), version 2.1 (http://stnava.github.io/ANTs/). I have used a scalped and smoothed at 8mm kernel version of the 152T1 template available in the SPM12, 2x2x2mm³. The pre-processed functional images were smoothed with an isotropic Gaussian kernel of 8 mm full width at half maximum (FWHM) smoothing kernel, using Matlab and statistical parametric mapping (SPM12; Welcome Department of Cognitive Neurology, London; http://www.fil.ion.ucl.ac.uk/spm/).

1.5. Statistical analysis

1.5.1. Whole-Brain Analysis

The time series analysis was carried out using SMP12. Activated voxels were identified by a fixed-effects General Linear Model approach (Friston et al., 1994). The analysis steps included the convolution of the time series with the modeled hemodynamic response function (HRF, we used double gamma) and a high pass filter was applied followed by pre-whitening to deal with low-frequency noise and temporal autocorrelation. Each language trial was preprocessed separately - one regressor for each language task (i.e. processing in L1, L2, and

L3) and one for each baseline (processing of L1 degraded, L2 degraded and L3 degraded) were modeled for both groups. The extended motion parameters from each language trial were included in the model as nuisance regressors. To test hypotheses about regionally specific condition effects, these estimates were compared using linear contrasts. In order to investigate global patterns of activation for comparison with previously reported results (i.e.Scott et al., 2017). I first performed a whole-brain analysis, in which all 30 subjects were included. For this analysis contrasts subtracting the baseline control activation from the native language processing were performed. Additionally, contrasts subtracting the language task minus baseline control (namely, L1processing minus degraded L1, L2 processing minus degraded L2 processing, L3 processing minus degraded L3 processing) baseline "degraded speech" were performed for each condition for each group, to generate SPM (t) maps of condition differences. Significant regions of activation were identified using a cluster size of k = 8, significant at a family-wise error rate (FWE), one-tailed threshold of $p < 0.05$. Thresholded statistical parametric maps (SPMs) were overlaid on the mean anatomical image of the study sample (an additional T1-weighted image template from the MRIcro software was used) using MRIcro software (Rorden & Brett, 2000).

1.5.2. ROI Analysis

I made use of the core language areas identified in Scott et al., 2017, and the control areas proposed by the ACH to guide the regions of interest that will be investigated in this study. The ROI analysis was performed with Matlab (MathWorks.com) and R (R Core Team, 2020; www.R-project.org) tools. Anatomical regions of interest were identified using both coordinates extracted from the (Fedorenko et al., 2010, 2013) as well as using the Wake Forest University Pick Atlas (UWFpick_atlas)[20] (Maldjian et al., 2003) and the MarsBar tool in SPM12 (Brett et al., 2002); http://marsbar.sourceforge.net/). I created 10mm and 8mm

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[20] The AAL atlas was used to create the cerebellum masks. The UWFpick_atlas was also used to identify MNI coordinates for the manually created spherical ROIs as well as further check if the sphere ROIs matched the anatomical space of the manually created spheres.

spheres, for the cortical areas, and 5mm[21] spheres for subcortical areas for the regions of interest. The size of the spheres was decided upon depending on the size of the anatomical area. P*arameter estimates (i.e. betas) were* extracted from the mean BOLD signal of all voxels comprised by the ROI mask, for each contrast (namely processing of L1 in monolinguals and multilinguals, and L2 and L3 for the multilingual group). For this step, we used the nee_ROI function in MatLab (written by Derek Evan Nee, University of California, Berkeley, 2015). This function outputs a time-course value for each subject, representing the contrast estimate averaged over all the voxels in the ROI. The obtained average for each contrast was saved in a .csv file for further analysis in R. T test, and group averages were performed in the R software (R Core Team, 2020; www.R-project.org.). Other summary statistics were performed in Excel. Tables and figures were also created in Excel and R.

1.5.3. Regression Analysis/Correlation Analysis.

The obtained average beta values for the selected ROIs were saved in .csv files. These were used to perform correlation analysis with the behavioral variables, in the R software (R Core Team, 2020; www.R-project.org.). Plots, tables, and figures for the correlation analysis were also created in Excel and R.

Section I

In this section, I provide exploratory and hypothesis-driven investigations of functional activation in multilingual and monolingual individuals. The theoretical framework for these initial investigations is built upon fundamental predictions of the newer models of language processing, that reframe the issues of language brain architecture in terms of a *language*

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[21] Note: a 4mm sphere was created for the Caudate tail, due to the small anatomical size of this brain area.

network (i.e. Fedorenko & Thompson-Schill, 2014s), including core and periphery brain regions, that together support language function in both monolinguals and bi/multilinguals.

I start the discussion by describing the core and periphery component nodes (i.e.brain regions) of the language network. Following, an exploratory investigation that seeks to address whether our paradigm, modeled after Scott et al., 2017, results in similar activation within the language network brain regions identified by the authors in several studies (Fedorenko, 2014; Fedorenko et al., 2010, 2012, 2013). Initial whole-brain analysis for a contrast of language processing task minus baseline condition including all 30 participants of the study is described, followed by an ROI analysis focusing on a priory defined language and cognitive control ROIs. Finally, we investigate whether activation in the language network changes as a function of AoA of a second language.

1. The Language Network

1.1. Core Language Brain Areas

Fedorenko & Thompson-schill, 2014, propose a language network that comprises both "core" component nodes (i.e. brain regions) that are specialized for language processing, and "periphery" brain regions that are recruited during both language processing tasks but also tasks from other so-called general domains (i.e. sensory, motor, memory, and control systems). In a few studies, (e.g. Fedorenko et al., 2010; Fedorenko & Thompson-schill, 2014; Scott et al., 2017) this research group identified eight language "core" regions (focusing on the left hemisphere) that showed consistent activation for language processing with different paradigms (i.e. Fedorenko et al., 2010; Scott et al., 2017). They include the lateral surface of the left frontal cortex (lIFG), the orbital regions of the inferior frontal gyrus (lIFGorb), the middle frontal gyrus (lMFG), the left lateral anterior temporal cortex (lLatAntTemp), left middle anterior temporal cortex (lMidAntTemp), the left middle posterior temporal cortex (lMidPostTemp), the left posterior temporal cortex (lPostTemp), and the left angular gyrus (lAG). Table 8 (A) provides a list of the functional regions of interest (fROIs). These core language areas have been documented in numerous studies in the last two decades to be involved in language processing (e.g.Mashal et al., 2009; Vigneau et al., 2006; Wagner et al., 2000, etc).

1.2. Language Periphery Regions

Along with areas emerging in investigations of the language system, the Fedorenko & Thompson-schill, 2014, language network, propose a set of "periphery" brain regions that may co-activate during language tasks but are also consistently implicated in non-linguistic, goal-directed behaviors (e.g. Duncan, 2010; Fedorenko, 2014). Fedorenko et al., 2013, using a large number of tasks that involved different representations (e.g. four tasks included verbal representations, two tasks included Arabic numerals, and one task used spatial location) identified a set of regions that are involved in all these tasks, hence suggesting a broader domain-general functionality. The regions identified by the authors include the dorsolateral frontal lobes (along the inferior sulcus/middle frontal gyrus; DLPFC), parts of the insular cortex, precentral gyrus, pre-supplementary and supplementary motor area (preSMA, SMA), parts of the anterior and middle cingulate gyrus (ACC/MCC), the intraparietal sulcus (IPS). A list of these regions is provided in Table 8 (B).

Although not all psycholinguistic models agree on whether the same mechanisms are involved in linguistic and non-linguistic control processes, these have been argued by scholars to be part of the executive control systems (a.k.a. cognitive control system; Piai et al., 2013; Roelofs & Piai, 2011). The cognitive control system consists of two subnetworks, the fronoto-parietal network (including the inferior frontal regions, and the intraparietal sulcus (IPS)) and a cingulo-opercular network (including the cingulate cortex (ACC), the anterior insular cortex (AIC), and the subcortical regions of the basal ganglia and the thalamus (Dosenbach et al., 2008; Wu et al., 2021). The main functions described in executive control models are response inhibition (self - control - resisting), interference control (selective attention), working memory, and cognitive flexibility (i.e. thinking 'outside the box'; Diamond, 2013;

Duncan, 2010). These two systems, working in concert with language-specific brain areas, likely manage inhibitory control as well as language selection, both of which are necessary for the effective management of language (Jenny Crinion et al., 2006; Hervais-adelman et al., 2011).

Table 8. List of Core and Periphery Language Regions

A. 'Core' Language Brain Regions (Fedorenko et al., 2010)
Brain Area
Lateral Surface of the Left Frontal Cortex (lIFG)
Left Inferior Frontal Gyrus Orbital (lIFGorb)
Left Middle Frontal Gyrus (lMFG)
Left Lateral Anterior Temporal Cortex (lAntTemp)
Left Middle Anterior Temporal Cortex (lMidAntTemp)
Left Middle Posterior Temporal Cortex (lMidPostTemp)
Left Posterior Temporal Cortex (lPostTemp)
Left Angular Gyrus (lAngG)
B. Language Control 'Periphery' Brain Regions (Fedorenko et al., 2012, 2013)
Dorsolateral Prefrontal Cortex (DLPFC)
Insular Cortex (regions along the precentral gyrus)
Posterior inferior Frontal Gyrus (IFG)
Pre-supplementary Motor Area (pre SMA)
Supplementary Motor Area (SMA)
Parietal Lobule (PL)
Anterior Cingulate Cortex (ACC)

A prominent question in the field of neurolinguistics is whether the neural representation within brain regions that are specialized for language processing changes as a function of the number of languages one knows. As described in the introductory chapter, two general hypotheses in terms of brain representation of languages in the multilingual brain have been proposed. One is that when a second language is acquired early in life, or simultaneously, the neural representation of the two languages will be shared, while a later learned language will recruit additional adjacent areas (e.g. Bello et al., 2006; Blackburn, 2019; Golestani et al., 2006; Johanne et al., 2013; Lubrano et al., 2012; Ullman, 2001). Another hypothesis posits that by learning two languages simultaneously, a bilingual language system develops that can accommodate both languages, as well as later learned languages, that converge onto the already developed bilingual language system (e.g. Abutalebi, 2008; Blackburn, 2019; Bloch et al., 2009; Bruin et al., 2014; Perani & Abutalebi, 2005). Based on the background characteristics of the multilingual participants in this study (i.e. similar proficiency in two secondly learned languages, with a first second language learned early in life (mean of 5.8 years of age) and a second L2 learned later in life (mean age of 15.2 years old); see Chapter I for details. I will be able to investigate whether second language neural representation, as measured by mean activation in language core regions, differs as a factor of AoA. If neural representations are shred between the native language and early learned second languages, white a third later learned second language recruits adjacent brain regions (i.e. according to the first view described above) similar mean activation should be observed in Native L1 (Romanian) and the early L2 (Russian), but not the later learned L3 (English) in the language core regions. On the other hand, if an early second language leads to a bilingual language system that can accommodate all languages (i.e. premises of the second hypothesis described above), similar activation in language core regions should be observed in all three languages (i.e. L1 native, early L2, and late L3).

2. Results

2.1. Whole Brain Analysis

The first exploratory hypothesis I investigated in this chapter was whether similar patterns of activation are observed in left hemisphere core language areas, as predicted by Scott et al., 2017, in our task. I first report a whole-brain analysis (including all 30 participants) to investigate global patterns of activation. Following, an ROI analysis across groups, of the core and periphery language regions (identified by Fedorenko et al., 2010, 2012, 2013) is discussed.

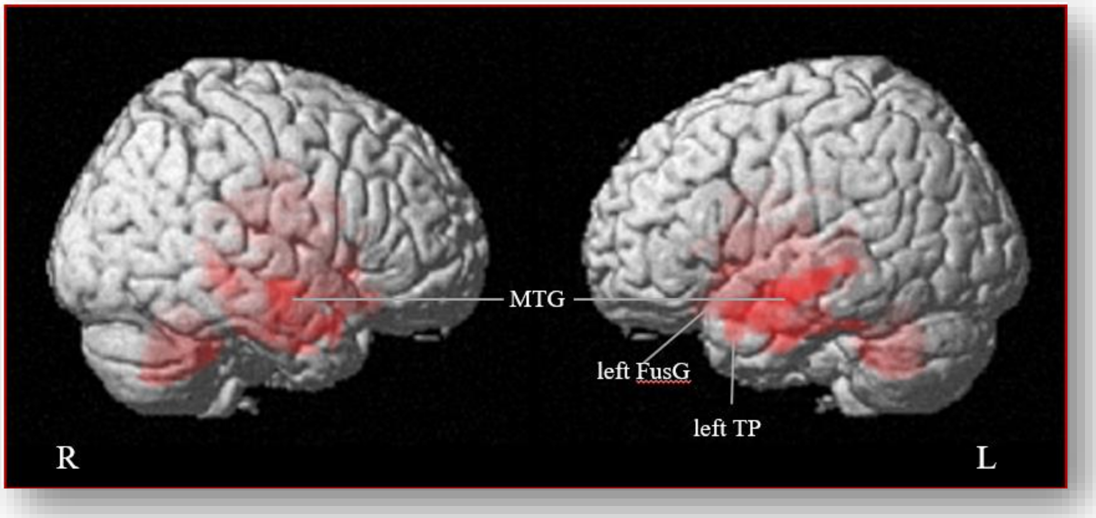

Figure 10. Whole-Brain Analysis Results for Native Language Processing (all 30 subjects)

Figure 10. Description: The 2nd level T-maps are overlaid and rendered on a T1-weighted image provided in MRIcron [http://www.sph.sc.edu/comd/rorden/mricron/]. MTG: middle temporal gyrus; FusG: fusiform gyrus; TP: temporal pole.

Overall, whole-brain analysis maps show robust activation in cortical areas typically associated with language processing, specifically, in the middle, inferior, and superior temporal gyrus (MTG, ITG, STG), and the temporal pole (TP). Figure 10 displays the combined activation of all 30 participants during passive L1 sentence processing compared to the associated baselines controls (backward degraded speech). The results are displayed on a standard MNI 3D volume template (provided by the SPM12 software), at FWE correction of $p< .001$. These patterns of activation are very similar to Scott et al., 2016 whose “localizer” paradigm was also used in this study. However, compared to the original study, the activation in the frontal regions did not reach significance and was limited to activation in the fronto-orbital gyrus. Table 9 provides a list of all areas that were statistically significant at FWE correction of $p < 0.05$, at a voxel extended threshold of 8 consecutive voxels, for a contrast of language processing versus baseline for all 30 participants along with the Broadman’s Areas and the MNI coordinates for the cluster peaks. For details of results for whole-brain analysis per group (i.e. monolinguals (A) task > baseline, and multilinguals (B) task > baseline) see Appendix C.

Table 9. Stereotactic Coordinates of Peak Activations for Whole-Brain Analysis

Region	**BA**	***MNI Coordinates***			***t stats***
		x	***y***	***z***	
Native (L1) > Baseline (Both Groups)					$t_{(30)}$
L. Middle Temporal Gyrus	BA 21	-58	-10	-14	13.12
		-52	4	-28	10.43
L. Inferior Temporal /Fusiform Gyrus	BA20/BA37	-38	-12	-34	11.63
L. Posterior Cingulate Gyrus	BA31	4	-42	10	9.65
L. Thalamus		-14	-28	-6	13.27
R. Middle Temporal Gyrus	BA21	50	4	-28	11.63
R. Superior Temporal Gyrus	BA22	60	-8	-10	11.42
R. Middle Cingulate Cortex	BA 24	10	-4	28	9.13
R. Posterior Cingulate Gyrus	BA23/31	10	-32	22	8.97
R. Putamen/R. Pallidum		14	6	-8	15.02
R. Caudate/Insula	/BA13	24	-12	24	8.62
R. Cerebellum		6	-50	-30	11.4

Table 9. Description: L. left. R. right; BA: Brodmann's Area. MNI coordinates determined by the SPM 12 anatomical toolbox $p < 0.05$ (FWE corrected). Voxel extent threshold of 8 consecutive voxels.

2.2. ROI Analysis

2.2.1. Core Language Areas

Table 10 gives a list of the core language brain regions, with the MNI coordinates (taken from Fedorenko et al., 2010) for which two-sample t-tests were performed. Figure 11 shows the average beta values for each ROI for the multilinguals versus the monolinguals. As expected, these are very similar.

Table 10. Core Language ROIs

Region	***MNI Coordinates***			***Sphere Size***
	x	***y***	***z***	
Cortical Regions				
Left inferior frontal gyrus (IFG)	-48	16	24	8
Left inferior frontal gyrus Pars orbitalis	-48	33	-4	8
Left middle frontal gyrus (MFG)	-40	-2	53	8
Left middle anterior temporal pole	-55	-18	-13	8
Left middle posterior temporal gyrus (MidPostTemp)	-56	-40	10	8
Left posterior temporal gyrus (MidPostTemp)	-48	-62	15	8
Left Angular Gyrus (AG)	-37	-76	30	8

Table 10. Description: MNI coordinates from the Fedorenko et al., 2010

The t-test analysis in these regions did not yield any significant differences across the groups (see Appendix D for test results). Overall, the comparison between the native languages across groups corroborated with the prediction of the language network framework (Fedorenko & Thompson-schill, 2014), suggesting equal recruitment of language "core" areas across groups. There were no regions in which monolinguals showed greater activation than multilinguals.

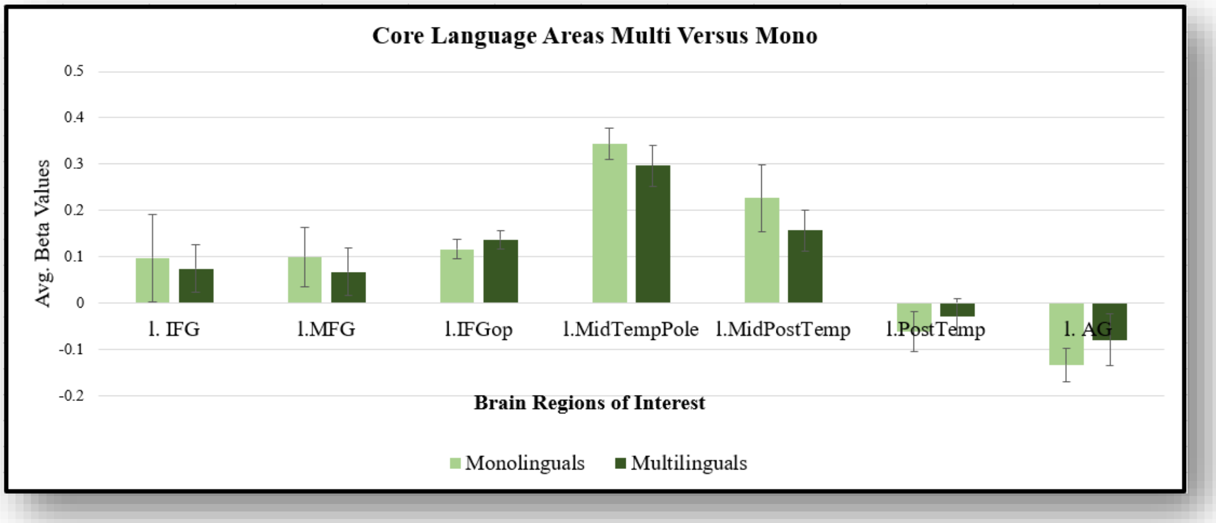

Figure 11. Average Beta-Values in A Priori Defined Core Language ROIs Mono vs Multi

2.2.1.1. Core Language Areas and AoA

A second question related to the activation patterns in language core regions was whether a similar activity will be observed within these regions indifferent of AoA. Figure 12 shows average beta values in these areas for the early L2 (Russian) and the late L3 (English).

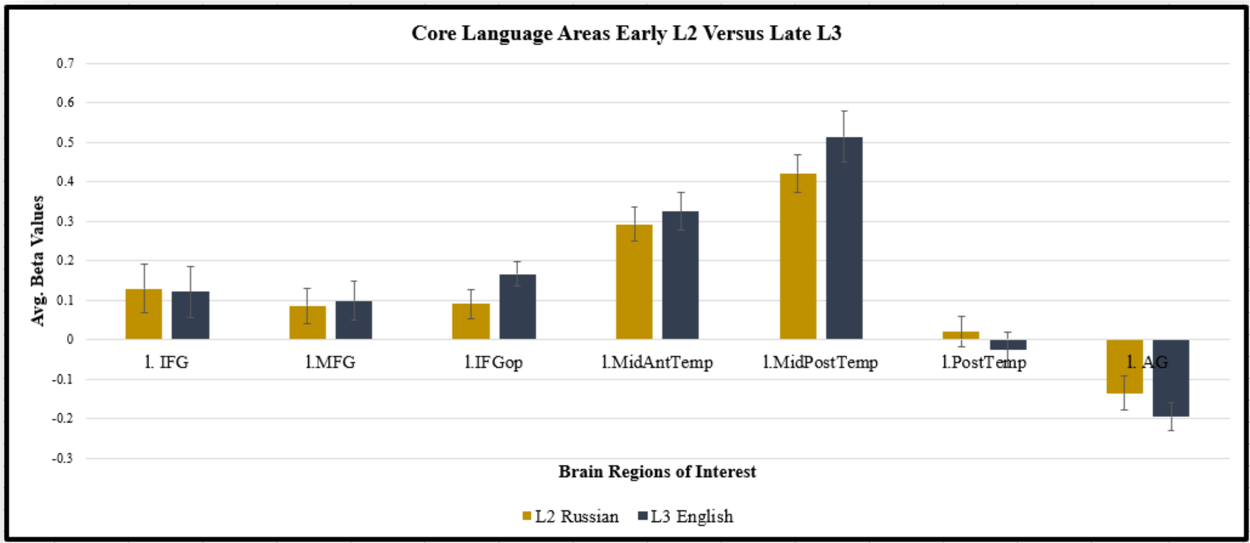

Figure 12. Average Beta-Values in A-Priori Defined Core Language ROIs, L2 vs L3

Two-sample t-test between activation in core regions between the three languages spoken by the multilinguals did not yield any significant differences, suggesting similar overall recruitment of areas specialized for language processing indifferent of age at which the language was learned. These patterns of results are in line with the predictions of a shared neural system that can accommodate all languages learned, once a second language was learned early in life (e.g. Abutalebi, 2008; Perani & Abutalebi, 2005).

In addition, similar to Fedorenko et al., 2010 that have noted a set of regions (including an area in the posterior temporal cortex) to show relative deactivation for the task (these will be succinctly discussed below in a post-hock analysis. These have been shown to be involved in off-task thought processes, and have been attributed to the default mode network (Fedorenko et al., 2013; Sormaz et al., 2018). Noteworthy, in this study, another area that has been proposed as part of the core language regions, namely the angular gyrus, showed the same pattern. The AG, situated at the junction of the temporal, parietal, and occipital lobes, has been described as a heterometal region that has been mostly documented for its role in language and semantic processing (e.g. Binder et al., 2009; Vigneau et al., 2006). More recently, however, it has been shown to be involved in focusing attention on memory when information in memory has to be evaluated internally (Dennis & Cabeza, 2011; Sormaz et al., 2018). Murphy et al., 2018 showed right AG activation in tasks that required decisions on no longer present information and have associated this region with the default mode network.

2.2.2. Periphery Brain Areas

Table 11 shows a list of all the language control ROIs[22] for which two-sample t-tests were performed. Similar activation was observed for both groups, with increase recruitment of areas of language control overlapping with bilingual language control regions (see next section of this chapter for a discussion about bilingual language control). Figure 13 provides the ROI analysis results for native language processing across the groups in the brain areas associated with language control. Note that the figure includes only areas that were statistically significant (for details of results for all tests performed, see Appendix D). Two sample t-tests between the multilingual (n=15) and the monolingual(n=15) group showed significantly higher activation in the right mid/anterior insula (r.insula $t_{(30)} = 3.06$, $p < .005$, (95% CI, -0.23 to 0.04) and bilateral ACC (right vACC, $t_{(30)} = 2.51$, $p < .01$, (95% CI, -0.15 to 0.001); left vACC, $t_{(30)} = 2.29$, $p < .05$, (95% CI, -0.27 to -0.01); right dACC $t_{(30)} = 2.79$, $p < .01$,

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[22] In addition to the coordinates extracted from the Fedorenko et al., 2013, I have used separate ROIs for the ventral versus the dorsal ACC and for the bilateral pre-SMA.

(95% CI, -0.27 to -0.01); left dACC $t_{(30)} = 3.14$, $p < .01$, (95% CI, - 0.15 to -0.03). There were not statistically significant differences in the pre-frontal cortex brain regions involved in control (i.e. the dorsolateral prefrontal cortex (DLPFC) and the pre-supplementary motor area (preSMA)), across groups. Considering the comprehension task used in this study, the lack of differences in these areas is not surprising. Previous studies have attributed the control mechanisms involved in language production to the DLPFC, involved in the retrieval of the relevant lexical candidates in bilinguals (Blanco-Elorrieta & Pylkkänen, 2016). On the other hand, language comprehension in bilinguals relies on recruitment of the ACC for tuning the control system to the linguistic features that enable the discrimination between the languages spoken by the multilinguals, in order to selectively activate the relevant language (Green & Abutalebi, 2013; Krizman et al., 2012).

Table 11. Language Control ROIs

Region	***MNI Coordinates***			***Sphere Size***
	x	***y***	***z***	
Language Periphery Regions				
L. Dorsolateral Prefrontal Cortex	-21	43	-10	8
R. Dorsolateral Prefrontal Cortex	21	43	-10	8
L. Pre-supplementary Motor Area	-1	1	57	8
R. Pre-supplementary Motor Area	1	1	57	8
L. Inferior Parietal Lobe (IPL)	-37	-56	41	8
R. Inferior Parietal Lobe (IPL)	37	-56	41	8
L. Insula	-35	18	-2	10
R. Insula	35	18	-2	10
R. ACC (dorsal)	-4	38	28	8
L. ACC (dorsal)	4	38	28	8
R. ACC (ventral)	-2	46	-6	8
L. ACC (ventral)	2	46	6	8

Table 11. Description: MNI coordinates from the Fedorenko et al., 2013.

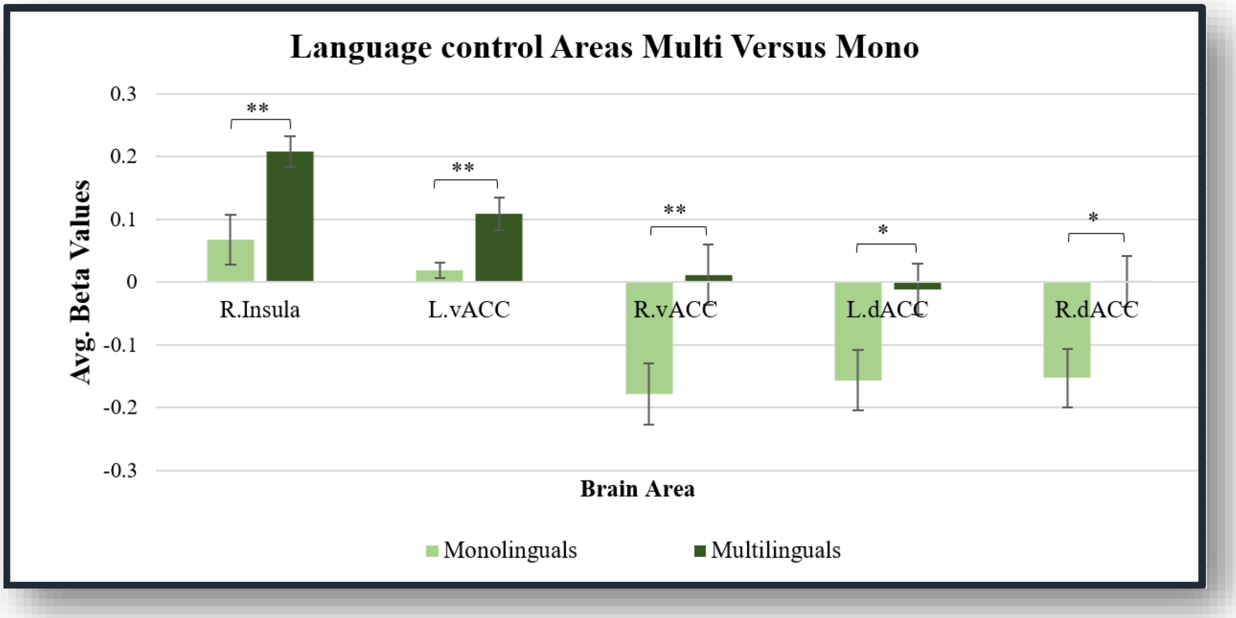

Figure 13. ROI Analysis Results for Language Control Areas Across Groups

2.3. Discussion

Overall, the results of this exploratory analysis for native language processing across groups corroborated with the language network framework (Fedorenko & Thompson-schill, 2014), suggesting equal recruitment of language "core" areas across groups and language control regions. There were no differences observed in the left hemisphere (frontal- inferior pars orbitalis) and temporal regions (Scott et al., 2017) involved in language processing. Table 12 shows a list of all ROIs investigated in this study, whit the comparison between the original studies identifying language core and periphery language areas (Fedorenko et al., 2010, 2012, 2013).

Table 12. List of Core and Periphery Language Regions and Result Comparisons

'Core' Language Brain Regions (Fedorenko et al., 2010)	
Brain Area	*Comparison With the Original Study*
Lateral Surface of the Left Frontal Cortex (lIFG)	similar recruitment
Left Inferior Frontal Gyrus Orbital (lIFGorb)	similar recruitment
Left Middle Frontal Gyrus (lMFG)	similar recruitment
Left Lateral Anterior Temporal Cortex (lAntTemp)	similar recruitment
Left Middle Posterior Temporal Cortex (lMidPostTemp)	similar recruitment
Left Middle Posterior Temporal Cortex (lMidPostTemp)	similar recruitment
Left Posterior Temporal Cortex (lPostTemp)	Relative deactivation
Left Angular Gyrus (lAngG)	Relative deactivation
Language Control 'Periphery' Brain Regions (Fedorenko et al., 2012, 2013)	
Dorsolateral Prefrontal Cortex (DLPFC)	Relative deactivation
Insular Cortex (regions along the precentral gyrus)	Increase recruitment in multilinguals
Posterior inferior Frontal Gyrus (IFG)	Relative deactivation
Pre-supplementary Motor Area (pre-SMA)	Relative deactivation
Supplementary Motor Area (SMA)	Relative deactivation
Parietal Lobule (PL)	Relative deactivation
Anterior Cingulate Cortex (ACC)	Increase recruitment in multilinguals

Multilinguals showed stronger recruitment of regions often involved in language control, referred to as periphery language regions (Fedorenko & Thompson-schill, 2014). These included regions of the cingulo-opercular cognitive control subsystem, such as the right anterior insula and regions of the anterior cingulate cortex.

The role of the insula in language processing has been attributed to the motor aspect of speech production and articulatory control. A meta-analysis of 42 fMRI studies with adults showed right anterior and mid-insula involvement in speech perception (Oh et al., 2014). Generally, activation in the anterior insula has been related to this area's direct anatomical connections to the lateral frontal brain regions (IFG), involved in different aspects of language processing, including semantic, phonological, and syntactic processing (Heim et al., 2009; Zaccarella et al., 2017, Friederici & Gierhan, 2013). Several studies, however, have related the role of the insula to integrative functions during auditory language processing, such as auditory attention allocation, auditory-visual integration, and auditory tunning in the presence of novel stimuli (Bamiou et al., 2007; Bamiou et al., 2004). The results of this study are collaborative of these suggestions, showing activation in the right mid-anterior insula during auditory sentence comprehension.

Most neuroimaging studies reporting activation in the ACC (Wang et al., 2009), and insula (for a review see Oh et al., 2014) are studies of bilinguals that use production and switching tasks. Remarkably, this study shows the involvement of similar brain networks for a passive auditory comprehension paradigm.

The involvement of the ACC in linguistic and non-linguistic activities that require conflict and error monitoring is unequivocal (Abutalebi & Green, 2016; Botvinick et al., 2004; Botvinick et al., 2001; Seo et al., 2018). However, the level of control at which the ACC is involved is still under debate. Some authors suggest its involvement in early selection processes at stimulus encoding, and others show the involvement of the ACC at the level of execution. For example, Seo et al., 2018, investigated the role of the preparatory processes implicated in language control and also the processes that are involved in global language selection versus the selection of words and morpho-syntactic rules concluded that the ACC was involved in the language preparation phase of the task, the authors suggest that the ACC may be particularly important for top-down target language preparation (or global language selection). The authors have described activation of selected ROI (namely the ACC, the DLPFC, and the Caudate) during three phases of control in a bilingual task: *preparatory activation* (highest activation during the preparation for a target language), the *execution activation* (highest activation during task execution) and the *stable activation* (consistent activation across all phases of the task). The results indicated that the ACC was more active during the preparatory phase, the DLPFC was more active during the execution phase and the caudate was constantly active during the whole task. The authors propose that the ACC may be involved in detecting conflict at the earliest possible stage and using that information to trigger the fronto-striatal circuit towards biasing language selection and use.

On the other hand, Van Veen et al., 2001, in an event-related fMRI study, investigated the levels of processing (namely at the stimulus encoding, target detection, response selection, or response execution level of processing) at which the ACC is recruited for monitoring of conflict. The authors suggest that the ACC detects competition between processes that conflict during task performance (namely, detection of conflict that occurs at the level of response), and signals to the prefrontal cortex the extent to which attentional control is required during a task. According to the authors, the ACC is not a top-down attentional control mechanism, rather detects conflict during task performance, in other words, the ACC is not responsive at the stimulus encoding (or target detection) level, but rather at the later response-related processing level. Similarly, Botvinick et al., 2001, suggest that the ACC is involved in evaluating the amount of conflict at the level of execution and action planning and signaling the degree to which top-down control is needed.

Consistent with this interpretation our data suggest that the anterior cingulate is involved in tonic ongoing activation during the length of the task. These interpretations are also in line with recent resting-state functional connectivity studies, that group regions of the cognitive control into a fronto-parietal component, that seems to be responsible for initiating and adjusting control settings, while the cingulo-opercular component provides tonic, ongoing control over the length of the task (e.g. Dosenbach et al., 2008). These organization patterns in the control network are consistent with the data in this study.

Lastly, the differences in these previous studies showing evidence of the involvement of the ACC in both the initial preparatory and stable set-maintenance control tasks may be due to different functional specializations within subregions of the ACC (Braver et al., 2001; Wu et al., 2021). In this study, the statistical difference in the dorsal ACC across groups was mainly driven by greater deactivation in this region for the monolinguals versus the multilinguals. Relative deactivation in the dorsal ACC during the task may be related to the specific role of this subregion in preparatory processes, that are not as taxed during tasks that do not require language interference control, selection, and switching. On the other hand, the ventral ACC showed increased activity in the multilinguals (bilaterally), along with relative deactivation in the right hemisphere and slight activation in the left hemisphere for the monolinguals. The involvement of the ventral ACC during the task reflects tonic ongoing activity, that is detectable even during passive language comprehension within this region.

Section II

The brain correlates of passively listening to language (extracting the meaning from the spoken utterances) have been extensively studied in monolinguals (Crinion et al., 2003; Spitsyna et al., 2006) whereas most of the behavioral and functional magnetic resonance imaging data looking at bilinguals come from production or/and studies using switching paradigms (Calabria et al., 2019; Del Maschio & Abutalebi, 2019; Golestani et al., 2006; Indefrey, 2006, for a review on non-linguistic cognitive control tasks, see Pliatsikas & Luk, 2016).

In this section, I investigate functional differences in a group of multilinguals, in brain regions associated with cognitive control, using a passive language task. The questions addressed in this study are based on the adaptive control hypothesis (ACH) framework (Green & Abutalebi, 2013), which predicts the extent to which control mechanisms adapt to the interactional context of the bilinguals and gives a neural account of the areas involved in such processes. Through an ROI approach, differences in activation for L1 versus early L2 versus late L3 are tested. Following, correlation analysis testing the relationship between activation in regions of interest and AoA and proficiency is further performed. Here, I limit my investigation to one region that has most often shown to be involved in language control tasks (e.g. Abutalebi & Clahsen, 2017; Mohades et al., 2014), namely the caudate.

1. Relevant Background Literature

1.2 Language Competition and Inhibition

Studies looking at multilinguals highly proficient in more than two languages are very few. Consequently, while multiple psycholinguistic models try to explain the processes of language control in bilinguals, these are yet to be scaled to speakers of multiple languages. Consider, one of such models, namely the inhibitory control model (ICM; Green, 1986, 1998) that assumes that both languages compete during language selection. These assumptions have been greatly investigated and documented in studies looking at speakers of two languages (e.g. Dijkstra & Kroll, 2005; Gullifer et al., 2013; Kroll et al., 2008; Kroll & Gollan, 2014; Marian & Spivey, 2003). Whether all languages compete during language selection for speakers of more than two languages is not known.

Following, in order to resolve competition and maintained activation in one of the languages, the ICM suggests that the bilingual has to inhibit the inappropriate language. It is poorly understood whether the same mechanisms of language inhibition are recruited in multilinguals. For example, are all languages active to the same extent, are they all inhibited to the same extent? Questions as such are still to be answered in the literature.

A further assumption of the model is that recovery (i.e. reactivation) from previously inhibited languages is modulated by the strength of inhibition, with more dominant[23], usually the native languages requiring more time to recover. Most studies suggest that switching from the L2 into an L1 is more costly than switching in reverse, due to the need to recover from stronger inhibition of the more dominant native language (e.g. Costa & Santesteban, 2004; Macizo et al., 2010, 2012, etc). However, a recent study used a language comprehension task, that examined the effects of language switching in a group of Italian/French bilinguals from Italian families living in Switzerland showed more robust brain activation while switching from the more dominant (L2) into the less exposed (L1) and not vice versa. They reported involvement of language-related interior frontal (BA 45 BA9), parietal (Ba39, Ba7 and temporal regions (Ba 22, Ba 21, Ba 37), along with regions of the cingulo-opercular control subnetwork (including the anterior cingulate (BA32) as well as basal ganglia (head of caudate and putamen), and the thalamus, when bilingual speakers heard

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[23] Language dominance - the relative level of proficiency and use in each of the languages spoken by the bilinguals, with the more proficient/used language being the most dominant.

sentences with inter-sentential switches from dominant L2 into weaker L1. The cortical effects however were not as robustly observed during switches from L1 into L2. While activation of the anterior cingulate cortex (ACC) and subcortical regions were not detected in this direction of the switch (Abutalebi et al., 2007). The authors interpreted the results in relation to previous studies as evidence of a change in language dominance, here L1 was considered the currently less-exposed (a.k.a. less dominant) language.

This is in line with the ICM that proposes that the amount of inhibition that is necessary for a more dominant language (generally, the L1), is greater than for a less dominant language (generally, an L2). Hence, more control mechanisms should be required for both inhibiting the more dominant language and reactivating it after inhibition. Therefore, when a speaker is processing their native language (L1), competition from L2 is weaker, than vice versa (Mosca & de Bot, 2017).

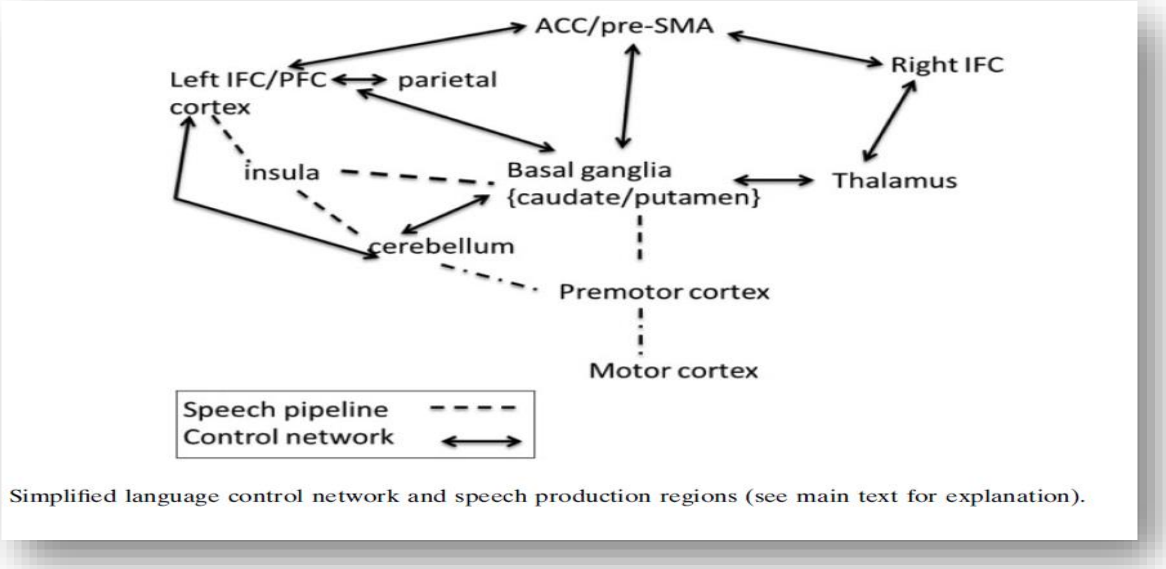
1.3. Factors Influencing Brain Activation Patterns in Bilingual

Neuroimaging research shows that a number of different linguistic background factors influence the degree of brain representation overlap between the languages spoken by a bi/multilingual (for a detailed description see the introductory chapter), these differences should be reflected in brain activation during language tasks. Age of acquisition (AoA), language immersion and exposure, language use, language proficiency, language attrition, and language structure, have all been shown to influence brain activation patterns in bi/multilinguals (Blackburn, 2019; de Bot, 2019). Two of these factors have been mostly investigated in studies of bilingual language control, namely, the age at which a language was learned (AoA), and proficiency in the second language (Blackburn, 2019; de Bot, 2019). Generally, the earlier the age of acquisition (AoA) of a second language, the smaller the differences in brain activation patterns between the native and the second learned language(s) (Cherodath & Singh, 2015; Wattendorf et al., 2014). Similarly, the greater the proficiency in a second language, the more overlap between the brain areas recruited during the processing of the native versus the second languages (Abutalebi et al., 2001, 2013; Videsott et al., 2010).

1.4. The Adaptive Control Hypothesis

Recent resting-state studies identify two subnetworks within the cognitive control network, the fronto-parietal network (including the inferior frontal regions, and the intraparietal sulcus (IPS)) and a cingulo-opercular network (including the cingulate cortex (ACC), the anterior insular cortex (AIC) and the subcortical regions of the basal ganglia (which includes the caudate and putamen) and the thalamus (Dosenbach et al., 2008; Wu et al., 2021). These two systems, working in concert with language-specific brain areas, likely manage inhibitory control as well as language selection, both of which are necessary for the effective management of language (Jenny Crinion et al., 2006; Hervais-adelman et al., 2011).

One of the most comprehensive models describing the neural correlates of the processes involved in bilingual language control is the Adaptive Control Hypothesis (Abutalebi & Green, 2016; Green & Abutalebi, 2013). The model proposes that the language context in which the bilingual is situated affects the adaptation of the brain regions involved in the possible control processes required by each language interactional context. The language context of a bilingual may be a *single* language context, in which one of the languages known by the bilingual is used exclusively in one environment while the other language is used in another environment (with no switching within the same environment), a *dual*-language context in which the bilingual uses their languages with separate speakers, and switching occurs only if a different speaker is encountered (i.e. low switching occurrences), and a dense *code-switching* language contexts in which bilinguals use both languages with the same interlocutor and in the same utterance.



Simplified language control network and speech production regions (see main text for explanation).

Figure 14. Simplified Language Control Network and Speech Production Regions

Figure 14. Description: Reprinted from Journal of Cognitive Psychology, Vol 25., David W. Green & Jubin Abutalebi, Language control in bilinguals: The adaptive control hypothesis., Pages 515-530., Copyright (2013)., Open Access.

The ACH proposes eight control processes that are taxed differently depending on the interactional context. They include a *goal maintenance* process (e.g., the goal of speaking in one language), that requires two additional *interference control* top-down processes (conflict monitoring and interference suppression). Another controlling process is *salient cue detection* (e.g. detecting the need to change a task goal when a new interlocutor arrives, or the context changes), which may be necessary for *selective response inhibition* (e.g. switching the task goal (*task disengagement*) to a more appropriate goal (*task engagement*). *Opportunistic planning* is the last control process in the model, and it refers to using whatever comes most readily available in a bilingual context, to ensure the communication goal. Based on its architecture (see Figure 14), the ACH makes certain predictions related to the neural adaptation that should be observed on specific brain regions, based on the interactional context of the bilingual. The *single language* context imposes demands on subregions of the cingulo-opercular network, namely the ACC/pre-SMA related to the processes of control monitoring, and subregions of the fronto-parietal network, including the inferior frontal and prefrontal cortex (felt IFC/PFC) involved in interference suppression, as well as the inferior parietal lobule, (IPL) involved in goal maintenance. A *dual-language* context relies on the fronto-parietal network, but also the subcortical regions of the cingulo-opercular network, namely the basal ganglia regions involved in switching (specifically selective response inhibition, task engagement, and task disengagement), and the thalamus/right IFC circuit is involved in salient cue detection. A *dense code-switching language* context is predictive of changes in the connectivity of the left inferior frontal regions and the right cerebellum (both part of the cingulo-opercular network), involved in processes of opportunistic planning (see Figure 14 for a schematic architecture of the model). A list of the specific areas related to language control identified[24] by the ACH is provided in Table 13. Note that table one divides the regions involved in control within the recently proposed subnetworks of control, however, these were not originally stated as such in the ICH model. These will be used to define the regions of interest in the statistical analysis. Appendix E provides the list of ROIs used in the statistical analysis with the MNI coordinates.

Table 13. List of Cognitive Control Language Regions

Regions of the Cognitive Control Network
Fronto-Parietal Components
Dorsolateral Prefrontal Cortex (DLPFC)/Inferior Frontal Cortex
Pre-supplementary Motor Area (pre-SMA)
Parietal Lobule (PL)
Cingulo-Opercular Components
Anterior Cingulate Cortex (ACC)/pre-supplementary Motor Area (SMA)
Basal Ganglia (including the Globus Pallidus, Caudate/Putamen)
Right Cerebellum /Frontal Cortex

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[24] The ACH does not include/specify the globus pallidus within the basal ganglia control regions. Based on previous literature of structural differences in bilinguals compared to monolinguals (e.g. Pliatsikas 2020), I selected this area as a region of interest.

There are two important aspects of the model architecture that should be considered in relation to this study. One is that while the interactional contexts described by the ACH may be characteristic for most bilingual populations, the language contexts of a multilingual may not be a unique specific international context. In most cases, the multilinguals may find themselves in a mixture of these contexts. For example, the multilinguals in this study are speakers of four or more languages. All participants use the native language at home, with some family members, and interchangeably the native language and the later learned L2 (English) with other family members. They also use the later learned L2 (English) in other social contexts, such as school, work, etc. They may additionally use a third or fourth language with friends, or specific work environments (namely the earlier learned L2s), requiring some switching and a mixture of two or three of these languages with friends that come from the same background. Consequently, the demands for each of these contexts may vary greatly for multilinguals compared to bilinguals. In a single language context, a speaker of three or more languages may need to control for interference from more than one language, in a dual-switching context where the bilingual speaker does not need to maintain a task goal, a multilingual, whose addressee may only speak few but not all of the languages spoken by the multilingual, may still need to have to maintain the task goal by controlling for interference from the languages not needed in the specific environment. Similarly, the multilingual may not take advantage of the opportunistic planning to the same extent, needing to only select the appropriate information for the languages shared by the interlocutors - whatever comes most readily available may not be most appropriate. Therefore, specific predictions as related to modulations resulting from ideal interactional contexts are challenging for this population. While the interactional context may be more fixed for bilinguals, resulting in specific brain changes, due to the complex interactional contexts of the multilinguals, adaptations in all areas of control should be expected in this population.

Second, due to the overwhelmingly larger literature on bilingual language production and switching, the ACH is built to explain control processes involved in language production.

Whether the same underlying brain regions are recruited during control in production or comprehension is not specified by the authors. Some scholars argue that the processes involved in language control during production are not the same as during comprehension. For example, Blanco-Elorrieta & Pylkkänen, 2016, using MEG, investigated the difference between control networks during language production and comprehension in Arabic/English bilinguals. Using a playing-card task, which required participants to make and detect numbers and color and suit judgments. This study reported a dissociation between language control mechanisms in production and comprehension, localizing the switch effect in language production in the fronto-parietal network, specifically, the dorsolateral prefrontal cortex DLPFC, and incomprehension in the cingulo-opercular network, namely, the left ACC. They suggested that in incomprehension, bilingual individuals' attention may focus on tuning the system to detect critical features that discriminate one language from another (Green & Abutalebi, 2013; Krizman et al., 2012; Kuipers & Thierry, 2010) and on monitoring relative language activation. Whereas, in production, individuals may require a biasing mechanism that enables the retrieval of the target representation among other irrelevant candidates. While the presence of differential cortical and subcortical neural circuits has been observed during bilingual language use, prominent questions remain concerning the functional role and temporal properties of these regions and the extent to which these circuits are engaged during language comprehension (Abutalebi et al., 2007; Blanco-Elorrieta & Pylkkänen, 2016).

2. Predictions

First, a main question of interest is whether we observe the involvement of these control regions in the present passive listening task, given that these are mostly predicated on switching and production studies.

Considering the MRI task used in this study (see methods section) I expect increased activation in language control areas related to monitoring and interference control (namely the ACC/pre-SMA), goal maintenance (namely, the IPL), and salient cue detection (the circuit involving the inferior frontal cortex and the thalamus). Smaller differences in brain regions involved in language switching and task engagement and disengagement (namely, subcortical regions of the basal ganglia (i.e. the Caudate, Putamen, Globus Pallidus)) are expected since the task does not require participants to switch within language trials. Additionally, since the participants are only hearing one language at a time, the right cerebellum and right inferior cortex, involved in opportunistic planning processes, are not expected to show activation differences across languages. In the same vein, activation in the DLPFC is not expected for this task.

Four factors may influence the recruitment of cognitive control regions during the task. One of these factors is the age of acquisition of the language. If AoA modulated language activation in control brain areas, stronger activation will be observed for the late L3 (learned at a mean age of 15.2 years old, see Chapter I for details) versus the early L2 (learned at a mean age of 5.8 years old), and L1, as well as stronger activation in the early second language (L2) versus the L1 (de Bot, 2019; DeLuca et al., 2019; Marian et al., 2007; Blackburn, 2019; Segalowitz & Hulstijn, 2005) (see Table 14).

A second factor that may modulate brain activation is language dominance. The participants in this study showed equal proficiency in both second languages, which were very close to proficiency in the native language. They also reported living in an English (L3) speaking country for more than ten years before the scanning. During this time, the early L2 (Russian) was only used on occasions. The native language and the L3 were both used to the same extent. If dominance modulates activity in brain regions related to control, similar recruitment should be observed for the L3 and L1, and L2 (less used, less dominant) should show stronger activation (e.g. Abutalebi et al., 2007) (see Table 14).

The last factor that we investigate in this analysis is language proficiency. Since similar proficiency was shown by the participants in both second languages, with slightly higher proficiency in the native language, if language proficiency modulated brain activity, equal recruitment of control brain regions should be observed for the L2 and L3 and stronger activation in these should be noted compared to the more proficient L1 (Abutalebi et al., 2001, 2013; Videsott et al., 2010) (see Table 14).

Table 14. Predictions Based on Previous Literature

Brain Region	***Comparison***		
	L1 versus L2	*L1 versus L3*	*L2 versus L3*
	Language Age of Acquisition		
ACC, IPL, IFG/Thalamus	Stronger activation in the L2 > L1	Stronger activation in the L3 > L1	Stronger activation in the L3 > L2
Caudate, Putamen, Globus Pallidus, DLPFC, r.IFG/r.Cerebellum	Similar activation across all languages	Similar activation across all languages	Similar activation across all languages
	Language Dominance		
ACC, IPL, IFG/Thalamus	Stronger activation in the L2 > L1	Equal activation in the L3 = L1	Stronger activation in the L2 > L3
Caudate, Putamen, Globus Pallidus, DLPFC, r.IFG/r.Cerebellum	Similar activation across all languages	Similar activation across all languages	Similar activation across all languages
	Language Proficiency		
ACC, IPL, IFG/Thalamus	Stronger activation in the L2 > L1	Stronger activation in the L3 > L1	Equal activation in the L3 = L2
Caudate, Putamen, Globus Pallidus, DLPFC, r.IFG/r.Cerebellum	Similar activation across all languages	Similar activation across all languages	Similar activation across all languages

3. Results:

3.1. Native L1 (Romanian) versus early L2 (Russian)

Paired sample t tests results for a comparison between the native L1(Romanian) and the early L2 (Russian) showed significant stronger activation for the native language (see Figure 15), in the right ventral ACC($t_{(15)} = 2.2$, $p < .05$, (95% CI, 0.01 to 0.3), bilateral dorsal ACC (left dACC, $t_{(15)} = 3.1$, $p < .01$, (95% CI, 0.052 to 0.29); right dACC, $t_{(15)} = 4.4$, $p < .001$, (95% CI, 0.09 to 0.26), bilateral caudate head (left CNh, $t_{(15)} = 2.3$, $p < .05$, (95% CI, 0.004 to 0.1); right CNh, $t_{(15)} = 2.2$, $p < .05$, (95% CI, 0.001 to 0.1)), and body (left CNb, $t_{(15)} = 3.1$, $p < .01$, (95% CI, 0.03 to 0.1); right CNb, $t_{(15)} = 3.7$, $p < .005$, (95% CI, 0.04 to 0.2)), putamen (left putamen, $t_{(15)} = 4.3$, $p < .001$, (95% CI, 0.03 to 0.1); right putamen, $t_{(15)} = 3$, p < .01, (95% CI, 0.01 to 0.09)), and the globus pallidus ((left GP, $t_{(15)}$ = 3.2, p < .01, (95% CI, 0.04 to 0.2); right GP, $t_{(15)}$ = 4.1, p < .001, (95% CI, 0.03 to 0.1)), as well as left anterior thalamus ($t_{(15)}$ = 3, p < .01, (95% CI, 0.03 to 0.2)), Additional differences (see Figure 17) were observed in the left cerebellum (left Cer Crus II , $t_{(15)}$ =2.65, p < .05, (95% CI, -0.02 to 0.2); left Cer VIIb, $t_{(15)}$ = 3.1, p < .01, (95% CI, 0.3 to 0.1); left Cer VIII, $t_{(15)}$ = 3, p < .01, (95% CI, 0.2 to 0.1)).

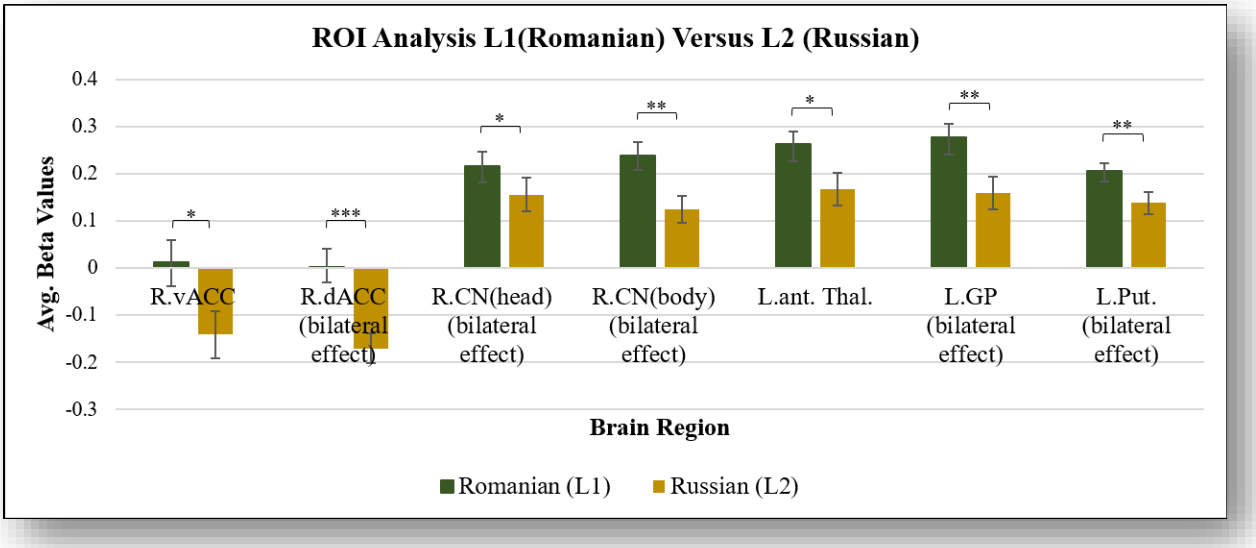

Figure 15. ROI Analysis L1 (Romanian) vs L2 (Russian)

3.2. Late L3 (English) versus native L1 (Romanian)

There were no regions in which processing the late L3 (English) versus the native L1 (Romanian) showed significantly greater activation. Overall, activation was similar across L3 and L1.

3.3. Late L3 (English) versus Early L2 (Russian)

Figure 16 shows the region of interest analysis results for a comparison between the early L2 (Russian) and the late L3 (English), paired sample t tests showed significant differences in the left ventral ACC ($t_{(15)}$ = 2.1, p < .05, (95% CI, -1.4 to 0)), right anterior and posterior

thalamus (anterior thalamus, $t_{(15)} = 2.2$, $p < .05$, (95% CI, 0.002 to 0.1); posterior thalamus $t_{(15)} = 2.7$, $p < .05$, (95% CI, 0.02 to 0.2)), as well as bilateral caudate body (left CNb, $t_{(15)} = 2.13$, $p < .05$, (95% CI, -0.2 to 0.002); right CNb, $t_{(15)} = 2.5$, $p < .05$, (95% CI, -0.2 to -0.02)), globus pallidus(left GP, $t_{(15)} = 2.2$, $p < .05$, (95% CI, -0.2 to -0.001); right GP, $t_{(15)} = 3.1$, $p < .01$, (95% CI, -0.2 to -0.03)), and putamen(left putamen, $t_{(15)} = 2.2$, $p < .05$, (95% CI, 0.001 to 0.1); right putamen, $t_{(15)} = 2.3$, $p < .05$, (95% CI, 0.004 to 0.2)).
The opposite comparison (L2 > L3) did not yield any significant results.

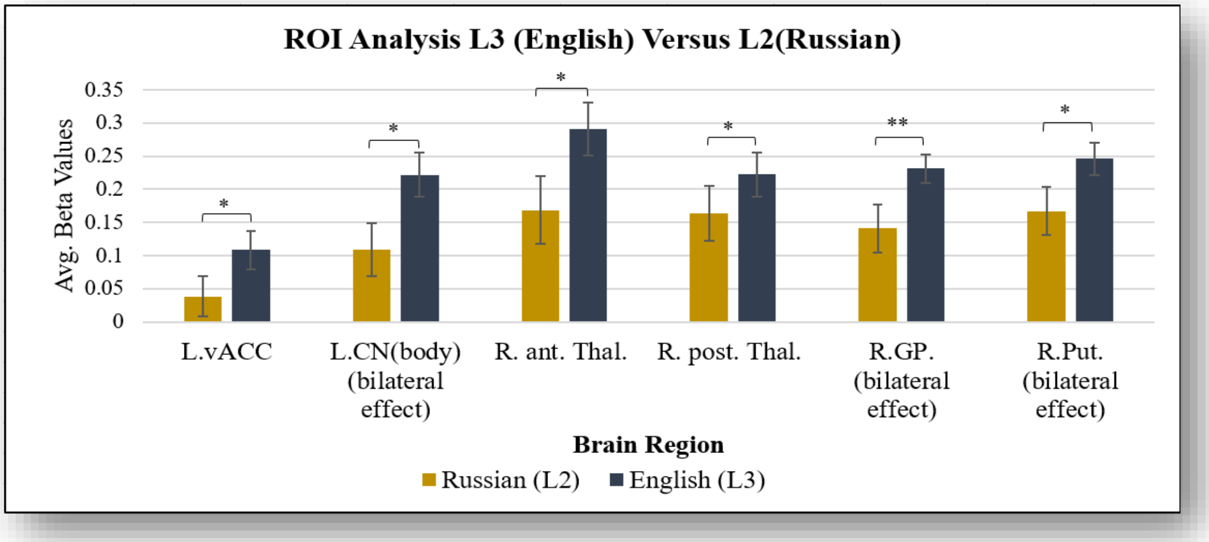

Figure 16. ROI Analysis L3 (English) vs L2 (Russian)

In the cerebellum, the significant differences were observed in the left cerebellum VIIb ($t_{(15)} = 2.4$, $p < .05$, (95% CI, -0.13 to -0.01)), and bilateral cerebellum VII and IX (left Cer VIII, $t_{(15)} = 2.2$, $p < .05$, (95% CI, 0.1 to .001); right Cer VIII, $t_{(15)} = 2.4$, $p < .05$, (95% CI, -0.2 to -0.01); left Cer IX, $t_{(15)} = 2.8$, $p < .01$, (95% CI, 0.02 to 0.1); right Cer IX, $t_{(15)} = 3$, $p < .01$, (95% CI, 0.3 to 0.2)). Figure 17 shows the activation differences across the three languages within the multilingual group in the cerebellum.

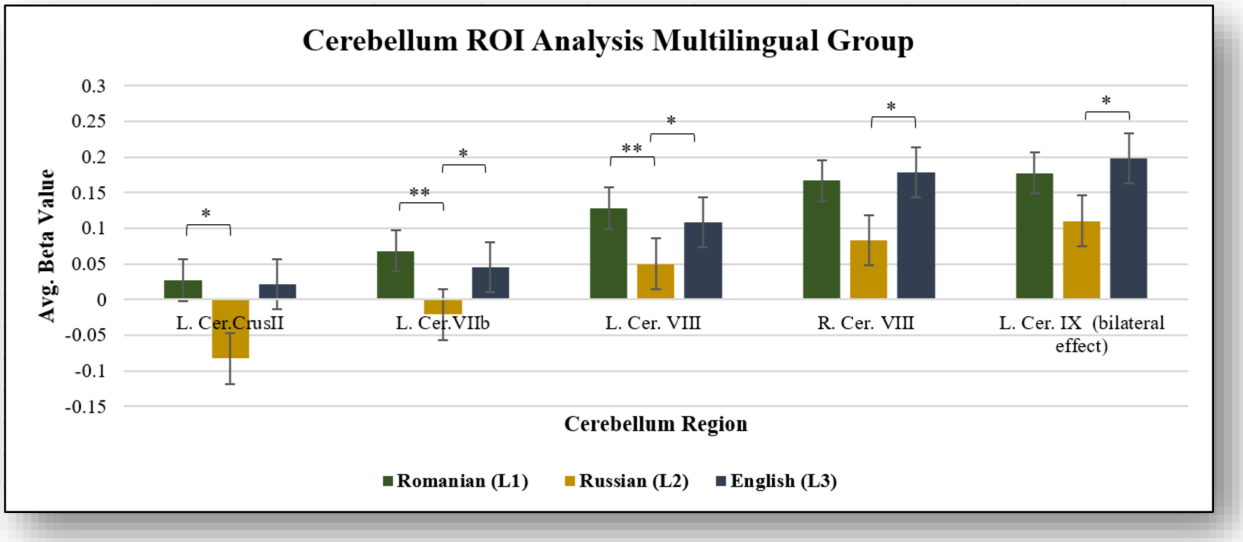

Figure 17. Cerebellum Activation for the Multilinguals Across the Three Languages

4. Discussion:

4.1. Cognitive Control Brain Regions

The first question we addressed in this analysis was whether language comprehension recruits similar brain regions involved in language control. Surprisingly, stronger recruitment in the brain regions identified by the ACH for language production was also recruited for the passive language listening task in this study. Additionally, similar to the observation in Blanco-Elorrieta & Pylkkänen, 2016, the dorsolateral prefrontal cortex, although shown to be bilaterally involved in language production in bilinguals (e.g. Pang, 2012), did not show a significant activation during the task. On the other hand, the anterior cingulate cortex showed greater activation in the native language compared to the early L2 (Russian), as well as in the comparison between the later learned L3 versus the early L2. Overall, these patterns of results suggest that although similar brain regions are recruited for bilingual language control, they are not entirely overlapping in production and comprehension. Namely, while the dorsal inferior frontal regions are involved in control during language production, they do not show increases in activation during passive language comprehension.

Contrary to the ACH predictions of the inferior parietal lobe's involvement in goal maintenance (keep speaking in one of the languages), this region did not show increased activation across languages. On the other hand, an unexpected difference in inactivation of the cerebellum was observed across languages. The ACH proposes a role of the right cerebellum in opportunistic planning, the process of using what is most readily available when there is no need to speak in one of the languages spoken, rather any of the languages can be interchangeably used. Here, stronger recruitment of the left cerebellum was observed for the processing of the L1 native versus the early L2 (Russian), and bilateral cerebellum involvement for processing the later learned L3 (English) versus the early L2 (Russian) when processes of opportunistic planning were not part of the task.

The cerebellum function in bilingual language control is yet to be understood (Calabria et al., 2018, 2019; Cattaneo et al., 2015), however numerous studies have demonstrated its involvement in language processing tasks (De Smet et al., 2013; Fabbro, 2001b; Pliatsikas et al., 2014; Ullman, 2004). Also, cerebellar damage has been associated with a wide range of language impairments, including agrammatism, cerebellar-induced aphasia (with symptoms at the level of speech production, comprehension, naming, repetition, reading, and writing), alexia, dyslexia, agraphia, etc. (for a review, see De Smet et al., 2012). The cerebellum is linked to all the key regions of the language control network, including the striatum, the basal ganglia, the thalamus, and the IFC (Cattaneo et al., 2015). Not surprisingly, language processing research shows its involvement in different aspects of language processing, including verbal fluency and lexical retrieval (e.g. Pliatsikas et al., 2014), syntax (e.g. Strelnikov et al., 2006), and high-level linguistic processing (Murdoch & Whelan, 2007), either in language production or language comprehension and inner speech.

Greater activation in the different lobules of the posterior cerebellum for the processing of the L1 and L3 versus L2 may be related to two aspects of the task. First, the participants had to decode the morphological and syntactic information from the sentences they have heard. Structural MRI studies show a significant correlation between verbal fluency and GM density in the bilateral inferior cerebellum (Alice Grogan et al., 2009). For example, Pliatsikas, Johnstone, and Marinis, 2014 showed increased cerebellum grey matter (GM) volume for the bilinguals versus monolinguals, in a study that used voxel-based morphometry to look at language processing in Greek- English bilinguals. The results showed a positive correlation between the cerebellar GM volume and the speed of processing morphological complex forms in an L2, thus, suggesting this structure's involvement in L2 morphological processing and processing of grammatical rules in a second language (Pliatsikas et al., 2014).

Second, the task asked participants to pay attention as they will be asked at the end of the trial to identify whether the sentences in the test trial were familiar or unfamiliar. Participants might have silently rehearsed or tried to maintain in working memory the trial sentence, for a better recall after the trial. The cerebellum has been shown to be involved in tasks that require inner speech (e.g. Zago et al., 2001 suggest right cerebellum involvement in the verbal association between an arithmetic fact and its appropriate response; Marvel & Desmond, 2010a, 2010b; Tyson et al., 2014). Strick et al., 2009, propose that the involvement of the cerebellum in inner speech may be particularly important in representing, organizing task-related information, and maintaining conscious thoughts. The authors highlight the role of the cerebellar lobule VII (medial and lateral) in the maintenance and manipulation of information in verbal working memory. During the task for the present study, participants needed to maintain the representation of the sentences they have heard to map it to their intended meaning, in the appropriate language. Considering the variability in the data reporting topographically different results, related to language tasks, it is possible that different areas of the cerebellum are involved in specific aspects of the language processing tasks and further evidence is needed in order to appropriately interpret the specifics of the cerebellar involvement in language control.

In addition, inconsistent with the predictions of lower activation in regions proposed by the ACH to be involved in switching (i.e. basal ganglia regions) stronger recruitment of the subcortical basal ganglia regions were observed for the native L1 versus the early L2, as well as for the later learned L3 compared to the early L2. The ACH suggests the implication of the basal ganglia in selective response inhibition processes. A specific role is proposed for the caudate, of involvement in language switching, in processes of task engagement, and task disengagement (Green & Abutalebi, 2013). Surprisingly, in this study, greater activation in the caudate, along with putamen and globus pallidus, was observed even in the absence of switching.

While the role of the basal ganglia regions (specifically the caudate) have been attributed to language switching in bilingual literature (e.g. Crinion et al., 2006; Haber, 2016), the specific role of these regions in language control is not well understood. Ali et al., 2010, examined the neural correlation of Stroop and Simon tasks in fMRI and concluded that the head of the caudate is specifically involved in interference suppression at the level of action plans. On the other hand, the putamen was active at a change in response in both tasks and the globus pallidus in the control of both. Similarly, Hervais-Adelman et al., 2014, investigated the neural correlates of multilingual language control during extremely demanding language control tasks (i.e. simultaneous interpreting) versus simultaneous repetition (shadowing). They have identified the left caudate nucleus to engage in high-level monitoring and selection of the "language set" with its lexico-semantic system appropriate in the interactional context. In contrast, they propose the lower-level inhibiting of the nontarget language motor-representation to the putamen. The present results demonstrate activation in the basal ganglia regions in the absence of switching, suggesting a more general role of these nuclei in tonic ongoing activity. In line with this, the results suggest that passive language listening in different languages seems to differentially engage the cingulo-opercular subsystem of the cognitive network (including the cingulate cortex (ACC), the subcortical regions of the basal ganglia, the thalamus, and cerebellum (Dosenbach et al., 2008; Sadaghiani & D'Esposito, 2015; Wu et al., 2021)). None of the areas that have been identified to be part of the fronto-parietal subsystem (including the inferior frontal cortex, and the inferior parietal sulcus) showed significant activation across languages. Robust activation was observed in the regions of the cingulate cortex, the cerebellum, and subcortical areas (namely, the putamen, caudate, globus pallidus, and the thalamus). The cingulo-opercular network has been shown to be involved in tonic alertness (Coste & Kleinschmidt, 2016; Fox et al., 2006; Sadaghiani & D'Esposito, 2015), while the fronto-parietal network is described as "phasic alertness" acting on an event-by-event basis (Sadaghiani & D'Esposito, 2015). Consistent with this characterization, the resulting activation in this study's task reflects tonic, ongoing activation related to maintaining alertness during task processing. Recall the blocked structure of this experiment, where subjects were instructed as to the language that was to be presented and listened to a specific block of language for 20 seconds.

4.2. Factors Influencing Language Control Mechanisms in Multilinguals

The second set of hypotheses were whether activation in areas involved in language control is modulated by AoA, language dominance, and proficiency.

If AoA modulates recruitment of control areas, activation was expected to increase with an increase in AoA. Hence L3 processing should have shown the strongest activation, followed by lower activation in the early L2. The least activation should have been noted in the native language (see Table 14).

Contrary to these predictions, the strongest recruitment of control regions was observed for the native language (the recruited areas included right dorsal and bilateral ventral anterior cingulate, bilateral head and body of the caudate nucleus, bilateral globus pallidus, left anterior thalamus, and left cerebellum). Similar activation was observed for a difference between the later learned L3 versus the early L2 (stronger activation was observed in the left ventral anterior cingulate cortex, bilateral caudate head, putamen, globus pallidus, right thalamus, and right cerebellum VII, and bilateral inferior cerebellum (IX)). Overall, age of acquisition did not show to modulate the recruitment of control regions.

Additionally, predictions of language proficiency effects on activation patterns were inconsistent with the results of this study. While similar proficiency was exhibited for both second languages spoken by the multilinguals, activation across these languages was lower for the early L2 (Russian) compared to the late L3 (English).

I further investigated the effect of language dominance on control processes. Language dominance seems to affect activation patterns in multilingual. The results showed equal recruitment of control regions for the two most dominant languages (L1 and L3). This interpretation is corroborated by the lack of significant differences in any of the ROIs across these two languages. However, contrary to predictions of stronger recruitment of control processes for the less dominant languages, lower activation was observed in the early, less dominant L2. One possible reason for such patterns of results may be a difference in the level of activation/inhibition across the second learned languages for speakers of more than two languages. Evidence from bilingual research suggests that both languages are active and compete for output (Dijkstra et al., 2013; Gullifer et al., 2013; Kroll et al., 2008a, 2012; Rossi et al., 2018). The competing occurs at all levels of linguistic processing (Kroll & Gollan, 2014; Marian et al., 2003), independent of language modality (Morford et al., 2011), language family (Thierry & Wu, 2007), or the languages known by the bilinguals (Hoshino & Kroll, 2008). An increase in the recruitment of control mechanisms was observed in the native L1 (Romanian) and the late (equally dominant) L3 (English) compared to the early L2 (Russian), in which participants demonstrated equally high proficiency, but reported limited usage. These patterns of activation suggest that compared to bilinguals, in which both languages seem equally active and competing to control output at all levels, the activation of the multiple languages spoken by multilinguals is differential and so is the need for control while processing one language versus another. The results suggest greater activation of the languages that are more dominant and more used, as well as greater interference and need for control while processing these compared to less dominant, less used languages.

Overall, the results of this study, challenge equal activation and interference from 'all' languages spoken by the multilingual, generally reported in bilinguals.

4.3. Post-hoc Investigations:

In addition to the regions involved in language control, Fedorenko 2010, identified a set of regions that showed deactivation during the task, that has been shown to be generally involved in off-task thought processes, attributed the default mode network (Fedorenko et al., 2013; Sormaz et al., 2018). I further investigated whether a similar pattern was observed during the task in this study. A similar set of regions showed stronger relative deactivation during language processing, in the monolinguals compared to the multilinguals. These included the posterior cingulate cortex, a small section of the posterior temporal cortex, and the precuneus. Noteworthy another area that has been proposed as part of the core language regions, namely the angular gyrus, showed the same pattern (see discussion in the first section of the chapter). For a better appreciation of these regions the second level uncorrected (p < .001) whole-brain maps were overlayed on the mean structural image of the study template. Figure 18 shows the uncorrected (p<.001) activation maps between the groups.

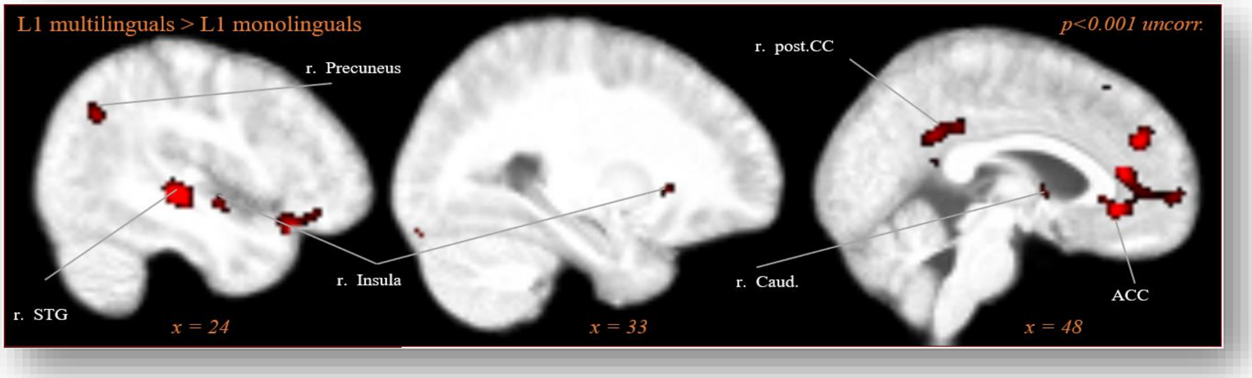

Figure 18. Group Differences for Native Language Processing

Figure 18. Description: The 2nd level uncorrected (p<.001) T-maps, are overlaid on the mean structural image of the study sample (30 subjects) with MRIcron [http://www.sph.sc.edu/comd/rorden/mricron/], including some of the predefined ROIs, for visualization purposes. r: right; l: left; Caud: caudate nucleus; STG: superior temporal gyrus; ACC: anterior cingulate cortex.

Conclusion

This study investigated the neural correlates of language comprehension in a group of multilinguals compared to monolinguals. In the first section of the paper, I discuss an exploratory analysis, looking at the core and periphery language regions predicated on the language network framework (Fedorenko & Thompson-Schill, 2014). I started the discussion by presenting a whole-brain analysis for a comparison of activation during the language task in all 30 participants. The aim of this analysis was to test whether similar brain regions will be recruited in this study's participants, similar to the Scott et al., 2017, after which the task was modeled. Overall similar activation was found; however, there was a lack of recruitment of frontal brain regions (i.e. IFG, IFGorb), compared to the original study.

Following, an ROI analysis was presented. Here, brain representation in multilinguals versus monolinguals was investigated within core and periphery language regions. As expected, similar activation was observed across the groups and within the multilinguals group between languages, in core language regions. Contrary to the generally proposed regions, the angular gyrus, showed deactivation during the task. This is in line with recent resting-state studies associating this brain region to the default mode network. Compared to monolingual multilinguals showed stronger recruitment of regions part of the periphery language regions. These included regions of the cingulo-opercular cognitive control subsystem and included the right anterior insula and regions of the anterior cingulate cortex.

In the second section, I describe an ROI analysis, looking at activation patterns across languages of the multilingual speaker. Here I identify regions of interest, based upon prior studies from the bilingual neuroimaging literature. Predictions were also built based on a language production model of bilingual control, namely the adaptive control hypothesis (ACH; (Abutalebi & Green, 2016; Green & Abutalebi, 2013). A main question of interest is whether we observe the involvement of a similar control region in the present task, which is a passive listening task. I then asked if activation in cognitive control structures is modulated by various multilingual background factors. The analysis focused on the effects of AoA, proficiency, and language dominance. Contrary to predictions of the ACH, proposing involvement of specific brain regions in certain aspects of the task, the results of this study show that during language comprehension, multilinguals involve regions of the cingulo-opercular subnetwork, reflective of tonic activation related to maintaining alertness during task processing.

Additionally, these results contradict popular psycholinguistic model predictions of bilingual language inhibition and control, stating that both/all languages are active and compete for output. Rather, the results show a pattern of graded activation across the multilinguals' languages modulated by language dominance, with the most dominant languages showing stronger interference, and requiring most control during processing.

Chapter III

Brain Grey Matter Restructuring Related to Multilingual Practice

Abstract

A general principle is observed in bilingual studies investigating experience-related microstructural brain changes. Initially, there is an overshoot (i.e. expansion or increased number of connections), considered to be necessary for learning (demand and supply principle), but the actual learning results in a renormalization process, when the newly created structures are maintained and stabilized. These trajectorial changes in brain restructuring have been described in recent models of bilingualism-induced plasticity. This study investigates predictions of the dynamic restructuring model (DRM) model, regarding GM thickness changes associated with high proficiency for multiple languages in a group of multilinguals compared to a group of monolinguals. ROI analysis revealed brain restructuring patterns highly similar to those previously reported in simultaneous interpreters and high proficient bilinguals. Decreases in GM were observed in several brain regions, namely the primary auditory cortex (Heschl's Gyrus), the brain regions involved in semantic processing (the SPL, and the inferior part of the AG in the inferior parietal lobe), along with several areas involved in conflict monitoring and interference control (left insula, and the putamen) as well as the middle and posterior part of the CC, supporting communication between brain hemispheres. Additionally, increased GM thickness was observed in the right ACC for the multilinguals compared to monolinguals, and these were correlated with the amount of time participants were immersed in the L2 speaking environment. Previous neurolinguistic models of multilingualism (the dynamic restructuring model (DRM; Pliatsikas, 2020), and the bilingual anterior to posterior shift (BAPSS; Grundy et al., 2017) have predicted and an inverted U-

shaped trajectory of brain matter changes related to bilingualism; this study provides unique evidence of the 'tail' of its function. Namely, GM tissue shows increases as the bi/multilingual is first exposed and starts gaining proficiency in a second language, however, peak level second language expertise is associated with GM reductions.

1. Introduction

Chapter III provides evidence for the additional recruitment of areas involved in language control (namely, areas involved in the executive control network (ECN) and the salience network (SN) for the multilinguals compared to monolinguals. These activation patterns are indicative of changes in the responsiveness of brain regions related to language processing and control in multilinguals compared to monolinguals. However, they do not provide information on the changes in the brain's structural resources, or capacity. Hence, in this chapter, I describe a study investigating these additional changes within the same multilingual group.

1.1. Background Information

The brain's capacity to restructure for more efficient functionality and connectivity, as a result of a learned skill, has been increasingly researched in the last few decades (Maguire et al., 2003; Park et al., 2018). Accordingly, neurolinguistic studies have reported changes in grey matter associated with bi/multilingual experience. The literature, however, depicts a complicated picture of the effects of learning additional languages on brain structure. Bilingual research undoubtedly suggests that the cognitively demanding task of juggling multiple languages "forces" the brain to restructure in areas recruited for language processing and control. However, the temporality of the expansion and normalization effects due to pruning, or other brain adaptation mechanisms, are yet to be understood. Despite the different techniques (i.e. volume-based morphometry (Ashburner & Friston, 2000) or surface-based methods (Fischl et al., 2008) used, it is important to note that the MRI technique, to date, cannot identify the specific microstructural changes that occur in grey matter, resulting in the observed changes in volume, thickness or surface area. These may be due to a change (i.e. increase or decrease) in the number of synapses, the tissue surrounding the neurons including the astrocytes and the glial cells, as well as changes in dendritic branching, synaptic spines, and capillaries (Lövdén et al., 2013), for a more detailed review, see the discussion in Appendix H. Additionally, the cyclical nature of brain restructuring as a result of language acquisition beyond three languages is yet to be investigated, since evidence from multilinguals is scarce.

Recent neurolinguistic models of bilingualism try to account for the difference in the results across studies, by emphasizing the importance of linguistic experience as a modulating factor. In these models, the general brain restructuring patterns seem to shape a trajectory of increasing GM size at the initial stages of second language learning, followed by a re-normalization process with increasing linguistic experience. The majority of the data on which these models are built stems from studies looking at sequential and simultaneous bilinguals. These include a large number of training studies (e.g. Della Rosa et al., 2013; Richardson et al., 2010) and cross-sectional studies looking at bilinguals (in vast majority sequential bilinguals) with limited exposure to a second language (Golestani, 2014; Klein et al., 2014; Olulade et al., 2016). Consequently, the brain adaptations to the initial stages of language learning are better understood. Although this data is an important source of evidence, it only identifies changes occurring at a certain point in time. Bilingual cross-sectional studies do not allow for inferences about the extent of structural changes over time, and the effect of "re-restructuring" with learning an additional second language. In contrast, the effects of peak efficiency second language expertise are much less understood. Even less is known about the changes that occur when the brain has to re-adapt to a new second language after high proficiency was achieved in a previously learned L2. Although longitudinal and multilingual studies are ideal for investigating restructuring patterns over time at advanced stages of language expertise, they are very rare. Multilingual groups provide a unique platform for testing hypotheses related to the long- and short-term effects of linguistic experience, as well as the processes of additional restructuring associated with a third or fourth L2.

1.2. Evidence of Restructuring at Initial Stages of Language Learning

Starting with Mechelli et al., 2004, which showed increased grey matter[25] density in the left supramarginal gyrus (SMG) correlated with AoA and proficiency for bilinguals versus monolinguals, many other studies consistently reported similar differences in grey matter. Several different regions implicated in language processing and control have been reported to undergo structural changes. These include the inferior frontal gyrus (IFG), inferior parietal lobe (IPL) and the middle (MTG) and superior temporal gyrus (STG) – regions that have been implicated in lexico-semantic and syntactic processing (Hickok & Poeppel, 2007; Hofstetter et al., 2017; Stein et al., 2012), as well as brain areas that have been shown to be involved in language control (Abutalebi & Green, 2016; Luk et al., 2011), including the dorsolateral prefrontal cortex (DLPFC), the anterior cingulate cortex (ACC), the anterior insula, and subcortical regions as the caudate nucleus (CN), thalamus and putamen(Abutalebi & Green, 2016). Lastly, another area that has been implicated in several different functions related to language control and interhemispheric communication is the cerebellum (Li et al., 2020).

Nonetheless, the findings to date remain noisy and seemingly random across studies. Both increases (Becker et al., 2016; A. Grogan et al., 2012; Olulade et al., 2016) and decreases (Elmer et al., 2014; Kaiser et al., 2015) in density and volume have been reported in cortical and subcortical regions. For example, Olulade et al., 2016, reported increased GM volume in the left dorsolateral prefrontal cortex (DLPFC), posterior cingulate gyrus (PCG), middle occipital gyri, and cuneus, as well as right superior frontal gyrus (SFG), frontal operculum, inferior parietal lobe (IPL), bilateral middle frontal gyrus (MFG), inferior frontal gyrus (IFG), and posterior middle and superior temporal gyrus (MTG; STG), for bilinguals compared to monolinguals. The same study also reported reduced GM volume for bilinguals, in the occipital

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[25] Grey matter – high concentrations of the neuronal cell bodies, the parts of the neuron responsible for the collection and the synthesis of information in form of electrical signals.

and parahippocampal regions, bilaterally. Similarly, Felton et al., 2017 - reported greater thickness values in the right ACC for bilinguals versus monolinguals (which they interpreted as plasticity), and the opposite pattern in the left ACC, which they interpreted as bilingualism-induced “maturity” in this area, largely involved in tasks of executive control (Abutalebi et al., 2012). Another study showed decreased GM volume in the ACC, IFG pars triangularis (IFGtr), and Insula for bilingual interpreters, and these were negatively correlated with the number of interpreting hours (Elmer et al., 2014).

There are multiple reasons for the variability of the results such as the method used, anatomical quantification and statistical procedures, participant characteristics, etc. However, a recent theoretical model of brain restructuring (i.e. the dynamic restructuring model; DRM) makes testable predictions of grey matter changes related to bilingualism (Pliatsikas, 2020). The DRM (predicated on the expansion partial renormalization hypothesis (Lövdén et al., 2013) – see Appendix H for a brief description of the hypothesis) tries to explain the available evidence by suggesting that the seemingly random findings in literature follow a specific trajectory and are a reflection of the ‘bilingual experience’. According to this model, an earlier age of second language acquisition will result in fewer changes in the cortical grey matter (including the ACC, IPL, anterior temporal lobe (ATL), IFG, MFG, the Heschl’s gyrus, STL, and IPL) but more pronounced changes in subcortical areas (including the basal ganglia, namely the putamen, caudate and globus pallidus, along with the thalamus), and the cerebellum. On the other hand, sequentially learning a language will result in different adaptation patterns depending on the learning stage at which the bilingual is found. Namely, at initial stages (related to lower proficiency and /or limited exposure), restructuring should be observed in cortical areas; as higher proficiency is gained, or/and language exposure increases, changes in subcortical areas along with anterior white matter adaptations should be observed. Hence, bilinguals that are at a consolidation stage of second language learning should show an increase in grey matter integrity in subcortical areas (namely the putamen, globus pallidus, thalamus, and caudate nucleus) and less differences (a.k.a. normalization) should be observed in the cortex.

Evidence for the earliest stages of brain adaptation in cortical regions comes from longitudinal training studies, in which cohorts of participants are enrolled in intensive second language learning programs. Participants are scanned before and after the training. Osterhout et al., 2008 reported significant increases in the volume of the left SMG, only after a nine-week training course for a group of second language learners. Cortical thickness was also shown to increase in a group of conscript army interpreters at the initial stages of learning a new language (Arabic, Dari, and Russian). A contrast of before and after three months of intensive language learning revealed increases in GM cortical thickness in the MFG, IFG, and STG, as well as volumetric increases in the hippocampus, post-training. The subcortical evidence is more limited but more robust. The areas that show restructuring are generally the basal ganglia (putamen, caudate, and pallidum) and the thalamus. Grogan et al., 2012, was the first study to use voxel-based morphometry (VBM) and show increased volume in the left CN (head) correlated with L2 phonemic fluency in a group of bilinguals compared to monolinguals. Following that, several studies showed volumetric increases in few subcortical regions and the CC (e.g. Burgaleta et al., 2016; Pliatsikas et al., 2017). Interestingly, most of these studies reported changes in the left putamen. For example, Abutalebi et al., 2013 showed volumetric increases in the left putamen of female multilinguals compared to monolinguals. The volumetric changes in this area were correlated with performance on a picture-naming task in the multilingulals’ L3. Similarly, Berken et al., 2016 showed grey matter increases in the left putamen for simultaneous but not sequential bilinguals. This evidence suggests that the putamen may be crucial for language acquisition and the processing of languages later in life, whereas an increase in its size may be related to the need to reorganize and accommodate the increased articulatory demands of a later-acquired language (Abutalebi & Green, 2016; Green & Abutalebi, 2013).

1.3. Factors Associated with Brain Restructuring in Bilinguals

Different factors have been correlated with structural changes in bilinguals/multilinguals. Most of the literature focuses on the effects of AoA. For example, Klein et al., 2014 looked at simultaneous (i.e. L2 learned within 0-3 years of age) versus early (i.e. L2 learned within 4-7 years) versus late bilinguals (i.e. learned L2 within 8-13 years). They reported greater GM thickness in the left IFGop and IFGtr for both groups of sequential bilinguals compared to monolinguals. Additionally, reduced cortical thickness in the right IFGorb was observed in the late sequential bilinguals compared to the other groups, and early bilinguals compared to monolinguals. Interestingly, the AoA was positively correlated with thickness in the right IFGorb and SPL and negatively correlated with thickness in the right IFG, across all bilingual subjects. The authors did not observe any statistical differences between the simultaneous bilinguals and monolinguals, suggesting that the simultaneous acquisition of a second language does not have an additional effect on brain development. However, another study comparing simultaneous versus sequential bilinguals reported a GM density increase in the left putamen and posterior insula, the right DLPFC, mid occipital gyrus, and lateral occipital cortex, for the simultaneous compared to the sequential group. In contrast, sequential bilinguals demonstrated denser gray matter in the bilateral premotor cortex. While the left putamen, cerebellum, IFG, PMC, right primary motor cortex (PMC), and bilateral IPL correlated with speech production proficiency in the sequential bilinguals (Berken, Gracco, et al., 2016). Proficiency in a second language was also shown to correlate with grey matter density in a study comparing sequential trilinguals versus bilinguals. For bilinguals, grey matter density in the left IFG pars opercularis positively correlated with lexical efficiency in L2 (measured by speed and accuracy in a lexical decision task, and the number of words produced in a timed verbal fluency task). Grey matter, in the same region, was negatively associated with AoA. The authors attribute the results in the pars opercularis to the involvement of this area in phonetic expertise. Additionally, increases in grey matter density were noted in the right IPL and right posterior SMG, for sequential trilinguals versus bilinguals (A. Grogan et al., 2012).

1.4. Evidence of Restructuring Beyond One Second Language

Although the DRM is mostly based on available evidence from bilingual studies, the authors suggest that a sequentially acquired third language seems to follow a pattern of restructuring similar to a second L2. Hence, the brain areas that went through a process of re-adaptation while a second language was acquired will undergo a similar restructuring process with the acquisition of an additional language. Thus, the model suggests that these processes are additive in multilinguals. Namely, at the initial stages of learning a second language, effects of re-adaptation will be observed in cortical grey matter (i.e. increases in grey matter volume and thickness). These changes will be followed and replaced by changes in subcortical volume and white matter at future stages of language learning and with increasing experience. When a multilingual learns a third language, the same cycle of processes will take place. Evidence in favor of the cyclical nature of changes comes from studies of multilinguals speakers. Kaiser et al., 2015, compared two groups of trilinguals; one group learned two languages simultaneously early in life, and the second group learned two sequential languages later in life. Compared to the sequential group, simultaneous multilinguals showed lower gray matter volume in the bilateral middle and inferior FG (MFG, IFG), left inferior temporal gyrus (ITG), as well as the right middle temporal gyrus (MTG) and inferior posterior parietal gyrus (IPG). Another study showed that acquiring a third language sequentially after a simultaneously learned L2, compared with two sequentially acquired languages, leads to increases in GM in left IFG and STG, accompanied by increased fractional anisotropy (FA) and decreased medial diffusivity (MD) in the inferior fronto-occipital fascicle (IFOF; Hämäläinen et al., 2018). Additional evidence comes from longitudinal training studies that report a re-normalization of effects after the training program ends. Hosoda et al., 2013 investigated white and grey matter changes in a group of Japanese–English bilinguals, enrolled in a 16 week, L2 training course. The participants were scanned before the start of the program after the training ended, and one year after the training. They reported increases in the right IFGop volume for the training group, between the pre and post-training. However, these increases were reduced a year after the training course ended, and were not different from the values obtained at the first measurement (i.e. pre-training).

1.5. Evidence of Restructuring Beyond Initial Stages

The changes related to expertise in a second language are the least understood in the literature, due to the scarcity of appropriate evidence. The prediction of the DRM for a final peak efficiency stage suggests that the adaptation observed in the previous stages should be starting to fade and be less noticeable, and a shift from the anterior to more posterior adaptations should be noted (for a detailed description of the stages and restructuring associated with these, see the introductory chapter, also (Pliatsikas, 2020)). These changes should be occurring along with increases in white matter (WM) diffusivity anteriorly and reductions posteriorly, accompanied by changes in the cerebellum (in line with the Bilingual Anterior to Posterior and Subcortical Shift (BAPSS; Grundy et al., 2017)). At this stage, anterior cortical and subcortical regions should show full re-normalization.

Literature investigating bilinguals at peak efficiency stages is scarce. It is not known whether the brain keeps adapting as the proficiency of a language increases, even less is known about these changes for multilinguals that have reached peak efficiency in a third or fourth language. The most appropriate evidence of brain adaptations for highly proficient, highly immersed bilinguals comes from studies investigating interpreters who have high proficiency in their L2s and are highly immersed in a dense code-switching environment. Importantly, reductions rather than increases in cortical grey matter (e.g. ACC, IPL, and IFG) have been reported for interpreters versus multilinguals, along with reductions in the white matter of the corpus callosum (CC) and the forceps minor (FM) for the same group (Pliatsikas, 2020). However, it is unknown whether these changes reflect the effects of language proficiency or the increased demands of the act interpreting.

2. This Study

In this study, I investigate specific hypotheses within the DRM framework at the peak efficiency stage and whether these are additive for a thirdly learned second language. Grey matter differences in a group of multilinguals, native speakers of Romanian, and speakers of four or more languages, compared to a group of English monolinguals will be investigated. The participants in our study have acquired a second language (Russian) early in life (mean age 5.5 years) and have been highly immersed in a Russian environment for a mean of 9.5 years. The results of the proficiency test showed high-level proficiency in this early acquired language for the group. Additionally, the participants have learned a third or fourth language in middle/high school, which vary across participants but reported low immersion, varying proficiencies, and no usage of these for at least 12 years before scanning (see Table 2, in Chapter I). Therefore, I did not expect to see GM effects related to these additional languages. Finally, all participants started learning English, as a later acquired language (mean age, 12.5 years), for which they showed high immersion at the time of the study. Their proficiency in English was very similar to the secondly acquired Russian. Based on these characteristics, participants should roughly be at a normalization stage in terms of brain restructuring patterns (specifically, passed the consolidation stage, and toward a peak efficiency stage for both the early and later learned languages DRM). Accordingly, there are a few specific questions that I wanted to address in this study. First, if learning a third or fourth language leads to a “re-restructuring” process, similar brain regions should be undergoing these processes. Hence, I am expecting that the changes associated with the initial stages of learning should be starting to fade, and a shift from the anterior to more posterior adaptations should be noted in our multilingual group, associated with high proficiency in the later learned second language. Second, adaptations similar to the ones observed in simultaneous interpreters (namely a reduced GM thickness), related to peak efficiency stages of learning should be indicative of continuing adaptations as language proficiency increases.

A few regions of interest were selected, in which I investigated differences in cortical thickness. These include regions involved in language processing (sometimes also referred to as "core" language areas (Fedorenko et al., 2010)), namely, the inferior frontal brain regions (IFG), the regions of the temporal lobe, including the middle and superior temporal gyrus (MTG, STG), and the Heschl's gyrus. Based on previous literature and the language network framework, (described in Chapter II), differences in grey matter thickness within language core regions are not expected to differ across groups.

Table 15. Regions of Interest with Grey Matter Changes Predicted

Region	Predictions
Language Processing ('Core') Regions	
L. & R. Inferior Frontal Gyrus L. & R. Interior Frontal Gyrus Op. L. & R. Transverso-temporal (Heschl's) Gyrus L. & R. Middle Temporal Cortex L. & R. Superior Temporal Cortex	Similar grey matter thickness across groups
Periphery Brain Regions	
Cortical Regions ROIs	
L. & R. Inferior Parietal Lobe L. & R. Anterior Insula L.& R. Anterior Cingulate Cortex	Decreases in grey matter thickness for the multilinguals compared to the monolinguals
Subcortical Regions ROIs	
L. & R. Caudate L. & R. Thalamus L. & R. Globus Pallidus L. & R. Putamen Corpus Callosum	Decreases in grey matter volume for the multilinguals compared to the monolinguals
Off-Task Brain Regions	
L. & R. Angular Gyrus L. & R. Supramarginal Gyrus L. & R. Precuneus	Decreases in grey matter thickness for the multilinguals compared to the monolinguals

Table 15. Description: L & R: left and right.

Additionally, I investigate grey matter changes in areas involved in language control, such as the anterior cingulate cortex (ACC), the insula, as well as subcortical areas as the putamen, globus pallidus, thalamus, caudate nucleus, and the corpus callosum. For the control brain regions, I expect to see decreases in grey matter thickness and volume. Lastly, areas that have been shown to be related to off-task brain processes (e.g. see the previous chapter, as well as Fedorenko et al., 2012, 2013) will be included in our analysis, for which decreases in the grey matter are also expected. Here we include the angular (AG) and supramarginal gyri (SMG) in the inferior parietal lobe (IPL), and the superior parietal lobule, (SPL). Table 15 provides a list of the regions to be investigated with the direction of the predictions. However, it is worth noting that such investigations are limited by the parcellations available in the selected atlases provided by the software used.

2.1. Material and Methods

2.1.1. Participants

The structural data were acquired from 30 right-handed participants, 15 native Romanian multilinguals (speakers of four or five languages), and 15 English monolinguals. Participants completed several language proficiencies tests and a background questionnaire. The set of tests used in this study included a *Self-Rating Questionnaire*, a *Language Production and Vocabulary Assessment*, a *Semantic and Vocabulary Knowledge Test*, and a *Grammatical Assessment Test*. For a detailed description of the test and the test results, see Chapter I.

2.1.2. Data Acquisition

All participants were scanned with a Siemens 64-channel 3-Tesla "Skyra" MRI System (Siemens Healthcare, Erlangen, Germany) at the Imaging Research Center of the University of California, Davis. For the structural data, I used a T1-weighted imaging sequence using an MR-RAGE (TfL) sequence with a voxel size = 0.9x0.9x0.9 mm3, FOV = 243 × 243 mm, 208 sagittal slices, 7-degree flip angle, TR = 2500msec, TI = 1100 msec, and TE = 4.44 msec, Bandwith 160 Hz/Px; GRAPPA = 2, 32 reference lines.

2.1.3. Data Processing

MRI structural data preprocessing: The raw MPRAGE images (.dcm) were converted to NIFTI format using the "*dcm2nii"* function obtained from www.nitrc.org. We used a Debian-based Ubuntu distribution to run the different command tools in *bash* (Bourne-Again Shell) command language interpreter. Data preprocessing, image reconstruction, and volumetric and cortical parcellation were carried out with the "*recon-all"* command from the *Freesurfer* image analysis suite, which is freely available software that can be found and downloaded online (http://surfer.nmr.mgh.harvard.edu/). The preprocessing steps performed were non-brain removal, referred to as "*skullstripping",* using a hybrid watershed/surface deformation procedure (Ségonne et al., 2004), automated Talairach transformation and segmentation of the subcortical white matter and deep gray matter volumetric structures (Fischl et al., 2002; Fischl, Salat, et al., 2004). Intensity normalization was performed, as described in Sled et al., 1998. An estimation of the delimitation between white and grey matter and subcortical structures was then performed, followed by automated topology correction (Fischl et al., 2001; Ségonne et al., 2007). From these estimates, the pial surface of the brain is estimated. Surface deformation following intensity gradients was estimated (Dale et al., 1999; Dale & Sereno, 1993; Fischl & Dale, 2000). This procedure produces optimal placement of the border between the gray-white and gray-cerebrospinal fluid where there is a shift in the intensity, defining the transitions to another class of tissue. After the computation of cortical models, deformable procedures are performed, including two steps of surface inflation and flattening (Fischl et al., 1999)and registration to a spherical atlas based on individual cortical folding patterns to match cortical geometry across subjects (Fischl et al., 1999). Cortical parcellations into units based on gyral and sulcal structure are performed, based on two atlases (i.e. Desikan-Killiany atlas, and the Destrieux atlas; (Desikan et al., 2006; Fischl, Salat, et al., 2004). The parcellations from the template are matched back to the individual subjects and adjusted for small variations depending on the individual’s anatomy. These steps output several surface-based maps of curvature and sulcal depth, by using the spatial intensity gradients across tissue classes. Cortical thickness is calculated as the closest distance from the gray-white boundary to the gray-CSF boundary at each vertex on the tessellated surface (Fischl & Dale, 2000). The algorithms are not restricted by voxel resolution (i.e. from the raw original data) and can detect submillimeter differences between groups. Procedures for the measurement of cortical thickness have been validated against histological analysis (Rosas et al., 2002) and manual measurements (Fischl, Van Der Kouwe, et al., 2004; Kuperberg et al., 2003). FreeSurfer morphometric procedures have been demonstrated to show good test-retest reliability across scanner manufacturers and different field strengths (Reuter et al., 2012).

2.1.4. Data Analysis.

FreeSurfer offers a rich set of data outputs that can be further analyzed for comparisons across groups. The outputs include an average number of the metrics calculated (i.e. thickness, area, and volume) for each region in which the brain is segmented and parcellated. This is achieved by averaging the metric values of each voxel across all voxels in a brain region. The assignment of voxels into regions is based on information from the atlas, information based on the location of the voxel compared to other voxels, and the intensity of each voxel for a particular subject. The data on the statistical outputs include volume information of subcortical structures, achieved through subcortical segmentation (‘*aseg*’-automated segmentation) of brain regions based on a probabilistic atlas of 39 subjects (Fischl et al., 2002), and white matter parcellations, achieved through labeling each point of white matter based on its nearest cortical label[26] (Salat et al., 2009). Along with subcortical structures, parcellations of the cortical areas are computed. These are based on two atlases:

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[26] Note that the parcellation of the white matter is not based on the white fiber bundles/tracts.

the *Desikan/Killiany Atlas* (Desikan et al., 2006), which is based on 40 subjects, includes 35 regions for the hemisphere, and it is a measure of the gyri parcellation; the second atlas is the *Destrieux Atlas* (Fischl, Van Der Kouwe, et al., 2004), which is based on 12 subjects and has 58 ROIs per hemisphere. It is also possible to draw and create customized labels for an individual study. Three metrics are provided for each cortical ROI average cortical thickness (the average of the entire region), the total area for the ROI (based on the surface mesh model, in which each triangle is assigned to a brain area), and total volume (which is a measure of thickness multiplied by the area).

For this analysis, the volume information for the ROI was extracted from the subcortical segmentation and white matter parcellation outputs. I restricted the measures of cortical structural changes to the "thickness" measure for the cortical ROIs. Thickness is one of the most sensitive metrics to certain clinical conditions and group differences. It is measured as the distance between white and pial surfaces (1-5mm) (see Figure 19).

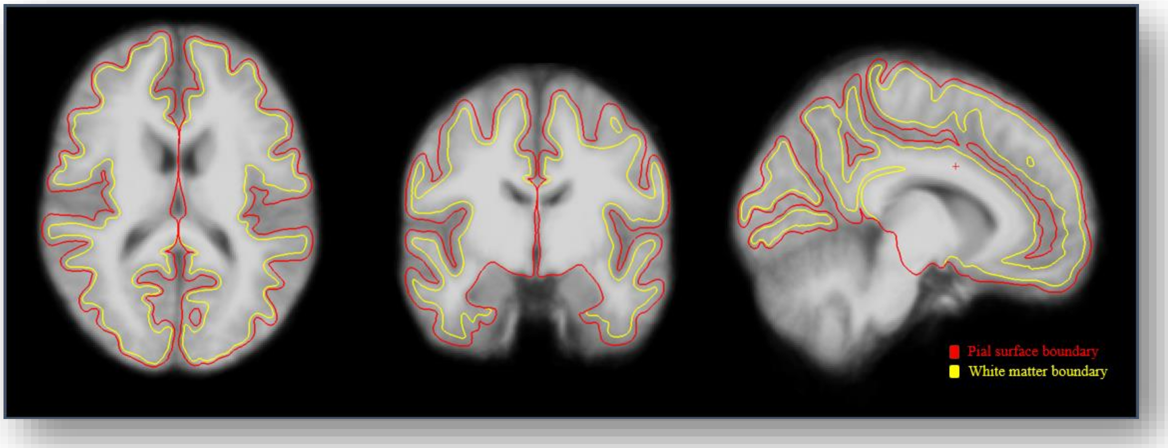

Figure 19. White Matter and Pial Surface Boundaries

Figure 19. Description: The surfaces are overlayed on the study anatomical template volume. Figure created with the FreeView tool in FreeSurfer

The data used for the analysis was provided by parcellations on the *Destrieux Atlas* (Fischl, Van Der Kouwe, et al., 2004). Figures 20 and 21 show the ROIs extracted for the analysis based on the subcortical segmentation and cortical parcellations, overlayed on the study template.

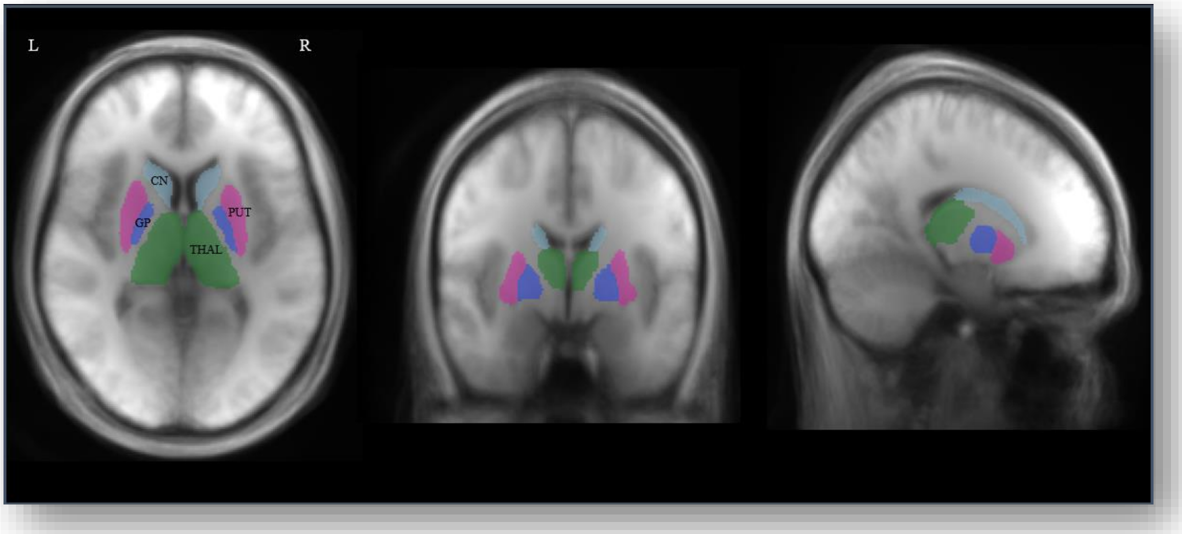

Figure 20. Subcortical ROIs Selected for the Structural Analysis

Figure 20. Description: The labels are overlayed on the study anatomical template volume. Figure created with the FreeView tool in FreeSurfer.

Welch's two-sample t-tests were performed for all areas of interest across the groups. Below, I provide the uncorrected p values, as well as the FDR (false discovery rate), corrected p-values by the number of tests performed.

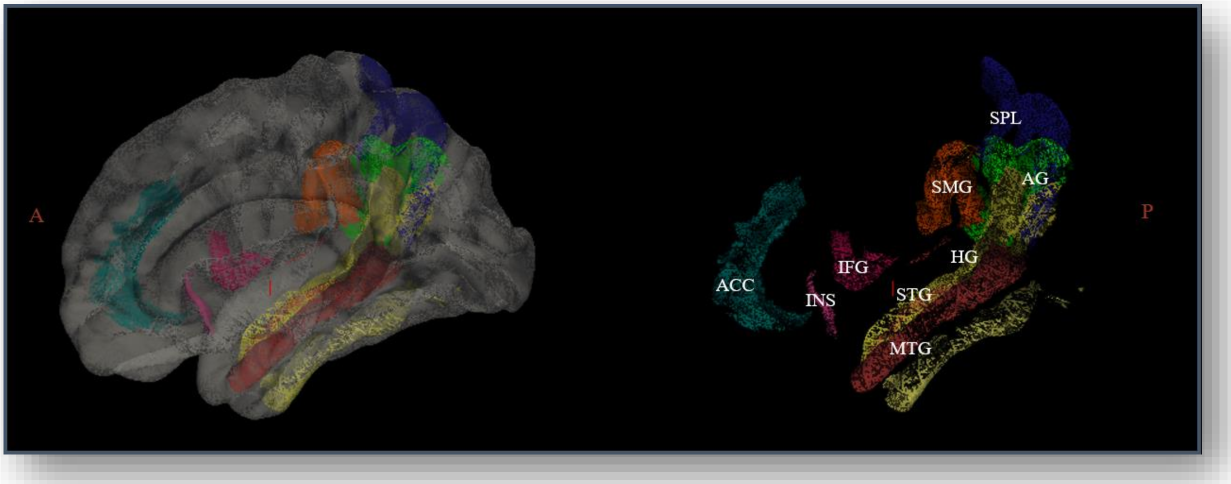

Figure 21. Cortical ROIs Selected for the Structural Analysis

Figure 21. Description: The labels are overlayed on the study anatomical template volume. Figure created with the FreeView tool in FreeSurfer.

2.2. Results

Overall, the multilingual group showed decreased thickness in cortical areas, as well as decreased volume in the subcortical areas (see Table 16) relative to monolinguals. Statistically significant differences within the cortical core language areas (Fedorenko et al., 2012) as shown in Figure 22, were limited to the transverse temporal gyrus, namely the Heschl's gyrus, $t_{(30)} = 2.3$, $p < .05$ (95% CI, 0.006 to 0.34). Additionally, decreases in right hemisphere homologues in the superior temporal sulcus were observed, $t_{(30)} = 2.1$, $p < .05$ (95% CI, 0.0005 to 0.25). These results are in line with previous literature suggesting bilinguals employ the brain more efficiently by distributing effort across brain networks (e.g. the bilingual anterior to posterior and subcortical shift hypothesis (BAPSS; Grundy et al., 2017).

Amongst the regions of interest, part of the cognitive control, decreases in cortical grey matter were observed in the left anterior insula, $t_{(30)} = 2.77$, $p < .01$ (95% CI, 0.0005 to

0.25) and the right anterior cingulate cortex (ACC), $t_{(30)}$ = -2.1, $p < .05$ (95% CI, 0.0005 to 0.25). Interestingly, the right ACC was the only brain region that showed a significant increase in thickness in the multilingual group compared to the monolinguals. Volume differences in subcortical regions (see Figure 23) were limited to the left putamen, which showed a decrease in volume in the multilinguals compared to the monolingual group, $t_{(30)}$ = 2.3, $p < .05$ (95% CI, 34.3 to 766.3), as well as in the white matter volume of the posterior ($t_{(30)}$ = 2.2, $p < .05$ 95% CI, 4.79 to 144.6) and mid-posterior corpus callosum ($t_{(30)}$ = 2.2, $p < .05$ 95% CI, 1042.9 to 916.3).

Lastly, two areas involved in off-task brain processes showed decreases in the multilinguals relative to the monolinguals, the left precuneus of the superior parietal lobe (SPL), $t_{(30)}$ = 3.37, $p < .01$ (95% CI, 0.4 to 0.18) and the left angular gyrus (AG), $t_{(30)}$ = 3.24, $p < .01$ (95% CI, 0.08 to 0.35). See Figure 22 for a histogram of all the ROIs that showed significant statistical differences across groups.

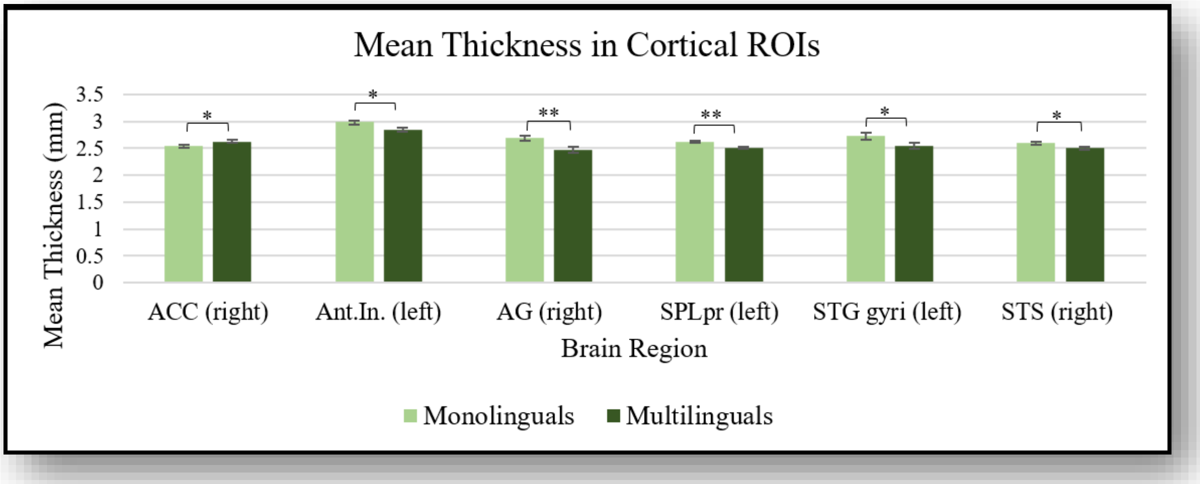

Figure 22. Significant Mean Grey Matter Thickness Cortical ROIs

Figure 22. Description: Results are based on uncorrected p values. ACC: anterior cingulate cortex, Ant.In: anterior insula, AG: angular gyrus, SPLpr: precuneus in the superior temporal lobe, STG: superior temporal gyrus, STS: superior temporal sulcus.

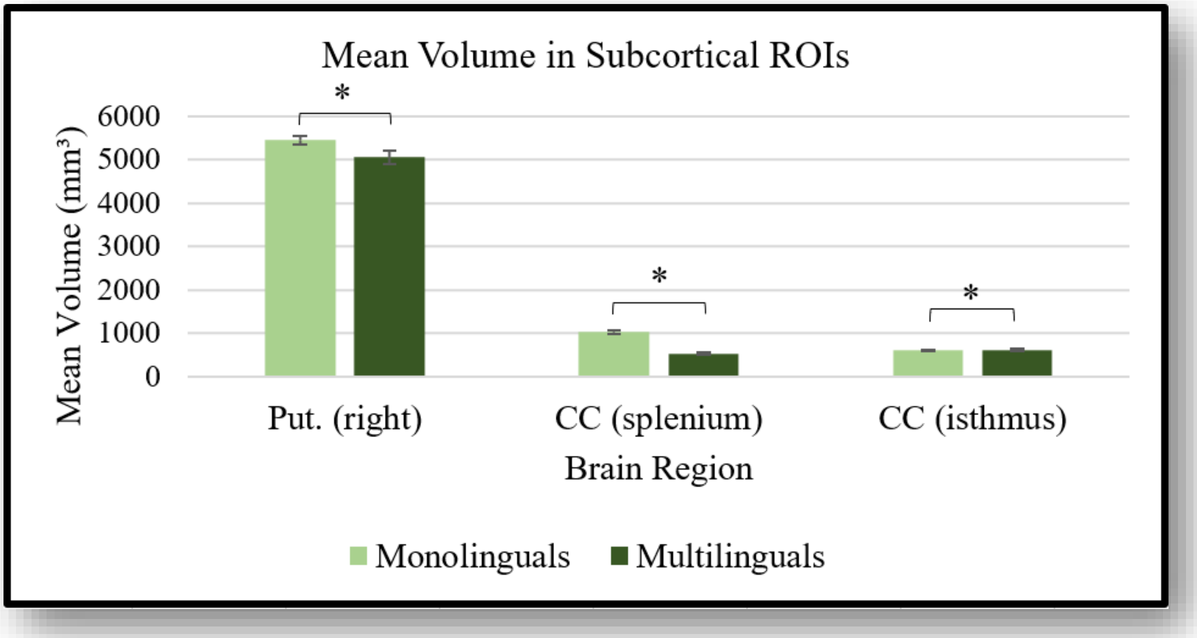

Figure 23. Significant Mean Grey Matter Volume

Figure 23. Description: Put: putamen, CC: corpus callosum

Lastly, the ROIs that remained significant at $p<.05$ (FDR) were the left AG and the precuneus in the STL (see Table 16).

Table 16. List of ROI Results for Cortical and Subcortical Grey Matter

Region	**BA**	***Monolinguals > Multilinguals***			
Cortical Regions		$t_{(30)}$	***p value***	***95% CI***	***Corrected p-value***
R. Transversotemporal G.	BA41/42	0.35	0.73	-116.34 to 163.41	0.8
L. Transversotemporal G.		2.13	0.04*	0.006 to 0.34	0.14
R. Middle Temporal G.	BA21/22	1.4	0.17	-0.03 to 0.17	0.3
L. Middle Temporal G.		1.96	0.06	-32.22 to 1380.35	0.16
R. Superior Temporal S.		2.1	0.05*	0.0005 to 0.12	0.15
L. Superior Temporal S.		-0.05	0.95	-0.08 to 0.08	0.98
R. Superior Parietal Lobe (precuneus)	BA7	1.8	0.08	-0.008 to 0.12	0.19
L. Superior Parietal Lobe (precuneus)		3.37	0.002*	0.04 to 0.18	0.04*
R. Inferior Frontal G. (op.)	BA47	1.8	0.09	-0.02 to 0.22	0.19
L. Inferior Frontal G. (op.)		0.91	0.37	-0.08 to 0.19	0.5
R. Supramarginal G.	BA40	1.67	0.1	-0.02 to 0.16	0.2
L. Supramarginal G.		0.63	0.53	-0.103 to 0.19	0.7
R. Angular G.	BA39	3.24	0.003*	0.08 to 0.35	0.04*
L. Angular G.		1.14	0.27	-0.07 to 0.23	0.4
R. Insula (anterior)	BA13	1.74	0.09	-0.02 to 0.22	0.19
L. Insula(anterior)		2.77	0.01*	0.04 to 0.25	0.09
R. ACC	BA32/33	-2.1	0.04*	-0.17 to -0.002	0.14
L. ACC		0.28	0.78	-0.07 to 0.09	0.14
Subcortical Regions ROIs					
R. Caudate		-0.44	0.66	-457.6 to 296.5	0.8
L. Caudate		0.02	0.99	-427.6 to 434.6	0.99
R. Thalamus		1.01	0.31	-338.9 to 1007.2	0.45
L. Thalamus		0.31	0.76	-526.1 to 712.5	0.84
R. Globus Pallidus		0.57	0.57	-124.1 to 218.6	0.72
L. Globus Pallidus		1.1	0.3	-98.4 to 305.7	0.45
R. Putamen		2.3	0.03*	34.3 to 766.3	0.14
L. Putamen		1.42	0.17	-123.1 to 671.3	0.29
Corpus Callosum (Isthmus)		2.2	0.04*	4.79 to 144.6	0.14
Corpus Callosum (Splenium)		2.24	0.03*	1042.9 to 916.3	0.14

Table 16. Description: The p-values were corrected using a factor of 28 (number of ROIs) with the false discovery rate method (FDR).

2.3. Post-hoc Correlation Analysis

The only region that showed increased GM thickness in the multilinguals was the right ACC. Generally, increases in ACC GM have been reported for bilinguals that have lower immersion or lower proficiency; while reductions have been reported for highly proficient, highly immersed bilinguals (Abutalebi et al., 2012, 2015). One potential explanation for this effect was that this area has not attained full renormalization from the initial stages of learning. To test a relation between the time spent practicing the second language and the changes in GM in this area, I initially conducted a correlation analysis between the GM thickness and the age of acquisition in the multilingual participants. Figure 24 shows the plots representing the correlation analyses between the AoA of the second and third L2s with the GM thickness in the ACC. The thickness in the right ACC was negatively associated (the correlation test approached significance) with the AoA of English (the earlier the participants started acquiring English, the greater the GM thickness in the ACC), $r_{(15)} = -.47$, $p < .08$, in the multilinguals group. No correlation was observed between the AoA of Russian (early acquired L2) and the GM thickness in the rACC, $r_{(15)} = 0.1$, $p < .07$, in this group. These results suggest that the changes in the GM ACC are associated with the learning of the additional second language (English) rather than the learning of the first L2. However, it is important to note the tighter range in the AoA of the early second language (Russian) – a factor that may have led to the inability to detect significance in associations in these correlation analyses.

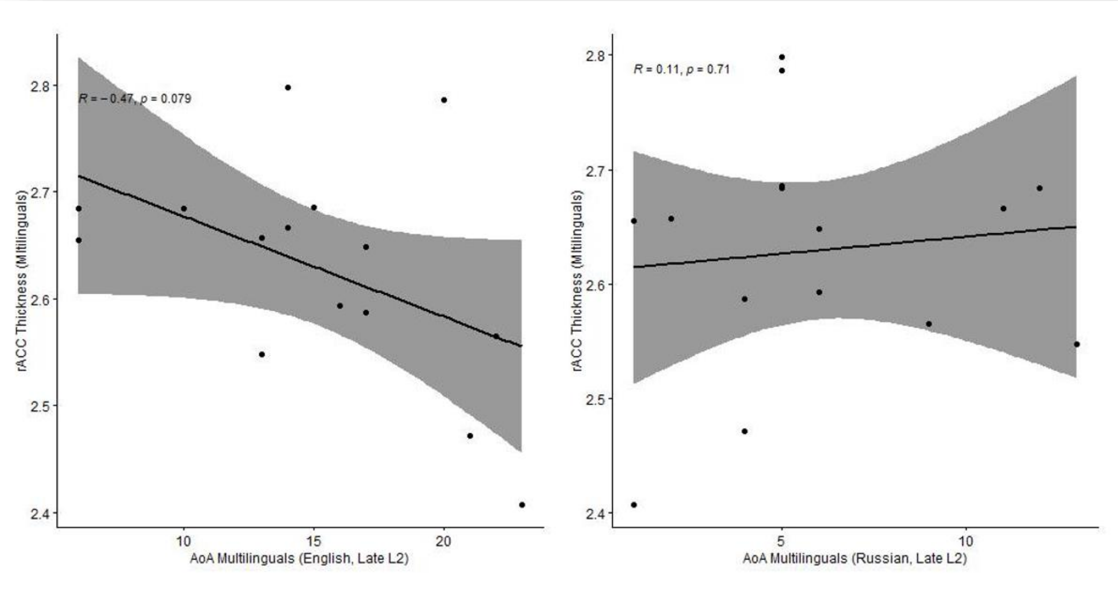

Figure 24. Correlation Analysis Between the AoA and the GM Thickness in the Right ACC

Figure 24. Description: AoA: age of acquisition, rACC: right anterior cingulate cortex

To establish a relationship between the temporality of the changes in the GM ACC and the practicing of a second language, I conducted a correlation between the time of immersion (i.e., amount of time the participants spent using their second languages daily, measured in years) and the GM thickness in the right ACC. The test showed a positive correlation that approaches significance; $r_{(15)} = .49$, $p < .07$; while the correlation with the amount of Russian immersion showed a significant negative correlation, $r_{(15)} = .53$, $p < .04$. The direction of the correlation corroborates with the initial hypothesis that the increased GM in the rACC may be suggestive of an ongoing adaptation process in this area, that has not reached full normalization. Namely, at the initial stages of learning, increases in GM are noticed, while higher immersion (and amount of language experience) is negatively correlated with GM thickness.

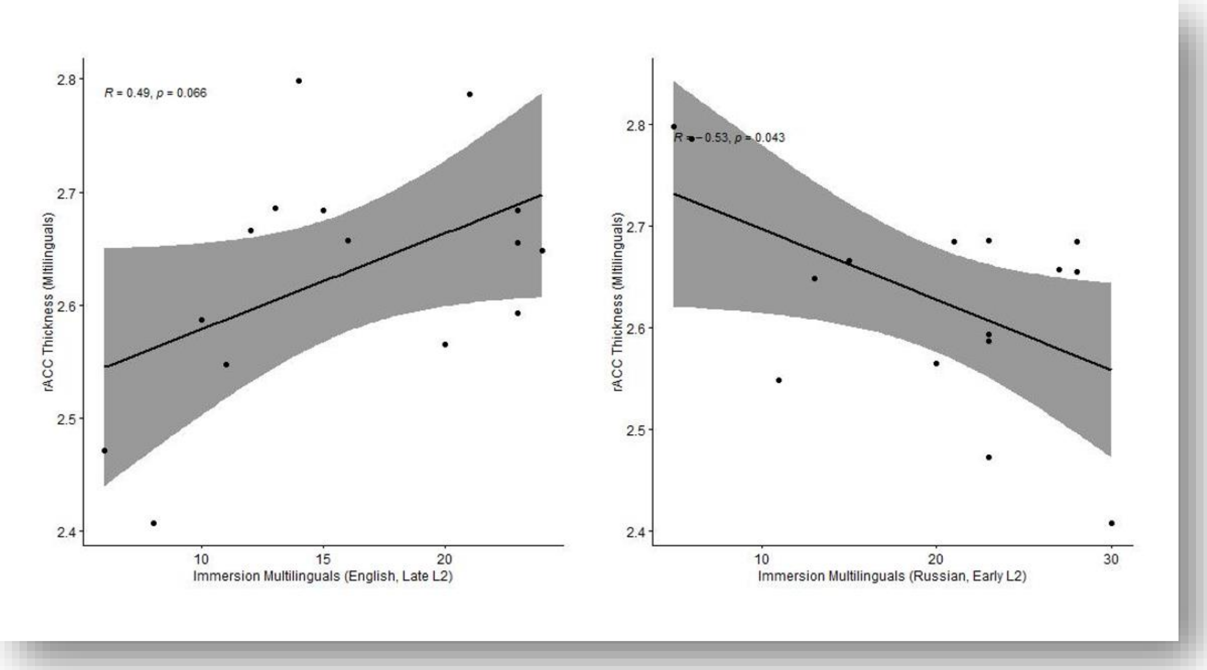

Figure 25. Correlation Analysis Between the Amount of Immersion and Right ACC Thickness

Figure 25. Description: rACC: right anterior cingulate cortex.

2.4. Discussion

This study investigated grey matter brain differences in a group of multilinguals compared to monolinguals. Results showed an overall decrease in grey matter thickness and volume for multilinguals compared to monolinguals. The first prediction investigated in this study was whether areas related to language processing (also referred to as "core" language regions) will show similar grey matter thickness across groups. Overall, the results are in line with this prediction, there were no significant differences in the left inferior frontal gyrus, as well as middle temporal regions. However, decreases were observed in the left Heschl's gyrus and left STG. These regions are part of the dorsal audio-motor pathways that subserve audio-motor mapping of sounds onto articulatory-based representations (Hickok & Poeppel, 2007; Poeppel & Hickok, 2004; Saur et al., 2008). The Heschl's gyrus has been previously linked to the ability to perceive non-native sounds (Goldstein et al., 2007; Wong et al., 2008), and has been demonstrated to show volumetric increases related to bilinguals. A training study reported greater Heschl's gyrus volume correlated with accuracy and speed of learning of non-native pitch for a group of English native speakers. The training required that participants learn to associate a list of 18 monosyllabic pseudowords with pictures, for which the meaning was encoded in pitch differences similar to mandarin tones. The authors provided further evidence of the implication of the temporo-parietal areas in non-lexical phonetic learning but also learning of lexically relevant cues (see Wong & Dras, 2011). Ressel et al., 2012, investigated the effects of early language acquisition on the volumetric changes in the primary auditory cortex, comparing a group of Spanish monolinguals to Spanish-Catalan bilinguals. Compared to the monolinguals, the bilinguals had a larger volume of the Heschl's gyrus. The authors have attributed the changes in this area to the daily processing of phonological features in the two languages by the participants in the bilingual group. The STG has been linked to low-level phonological processing (Ressel et al., 2012). Increases in the volume and thickness of the STG related to bilingualism have been reported in training studies, especially related to tasks requiring greater attention to phonological detail in non-native speech, such as simultaneous interpreting. For example, Hervais-Adelman et al., 2017, in a longitudinal study, investigated the effects of simultaneous interpreter training on the grey matter of the brain. The training group showed increases in the volume of the left posterior STG, SMG, planum temporale, and right SPL, AG, the intraparietal sulcus, inferior parietal cortex (IPC), and the superior frontal gyrus (SFG). Expansions of the STG have been associated with performance in an L2 learning training study, in which highly proficient L2 students showed increases in the STG while struggling students showed greater expansion in the MFG and IFG (Mårtensson et al., 2012).

A second prediction we investigated through these data was predicted decreases in grey matter thickness in areas involved in language control. Two cortical brain areas related to language control showed significant differences across groups. Namely, the right ACC and the left insula. The involvements of the insular cortex, as well as the ACC in tasks requiring language control, have been consistently reported. The ACC has been identified in numerous studies using verbal and non-verbal tasks, to play a crucial role in mediating the monitoring of conflicting information and detecting conflict, such as choosing between translation equivalents between different languages (Abutalebi & Green, 2016; Branzi et al., 2016; Calabria et al., 2012; Green & Abutalebi, 2008). More recent work in brain network analysis suggests the membership of these areas to the salience network (SN). The fronto-insular cortex (rFIC) is posited in these as the 'hub' of the network that enables task-related information processing, by initiating control signals to engage the ACC and further the central executive network (CEN) in response to salient stimuli or events. Multilinguals' constant need to pay attention to salient cues, such as the speaker's voice, face, environment, and context may lead to an increased involvement of the salience network in order to engage other control networks (e.g. CEN) in response to the contextual demands. The implication of the SN and the CEN in multilingual practice requires further testing and investigation through multivariate analysis methods.

Along with reductions in the cortical regions, the results of this study showed a reduction in the volume of the right putamen for the multilinguals compared to the monolinguals. The basal ganglia circuits have been largely implicated in the linkage between the pre-frontal cortex, involved in language control and interference monitoring, and the posterior cortical regions, involved in maintaining the task information (Cools & D'Esposito, 2011; Green & Abutalebi, 2013). The putamen has been one of the regions showing bilingualism induced restructuring the most often, amongst the subcortical regions. Generally, larger volumes of the putamen have been reported for bilinguals versus monolinguals (Burgaleta et al., 2016) multilinguals versus monolinguals (Abutalebi et al., 2013), and positive correlations of its size with the amount of immersion have been reported (e.g. Pliatsikas & Luk, 2016). The role of the putamen in language control has been linked to controlling and monitoring the initiation of motor programs as a response to cognitive and sensorimotor environments, and control for verbal interference (Abutalebi & Green, 2008; Ali et al., 2010). Due to the highly complex interactional contexts of the multilinguals in this group (e.g. speaking in one of the languages while controlling for the additional two or three languages in certain environments, such as speaking only English in school; speaking with one interlocutor in one language (Romanian with parents) and with another interlocutor in another language (e.g. English with younger siblings, spouses or children, within the same environment) the processes of monitoring for the appropriate motor-programs depending on the sensorimotor environment, as well as controlling for verbal interference are highly taxed, possibly leading to restructuring patterns in this population.

Finally, I investigate whether changes in grey matter are observed in areas that have been often documented to show grey matter differences in highly proficient bilinguals, however, are often associated with off-task activation (Fedorenko et al., 2012), and part of the default-mode network (Goulden et al., 2014). The changes in these regions as an effect of multilingual practice are yet to be understood. Decreases in the right SPL (precuneus) and the AG thickness were also observed in this study. The inferior parietal regions have been shown to be involved in lexico-semantic processing and processing of the phonological features of newly acquired lexical items (e.g. Pliatsikas, 2019; Richardson et al., 2010). Activation in the AG has been reported in tasks involving semantic processing, such as processing semantically plausible versus implausible sentences (Mashal et al., 2009), processing of sentences that have an easy meaning versus harder to predict (Obleser & Kotz, 2010). Obleser and Kotz, 2009 suggest that this area facilitates sentence comprehension via top-down activation of semantic concepts. Along the same lines, Vigneau et al., 2006, in their meta-analysis suggest that the AG (along with the Fusa- region in the temporal lobe) is involved in the processing of word meaning. The authors suggest that the two areas (i.e. the AG and the Fusa) are amodal regions that are devoted to meaning. Mesulam, 2000, defines this area (Wernicke's area) as the transmodal[27] area that allows the interactions between the sensory

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[27] Transmodal brain areas - areas of the brain with integration functions, creating the connection between modalities, i.e. creating the linkage between words and their meaning (Mesulam, 2000)

representation of words or objects and the symbolic association that gives them meaning. In bilingual studies, the volume of the left inferior parietal lobe correlated with L2 proficiency, while the right SPL correlated with L2 exposure and AoA (Abutalebi et al., 2014; Wei et al., 2015). The precuneus has been previously reported to be associated with semantic and lexico-phonological processing (Burgaleta et al., 2014). Activity in the precuneus in lexical tasks was sensitive to word imageability (Bedny & Thompson-Schill, 2006), and word frequency (Carreiras et al., 2009).

Based on the demographic characteristics and the results on the proficiency tests (see Chapter I) we classified the multilinguals to be at the peak efficiency stage of language expertise (based on the DRM model). As such, an important contribution of this study is the investigation of brain changes at this stage, poorly documented in the literature (Pliatsikas, 2020). Based on the predictions of the DRM, highly proficient/highly immersed bi/multilinguals should show continuous restructuring until reaching a maximum limit (Pliatsikas, 2020). These would demonstrate patterns or brain adaptation characteristics to the peak efficiency stage of language expertise. There are very few studies to date that provide evidence for further adaptation of brain areas with continuous language practice. Ideally, such evidence should arise from longitudinal studies looking at the trajectory of changes occurring at different levels of language expertise. One such longitudinal study reported increases in the lower-left cerebellar grey matter, along with decreases in the left caudate, amygdala, and bilateral hippocampus for highly immersed bilinguals. Furthermore, the amount of immersion and AoA were predictors of the grey matter changes observed in the cerebellum. The authors interpreted the effects observed as evidence for the more efficient and automatic language control, due to the high immersion and proficiency, consistent with predictions of the anterior to posterior and subcortical shift (BAPSS; Grundy et al., 2017). Additional evidence comes from studying interpreters. The patterns of restructuring, observed in the multilingual group investigated in this study, highly resemble changes observed in interpreters, that generally seem incompatible with bilingual literature

(Pliatsikas, 2019, 2020). Interpreters, compared to bilinguals, are highly immersed in the bilingual environment, have high proficiency in their second languages, and are required to use second languages consistently, within high code-switching environments. These tasks do not only require the processing of the second language but impose high demands on the control networks. In contrast to bi/multilinguals with low immersion and who generally show increases in GM volume and thickness, interpreters have been reported to have decreased GM volume and thickness in several different areas, including the left IPL, ACC, IFG, and bilateral caudate nucleus (Elmer et al., 2011, 2014). For example, Elmer and colleagues revealed reduced grey matter volumes for interpreters in the left IFG pars opercularis and SMG, in the middle ACC, and bilateral pars triangularis of the IFG and middle-anterior insula (Elmer et al., 2014). These reductions in the GM are generally interpreted as increased efficiency of language control and switching. Interestingly, a study reported increased GM in the frontal pole FP for interpreters, compared to highly proficient multilinguals (Becker et al., 2016).

The only observed increases in the ACC for the multilinguals may be related to a simultaneous renormalization related to stage three (according to the DRM) of peak efficiency, before a complete renormalization of cortical areas related to the second consolidation stage. Most studies looking at participants with lowered proficiency or lack of immersion report increases in the GM of the ACC. This is further corroborated by the correlation of the GM thickness with the amount of immersion and AoA. The results of the correlations show an increase in the GM of the rACC during the initial stages of learning a language (see Figure 25 (right)) and an increase in immersion in the later acquired L2 (English) leading to increases in the GM thickness. However, decreases in GM reductions were associated with greater immersion in the early L2 (see Figure 25 (left)). These results are in line with the DRM (Pliatsikas, 2020) which predicts further restructuring effects until the speaker reaches peak efficiency, as well as with predictions of the BAPSS hypothesis (Grundy et al., 2017), that note that the relationship between the GM size and language expertise follows an "inverted U shape": GM tissue size increases as the bilinguals start gaining proficiency in a second language, however, once high-level proficiency is reached, the tissue reduces compared to monolinguals and non-expert bilinguals. The reductions in tissue size are believed to be a result of tissue specialization. Compared to monolinguals and non-expert bilinguals, multilinguals show additional reductions and adaptations in GM, that exceed values reported before second language learning (noted is well controlled monolingual control groups). Hence, the shape of the trajectory observed in GM restructuring follows a double-gamma shape, similar to an action potential, (see schematic Figure below) with a tail that is below the baseline.

Schematic Double-Gamma Shaped Trajectory of Grey Matter Changes in Highly Proficient Multilinguals.

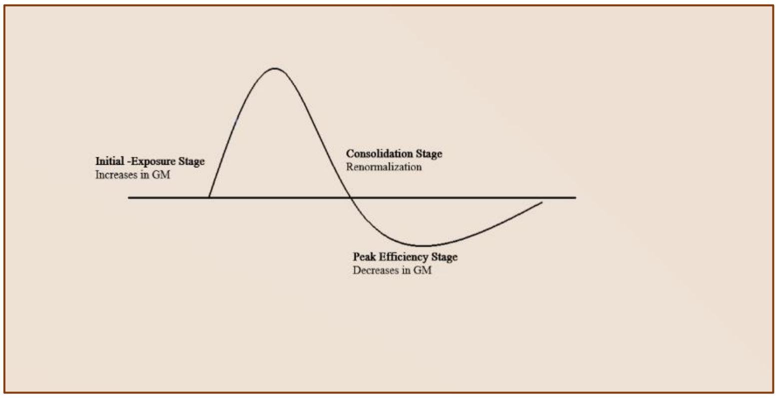
Note:

Compared to monolinguals and non-expert bilinguals, multilinguals and simultaneous interpreters show additional reductions and adaptations in GM, that exceed values reported before second language learning (noted is well controlled monolingual control groups). Hence, the shape of the trajectory observed in GM restructuring follows rather a double-gamma shape, similar to an action potential, (see Figure below).

At **Initial - Exposure Stage** of language exposure, bilinguals show increases in GM size.

At **Consolidation Stages** of language learning, a re-normalization process in observed, with GM restructuring showing an anterior-to-posterior and subcortical shift.

At **Peak Efficiency Stages**, bilinguals show reduced GM, below the baseline levels.



3. Conclusion

This study provided unique evidence of the trajectorial changes effects on grey matter thickness, within a highly proficient sample of multilinguals. The group of multilinguals investigated in this study provided two unique characteristics that allowed specific predictions testing, of recent models of brain restructuring associated with second language practice, that are not possible in studies looking at bilinguals. One was related to equal high proficiency for at least two second languages, although they were acquired at different ages. Additionally, due to the life-long practice of the early acquired language and higher immersion, I was able to identify a predicted trend of decrease GM with increase practice in a second language. Altogether, the results of this study are providing evidence for changes associated with peak efficiency in multiple languages, suggesting that with continuous usage of a second language, and exposure to highly complex multilingual interactional contexts, additional brain restructuring effects are observed, that perhaps contribute to better language control and usage in multilinguals. These changes, associated with the peak efficiency stage, are predicted to be accompanied by increases in anterior white matter diffusivity, that have been reported in most proficient bilinguals. These hypotheses are in line with the BAPSS hypothesis, which predicts increases in anterior white matter diffusivity as proficiency increases and a shift from using the anterior brain regions to more posterior and subcortical networks. Further discussion of the white matter changes associated with multilingualism will be provided in the following chapter.

4. Limitations and Further Steps

Although generally interpreted as such, a lack of observed changes in certain brain areas may not be interpreted as a lack of change. Due to the limitation of the MRI techniques, the changes may not be observable with the investigative tool (i.e. structural morphometry). For example, while investigating structural differences for simultaneous versus sequential bilinguals, Klein et al., 2014, did not note a difference between monolinguals and simultaneous bilinguals, Berken, Chai, et al., 2016, reported differences in brain connectivity, modulated by AoA, between areas that showed decrease neural activation during task performance for a comparable group of participants. Therefore, combined methods (i.e. structural, functional, network-based, etc.) should be used for a more comprehensive account of experience-based effects of brain function and structure.

It is important to note that one of the disadvantages of finer-grained atlases is the possibility of more inaccurate parcellation; larger ROIs may not give enough detail about the changes that occur in certain brain areas. As mentioned above, the output value in the morphometry analysis provided by the software is an average of all voxels included in the area, although it may not be representative of changes in larger brain areas. Additionally, one of the downfaults of automated segmentation is that the researcher is limited by the areas that are provided by the atlas. These are sometimes large and do not provide great spatial detail. For a more comprehensive analysis of the specific microstructural changes in the gray matter, newer methods should be used (De Santis et al., 2016; Metzler-Baddeley et al., 2017). Additionally, manual drawing of specific regions of interest that allow for investigating subregions of anatomical parcellations provided by the atlases, is necessary.

Another considerable limitation of this study is the number of participants. Limitation in power in MRI studies often leads to inconsistencies of findings (Munson & Hernandez, 2019) as well as limitations in identifying effects of interest. Hence, further research for groups comparable to this study’s participants is needed for a better understanding of grey matter changes associated with high proficiency in multiple languages.

Chapter IV

Brain White Matter Restructuring Related to Multilingual Practice

Abstract

Language processing relies on white matter (WM) for effective communication between brain regions. This chapter investigates white matter differences in a group of multilinguals compared to a group of monolinguals. White matter morphometry was quantified using a recently developed method, namely fixel-based analysis (Raffelt et al., 2015, 2017; Tournier et al., 2007). Here, I investigate predictions of increased fiber density (FD), fiber cross-section (FC), and fiber density and cross-section (FDC) in the anterior segments of the dorsal and ventral pathways for language processing for multilinguals compared to monolinguals, based on the bilinguals anterior to posterior and subcortical shift (BAPSS) hypothesis (Grundy et al., 2017). Higher fiber cross-section (higher fiber bundles size perpendicular to the length of the fiber) and lower fiber density (FD; a lower volume of intra-axonal restricted water) values were observed for the multilinguals, in the dorsal pathway, including superior longitudinal fasciculus and arcuate fasciculus (SLF/AF)) and the ventral pathway, including the inferior fronto occipital fasciculus (IFOF), inferior longitudinal fasciculus (ILF), and the uncinate fasciculus (UF). Additionally, I investigated changes in the corpus callosum (CC), where decreases in fiber density (FD), fiber cross-section (FC), and fiber density and cross-section (FDC) were expected, based on predictions of the dynamic restructuring model (DRM). In line with this prediction, two different segments (i.e. middle body and the isthmus) of the corpus callosum showed decreases in all three morphometry measures FD, FC, and FDC, for multilinguals compared to monolinguals. Finally, a positive correlation was observed between the bilateral ICP and the AoA of an early learned L2, and a negative correlation between the bilateral ICP and AoA in a later learned L2. These results corroborate with changes observed

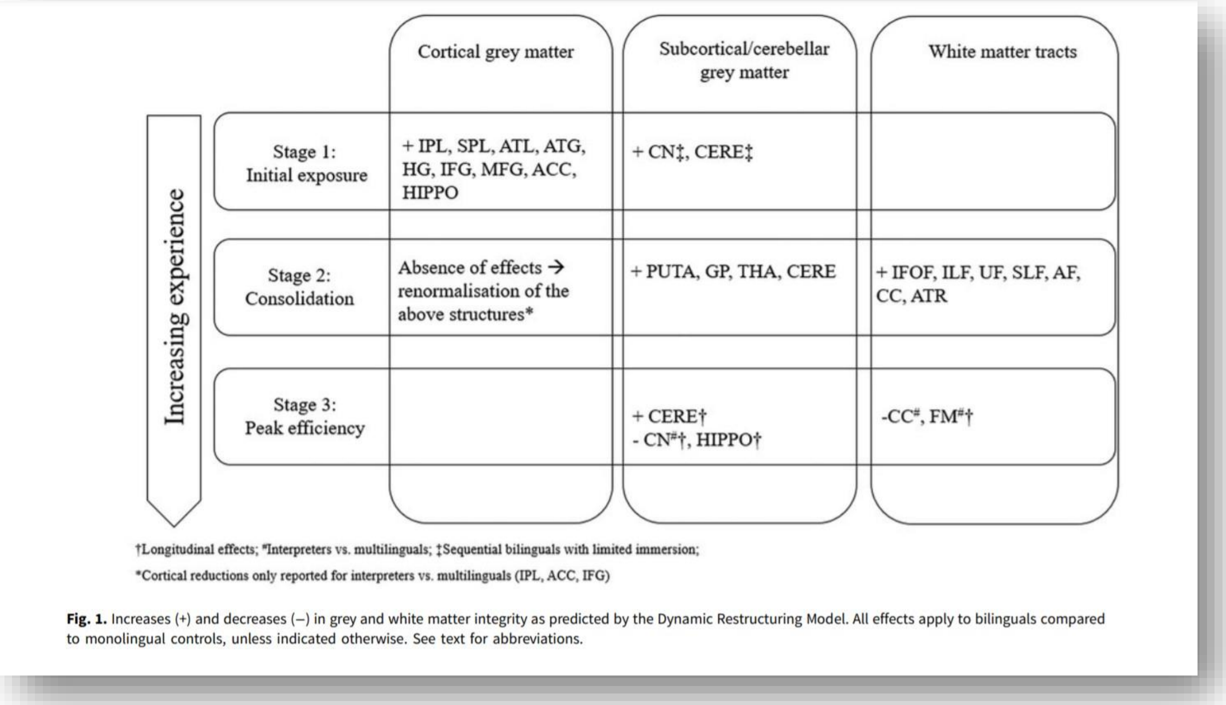
in the grey matter at peak efficiency experience in a second language of multilinguals speakers (Grundy et al., 2017; Pliatsikas, 2020).

1. Introduction

Acquisition of diffusion-weighted imaging (DWI) has been the subject of interest in many research fields, including bi/multilingualism. By applying controlled gradient magnetic fields and using the diffusive characteristics of the water within (un)restricted cellular environments such as the white matter of the brain (e.g. water can diffuse more freely along fibers than across them) across time, the diffusion MRI technique enables the three-dimensional mapping of the brain's white matter bundles (Heuvel & Pol, 2010).

Language processing relies on effective communication between brain regions that is achieved through several white matter (WM) tracts (Friederici, 2009) that are part of the dorsal and ventral language pathways, and a medial pathway involved in language control (Friederici & Gierhan, 2013; Hickok & Poeppel, 2007). The first study to report white matter changes related to bilingualism (Coggins et al., 2004) showed a larger volume of the anterior mid-body of the corpus callosum (CC) for the bilinguals, compared to the monolingual group. This is a pathway consider to be involved in aspects of language control. Following, (Luk et al., 2011) showed higher fractional anisotropy (FA) and radial diffusivity (RD) values in the CC as well as the dorsal pathway SLF(II), and the ventral pathway, in the inferior front-occipital fasciculus (IFOF) and the uncinate fasciculus (UF), for a group of life-long older bilinguals. The same tracts identified in Luk et al., 2011, have been consequently reported in the majority of the bilingual studies. The multilingual literature looking at white matter changes is very small. One study compared to grey and white matter density in a group of multilinguals (speakers of two or more languages, in addition to the native language) compared to a group of bilinguals (speakers of one additional (to a native) language). The multilinguals showed greater white matter density in the right posterior SMG, compared to bilinguals. The authors suggested that the difference was due to the number of words known by the multilingual compared to the monolinguals (Kaiser et al., 2015). Another group of authors looking at speakers of multiple languages showed that acquiring a third language later in life, after two languages have been acquired leads to an increase in FA and decreased in MD in the inferior longitudinal fasciculus, compared to acquiring two languages sequentially, besides the native language (Hämäläinen et al., 2017).

In the previous chapter, grey matter differences in a group of multilinguals compared to monolinguals were described. The results showed that highly proficient multilinguals exhibit restructuring patterns characteristic of the peak efficiency stage predicted by the dynamic restructuring model (DRM; Pliatsikas, 2020). According to the DRM, the changes associated with the peak efficiency stage (see Figure 26, namely decreases in GM volume and thickness), are to be accompanied by increases in anterior white matter diffusivity and enhancement of the cerebellum. Similar patterns were reported in the present research as outlined in Chapter III. In this chapter, I investigate these predictions by characterizing white matter differences in multilinguals and monolinguals, using one of the recently developed methods of investigating white matter morphometry in different populations, namely the fixel-based analysis approach (Raffelt et al., 2015, 2017; Tournier et al., 2007). I first give a short overview of traditional DWI methods used to study and characterize changes in white matter across groups and motivate the use of the fixel-based analysis (FBA) method used in this study. I then summarize observations of recently proposed white matter bundles in neurolinguistic models of language processing. I further describe restructuring patterns in the pathways observed in speakers of multiple languages and provide hypotheses for this study based on the DRM model developed to account for trajectorial neurostructural effects of multilingualism on the brain (Pliatsikas, 2020). Finally, white matter differences are characterized in our participant groups.



	Cortical grey matter	Subcortical/cerebellar grey matter	White matter tracts
Stage 1: Initial exposure	+ IPL, SPL, ATL, ATG, HG, IFG, MFG, ACC, HIPPO	+ CN‡, CERE‡	
Stage 2: Consolidation	Absence of effects → renormalisation of the above structures*	+ PUTA, GP, THA, CERE	+ IFOF, ILF, UF, SLF, AF, CC, ATR
Stage 3: Peak efficiency		+ CERE† - CN$^{\#}$†, HIPPO†	-CC$^{\#}$, FM$^{\#}$†

†Longitudinal effects; $^{\#}$Interpreters vs. multilinguals; ‡Sequential bilinguals with limited immersion;

*Cortical reductions only reported for interpreters vs. multilinguals (IPL, ACC, IFG)

Fig. 1. Increases (+) and decreases (–) in grey and white matter integrity as predicted by the Dynamic Restructuring Model. All effects apply to bilinguals compared to monolingual controls, unless indicated otherwise. See text for abbreviations.

Figure 26. The Dynamic Restructuring Model

Figure 26. Christos Pliatsikas, Understanding structural plasticity in the bilingual brain: The Dynamic Restructuring Model. Bilingualism: Language and Cognition, 23(2), 459-471, reproduced with permission.

2. Common Methods Used for Quantifying White Matter in the Brain

Water diffusion is an indirect indicator of the local microstructure of the environment in which it is found (usually referred to as a measure of white matter "integrity", or structure). Since the patterns of displacement of the water molecules are restricted by the environment, these can be used as markers of the type and characteristics of the tissue in which the water molecules are measured. For example, water can have free diffusion in tissues in which the molecules can freely move in the environment, such as the cerebrospinal fluid in the brain. This type of random, unrestricted diffusion is called "*isotropic"*. On the other hand, restricted diffusion is a regime or diffusion in which the water is restricted by the compartment in which it is found but cannot exist (such as white matter axonal tissue) and can freely move along but is restricted across the compartment, creating a preferred diffusion direction, along with the compartment, called *"anisotropic"* diffusion. Another type of water diffusion regime is hindered diffusion, in which the water molecules are not restricted within a compartment but hindered by it, such as the extracellular space in fiber bundles in the brain, or within the grey matter. The displacement of water molecules in this environment is reduced, however still arbitrary, or isotropic.

Several methods have been used to investigate white matter differences in bi/multilinguals. The traditional methods are voxel-based, and test diffusion images for voxel-wise differences across different groups (Ashburner & Friston, 2000; Schwarz et al., 2014). A traditional, powerful model for estimating the signal at each voxel, is the tensor model, which approximates the displacement of water by fitting an ellipsoid-shaped model to each voxel. This model allows for the calculation of several useful measurements, such as eigenvectors and eigenvalues. Eigenvectors are a measure of the displacement along the primary axes of the fitted ellipsoid (first eigenvector) and the displacement perpendicular to the primary axes (second and third eigenvector); these are indicative of the direction of the white fiber. Eigenvalues provide a measure of the sizes of the axes, indicating the strength of diffusion within a fiber bundle.

These quantities provide indirect measures of tissue structure. They include the apparent diffusion coefficient (ADC), a measure of the mean diffusion across all directions, which is an indirect measure of the tissue integrity (i.e. usually, the more restricted the water diffusion is an indicator of greater tissue integrity). Another, most commonly used measurement, is fractional anisotropy (FA), which is a measure of how elongated the ellipsoid is, the directional asymmetry of water diffusion (i.e. it is scaled between 0 and 1, with an FA = 1 meaning fully anisotropic, and an FA = 0, fully isotropic), giving a measure of fiber integrity. Lastly, one can obtain a measure of the principal diffusion direction (PDC), which is a measure of the direction of the greatest diffusion, and it is an indirect characterization of the fiber orientation. The PDC information can be further used in order to trace (also called diffusion tractography), the fiber bundles (a.k.a. fascicles). Diffusion tractography aims to obtain information about the connection between different regions of the brain by reconstructing the white matter pathways (a process also referred to as streamline[28] creation within certain software packages). Tensor-based models offer additional tensor-driven scalar quantities (e.g. FA, MD, AD, RD; (Johansen-Berg & Behrens, 2013), that has been largely used in studies of brain plasticity in bi/multilinguals. These include axial diffusivity (AD) – a measure of water diffusion along the WM tract (i.e. greater AD values reflecting greater ellipsoid relative to spherical geometry), radial diffusivity (RD) – water diffusion perpendicular with the WM tract (i.e. higher RD reflect faster diffusion of water molecules), mean diffusivity (MD) - a measure of average diffusivity across all directions at a given tract, characterizing the magnitude or strength of tract diffusion (i.e. higher MD values implies higher rate of diffusion), and the most used, fractional anisotropy (FA), measures the directional asymmetry of water diffusion and quantifies the degree of diffusivity, described above.

2.1. Issues Related to Traditional Models:

2.1.1. Issues With Fitting the Data:

Despite the great advantages of the diffusion tensor model, the model suffers from inherent limitations. These encompass issues of partial volume effects (voxels containing several types of tissues (Papadakis et al., 2002), non-Gaussian diffusion - one of the main assumptions of the model (Assaf et al., 2005), and averaging effects (voxels containing more than one fibers). The diffusion tensor model is appropriate for voxels containing one single type of tissue and fibers of only one direction. It is however very well known that brain tissue microstructure is much more complex than the tensor model implies. Constructs obtained with tensor models are heavily confounded by both, issues of partial volume effects, as well

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[28] Streamlines - lines along which the flow of a moving fluid is least restricted. In diffusion, these are lines representing the white matter fiber bundles.

as bias in tract estimation due to crossing fibers. A study looking at the proportion of voxels affected by crossing fibers, and fiber orientation within a voxel estimated that approximately 90% of the white matter contains crossings fibers (Jeurissen et al., 2013). This makes the interpretation of measures obtained with such methods (i.e. FA, MD, AD, RD) challenging.

Several models have been proposed to account for the partial volume effects issues. The most popular model, tract-based spatial statistics (TBSS) allows for whole-brain analysis by estimating a "group mean FA skeleton" (Smith et al., 2006, 2007). This mean image encompasses the centers of all the fiber bundles present in all the subjects in the study that is warped to each subject's FA image space. This method provides more robust cross-subject statistical comparisons, by avoiding the voxels at the edges of the bundles that may be misaligned across participants, due to tissue boundaries, however, it does not provide fiber-specific information, rather analysis is limited at the voxel level.

2.1.2. Issues With Biological Interpretability:

These are further complicated by the biological interpretability issues, posed by the MRI technique. The tensor measures are often used to describe tissue "integrity". In the bilingual literature, these metrics have been interpreted to reflect the efficiency of processing, due to a change in myelination (Pliatsikas, 2019; Singh et al., 2018). However, these constructs are poorly defined, as well as substantiated. At the biological level, a change in fractional anisotropy (FA) can reflect a range of different processes, including changes in the myelin sheath, permeability of cellular membranes, axonal density, glial density, fiber coherence, etc. From animal testing experiments, investigating the effect of myelin on the fractional anisotropy (FA) metric, it is known that myelination has a limited contribution to FA values, and other sources as axonal structure and health are equally and undifferentiable contributing to the FA measure (De Erausquin & Alba-Ferrara, 2013; Song & Huganir, 2002). Indeed, many studies looking at the role of axonal membranes (e.g. Beaulieu, 2002, 2013; Beaulieu et al., 1996), independent of myelination, are sufficient barriers for water to diffuse along the white matter axon. Gilmore et al., 2007, showed that the anisotropy index in the non-myelinated splenium of the corpus callosum was higher than the myelinated corticospinal tract in healthy neonates suggesting that diffusion anisotropy is not myelin-specific (for further detail on factors that influence the fractional anisotropy measure, see Appendix I).

Another issue that is poorly understood related to the biological changes associated with white matter in both linguistic and non-linguistic studies is whether the same underlying mechanisms are responsible for changes observed after a short time period (e.g. one-hour training in a second language) versus longer periods (e.g. after years of practicing experience with a second language). Changes in FA have been reported in training studies that included a wide range of time durations. Surprisingly, increases in FA in the superior longitudinal fasciculus (SLF) and decreases in medial diffusivity MD in parietal regions close to the SMG were observed in as short as one hour, in a training study, in which participants learned 20 new words (flower names) in Hebrew, their native language (Hofstetter et al., 2017). Longer training periods also seem to lead to increases in FA. Hosoda et al., 2013, reported increases FA in a tract connecting the IFG opercularis to the caudate nucleus, and the STG/SMG was correlated with the "*Extensive Vocabulary"* training score only after a 16-week training in native Japanese learning English. The training consisted of learning 60 idioms with their meaning, pronunciation, spelling, and usage. Interestingly, a follow-up scan (after one year) revealed decreases in the FA values within the group compared to the post-training scan. Such results suggest that both short and long training in lexical learning can lead to microstructural changes in the brain. Additionally, some of these changes following relatively short training periods seem to be reversible. However, whether the same or different biological changes lead to these differences is not known. These may be the result of very different biological changes. For example, decreases in mean diffusivity have been shown when the cellular index of the tissue increases (Gauvain et al., 2001) as well as when the cell swells (Benveniste, 1992).

Trying to overcome some of the shortcomings of the issues related to interpretability and model fit, in this study, I would like to make use of a novel method that allows voxel-wise fiber specific analysis, called fixel[29]-based analysis (FBA; Raffelt et al., 2017). This newer method uses a higher-order diffusion model (i.e. spherical deconvolution (CSD), and is able to characterize specific fiber population orientations within voxels (e.g. Raffelt et al., 2012; Raffelt et al., 2017; Tournier et al., 2007; Tournier et al., 2011). It provides a more sensitive and interpretable alternative to the traditional models described above. The FBA provides a characterization of the volume fraction of individual fiber populations. The local capacity of transfer information of a fiber bundle is inferred through measures of apparent fiber density (AFD). A change in the intra-axonal volume of restricted water manifested at the microstructure[30] level as decreased fiber density (FD; Raffelt et al., 2012), fiber cross-section (FC) - macrostructural[31] volumetric change manifested as a change in the bundle size, and a combination of both fiber density and cross-section (FDC; Raffelt et al., 2015, 2017). These metrics aim at providing quantities that have greater interpretability and are closer to characterizing biological differences across groups. Therefore, this novel method offers an additional approach to understanding brain restructuring patterns in bi/multilinguals (for a detailed description of the metrics and their interpretation, see Raffelt et al., 2017). It is important to note that these constructs (i.e. FD, FC, FDC) although more specific to the type of change, are not a direct measure of the underlying biological restructuring occurring at the cellular level. Changes in FD and FC cannot inform us whether these differences are due to myelination, axonal density, or other microstructural changes, although all or any combination of these factors are possible underlying changes in white matter structure. For example, a decrease in fiber density (FD) may be the result of axonal loss, an increase in axonal diameter, or axonal myelination (that may lead to restrictions in the total volume of restricted water

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[29] Fixel - refers to a specific fibre population within a voxel (Raffelt et al., 2015).

[30] Microstructure – the fine structure of the tissue, in white matter, the changes that occur at the cellular level.

[31] Macrostructure – the gross structure of the tissue, in terms of axons, the whole bundle tissue

within any given voxel), etc. However, differences in fiber density (FD) should be mainly observed due to within-voxel changes of volume of restricted water (i.e. intra-axonal volume). These differences in FD can be due to many biological changes, including a change in the number of axons, axonal diameter, axonal membrane packing, etc., however, little influence from the myelin should be observed in the FD, due to the long echo time (TE) of the DWI method that makes the myelin indistinguishable from extra-cellular space, and the intracellular diffusion restriction, independent of myelin (see Appendix I for details). Whereas changes in fiber cross-section (FC) should mainly be driven by the fraction volume occupied by the bundle, perpendicular to the fiber orientation (fiber "width"), implying a change in the number of axons (Raffelt et al., 2017), but also axonal diameter, axonal membranes packing, as well as myelination.

3. White Matter in Neurolinguistic Models of Language Processing

Experience with different skills, such as learning a second language, leads to changes in the brain's white matter (WM). These changes are believed to be the result of an increased need for additional resources to cope with the demands of the new skill and are usually interpreted as reflecting higher computational ability and efficiency of communication between brain regions. In recent years, there has been an interest in quantifying and contrasting white matter tracts in multilinguals that are involved in core language processing and language control.

Two main language processing pathways have been proposed - a ventral and a dorsal pathway (see Figure 27, Friederici & Gierhan, 2013). A ventral pathway, involved in semantic processing (Hickok & Poeppel, 2007; Leclercq et al., 2010; Martino et al., 2013), is supported by the inferior fronto-occipital fascile (IFOF), as well as the uncinate fasciculus (UF), a bundle connecting areas involved in basic syntactic processing (Friederici & Gierhan, 2013; Pliatsikas, 2019; Tremblay & Dick, 2016). Finally, the inferior longitudinal fasciculus (ILF) was proposed as an additional ventral fiber bundle involved in semantic and syntactic processing

(Mandonnet et al., 2007). The IFOF connects the inferior frontal gyrus (IFG) with the middle temporal gyrus (MTG), the angular gyrus (AG), and the posterior temporal gyrus (pTG). The uncinate fasciculus (UF) connects the inferior frontal gyrus (IFG) with the anterior regions of the temporal cortex, including the temporal pole (TP), and the inferior longitudinal fasciculus (ILF) connects the occipital lobe with the temporal and middle anterior and inferior temporal gyri, the parahippocampal gyri, the hippocampus and amygdala (Tremblay & Steven, 2016).

A dorsal pathway is believed to be involved in top-down complex syntactic processing and it is anchored by the arcuate fasciculus (AF; Friederici, 2012), and input-driven auditory-to-motor mapping, supported by superior longitudinal fasciculus (SLF; Friederici, 2012; Tremblay & Dick, 2016). The arcuate fasciculus (AF) connects the opercular region of the IFG with the middle and posterior temporal gyri. The superior longitudinal fasciculus (SLF) connects the MTG and the STG to the dorsal premotor cortex (dPMC), via the parietal cortex, as well as frontal regions to the angular gyrus (AG) and supramarginal gyrus (SMG; Friederici, 2012). Tremblay & Dick, 2016, propose a division of the SLF into three components, parallel to the studies reported in macaque brains: the SLFII that connects the frontal cortex (FC) to the AG; the SLFIII connecting the FC to the SMG, and the SLFtp (temporo-parietal bundle, also referred to as the 'perisylvian - SLF', or SLF IV) connecting the AG to the temporal cortex. It is worth mentioning that the SLFtp and portions of the SLFII have been identified to overlap with the arcuate fasciculus (AF; Catani & De Schotten, 2008; Makris et al., 2005; Study et al., 2009). Lastly, another fiber tract that is crucial for interhemispheric communication and creates a medial pathway, related to executive control in language processing is the corpus callosum (CC; Just et al., 2007; Schlegel et al., 2012; Stein et al., 2012).

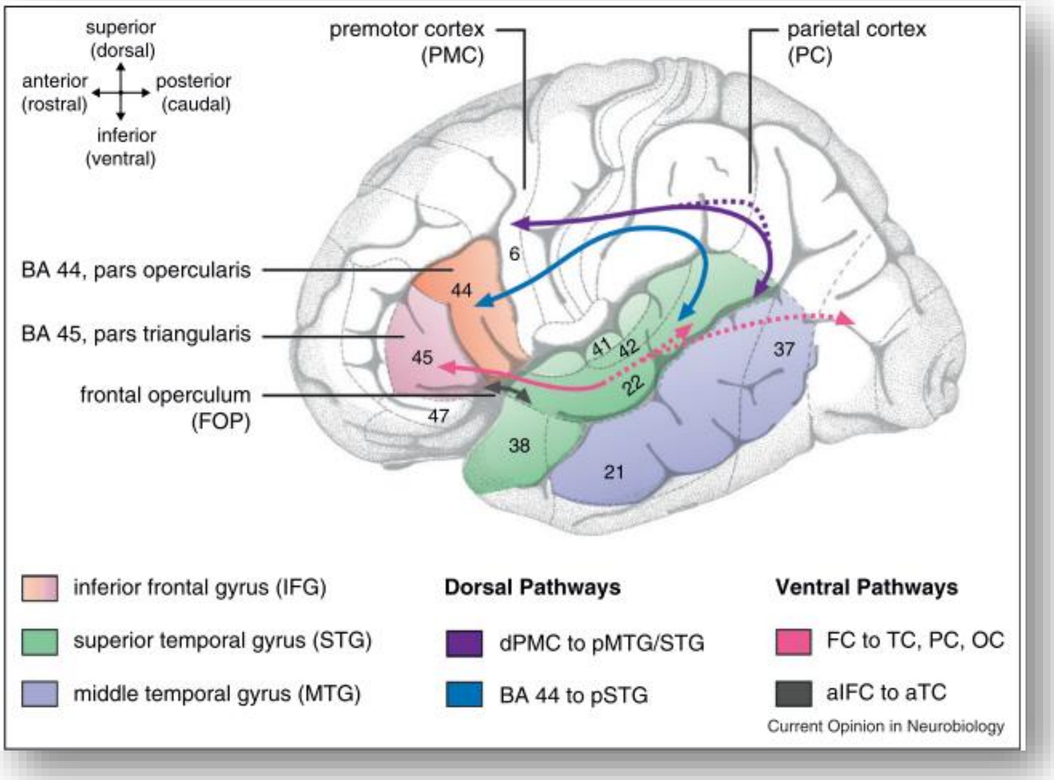

Figure 27. A Dual-Stream Model of Language Processing

Figure 27. Reprinted from Current Opinion in Neurobiology, Vol. 23., Angela D. Friederici & Sarah ME Gierhan, The Language Network, Pages 250-254., Copyright (2013), with permission from Elsevier.

The dorsal pathway connects the dorsal premotor cortex with the posterior temporal cortex/superior temporal cortex (pMTG/STG), through the second and the temporal segment of the superior longitudinal fasciculus (SLFII, SLF-tp/IV), the dorsal pathway connects the Broadman's area (BA) 44 with the posterior superior temporal gyrus (STG) by the arcuate fasciculus (AF). The ventral pathway connects the frontal cortex (FC), including the BA45, to the temporal, parietal, and occipital cortex (TC, PC, OC) through the inferior longitudinal fasciculus (IFOF), also a ventral pathway that connects the anterior inferior frontal cortex (aiFC), including the BA47, and the fronto opercular regions with the anterior temporal cortex (aTC) by the uncinate fasciculus (UF).

3.1. White Matter Restructuring Patterns in Multilinguals

The available evidence coming from white matter changes in studies of bi/multilinguals is very mixed. In general, the white matter restructuring patterns observed in bi/multilinguals studies are decreased diffusivity, measured by increases in fractional anisotropy (FA; the directional/preferential asymmetry of water diffusion along a specific axis) and axial diffusivity (AD; i.e. greater water displacement along with the fiber rather than perpendicular to the principal axis). These are generally accompanied by decreases in radial (RD; diffusion perpendicular to the fiber) and mean diffusivity (MD; mean water displacement along all axes) metrics interpreted as increases in water diffusion, for bilinguals compared to monolinguals (Pliatsikas, 2019). However, there are several studies (e.g. Cummine & Boliek, 2013; Elmer et al., 2011; Kuhl et al., 2016), that report contradictory or mixed results (e.g. increases in FA, accompanied by reduced MD, in monolinguals versus bilinguals). Surprisingly, both directions of results are usually interpreted to indicate an increase in myelination or axonal density, although the biological underlying changes are impossible to be described by any of the above-mentioned constructs (i.e. FA, AD, MD, RD). Hence, although it is indisputable that experience with practicing second languages leads to brain restructuring, the patterns of change with their underlying mechanisms are yet to be understood and substantiated.

Most of the evidence of language acquisition-induced white matter changes comes from bilingual longitudinal and training studies. For example, a study looking at a group of simultaneous bilinguals, sequential bilinguals, and monolingual children (8 – 11 years old), reported higher FA values in the ventral pathway for language processing (left IFOF) for the simultaneous bilinguals compared to the sequential bilinguals and the monolinguals. Additionally, they reported reduced FA for the same group (i.e. simultaneous bilinguals) versus monolinguals in the medial pathway involved in language control (anterior radiation of the corpus callosum (CC)) towards the frontal lobe (Mohades et al., 2012). In a follow-up study, after two years, looking at the same participants, the authors reported increased FA values relative to the initial scans, in the left IFO fasciculus for all three groups, with the highest values for the sequential bilinguals. These results were interpreted as a maturation effect associated with semantic processing, which is claimed to be more demanding for bilinguals versus monolinguals (Mohades et al., 2015). Similarly, (Pliatsikas et al., 2015) compared sequential bilinguals of different language backgrounds, in a high immersed in an L2 (English) environment group, to native speakers of English and reported increases in FA for the bilateral CC (genu, body, and splenium), IFOF, UF (both part of the ventral pathway) and SLF (part of the dorsal language pathway) for the bilinguals compared to native monolinguals. The authors suggested that the CC is related to interhemispheric communication while the other fiber bundles are related to the processing of semantics and syntax. Note that both ventral and dorsal pathways, as well as the pathway involved in language control, are implicated in these changes and that bilingual language status (i.e. simultaneous versus sequential 2nd language acquisition) modulates these effects, with the greatest chances for sequential bilinguals. Additionally, both increases and decreases in FA have been reported in segments of the corpus callosum between the two studies.

Along with a limited number of multilingual studies, restructuring of white matter in highly immerse, highly proficient bi/multilinguals are also little described in the literature. One group of high proficient bilinguals that have received attention are interpreters. One such study comparing simultaneous interpreters with a group of low immersion multilinguals reported reduced FA (associated with reduced axial and enhanced radial diffusivity) in a few regions, specifically, the left anterior insula, basal ganglia, inferior parietal lobe, the upper part of the corticospinal tract, and the anterior (genu) middle (body) and the posterior (splenium) corpus callosum (Elmer et al., 2011). The authors suggested that these changes are related to the specific language-related demands that are associated with interpreting. In the same vein, Kuhl et al., 2016 reported lower FA and higher RD and MD values in bilinguals versus monolinguals in multiple tracts, of dorsal (SLF), ventral (IFOF, ILF, UF), and medial pathways, including the CC; as well as the Anterior Thalamic Radiation (ATR). The same study showed positive correlations between the FA and the tracts mentioned here, as well as negative correlations between RD and MD, associated with experience listening or speaking the L2. Note that these are the opposite patterns of results with reduced rather than increases in FA.

Finally, several studies have reported an association between different background factors (i.e. AoA, immersion, and extent of daily use) with microstructural changes observed in white matter. For example, Rossi et al., 2017, showed higher FA in the anterior and posterior thalamic radiation (ART, PRT), the IFOF, the UF, and the ILF for a group of native English speakers, learners of L2 (Spanish), versus monolingual English speakers. They further showed that the FA values were negatively correlated with the AoA of the L2. Similarly, Mamiya et al., 2016, investigated the role of short intensive language learning on neural changes. The study looked at before and after training diffusion-weighted data in 44 Chinese speakers learning English during a 16-day period. The results showed higher FA and lower RD values in the SLF, positively correlated with the number of days in the program, and a marginally negative correlation between the FA values and the days passed after the training ended. These suggest that the length of immersion and the amount of practice in a second language influences changes in white matter.

Overall, the evidence coming from neuroimaging studies indisputably shows, the restructuring of brain white matter related to bi/multilingualism, both long and short-term. These changes seem to be modulated by different experience-based factors, that co-occur with language learning. However, the evidence is still variable, showing both increases and decreases in bilinguals compared to monolinguals in metrics used to quantify these changes (i.e. FA, MD, RD, and AD). While different studies have examined the background and experience-based factors that influence white matter changes in bi/multilinguals, further research is needed to understand how these individual factors contribute to specific changes in these pathways.

4. Present Study

Several different hypotheses regarding expected patterns of brain restructuring as a result of multilingualism (and associated factors) have been proposed. Here we discuss the anterior to posterior hypothesis (BPASS; Grundy et al., 2017 Anderson et al., 2018) and dynamic restructuring model (DRM; Pliatsikas, 2020). The bilingual anterior to posterior hypothesis (BPASS; Grundy et al., 2017, Anderson et al., 2018) suggests that with increased proficiency in a second language, bilinguals become more efficient by increasing important grey structure and facilitating communication between anterior and subcortical/posterior regions through white matter diffusion increases. With high proficiency, a shift from devoting resources to the anterior regions should be noted towards more subcortical and posterior brain regions, with enhanced white matter integrity in the anterior parts of the brain. The dynamic restructuring hypothesis (DRM; Pliatsikas, 2020), predicts trajectorial brain changes depending on the language learning stage at which the bilingual is found, the interactional context of the bilingual, and the quality, quantity, and exposure to the second language (see Figure 26). The DRM is a three-stage model (i.e. initial stage, consolidation stage, and peak efficiency stage) that predicts specific grey and white matter changes for each of the stages (for a detailed description see the introductory chapter).

In the present study, predictions based on these two hypotheses/models will be tested, for a group of high proficiency multilinguals compared to a group of monolinguals (see Chapter I for further details on participants' background). Based on the DRM that predicts higher white matter integrity in the cerebellum at the peak efficiency stage, I will investigate whether fiber density and cross-section will show increases compared to the monolinguals. Additionally, I will test whether white matter integrity is reduced in the corpus callosum (CC), another change predicted by the DRM. The white matter changes associated with high proficiency predicted by the BAPSS are increased white matter integrity in the frontal brain regions, in the pathways associated with language processing. Increases in FD, FC, and FDC are expected in the dorsal pathway (including the SLF, and AF), as well as the ventral language pathway (including the IFOF, ILF, and UF). Increases in these fiber bundles should be mainly observed in the anterior segments, passing through the anterior temporal and frontal regions. It is important to note that, as mentioned before, the terms used in the model predictions (i.e. “integrity”) are poorly defined and biologically unsubstantiated. Additionally, the interpretation of the contradictory changes observed in diffusion studies attributed all to white matter ‘integrity’ is still to be understood. Thus, here I test whether constructs as fiber density and fiber cross-section can be used to better understand white matter structural changes across groups and help better describe and interpret the changes observed.

Additionally, Since I am looking at a population with a very unique linguistic background, I will examine whether factors such as AoA, language proficiency (at different linguistic levels, (see Chapter I for details on proficiency testing) or the number of years practicing one’s language are associated with observable changes in white matter bundles. Here, I am focusing on the association between the linguistic background and changes in the cerebellum white matter. Table 17 summarizes the above predictions.

Table 17. Predicted White Matter Restructuring Patterns

White Matter Restructuring Predicted in the Multilinguals Compared to Monolinguals

Based on the BAPSS Hypothesis

1. Greater white matter integrity in the language pathways in the anterior brain regions
    - Increased FD, FC, and FDC in the *dorsal pathway* fiber bundles (SLF, AF)
    - Increased FD, FC, and FDC in the *ventral pathway* fiber bundles (IFOF, ILF, UF)

Based on the DRM

1. Decreases in the white matter integrity in the Corpus Callosum
2. Decreases in FD, FC, and FDC in the CC
3. Increases in the white matter integrity in the Cerebellum
    - Increases in FD, FC, and FDC in the inferior cerebellar peduncle (ICP)
    - Is Age of Acquisition, Immersion, and Proficiency associated with mean FD, FC, and FDC in the ICP.

Table 17. Description: FD: fiber density, FC: fiber cross-section, FDC: fiber density and cross-section, SLF: superior longitudinal fasciculus, AF: arcuate fasciculus, IFOF: Inferior fronto-occipital fasciculus, ILF: inferior longitudinal fasciculus, UF: uncinate fasciculus, ICP: inferior cerebellar peduncle.

4.1. Methods

4.1.1. Participants

The diffusion data were acquired from 30 right-handed participants, 15 native Romanian multilinguals (speakers of four or five languages), and 15 English monolinguals. Participants completed a background questionnaire and several language proficiency tests. For a detailed description of the test and the test results, see Chapter I.

4.1.2. Image Acquisition

A diffusion single-shot EPI scan with 64-directions, b = 1000 s/mm2, 106x106 matrix, FOV =212x182 mm, isotropic 2mm voxel size was acquired. A multiband acceleration factor of 2, allows the acquisition of 64 slices in a time TR = 4520ms. The echo time was kept to a minimum of 84ms by using a partial Fourier factor of 6/8. A single acquisition with the opposed phase encoding direction was used to correct the geometrical distortion induced by the inhomogeneous B0 field.

4.1.3. Data Preprocessing

DWI images were preprocessed with the MRtrix3 software package, using the pre-processing pipeline recommended for FBA analysis of single shell DWI images (Raffelt et al., 2017). The pre-processing steps included denoising (Veraart et al., 2016), eddy-current correction using FSL's eddy (Andersson & Sotiropoulos, 2016; Smith et al., 2004), and bias field correction (Tustison et al., 2019). Global intensity normalization was performed using a group-wise (within study) registration. To increase the anatomical contrast and improve spatial normalization and statistics, the images were upsampled to a voxel size of 1.25 mm^3. A whole-brain mask was computed from the upsampled images. In order to enable an analysis of differences in the apparent fiber density (AFD) across subjects, a representative of study population group average response was generated using the "*tournier*" function (Tournier et al., 2004), which enables the creation of a single-fiber white matter function. Fiber orientation was estimated using the fiber orientation distribution (FOD) images (Raffelt et al., 2011) of all participants in the study. The group average response was computed by estimating the response function per subject with the multi-shell, multi-tissue constrained spherical deconvolution CSD algorithm (*msmt_csd*) recommended even for single-shell data (Dhollander et al., 2016). These were then averaged across subjects. The location and orientation of the white matter fixels are computed through the segmentation of each FOD lobe in the template (method described in Smith et al., 2013). All 29 participants' FOD images were registered to the study FOD template using FOD guided non-linear registration (Raffelt et al., 2011, 2012) and then segmented to produce a set of discrete fixels (Raffelt et al., 2017; Smith et al., 2013). The resulting template is an unbiased group average and the estimated fixels are representative of the subjects in the study. Fiber cross-section (FC), fiber density (FD), and a combined measure of fiber density and cross-section (FDC) were computed for each subject. Log (FC) was used, to ensure that data is centered at about zero and is normally distributed. Finally, whole-brain fiber tractography was performed on the FOD template on 20 million streamlines that were further sifted to two million streamlines, using spherical deconvolution informed filtering (SIFT; Smith et al., 2015), to reduce bias in tractogram[32] densities. All preprocessing steps were conducted using commands implemented in MRtrix3 (www.mrtrix.org) or using MRtrix3 scripts that interfaced with external software packages (Tournier et al., 2019).

For the correlation analysis, the cerebellar tracts that showed significant differences between the groups were categorized into white matter tracts, using DWI atlases to guide categorization (Oishi et al., 2009; Wakana et al., 2004), and tracks were generated using the automated TractSeg method on the population's FOD template. This method has been shown to provide reliable and accurate tract segmentation (Wasserthal et al., 2018). Tract segmentation and orientation maps were created for specific bundles (namely, the inferior

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[32] *Tractograms* - virtual representations of the white matter fibers of the brain.

cerebellar peduncle). The resulting pictograms were converted to fixel maps by cropping the template fixel mask to the fixels that were traversed by the streamlines that were part of the tract of interest, using the *tck2fixel* command in MRtrix (Tournier et al., 2019). The *mrstats* command in MRtrix (Tournier et al., 2019) was used to compute mean FD, FC (log), and FDC on each participant's data, by providing the *'-mask'* option to the command. The output of the command gives a range of statistics (including the mean) that can be further used for statistical analysis.

4.1.4. Statistical Analysis:

4.1.4.1. Whole Brain Analysis

Fixel-based statistical analysis and inferences were performed across groups for each white matter fixel by a GLM, comparing monolingual to multilingual participants. Mean-centered intracranial volume (ICV), computed using *Freesurfer* image analysis suite (http://surfer.nmr.mgh.harvard.edu/), was included as a nuisance covariate in the GLM. The connectivity-based smoothing and statistical inference were done using connectivity-based fixel enhancement (CFE), (Raffelt et al., 2015) on the template tractogram (i.e. 2 million streamlines). The default smoothing parameters described in Raffelt et al, 2015 were used (smoothing = 10mm full-width and half maximum, C=0.5, E=2, and H =3). Family-wise error (FEW) corrected P-values were computed using non-parametric permutation testing of 5000 permutations (Nichols & Holmes, 2002). Significant fixels ($p < 0.05$, FWE corrected) were displayed using the *mrview* tool in MRtrix3. Streamlines corresponding to significant fixels were additionally displayed on the template tractograms and were color-coded by effect size in terms of percentage relative to the control group (monolinguals) and by direction (left-right: red, inferior-superior: blue, anterior-posterior: green, at FWE-corrected *p*-value of $p < 0.05$ (see Figure 30 and 33). All whole-brain fixel-based statistical analyses and visualizations were performed in MRtrix3.

4.1.4.2. Correlation analysis

For the correlation tests, I limited my analysis to the bilateral inferior cerebellar peduncle (ICP) which has been predicted to be associated with high proficiency and high immersion in a second language. Pearson's correlation tests of mean FD, FC (log), and FDC and behavioral measures for the multilingual group were performed. Correlation analyses were performed for bilateral ICP and proficiency (as measured by the average score of the proficiency tests for each language), immersion (as measured by the amount of time the participants spend practicing the L2 daily), AoA, and participants' chronological age (see Chapter I for a detailed description of these variables). The correlation analysis and creation of plots were done in the R software (R Core Team, 2020; *www.R-project.org)*.

4.2. Results

4.2.1. Whole-Brain Analysis Results

4.2.1.1. White Matter Differences for a Comparison of Multilinguals > Monolinguals.

Figure 28. shows fixels that had significantly higher FC, FD, and FDC for the multilinguals compared to the monolinguals. The microstructural differences in fiber density (FD) were limited to a segment of the right anterior cingulum bundle. The differences were up to > 15% higher for the multilingual group (see Figure 29).

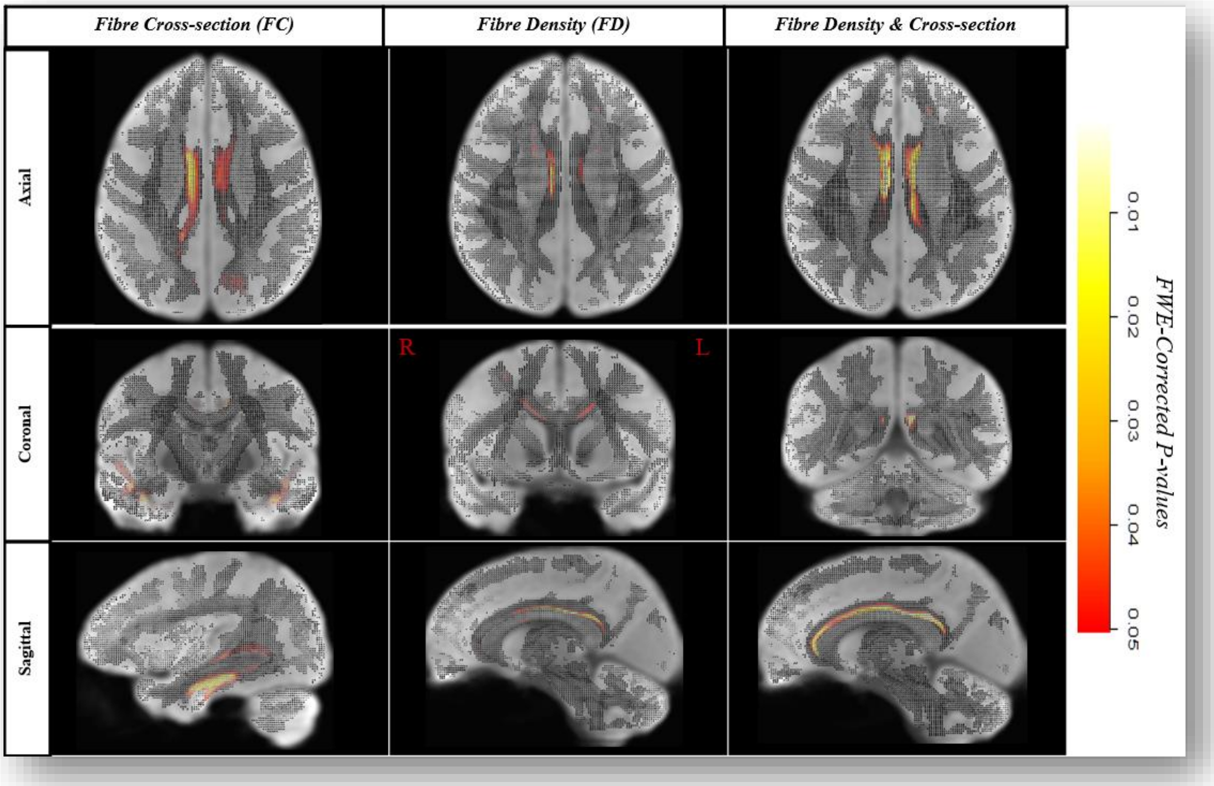

Figure 28. White Matter Significant Fixels - Multilinguals > Monolinguals

Figure 28. Description: Fixels with a significant ($p<0.05$) higher fiber-bundle cross-section (FC), fiber density (FD), and fiber density and cross-section (FDC) for multilinguals compared to monolinguals. Fixels are color-coded by family-wise error (FWE) corrected p-values and overlaid on the white matter study template map

On the other hand, macrostructural differences in fiber bundle cross-section (FC) were more pronounced (up to > 40%) and included the bilateral cingulum (CG), the anterior sections of the ventral language pathway, including the IFOF and the ILF and UF. The segments that showed significant differences were the anterior sections, passing through the anterior temporal lobe. All fiber bundles showed right hemisphere dominance asymmetries. Additionally, one small segment of the dorsal pathway, namely the SLFtp, overlapping with the posterior segment of the angular gyrus (AF) showed higher FC values for the multilinguals compare to the monolinguals. Lastly, the right inferior cerebellar peduncle (ICP) showed a higher fiber cross-section in the multilinguals.

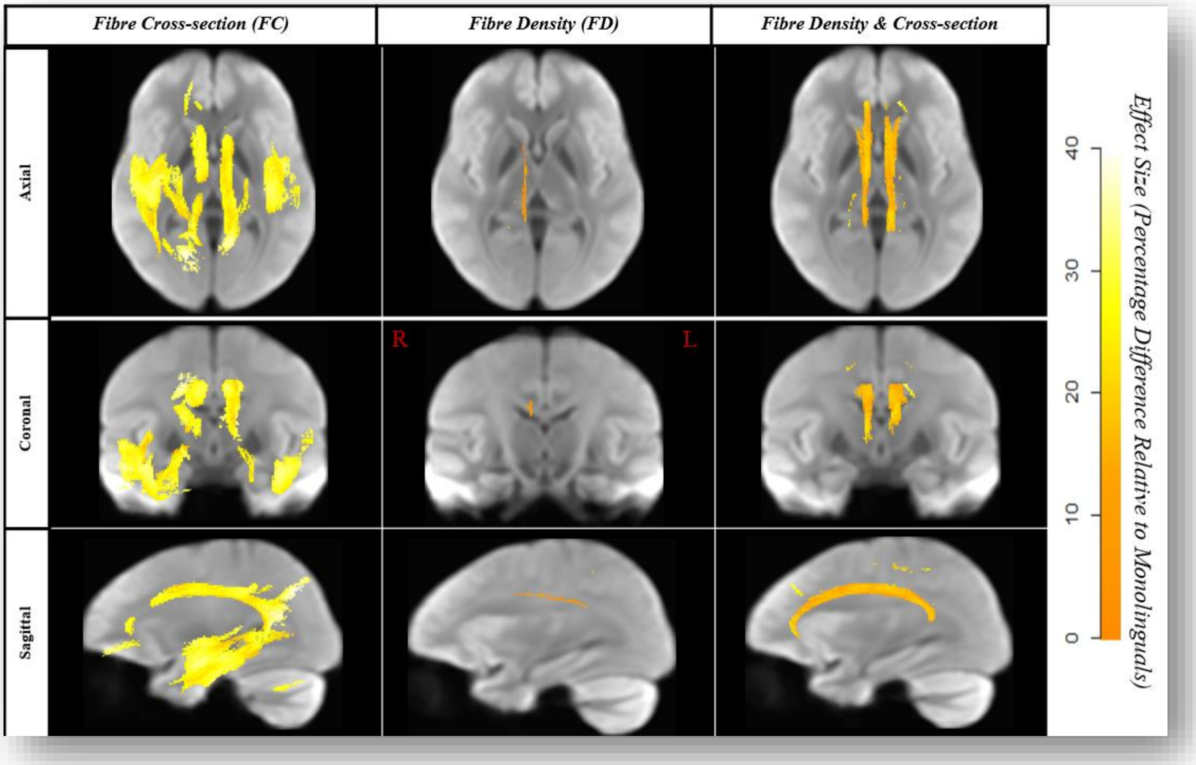

Figure 29. Tractograms of Significant White Matter Fixels – Multilinguals > Monolinguals

Figure 29. Description: Effect sizes are expressed as a percentage increase relative to the monolingual group. To enable the visualization of all the significant fixels in 3D, streamlines from the template-derived whole-brain tractogram were 'cropped' to include streamline points that correspond to significant fixels (FWE-corrected p-value <0.05). For a direct comparison of effect sizes across FD, FC, and FDC, streamlines shown correspond to significant fixels from all three analyses combined (i.e. the union of FD, FC, FDC).

The combined fiber density and cross-section (FDC) measure was significantly higher (effect size of > 30%) for the multilinguals in the bilateral cingulum (CG), a small segment of the dorsal language pathway (bilateral SLF I), and the most anterior aspect of the inferior fronto-occipital fasciculus (IFOF), in the frontal lobe, part of the ventral language pathway. The largest differences for this comparison were noted in the FC (see Figure 29). Figure 30 displays the streamlines corresponding to the significant fixels, derived from the whole brain tractogram, color-coded by direction (red: left-right, blue: inferior-superior, green: anterior-posterior).

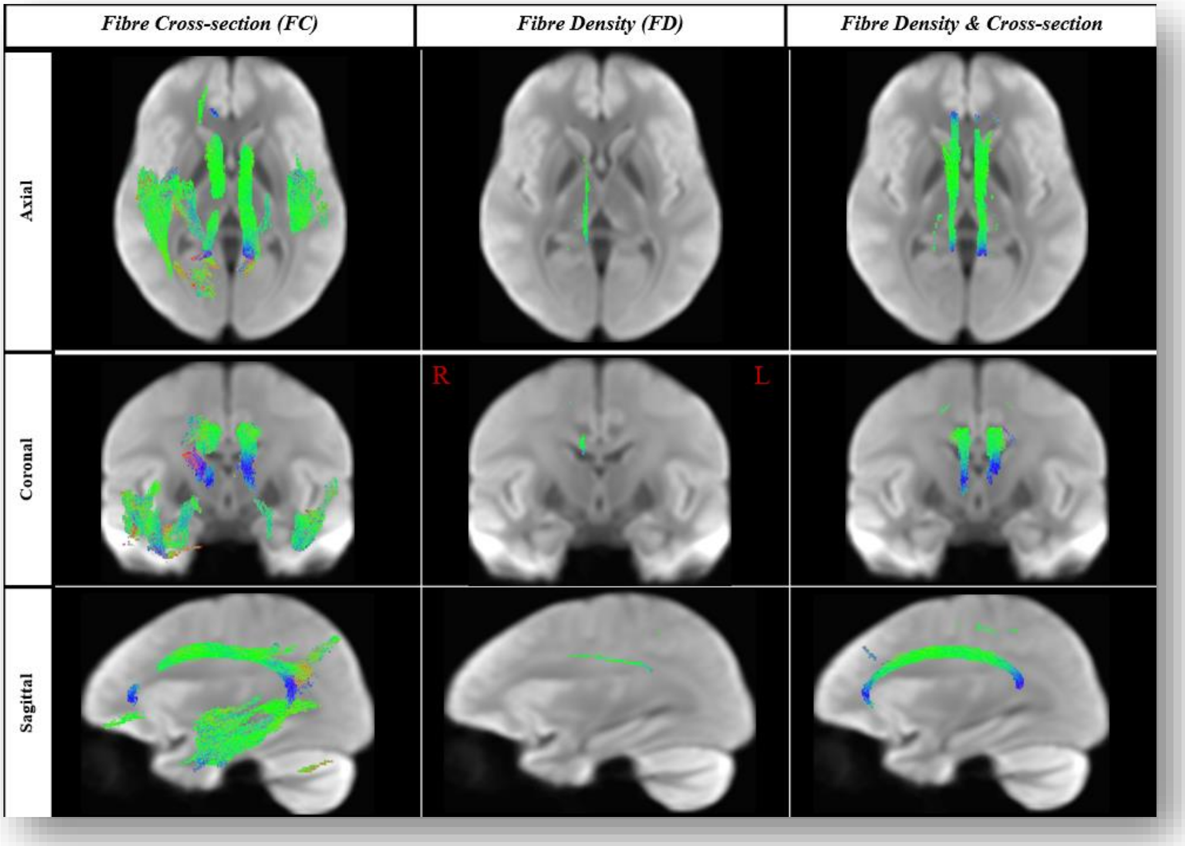

Figure 30. Reconstructed White Matter Pathways of Significant Fixels – Multi> Mono

Figure 30. Description: White matter pathways that showed significantly higher FD, FC, and FDC in the multilinguals compared to the monolinguals. To enable the visualization of all significant fixels in 3D (i.e. not just a 2D slice), streamlines from the template-derived whole-brain tractogram were 'cropped' to include streamline points that correspond to significant fixels (FWE-corrected p-value <0.05), and colored by direction (red: left-right, blue: inferior-superior, green: anterior-posterior).

4.2.1.2. White Matter Differences for a Comparison of Multilinguals < Monolinguals

Figure 31 shows plots of fixels that had significant (FEW, $p< 0.05$) lower FC, FD, and FDC for the multilingual group compared to the monolinguals. These are color-coded by family-wise error (FWE) corrected p-values and overlaid on the white matter template map. Differences were observed across different fiber bundles, with some fibers (e.g. the CC (splenium) exhibited lower fiber density (FD) up to < 46% (see Figure 32) in the multilinguals compared to monolinguals. Lower fiber density (FD) was observed in the medial pathway involved in language processing and control, namely, the corpus callosum (CC) radiation that passes through the splenium and the midbody, as well as anterior portions (passing through the temporal lobe) of bundles of the ventral pathway, namely the bilateral IFOF, ILF, and left UF. Fiber density was also lower in the left inferior cerebellar peduncle (ICP), medial segments of the right corticospinal tract, and bilateral fornix (see Figures 32 and 33).

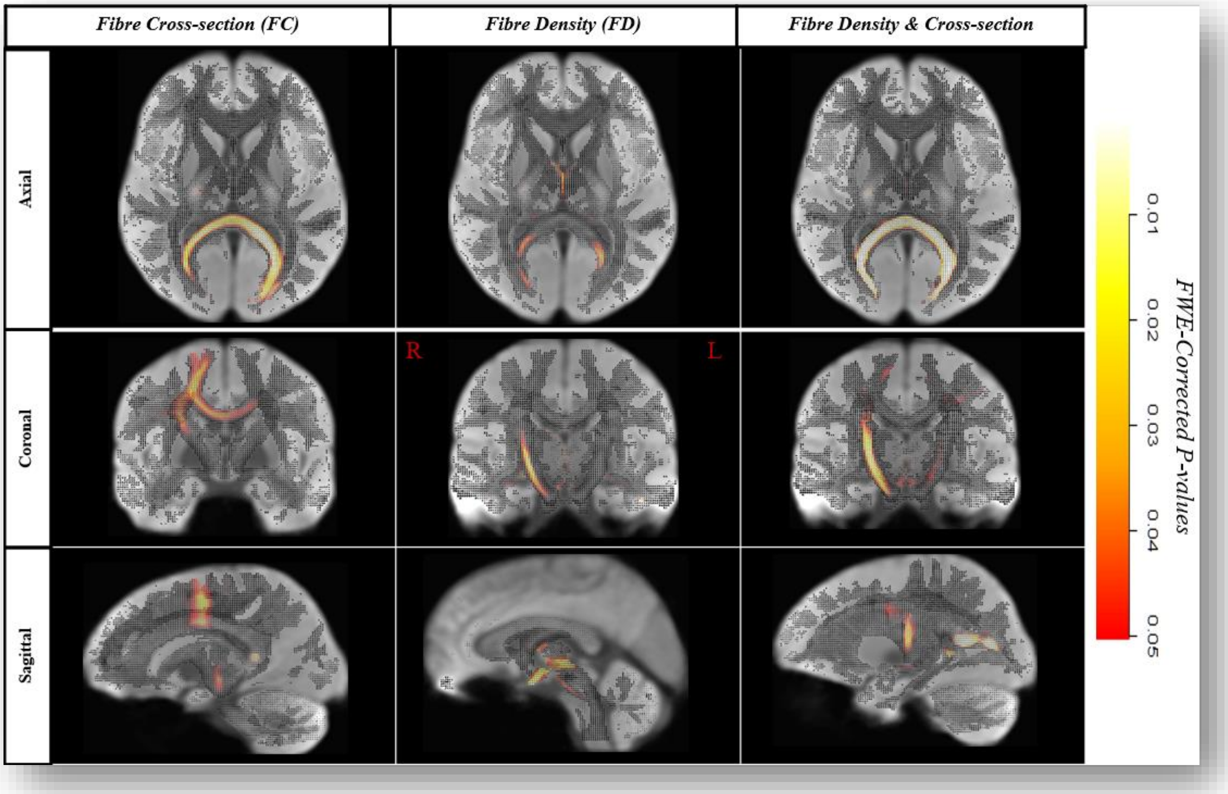

Figure 31. White Matter Significant Fixels - Monolinguals > Multilinguals

Figure 31. Description: Fixels with a significant ($p<0.05$) lower fiber-bundle cross-section (FC), fiber density (FD), and fiber density and cross-section (FDC) for multilinguals compared to monolinguals. Fixels are color-coded by family-wise error (FWE) corrected p-values and overlaid on the white matter template map.

Macrostructural differences in the fiber bundle cross-section (FC) were more pronounced and more spatially restricted to delimited fiber bundles, including the right cortico-spinal tract, the radiation passing through the central body of the CC, and the segment passing through the CC splenium. Figure 32 presents the results using template-driven streamlines. The streamlines presented here correspond to all the white matter fixels that exhibited significantly lower FD, FC, and FDC in the multilinguals. These are projected on top of the white matter population template and are color-coded by percent effect size relative to the monolinguals. Figure 33 displays the same streamlines, color-coded by direction (i.e. red: left-right, blue: inferior-superior, green: anterior-posterior).

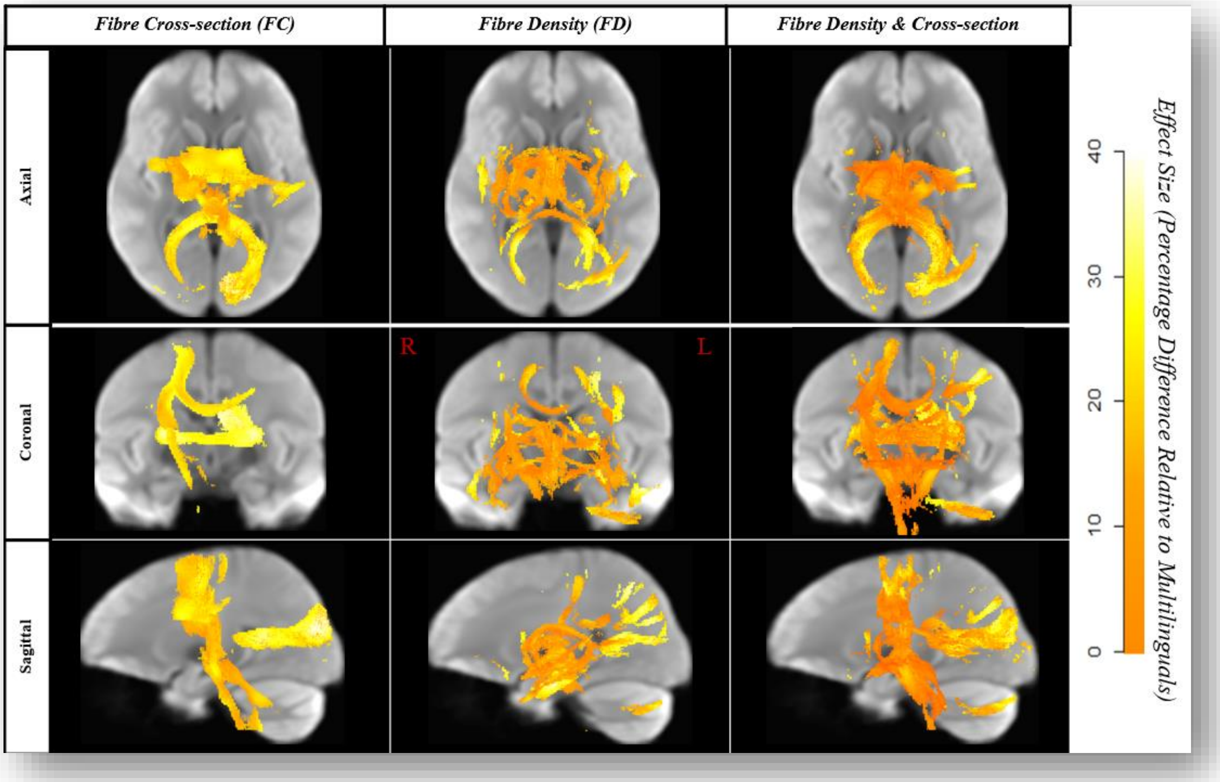

Figure 32. Tractograms of Significant White Matter Fixels – Monolinguals > Multilinguals

Figure 32. Description: Effect sizes expressed as a percentage decrease relative to the control group. To enable the visualization of all the significant fixels in 3D, streamlines from the template-derived whole-brain tractogram were 'cropped' to include streamline points that correspond to significant fixels (FWE-corrected p-value <0.05). For a direct comparison of effect sizes across FD, FC, and FDC, streamlines shown correspond to significant fixels from all three analyses combined (i.e. the union of FD, FC, FDC).

Combined macro - and microstructural differences (FDC) showed a combination of both bundles that showed a difference in the FD and the ones that were different in FC, with the exception of the ventral pathway bundles that showed a difference in FD (i.e. anterior portions of the IFOF, ILF, and UF). These include in the bilateral corticospinal tract (with leftward asymmetry), the radiation passing through the central body of the CC and the CC isthmus, as well as the fornix, and the left inferior cerebellar peduncle.

Noteworthy, the ventral pathway fiber bundles (i.e. IFOF, IFL, UF) showed a very specific change with higher FC (higher fiber bundles size perpendicular to the length of the fiber) and showed lower FD (lower volume of intra-axonal restricted water) values for the multilinguals. They did not show significant differences in the combined FDC measure. Table 18 lists a summary of the significant results.

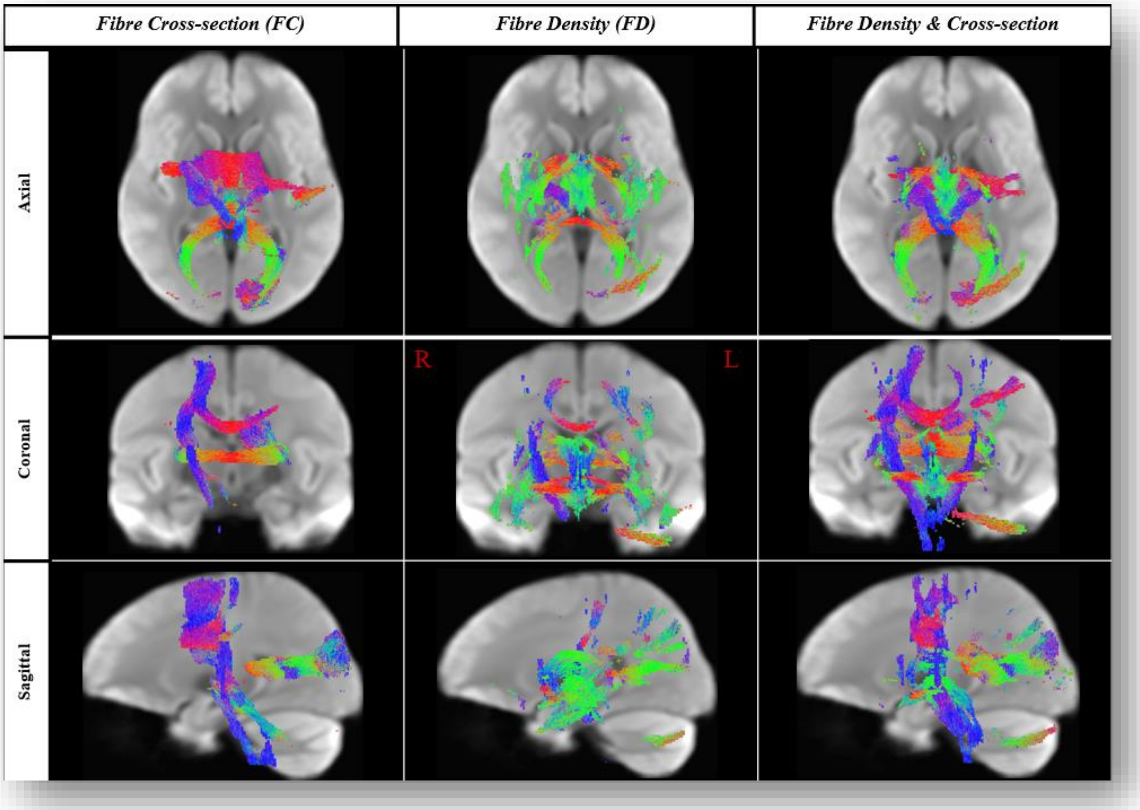

Figure 33. Reconstructed White Matter Pathways of Significant Fixels – Mono > Multi

Figure 33. Description: White matter pathways that showed significantly higher FD, FC, and FDC in the multilinguals compared to the monolinguals. To enable the visualization of all significant fixels in 3D (i.e. not just a 2D slice), streamlines from the template-derived whole-brain tractogram were 'cropped' to include streamline points that correspond to significant fixels (FWE-corrected p-value <0.05), and colored by direction (red: left-right, blue: inferior-superior, green: anterior-posterior).For a direct comparison of effect sizes across FD, FC, and FDC, streamlines shown correspond to significant fixels from all three analyses combined (i.e. the union of FD, FC, FDC).

Table 18. Fixel-Based Analysis Results of White Matter Morphometry

Multilinguals > Monolinguals		
FD		- right cingulum (CG)
FC		- bilateral cingulum (CG)
	ventral pathway	
		- inferior fronto-occipital fasciculus (IFOF)
		- inferior longitudinal fasciculus (ILF)
		- uncinate fasciculus (UF)
	dorsal pathway	
		- superior longitudinal fasciculus, temporo-parietal segment SLFtp (AF)
		- right inferior cerebellar peduncle (ICP)
FDC		- bilateral cingulum (CG)
	dorsal pathway	
		- superior longitudinal fasciculus (SLF I)
	ventral pathway	
		- inferior fronto-occipital fasciculus (IFOF)
Multilinguals < Monolinguals		
FC	*medial pathway*	
		- corpus callosum (CC) midbody
		- corpus callosum (CC) splenium
		- right corticospinal tract (CST)
		- Fornix (FX)
FD		- corpus callosum (CC) midbody
		- corpus callosum (CC) splenium
		- right corticospinal tract (CST)
		- Fornix (FX)
	ventral pathway	
		- inferior fronto-occipital fasciculus (IFOF)
		- inferior longitudinal fasciculus (ILF)
		- uncinate fasciculus (UF)
		- left inferior cerebellar peduncle (ICP)
FDC		- corpus callosum (CC) midbody
		- corpus callosum (CC) splenium
		- right corticospinal tract (CST)
		- Fornix (FX)
		- left inferior cerebellar peduncle (ICP)

Table 18. Description: Fiber bundles showing significant results in the FBA analysis for the multilinguals versus the monolingual group. CG: cingulum, IFOF: inferior fronto-occipital fasciculus, ILF: inferior longitudinal fasciculus, UF: uncinate fasciculus, SLFtp/AF: superior longitudinal fasciculus, temporal segment/Arcuate fasciculus, ICP: inferior cerebellar peduncle, CC: corpus callosum, FX: fornix, CST:cortico-spinal tract.

4.2.2. Correlation Analysis Results:

The DRM predicts an enhancement of the cerebellum associated with high proficiency/high immersion for bi/multilinguals. In order to test whether there is a relation between the white matter morphometry differences in the inferior cerebellar peduncle and multilingual background, I performed correlation analysis between the mean log FC, FD, and FDC, for the bilateral inferior cerebellar peduncle (ICP) and three background variables associated with the native language, the early and, the late L2s (i.e. age of acquisition, years of immersion, and average proficiency). A correlation between the background variables was also tested. The only significant positive correlation was between age and immersion in late L3 (English). There were no significant correlations between proficiency in Romanian (native language) and the ICP. Appendix J lists all the variables for which correlations were performed in Romania, Russian, and English, along with the computed *p* and the *r* values.

There was no significant correlation between mean FC (log) in the ICP and any of the behavioral variables. However, the FD metric showed a strong correlation with both Russian and English. A significant positive correlation (left ICP, $r_{(15)} =.51$, $p <.06$; right ICP $r_{(15)} = .71$, $p < .005$) was observed in the bilateral ICP (left ICP, $r_{(15)} =.51$, $p <.06$; right ICP $r_{(15)} = .71$, $p < .005$) with AoA of Russian (Early L2), while the AoA of the later learned English showed a negative correlation with the ICP that approached significance in the right hemisphere ($r_{(15)} = -0.46$, $p < .07$).

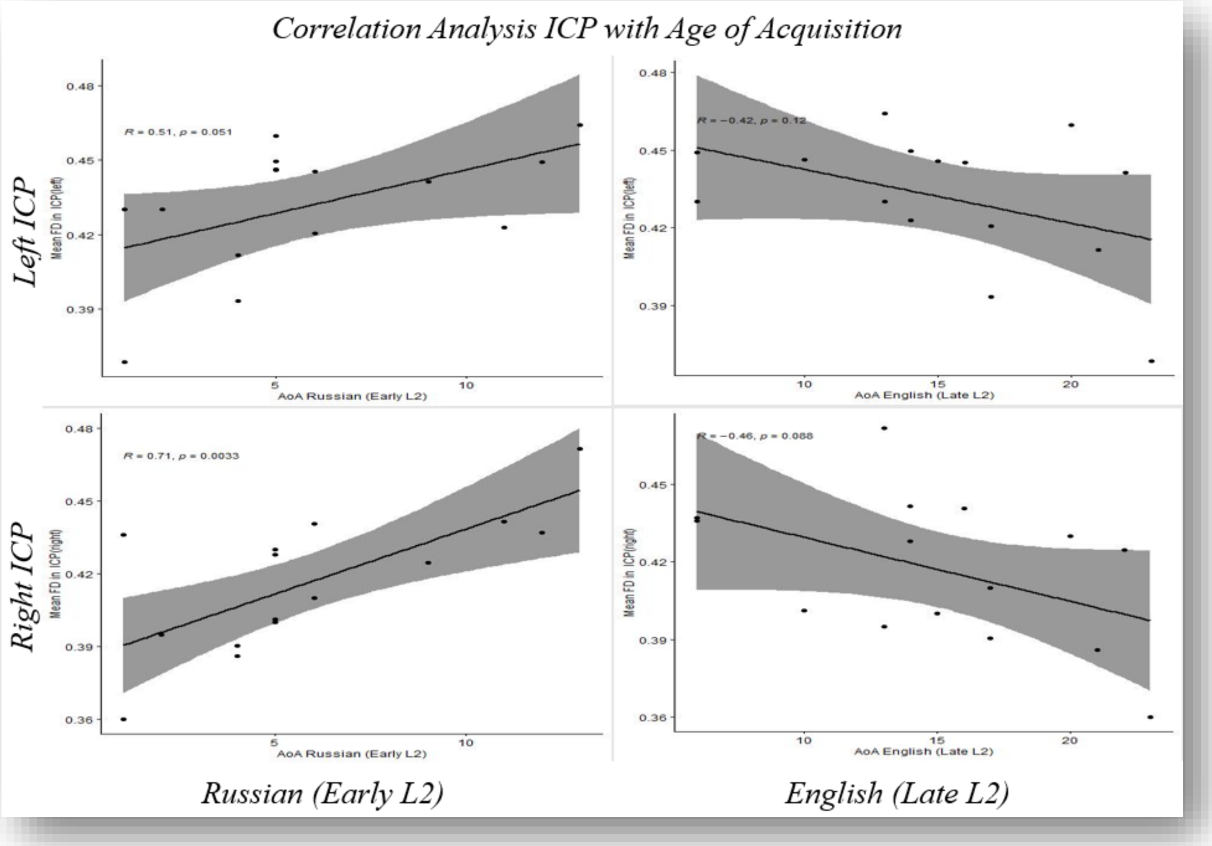

Figure 34. Correlation Plots Between AoA and Fiber Density in the Inferior Cerebellar Peduncle

Figure 34. Description: AoA: age of acquisition, ICP: inferior cerebellar peduncle, FD: fiber density

These data show that participants who learned Russian (early L2) later in life (approximately 10 years) show greater FD than participants who learned Russian earlier (approximately 5 years). In contrast, participants who learned the late L2 (English) late in life (approximately 20 years) show lower FD compared to those who learned English earlier in their life (approximately 10 years). Figure 34 shows the plots representing the correlation analyses between the AoA and the FD metric in bilateral ICP, for both second languages. It is important to note that the age range between the two languages differs, the early L2 (Russian) was learned during an approximately 10 years span across participants, while the late L2 (English) during approximately 20 years.

To test whether fiber density is associated with age, a correlation analysis between bilateral ICP and chronicle age was performed. Figure 35 shows the correlation plots between age and fiber density in the ICP.

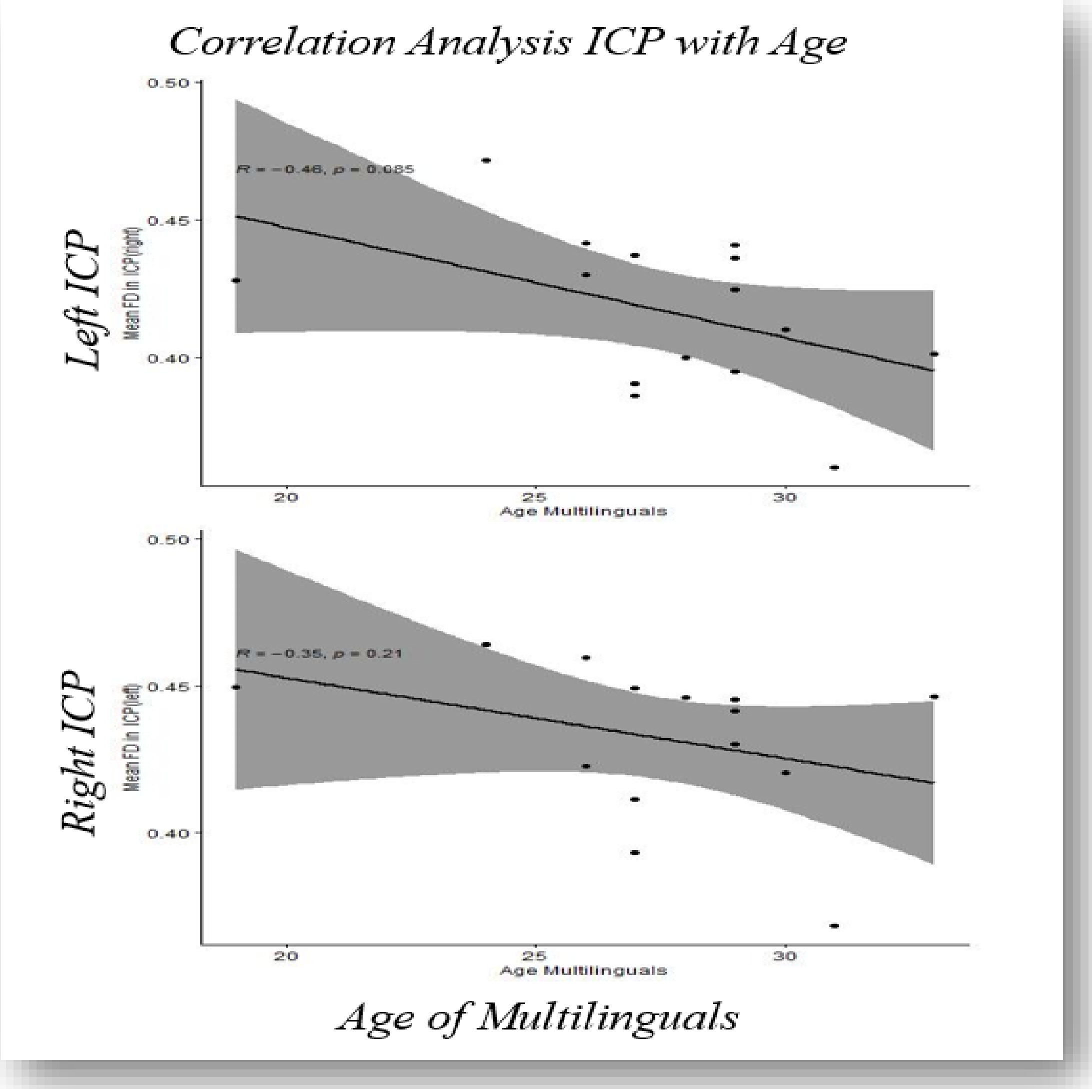

Figure 35. Correlation Plots for Age and Fiber Density in the Inferior Cerebellar Peduncle

Figure 35. Description: ICP: inferior cerebellar peduncle, FD: fiber density

The correlation analysis between age and left and right ICP was not significant (left ICP, $r_{(15)}$ =-.46, p <.09; right ICP $r_{(15)}$ = -.35, p < .2), although displayed an overall negative trend, with an overall decrease in fiber density (FD) as the participants become older.

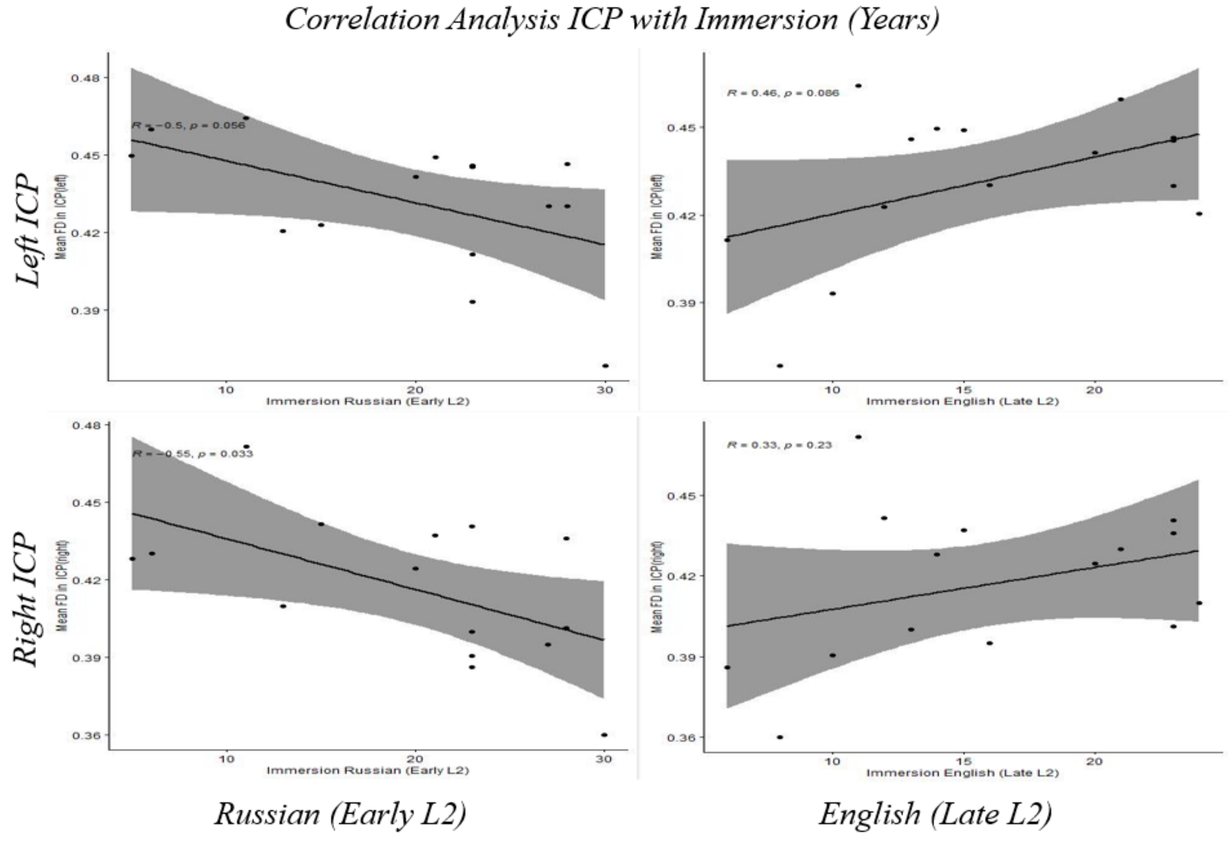

Figure 36. Correlation Plots Between Immersion and Fiber Density in the ICP

Figure 36. Description: ICP: inferior cerebellar peduncle, FD: fiber density

Correlation analyses between the amount of immersion the participants had in each of the second languages showed a negative correlation between the years of immersion in the early L2 (Russian) and bilateral ICP (left ICP, $r_{(15)}$ =.5, p <.06; right ICP $r_{(15)}$ = .55, p < .005). The amount of immersion in the later acquired L2 (English) showed an overall positive correlation with the ICP and approached significance in the left ICP ($r_{(15)}$=.46, p < .07). Figure 36 shows the correlation plots for bilateral ICP and immersion. These results suggest that using the early L2 (Russian) longer is associated to lower FD while using the late L2 (English) longer is associated with higher fiber density in the inferior cerebellar peduncle (ICP).

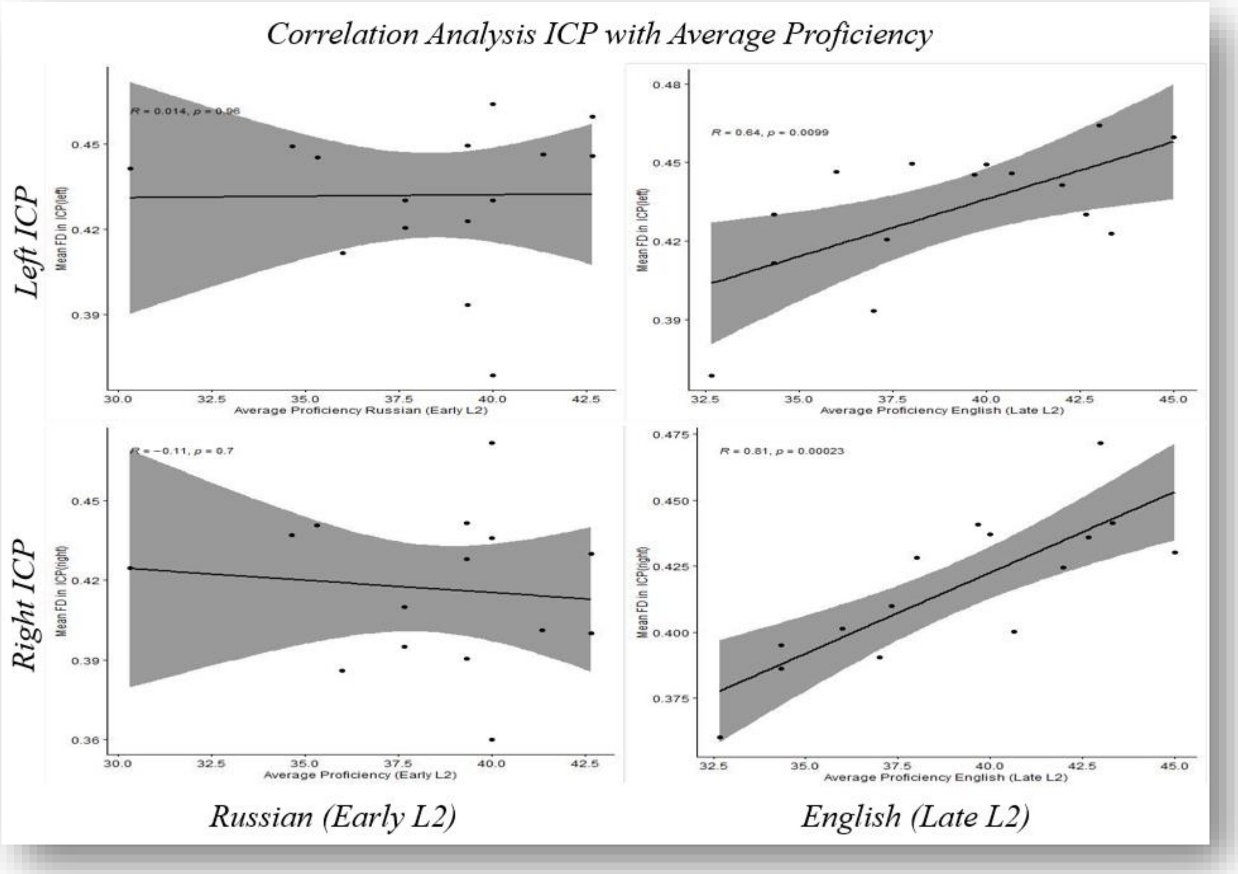

Figure 37. Correlation Plots Between Average Proficiency and Fiber Density in the ICP

Figure 37. Description: ICP: inferior cerebellar peduncle, FD: fiber density

Finally, a third correlation analysis was performed between the average proficiency and ICP fiber density (see Figure 37). These revealed significant positive correlations bilaterally in the ICP, with the proficiency in the later learned L2 (left ICP, $r_{(15)} = .64$, $p < .01$; right ICP, $r_{(15)} = .81$, $p < .0005$). There was no statistically significant correlation between the proficiency in the early acquired L2 and the ICP (left ICP, $r_{(15)} = .014$, $p < .97$; right ICP, $r_{(15)} = -.11$, $p < .7$). These correlation results suggest that participants with higher proficiency in the later learned a second language (English), show an increase in fiber density in the ICP, while there is no association between the density in this fiber and the early L2 (Russian) proficiency.

5. Discussion

In this study, I applied a recently developed method to investigate white matter changes in clinical and non-clinical populations. Fixel-based analyses do not only account for crossing-fiber populations within voxels but can also give a more comprehensive and biologically interpretable picture of ways in which intra-axonal volume changes may manifest (Raffelt et al., 2017). Compared to other diffusion metrics, the FBA can account for both microstructural changes (manifested in fiber density), macrostructural (expressed in the FC metric), and total axonal-volume changes (expressed by the FDC metric).

The first hypothesis I investigated was based on the BAPSS hypothesis (Grundy et al., 2017) predicting increases in white matter integrity in the anterior regions of the brain, in pathways involved in language processing, namely the *dorsal pathway* fiber bundles (SLF, AF) and the ventral pathway fiber bundles (IFO, ILF, UF). The results are in line with these predictions. Higher fiber cross-section (higher fiber bundles size perpendicular to the length of the fiber) and lower fiber density (FD; a lower volume of intra-axonal restricted water) values were observed for the multilinguals. These results are indicative of the same underlying structural changes and may be due to a change in axonal myelination, leading to greater intra-axonal space, an increase in axonal membrane packing, or a combination of these (which can result in both decreases in FD, and increases in FC) within these fibers for the multilingual compared to the monolingual group. These interpretations are consistent with bilingual neuroimaging literature, describing increases in FA and AD along with decreases in RD and MD (effects largely attributed to increased myelination), as indicators of increased communication efficiency (Luk et al., 2011; Pliatsikas, 2019, 2020; Pliatsikas et al., 2015).

Similarly, higher fiber cross-section (FC) and fiber density and cross-section (FDC) values were observed in bundles of the dorsal pathway, namely the SLF. Higher FDC values are associated with higher efficiency of communication within a fiber bundle (Raffelt et al., 2012; Raffelt et al., 2015, 2017).

The ventral pathway for language processing is involved in semantic and syntactic processing (Brauer et al., 2013; Hickok & Poeppel, 2007; Leclercq et al., 2010; Mandonnet et al., 2007; Martino et al., 2013; Pliatsikas, 2019; Tremblay & Dick, 2016). Multilingual speakers have to process semantic and syntactic information in all languages known. Differences observed in white matter bundles that realize the communication between regions involved in syntactic and semantic processing are suggestive of an increase in the efficiency of information transfer across such brain regions. The dorsal pathway connects areas involved in processing the spectro-temporal aspects of the speech (i.e. in the dorsal superior temporal gyrus (dSTG)), phonological processing (involving the mid-posterior superior temporal sulcus (mpSTS)), and mapping the sensory or/and phonological representations (anchored in the inferior parietal/superior posterior temporal sulcus) into articulatory representations (involving the pre-motor areas, posterior inferior frontal gyrus (pIFG) and the anterior insula). Speakers of multiple languages have an increased demand for extracting speech characteristics from multiple languages and mapping the important information from the acoustic signal to the appropriate articulatory gesture in the intended language, hence driving brain adaptations to increase communication between regions involved in these processes.

Noteworthy, the pattern of results observed in language processing pathways, namely, increased white matter macrostructure in the frontal regions of the brain, are in agreement with the predictions of the DRM, and the BAPSS, and further corroborate the patterns of change observed in most efficient language switchers, associated with a peak efficiency stage of second language expertise (Pliatsikas, 2020). Namely, the results from this study suggest that multilinguals exhibit a similar pattern of increased frontal white matter diffusivity (along with decreases in subcortical and cortical grey matter, see Chapter II) similar to simultaneous interpreters.

A second hypothesis investigated in this study was reduced FD, FC, and FDC values in the corpus callosum. Two different segments of the corpus callosum showed decreases in all three morphometry measures FD, FC, and FDC, for multilinguals compared to monolinguals. The splenium of the corpus callosum contains fibers connecting the parietal temporal language-related regions (Coggins et al., 2004). It has previously been reported to be involved in the interhemispheric transfer of auditory (Pollmann et al., 2002) and the development of verbal abilities (Nosarti et al., 2004). A previous electroencephalography (EEG) study discusses the functional relevance of the posterior corpus callosum in the processing of suprasegmental prosodic information and syntactic information in patients with posterior callosal lesions. Compared to the controls, which displayed an N400 effect for prosodically mismatched verb-argument structures, signaling lexical integration difficulty for unexpected argument structure for certain verb classes (e.g. a structure in which a direct object follows an intransitive verb) the patients did not exhibit a prosody-induced N400 effect (Friederici et al., 2007). These results are suggestive that syntactic processing during language comprehension in these patients is not influenced by prosodic information. The authors underly the relevance of the posterior part of the CC (including the isthmus) in the interplay between the verb argument and the relevant syntactic information provided by the prosody. Multilinguals, compared to monolinguals, have increased demand in computing the syntactic structure by using different prosodic information (i.e. appropriate for the language spoken in a specific interactional context) for adequate interpretation of the sentences' meanings.

Surprisingly, multilinguals showed overall lower FD, FC, and FDC compared to the monolinguals in several additional fiber bundles, including the right corticospinal tract (CST), and the fornix. Differences in these seem to be mostly related to changes in the fiber cross-section. Importantly, similar fiber bundles have been shown to exhibit reduced FA in simultaneous interpreters compared to multilinguals (Elmer et al., 2011; see details of the study described above). The authors suggested that these changes are related to the specific language-related demands associated with interpreting and are indicative of enhanced efficiency in these tracts. The corticospinal tract and the mid-body of the CC contain fibers related to motor control. The changes observed in these bundles may be related to adaptations supporting speech-articulation and sensory-motor planning (sound-to-motor mapping), fundamental in language production and perception. Thus, these may be the result of adaptations to the greater demands of mapping sounds to the multiple articulation gestures in all languages spoken by the multilingual.

In the bilingual neuroimaging literature decreases in FA are interpreted as decreases in white matter diffusivity, as a result of increased myelination. These are also believed to be associated with increases in the overall efficiency of communication between the hemispheres, for the multilinguals compared to monolinguals. As mentioned before, although these changes have been attributed to the process of myelination, the biological underpinnings of fractional anisotropy are poorly substantiated. The results of this study, namely, decreases in FD, FC, and FDC, with the largest effects in fiber cross-section (FC) in the above-mentioned fiber bundles, are not favoring interpretations of changes in myelination. An increase in myelination is most likely to be associated with an overall increase in the total volume fraction occupied by the fiber bundle, as measured by FC, here an overall lower FC is observed in these bundles. If increased efficiency in these fiber bundles is to be assumed, a potential mechanism leading to overall decreases in these pathways may be a change in axonal membrane packing, that restrict water diffusion perpendicular to the axonal length, yet the intra-axonal volume of restricted water, along with its width show overall reductions.

Unrelated to the hypothesis I investigated in this study, a fiber bundle that showed consistent higher morphometry values (for FD, FC, and FDC) in multilinguals compared to monolinguals was the bilateral cingulum. The cingulum bundle is a white matter tract that connects the frontal, temporal and parietal regions, as well as the cingulate gyrus to the subcortical regions (Bubb et al., 2018). Accumulative evidence from both non-human and healthy and clinical human populations has implicated the cingulum in tasks involving working memory, attention, and executive functions. (for a detailed review, see Bubb et al., 2018). The cingulum has been documented in only very few studies, to show white matter differences in bilinguals. Rahmani et al., 2017, looked at a group of bilinguals compared to monolinguals and found an increase in quantitative anisotropy in the bilingual group. Another study reported an association between the fractional anisotropy in the dorsal and anterior segments of the cingulum with the level of L2 usage, of a group of native Italian adults, speakers of English as a second language. There is ample evidence showing the involvement of the anterior cingulate cortex (ACC) in linguistic and non-linguistic activities that require conflict and error monitoring is unequivocal (Abutalebi & Green, 2016; Seo et al., 2018) Similarly, the involvement of the subcortical areas (linked to the ACC through the cingulum) in language control processes is unequivocal (e.g. Abutalebi & Green, 2016; Pliatsikas, 2019). The increased demands in control in multilinguals related to juggling multiple languages and resolving interference from the inappropriate languages, impose increases in recruitment of areas related to control in bi/multilinguals and may lead to restructuring in pathways related to language control, such as the cingulum bundle.

A final hypothesis that I investigated was the DRM prediction of increase white matter integrity in the cerebellum. I expected that this will manifest as an increase in the fiber density (FD), cross-section (FC), and density and cross-section (FDC) in the inferior cerebellar peduncle (ICP). The results of this study show higher fiber cross-section (FD) values in the right inferior cerebellar peduncle for the multilinguals compared to the multilinguals. However, lower fiber density and fiber density and cross-section in the right inferior cerebellar peduncle (ICP).

The cerebellum does not appear often in discussions of white matter diffusivity differences in the bilingual literature; however, the limited available evidence suggests that the cerebellum plays a crucial role in multilingual language processing. Most of the available studies show volumetric increases in the cerebellum for bilinguals versus monolinguals (e.g. Burgaleta et al., 2016; Filippi et al., 2011; Pliatsikas et al., 2014). Grey matter volume and density in the cerebellum are associated with the efficiency of language interference suppression in bilinguals (Filippi et al., 2015, 2020) and how native-like the L2 accent of bilinguals sounds (Berken, Gracco, et al., 2016). A recent longitudinal study, looking at highly immersed and highly proficient bilinguals, reported restructuring in the cerebellum, modulated by the amount of time the bilinguals had been using their second language before being immersed, as well as the amount of immersion (Deluca et al., 2019). Pliatsikas, 2019, suggest that structural changes (i.e. increases in grey matter volume and white matter integrity) in the cerebellum may only occur with high proficiency and high immersion.

Evidence suggests the involvement of the different areas of the cerebellum in different aspects of language processing. For example, the right cerebellar hemisphere has been shown to be activated during the processing of suprasegmental features of speech processing. Right medial posterior cerebellar activation was associated with the processing of speech and prosodic cues in a phrase processing task, in which the meaning of the phrase changed depending on the segmentation of the phrase (Strelnikov et al., 2006). Similarly, Chen & Desmond, 2005, propose two anatomically distinct roles during verbal memory: articulatory rehearsal (involving the right lateral cerebellar hemisphere/Broca's area loop) and the phonological maintenance and storage (supported by the right posterolateral cerebellar hemisphere and the left inferior Parietal Lobule). On the other hand, the left cerebellum was associated with syntactic processing. For example, Murdoch & Whelan, 2007, described 10 patients with primary left cerebellar strokes compared to a group of non-neurologically impaired controls. Their findings suggest that left cerebellar lesions lead to impairment in sentence re-creation tasks, word association tasks, word relation tasks (antonym/ synonym generation), and sentence interpretation of semantic absurdities, as well as ambiguous and figurative speech. The differences in the microstructure across the hemispheres for the multilinguals (greater fiber cross-section in the right hemisphere and lower fiber density (FD) and fiber density and cross-section (FDC) in the left hemisphere) may be the result of different involvement of the two hemispheres in language processing.

Similarly, the observed changes in the cerebellum may be the result of mixed adaptation patterns as a result of restructuring influenced by the early learned L2 and a later learned L3, which may result in different structural changes. The correlation analysis results corroborate these predictions. A positive correlation was observed between the bilateral ICP and the AoA of an early learned L2, suggesting greater FD with earlier age of acquisition. The AoA of a later learned L3 showed the opposite relationship with the FD values in bilateral ICP. As predicted by the DRM, these patterns of results suggest that restructuring in the cerebellum occurs as the time spent practicing a second language increases. These changes seem to be mostly associated with changes at the microstructural level (i.e. FD). This is followed by a renormalization of the white matter microstructure with a prolonged time of usage, increased immersion, and high proficiency for a second language. Changes associated with decreases in fiber density and cross-section. These interpretations are supported by the negative correlation between the AoA of the late L2 and the FD values in the ICP, along with the positive association between the FD values with both the amount of immersion in a second language environment and L2 proficiency, overall, suggesting that observable restructuring changes increase with earlier AoA, hence higher immersion time and higher proficiency.

Additionally, the positive correlation between the AoA (suggestive of increased restructuring with later age of acquisition), along with the negative association of the amount of immersion, and lack of association between the proficiency in an earlier learned L2, with the ICP fiber density, are suggestive of a renormalization process in the white matter structure of the cerebellum, with increased time spent practicing a second language.

These patterns parallel changes observed in the grey matter (results described in the previous chapter) at peak efficiency multilinguals speakers, namely increases in tissue density and volume at consolidation stages (when the speaker start gaining proficiency in a second language) with decreases when peak efficiency in a second language is achieved (Grundy et al., 2017; Pliatsikas, 2020). However, more research is needed to confirm these suggestions.

6. Limitations and Further Directions

One of the limitations of the FBA technique is its inability to identify the confounding potential contribution of the different factors for the metrics computed. The FBA metric does not provide a direct measure of axonal myelination, which has been proposed as the main factor resulting in the changes observed in bilingual studies. However, additional information is needed, such as the use of T1 relaxometry (De Santis et al., 2016), to estimate the fixel-specific myelin content. Another limitation related to the apparent fiber density within a voxel is that the measure is influenced by the fraction of the volume that is occupied by the crossing fibers within one single voxel. Additionally, the fiber density measure is confounded by the changes in axonal density, axonal diameter, and membrane permeability which all influence the FD metric (for additional details, see Raffelt et al., 2017).

Many studies have reported white matter interhemispheric asymmetries for specific fiber bundles with both studies using the DTI (e.g De Schotten et al., 2011; Park et al., 2004) and FBA techniques (Arun et al., 2021; Bokde et al., 2001). Although the results remain contradictory, the many asymmetries within the white matter of healthy populations which have been reported with both left>right and right>left dominances may play an important role in the interpretation of the results in studies looking at healthy human subjects. Nonetheless, more research is needed to identify the contribution of the specific metrics for the asymmetries in specific fibers.

This study used a novel method for estimating white matter morphometry adaptations in a group of multilinguals compared to monolinguals. More research is necessary, using the fiber-specific characterization of WM changes, in order to better understand micro-and-macro-structural changes related to bi/multilinguals practice.

Chapter V

Conclusions

This dissertation project aspired to be one of the few attempts to integrate behavioral, functional, structural, and diffusion MRI for a wholistic characterization of functional and anatomical differences in a group of highly proficient multilinguals compared to a group of monolinguals. Brain neural adaptation can happen at various levels, including a change in the responsiveness of the neural population (best measured through BOLD), a change in the structural resources (best described by investigations of grey matter thickness and volume), or a change in the connectivity between brain regions (investigated through white matter diffusivity and network analyses). This project combined different MRI imaging modalities to target changes in function, structure, and connectivity, and obtain complementary information related to brain adaptations in the groups investigated. In doing so, I attempted to get a more comprehensive picture of brain plasticity as an effect of multilingual experience. The general contribution of such nuanced descriptions of brain adaptations from both functional and structural data is to help build a more comprehensive theoretical framework of multilingual brain plasticity.

In this project, I was able to investigate important questions related to models of language processing and language control. The theoretical framework was built upon fundamental predictions of recent dual-stream models of language processing, proposing a *dorsal pathway*, involved in planning, auditory-to-motor mapping, and syntactic processing, and a *ventral pathway*, delivering the interdependence between sound and meaning and supporting basic syntactic processing (Friederici & Gierhan, 2013; Hickok & Poeppel, 2007; Saur et al., 2008). Together these pathways compose the core brain nodes of the language network (Fedorenko & Thompson-schill, 2014). Along with these, another set of brain regions involved in language control, that are particularly recruited during language processing in bi/multilinguals (Del

Maschio & Abutalebi, 2019; Golestani et al., 2006; Indefrey, 2006; Pliatsikas & Luk, 2016a), create the periphery language nodes of the language network. Through these rich data, I was able to ask questions of language representation in the brain, within the core and periphery brain regions, as well as investigate brain restructuring patterns related to previously predicted trajectorial changes (i.e. the dynamic restructuring model (DRM); Pliatsikas, 2020); and the bilinguals anterior to posterior and subcortical shift hypothesis (BAPS; Grundy et al., 2017), associated with specific stages of second language learning.

I start my discussion with an introductory chapter providing a state-of-the-art description of the main issues related to the neurolinguistics aspects of multilingualism. Bi/multilinguals research through the last two decades showed time and time again that multilingual practice affects brain organization that starts as soon as a second language is learned (sometimes early infancy) and continues into adulthood. Generally, acquiring and using a/or more second languages has been shown to promote more efficient language learning in children (Bradley et al., 2013; Petitto et al., 2012), seems to have a positive effect of developing control mechanisms and executive function (e.g. Bialystok et al., 2009; Dong & Li, 2015; Festman et al., 2010; Green & Abutalebi, 2013; Poarch & Bialystok, 2015), in both children and adults and is associated with a later onset of dementia in older adults (e.g. Alladi et al., 2013; Bialystok et al., 2007; Pliatsikas et al., 2020). Hence, understanding the neurobiology of multilingualism has great implications for gaining insight into the hallmarks of cognitive and language development throughout life (that have a further consequence for educational and public health policies in a given society), as well as predicting a loss of function and developing appropriate therapies for individuals that have lost language abilities.

Different background factors modulate restructuring patterns of the multilingual brain and need to be appropriately described when investigating brain adaptation differences across groups. In Chapter I, a description of the multilinguals background characteristics is provided, obtained through multiple proficiency and self-evaluation measures. A self-rating questionnaire was used to assess language use, age of acquisition, and language immersion metrics, a language production and vocabulary test (MINT) was used to assess vocabulary and production, a semantic and vocabulary knowledge (SHIPLEY), as well as a grammatical assessment test (GAT) were also performed. Together these tests showed that the participants are highly proficient in all three languages assessed. Additionally, background measures of language use showed that the participants have high immersion in both languages (over 10 years of everyday usage for both second languages), and two of the languages (namely the later acquired L2 (English), and the native Romanian) were used on everyday life to the same extent, at the time of the scanning. The unique background characteristics of this group, specifically the differences in age of acquisition and language use, along with equal proficiency for the second languages, allowed for further correlation analysis to investigate associations with the MRI obtain metrics.

I start the discussion on multilingual brain plasticity in Chapter II with an investigation of functional activation differences across the groups. I first section of the chapter tested whether our functional task, modeled after Fedorenko & Thompson-Schill, 2014, revealed similar activation patterns in language 'core' areas, and brain regions involved in language control. I further investigate the effect of different background factors on native versus early and late second language representation within periphery language regions, a priori defined, based on the adaptive control hypothesis (ACH) framework. The results of this study suggested similar recruitment of core language regions across both groups, and stronger recruitment of the control regions, part of the cingulo-striatal network. Additionally, activation levels within these control regions seem to be modulated by language dominance.

Chapter III describes a study investigating grey matter changes within both core and periphery control regions. Here I provide unique evidence of grey matter thickness adaptations at peak efficiency stages of language expertise, supporting predictions of the dynamic restructuring model (DRM; Pliatsikas, 2020), which suggests that with continuous usage of a second language, and exposure to highly complex multilingual interactional contexts, additional brain restructuring effects are observed. The results show that compared to monolinguals and non-expert bilinguals, multilinguals and simultaneous interpreters show additional reductions and adaptations in grey matter, that exceed values reported before second language learning. These changes seem to be associated with differences in the amount of time the multilinguals have used a language and have been immersed in a bilingual environment (a.k.a. language immersion).

Changes in grey matter, associated with peak efficiency stages have been predicted to be associated with white matter adaptation in anterior brain regions (i.e. bilingual anterior to posterior and subcortical shift (BAPSS) hypothesis; Grundy et al., 2017). These predictions were tested in Chapter IV, using a recently developed method to investigate white matter changes (Raffelt et al., 2017). Increases in anterior brain white matter connectivity (expressed in decreases in fiber density and increases of fiber-cross section) were observed in the anterior section of fiber bundles creating the ventral pathways of the language processing network. Additional differences observed across groups in the corpus callosum, corticospinal tract, and the fornix suggest overall decreases in the efficiency of communication within these bundles for the multilingual compared to monolinguals. Such unexpected results are yet to be understood and framed within the current models of bi/multilingualism. Table 19 below, list the results obtained from the MRI data in studies described in this thesis.

Table 19. Combined Results Obtained from the MRI Data, all Three Studies

Results from Three MRI Measures						
Functional Data			**Structural data**		**White Matter**	
Multi > Mono	***L1> L2***	***L3 > L1***	***Mono > Multi***	***Multi >Mono***	***Mono > Multi***	***Multi > Mono***
r. STG			r. STG			
	r. AG		r. AG			
			l. Hesghl's Gyrus			
r. precuneus			l. Precuneus (PL)			
			l. Insula			
r. Insula						
r. ACC	r. ACC			r. ACC		
	l. ACC	l. ACC				
r. Posterior CC						
l. Caudate (head)	l. Caudate (head)					
	r. Caudate (head)					
l. Caudate (body)	l. Caudate (body)	l. Caudate (body)				
	r. Caudate (body)	r. Caudate (body)				
	l. Putamen	l. Putamen				
	r. Putamen	r. Putamen	r. Putamen			
	l. GP	l. GP				
	r. GP	r. GP				
	l. Ant Thal					
	l. Cer. Crus II					
	l. Cer.VIIb	l. Cer.VIIb				
	l. Cer.VIII	l. Cer.VIII				
		r. Cer.VIII				
		l. Cer.IX				
		r. Cer.IX				
					CC (midbody)	
			CC (isthmus)			
			CC (splenium)		CC (splenium)	
					FX	
					r. CST	
					l. ICP	
						r. ICP
						r. Cingulum
						l. Cingulum
						l. IFOF (ant)
						r. IFOF (ant)
						l. ILF (ant)
						r. ILF (ant)
						r. SLF

Table 19. Description: Mono: monolinguals, Multi: Multilinguals, r: right, l: left, STG: superior temporal gyrus, AG: angular gyrus, ACC: anterior cingulate cortex, PL: parietal lobe, GP: globus pallidus, Ant Thal: anterior thalamus, Cer: cerebellum, CC: corpus callosum, FX: fornix, CST: corticospinal tract, ICP: inferior cerebellar peduncle, IFOF: inferior fronto-occipital fasciculus, ILF: inferior longitudinal fasciculus, SLF: superior longitudinal fasciculus, ant: anterior.

1. Framing The Results Within Current Literature

The findings from these three different MRI measures are complementary, reflecting the same underlying brain plasticity adaptations related to multilingual language function. As shown in Table 19, similar brain regions that have shown increases in recruitment during the functional task (e.g. right superior temporal gyrus, insula, anterior cingulate cortex, and the putamen), show differences in grey matter thickness across groups. Similarly, regions that showed significant relative deactivation across groups during the functional task, such as the angular gyrus (AG) and the precuneus showed changes in grey matter density. Along with changes in function and grey matter structure, white matter morphometry differences suggest stronger connectivity patterns between similar brain regions (e.g. increases in white matter fiber cross-section in anterior brain regions, with decreases in fiber density (FD), fiber cross-section(FC), and fiber density and cross-section (FDC) for posterior brain regions, including the precuneus and the angular gyrus, connected by the corpus callosum and posterior cingulum).

Overall, three main findings surface from these complementary investigations. First, monolinguals and highly proficient multilinguals similarly recruit core language brain regions during the processing of the native language, as well as the second languages (either learned early or later in life). This observation is corroborated with the results from the functional study (see Chapter II) that show similar recruitment of left hemisphere language areas, as well as the structural gray matter study showing similar matter thickness within left hemisphere core regions across both groups (see Chapter III).

Second, compared to monolinguals, highly proficient multilinguals strongly rely on the cingulo-striatal subnetwork implicated in cognitive control during language comprehension, and on the default-mode network regions (containing the precuneus, angular gyrus, and posterior cingulate cortex (PCC)) for off-task brain processes. Changes in two areas that anchor the cingulo-striatal network showed statistically significant differences in all three studies for the multilinguals compared to the monolinguals. An increase in recruitment in the insula and the ACC were observed in the functional task (Chapter II), differences in grey matter thickness in these regions were also noted (Chapter III) across groups, and lastly, increases in white matter integrity were observed in the anterior brain regions (containing the insula and the ACC), as well as in bilateral cingulum white matter, connecting the cingulate cortex to the rest of the brain. Deactivation for the task relative to the baseline condition for multilinguals compared to the monolinguals was observed in areas of the default-mode network, including the AG, precuneus, and posterior cingulate cortex (proposed as a "hub" region of the default-mode network (e.g. Sridharan et al., 2008), and these same regions exhibited decreases in the grey matter density for the multilinguals. These results are further affirmed by white matter restructuring. Changes in the posterior corpus callosum, connecting posterior brain regions as well as changes in white matter bilateral cingulum suggest an increase in the connectivity of areas belonging to these networks.

Lastly, multilinguals are not bilinguals. Functional data show that while for bilinguals the known languages are similarly active and competing for output, multilinguals show different patterns of language activation and inhibition. For multilinguals, language dominance, defined here as the proportion of time a language is used compared to another language by a multilingual seems to act as a modulator on the activation thresholds for each language. Namely, these data suggest that activation/inhibition strength is greater for languages that gain usage and dominance (i.e. control mechanisms are more taxed while processing these). Languages that are similarly proficient but less relevant to the context of the multilingual show lower activation/inhibition levels and require less control in processing. Grey matter adaptations corroborate with these findings, suggesting an association between immersion in a second language (note that generally, with increased immersion, proficiency increases and so does language dominance - true of the multilingual investigated in this study) and changes in grey matter thickness (see Chapter III) and white matter fiber density (see Chapter IV). Contrasted to monolinguals, highly proficient multilinguals typify simultaneous interpreters rather than bilinguals, in brain restructuring patterns showing decreases in grey matter for posterior brain regions and increases in white matter diffusivity in anterior brain regions.

The results of this project attest to the importance of integrating different measures, that cumulatively provide a more comprehensive picture of language function in the brain and adaptations associated with multilingual experience.

2. Limitations and Further Steps.

2.1. Limitations of the Project

The limitations of this project are related to the theoretical framework that I adopted, the MRI method, and the limited number of subjects from which the data was collected. It is worth noting that the domain-specific/domain-general dichotomy is challenged by some authors. For example, (Paradis, 2004; Paradis et al., 2013) proposes that while L1 rule-based grammar is implicitly acquired (dependent on procedural memory), and involves a fronto-striatal brain network, L2 grammars acquired later in life, are more dependent on declarative memory (explicit learning). Evidence for this view comes from Wattendorf et al., 2014, that compared brain activity for early versus late multilinguals proficient in three languages. The results showed that early multilinguals activate more a fronto-striatal network in the left hemisphere (namely the right homolog of Broca's area, bilateral supplementary motor area, the left striatum, the left anterior cingulate gyrus, and the left precentral gyrus), and activate less the posterior superior temporal gyrus (pSTG). The authors interpret the findings suggesting that the early multilinguals rely more on executive control, thus recruiting the network involved in language control, whereas late multilinguals recruit the posterior superior temporal gyrus, pSTG since they cannot rely on the already established networks for their early learned languages.

Additionally, although neurolinguistic studies report changes in brain structure (both in grey and white matter) related to bi/multilingualism (i.e. Grogan et al., 2012; Stein et al., 2012, 2014, etc.,) the results reported in the literature remain hazy and have low biological interpretability. This is the consequence of the limitations of the MRI technique. Lack of sensitivity to small-scale temporal events and direct access to neural activity and cellular structure, do not only make interpretations challenging, but also may mask effects of interest.

Another important limitation of this study is the number of participants. Data were collected from 30 participants (15 in each group), which provided relatively weak statistical power. Nevertheless, the results are complemented by the different methods and provide more reliable patterns of differences, despite the power limitation.

2.1. Further Steps

Bilingual neuroimaging models (e.g. the adaptive control hypothesis, ACH; Abutalebi & Green, 2016; Green & Abutalebi, 2013), stemming from analysis of univariate task-based functional MRI, show that bi/multilinguals additionally engage brain areas outside of the perisylvian language network compared to monolinguals. In these models, brain regions are associated with specific functions and are described as independent of other brain regions to which they communicate. However, the human brain is a complex system in which higher-level processes are not performed by regions in isolation rather by a cascade of intricately interacting units. The results of this project suggest that the complex, sometimes contradictory results observed in the neuroimaging literature (DeLuca et al., 2019; Pliatsikas, 2020) may be better understood in terms of brain networks.

These regions have been described as part of the domain-general executive control network, composed of a fronto-parietal and a cingulo-striatal subnetwork. However, recent research, mainly stemming from analysis of resting-state functional connectivity, suggests the existence of few different networks (e.g. Bressler & Menon, 2010; Dosenbach et al., 2008;

Fornito et al., 2012, etc.,). Using independent component analysis, Seeley et al 2007, were able to identify two distinct networks, one anchored in the frontal dorsal ACC and orbital fronto-insular cortices, and another in the DLPFC. The first comprised regions of the fronto-insular cortices, anterior insula, dACC, STP, the sublenticular extended amygdala, the ventral striatopallidal, dorsomedial thalamus, hypothalamus, periaqueductal gray, and the substantia nigra[33]/ventral tegmental[34] area. The authors referred to the network as the Salience network. While the network anchored in the DLPFC, which is referred to as the executive control network (ECN) included the bilateral DLPFC, the VLPFC, dorsomedial PFC, the lateral parietal cortices, a region in the lest fronto-insula, the dorsal caudate, and the anterior thalamus.

Sridharan et al., 2008, identified activation in three different networks. The network that was active during the task was the central executive network (CEN) anchored in the dorsolateral prefrontal cortex (DLPFC) and the posterior parietal cortex (PPC); at the same time, they observed de-activation in the default - mode network (DMN) with the key nodes in the ventromedial prefrontal cortex (VMPFC) and posterior cingulate cortex (PCC); a third network, namely the SN, comprising the fronto-insular (rFIC) and the anterior cingulate cortex (ACC) was observed during the perception of salient auditory event boundaries. The CEN and the SN have been previously shown to be activated during the cognitively demanding task, along with a decrease in the DNM (Aron et al., 2007; Fox et al., 2006; Greicius et al., 2003) is observed. The SN, with the FIC – ACC complex has been to be proposed to be the responsible control mechanism mediating the concurrent activation and de-activation of the CEN and the DMN (Goulden et al., 2014; Sridharan et al., 2008). These brain regions have been previously reported to moderate arousal during cognitively demanding tasks (Critchley et al., 2004), activate in response to other salient events (e.g. empathy for pain (Danziger et al., 2009) face of loved ones (Bartels & Zeki, 2004) enjoyable music (Blood & Zatorre, 2001),

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[33] The substantia nigra (SN) is a basal ganglia structure located in the midbrain that plays an important role in reward and movement.

[34] ventral tegmentum, is a group of neurons located close to the midline on the floor of the midbrain

etc.), and are the only brain regions containing the von Economo neurons, proposed to provide a “rapid relay to other parts of the brain” (Watson et al., 2006) and hence seem to be especially fit to initiate control signals that modulate the activation-deactivation mechanism of the CEM and DMN. Areas of the CEN, on the other hand, have been shown to be involved in sustained working memory tasks (Curtis & D’Esposito, 2003) selection and suppression of a response (Lau et al., 2008; Ridderinkhof et al., 2004).

The results of these studies drive me to pursue network-based approaches looking at the interplay of different networks in multi-language processing and control. Namely, I plan to further investigate the potential involvement of the salience network (Sridharan et al., 2008), rather than the central executive control network in populations of highly proficient multilinguals, through a dynamic network analysis of task fMRI, as well as analyses of resting-state data acquired from the same participants. My long-term plans are to use these data to motivate an NIH-R21 or an R0-1 grant that aims to explore the experience-dependent plasticity of language systems. This larger proposal examines domain-specific versus domain-general components of the language network (Fedorenko & Thompson-schill, 2014). Such investigation can provide a better understanding of the affected mechanisms resulting in language loss in multilinguals (i.e. multilingual aphasia); and the development of therapies targeting specific aspects of such mechanisms (e.g. brain regions of domain-general or domain-specific membership). I plan to examine plasticity as a function of multilinguistic experience across groups of different linguistic backgrounds. The evaluation of these diverse linguistic experiences will provide a strong test case to examine the flexibility (or lack thereof) of neural networks underlying language function and the crosstalk between language and cognitive control.

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Appendix A

English		**Russian**		**Romanian**	
List of Items	*Normed Freq.*	*List of Items*	*Normed Freq.*	*List of Items*	*Normed Freq.*
TALK	33.08	ГОВОРИТЬ	9.94	VORBI	7.53
PERMIT	19.42	ПОЗВОЛИТЬ	3.21	PERMITE	3.98
PARDON	4.73	ПРОСТИТЬ	2.26	PARDON	3.3
COUCH	4.12	ДИВАН	1.46	CANAPEA	2.27
REMEMBER	2.21	ПОМНИТЬ	0.73	AMINTI	1.65
TUMBLE	2.07	СВАЛИТЬ	0.72	POTICNI	0.97
HIDEOUS	2.03	УЖАСНО	0.57	ÎNGROZITOR	0.84
CORDIAL	1.38	ДРУЖЕЛЮБНЫЙ	0.52	PRIETENOS	0.81
EVIDENT	0.99	ОЧЕВИДНЫЙ	0.44	APARENT	0.81
IMPOSTOR	0.79	САМОЗВАНЕЦ	0.29	ȘARLATAN	0.76
MERIT	0.6	ЗАСЛУГИ	0.18	MERIT	0.62
FASCINATE	0.52	ПРИВЛЕЧЬ	0.11	FERMECA	0.54
INDICATE	0.49	УКАЗАТЬ	0.1	SEMNIFICA	0.49
IGNORANT	0.35	БЕЗГРАМОТНЫЙ	0.1	IGNORANT	0.43
FORTIFY	0.31	УСИЛИВАТЬ	0.09	FORTIFICA	0.35
RENOWN	0.27	СЛАВА	0.07	RENUME	0.32
NARRATE	0.18	ПОВЕСТВОВАТЬ	0.06	RELATA	0.3
MASSIVE	0.12	ОГРОМНЫЙ	0.05	ENORM	0.27
HILARITY	0.11	ВЕСЕЛОСТЬ	0.04	VESELIE	0.24
SMIRCHED	0.1	ГРЯЗНЫЙ	0.03	MÂNJI	0.24
SQUANDER	0.09	РАЗБАЗАРИВАТЬ	0.01	RISIPI	0.22
CAPTION	0.08	ТИТУЛ	0.01	DENUMIRE	0.19
FACILITATE	0.07	СОДЕЙСТВИЯ	0.01	ÎNLESNI	0.19
JOCOSE	0.07	ШУТЛИВЫЙ	0.01	JUCĂUȘ	0.19
APPRISE	0.06	ИЗВЕЩЯТЬ	0.01	AVERITIZA	0.16
RUE	0.06	РАСКАИВАТЬСЯ	0	REGRETA	0.14
DENIZEN	0.06	АБИТАТЕЛЬ	0	LOCĂTAR	0.11
DIVEST	0.05	ЛИШАТЬ	0	DESPUIA	0.11
AMULET	0.05	АМУЛЕТ	0	TALISMAN	0.11
INEXORABLE	0.04	НЕУМОЛИМЫЙ	0	NEÂNDUPLECAT	0.08
SERRATED	0.04	ЗАЗУБРЕННЫЙ	0	ZIMŢAT	0.08
LISSOM	0.03	ГИБКИЙ	0	MLĂDIOS	0.08
MOLLIFY	0.03	УМИРОТВОРИТЬ	0	DOMOLI	0.05
PLAGIARIZE	0.02	ПЛАГАТОРСТВО	0	PLAGIA	0.05
ORIFICE	0.02	ОТВЕРСТИЕ	0	ORIFICIU	0.05
QUERULOUS	0.01	ВОРЧЛИВЫЙ	0	PLÂNGĂREŢ	0.03
PARIAH	0.01	ПАРИЯ	0	IZGONIT	0.03
ABET	0	ПОДСТРЕКАТЬ	0	SPRIJINI	0.03
TEMERITY	0	БЕЗРАССУДСТВО	0	ÎNDRĂZNEALĂ	0
PRISTINE	0	БЕЗУПРЕЧНЫЙ	0	IMPECABIL	0

Appendix B

Grammatical sentence Type	Ungrammaticality	Example in English, Romanian and Russian
Type I	Ungrammaticality comes from the fact that the intransitive verb is followed by a direct object:	* James went the campus coffee shop. James went to the campus coffee shop. * Anca a venit casa bunicilor Anca a venit la casa bunicilor. * Девушка спала диване Девушка спала на диване
Type II	Ungrammaticality comes from the position of the empty NP elements in the sentences.	* That day was promised to be given the test _ The test was promised to be given that day. * Aceste lucrări urmează să finiseze Maria_ Maria urmează să finiseze aceste lucrări. * Эту роль, корее всего Райан сыграет_ Скорее всего, Райан сыграет эту роль
Type III	Ungrammaticality comes from the fact that the specifier of an NP is fronted by Wh-movement; the head noun of the NP is left behind. The fronted material is interpreted as an NP with the ellipsis of the head.	* Which good did you see friends at the party? Which good friends did you see at the party? * Câţi ai invitat prieteni la ziua de naştere? Câţi prieteni ai invitat la ziua de naştere? * Какие интересные вы услышали истории в детстве? Какие интересные истории вы услышали в детстве?
Type IV	Ungrammaticality comes from the fact that the gap is absent in the relative clause.	* John knew the poems that she assigned the homework. John knew the poems that she assigned. * Vadim nu mi-a adus cartea pe care i-am dat caietul. Vadim nu mi-a adus cartea pe care i-am dat-o. * Вы не убирали комнату которую я обычно убираю комнату Вы не убрали комнату, которую я обычно убираю?
Type V	Ungrammaticality comes from the violation of phrasal structures (NP – NP NP vs NP – NP PP).	* The newspaper this boutique is from yesterday. The newspaper this boutique sells, is from yesterday. * Cartea prietenul meu e foarte interesantă. Cartea pentru prietenul meu e foarte interesantă. * Машина мой друг очень чиста. Машина для моего друга очень чиста
Type VI	Ungrammaticality comes from the violation of gender, number or person, or both number and person).	* The neighbors herself were very surprised! The neighbors themselves were very surprised! * Ei înşivă vor regreta pierderile suferite. Ei înșiși vor regreta pierderile suferite. * В эти выходные мы сам не были дома В эти выходные мы сами не были дома
Type VII	Morphological case violations (specifically, the morphological form of the pronoun that is incompatible with the *-agrt* feature required).	* The list had they in the first place The list had them in the first place. * Mama pasăre le-a adus şi ţie nişte viermişori. Mama pasăre le-a adus şi lor nişte viermişori. * Маленькая девочка принесла мы кошку. Маленькая девочка принесла ему кошку.
Type VIII	English: Particle Movement construction. Ungrammaticality	* Liz ran the stairs down when she heard the noise. Liz ran down the stairs when she heard the noise.

	comes from the fact that the preposition is put after rather than before the NP. Romanian: Ungrammaticality comes from the violation of adjective agreement in number and gender with the noun. Russian: Ungrammaticality comes from the violation of the required complementizer for the subordinate clause (i.e. interrogative, subjunctive, and indicative).	* Ele au cumpărat cămaşă albastre. Ele au cumpărat cămaşe albastre. * Она спросила, чтобы вы потратили столько денег. Она спросила, почему вы потратили столько денег.
Type IX	English: Ungrammaticality comes from either the existence of a copy of the *aux* in its initial position within the yes/no question formation structures or the *aux* is not appropriate with the verb form. Romanian: Ungrammaticality comes from *double-case* violation. Russian: Ungrammaticality comes from the fact that infinitival clauses do not allow for overt subjects.	* Is the boy is having a good time? Is the boy having a good time? * Ilincuţa l-a ajutat pe bunicului să ducă lemne. Ilincuţa l-a ajutat pe bunicul să ducă lemne. * Танцору танцевать - это хороший вид спорта. Танцевать - это хороший вид спорта.
Type X	English: Ungrammaticality comes from the fact that the subject pronouns cannot be coindexed with the subject of the sentence. Romanian: The ungrammaticality comes from the usage of inappropriate auxiliary verbs. Russian: Ungrammaticality case violation.	* Ana broke her arm climbing the tree, didn't they? Ana broke her arm climbing the tree, didn't she? * Femeia am adus multe cărţi in bagajele ei grele. Femeia a adus multe cărţi in bagajele ei grele. * Ребенок был отправлен новая книга. Ребенку была отправленна новая книга.
Type XI	English: Ungrammaticality comes from wrong word ordering. (Note: All word orderings are possible in Romanian and Russian, just two are possible in English (SOV, OVS). Romanian: Ungrammaticality comes from the fact that the sentences contain a copy of the aux in its initial position for question formation. It is possible in English, but not Russian. Russian: Ungrammaticality comes from the wrong ordering of interrogative pronouns.	* Are always worried for their children parents. Parents are always worried for their children. * Suntem oare şi noi suntem bineveniţi la sărbătoare? Suntem oare şi noi bineveniţi la sărbătoare? *Кто-то кого-то узнал но мы не знаем, кого кто. Кто-то кого-то узнал но мы не знаем, кто кого.
Type XII	English: Ungrammaticality comes from the violation of "real-world-knowledge" sentence structure. Romanian: Ungrammaticality comes from the missing verb "to	* I was surprised for you to get married. I was surprised that you got married. * Eu am răspuns la mesaj dar tu nu ai. Eu am răspuns la mesaj dar tu nu ai răspuns.

	have", which needs to be repeated with the auxiliary in negative constructions; note that the verb "to be" does not need to be repeated in such constructions. Russian: Ungrammaticality comes from the missing noun after extraction from inside the DP (note that extraction from inside the DP is grammatical in Russian but not in Romanian or English).	Soţia era gata de plecare iar soţul nu era. Soţia era gata de plecare iar soţul nu. * Хорошую Александра купила. Хорошую Александра купила книгу.

Appendix C

Region	**BA**	***MNI Coordinates***			***t stats***
		x	***y***	***z***	
(A). *Native (L1) > Baseline Monolinguals*					*t(15)*
L. Middle Temporal Gyrus	BA 21	-56	-12	-16	14.69
L. Frontal/Middle Orbital Gyrus	BA11	-12	14	-16	9.98
L. Fusiform Gyrus	BA37	-36	-20	-26	9.16
L. Thalamus		-14	-28	-6	9.1
L. Thalamus/L. Pallidum		-12	-8	-4	7.88
L. Hippocampus/L. Pallidum	BA34	-14	-6	-10	8.09
L. Cerebellum (IX)		-18	-50	-38	9.41
R. Middle Temporal Pole	BA38	52	6	-26	8.77
R. Parahippocampal/R. Fusiform Gyrus	BA36/BA37	24	-40	-10	8.76
R. Hippocampus	BA34	-12	-14	-14	9.47
R. Caudate/Pallidum		14	6	-8	9.42
R. Cerebellum (VIII/IX)		20	-52	-38	9.65
R. Cerebellum (IV/V)		8	-48	-24	8.45
R. Cerebellum (VIII)		20	-68	-44	8.34
(B). *Native (L1) > Baseline Multilinguals*					*t(15)*
L. Middle Temporal Gyrus	BA21	-46	-26	-8	9.39
L. Middle/Superior Temporal Gyrus	BA21/BA22	-58	-4	-12	9.6
L. Fusiform Gyrus	BA37	-36	-14	-34	12.1
L. Cerebellum (IX)		-14	-48	-44	8.1
R. Superior Temporal Gyrus	BA22	60	-8	-10	10.88
R. Middle Temporal Gyrus	BA21	48	-34	-6	9.77
R. Middle/Inferior Temporal Gyrus	BA21/BA20	50	2	-30	8.62
R. Middle Temporal Pole	BA21	46	14	-22	8.38
R. Posterior Orbital Gyrus/Insula	BA11/BA13	22	24	-14	11.15
R. Anterior Cingulate Cortex	BA32/33	8	22	14	8.29
R. Middle Cingulate Cortex	BA24	12	-8	32	7.83
R. Posterior Cingulate Cortex	BA31	8	-36	20	8.46
		4	-40	10	9.53
R. Thalamus		4	-22	10	8.7
		20	-22	16	8.46
R. Hippocampus	BA34	26	-34	2	8.76
R. Pallidum		16	0	-6	14.33
R. Cerebellum(X)		4	-48	-30	10.2
R. Cerebellum (IX)		-14	-48	-44	8.1

Stereotactic coordinates of peak activations for whole-brain analysis. Note: L. left. R. right; BA: Brodmann's Area. MNI coordinates determined by the SPM 12 anatomical toolbox $p < 0.05$ (FWE corrected). Voxel extent threshold of 8 consecutive voxels.

Appendix D

ROI Analysis Results for a 2- Sample T-Test Between the Monolingual and Multilingual Group.

Region	***L1(multi) > L1 (mono)***		
Language Core Regions	$t_{(30)}$	*p-value*	*95% CI*
L. Inferior Frontal Gyrus	-0.2	0.8	-0.19 to 0.24
L. Interior Frontal Gyrus Op.	0.67	0.5	-0.08 to 0.04
L. Middle Frontal Gyrus	-0.3	0.7	-0.13 to 0.2
L. Anterior Temporal Cortex	0.8	0.41	-0.06 to 0.1
L. Middle Posterior Temporal Cortex	0.8	0.4	-0.12 to 0.2
L. Posterior Temporal Cortex	0.54	0.6	-0.15 to -0.08
L. Angular Gyrus	0.82	0.42	-0.13 to -0.08
Periphery Brain Regions			
Cortical Regions ROIs			
L. Dorsolateral Prefrontal Cortex	-0.18	0.85	-0.14 to 0.17
R. Dorsolateral Prefrontal Cortex	0.5	0.6	-0.19 to 0.11
L. Pre-supplementary Motor Area	0.8	0.43	-0.06 to 0.15
R. Pre-supplementary Motor Area	0.7	0.49	-0.15 to 0.07
L. Inferior Parietal Lobe	0.23	0.8	-0.08 to 0.1
R. Inferior Parietal Lobe	0.5	0.66	-0.13 to 0.08
R. Insula	3.06	0.005*	-0.23 to 0.04
L. Insula	1.01	0.32	0.1 to 0.15
R. ACC (dorsal)	2.51	0.01*	- 0.15 to 0.001
L. ACC (dorsal)	2.29	0.02*	-0.27 to -0.01
R. ACC (ventral)	2.79	0.009*	-0.18 to 0.05
L. ACC (ventral)	3.14	0.005*	- 0.15 to -0.03

Note: L. left. R. right; Mono: monolinguals; Multi: multilinguals; ACC: anterior cingulate cortex.

Appendix E

Region	***MNI Coordinates***			***Sphere Size***
	x	***y***	***z***	
Cortical Regions				
L. Dorsolateral Prefrontal Cortex	-21	43	-10	8
R. Dorsolateral Prefrontal Cortex	21	43	-10	8
L. Pre-supplementary Motor Area	-1	1	57	8
R. Pre-supplementary Motor Area	1	1	57	8
L. Inferior Frontal Gyrus (IFG)	-48	16	24	8
R. Inferior Frontal Gyrus (IFG)	48	16	24	8
L. Inferior Parietal Lobe (IPL)	-37	-56	41	8
R. Inferior Parietal Lobe (IPL)	37	-56	41	8
L. Insula	-35	18	-2	10
R. Insula	35	18	-2	10
R. ACC (dorsal)	-4	38	28	8
L. ACC (dorsal)	4	38	28	8
R. ACC (ventral)	-2	46	-6	8
L. ACC (ventral)	2	46	6	8
Subcortical Regions ROIs				
R. Caudate (head)	12	16	2	5
L. Caudate (head)	-12	16	2	5
R. Caudate (body)	6	6	14	5
L. Caudate (body)	-6	6	14	5
R. Caudate (tail)	32	-32	-4	4
L. Caudate (tail)	-32	-34	-4	4
R. Thalamus (anterior)	10	-2	8	5
L. Thalamus (anterior)	-10	-2	8	5
R. Thalamus (posterior)	16	24	8	5
L. Thalamus (posterior)	-16	-24	8	5
R. Globus Pallidus	16	4	-6	5
L. Globus Pallidus	-16	4	-6	5
R. Putamen	14	4	-8	5
L. Putamen	-14	4	-8	5

Appendix F

Region	***L1 Romanian > L2 Russian***		
Cognitive Control Brain Regions	$t_{(30)}$	*p-value*	*95% CI*
Cortical Regions ROIs			
L. Dorsolateral Prefrontal Cortex	-0.48	0.6	-0.19 to 0.12
R. Dorsolateral Prefrontal Cortex	-0.38	0.7	-0.24 to 0.17
L. Pre-supplementary Motor Area	-0.9	0.4	-0.14 to 0.05
R. Pre-supplementary Motor Area	-0.06	0.9	-0.16 to 0.15
L. Inferior Parietal Lobe	0.08	0.9	-0.08 to 0.09
R. Inferior Parietal Lobe	0.35	0.7	-0.13 to 0.09
R. Insula	0.94	0.36	-0.05 to 0.11
L. Insula	0.44	0.66	-0.07 to 0.1
R. ACC (dorsal)	4.41	0.0005*	0.09 to 0.26
L. ACC (dorsal)	3.1	0.007*	0.05 to 0.29
R. ACC (ventral)	2.24	0.04*	0.01 to 0.3
L. ACC (ventral)	1.73	0.1	- 0.02 to 0.16
Subcortical Regions ROIs			
R. Caudate (head)	2.11	0.05*	-0.001 to 0.12
L. Caudate (head)	2.26	0.04*	0.004 to 0.18
R. Caudate (body)	3.74	0.002*	0.04 to 0.18
L. Caudate (body)	3.12	0.007*	0.03 to 0.15
R. Caudate (tail)	1.15	0.27	-0.03 to 0.1
L. Caudate (tail)	1.36	0.19	-0.02 to 0.07
R. Thalamus (anterior)	1.64	0.12	-0.02 to 0.17
L. Thalamus (anterior)	2.93	0.01*	0.03 to 0.16
R. Thalamus (posterior)	-0.55	0.59	-0.09 to 0.05
L. Thalamus (posterior)	0.46	0.65	-0.04 to 0.06
R. Globus Pallidus	4.12	0.001*	0.03 to 0.1
L. Globus Pallidus	3.22	0.006*	0.04 to 0.19
R. Putamen	2.97	0.01*	0.01 to 0.09
L. Putamen	4.31	0.0007*	0.03 to 0.1
Cerebellum			
L. Cerebellum (Crus I)	1.23	0.24	-0.05 to 0.2
R. Cerebellum (Crus I)	0.9	0.36	-0.07 to 0.2
L. Cerebellum (Crus II)	2.65	0.02*	0.02 to 0.2
R. Cerebellum (Crus II)	0.36	0.7	-0.07 to 0.1
L. Cerebellum (VII b)	3.12	0.007*	0.03 to 0.15
R. Cerebellum (VII b)	0.83	0.42	-0.05 to 0.11
L. Cerebellum (VIII)	3.03	0.008*	0.02 to 0.13
R. Cerebellum (VIII)	2.01	0.06	-0.01 to 0.17
L. Cerebellum (IX)	1.84	0.09	-0.01 to 0.14
R. Cerebellum (IX)	1.81	0.09	-0.01 to 0.17
L. Cerebellum (X)	1.86	0.08	-0.01 to 0.15
R. Cerebellum (X)	1.32	0.21	-0.04 to 0.16

Note: L. left. R. right; Multi: multilinguals.

Appendix G

Region	*L2 English > L2 Russian*		
Cognitive Control Brain Regions	$t_{(30)}$	*p-value*	*95% CI*
Cortical Regions ROIs			
L. Dorsolateral Prefrontal Cortex	-1.24	0.23	-0.04 to 0.17
R. Dorsolateral Prefrontal Cortex	-0.45	0.65	-0.15 to 0.2
L. Pre-supplementary Motor Area	-0.09	0.9	-0.07 to 0.06
R. Pre-supplementary Motor Area	-0.3	0.8	-0.14 to 0.11
L. Inferior Parietal Lobe	-0.2	0.8	-0.11 to 0.09
R. Inferior Parietal Lobe	0.65	0.5	-0.09 to 0.17
R. Insula	1.38	0.19	-0.15 to 0.03
L. Insula	0.92	0.37	-0.13 to 0.05
R. ACC (dorsal)	1.03	0.32	- 0.21 to 0.07
L. ACC (dorsal)	0.76	0.46	- 0.2 to 0.1
R. ACC (ventral)	1.61	0.13	-0.23 to 0.03
L. ACC (ventral)	2.14	0.05*	-1.4 to 0.00007
Subcortical Regions ROIs			
R. Caudate (head)	1.75	0.1	-0.2 to 0.02
L. Caudate (head)	1.01	0.33	-0.19 to 0.07
R. Caudate (body)	2.51	0.02*	-0.25 to -0.02
L. Caudate (body)	2.11	0.05*	-0.23 to 0.002
R. Caudate (tail)	1.88	0.08	-0.17 to 0.01
L. Caudate (tail)	1.29	0.21	- 0.07 to 0.02
R. Thalamus (anterior)	2.19	0.04*	0.002 to 0.14
L. Thalamus (anterior)	1.76	0.09	-0.021 to 0.22
R. Thalamus (posterior)	2.68	0.01*	0.02 to 0.21
L. Thalamus (posterior)	1.6	0.13	-0.02 to 0.14
R. Globus Pallidus	-3.1	0.008*	-0.2 to -0.03
L. Globus Pallidus	2.15	0.04*	-0.22 to -0.001
R. Putamen	2.28	0.04*	0.004 to 0.15
L. Putamen	2.18	0.05*	0.001 to 0.14
Cerebellum			
L. Cerebellum (Crus I)	1.47	0.16	-0.2 to 0.04
R. Cerebellum (Crus I)	1.1	0.29	-0.18 to 0.06
L. Cerebellum (Crus II)	1.52	0.15	-0.17 to 0.03
R. Cerebellum (Crus II)	-0.1	0.92	-0.12 to 0.13
L. Cerebellum (VII b)	2.4	0.03*	-0.13 to -0.006
R. Cerebellum (VII b)	1.24	0.23	-0.15 to -.04
L. Cerebellum (VIII)	2.16	0.04*	0.11 to 0.001
R. Cerebellum (VIII)	2.4	0.03*	-0.18 to -0.01
L. Cerebellum (IX)	2.83	0.01*	0.02 to 0.15
R. Cerebellum (IX)	3.02	0.008*	0.03 to 0.19
L. Cerebellum (X)	2.72	0.01*	-0.15 to -0.02
R. Cerebellum (X)	2.6	0.02*	-0.21 to -0.02

Note: L. left. R. right; Multi: multilinguals

Appendix H

Microstructural Changes Influencing Brain Plasticity

The seminal work providing evidence of experience-related brain plasticity comes from studies looking at cellular changes as a result of "environmental enrichment" paradigms, found in studies on rodents. Such paradigms compare microstructural brain changes that occur in rodents that for a certain amount of time have been placed in an environment (i.e. cage) containing novel objects (e.g. toys) versus rodents that develop in regular cages. Initially, studies have reported changes in the rodents' cortical weight and thickness (Rosenzweig et al., 1962, 1964). As a consequence, an interest in understanding the underlying microstructural changes resulting in observable macrostructural volume and thickness differences has increased (e.g. Anderson & Pessoa, 2011). The increases in brain regions are the result of various factors, such as dendritic length and branching (Green et al., 1983; also in non-human primates (Kozorovitskiy et al., 2005), changes in the number of synapses and dendritic spines per neuron (e.g. Briones et al., 2004), and both synapse number and dendritic branching (such as motor training and spatial learning (Whishaw et al., 2008). More recent literature (using in vivo two-photon microscopy in mice, rather than the Golgi method) suggests that the dendrites' structure is relatively stable, and the experience-dependent effects are restricted to alterations in synaptic connectivity (observed in the elimination of dendritic spines and axonal boutons (Fu & Zuo, 2011). In addition, a growth of capillaries and glia[35] may accompany changes in dendrites and synapses. For example, (Black et al., 1990)showed that physical exercise may increase capillaries, without increasing the neural tissue, while glial cells are believed to increase as a compensatory mechanism for the decrease in synapses, to support the energy demand of the new synapses (Tata et al., 2006).

It is important to consider that a change observed in a specific region has likely not occurred in isolation from other changes, but rather is part of a chain of different microstructural changes that may contribute to global density/thickness (the number of cells per unit volume) and volume (total regional volume). It is also important to note that these are independent measures of brain restructuring and may be considered "complementary" in studies investigating brain structure. For example, increases, unaltered or decreases in regional volume may be accompanied by: 1. a decrease in certain aspects of cell density (i.e. changes in dendrites[36], synapses[37], synaptic spines[38], and capillaries[39]); 2. stable cell tissue density (implying growth of a certain aspect of the cell, such as the dendrites) or 3. an increase in cell density (implying disproportional growth of cell tissue; Lövdén et al., 2012). Additionally, the relative extra and intra-cellular space may change during learning due to activation-related dynamics (i.e. swelling or shrinking of cells; Le Bihan, 2007; Syková & Nicholson, 2008).

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[35] Glia, also called glial cells or neuroglia, are non-neuronal cells in the central nervous system (brain and spinal cord) and the peripheral nervous system that do not produce electrical impulses. They maintain homeostasis, form myelin in the peripheral nervous system, and provide support and protection for neurons.

[36] Dendrites are projections of a neuron (nerve cell) that receive signals (information) from other neurons.

[37] Synapses are junctions between two nerve cells, consisting of a gap across which impulses pass by diffusion of a neurotransmitter.

[38] A dendritic spine (or spine) is a small membranous protrusion from a neuron's dendrite that typically receives input from a single axon at the synapse. Dendritic spines serve as a storage site for synaptic strength and help transmit electrical signals to the neuron's cell body.

[39] Capillaries are very tiny blood vessels. In the human body, these help to connect arteries and veins, and facilitate the exchange of certain elements between blood and other tissues.

The Time-Course and trajectory of Experience - Related Brain Plasticity

Research in non-human primates and rodents suggests that experience-related plasticity can be observed in a very short amount of time. For example, Lerch et al., 2011, showed that as long as five days of Morris maze training can result in GM restructuring in mice. These changes were correlated with learning amount and speed and were followed by a partial - normalization. Lövdén et al., 2012 propose the expansion partial renormalization hypothesis (EPR), which suggests that the trajectory of the GM cortical expansion is initially marked by a rapid expansion but follows a long process of normalization. The mechanism behind this pattern is believed to be an initial increase in the number of neural pathways in the brain. Out of the pool of existing circuits, the most efficient is selected, resulting in a region normalization (e.g. Reed et al., 2011). This hypothesis is supported by evidence looking at cell structure. For example, Fu & Zuo, 2011 observed that at early stages of learning, new dendritic spines are generated, although this fast process is followed by a slower elimination process of the previous-to-training existing spines, while the newly created spines are selectively stabilized. The same authors suggest that spine formation is related to learning and the surviving spines will depend on practicing the newly acquired skill.

Appendix I

Overview of White Matter Structure That Might Influence the Diffusion of Water

The white matter (constituting the bundles of axons that extend from one region of the central nervous system to another) accounts for ~40% of the central nervous system (Morrell et al., 1984). The vast majority of the axons are surrounded by myelin. Besides the axons, the white matter contains glial cells, ependymal, endothelial, pericytes, and fibroblasts cells. All these constituents can affect the white matter diffusivity and therefore influence metrics obtained from diffusion imaging. A short description of the constituents affecting the white matter are:

1. *Axonal structure and constituency.* The axon is the fiber that extends from the cell body. It contains: actin filament – found beneath the axonal plasma membrane and providing scaffolding, are important for maintaining the membranal integrity; microtubules – are the tracks allowing the organelle transportation, microtubules-associated proteins; neurofilaments – are the main determinant of the axonal diameter, and provide structure (J. M. Dale & Garcia, 2012), and mitochondria – a membrane-bound organelle, that seems to be denser in de/non-myelinated axons (Campbell and Mahad, 2012). All these longitudinally oriented structures contain water and are a potential contributor to the diffusion metrics. However, there is evidence to suggest only a limited influence of these on the Fractional Anisotropy measure (e.g. Beaulieu & Allen, 1994; Takahashi et al., 2002).
2. *Axonal Diameter.* The diameter of the axons seems to be constant throughout their length, but are different in different fiber bundles (e.g. the optic nerve contains predominantly small in diameter axons; while the corticospinal tract contains a mixture of both large and small in diameter axons (Johansen-Berg & Behrens, 2013).
3. *Axonal Packing densities.* The packing of the axons is influenced both by the axonal diameter and the number of myelinated axons. Densities can vary greatly from bundle to bundle. Similarly, a great variation is estimated across individuals. For example, the human corpus callosum is estimated to contain between 200 million (Aboitiz et al., 1992) to 800 million (Koppel & Innocenti, 1983).
4. *Myelination.* Myelin is a lipid-rich sheath that supports faster information transmission (from 10 to 100 times faster rate of electrical conduction (Trapp & Kidd, 2004). Generally, studies suggest that the perpendicular diffusion (RD) shows higher increases than parallel diffusion (AD) from myelinated to de/non-myelinated axons, also the perpendicular increases (RD) are usually greater than the percentage decreases observed in fractional anisotropy (FA) (for a detailed review of several of these studies see(Beaulieu, 2013). Also, studies looking at the effect of re-myelination through transplant (e.g. Uchida et al., 2012 in shiverer mice; Gupta et al., 2012, in children with genetic disorders affecting the myelin formation oligodendrocyte cells), report an increase of up to 12% in the FA measure, mostly driven by the reductions in perpendicular diffusion. These results are suggestive of the fact that perpendicular diffusion (RD) is more sensitive to myelination than the FA, and the role of the myelin is only secondary in affecting the fractional anisotropy value.
5. *Axonal Membranes.* The axonal membranes are active and passive participators in the regulation of the molecules (including water) between cellular compartments. One of the first studies to show the importance of membranes in water diffusion was by Beaulieu & Allen, 1994, which has looked at the diffusion anisotropy in the non-myelinated olfactory nerve, a Schwann cell myelinated trigeminal nerve, and the oligodendrocyte myelinated optic nerve of the garfish. They found that the degree of anisotropy was similar across the three, suggesting that the critical component needed for anisotropic diffusion are membranes, rather than myelin (see also Seo et al., 1999 for similar results in the rat vagus nerve; Larvaron et al., 2007, for baby mice, (Kasprian et al., 2008, 2013), in human white matter in utero).

Appendix J

White Matter Correlation Analysis Results. Results (p-values: in black; and r-values: in orange) from the correlation analysis between fiber density (FD), fiber cross-section (FC), and fiber density and cross-section (FDC) and language background variables.

Romanian Native Language

	Age	Prof.	l.ICP (FD)	r.ICP (FD)	l.ICP (FDC)	r.ICP (FDC)	l.ICP (FC)	r.ICP (FC)
Age		0.97	0.21	0.08	0.83	0.33	0.26	0.93
Prof.	0.01		0.36	0.43	0.24	0.19	0.37	0.16
l.ICP (FD)	-0.35	0.25		0.00	0.00	0.02	0.38	0.60
r. ICP (FD)	-0.46	0.22	0.76		0.05	0.00	0.59	0.41
l.ICP (FDC)	0.06	0.33	0.69	0.51		0.00	0.00	0.00
r. ICP (FDC)	-0.27	0.36	0.59	0.80	0.78		0.01	0.00
l. ICP (FC)	0.31	0.25	0.24	0.15	0.87	0.63		0.00
r. ICP (FC)	0.02	0.38	0.15	0.23	0.70	0.77	0.83	

Russian Early L2

	Age	Prof.	Imm.	AoA	l.ICP (FD)	r.ICP (FD)	l.ICP (FDC)	r.ICP (FDC)	l.ICP (FC)	r.ICP (FC)
Age		0.23	0.19	0.87	0.21	0.08	0.83	0.33	0.26	0.93
Prof.	-0.33		0.22	0.41	0.01	0.00*	0.15	0.01*	0.75	0.47
Imm.	0.36	0.33		0.27	0.09	0.23	0.05*	0.40	0.20	0.99
AoA	0.05	-0.23	-0.30		0.12	0.09	0.08	0.09	0.26	0.46
l.ICP (FD)	-0.35	0.64	0.46	-0.42		0.00	0.00	0.02	0.38	0.60
r. ICP (FD)	-0.46	0.81	0.33	-0.46	0.76		0.05	0.00	0.59	0.41
l.ICP (FDC)	0.06	0.39	0.51	-0.46	0.69	0.51		0.00	0.00	0.00
r. ICP (FDC)	-0.27	0.66	0.24	-0.45	0.59	0.80	0.78		0.01	0.00
l. ICP (FC)	0.31	0.09	0.35	-0.31	0.24	0.15	0.87	0.63		0.00
r. ICP (FC)	0.02	0.20	0.00	-0.21	0.15	0.23	0.70	0.77	0.83	

English Early L2

	Age	Prof.	Imm.	AoA	l.ICP (FD)	r.ICP (FD)	l.ICP (FDC)	r.ICP (FDC)	l.ICP (FC)	r.ICP (FC)
Age		0.77	0.00*	0.24	0.21	0.08	0.83	0.33	0.26	0.93
Prof.	-0.08		0.66	0.26	0.96	0.70	0.63	0.69	0.60	0.31
Imm.	0.76	-0.12		0.08	0.06	0.03*	0.94	0.40	0.16	0.52
AoA	-0.32	-0.31	-0.46		0.05*	0.00*	0.66	0.08	0.49	0.87
l.ICP (FD)	-0.35	0.01	-0.50	0.51		0.00	0.00	0.02	0.38	0.60
r. ICP (FD)	-0.46	-0.11	-0.55	0.71	0.76		0.05	0.00	0.59	0.41
l.ICP (FDC)	0.06	0.14	0.02	0.12	0.69	0.51		0.00	0.00	0.00
r. ICP (FDC)	-0.27	0.11	-0.23	0.47	0.59	0.80	0.78		0.01	0.00
l. ICP (FC)	0.31	0.15	0.39	-0.19	0.24	0.15	0.87	0.63		0.00
r. ICP (FC)	0.02	0.28	0.18	0.05	0.15	0.23	0.70	0.77	0.83	

Note Prof: proficiency, Imm: Immersion (years), AoA: age of acquisition, l: left, r: right. ICP: inferior cerebellar peduncle; FD: fiber density, FC: fiber cross-section, FDC: fiber density and cross-section.

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