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A Longitudinal Structural Covariance Study

Age-Related Sex Differences in Healthy Controls

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ABSTRACT

Historically, human beings have been categorizing themselves into males and females. Here, I will use a large longitudinal database of healthy female and male brain data (298 subjects, 700 brain scans) with an age range of 17 – 68 years to analyze developmental brain differences between the sexes. It is common knowledge that age has a strong effect on metrics of brain structure (for example, a decrease of cortical thickness (CT) over time). Instead of trying to eliminate age variance I intend to model the age effect when assessing sex differences. In practice this means that we assess whether sex differences are constant across the life span or change with time. I have chosen a structural covariance approach for assessing sex differences. Structural covariance deals with correlation-matrices where, in our case, each cell represents the change of CT over time for a given cortical region. These 'developmental matrices' can be statistically compared between female and male groups to assess whether covariance-patterns are similar or not. We created a statistical pipeline in the statistical programming language 'R' to do just that. This work thus presents a novel longitudinal structural covariance approach to assess differences between females and males.

INDEX

ABBREVIATIONS

APC	Annual Percentage Change
bankssts	Banks Superior Temporal Sulcus
CSF	Cerebrospinal Fluid
CT	Cortical Thickness
Ed Lvl	Social Economic Status based on Education Level
Ed Years	Social Economic Status based on Education Years
Fsc	Structural Covariance Matrix of Female Subjects
FDR	False Discovery Rate
Fz	Z- Transformed Structural Covariance Matrix of Female Subjects
GM	Grey Matter
MRI	Magnetic Resonance Imaging
Msc	Structural Covariance Matrix of Male Subjects
Mz	Z- Transformed Structural Covariance Matrix of Male Subjects
Occ Status	Social Economic Status based on Occupational Status
QA	Quality Assurance
ROI	Region of Interest
SBM	Surface-Based Morphometry
SC	Structural Covariance
SES	Social Economic Status
subjF	Number of Female Subjects
subjM	Number of Male Subjects
VBM	Voxel-Based Morphometry
WM	White Matter

CHAPTER 1: INTRODUCTION

1.1. Motivation

The categorization of human beings into different sexes, i.e. females and males has been subject to scientific analyses, one of which focuses on the brain. Nevertheless, we are still far from answering the question of what impact sex differences have on the human brain. Some researchers point out that this lack of knowledge could affect our understanding of the human brain [1, 2, 3].

Differences between females and males can be observed in human behavior and cognition [4, 5]. Sex-related brain differences have also been reported at micro- and macroscopic levels of brain structure and function (i.e. connectome organization, morphology, activation patterns and neurochemistry) [6]. However, we do not know yet to what extent sex categorization is relevant [7] to explain human variability on behavioral and brain-related properties. Thereby, the question of whether these sex-related differences at the brain level explain behavioral sex differences remains unsolved.

Sex differences have also been reported for brain development [8, 9], i.e. age-related changes in the brain's structure and function appear to differ by sex. Furthermore, some studies [10] have demonstrated the importance of age when studying sex-related differences at the brain level. Many studies investigating sex differences in the brain still consider age as a so-called nuisance variable. That is, hypotheses about sex differences in the brain do not assess the effect of age. Instead, the effect of age is eliminated, or at least minimized, by, for instance, statistically regressing out its effect. Thus, the sex differences reported at brain level are usually statistically independent of age. But age has an important effect on the brain. Our brain structure and function changes as we grow older. This is, to a certain degree, common knowledge. Therefore, it is not entirely clear why research does not try to assess the effect of age when dealing with brain-sex differences instead of trying to take it out of the equation. Thus, not only is there a need for contemplating age as a nuisance variable, but also a need of addressing how sex-related differences vary across the life span.

To study sex-related differences in human brain, various magnetic resonance techniques appear as an alternative to post-mortem studies. Magnetic resonance imaging (MRI) development provides the opportunity of analyzing morphological brain characteristics at unprecedented spatial levels in vivo. Moreover, as increasingly faster development of biomedical methods takes place, more and more technologies are becoming available to study human brain features.

After the introduction of improved resolution and tissue contrast in T1-weighted images, an approach to study structural brain attributes is introduced: voxel-based morphometry (VBM). VBM substitutes the manual tracing of brain images. The VBM technique allows the study of the brain based on the voxel-wise comparison of image intensities. The voxels are divided into classes depending on brain tissue: cerebrospinal fluid (CSF), white matter (WM) and grey matter (GM). Brain metrics obtained using VBM for which sex-differences have been reported are GM and WM volumes [11, 12]. As mentioned before, voxel-based morphometry is based on intensities not on surfaces itself, thus, it does not offer complete information of brain tissue boundaries. To overcome this problem, surface-based morphometry (SBM) emerges (see Figure 1). This novel technique improves image processing and allows the obtention of a greater number of morphometric attributes. This provides new information when studying genetics which can be of use when analyzing genetic and developmental motives [13, 14]. The SBM technique allows the estimation of surface-based metrics shape. Brain metrics which can be obtained using SBM allow a more complete understanding of brain cortex morphometry, based on cortical folding and curvature parameters. Such metrics are, for instance, cortical thickness, cortical surface area and cortical volume (see Figure 2). Nevertheless, due to the novelty of this technique, not many studies have reported sex differences using these morphometric metrics.

Among a large variety of brain morphometric metrics, cortical thickness (CT) stands out. This is due to the relation found between CT, brain development and brain disorders, both psychiatric and neurologic [15]. Furthermore, aging has a strong effect on CT. However, to date, no studies have examined age-related sex differences in CT. The current project provides a wonderful opportunity to assess the importance of region-specific change in CT modelling both the effect of sex and age.

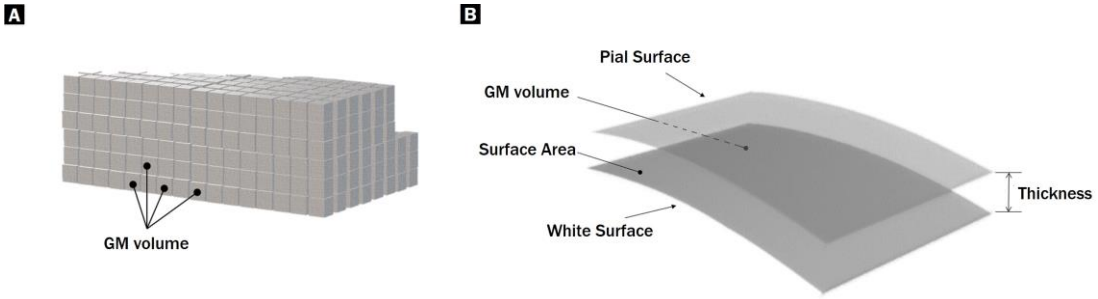

Figure 1. Difference between VBM and SBM techniques*. (A) VBM: the volumes can only be measured directly and require partial volume-effects (not depicted) to be considered. (B) SBM: GM volume is a quadratic function of distances in the surfaces and a linear function of the thickness.*

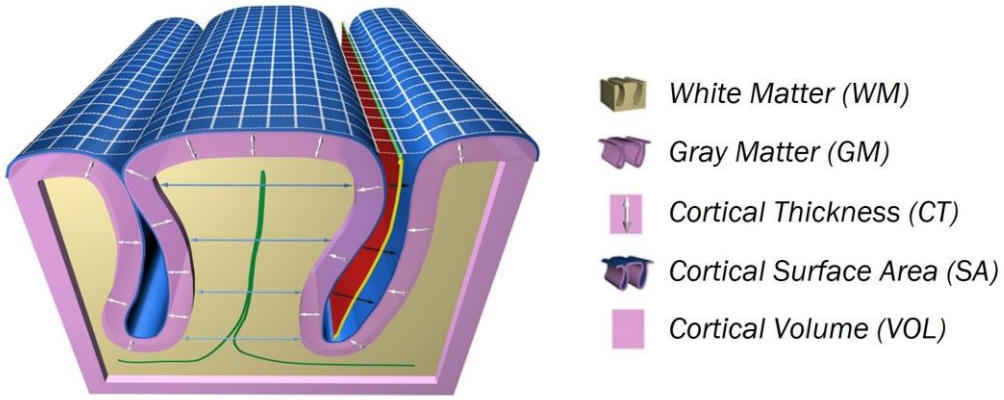

Figure 2. Schematic representation of brain metrics.

With regard to the previous literature on brain morphometry sex-differences, results of a great number of studies contradict each other. Besides, there is not a specific outline that explains these sex differences and within which brain features these differences are more notorious. Consequently, many of the findings regarding sex-differences in brain morphometry remain inconsistent. Studies differ, and among the greatest sources of variability across such studies can be said to be the age of the participants, the brain parcellation system (see below), the nuisance variables and the image processing method.

For neuroimage analyses, brain parcellations appear as means for segmenting the brain, in particular the brain cortex, into subregions. The boundary of each region can be based on neuroanatomy or other features [16]. Once a brain parcellation system is chosen, following the boundaries set by the system, the brain is segmented into regions of interest (ROIs) for subsequent feature measurement and analysis. The researcher has the option to choose different features of the parcellation system, for example the scale. A lower scale parcellation will have a smaller number of ROIs compared to a higher scale parcellation. A higher scale parcellation will have higher topological precision, which may change the statistical effect of sex on the brain. For example, if a statistical effect of sex is very 'local', then, this effect may be better picked up in a higher scale parcellation. Thus, the effect of parcellation system selection is important as it will influence the data. However, not many sex-related-brain studies analyze the effect of parcellation system and ROI selection. Accounting for all the reasons mentioned above, there is a need of observing the impact different parcellation systems have on data and, consequently, on results.

Moreover, many papers state that future research on sex-related-brain differences should include *longitudinal* analyses. With "longitudinal" meaning that multiple brain metrics are collected within the same subject over time. There is a clear lack of longitudinal studies [17]. The value of longitudinal analyses builds on the shortage of longitudinal data availability and the opportunity longitudinal data presents to analyze change in brain metrics *over time*. What is more, the relation between change in brain metrics (e.g. CT) and age-related sex differences can be analyzed. The importance of all what is mentioned above is related to the fact that we also want to study the aging effect in CT. Time is a dynamic variable, age depends on time. Thus, if only one image at one time point is analyzed (cross-sectional data), the aging effect is not completely accounted for. Therefore, a longitudinal analysis will certainly be more suitable in the analysis of age-related sex differences. This can be done by firstly, analyzing change directionality in CT (cortical thinning or thickening), and secondly, among which age group this change in CT is more drastic.

Concluding, the main goal of this project is to create a novel longitudinal analysis evaluating changes in CT. This change will be compared among age groups and among different sexes to analyze the effects of age and sex in the alterations of CT on the human brain.

1.2. Hypothesis and Objectives

1.2.1. Hypothesis

Given the previous literature described, I expect to see age-related sex brain differences and such differences will not be consistent among different parcellation systems.

1.2.2. Objectives

The development of a pipeline that analyzes the effect of phenotypical variables to determine age-related sex differences using a methodology as non-variable as possible and CT as a biomarker. In this project, four main objectives can be outlined.

- Longitudinal study of age-related sex differences and its directionality (males > females or vice versa) to evaluate change in CT accounting for sex and age.
- Analyze the effect of brain parcellation system or scale effect.
- Study whether sex-related brain differences in longitudinal study of change in CT are consistent among different age groups.

- Examine the importance of longitudinal studies and the additional information longitudinal data provides to cross-sectional data.

1.3. Regulatory Framework

As my project involves the use of data from human subjects, different legal principles regulate the acquisition, manipulation and distribution of such data, as well as, the regulations regarding intellectual property and copyright. This research project operates within the Spanish law and regulations of Biomedical Research:

- **Royal Decree 1/1996**, of April 12th, Intellectual Property Legislation.
- **Council of Europe Convention** ratified by Spain on July 23rd, 1999, Convention for the protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine.
- **Organic Law 15/1999**, of December 13th, Protection of Personal Data.
- **Law 41/2002**, of November 14th, regulating patient autonomy and rights and obligations of information and clinical documentation.
- **Law 14/2007**, of July 3rd, on Biomedical Research
- **Royal Decree 1720/2007**, of December 21th, which approves the regulation implementing Organic Law 15/1999 of December 13th on the protection of personal data in any type of clinical research.
- **Law 14/2011**, of June 1st, on Science, Technology and Innovation.
- **Royal Decree 1716/2011**, 18 November 18th, legislation related to the basic requirements of biobank authorization and functioning with biomedical research purposes.

1.4. Financial Implications

This project was carried out at the Hospital Gregorio Marañón on the Department of Psychiatry. Financial implications of the project involve material and human resources costs (Table 1). Within the latter ones, there are included the cost of a student, my supervisor (PhD. Joost Janssen) and other personnel involved in the project from the Department of Psychiatry (engineers and psychologists). Regarding the material costs, in the heading “other goods and services” facilities, internet costs and office supplies are included.

Financial implications regarding the sample (recruitment, follow-up and image acquisition) are not included as the sample was acquired from University Medical Center Utrecht in the Netherlands.

Concept	Amount	Cost (€/month)	Dedication	Total (€)
Human resources Costs				
Student	1	1,000	6 months	**6,000**
Supervisor	1	3,000	6 months	**18,000**
Personal involved	5	2,000	2 months	**20,000**
Material costs				
Computer	2	200 per month + 2,000 initial	6 months	**6,400**
Other goods and services	1	10,000	6 months	**60,000**
Total Human resources				**44,000**
Total Material Costs				**66,400**
Total direct costs				**110,400**
Taxes (21%)				**23,184**
Total costs				**133,584 €**

Table 1. Total Costs of the Project.

1.5. Socioeconomic Background

Psychiatric and neurological diseases affect at least one out of four people worldwide, according to the World Health Organization (WHO). More specifically, in developed countries, mental disorders concern more than 50% of the population at least once in their lifetime. Not only is this population percentage increasing, but also there exists a vicissitude of mental health diseases. This involves elevated cost of therapies and lower efficiency of the population at their workplaces. In 2010, the cost destinated to mental disorders in the US was higher than the cost destinated to other diseases, such as cancer and diabetes [18]. This society's economic burden is expected to increase with time.

All these issues surrounding mental health disorders are caused by a scarcity of knowledge about patient conditions. A solution to this problem is to achieve a personalized medicine by optimizing the clinical diagnosis and illness understanding.

Regarding sex-related brain differences, these are important to take into account for creating a personalized medicine depending on sex. Sex has influenced the predisposition of certain brain disorders [19], such as, multiple sclerosis in females and autism in males. To do so, neuroimaging has aided the understanding of the brain by facilitating the study and identification of brain disorders. Functional MRI (fMRI) allows the study of functional brain networks. Sex-related brain differences have been studied

for these networks [20]. Structural networks have also displayed sex-related differences [6].

Having all the aforementioned statements in mind, further study of brain networks and other brain features will help create a sex-personalized therapy for certain sex-dependent diseases. The methodology used in this project can definitely facilitate the better understanding of brain networks, both functional and structural, and developmental processes. Thereby, these brain attributes can be compared among disease (case/control), sex and age groups to develop new cost-effective therapies for each patient.

1.6. Author's Contribution

In this project, the tasks carried out by the student are:

- Research on sex-related differences in brain and other biological aspects (chemistry, hormones, genetics) to provide background and state the motivation of the study of these differences.
- Basics of image processing software.
- Understand and study the basics of R programming language. Research of R packages and implementation of those packages.
- Code creation to carry out the data analysis (section 3.3.), image processing (section 3.4.), statistical analysis (section 3.5.) and figure creation (figures in chapters 4 and 5). For this tasks, more than 1,500 lines of code where written which can be seen **here**.

1.7. Project Description

The project has six different sections.

Introduction. Covers the project motivation, hypothesis and main objectives to cover during the project, as well as, the regulatory framework, financial implications, and socioeconomic background.

Approach of the problem. Provides a brief background of the main concepts included in the project and literature review of those concepts (sex-related brain differences, MRI, image processing, structural covariance, longitudinal analysis). Its purpose is to provide the reader with some basic knowledge, in case needed, for a better understanding of the project.

Methodology. Discusses about the methods used for the study: sample characteristics, image acquisition, data analysis, image processing and statistical analysis carried out.

Results. Addresses the findings of the study conducted in this project.

Discussion. Exposes the significance of the project results in the neuroimaging field by comparing the results obtained with the existing literature. Strengths and limitations of the project are also presented.

Conclusion and Future Work. Presents the conclusions that can be drawn from this project and future work suggestions on the project's topic.

Finally, the **Bibliographic References, Figure Index and Annexes** make up the final part of the project.

CHAPTER 2: APPROACH OF THE PROBLEM

2.1. Brain Anatomy

Sex-related differences in brain anatomy have been reported [21, 22]. Some studies claim these differences are a consequence of genetics, neurotransmitters and environmental factors [2], whilst others attribute such differences to sexual differentiation [23, 24, 25]. Nevertheless, our knowledge about why these sex-related differences in brain anatomy exist remains surprisingly sparse.

Researchers point out the existence of sex-related differences in brain morphometry demonstrating that male brains, on average, are larger than female brains [26]. Such difference in brain size, related to body mass [27], results in males having 16% more neurons than females [28]. Furthermore, when correcting for body size, the difference brain size adds up to a 10% [29] favoring males. This sexual dimorphism has been reported to be prominent in frontal [30, 31] and occipital lobes [31].

Attending to sex-related differences in neural tissues, *in vivo* and post-mortem studies show that males have a higher percentage of white matter (WM) and cerebrospinal fluid (CSF), whereas woman have higher grey matter (GM) percentage [32, 20]. Moreover, it has been proven that males, on average, have better performance on motor and spatial cognitive tasks whereas females show higher average scores on memory, verbal and social tasks. Thus, spatial memory has been related to bigger brain size and verbal abilities to higher brain efficiency [33].

Sex-related morphometric differences in brain regions have been reported to be more prominent in the hippocampus [34] and the amygdala [34, 35, 36, 37]. Other features, such as, sex differences in hemispheric asymmetries [38, 26, 39] have been described.

All the findings mentioned above consider the brain as a structure divided in independent regions. However, the brain is a network that connects different brain regions. This network is known as the human brain connectome [40]. Only a few studies examine sex-related differences in brain anatomy interpreting the brain as a complex network [41, 42, 20]. In section 2.5 of this project, the importance of such change of perspective when analyzing the brain structural metrics will be further explained.

2.2. Aging

Aging leads to a reduction of brain abilities along with other cognitive functions [43]. Moreover, at a microscopic level, aging causes shrinkage of cells and loss of cell

mechanisms quality [44]. Thus, leading to some neurodegenerative diseases, e.g. Alzheimer. When analyzing brain morphology, aging is said to be responsible for the thinning of the brain cortex [45] and the decrease in brain volume [32]. Due to all of the above, some studies consider aging effect when examining sex-related brain differences [10].

Sex-related brain differences have been found at certain structures. Brain morphometry changes caused by aging are more drastic in males [10]. For instance, the loss of GM volume has been shown to be more severe in males [46].

Many questions still arise about whether sex differences in aging are relevant or not and the effect they have on the brain.

2.3. MRI to Quantify Brain

Magnetic Resonance Imaging (MRI) is a non-invasive medical imaging modality that uses non-ionizing radiation (radiofrequency). MRI is also known as magnetic resonance tomography and Nuclear Magnetic Resonance Imaging. Its main applications are disease detection, diagnosis and follow-up. Furthermore, MRI is highly used in neuroimaging, as it provides high tissue resolution and contrast.

2.3.1. MRI Basics

The properties imaged in MRI are proton density and relaxation times. Thus, MRI combines magnetic and radiofrequency fields. The imaging data is obtained by the changes in proton directionality produced by the magnetic field (BOX 1).

BOX 1 | IMAGE ACQUISITION ON MRI

The main steps of MRI are polarization, excitation and spatial differentiation. To undergo these processes, three magnetic fields are present.

Polarization. A steady field (B0) separates the proton pin states, i.e. proton spins align with B0.

Excitation. A pulsed radiofrequency (rf) field (B1) is applied. Such field produces proton state transitions, causing a 90º phase shift of proton spins.

Spatial differentiation. A switched gradient (Gx, Gy, Gz) field, applied on B0, modulates signal depending on location. Therefore, the frequencies that will be recorded by the scanner will depend on the 3-dimenstional location (x, y and z coordinates) of each structure.

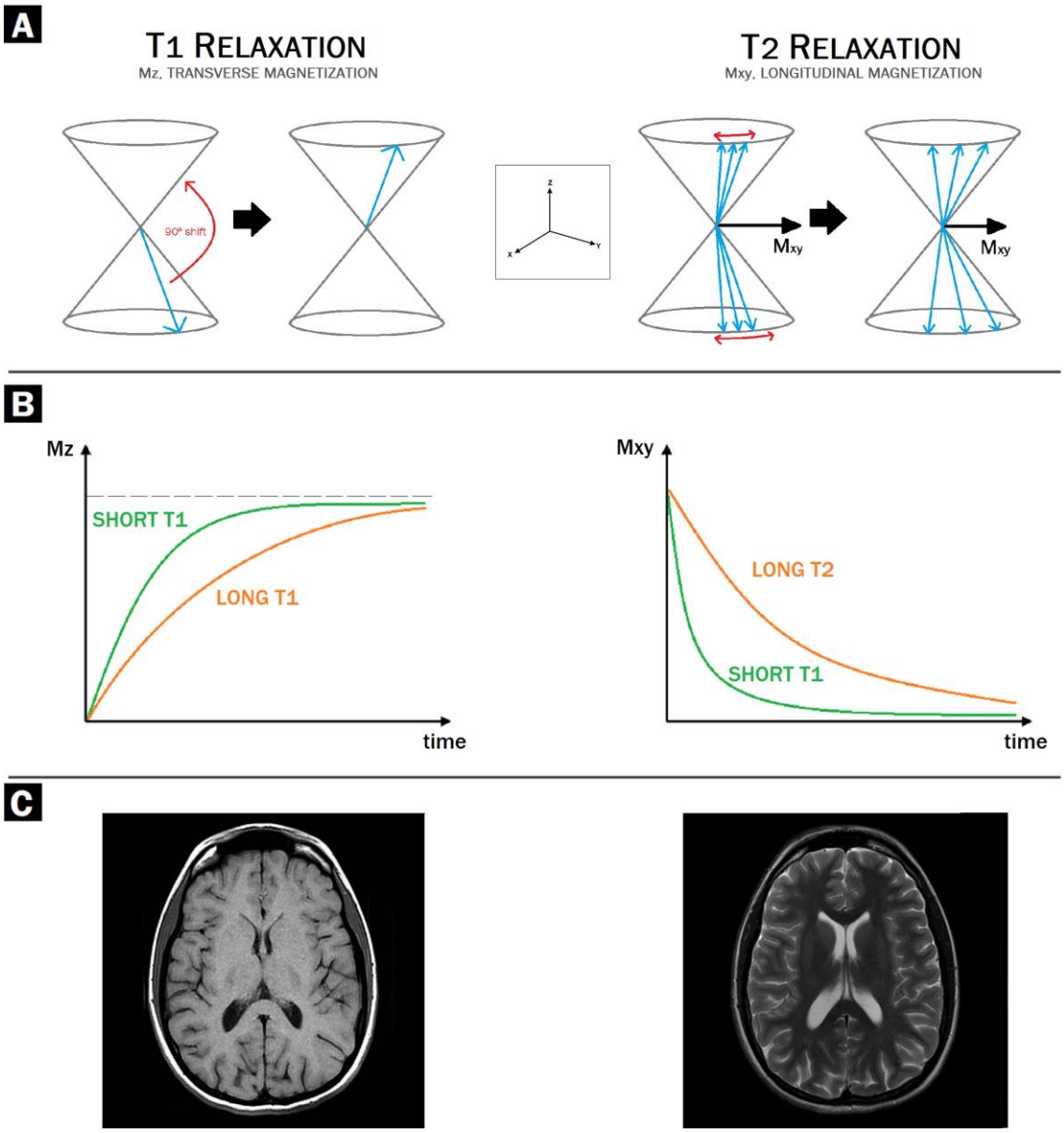

Figure 3. T1 and T2 weighted MRI. (A) *Visual Representation of Mz and Mxy and their relation to T1 and T2 values.* ***(B)*** *Graphical Representation of Mx and Mxy with time for low (short) and high (long) values of T1 and T2* ***(C)*** *Axial MRI images, left: T1-weighted, right: T2-weighted.*

The collected data in MRI is composed by frequencies. Thus, as the data is on the frequency domain, to reconstruct the image, the Fourier transform is used. Tissue contrast is given by relaxation times, i.e. the deterioration of the signal from each data point (x, y, z coordinates). Relaxation occurs due to signal decay caused by spin-spin and spin-lattice interactions, chemical shift, rf gradients and the nonuniformity of B0. Two different time constants are obtained by measuring relaxation times: T1 and T2 (see Figure 3A & 3B). The former evaluates longitudinal directions (magnetization in z, Mz), whereas the latter accounts for the transverse ones (magnetization in xy, Mxy). The contrast of the image depends of these time constants (see Figure 3C).

2.3.2. Basics of T1-weighted MRI

For this project, T1-weighted images have been used. T1-Weighted MRI is commonly used for cortical surface and cortical biomarkers extraction.

T1 is caused by spin-lattice interactions. When the system is at equilibrium (no magnetic field applied) magnetization is in equilibrium, Mo. Once the magnetic field is applied, the nuclear spins of protons change, and the system is no more in equilibrium. T1 explains how the longitudinal magnetization recovers to Mo, i.e. the time Mz takes to recover 63% of its initial value. T1 constant is calculated according to the formula below (Equation 1).

$$M_z = M_o\ (1 - e^{-\frac{t}{T_1}})$$

Equation 1. Formula to calculate T1.

By applying this formula (Equation 1), values for T1 will differ by tissue properties. Higher amount of air, gas and fast-flowing blood, i.e. higher proton density, correlates with higher T1 values. Some literature values of T1 in neural tissues can be seen in Table 2. The higher the value of the constant, the darker it will be in the image, i.e. air and CSF will be black, and GM will be darker than WM.

WM	GM	CSF
652 ± 49 ms [47]	1093 ± 101 ms [47]	3700 ± 500 ms [48]
608 ± 23 ms [48]	1065 ± 51 ms [48]	3940 ± 340 ms [50]

Table 2. T1 reported values for WM, GM and CSF.

2.4. Image Processing

Traditionally, images were processed manually by radiology experts. This method is time-consuming and not especially precise when examining small regions. Nowadays, automatized techniques are used in image processing. Two of these techniques used for MRI image analysis are voxel-based morphometry (VBM) and surface-based morphometry (SBM). The latter one is the technique used in this project for image processing.

Image processing techniques require T1-weighted images. The platform used to process these T1 images with SBM is FreeSurfer. FreeSurfer is an open source software used to analyze the data from the MRI images.

Within the steps followed for image processing, one of the most critical ones is tissue segmentation. The objective of this step is to divide the image voxels into classes. In the case of the brain, these classes correspond to the brain tissues (Figure 4). The

tissues that are considered in brain image segmentation are CSF, GM, WM and absence of brain tissue [47], which have different T1 values (as shown in Table 2).

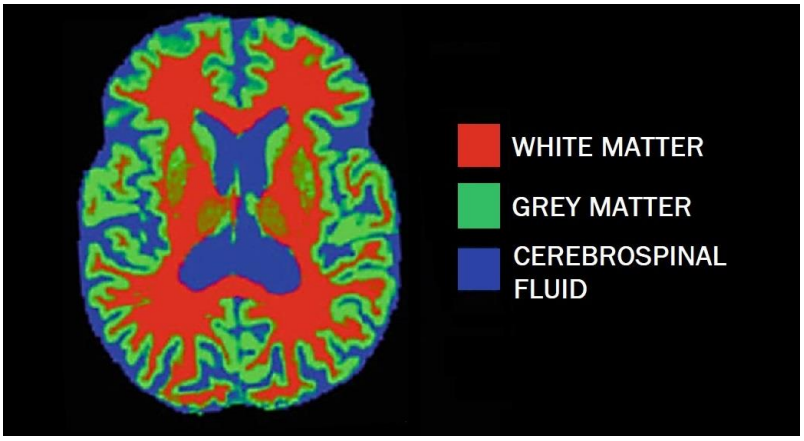

Figure 4. Tissue Segmentation of Brain Tissues. *White matter (red), grey matter (green) and cerebrospinal fluid (blue).*

Once the brain is segmented, the cortical surfaces can be extracted (see Figure 5). These cortical surfaces are the pial surface and the white surface. The latter is obtained by modeling the information obtained during the WM segmentation as a mesh into the original surface. The white surface is obtained from the boundary between WM and GM. The pial surface is created by nudging the white surface towards the CSF-GM boundary. Thus, the pial surface represents the boundary between CSF and GM.

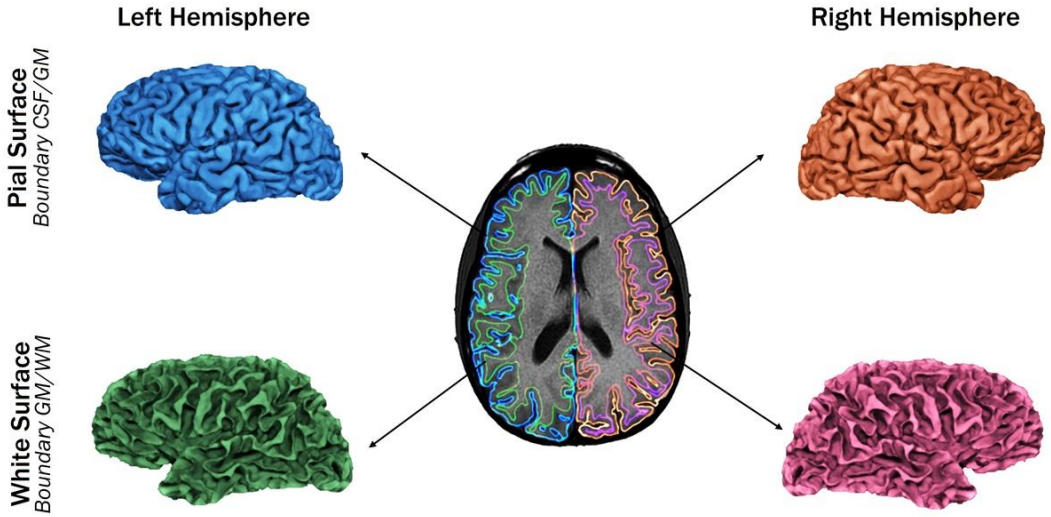

Figure 5. Extraction of White and Pial Surfaces*. Extracted from T1-weighted MRI image. Pial surfaces are shown in blue (left hemisphere) and orange (right hemisphere). White surfaces are shown in green (left hemisphere) and pink (right hemisphere).*

2.4.1. Cortical Thickness as Biomarker

When analyzing populations, biomarkers or biological markers are used. Biomarkers can be used for diagnosis, detection or research of diseases. With image processing, brain biomarkers can be extracted. Furthermore, by using SBM, local and global cortical brain biomarkers can be extracted from the cortical surfaces. To study age-related sex-brain differences in this project, the cortical biomarker used is CT.

CT measures the amount of GM present in cortical layers. This cortical brain biomarker is obtained by calculating, for each point or vertex, the distance between white and pial surfaces (see Figure 6).

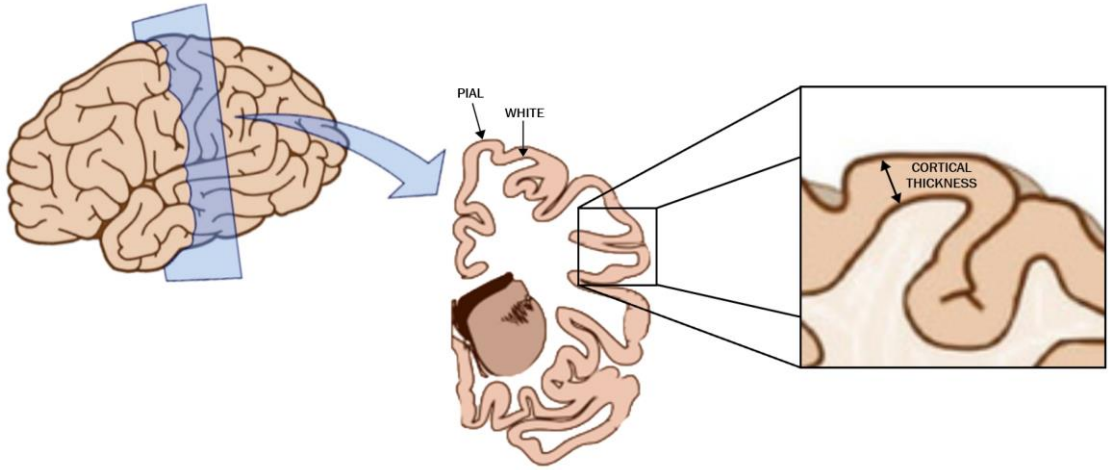

Figure 6. Cortical Thickness (CT). *CT is obtained by measuring the distance between pial and white surfaces and quantifies the among of GM in cortical layers.*

2.5. Structural Covariance Based in Cortical Thickness

In order to compare the data and conclude if there exist sex-related brain differences, a statistical analysis is needed. Studies use statistical tests (t-test, z-test, etc.) and regression analyses to assess these differences [32, 39, 6]. Nevertheless, such analyses do not account for brain connectivity patterns (neither functional nor structural). A new approach considers such brain networks in structural measures: structural covariance.

Structural covariance (SC) is a phenomenon that explains the variability relation in structural differences of two or more brain regions (see Figure 7). Some researchers have interpreted covariation between 2 regions as an indication that the regions belong to the same *brain network*. This has been corroborated by DTI and fMRI studies that have shown increased structural and functional connectivity between regions that show increased covariation from SC [41].

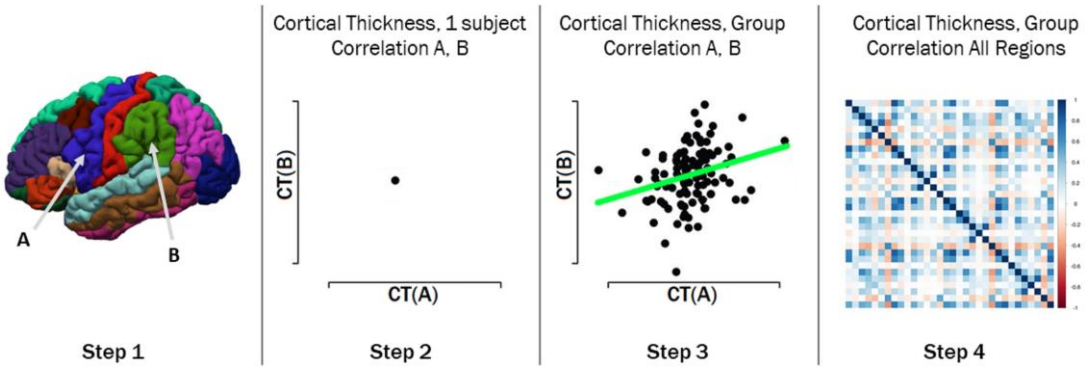

Figure 7. Structural Covariance (SC). *The schematics followed to obtain SC matrix: choose two regions A and B (Step 1) and calculate the correlation between the CT values of A and B for one subject (Step 2). Do this same two steps for all subjects of a group (Step 3) and obtain the regression line (green line in step 3), whose slope is the correlation value for the group between CT of A and B. Finally, repeat these three steps for every pair or regions (Step 4), obtaining a matrix, which is the SC Group Matrix.*

2.6. Longitudinal Structural Covariance

In this section, I will explain why is important to analyze structural covariance using a longitudinal approach, as well as, the advantages of longitudinal designs compared to cross-sectional ones.

The vast majority of studies use cross-sectional data for their SC analyses, however longitudinal designs provide a number of advantages for this type of analyses. First of all, when using a longitudinal design instead of cross-sectional one, a far smaller sample is needed to obtain the same statistical power. In 2007, a paper [53] reported that, in order to detect group-differences in brain volume, 146 subjects were required in cross-sectional designs, whereas only 4 subjects were needed in longitudinal designs to obtain similar results. This change in sample size requirement is based on the low precision of cross-sectional studies, which is caused by high variation of brain size among individuals. These inter-individual differences have been reported [54, 53], stating that variability can add up to a value of 81% in adults [55].

Furthermore, regarding the study of brain developmental processes, cross-sectional studies have limited precision to draw conclusions among these processes [56]. The brain is a dynamic organ, i.e. all its characteristics (macroscopical, microscopical) are likely to change over time. In order to take into account the intra-individual variability, i.e. how does the brain of a person change over time, longitudinal data is necessary. Moreover, depending on the age and the period of time of the image acquisition, aging and developmental brain effects can be studied. There have been many studies that study brain developmental processes with intra-individual change in

brain structures [57, 58]. In this project, to study the aging effect in sex-related brain differences in a more precise way, a longitudinal SC design will be used.

As explained in the anterior section, conventional SC (cross-sectional data) allows the analysis of covariance patterns for a certain group. Nevertheless, it is known that there exists a synchronized change between certain brain regions, i.e. brain regions that thicken or thin similarly in a certain time period [41]. To account for such change in brain regions, longitudinal data is required. Thus, the synchronization (i.e. covariance) of the CT change over time in brain regions can be studied.

One way of studying this CT change is to calculate the CT Annual Percent Change (APC) [59, 60]. In our case, sex-related brain differences in change of CT are studied, as well as, the consistency of these differences among age groups (young, middle, elder).

CHAPTER 3: METHODOLOGY

3.1. Sample

The sample used in this project corresponds to a subset from a large, single-site longitudinal sample with T1-weighted magnetic resonance imaging obtained through an international collaboration between the Department of Psychiatry at the Hospital Gregorio Marañón and the University Medical Center Utrecht in the Netherlands [61, 62].

The inclusion criteria for the sample are described below. The sample included subjects that met the research diagnostic criteria of “never [being] mentally ill”. For every subject age at scan, sex, and handedness were reported as well as clinical assessments [61]. IQ scores were estimated based on subtests (digit-symbol coding, information, arithmetic, and block design) of the Dutch version of the Wechsler Adult Intelligence Scale III (WAIS-III). Social economic status (SES) was specified as the highest completed level of education from one of the parents.

From the initial dataset, 298 unique subjects were included, all of whom had a baseline and at least one follow-up T1-weighted MRI scan. From this subset, subjects were excluded if they: (1) had an additional diagnosis not in the interest of the study; (2) or those whose diagnosis changed during follow-up and after being reassessed met the criteria for other diagnosis than healthy on the follow-up scan, (3) did not have all their scans acquired on the same scanner or only had one available baseline scan . This led to the exclusion of a number of participants (see Figure 8). Further exclusion of subjects due to image quality assurance procedures is described in more detail below (BOX 2).

Figure 8. Sample. *Flowchart followed to obtain the final scans to work with.*

Detailed demographic information for the final longitudinal sample can be found in Annex A, as well as imaging variables information for two dimensions analyzed: global measures and lobar measures.

BOX 2 | IMAGE QUALITY ASSURANCE

QUALITY ASSURANCE (QA)

After the completion of the preprocessing pipeline for all T1 MRI scans in FreeSurfer, we rigorously assessed the quality of the data using a combination of visual inspection and the examination of several quantitative metrics following recent recommendations from the literature [63].

First, we calculated five quality measurements proposed by the authors of the Preprocessed Connectome Project (http://preprocessed-connectomes-project.org/quality-assessment-protocol/) to identify images that were unusable: signal-to-noise ratio (SNR), contrast to noise ratio (CNR), foreground to background Energy Ratio (FBER) and percent artefact Voxels (Artifacts) and Entropy Focus Criterion (Entropy). We defined the threshold for outliers as [mean-(2.698*SD)] for SNR, CNR and FBER metrics; and Artifacts and Entropy were only tested for [mean-(2.698*SD)] as based on the exclusion criteria explained by the ENIGMA QA (www.enigma.ini.usc.edu) protocol. Next, for each scan the whole brain mean CT, total SA, total WM volume, total GM volume, subcortical GM volume and ICV were calculated. These quality control steps resulted in the below exclusions.

QA-BASED EXCLUSIONS

Following the exclusion of subjects that met our exclusion criteria (855 scans) a sample of 924 scans was obtained. After the evaluation of the computed quantitative measures of image quality, 257 scans were considered of insufficient quality and were excluded from our analysis (see Figure 8). These excluded scans were validated by manually assessing image quality after preprocessing to assure that all preprocessing steps worked and to avoid the dissemination of error along the analysis. The parameters that were most useful for objectively detect artefacts visually were agreed on between researchers, e.g. incorrect sulcal labelling or insufficient quality of sulcal segmentation resulting in gross anatomical abnormalities. These visual checks were performed in BrainVisa. In addition, 12 scans were excluded after this manual inspection.

Based on these exclusion criteria, the final sample comprised of 700 scans: including at least two scans for the 298 females and males (of which 102 had an additional third scan).

3.2. Image Acquisition

Two scanners (same vendor, field strength and acquisition protocol) were used. All subjects had their baseline and follow-up scans on the same scanner. Subjects were scanned on either a Philips Intera or Achieva 1.5 T and a T1-weighted, 3-dimensional, fast-field echo scan with 160-180 1.2 mm contiguous coronal slices (echo time [TE], 4.6 msec; repetition time [TR], 30 msec; flip angle, 30°; field of view [FOV], 256 mm; in-plane voxel size, 1x1 mm) was acquired (Table 3).

Scanner	Field strength	Orientation	Voxel Size (mm)	FOV	Slices	Acquisition matrix	TR (ms)	TE (ms)	Flip Angle	Sequence
Philips Achieva	1.5 T	T1 Coronal	1x1x1.2	256x256	160-180	256x192	30	4.6	30	Fast field echo
Philips Intera	1.5 T	T1 Coronal	1x1x1.2	256x256	160-180	256x192	30	4.6	30	Fast field echo

Table 3. Scanner and sequence characteristics of the sample.

3.3. Data Analysis

To analyze the data, a pipeline in R (www.r-project.org/) was created. R was used in order to be consistent with existing pipelines at the Imaging Group of the Department of Psychiatry, leaving the possibility of interfacing various pipelines in the future. R is an open source programming language which is widely used in research to run statistical analysis and for graphic creation. The pipeline followed for the data analysis (see Figure 9) will be further explained in this section. The scripts used for this project are available **here**.

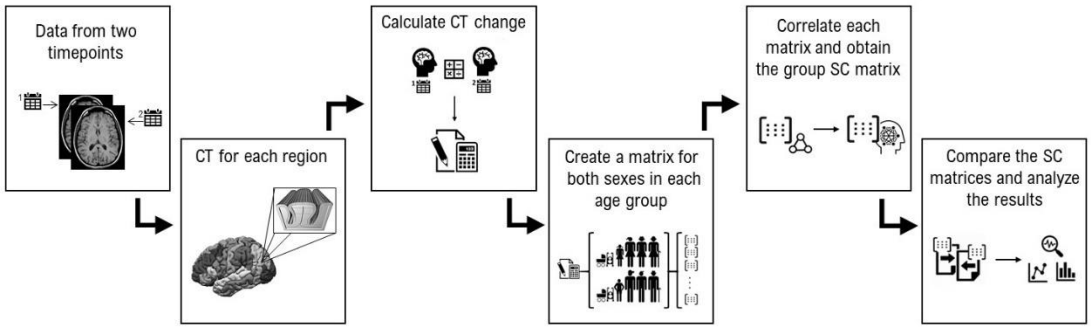

Figure 9. Steps followed in the data analysis.

Firstly, the data of the scans (T1-weighted MRI images) is gathered. In total 596 final scans (2 scans per subject) belonging to 298 included healthy controls. Then, for

each scan, CT is measured in different brain regions (section 3.4.1). With the CT value for each brain region, I calculated the CT change (section 3.4.2). Then, the data is divided into groups (section 3.5.1). For each group, a structural covariance matrix is obtained (section 3.5.3). Finally, the group-SC-matrices of males and females will be compared (section 3.5.4) in each age group.

The first three steps (Figure 9) will be explained in the image processing section (3.4) and the three last ones in the statistical analysis section (3.5).

3.4. Image Processing

After the images are acquired, they are processed. The images processed are the ones which belong to the final included scans dataset (596 scans). The goal of this processing is to measure CT. Note that to measure CT, the pial and white surfaces are needed. To extract the required surfaces the FreeSurfer outline followed is shown in Figure 10.

After the surface extraction, for each of the final included scans, CT is measured at the regions of interest. This brain division into is the last step of image processing (Figure 10). The ROIs are set depending on the parcellation system.

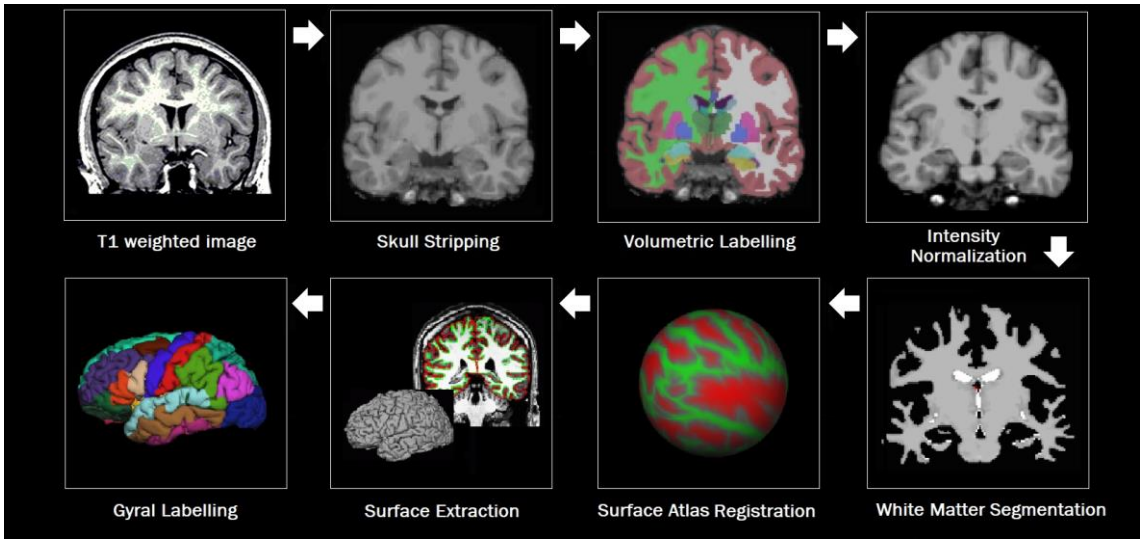

Figure 10. Image Processing Pipeline.

3.4.1. Brain Parcellations: Establishing the Regions of Interest

In this project, the brain parcellation system used is the Desikan-Killiany parcellation system [64] (BOX 3). This brain atlas divides the brain into 35 ROIs.

BOX 3| DESIKAN-KILLIANY ATLAS

The Desikan-Killiany (dk) parcellation system or brain atlas is one of the most common atlas used in neuroimaging. The brain is divided into 35 gyral-based ROIs. By “gyral” meaning the brain gyrus (see Figure 11), which are the folds of the cerebral cortex. Oppositely, the spaces between folds of the cerebral cortex are known as sulci.

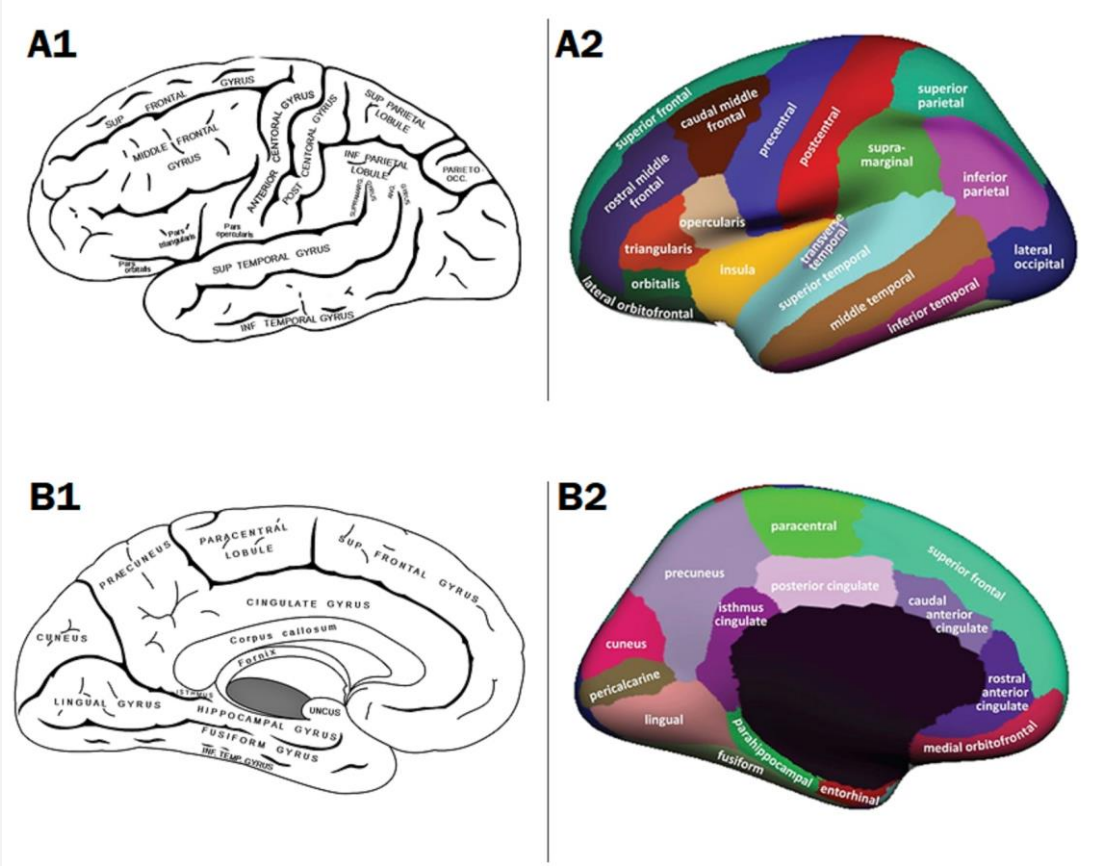

***Figure 11. Desikan-Killiany atlas**. **(A) Lateral view** of brain gyrus (A1) and ROI division with dk atlas (A2). **(B) Medial view** of brain gyrus (B1) and ROI division with dk atlas (B2).*

3.4.2. Annual Percentage Change

Once the CT data is measured for each ROI and scan, the CT change can be computed. Change in CT is calculated with the formula of symmetrized annual percentage change (APC) (BOX 4).

The formula used to calculate APC (Equation 2) removes both the effect of brain size and time interval between scans. The former is removed by dividing by the mean of both CT values, the latter is removed by dividing by the time between scans. In the APC formula, CT change is weighted by the mean CT over time, thus eliminating bias of change due to higher/lower CT values (a thicker cortex might be related to larger change in CT [65]. Regarding the need of controlling time between scans is due to the fact that not all subjects have the same time between the acquisition of both scans.

BOX 4 | SYMMETRIZED ANNUAL PERCENTAGE CHANGE

To analyze the longitudinal data of CT, I have used the annual percentage change (APC), i.e. the CT change percentage per year. To do so, a general formula has been applied to the data to compare it across subjects (Equation 2). APC is calculated at every ROI and in all of the included subjects, taking into account each subject's first and second scan.

$$APC = \frac{{}^{CT_2 - CT_1}/_{mean(CT_1, CT_2)}}{time\ between\ scans\ (years)} * 100$$

Equation 2. Annual Percentage Change (APC). *CT1 and CT2 stand for the CT values measured in a certain ROI at the first and second scan of the same subject, respectively.*

3.5. Statistical Analysis

Using the computed APC values, different statistical analyses were run on the data to obtain relevant results.

3.5.1. Age Groups

To study the effect of age in sex-related brain differences, the 298 subjects were divided into similar and sufficiently sized groups of females and males across the age range. The optimal solution was diving the sample into three age groups: young adults, middle adults and elder adults. Such nomenclature is employed to facilitate the age group referencing, I am aware that the terms "young", "middle" and "elder" are to some extent arbitrary. The descriptives of each age group are summarized in Table 4.

Group	Mean Age	Scan Interval	Female Subjects	Male Subjects	Age Range (years)
Young Adults	23.29 years	3.84 years	53	76	17.94 - 27.16
Middle Adults	34.51 years	3.96 years	59	69	27.17 - 45.00
Elder Adults	54.65 years	4.66 years	19	22	45.01 - 67.32

Table 4. Division of sample in age groups.

3.5.2. Statistical Sample Description

Once the sample was divided into three age groups, a statistical description of the sample was done to control there are no prominent differences between the demographic values of males and females in each age group. Therefore, each age group was statistically analyzed independently. The demographic data or variables of the subjects can be divided into two groups: descriptive variables and qualitative variables.

Regarding the descriptive variables, a two-sample t-test was run on them to assess mean differences between sexes. As mentioned before, each age group was

analyzed independently. Thus, for each age group, male's descriptive variables were statistically compared to female's. Descriptive variables included are age at first scan (age 1), age at second scan (2), subject's IQ, time between both scans (scan Interval) and socio-economic status (SES, based on the years of education (Ed Years)). The statistical parameters extracted from the two sample t-test of these variables are the mean value of females (F Mean) and males (M Mean), the t-statistics (t) and its corresponding p value (p).

Regarding the qualitative variables, a standard chi squared test (N > 5 for each cell) was run on them. As with the descriptive variables, male's qualitative variables were statistically compared to female's. Qualitative variables included are scanner used for acquisition (Scanner), handedness (Right-handed, Left-handed or Ambidextrous), SES education level (Ed Lvl 1-5) and SES occupational status (Occ Status 1-4). The statistical parameters extracted from the chi-test of these variables are the occurrence value in females (F) and males (M) of each case among the same qualitative variable, the X-statistics (X) and p value (p).

3.5.3. Obtaining the SC Matrices

Once the sample has been divided into groups, a matrix with the APC values of each sex (females, males) and age group (young, adults, elder) is created. Thus, 6 matrices are created. In these matrices, each row contains all APC values for subject and each column all APC values of a ROI (Figure 12). Thus, all matrices have the same number of columns (as many as ROI) but different number of rows (depending on the number of subjects for each group).

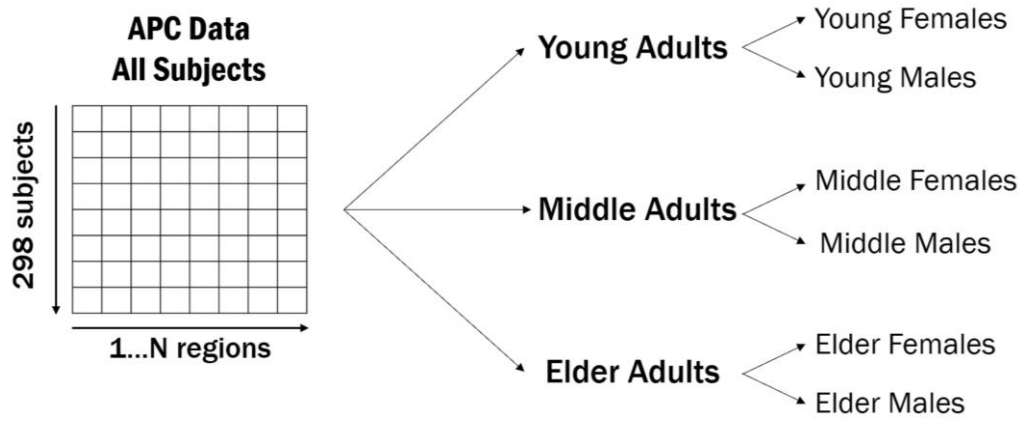

Figure 12. APC group-matrices. *Starting from the initial APC matrix. The final matrices will be as the one shown, but changing the number of subjects by the ones included in each of the six groups*

After the creation of these APC group-matrices, as the APC values have been calculated for both left and right hemisphere, a mean group matrix is created. This is the matrix that is used in the remaining steps. Each mean APC group-matrix is correlated, obtaining the SC group-matrix (see Figure 13). In the SC matrix, each cell represents

the Pearson's correlation (r) coefficient for each pair of regions. The closer such value is to 1, the more the CT change changes synchronously between regions. If the correlation value is 0, the change in both regions is unrelated. In case the correlation value is close to -1, represents that synchronous changes are in the opposite direction, i.e. while one region is growing, the other one is shrinking at a similar rate.

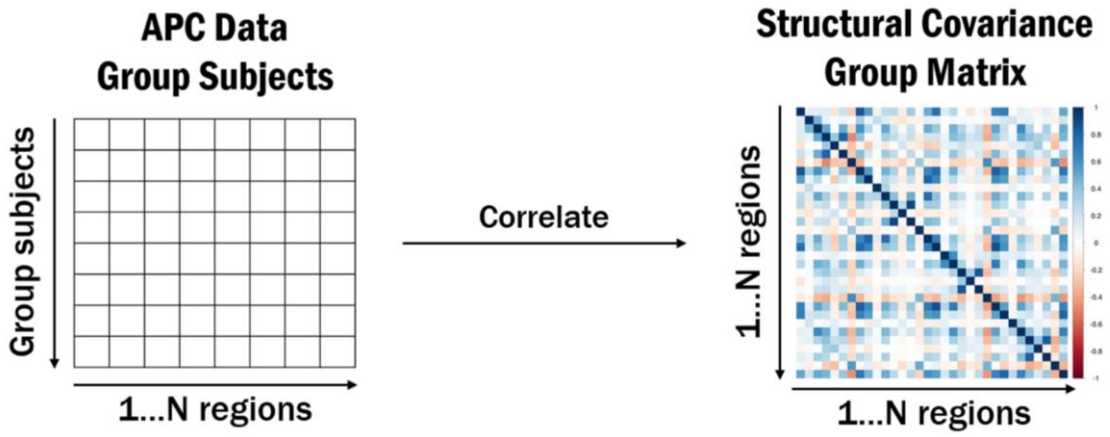

Figure 13. Structural Covariance Matrix. *The SC group-matrix is a symmetric matrix as it has the same regions in rows and columns. The SC matrix in the image is only a representation, it does not refer to any of the results.*

3.5.4. Comparing the SC Matrices

Once the six SC-group matrices have been obtained, the differences between them are computed. Therefore, to address the sex-differences with SC matrices, each age group is analyzed independently. This section will be explained for one group of age, but it has been done for the three of them.

$$Difference\ Matrix = Msc^2 - Fsc^2$$

Equation 3. Difference Matrix Calculation. *Subtraction of the males' SC matrix (Msc) squared to the females' SC matrix (Fsc) squared.*

To compare both matrices, the difference matrix is computed (Equation 3). After calculating the difference matrix, to analyze if there are statistically significant differences, further statistical analyses need to be done. As the SC matrices are based on correlation and are calculated for two groups (females and males) with different sample size, a comparison needs to be done. To compare both matrices, the correlation coefficients of the matrices are transformed into z-scores. Once both matrices are z-transformed, they can be compared and examined for statistical significance. To do so, the value of Z-observed needs to be calculated (Equation 4).

$$Z_{observed} = \frac{Mz^2 - Fz^2}{\sqrt{\frac{1}{subjM - 3} + \frac{1}{subjF - 3}}}$$

Equation 4. Calculation of Z-observed. *The Fisher-Z matrix of males (Mz) squared is subtracted to the Fisher-Z matrix of females (Fz) squared. Both matrices (M and F) belong to the same age group. That result is divided by the square root of the sum of two divisions, where subjM and subjF are the number of male and female subjects, respectively.*

Once the Z-observed matrix has been calculated, the p-values for each matrix component can be derived. As multiple comparisons have been made before obtaining the p-value matrix, a false discovery rate (FDR) correction [66] is required. Thus, a new FDR-corrected p-value matrix is obtained.

The obtained corrected p-value matrix can now assess with higher reliability and precision if the sex-related brain differences are significant or not. For this project the threshold for determining significance during FDR correction was set at 0.05 (q). Then, by applying this threshold to the difference matrix obtained before (Equation 3), only the values that have a lower p-value than the threshold are considered significant.

In the plotting of the matrix results, only the regions involved in a significant correlation are shown i.e. significant ROIs. Within these reduced matrices which contain only significant ROIS, only the significant correlations between them are plotted. The non-significant correlations will be white cells in the matrix. This subsetting of ROIs is done in order to facilitate interpretation of the matrices and focus only on the significant correlations. This is applied in the three matrices represented: the difference matrix, the Msc and Fsc. As all these matrices are symmetric, only the lower triangle of these matrices will be represented to avoid redundancies.

Nevertheless, the significant correlations themselves do not represent the magnitude of change, i.e. if the pair of regions have a large APC value (large change in CT) or a small one (small change). To assess this, a one sample t-test was run on the APC values of the significant ROIs. Then, the t-statistic value and p-value are extracted from that analysis. With the R package *ggseg* the t values of the significant ROIs are visualized. I have chosen *ggseg* because it provides a schematic way of plotting brain regions inside the R environment that aids in interpretation of the results. Moreover, *ggseg* can be easily integrated in any R workflow.

As mentioned before, this whole process (Figure 14) has been repeated in the three age groups (young, middle and elder adults) to assess the sex-related brain differences for each age group. Then, the results obtained for each age group can be compared to address the aging effect in sex-related brain differences.

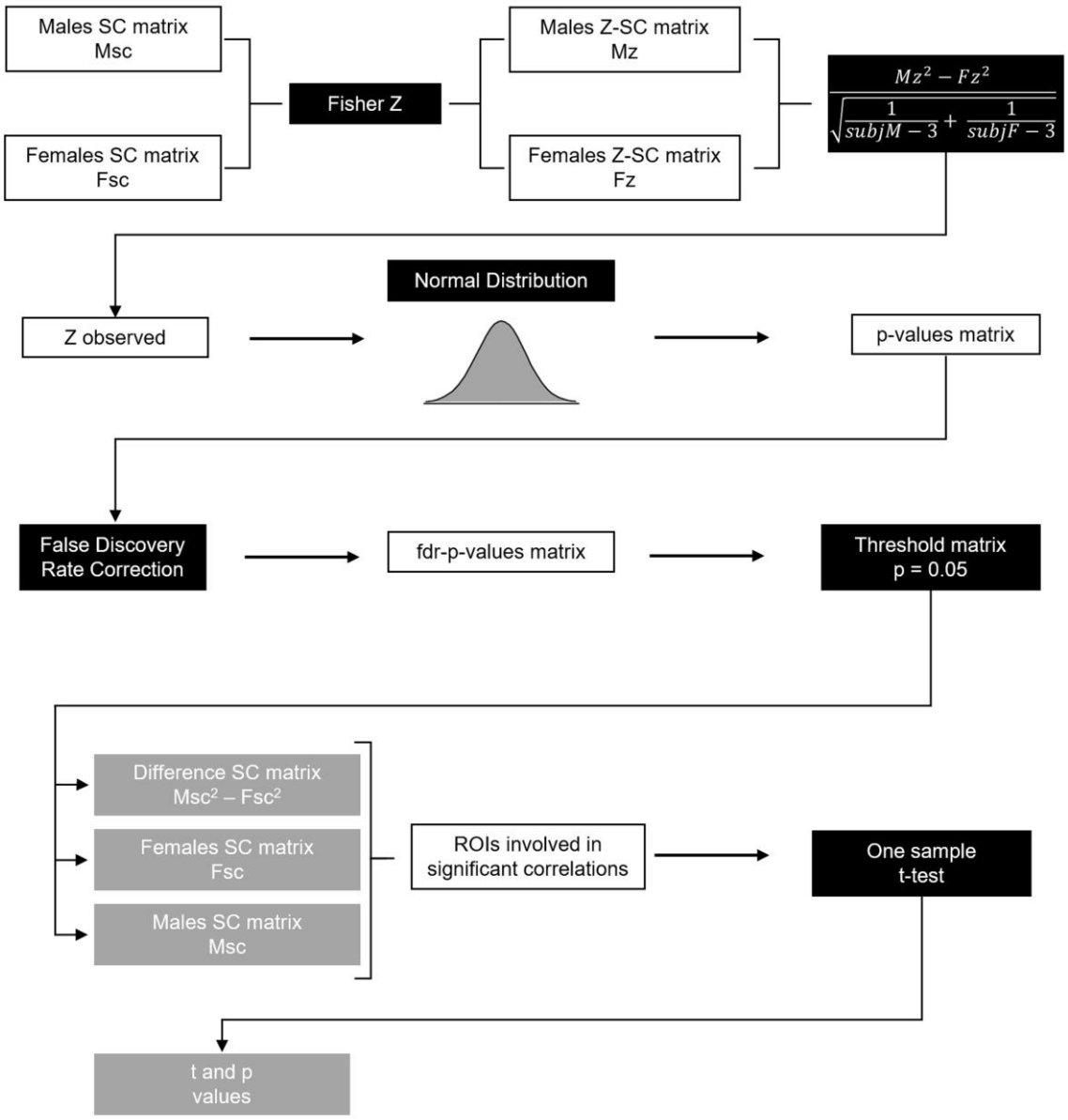

***Figure 14. Comparison of SC Matrices**. Grey boxes represent the values that will be represented by figures in the results section: the matrix of SC differences and the females and males SC matrices with the p value (p= 0.05) threshold; and the representation of the t and p values of the ROIs involved in those correlations to visualize the magnitude of the APC.*

3.5.5. Scale Effect

As explained before, larger regions will account for global differences, whereas smaller regions will determine local ones. To address this scale effect, the process explained for image processing and statistical analysis was also done for other three more parcellation scales. The baseline scale (scale 1) is the one explained in 3.4.1. Scales 2, 3 and 4 have the same region division, but the 35 ROIs established in scale 1 are subsequently divided into smaller similarly sized subregions. The higher the scale, the lower the region size and the more regions (see Table 5).

Furthermore, for a better visualization of the scale effect and how this effect changes the results, a figure with the correlograms, a graphical circular representation of correlation-strength, will be presented. This correlograms have been created using the R packages *circlize* and *chorddiag*.

ROI	Scale 2	Scale 3	Scale 4
Banks Superior Temporal Sulcus	-	2	3
Caudal Anterior Cingulate	-	-	2
Caudal Middle Frontal	-	3	6
Cuneus	-	-	3
Entorhinal	-	-	-
Frontal Pole	-	-	-
Fusiform	2	4	8
Inferior Parietal	2	5	10
Inferior Temporal	2	4	8
Insula	2	4	7
Isthmus Cingulate	-	-	3
Lateral Occipital	2	5	11
Lateral Orbitofrontal	2	4	7
Lingual	2	4	8
Medial Orbitofrontal	-	2	5
Middle Temporal	2	4	7
Para Central	-	2	5
Para Hippocampal	-	-	3
Pars Opercularis	-	2	4
Pars Orbitalis	-	-	2
Pars Triangularis	-	-	3
Pericalcarine	-	-	3
Post Central	3	7	14
Posterior Cingulate	-	2	4
Pre Central	4	8	16
Precuneus	2	5	11
Rostral Anterior Cingulate	-	-	2
Rostral Middle Frontal	3	6	12
Superior Frontal	4	9	18
Superior Parietal	3	7	14
Superior Temporal	2	5	11
Supramarginal	2	5	10
Temporal Pole	-	-	-
Transverse Temporal	-	-	2

Table 5. Number of ROI subdivision of parcellation scales 2-4. *As the first scale is based in the dk atlas, the table shows the number subdivision for the basal ROIs in each scale (2-4). A value of "-"means no subdivision of the basal ROI.*

CHAPTER 4: RESULTS

4.1. Statistical Sample Description

The sample description is shown including both descriptive (Table 6) and qualitative (Table 7) variables. Regarding the descriptive variables, values with significant differences between groups are IQ (p = 0.001) and Scan Interval (p= 0.008) and the distribution of Young Adults and age at the second scan (p= 0.045) of Middle Adults. With reference to the qualitative variables, significant differences can be found for scanner (p= 0.05) of Middle Adults and Occupational Status (p= 0.047) in Elder Adults.

	Group 1. Young Adults				Group 2. Middle Adults				Group 3. Elder Adults			
	F Mean	M Mean	t	p	F Mean	M Mean	t	p	F Mean	M Mean	t	p
Age 1	21.219	21.478	-0.611	0.543	31.771	33.182	-1.636	0.104	51.226	53.273	-1.216	0.232
Age 2	24.764	25.516	-1.864	0.065	35.537	37.304	-2.030	0.045	56.061	57.782	-0.956	0.345
IQ	103.915	114.095	-3.537	0.001	107.709	111.662	-1.450	0.150	122.588	124.714	-0.488	0.629
Scan Int.	3.546	4.042	-2.714	0.008	3.769	4.125	-1.909	0.059	4.839	4.511	1.328	0.192
Ed Years	24.062	26.652	-0.460	0.647	23.933	15.481	1.946	0.056	12.941	18.471	-1.073	0.299

Table 6. Sample Description of Descriptive Variables. *t and p values of the descriptive variables for the three age groups, as well as the mean value of females (F Mean) and males (M Mean) for these variables.*

	Group 1. Young Adults				Group 2. Middle Adults				Group 3. Elder Adults			
	F	M	X	p	F	M	X	p	F	M	X	p
Scanner 1	6	19	2.915	0.088	11	25	4.036	0.045	15	18	0.000	1.000
Scanner 2	47	57			48	44			4	4		
Right-handed	33	53			34	47			16	18		
Left-handed	3	6	0.361	0.835	3	3	1.138	0.566	0	1	1.349	0.509
Ambidextrous	1	3			1	4			2	1		
Ed Lvl1	1	1			0	4			1	1		
Ed Lvl2	1	1			4	7			4	1		
Ed Lvl3	1	8	8.104	0.088	2	0	6.515	0.164	0	0	2.685	0.443
Ed Lvl4	16	10			8	11			6	4		
Ed Lvl5	28	45			29	30			6	9		
Occ Status 1	12	17			41	46			15	8		
Occ Status 2	35	44	1.594	0.451	3	4	0.055	0.973	0	0	6.106	0.047
Occ Status 3	0	0			0	0			2	6		
Occ Status 4	0	2			1	1			0	2		

Table 7. Sample Description of Qualitative Variables. *X and p values of the qualitative variables for the three age groups. For Ed Llv (Education Level) the values are set according to the following grading system: 1=Low; 2 =Intermediate-low; 3 =Intermediate; 4=Intermediate-high; 5= High. Regarding Occ Status (Occupational Status), the values are set according to the following grading system: 1= currently working; 2 = student; 3= retired/early retired; 4=workless or unsuitable to work.*

4.2. Sex-related Brain Differences Across Age Groups in Structural Covariance

In this section, the results for sex-related brain difference for the three age groups (young, middle, elder), as well as, the scale effect in those findings will be presented.

Firstly, the results for the sex-related brain differences will be shown in detail for the brain parcellation based on scale 1. The other parcellation scales are used to examine the robustness of scale 1 findings against the effect of higher scale parcellations and this will be mentioned afterwards. Regarding these sex-related brain differences at the scale one, the results for each age group are presented independently: young (Figure 15), middle (Figure 17) and elder (Figure 19) adults. Each figure is divided into three images: females and males SC matrices, matrix of SC differences and t-values for the ROIs involved in significant correlations. In the second image of each figure (matrix of SC differences), all the values above 0 (blue color palette) indicate a higher coefficient of co-variation in males between the respective pair of regions. On the contrary, a value below zero (red color palette) indicates a higher correlation of females between the respective pair of regions. This is due to the formula used to obtain the value of the matrix (see Equation 3 in Methodology). The third figure in the image represents the APC t-test results of the significant ROIs for females and males. In a smaller size, represent only the t values below the statistical threshold ($p<.05$) i.e. the regions where the CT change is significant. Furthermore, for each age group: young (Figure 16), middle (Figure 18) and elder (Figure 20) adults, significant correlations are represented in a chord diagram. Blue correlations indicate higher coefficient of co-variation in males and, red correlations, in females. All these figures (Figures 15-20) will be later analyzed in section 4.2.2. in order to address the sex-related brain differences and the aging effect in these differences.

Regarding the robustness scale effect analysis, the results for sex-related brain differences at the four parcellation scales are presented (see Figures 21-27) and discussed in section 4.2.3. For each age group, two figures accounting for the scale effect are shown: young (Figures 21 & 22), middle (Figures 23 & 24) and elder (Figures 25 & 26) adults. In each group, the first of both figures represents the significant correlations for each scale. The second figure shows the value of these correlations and the APC in the significant ROIs among the four scales. Finally, all the age groups and scales are presented in one figure (Figure 27) showing the regions where the significant ROIs have a significant APC ($p<.05$). All the figures accounting for the scale effect have the same ROI legend or number code for the ROIs, independently of scale or age group.

4.2.1. Sex-related Brain Differences

Regarding the first age group, young adults, sex-related brain differences are found in 14 significant correlations. For both, females and males, the significant correlations involve positive correlations of APC (Figure 15A). When comparing the SC matrices of females and males, in all significant correlations, males have higher coefficients of co-variation than females (Figures 15B & 16). The difference values range from 0.26 to 0.47. These correlations comprise 12 ROIs out of the 34 initial ones. These significant ROIs are located in the frontal, parietal and temporal lobes (Figure 15C). Furthermore, regarding the APC values in those regions, these are far more significant in females compared to males. This can be seen in the schematic representation of the brains (Figure 15C), where males only have one region with significant APC whereas females display far more. Thus, indicating that within the significant correlations, females have synchronized CT loss, but males do not.

As for the second age group, middle adults, seven significant correlations show sex-related brain differences. As in the previous age group, all significant correlations are positive (Figure 17A), this is, the correlations are stronger in males than females (Figure 17B & 18). These correlations involve less number of regions, in this case 8, which are located in frontal and parietal lobes. Moreover, oppositely to young adults, males have greater negative APC in almost all regions thus being the group with more CT loss (Figure 17C).

Finally, for elder adults, the number of correlations decreases to four, being the lowest value among the three age groups. All significant correlations are positive (Figure 19A), as in the previous age groups. Nevertheless, there is a main difference when comparing the difference matrix of elder adults with the ones from other age groups. This is, there is one correlation, between the inferior parietal and supramarginal regions, which is stronger in females (red correlation in Figures 19B & 20). Regarding the regions included in significant correlations (Figure 19C), there are six. Significant ROIs are located in parietal and occipital lobes. Elder males show apparently no APC as the t values are around zero and there are no regions with significant change. Contrarily, elder females present negative values of APC with four regions indicating significant change. Thus, following the pattern discussed in the first age group, elder females, in general, exhibit CT loss whereas elder males do not.

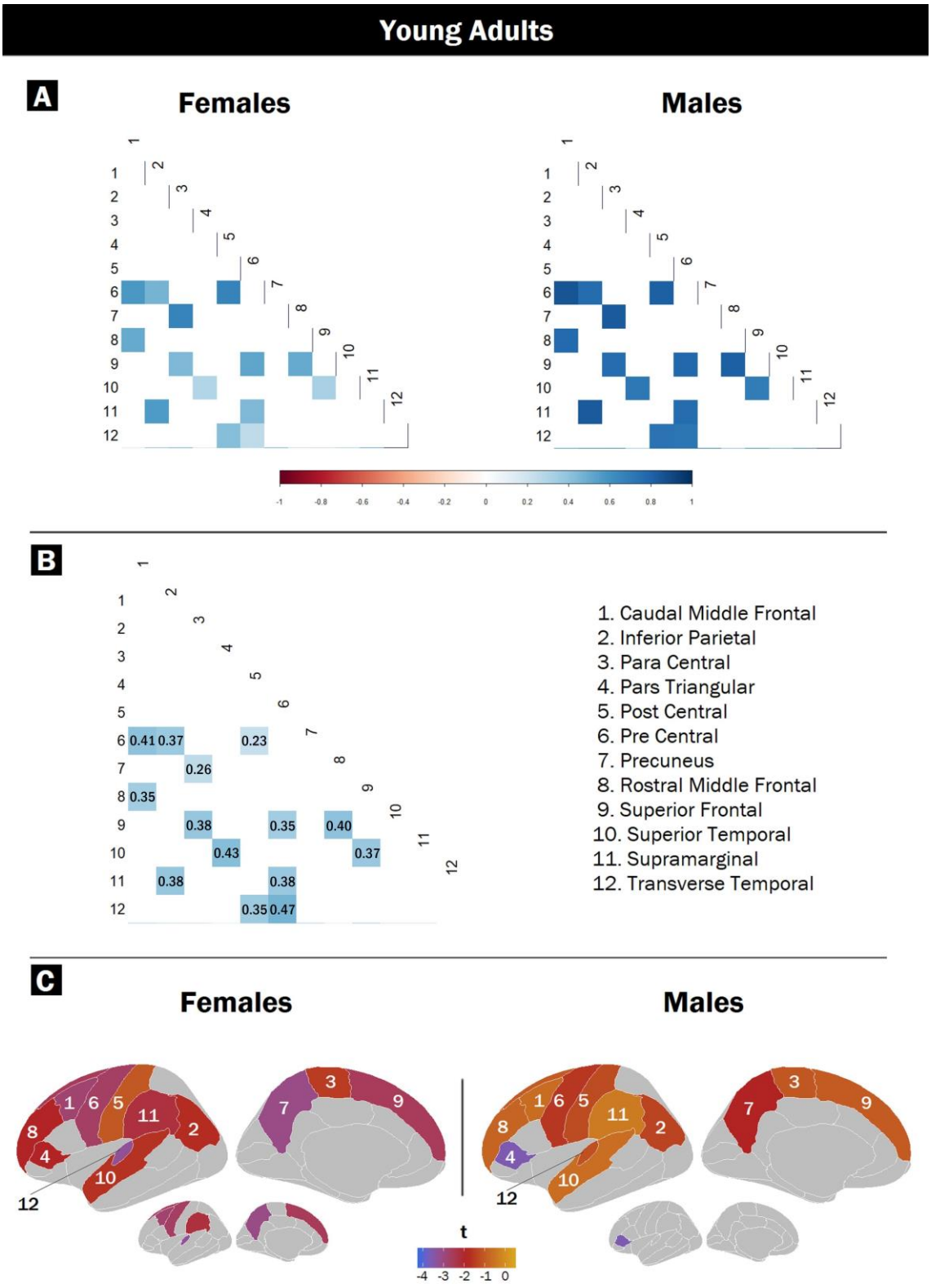

Figure 15. Young Adults. (A) Structural covariance matrices. *Females (left) and males (right) showing only the significant correlations (14) and the regions (12) involved in those correlations (number code in image B).* ***(B) Difference Matrix.*** *All values with a value above zero will mean a higher coefficient of co-variation in males, oppositely, a value lower than zero, will refer to a higher correlation value in females.* ***(C) Visualization of APC t values of significant ROIs.*** *In the small brains only the t values for significant results (p<.05) are shown.*

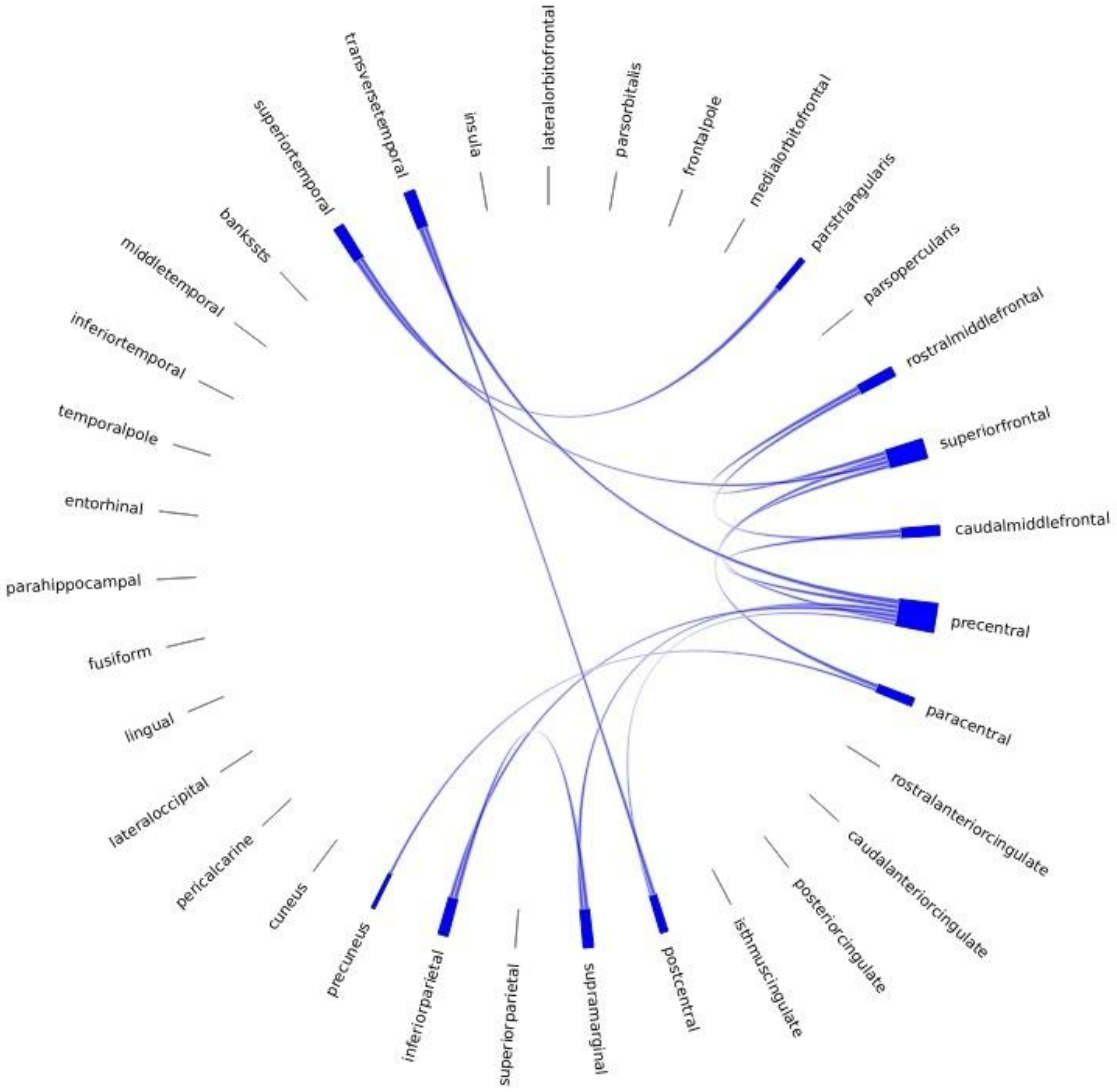

Figure 16. Significant Correlations for Young Adults. *The values that are plotted are the ones obtained in the difference matrix (Figure 15B), thus correlations with higher coefficient of co-variation of males are represented in blue.*

BOX 5 | YOUNG ADULTS RESULTS

- 14 significant correlations.
- 12 significant ROIs in frontal, parietal and temporal lobes.
- All significant correlations positive.
- Stronger co-variation coefficient in males for all correlations.
- Females have significant CT loss, males do not.

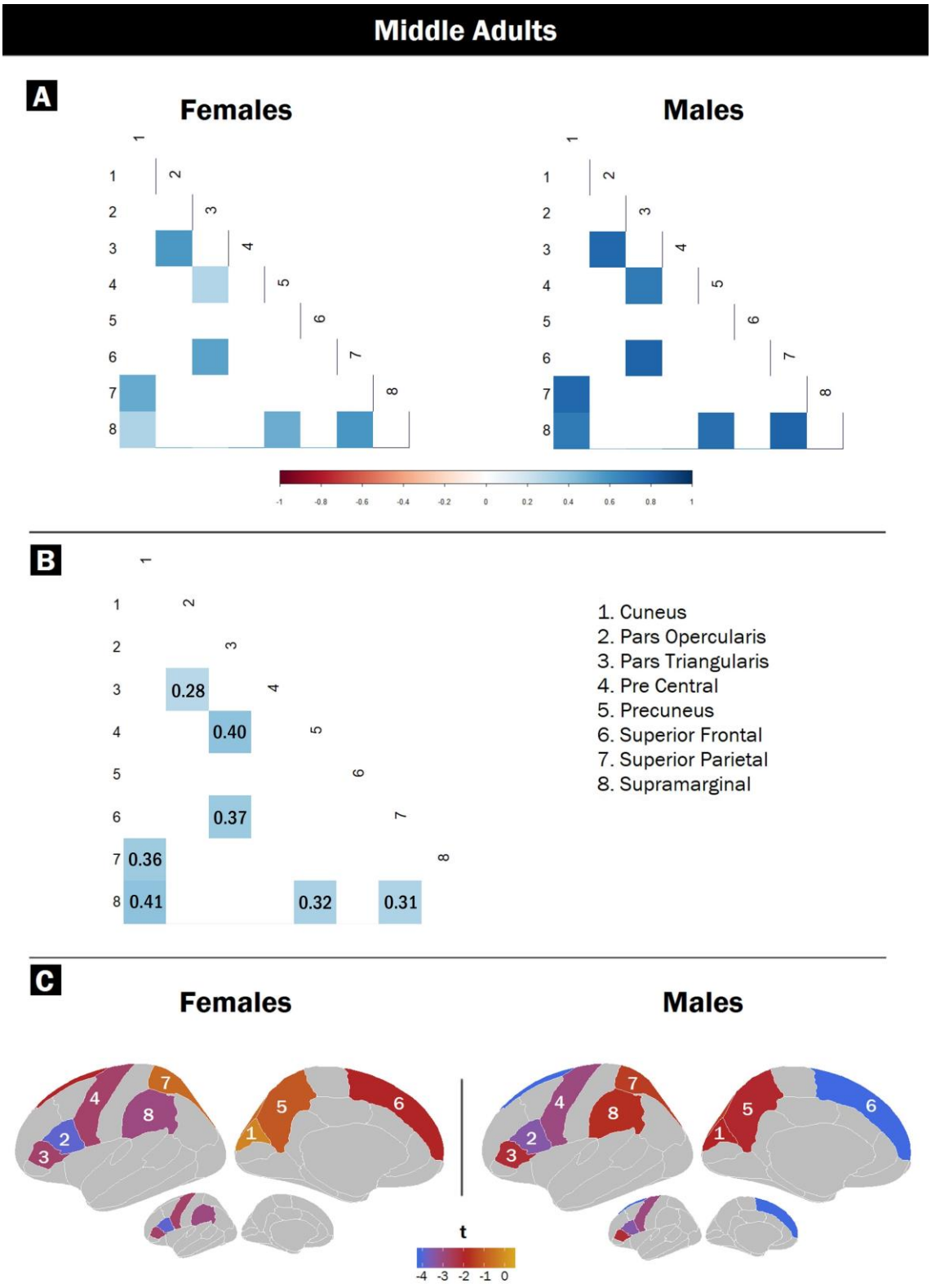

Figure 17. Middle Adults. (A) Structural covariance matrices. *Females (left) and males (right) showing only the significant correlations (7) and the regions (8) involved in those correlations (number code in image B).* ***(B) Difference Matrix.*** *All values with a value above zero will mean a higher coefficient of co-variation in males, oppositely, a value lower than zero, will refer to a higher correlation value in females.* ***(C) Visualization of APC t values of significant ROIs.*** In the small brains only the t values for significant results (p<.05) are shown.

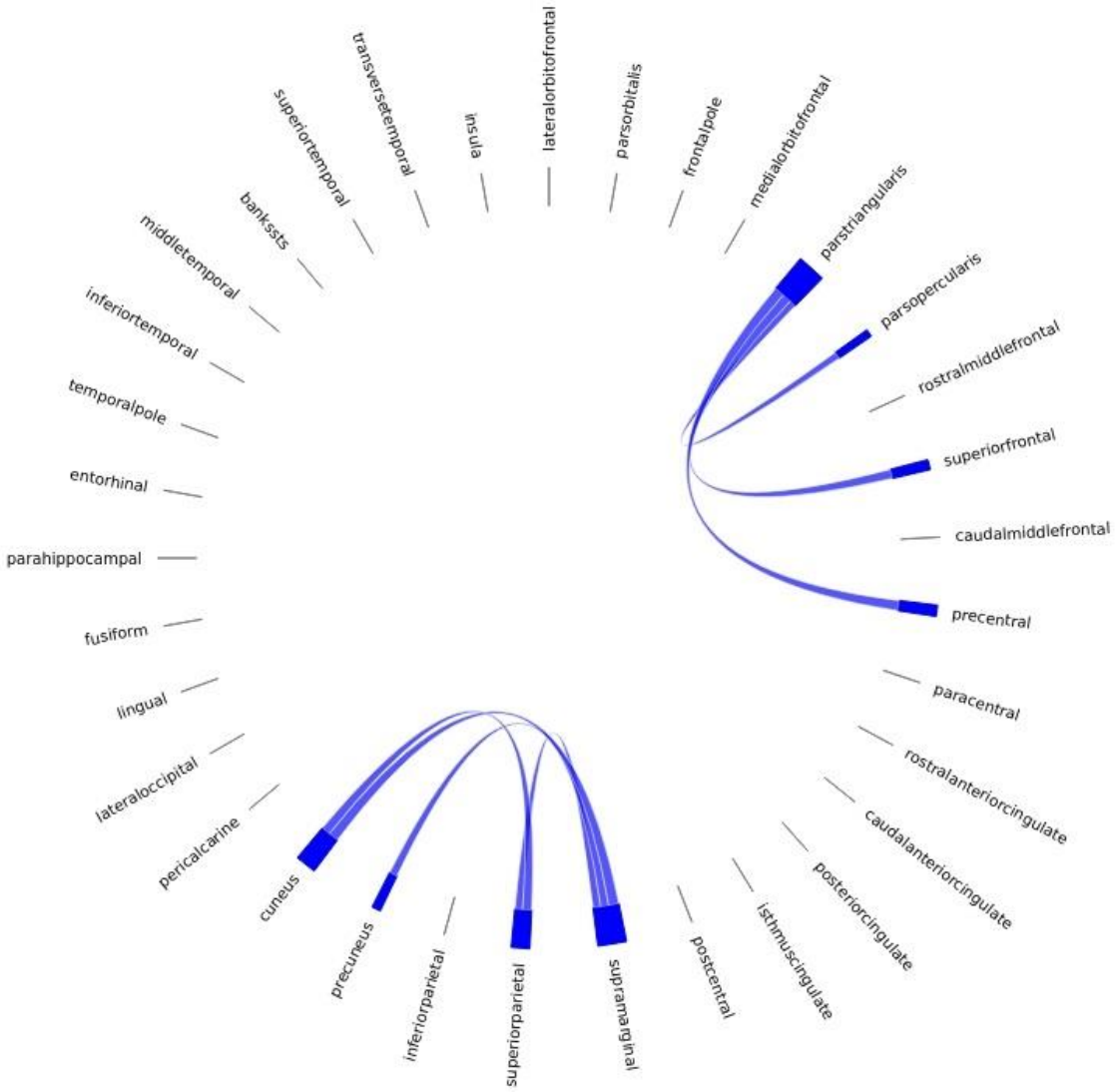

Figure 18. Significant Correlations for Middle Adults. *The values that are plotted are the ones obtained in the difference matrix (Figure 17B), thus correlations with higher coefficient of co-variation of males are represented in blue.*

BOX 6 | MIDDLE ADULTS RESULTS

- 7 significant correlations.
- 8 significant ROIs in frontal and parietal lobes.
- All significant correlations positive.
- Stronger co-variation coefficient in males for all correlations.
- Males have more significant CT loss than females.

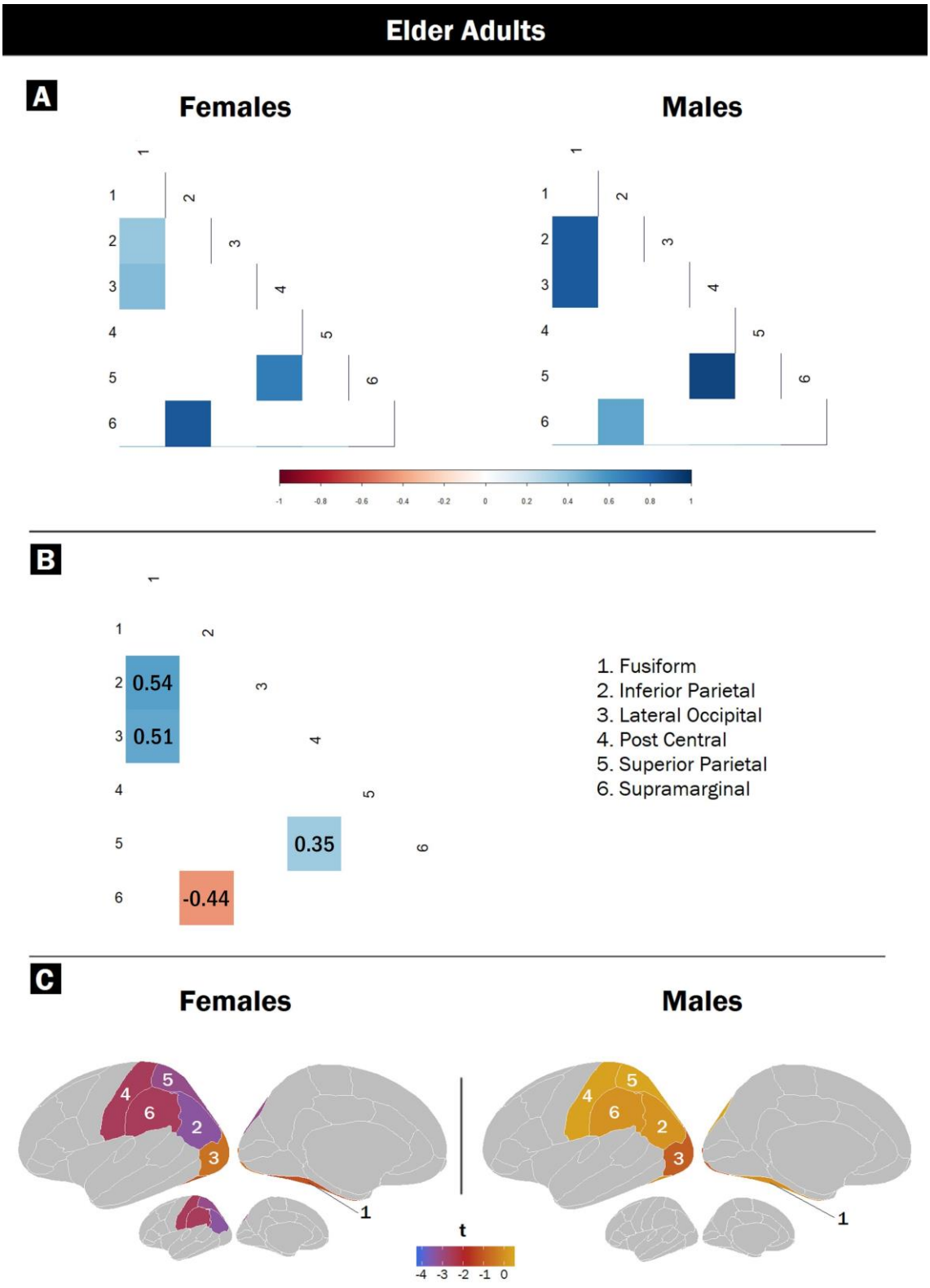

Figure 19. Elder Adults. (A) Structural covariance matrices. *Females (left) and males (right) showing only the significant correlations (4) and the regions (6) involved in those correlations (number code in image B).* ***(B) Difference Matrix.*** *All values with a value above zero will mean a higher coefficient of co-variation in males, oppositely, a value lower than zero, will refer to a higher correlation value in females.* ***(C) Visualization of APC t values of significant ROIs.*** *In the small brains only the t values for significant results (p<.05) are shown.*

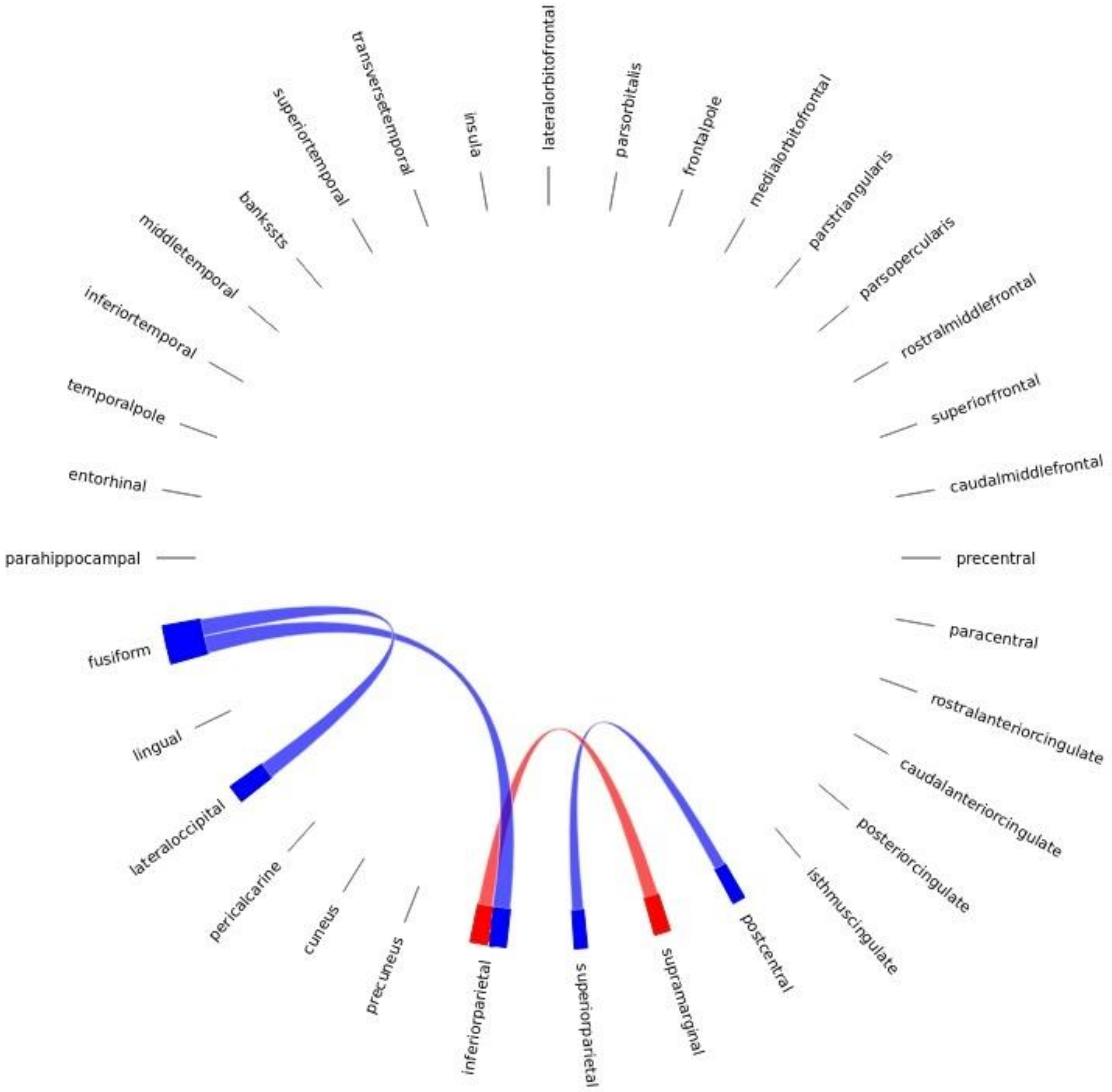

Figure 20. Significant Correlations for Elder Adults. *The values that are plotted are the ones obtained in the difference matrix (Figure 19B), thus correlations with higher coefficient of co-variation of males are represented in blue and, in the case of females, in red.*

BOX 7 | ELDER ADULTS RESULTS

- 4 significant correlations.
- 6 significant ROIs in parietal and occipital lobes.
- All significant correlations positive.
- Stronger co-variation coefficient in males for almost all correlations, except for the supramarginal-inferior parietal correlation, which is stronger in females.
- Females have significant CT loss, males do not.

4.2.2. Aging Effect in Sex-related Brain Differences

The number of Significant Correlations and Significant ROIs. Regarding the number of significant correlations, young adults show 14, middle adults 7 and elder adults 4. This indicates that the number of correlations for sex-differences lowers considerably with age group. Accounting for the number of regions involved in these correlations, young adults show 12, middle adults 8 and elder adults 6. Thus, following the same pattern as the number of correlations: the number of ROIs decreases with age group.

The significant ROIs involved in significant correlations and their location. The significant ROIs that change along the age groups not only in number but also in location. In young adults, these regions are located in frontal, parietal and temporal lobes. When moving on to middle adults, the temporal component does not appear. For elder adults, the frontal component disappears but there is an occipital component along with the parietal one. Regarding the significant ROIs involved, among the three age groups, there is only one common region: the supramarginal gyrus. Additionally, each pair of age groups have other common regions. Young and middle adults share the precentral, precuneus, superior frontal and pars triangularis regions. Middle and elder adults share the superior parietal region. Young and elder adults share the post central and inferior parietal regions.

Significant Correlations. Once the common regions have been derived from the data, the objective is to find if there is any correlation between a pair of regions that appears in more than one age group. Analyzing each pair of regions that have a significant correlation, the correlation between supramarginal and inferior parietal regions is significant in both young and elder adults. Note that this is the only correlation that appears stronger in females (Figure 19B) among the three age groups. The value of this correlation in elder adults has a value of -0.44 and in young adults (Figure 15B), it is a positive difference value of 0.38. This means that while for young adults, males had a stronger correlation of APC, in elder adults, females are the group with a stronger correlation. This clearly highlights a direct difference between age groups.

The APC values of significant ROIs. Based on the third image of figures 15, 17 and 19, the values of t for the regions change with age group. Moreover, the regions where the change of CT is significant also change. The sex-group with greater number of regions for each age group varies. In general terms, it can be said that the group that has more regions with significant CT loss are young and elder females and middle-aged males. To illustrate the first difference between young and middle adults, we look for

instance to the superior frontal region. The superior frontal region is significant for females but not for males in young adults. However, in middle adults is the other way around. In young adults, the APC t-value for females is around -3 and males it is approximately -1. In middle-aged adults, the value for females is around -2 and males it is approximately -4. In elder adults this region is not involved in a significant correlation. To illustrate the second difference between middle and elder adults, we look, for instance, at the superior parietal region. This region is significant for males but not for females in middle-aged adults. In elder adults is the other way around. In middle adults, the value for males is around -2 whereas for females it is approximately -1. Finally, in elder adults, the value for males increases to 0, but the value for females decreases to -3.

BOX 8 | AGE-RELATED SEX-BRAIN DIFFERENCES

- The number of Significant Correlations and Significant ROIs decreases with age, i.e. the sex-differences in SC decrease.
- The significant ROIs involved change location and regions. Parietal lobe is present in all age groups, i.e. parietal lobe's correlations always differ by sex.
- Significant Correlations show higher coefficient of co-variation for males in all age groups, except for one correlation that appears in elder adults. In general terms, the difference of correlations between females and males are caused by correlations that are stronger in males and weaker in females.
- The APC t values of significant ROIs change with age. Females show significant CT loss in young and elder adults, whereas males do not show CT loss. For middle adults, both groups have CT loss, but this loss is more significant in males.

4.2.3. Scale Effect in Sex-related Brain Differences

In this section, the results of scale effect will be addressed for each age group independently, as the main objective is to explain the effect of scale over the findings from the same sample. Attending to the scale effect, the results will be discussed according to three features:

Correlogram. All the correlograms (Figures 21, 23, 25) have the same ROI number code. The correlogram helps with the visualization of the significant correlations, as the regions which are correlated in each scale can be seen directly. A more detailed and interactive view of each correlogram is available **here**. By passing the cursor through a correlation, the correlation value is displayed on the screen.

After the correlograms, for each age group, another figure follows. This figure (Figures 22, 24, 26) addresses the other two aspects to consider: difference matrices (left side of the figure) and APC t-test results (right side). The significant APC t-test values ($p<.05$) for each age group and scale are shown in Figure 27.

Difference matrices. Regarding the difference matrices, as for the results of difference matrices for scale 1, all the values above zero (blue color palette) indicate a stronger correlation between a pair of regions in males. All the negative values (red color palette), indicate a stronger correlation in females. The exact values for the correlations are in Annex C.

APC t-test results for each scale. The figures show also the APC t-test values for scales 1 to 4. Note that the t scale is not identical for all scale, but it remains the same within a scale for the three age groups. I decided to do this to visualize the results with more precision, as for each scale the t values do not belong to the same value range. The exact values for the APC t-test results values are in Annex B.

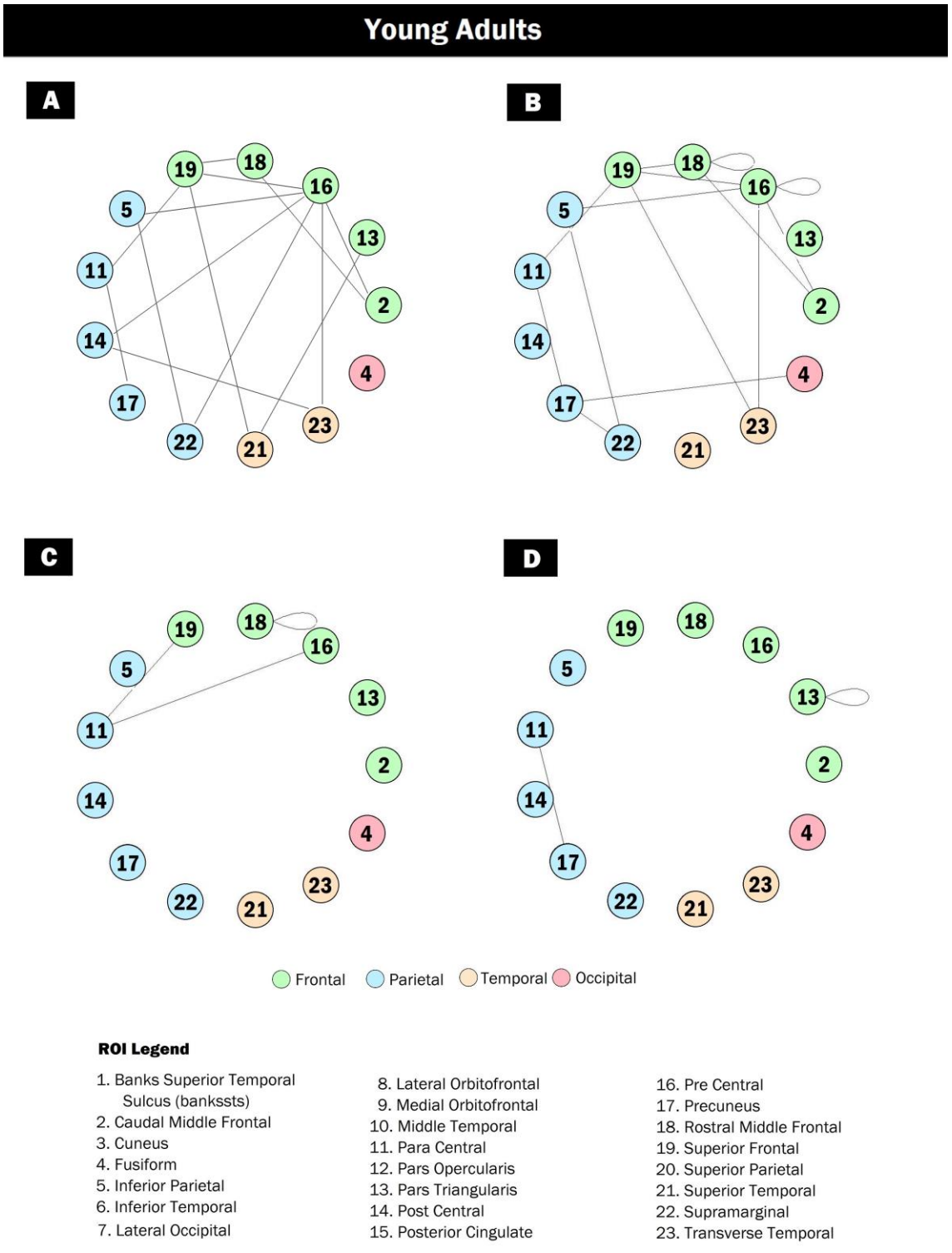

Figure 21. Scale Effect in Correlogram for Young Adults. *Visualization of the correlations for each scale. In scales that had subregions (scales 2-4), the correlations were assigned to the basal region, i.e. if a correlation involved pre central 4 subregion, such correlation was attributed to the basal pre central region for better visualization. (A) Scale 1 (B) Scale 2 (C) Scale 3 (D) Scale 4.*

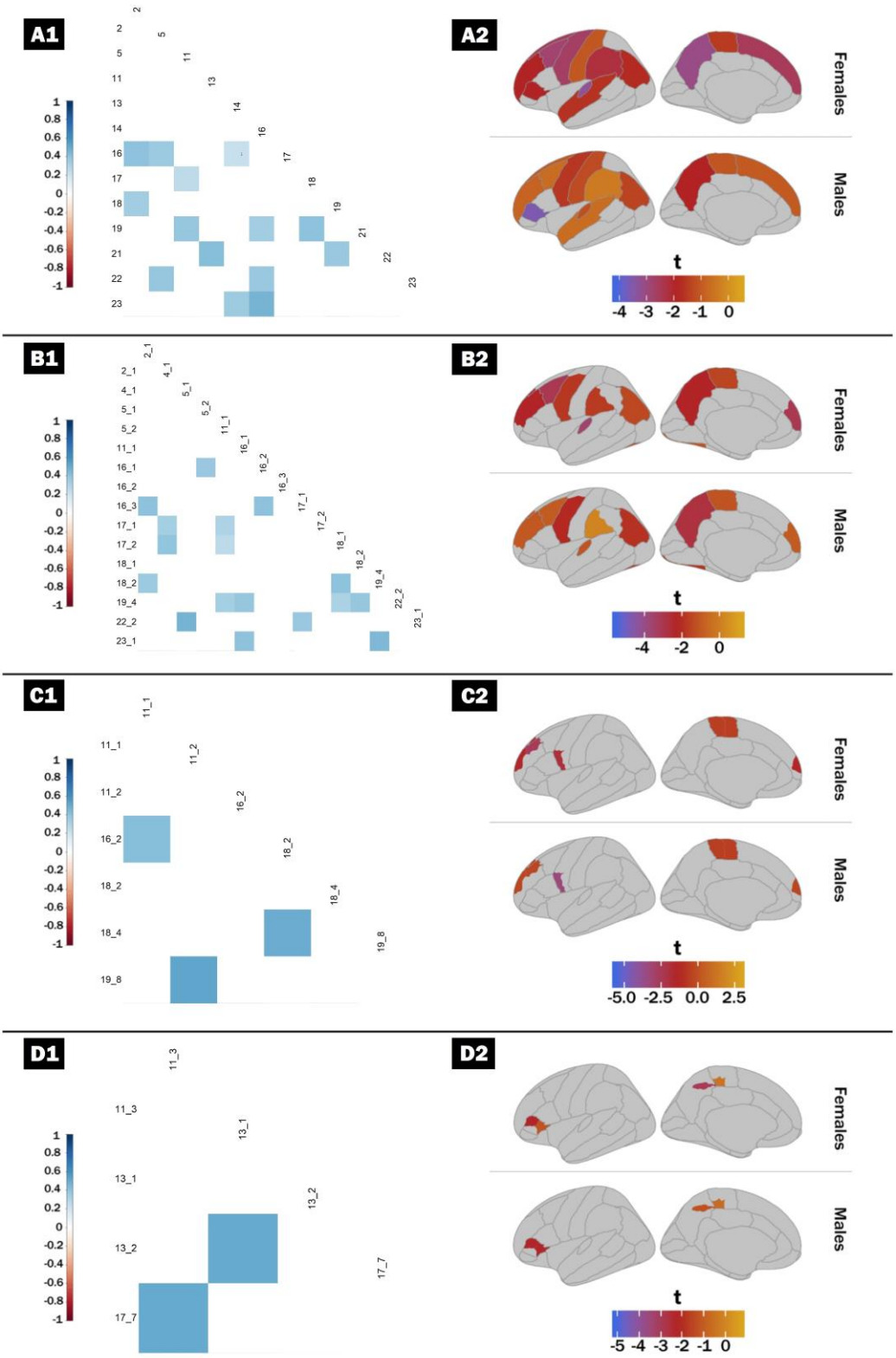

Figure 22. Scale Effect for Young Adults. *Results of the difference matrix (A1-D1) and one sample t-test of significant ROIs APC(A2-D2) through scales 1 to 4 (A to D, respectively). For the difference matrices, blue cells indicate higher coefficient of co-variation for males, in the case of females, this is represented with red cells.* ***ROI Legend****. 2: caudal middle frontal, 4: fusiform, 5: inferior parietal, 11: paracentral, 13: pars triangularis, 14: postcentral, 16: precentral, 17: precuneus, 18: rostral middle frontal, 19: superior frontal, 21: superior temporal, 22: supramarginal, 23: transverse temporal. The number after the underscore of each ROI represents the number of the subdivision of that region, e.g. "11_3" is subdivision 3 of paracentral region.*

Regarding the correlogram results for young adults (Figure 21), the four lobes (frontal, parietal, temporal and occipital) appear at least in one of the scales. The regions represented in this correlogram are 13. As the scale increases, the number of significant correlations decreases. Thus, for young adults, the sex-related brain differences can be said to be found in *global* measures. Analyzing the results scale by scale, the first two scales (Figure 21A-B) present one network totally connected, where the central node can be said to be the precentral region (ROI 16 in Figure 21). Furthermore, these two scales exhibit similar connections. Scale 2 (Figure 21B) is the only scale where a region from the occipital lobe (fusiform) is involved in a significant correlation. Referring to scales 3 (Figure 21C) and 4 (Figure 21D), the network-like appearance of scales 1 and 2 is not present anymore. The correlation between paracentral and superior frontal regions is present through scales 1 to 3 and the correlation between paracentral and precuneus is present in scales 1,2 and 4. In general terms, correlogram results are consistent among scales.

Moving on to the difference matrices and APC t-test (Figure 22), these results will be analyzed below. As for the former, for all scales, males show higher correlation than females. This shows consistency in the findings obtained. Furthermore, as it can be observed in the figures that present the APC t-test results (Figure 22, right) the regions do not significantly change location, therefore, it can also be said that the significant ROIs are consistent among scales. Nevertheless, the value of the significance of the APC of those ROIs does change with scale (Figure 27, young). For instance, take the pre central region in Figure 27B-young (scale 1) and 27C-young (scale 2). When the region is not divided into 4 subregions (scale 1), the APC is only significant for females (red color). Once the region is divided into 4 subregions, only 3 subregions are involved in significant correlations. Within these 3 subregions, one is significant for females (red), another not significant for any group (grey) and the last one is significant for males (blue). This indicates that even though the results were consistent in other aspects among the four scales, the findings present variability when examining *local* changes instead of *global* ones. However, the general tendency follow that females exhibit more regions with CT loss than males, as more regions present significant for females (Figure 27, young).

BOX 9 | SCALE EFFECT YOUNG ADULTS

- With scale, less significant correlations → sex-differences in global measures.
- Scales 1 and 2 follow a similar pattern.
- In all scales, males show higher co-variation coefficient.
- When changing scale, no significant change in the location of significant ROIs but there exits variability of the significance of change in these regions.
- In general, females show more significant ROIs with CT loss.

- Correlogram and difference matrix show consistency among scales.
- APC t-test results show inconsistency among scales.

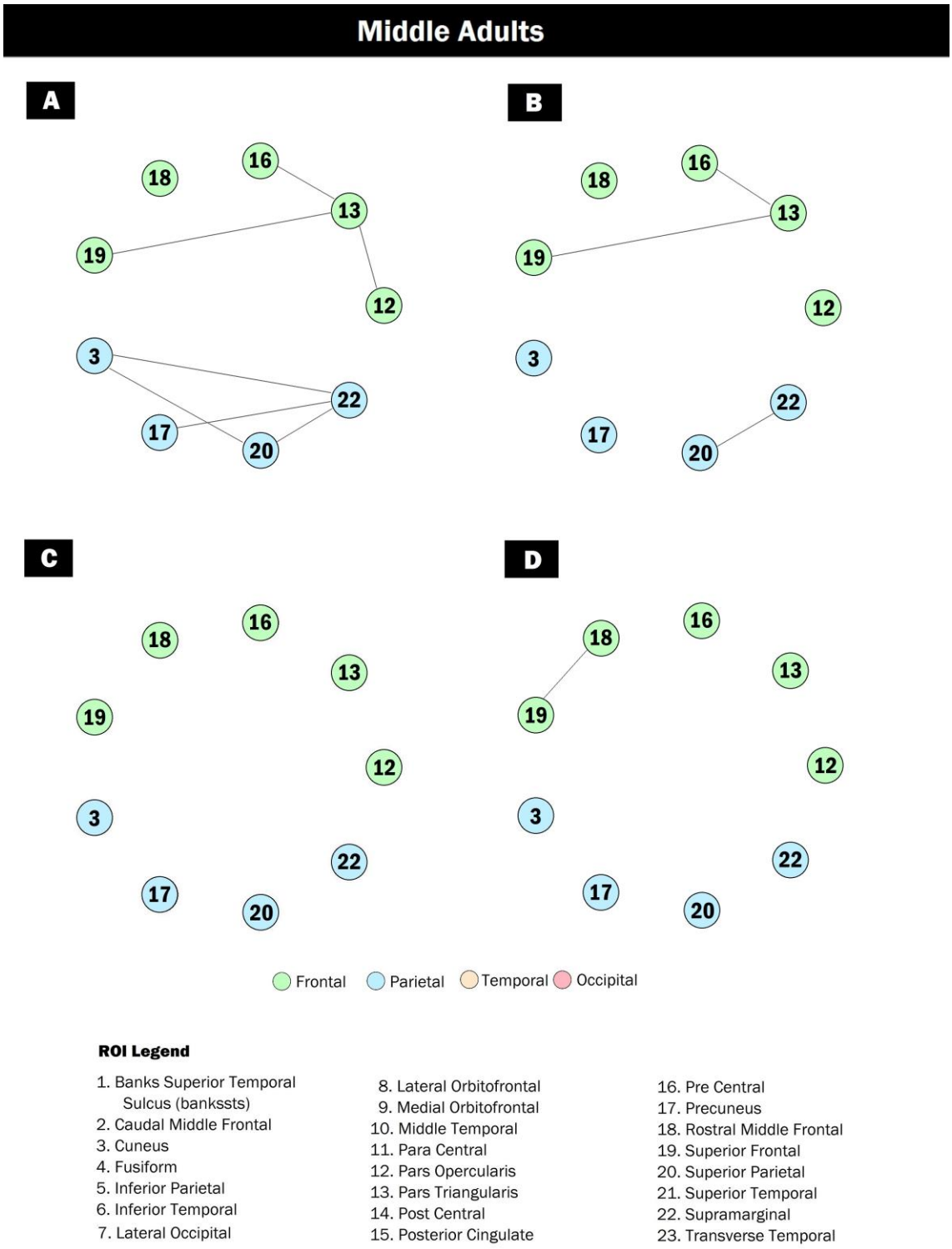

Figure 23. Scale Effect in Correlogram for Middle Adults. *Visualization of the correlations for each scale. In scales that had subregions (scales 2-4), the correlations were assigned to the basal region, i.e. if a correlation involved pre central 4 subregion, such correlation was attributed to the basal pre central region for better visualization. (A) Scale 1 (B) Scale 2 (C) Scale 3 (D) Scale 4.*

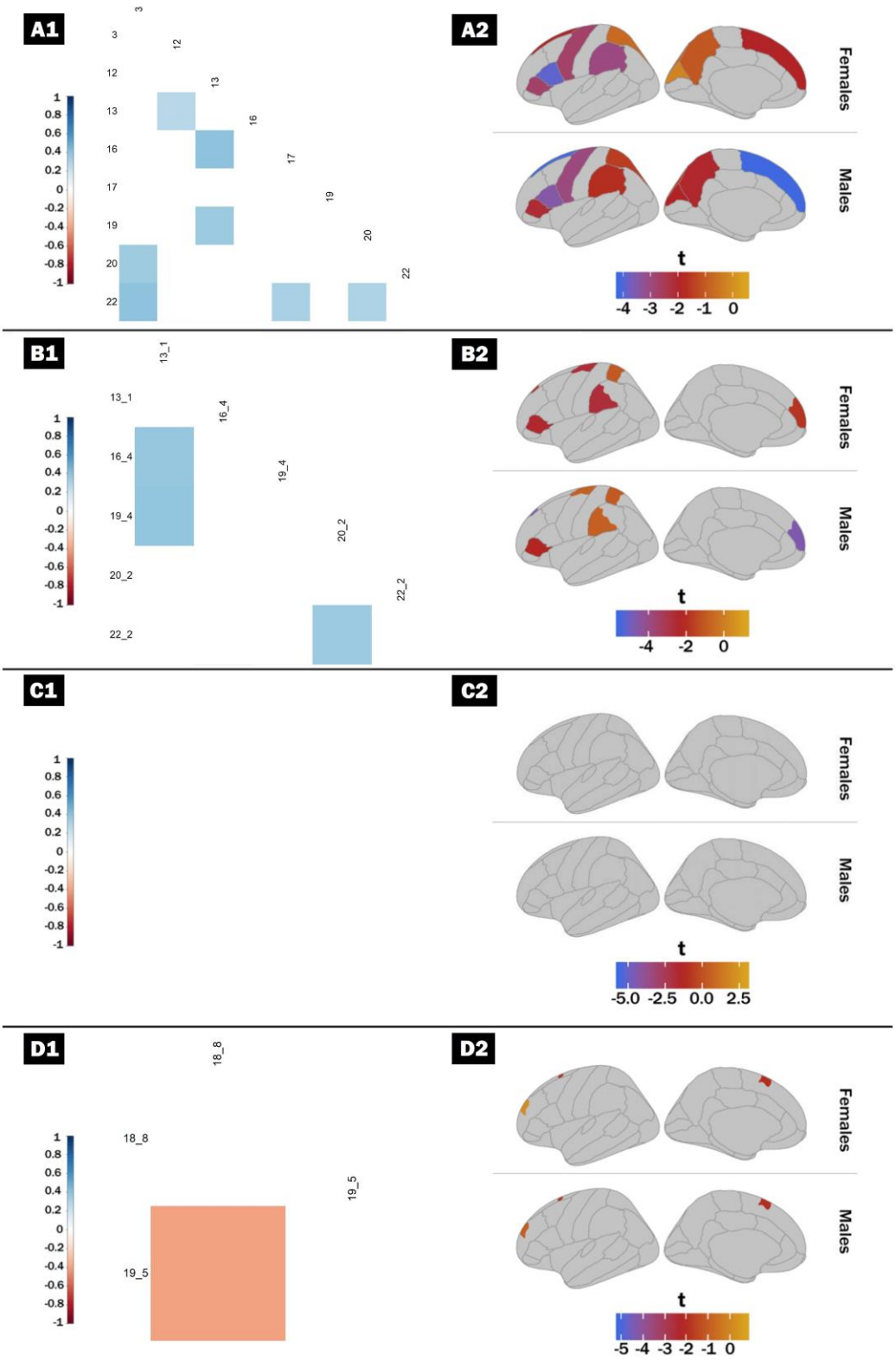

Figure 24. Scale Effect for Middle Adults. *Results of the difference matrix (A1-D1) and one sample t-test of significant ROIs APC(A2-D2) through scales 1 to 4 (A to D, respectively). In this age group, there are no significant correlations for scale 3 (C). For the difference matrices, blue cells indicate higher coefficient of co-variation for males, in the case of females, this is represented with red cells.* ***ROI Legend****. 3: cuneus, 12: pars opercularis, 13: pars triangularis, 16: precentral, 17: precuneus, 18: rostral middle frontal, 19: superior frontal, 20: superior parietal, 22: supramarginal. The number after the underscore of each ROI represents the number of the subdivision of that region, e.g. "19_5" is subdivision 5 of superior frontal region.*

Regarding the results of the correlogram for middle-aged adults (Figure 23), only regions from two lobes (frontal and parietal) are involved in a significant correlation among the four scales, thus sex-related brain differences only appear in intralobe correlations. Middle adults' results in scale 1 (Figure 23A) show two different networks, one for each lobe. Scale 2 (Figure 23B) follows the same pattern and, even though some correlations disappear, the ones that remain were already present in scale 1. In scale 3 (Figure 23C), the number of correlations disappears completely (i.e. no significant sex-related brain differences), while in scale 4 (Figure 23D) one new correlation appears. Oppositely to the consistency discussed in young adults through scales, middle adults' results only show consistency within the two first scales.

With regards to the difference matrix and APC t-test results (Figure 24), they differ from the ones obtained for the previous age group discussed. As explained above, scale 3 did not show significant correlations at $p<0.05$. The difference matrices of scales 1 and 2 show the same pattern, this is, males have stronger correlations than females. In the case of scale 4, there is only one correlation, which is stronger in females than males. Thus, middle adults' results are not consistent among scales as they present opposite findings. Regarding the APC t-test results, no major differences are found neither at the ROI localization (Figure 24, right) nor at the significance of APC value (Figure 27, middle). In this age group, the APC value in males is more significant than in females (Figure 27, middle) thus reinforcing what was stated before when analyzing the sex-related brain differences (section 4.2.1.), in middle adults, males have more regions with CT loss compared to females.

BOX 10 | SCALE EFFECT MIDDLE ADULTS

- With scale, less significant correlations → sex-differences in global measures.
- Scales 1 and 2 follow a similar pattern.
- Only frontal and parietal lobes and the correlations are intralobal.
- In Scales 1 and 2, males show higher co-variation coefficient. In Scale 3, no significant correlations. In Scale 4, females show higher co-variation coefficient.
- When changing scale, no significant change in the location of significant ROIs.
- In general, males show more significant ROIs with CT loss.
- APC t-test results show consistency among scales.
- Correlogram and difference matrix results do not show consistency among scales.

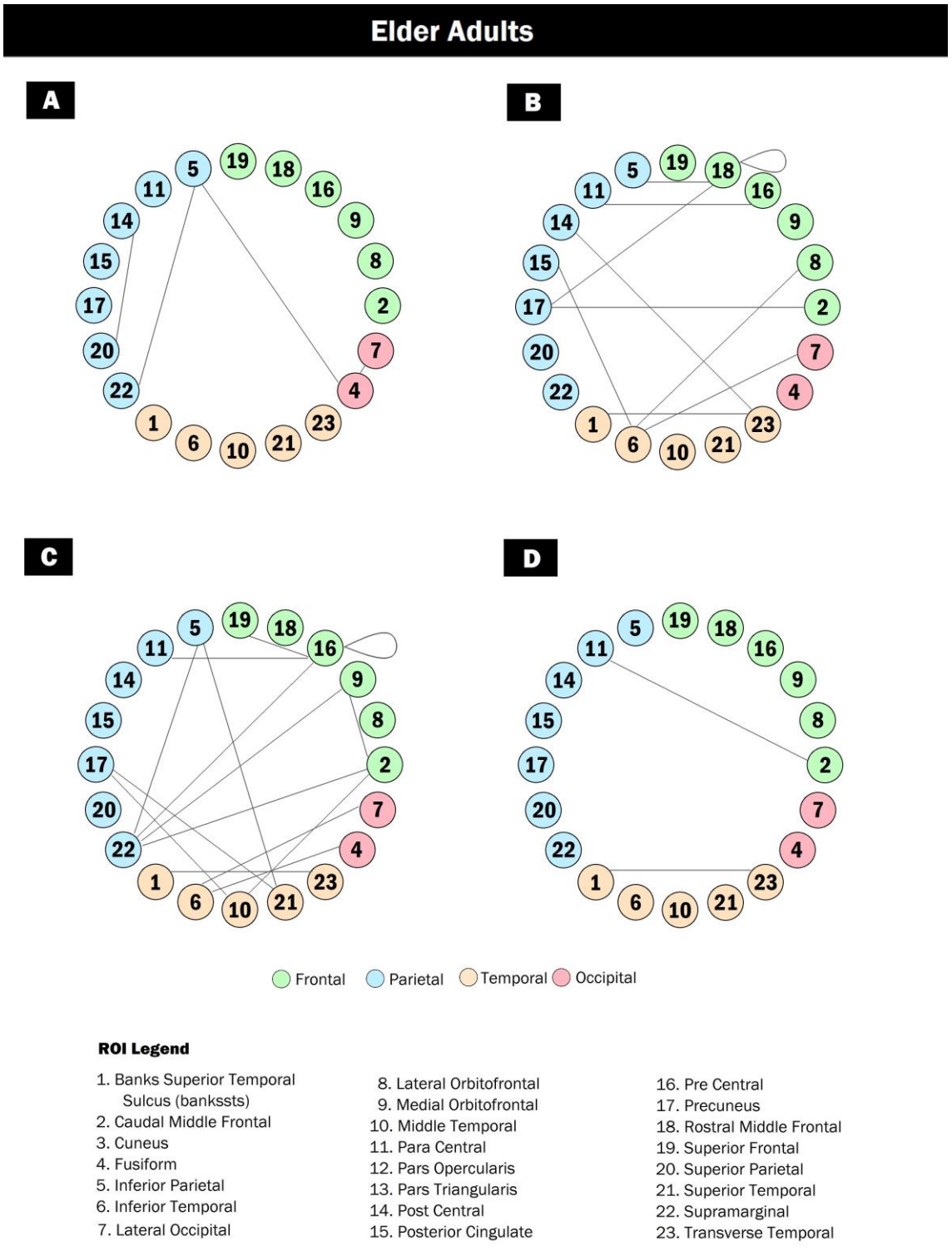

Figure 25. Scale Effect in Correlogram for Elder Adults. *Visualization of the correlations for each scale. In scales that had subregions (scales 2-4), the correlations were assigned to the basal region, i.e. if a correlation involved pre central 4 subregion, such correlation was attributed to the basal pre central region for better visualization. (A) Scale 1 (B) Scale 2 (C) Scale 3 (D) Scale 4.*

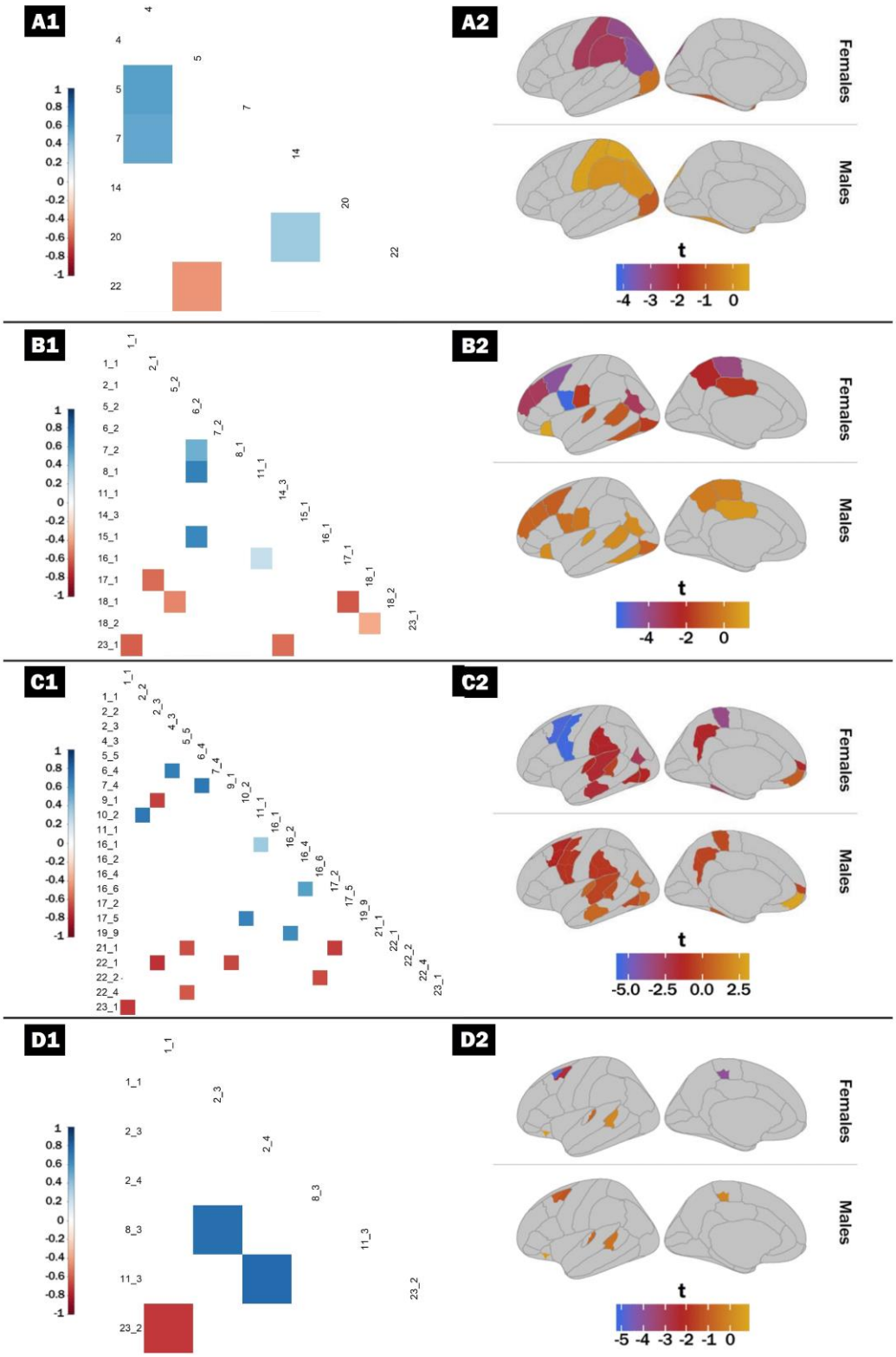

Figure 26. Scale Effect for Elder Adults. *Results of the difference matrix (A1-D1) and one sample t-test of significant ROIs APC (A2-D2) through scales 1 to 4 (A to D, respectively). For the difference matrices, blue cells indicate higher coefficient of co-variation for males, in the case of females, this is represented with red cells.* ***ROI Legend.*** *1: bankssts, 2: caudal middle frontal, 3: cuneus, 4: fusiform, 5: inferior parietal, 6: inferior temporal, 7: lateral occipital, 8: lateral orbitofrontal, 9: medial orbitofrontal, 10: middle temporal, 11: paracentral, 14: postcentral, 15: posterior cingulate, 16: precentral, 17: precuneus, 18: rostral middle frontal, 19: superior frontal, 20: superior parietal, 21: superior temporal, 22: supramarginal, 23: transverse temporal. The number after the underscore of each ROI represents the number of the subdivision of that region e.g. "1_1" is subdivision 1 of banksst region.*

With reference to the last age group, elder adults, the correlogram results (Figure 25) notably differ from the ones obtained for young and middle adults. While in previous age groups, with scale, the number of correlations decreased, in elder adults, the number of correlations increases with scale in scales 1 to 3. Among the four scales, all four lobes are present at least in one scale and in scales 2 (Figure 25B) and 3 (Figure 25C) all four lobes are involved in at least one significant correlation. For scale 1 (Figure 25A), the lobes connected are the parietal and occipital lobes. When analyzing scale 2, the number of significant correlations and the regions involved in such correlations, increases. For this scale, three small networks can be said to appear, with inferior temporal (number 6), rostral middle frontal (number 18) and transverse temporal (number 23) regions as nodes of these networks. In scale 3, two main networks can be established and the regions which are the nodes of these two networks are inferior temporal (number 6) and supramarginal (number 22). This last node creates a big network in which almost all regions are involved. Transverse temporal region can be said to not establish a network as it only has one connection. Nevertheless, this correlation between transverse temporal and banks superior temporal sulcus regions appears in scales 2-4. Finally, in scale 4 (Figure 22D), the number of correlations decreases to 3, being the group with most correlations for this scale. The correlogram results for elder adults do not show clear consistency.

To continue with, the results for the difference matrix and APC t-test results in elder adults (Figure 26) will be addressed. With regards to the former, all scales show both stronger correlations in females and in males. Some of them, are extended to more than one scale, for instance, the correlation between bankssts and transverse temporal regions is present trough scales 2 to 4. This correlation is in all scales stronger in females. Therefore, it can be said that there is no major scale effect in difference matrices for this age group. Oppositely, APC t-test results do show scale effect. Elder adults can be said to be the age group in which scale effect in APC t-test results is more evident. Firstly, the values of APC change with the scale completely, as well as the ROI location. Scale 1 (Figure 26, A2) shows regions in parietal and occipital lobe. In scale 2, the regions in parietal lobe start decreasing in size and number (Figure 26, B2) and frontal and temporal components appear. Scale 3 is similar to scale 2 attending to the ROI localization. Scale 4 has the same regions as scales 2 and 3 and one common correlation. Finally, the APC values show only significance in males in scale 3 (Figure 27D, elder), whereas females show significance at many regions among the four scales. Supporting the argument mentioned before, elder females present CT loss, whereas elder males do not.

BOX 11 | SCALE EFFECT ELDER ADULTS

- With scale, more significant correlations → sex-differences in local measures.
- Scales 2 and 3 follow a similar pattern.
- In all scales, males show higher co-variation coefficient for some correlations and females show higher co-variation coefficient for others.
- When changing scale, significant change in the location of significant ROIs.
- In general, females show more significant ROIs with CT loss.
- Difference matrix results show consistency among scales.
- Correlogram and APC t-test results are inconsistent among scales.

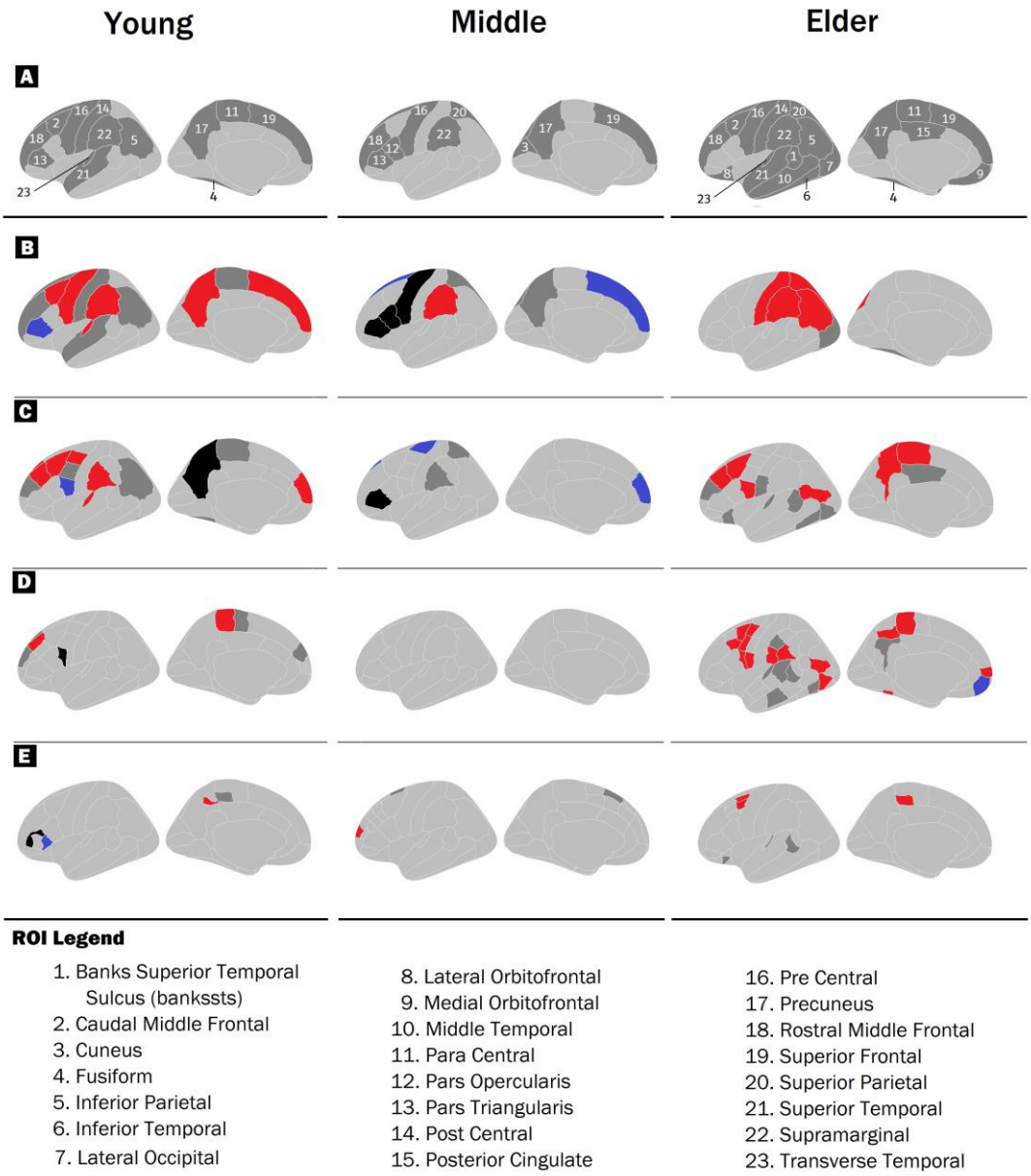

***Figure 27. Scale Effect accounting for the significant change in significant ROIs**. (A) All Scales. All the significant regions that appear among the four scales for that age group. (B-E) Scales 1-4 respectively. Significant regions of that age group for each scale. The regions where change is significant (p= 0.05) are red (significant change only in females), blue (only significant in males), black (significant in both) and dark grey (no significant change).*

CHAPTER 5: DISCUSSION

In this project I have analyzed the age-related sex-brain differences with a longitudinal structural covariance approach. To the best of my knowledge, this is the first longitudinal SC study assessing these sex-related brain differences in healthy individuals. Thereby, the interpretation of the findings in the context of the existing literature is complicated. The six main aspects of my project are: sex-related brain differences, aging effect, CT as biomarker, longitudinal data and SC analysis. With respect to these aspects, the overlap of my findings with the existent literature can be seen in Table 8. Note that none of the cited studies have assessed a scale effect.

	Sex Effect	**Aging Effect**	**Study Measure**	**Data Type**	**Statistical Analysis**
My Study	YES	YES 17 – 68 years	CT	Longitudinal	SC
Reported Literature					
Aboud, et al., 2019	NO	**YES** 7 – 90 years	GM volume	Cross-sectional	**SC**
Alexander-Bloch, et al., 2013a	NO	**YES** 9 – 22 years	**CT**	**Longitudinal**	**SC** and Graph Analysis
Coffey, et al., 1998	**YES**	**YES** 66 – 96 years	brain matter and CSF	Cross-sectional	Regression
DuPre, et al., 2017	NO	**YES** 9 – 94 years	GM volume	Cross-sectional	**SC**
Gautam, 2013	**YES**	**YES** 44 – 68 years	**CT**	Cross-sectional	ANOVA
He, et al., 2007	NO	NO 18 – 34 years	**CT**	Cross-sectional	**SC**
Im, et al., 2006	**YES**	NO 18 – 42 years	**CT**	Cross-sectional	Statistical Map of Differences
Ingalhalikar, et al., 2013	**YES**	**YES** 8 – 22 years	DTI	Cross-sectional	Graph Analysis
Salat, et al., 2004	NO	**YES** 18 – 93 years	**CT**	Cross-sectional	ANCOVA
Sowell, et al., 2007	**YES**	**YES** 7 – 87 years	**CT**	Cross-sectional	Statistical Map of Differences

Table 8. Previous Literature which partially overlaps with my project. *The features where other studies overlap with my project are in bold. Abbreviations: Diffusion-tensor imaging (DTI), analysis of variance (ANOVA), analysis of covariance (ANCOVA).*

5.1. Aging Effect in Sex-related Brain Differences

Previous literature has reported findings on healthy controls across the lifespan [67, 41, 68, 69, 20, 45, 31, 10].

Regarding an effect of aging, it has been reported to cause regional thinning of the cortex [45]. he regions where the cortical thinning is most drastic are reported to be inferior prefrontal cortex, supramarginal and precentral regions. Furthermore, in the reported findings the sex-related brain differences in such cortical thinning are not taken into consideration [45]. In fact, among the studies mentioned before, only four examine sex-related brain differences [69, 20, 31, 10].

The results obtained in my project are consistent with the findings regarding cortical thinning [45] as the negative APC in supramarginal, the prefrontal cortex and precentral regions is significant for all three age groups (Figure 28) but not for both sexes. Thereby, the significance of cortical thinning highly depends on sex and age group (BOX 12).

BOX 12| CORTICAL THINNING WITH AGE DEPENDS ON SEX

Here, the importance of the consideration of sex in the studies related to aging effect in the brain will be addressed by comparing the results of Salat et al. with my results dividing by sex (Figure 28).

WITH ASSESMENT BY SEX

- Supramarginal region: Cortical thinning is significant in all age groups, but only for female subjects.
- Prefrontal cortex: Cortical thinning is significant in middle-aged subjects for both sexes. For young adults, inferior prefrontal cortex thinning is significant for males and superior prefrontal cortex is significant for females. For elder adults, prefrontal cortex thinning is only significant for females.
- Precentral: Cortical thinning is significant in all age groups for female subjects and in middle-aged male subjects.

WITH NO SEX-DIVISION

- Supramarginal region: Cortical thinning is significant in middle-aged subjects.
- Prefrontal cortex: Cortical thinning is significant in all age groups.
- Precentral: Cortical thinning is significant in all age groups.

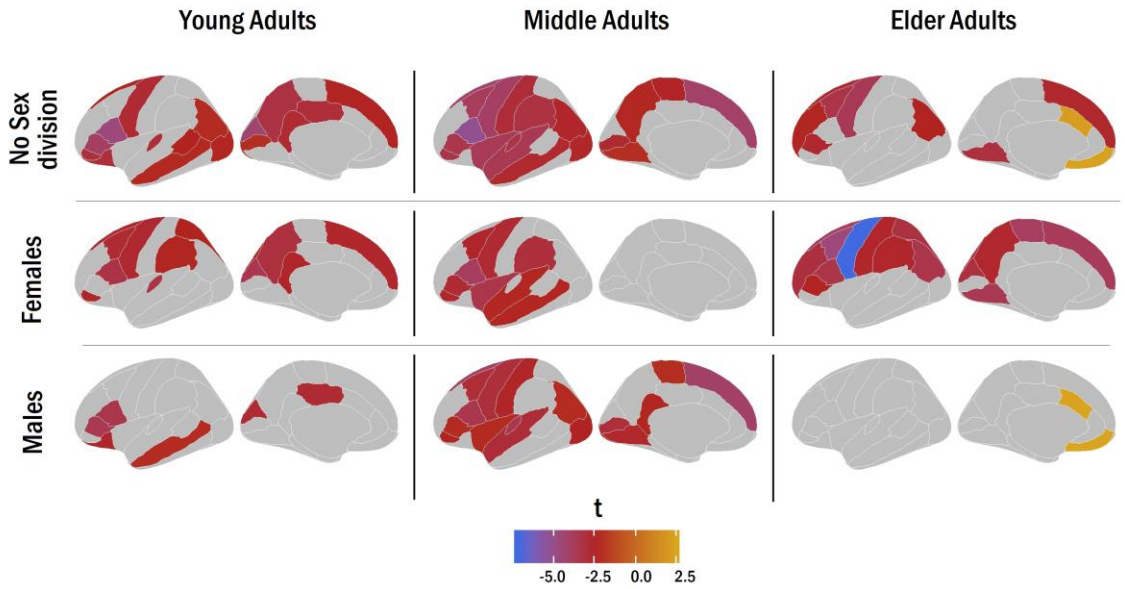

Figure 28. Regions where APC is significant for all age groups. *One sample t-test was run on all regions with no sex division (APC of all subjects from the respective age group), females and males. Then, only the ones showing significant APC change (p<.05) are plotted.*

Among the studies mentioned at the beginning of this section, only four of them examine sex-related brain differences [69, 20, 31, 10].

Age-related sex brain differences have been reported in cortical thinning, stating that the loss of CT is more drastic in males [10]. However, the results obtained in my study show that these aging effects depend on the age group analyzed. In the case of young and elder adults, cortical thinning is more significant for females, whereas in middle adults, cortical thinning is more significant for males (Figure 28). Thus, the incongruence of my results with the existing literature [10] could be caused by the fact that I have corrected for the effect of brain size, whereas these studies have not. The type of study design could also be another reason for this inconsistency as I used a longitudinal design and the prior studies use a cross-sectional design.

5.2. Sex-related Brain Differences in Structural Covariance

The paper, among the existing literature, that is most alike to my study is the study by Alexander-Bloch et al., where brain differences are studied with longitudinal SC. However, there are some differences with my study such as the subjects' age range and that sex-related brain differences are not taken into account in the reported study [41]. Another paper that can be said to be similar is the one from Ingalhalikar et al., where graph theory is used instead of SC and, alternatively to structural networks, functional ones are studies. Another difference is the type of design, in the reported study a cross-sectional design was used. Nevertheless, a relation between structural and functional

networks has been reported [41], as well as, the relation between studies examining the CT value (cross-sectional) and CT change (longitudinal). Thus, findings of CT cross-sectional studies should, in some way, be akin to mine. The regions where cross-sectional and longitudinal designs are more analogous are cingulate and frontal regions [41]

Both studies [41, 20], have examined subjects with < 22 years, thus the findings in these papers can be compared to my results obtained for young adults. In the case of Ingalhalikar et al, where graph theory is used, the attributes employed to compare brain SC networks are transitivity and modularity. Networks where neighbor regions are very connected (in SC connected implies correlated) are said to have high transitivity. Modularity explains how efficiently different subnetworks can be defined. Modularity has been reported to be highly influenced by aging [67] and, along with transitivity, graph measures can efficiently describe age-related structural differences [67, 41]. Thus, the findings in these studies can, in some way, be compared to my SC approach by studying the networks created by the correlations where sex-related brain differences are significant.

It has been reported that the thresholded (i.e. statistically significant) networks tend to have short-distance connections i.e. high transitivity [41] and both, modularity and transitivity, are stronger in males [20]. The former statement is replicated in my results for scale 1 of young adults, where the significant correlations are generally established within neighbor regions (see Figure 29). The latter statement is consistent with my findings as all the co-variation coefficients of the SC matrices are higher in males than in females for young adults (Figure 15A). Accordingly, as both parameters, modularity and transitivity, favor males in young adults, this indicates that sex-related brain differences occur at correlations where males show high transitivity and modularity and females do not.

Other characteristic reported concerns the regions that appear in the correlations (i.e. significant ROIs). The covariance between regions from frontal-parietal and frontal-temporal systems are reported to be the regions with strong SC, which are also observed to be predominant in functional networks [41]. This is related with my results for sex-differences in young adults that show significant sex-related brain differences at frontal, parietal and temporal regions (Figure 22). Thus, indicating that, for young adults, the systems that present higher correlation, also present significant sex-differences. Furthermore, due to the possible correlation of structural networks with functional networks, this can be related to other studies where sex-related brain differences at functional networks have been found [20]. Functional networks have been proven to be

stronger between hemispheres (inter-hemispheric) in females and along the same hemisphere (intra-hemispheric) in males [20]. In my study, only intra-hemispheric correlations are being examined, this could be related with the higher co-variation coefficient of young males.

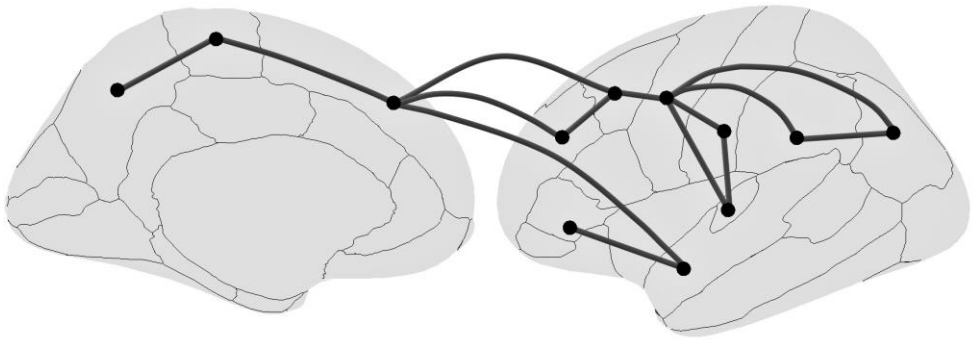

Figure 29. Young Adults Correlation Visualization. *The SC networks established with the significant correlations in young adults (scale 1) present high transitivity and modularity.*

5.4. Scale Effect in Sex-related Brain Differences

The reported literature regarding scale effect is limited. By *scale effect* meaning the effect of parcellation system and resolution. By analyzing it, the main purpose is to observe if the sex-related brain differences are *local* (more differences at higher scales where ROIs are small) or *global* (more differences at lower scales, larger ROIs) for each age group. In the hypothesis of the project I stated that the results obtained for sex-related brain differences will change with scale. This is because I understand that when changing from analyzing global differences to analyzing local ones, the results change.

From my results, the two first age groups (young and middle adults) showed differences at global level because with scale, the number of significant correlations decreased. Contrarily, elder adults exhibited more significant correlations with scale, i.e. local differences.

The paper from Alexander-Bloch et al. [41] reported that in adolescents, *global level networks* are more clustered and defined compared to *local* ones. This follows the pattern obtained in young adults' results, as an increase in scale resulted in a decrease of significant correlations and network-like patterns (Figure 21). This also indicates the differences in the brain *wiring* for young adults are significant. When looking at the results for middle adults (Figure 23), the number of significant correlations decreases, these has been reported to be caused by a decrease in modularity significance with age [67]. Furthermore, among this decrease in modularity in middle adults, *global level networks* are still more prominent than *local* ones. However, this pattern changes when looking at

the results of elder adults (Figure 25), where local level networks prevail over global ones. This could be caused by the disorganization of brain networks caused by aging [67], that is significantly different in female and male subjects.

5.4. Limitations and Strengths

This project has limitations which should be taken into consideration when interpreting the results. The limitations are listed (BOX 13), as well as the strengths of the project (BOX 14).

BOX 13| PROJECT LIMITATIONS

- Difficulties when comparing the results with reported literature, especially regarding scale effect.
- Low number of subjects for the third age group (n= 41).
- Minimum age was 17, no data on pre-adolescence, and maximum age was 68, no data for elder age subjects.
- No independent replication-sample.
- Difficult to establish a lifespan pattern for the subjects.

BOX 14| PROJECT STRENGTHS

- Novelty. No previous studies have studied sex-related brain differences, aging effect and scaling effect using both longitudinal data and structural covariance approach.
- Some results replicate similar studies.
- Future work can be destined to study the structural networks established by sex-related brain differences.
- Higher precision compared to other studies as I have corrected for brain size and time between scans when calculating the APC, then, when obtaining the significant sex-differences in SC matrices, FDR correction was applied. Moreover, several statistical analyses have been done to the initial data and results in order to support and provide further information of the findings.

CHAPTER 6: CONCLUSIONS AND FUTURE WORK

The literature on sex-related brain differences is far from consistent. The main reason of this inconsistency is the variability of the methods used to obtain the conclusions. As I have explained before, this project does not pretend to give a definitive answer to whether there are sex-related brain differences. Replication of the results in other independent studies/samples is needed to establish a solid conclusion regarding this topic. Nevertheless, this project has intended to use reliable and validated measurements of cortical thickness, and correction for multiple comparisons which may aid in the repeatability of the results.

Furthermore, the project results have been used to study two of the main sources of variability: age of the participants and the brain parcellation system. The hypothesis established in this project was: I expected to find age-related sex brain differences and such differences will not be consistent among different parcellation systems. The project results support this hypothesis, as the sex-related brain differences found vary amongst age groups and scale. This points out the fact that the variability in the published findings regarding sex-related brain differences can be explained, at least partially, by these two variability sources.

Future research should focus on studying aging effects (instead of controlling it) and the influence of the scale used for brain parcellation. This will provide more robust approaches when examining the sex-related brain differences, thus increasing the reproducibility of findings. Longitudinal designs should also be used in the study of these differences and their relationship with age. Ultimately, structural covariance has proved to be an appropriate statistical method to study brain attributes and measures, such as, cortical thickness. Thus, further research should use SC to study sex-related brain differences.

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FIGURE INDEX

ANNEX A. SAMPLE DEMOGRAPHICS

Characteristic	Females			Males		
N of scans	**Baseline** 131	**Follow-up 1** 130	**Follow-up 2** 50	**Baseline** 167	**Follow-up 1** 163	**Follow-up 2** 59
Sociodemographics						
Age, years: mean (SD)	30.32 (10.75)	34.05 (11.12)	33.685 (7.92)	30.50 (11.16)	34.60 (11.46)	34.52 (8.27)
Education (total years): mean (SD)	21.31 (24.48)	18.83 (20.54)	NA	2.29 (25.8)	22.14 (25.9)	NA
Clinical variables						
Full-scale IQ total: mean (SD)	109.24 (15.86), 23	110.19 (16.04), 18	111.72 (16.83), 3	113.02 (15.82),39	117.12 (16.56),34	119.31 (16.97),11
Global Imaging variables						
Cortical thickness: mean (SD)	2.529 (0.123)	2.506 (0.128)	2.525 (0.105)	2.505 (0.109)	2.484 (0.099)	2.491 (0.0998)

Table A1. Demographics, clinical and imaging characteristics with mean and variance comparisons for males and females healthy controls for the longitudinal sample included in the study: at each timepoint (baseline, first follow-up and second follow-up scans). The total sample

Brain Measures N of baseline scans	**Females** N = 131				**Males** N = 167			
Lobes	**Frontal**	**Parietal**	**Temporal**	**Occipital**	**Frontal**	**Parietal**	**Temporal**	**Occipital**
Cortical thickness: mean (SD)	2.633 (0.145)	2.329 (0.141)	2.813 (0.134)	1.974 (0.102)	2.60 (0.134)	2.283 (0.130)	2.833 (0.124)	1.967 (0.089)
Brain Measures N of baseline scans	**Young Females** N = 53				**Young Males** N = 76			
Lobes	**Frontal**	**Parietal**	**Temporal**	**Occipital**	**Frontal**	**Parietal**	**Temporal**	**Occipital**
Cortical thickness: mean (SD)	2.701 (0.112)	2.383 (0.132)	2.870 (0.126)	2.005 (0.088)	2.648 (0.121)	2.321 (0.116)	2.860 (0.128)	1.980 (0.080)
Brain Measures N of baseline scans	**Middle Females** N = 59				**Middle Males** N = 69			
Lobes	**Frontal**	**Parietal**	**Temporal**	**Occipital**	**Frontal**	**Parietal**	**Temporal**	**Occipital**
Cortical thickness: mean (SD)	2.625 (0.128)	2.326 (0.120)	2.802 (0.108)	1.970 (0.097)	2.587 (0.128)	2.272 (0.127)	2.820 (0.113)	1.964 (0.098)
Brain Measures N of baseline scans	**Elder Females** N = 19				**Elder Males** N = 22			
Lobes	**Frontal**	**Parietal**	**Temporal**	**Occipital**	**Frontal**	**Parietal**	**Temporal**	**Occipital**
Cortical thickness: mean (SD)	2.471 (0.145)	2.189 (0.130)	2.692 (0.139)	1.901 (0.117)	2.476 (0.111)	2.191 (0.111)	2.777 (0.126)	1.926 (0.080)

Table A2. Brain measures with mean and variance. Comparisons for males and females healthy controls for the healthy controls included in the study: at baseline. The total sample consisted of 298 unique subjects.

ANNEX B. APC t-test RESULTS FOR SIGNIFICANT ROIs

ROI	Females		Males	
	t	p	t	p
Scale 1				
caudal middle frontal	-2.807	0.007	-0.664	0.509
inferior parietal	-1.737	0.088	-1.395	0.167
para central	-1.523	0.134	-1.074	0.286
pars triangularis	-1.895	0.064	-3.578	0.001
post central	-1.059	0.295	-1.259	0.212
pre central	-2.665	0.010	-1.586	0.117
precuneus	-3.081	0.003	-1.874	0.065
rostral middle frontal	-1.895	0.064	-0.831	0.409
superior frontal	-2.532	0.014	-1.003	0.319
superior temporal	-1.640	0.107	-0.657	0.513
supramarginal	-2.198	0.032	-0.406	0.686
transverse temporal	-3.271	0.002	-1.176	0.243
Scale 2				
caudal middle frontal	-3.093	0.003	-0.960	0.340
fusiform 1	-1.226	0.226	-1.970	0.052
inferior parietal 1	-1.444	0.155	-1.936	0.057
inferior parietal 2	-1.578	0.121	-0.908	0.367
para central	-1.514	0.136	-1.175	0.244
pre central 1	-1.825	0.074	-2.102	0.039
pre central 2	-0.821	0.415	-0.584	0.561
pre central 3	-2.255	0.028	-0.654	0.515
precuneus 1	-2.300	0.025	-2.777	0.007
precuneus 2	-2.930	0.005	-1.818	0.073
rostral middle frontal 1	-2.317	0.024	-1.050	0.297
rostral middle frontal 2	-1.628	0.110	-1.263	0.211
superior frontal 4	-2.358	0.022	-0.967	0.337
supramarginal 2	-1.445	0.154	0.133	0.895
transverse temporal	-3.566	0.001	-1.154	0.252
Scale 3				
para central 1	-0.595	0.554	-0.556	0.580
para central 2	-2.290	0.026	-1.756	0.083
pre central 2	-2.134	0.038	-3.113	0.003
rostral middle frontal 2	-2.494	0.016	-0.390	0.698
rostral middle frontal 4	-1.403	0.167	-0.421	0.675
superior frontal 8	-1.916	0.061	-0.488	0.627
Scale 4				
para central 3	-0.464	0.645	-0.597	0.552
pars triangularis 1	-2.238	0.030	-2.111	0.038
pars triangularis 2	-1.160	0.252	-2.365	0.021
precuneus 7	-2.961	0.005	-1.121	0.266

Table B1. Young Adults APC t-test results.

ROI	Females		Males	
	t	p	t	p
Scale 1				
cuneus	-0.223	0.824	-1.896	0.062
pars opercularis	-3.926	0.000	-3.477	0.001
pars triangularis	-2.741	0.008	-2.098	0.040
pre central	-2.738	0.008	-2.996	0.004
precuneus	-1.052	0.297	-1.990	0.051
superior frontal	-1.908	0.061	-4.122	0.000
superior parietal	-0.722	0.473	-1.481	0.143
supramarginal	-3.003	0.004	-1.709	0.092
Scale 2				
pars triangularis	-2.421	0.019	-2.142	0.036
pre central 4	-3.955	0.000	-2.983	0.004
superior frontal 4	-1.860	0.068	-4.718	0.000
superior parietal 2	-1.012	0.316	-1.007	0.318
supramarginal 2	-2.680	0.010	-0.840	0.404
Scale 4				
rostral middle frontal 8	0.004	0.997	-0.863	0.391
superior frontal 5	-2.074	0.043	-1.966	0.053

Table B2. Middle Adults APC t-test results.

ROI	Females		Males	
	t	p	t	p
Scale 1				
Fusiform	-1.158	0.262	-0.008	0.994
inferior parietal	-3.384	0.003	0.092	0.928
lateral occipital	-0.571	0.575	-0.931	0.362
post central	-2.550	0.020	0.343	0.735
superior parietal	-3.137	0.006	0.426	0.674
supramarginal	-2.550	0.020	0.042	0.967
Scale 2				
bankssts	-0.995	0.333	0.376	0.710
caudal middle frontal	-4.405	0.000	-0.886	0.386
inferior parietal 2	-3.310	0.004	0.863	0.398
inferior temporal 2	-1.108	0.282	0.453	0.655
lateral occipital 2	-1.740	0.099	-0.747	0.464
lateral orbitofrontal 1	1.091	0.290	0.417	0.681
para central	-3.926	0.001	-0.014	0.989
post central 3	-1.817	0.086	-0.269	0.791
posterior cingulate	-1.855	0.080	0.717	0.481
pre central 1	-5.534	0.000	-0.446	0.660
precuneus 1	-2.357	0.030	-0.078	0.938
rostral middle frontal 1	-3.271	0.004	-0.719	0.480
rostral middle frontal 2	-2.947	0.009	-0.863	0.398
transverse temporal	-1.146	0.267	0.595	0.558
Scale 3				
bankssts 1	-0.527	0.605	-0.126	0.901
caudal middle frontal 2	-5.482	0.000	-1.211	0.239
caudal middle frontal 3	-2.750	0.013	-0.447	0.660
fusiform 3	-2.626	0.017	0.196	0.846
inferior parietal 5	-2.476	0.023	0.669	0.511
inferior temporal 4	-1.148	0.266	-0.219	0.829
lateral occipital 4	-2.120	0.048	0.082	0.935
medial orbitofrontal 1	0.437	0.667	2.991	0.007
middle temporal 2	-1.838	0.083	0.818	0.423
para central 1	-3.661	0.002	-0.192	0.85
pre central 1	-5.592	0.000	-0.871	0.394
pre central 2	-4.784	0.000	-0.036	0.972
pre central 4	-5.466	0.000	-1.128	0.272
pre central 6	-2.103	0.050	0.579	0.569
precuneus 2	-1.495	0.152	-0.386	0.704
precuneus 5	-3.401	0.003	-0.352	0.728
superior frontal 9	-3.671	0.002	-0.818	0.422
superior temporal 1	-1.676	0.111	-0.384	0.705
supramarginal 1	-1.445	0.166	-0.792	0.437
supramarginal 2	-2.447	0.025	0.344	0.735
supramarginal 4	-2.684	0.015	-0.140	0.890

transverse temporal	-1.142	0.268	0.594	0.559
Scale 4				
bankssts 1	-0.301	0.767	-0.614	0.546
caudal middle frontal 3	-2.608	0.018	-1.299	0.208
caudal middle frontal 4	-5.071	0.000	-1.002	0.328
lateral orbitofrontal 3	0.506	0.619	0.484	0.634
para central 3	-3.849	0.001	0.077	0.939
transverse temporal 2	-0.722	0.480	0.776	0.446

Table B3. Elder Adults APC t-test results

ANNEX C. SIGNIFICANT CORRELATION RESULTS

ROI 1	ROI 2	Diff	F Corr	M Corr
	Scale 1			
caudal middle frontal	rostral middle frontal	0.348	0.508	0.778
inferior parietal	pre central	0.369	0.468	0.767
inferior parietal	supramarginal	0.382	0.563	0.836
para central	superior frontal	0.382	0.444	0.761
post central	pre central	0.226	0.668	0.820
pre central	superior frontal	0.346	0.520	0.786
pre central	caudal middle frontal	0.409	0.571	0.857
precuneus	para central	0.259	0.664	0.837
superior frontal	rostral middle frontal	0.405	0.508	0.814
superior temporal	pars triangularis	0.428	0.293	0.717
superior temporal	superior frontal	0.374	0.322	0.691
supramarginal	pre central	0.380	0.447	0.761
transverse temporal	pre central	0.466	0.254	0.728
transverse temporal	post central	0.353	0.426	0.731
	Scale 2			
caudal middle frontal	rostral middle frontal 2	0.367	0.473	0.769
fusiform 1	pre cuneus 1	0.336	0.479	0.752
fusiform 1	pre cuneus 2	0.398	0.398	0.746
inferior parietal 1	supramarginal 2	0.463	0.385	0.782
inferior parietal 2	pre central 1	0.375	0.390	0.726
para central	superior frontal 4	0.332	0.558	0.802
pre central 1	superior frontal 4	0.386	0.386	0.732
pre central 3	caudal middle frontal	0.409	0.454	0.784
pre central 3	pre central 2	0.409	0.310	0.710
pre cuneus 1	para central	0.309	0.527	0.766
pre cuneus 1	supramarginal 2	0.370	0.405	0.731
pre cuneus 2	para central	0.266	0.582	0.778
rostral middle frontal 2	rostral middle frontal 1	0.409	0.553	0.845
superior frontal 4	rostral middle frontal 1	0.316	0.512	0.760
superior frontal 4	rostral middle frontal 2	0.387	0.327	0.703
transverse temporal	superior frontal 4	0.449	0.310	0.738
transverse temporal	pre central 1	0.409	0.249	0.687
	Scale 3			
para central 1	pre central 2	0.423	0.403	0.766
para central 2	superior frontal 8	0.525	0.229	0.760
rostral middle frontal 4	rostral middle frontal 2	0.493	0.265	0.751
	Scale 4			
pars triangularis 2	pars triangularis 1	0.505	0.349	0.792
precuneus 7	para central 3	0.504	0.352	0.792

Table C1. Young Adults Correlation Results. *Values of significant correlations for Females (F Corr) and males (M Corr) and values of the difference matrix (Diff).*

ROI 1	ROI 2	Diff	F Corr	M Corr
	Scale 1			
cuneus	supramarginal	0.409	0.307	0.710
cuneus	superior parietal	0.357	0.502	0.781
pars opercularis	pars triangularis	0.278	0.577	0.782
pre central	pars triangularis	0.403	0.298	0.701
precuneus	supramarginal	0.322	0.498	0.755
superior frontal	pars triangularis	0.365	0.534	0.807
superior parietal	supramarginal	0.307	0.587	0.807
	Scale 2			
pre central 4	pars triangularis	0.389	0.424	0.754
superior frontal 4	pars triangularis	0.398	0.452	0.776
superior parietal 2	supramarginal 2	0.365	0.468	0.765
	Scale 4			
superior frontal 5	rostral middle frontal 8	-0.408	0.797	0.476

Table C2. Middle Adults Correlation Results. *Values of significant correlations for Females (F Corr) and males (M Corr) and values of the difference matrix (Diff).*

ROI 1	ROI 2	Diff	F Corr	M Corr
	Scale 1			
fusiform	inferior parietal	0.543	0.384	0.831
fusiform	lateral occipital	0.511	0.431	0.834
inferior parietal	supramarginal	-0.442	0.848	0.526
superior parietal	post central	0.353	0.693	0.912
	Scale 2			
inferior parietal 2	rostral middle frontal 1	-0.487	0.842	0.471
inferior temporal 2	lateral orbitofrontal 1	0.678	0.190	0.845
inferior temporal 2	posterior cingulate	0.648	0.225	0.836
inferior temporal 2	lateral occipital 2	0.480	0.495	0.851
para central	pre central 1	0.231	0.771	0.909
precuneus 1	rostral middle frontal 1	-0.614	0.824	0.255
precuneus 1	caudal middle frontal	-0.567	0.822	0.33
rostral middle frontal 2	rostral middle frontal 1	-0.374	0.875	0.626
transverse temporal	post central 3	-0.553	0.838	0.386
transverse temporal	bankssts	-0.598	0.829	0.299
	Scale 3			
caudal middle frontal 3	medial orbitofrontal 1	-0.684	0.827	-0.001
inferior parietal 5	supramarginal 4	-0.616	0.896	0.433
inferior temporal 4	lateral occipital 4	0.717	0.136	0.858
inferior temporal 4	fusiform 3	0.689	0.203	0.854
middle temporal 2	caudal middle frontal 2	0.712	-0.248	0.880
middle temporal 2	precuneus 5	0.668	0.135	0.829
para central 1	pre central 1	0.352	0.662	0.889
pre central 2	superior frontal 9	0.621	0.268	0.832
pre central 6	pre central 4	0.546	0.461	0.871
superior temporal 1	inferior parietal 5	-0.649	0.863	0.310
superior temporal 1	precuneus 2	-0.699	0.863	0.216
supramarginal 1	medial orbitofrontal 1	-0.667	0.836	0.179
supramarginal 1	caudal middle frontal 3	-0.722	0.871	0.193
supramarginal 2	pre central 6	-0.659	0.878	0.335
transverse temporal	bankssts 1	-0.706	0.880	0.263
	Scale 4			
caudal middle frontal 3	lateral orbitofrontal 3	0.753	0.014	0.868
para central 3	caudal middle frontal 4	0.774	0.081	0.884
transverse temporal 2	bankssts 1	-0.705	0.899	0.322

Table C3. Elder Adults Correlation Results. *Values of significant correlations for Females (F Corr) and males (M Corr) and values of the difference matrix (Diff).*