A Comparison of White Matter Microstructure and its Relationship with Cognition in Younger and Older Adults

by

Abu-Bakar Sheriff
B.Sc. (Hons.), University of Victoria, 2019

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of the Requirements for the Degree of

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in the Division of Medical Sciences

Supervisory Committee

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Abstract

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Background: Given the growing aging population, it is crucial to better understand the neurobiological underpinnings of healthy aging and how changes in structure relate to changes in function. The current study derived diffusion tensor imaging (DTI) metrics of white matter microstructure in younger and older adults to simulate the healthy aging process.

Methods: The DTI metrics of fractional anisotropy (FA) and mean diffusivity (MD) as well as the cognitive domains of memory and executive function were examined in 34 healthy participants divided into older adults (17; Mean = 70.9, SD = 5.4) and younger adults (17; Mean = 28.1, SD = 2.8). Cognitive performance on the California Verbal Learning Test 2nd Edition (CVLT-II) and the trails making test (Trails-A & Trails-B) were used to evaluate memory and executive function, respectively. The differences in white matter microstructure between older and younger adults were analyzed using tract based spatial statistics (TBSS; $p < 0.05$, corrected for multiple comparisons) in FSL. Associations between the DTI metrics and cognition were then evaluated for each group.

Results: Older adults had lower FA and higher MD in diffuse brain regions, including major tracts such as the corticospinal tract, corpus callosum and superior and inferior

longitudinal fasciculi. There was a significant negative correlation between executive function and MD in the right superior and anterior corona radiata and the body of the corpus callosum of older adults. No significant relationship was detected between memory performance and DTI metrics in older adults. Furthermore, no significant relationships were detected between memory or executive function performance and FA or MD in younger adults.

Conclusions: The differences in DTI metrics between groups as well as the association between MD and executive function support further examinations into the healthy aging process. Future studies should use longitudinal designs with large sample sizes to better understand changes and trajectories during healthy aging.

Keywords: White matter microstructure, diffusion tensor imaging, healthy aging, cognition, memory, executive function

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Dedication

To Alpha Yahya Sheriff and Rosa Nyaama Yajo.

Chapter 1

Aging: A Brief Overview

Aging is a biological process that all living things undergo with the passage of time. According to the World Health Organization, aging is the culmination of a multitude of cellular changes ultimately leading to death (World Health Organization, 2021). These changes may consist of increased risk of disease, decreased ability to reproduce, hearing loss, chronic pain, inflammation, tissue degeneration, and decreased healing ability.

In recent years, it has been projected that the disparity in the Canadian population, in which individuals over age 65 currently outnumber children up to age 14, is projected to increase over time (Statistics Canada, 2022). By 2036, the number of seniors in Canada is expected to exceed 10 million (Statistics Canada, 2022). As such, the need to understand the changes involved with the aging process is paramount. As with all parts of the human body, the brain undergoes changes, both structural and functional, during the aging process. Although age increases the risk for certain neurodegenerative disorders (e.g. Alzheimer's disease, Parkinson disease), there are also normal change in the brain over time.

Although aging is associated with many detrimental factors, pursuing good health practices throughout the entirety of life can help stave off some of the deleterious effects (World Health Organization, 2021). Healthier diets, along with caloric restriction, has been shown to reduce chronic disease and mortality (Colman et al., 2013; Huffman et al., 2016; Omodei & Fontana, 2011). Regular exercise and cardiovascular activities have also

been shown to preserve longevity and prevent the onset of metabolic disorders and disabilities due to aging (Huffman et al., 2016; Mercken et al., 2012; Wang et al., 2002).

Based on the considerations of the National Institute on Aging, the Pan American Health Organization, and the World Health Organization, at large, healthy aging has been found to include the factors, such as genetics or controlled factors that allow for the maintenance or improvement of physical and mental health, independence, and quality of life throughout the lifespan. These factors, in the context of healthy aging, allow an individual to maintain a level of functional ability in order to continue being a resource to their community, family, and economy (National Institute on Aging, 2022; Pan American Health Organization, 2022; World Health Organization 2020).

Cellular Senescence

Somatic cells, or diploid cells, do not divide and replicate indefinitely under normal conditions. Cellular senescence, or aging, consists of the processes underlying the decreased replicability of cells. As humans, we age on both a cellular level and on a larger-scale organismic level. The $p15^{INK4b}$ (CDKN2B), $p16^{INK4a}$ (CDKN2A), $p21^{Kip1}$ (CDKN1A), and $p27^{Kip1}$ (CKDN1B) cyclin-dependent kinase (CDK) inhibitors cause the cessation of replication within cells through various mechanisms. The $p15^{INK4b}$ and $p16^{INK4a}$ CDK inhibitors (also known as p15 and p16, respectively) bind to CDK4 or CDK6 (Jafri et al., 2015; Rayess et al., 2012). This prevents the binding of Cyclin-D to CDK 4 or 6 and prevents the phosphorylation of retinoblastoma protein (pRB). If phosphorylated, pRB separates from an E2F transcription factor which then travels to the nucleus and triggers the transition from the G1 phase of the cell cycle to S-phase (Jafri et al., 2015; Rayess et al., 2012). Similarly, $p21^{Kip1}$ (also known as p21) can bind to all

cyclin-dependent kinases and, through binding to CDK 4 or 6 in a similar fashion to p15 and p16, can cause the cell cycle to halt at the G1 phase (Niculescu et al., 1998; Xiong et al., 1993). On the other hand, $p27^{Kip1}$ (also known as p27) can bind to CDK 2 or 4, as well as cyclins A, D, and E whether attached to a cyclin-dependent kinase or not. This binding causes a deformation in the CDK active site and halt the progression of the cell cycle at the G1 phase this way. Thus, p15, p16, p21, and p27 prevent the cell cycle from progressing beyond the G1 phase. This has been evidenced by investigations showing that a lack of these proteins leads to unrestrained cell proliferation. Telomeric shortening, increasing levels of the INK4a and ARF, reactive oxygen species (ROS), oncogene-induced (initiated by cancer-causing genes), and pro-inflammatory responses are all causes of cellular senescence (*Figure 1*) (Muñoz-Espín et al., 2014).

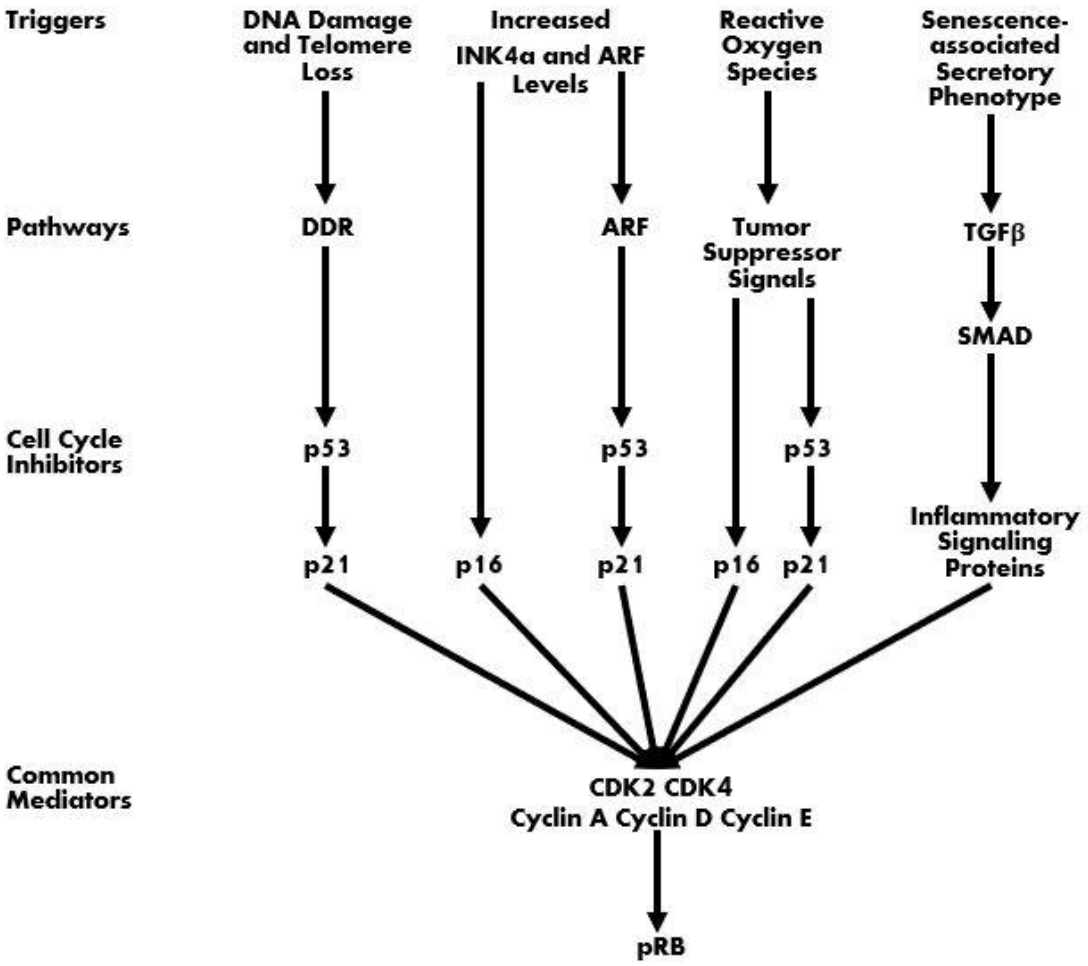

Figure 1. Model of cellular senescence pathways where specific triggers activate signaling pathways that therein activate certain cell cycle inhibitors and the tumour suppressor protein RB (pRB). DNA damage agents and telomere loss activate the DNA-damage response (DDR), which directly activates p53 and its downstream transcriptional target p21. Many types of senescence are associated with the increased presence of INK4a and ARF (encoding the cell cycle inhibitor p16 and p53 through ARF). Reactive oxygen species (ROS) activate p16 and p53 through tumor suppressor signals. Pro-inflammatory signals generated by transforming growth factor-β (TGFβ) is a notable component of the senescence-associated secretory phenotype (SASP) pathway, which upregulates cell cycle inhibitors through the SMAD complex. Information adapted from (Muñoz-Espín et al., 2014).

As a normal part of aging, cells undergo mitotic division as a means of replication. After each replication, telomeres (or segments of chromosomal DNA) that cap the ends of chromosomes shorten as a phenomenon known as the end-replication problem takes place (Campisi & d'Adda di Fagagna, 2007). Once these telomeres

become too short, a DNA damage response is triggered, and replication is halted through the activation of a DNA damage checkpoint protein known as checkpoint kinase 2 (CHK2). This protein activates tumor protein p53 (also known as the Guardian of the Genome), which causes a cessation of proliferation through the activation of p21 (Gire et al., 2004; Toufektchan & Toledo, 2018).

As seen in mouse models, as organisms age, expression of p16 and a protein known as ARF (or $p14^{ARF}$) within their cells increase (Krishnamurthy et al., 2004; Zindy et al., 1997). Similar to p16, ARF is also involved in ceasing cell replication. However, ARF does this by deactivating the HDM2 protein that normally inhibits p53 activation. This process allows the Guardian of the Genome to cease cell proliferation through p21 activation (Gil & Peters, 2006; Kim & Sharpless, 2006).

Reactive oxygen species (ROS) generated in the mitochondria of cells during aerobic cellular respiration have also been known to cause senescence (Balaban et al., 2005). ROS are oxygen-based free radicals that are highly reactive due to having an unpaired electron (Valko et al., 2006). With this high reactivity, when present within a cell, ROS can cause damage to the mitochondria in which they are formed, as well as DNA and other structures and organelles inside the cell through what is known as oxidative stress. This damage triggers a DNA damage response and initiates the CHK2 – p53 pathway, halting cellular proliferation. As such, antioxidants that bond with ROS to create less reactive chemicals are often seen as anti-aging tools (Macip et al., 2002).

Inflammation is a natural response to irritation or infection and is heavily involved in the healing of wounds and removal of pathogens as well as damaged tissue (Medzhitov, 2008). Cellular senescence has been shown to lead to inflammation and an

increase in the presence and activation of cytokines (or cell signaling proteins) involved in the immune response (David et al., 2007; Shelton et al., 1999). A pro-inflammatory response initiated by cells that have ceased cellular proliferation known as senescence-associated secretory phenotype (SASP) causes the secretion of cytokines necessary for inflammation and signal proteins that mark the cell for phagocytosis (or consumption by immune cells) (Muñoz-Espín et al., 2014).

Despite being somatic cells, neurons lack centrioles and, therefore, cannot undergo mitosis (Insolera et al., 2014). In the hippocampus, however, neural stem and progenitor cells (NSPCs) create new neurons through a process known as neurogenesis (Kirby et al., 2014). As neurons themselves cannot undergo mitotic division, senescence in neurons is not used as a mechanism for ceasing errant replication but is rather marked by a decrease or cessation of synaptic plasticity (Sikora et al., 2021). Synaptic plasticity is the ability for the connections between neurons to change in strength and efficiency based on their level of activity, over time (Gerrow & Triller, 2010). While still a topic of great interest, synaptic plasticity has been positively correlated with cognitive functioning (Lu et al., 2014). As such, neuronal senescence can be postulated to have a negative effect on cognition.

Magnetic Resonance Imaging (MRI)

Magnetic Resonance Imaging (MRI) is an imaging technique used in the medical field to non-invasively image tissue using high-strength magnetic fields. Developed in 1971 from the concept of nuclear magnetic resonance (NMR) that postulates that atoms in line with an initial magnetic field will resonate when influenced by a second magnetic field, MRI was initially invented to diagnose cancer (GE Healthcare, 2019). The main

magnetic field generated by an MRI scanner (known as the B_0 field) aligns the protons of participants largely in an orientation parallel to that of said field. The strength of the magnetic field will also have an effect on the frequency at which the protons undergo precession (known as the Larmor frequency). In a homogeneous magnetic field, all protons have the same rate of precession. In MRI, additional gradient coils within the scanner superimpose magnetic fields with an X, Y, and Z-axis alignment which, yield protons with unique precession frequencies, to help differentiate locations and create a 3-dimensional image.

To measure a signal, a radio frequency (RF) pulse is then applied, which causes the protons to flip from the longitudinal to the transverse plane. Protons in different tissues have different rates that they return to their original position when an RF pulse is applied. The B_0 field, still in effect, causes the protons to realign with it, generating voltage in a receiver coil, after which the protons go through a process known as longitudinal relaxation. Transverse relaxation, on the other hand, occurs when the protons start to fall out of phase with one another, due to the protons each experiencing slightly different magnetic fields. The protons in different tissue types of experience longitudinal and transverse relaxation at different rates, which allows for collection of images with different contrasts. The signals are picked up by the receiver coils that send the signal to computers that create an image.

Through the advent of MRI, it is possible to collect several types of imaging data for a more robust understanding of the aging brain (Soares et al., 2013). It should be noted, however, that since MRI involves the use of strong magnetic fields, complications can occur if participants or technicians in the room with the scanner have certain

contraindications, or conditions that may harm the participant during the use of the MRI scanner. Some of these contraindications include pacemakers and other metal defibrillators, metal piercings, wire catheters, DBS implants, cochlear implants, bullets or other metallic fragments, dental implants, and certain drug infusion devices (Inside Radiology, 2017). The powerful magnetic fields employed could cause heating, movement, or malfunction of these contraindications and so consultation before receiving an MRI scan is advised. That being said, since MRI provides the ability to discern between different types of tissue, structural changes within the brain, namely those in grey and white matter can be examined separately within a single MRI scan. For structural MRI images of the brain, T1-weighted images are typically used for their ability to visualize anatomical structures and cell density within the grey matter of the brain at a high resolution (*Figure 2*). This, along with the ability to perform several scans on an individual over a short period of time due to the lack of any radiation or harmful chemicals being applied to participants within the scanner, MRI is a very valuable tool in the field of neuroimaging and in the exploration of aging within the human brain.

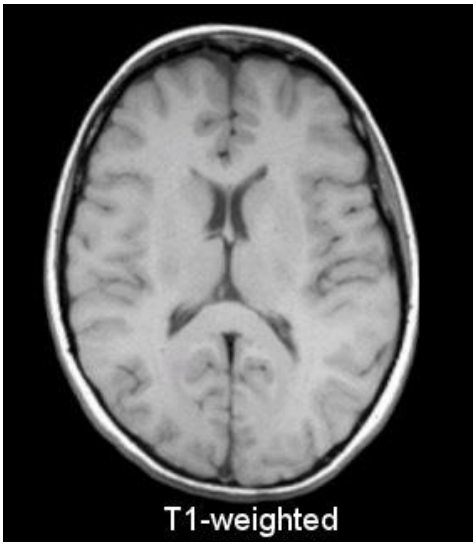

Figure 2. T1-weighted midsection MRI scan.

Diffusion Tensor Imaging: How Does It Work?

Diffusion-weighted magnetic resonance imaging (DW-MRI) is a form of neuroimaging acquisition derived from MRI in which the contrast is created by measuring the tissue water diffusion rate of within cubic sections of the brain, called voxels, on a microstructural level using what are known as T2-weighted images (Assaf & Pasternak, 2008). The diffusion-weighted images (DWI) produced through this method are highly sensitive to the movement of water molecules within the microstructure of the brain and, based on this movement different features regarding the integrity of said structure can be garnered (Soares et al., 2013). The technique operates under the assumption that the displacement of the water molecules is faster and parallel along white matter tracts than when measured moving in other orientations (Pierpaoli & Basser, 1996). The diffusion vector within a 3-dimensional space is calculated for each voxel based on the strength of the diffusion gradient created by the magnetic field B_0. This diffusion gradient is known as the b-value and, along with the diffusion vector, can be compiled to determine the overall directional information for a given space. For a single individual, a highly specific structural recreation, known as a tractography, can be created modeling the white matter tracts within the brain (Basser et al., 2000). Being a subset of magnetic resonance imaging, DTI carries the same benefits of MRI of being a non-invasive and repeatable approach to neuroimaging that can garner insight on the structural integrity of white matter within the brain that has been relatively overlooked in the field of neuroimaging.

Diffusion Tensor Imaging and White Matter

Using mathematical algorithms and physics as a foundation to determine the diffusion rate and directionality of water within the body, the diffusion tensors are calculated for the collected diffusion weighted data. For a given DTI scan, typically ~30 directions are measured and a typical b-value of 1000 s/mm^2 is used (Basser et al., 1994; Lope-Piedrafita, 2018). The strength of the gradient fields as well as the timing of their applications are indicated by the b-value. With these vectors (λ_1, λ_2, and λ_3) which consist of both a direction and magnitude of diffusion, an ellipsoid of diffusion can be created. An ellipsoid is a spherical shape that has been deformed due to transformational vectors. Transformational vectors with a magnitude of 0 will result in a perfect spherical shape (*Figure 3*).

Based on this diffusion ellipsoid, the diffusion metrics of fractional anisotropy (FA) and mean diffusivity (MD) are calculated and used to characterize the integrity of white matter. FA is calculated by taking the sum of squares of the diffusivity magnitudes as well as the sum of squares of the differences in diffusivity magnitudes to create a statistical estimate for the movement of water molecules along a preferred direction. This results in FA values ranging from 0-1 where they approach 0 in isotropic mediums with a diffusion spheroid and approach 1 in anisotropic mediums that cause a transformation in the diffusion ellipsoid. As such, FA can be used to infer the integrity of white matter based on the strength and orientation of diffusion (Table 1).

The greater the FA value, the greater the magnitude and directionality of the diffusion in a preferred direction (Assaf & Pasternak, 2008). This can often be best characterized in the central nervous system by white matter due to the restriction created

in regions that contain barriers to diffusion, such as the hydrophobic myelin found in axon bundles (Pierpaoli & Basser, 1996). Taking the diffusion tensors and calculating the average of their diffusion magnitudes on the X, Y, and Z-axes results in the MD value. This measures the average diffusion of water in a medium across all directions in 3-dimensional space without restrictive barriers and is often best characterized by isotropic mediums like cerebrospinal fluid within the central nervous system. Within each voxel, these diffusion metrics are analyzed, with the strength of the white matter microstructure being positively correlated to FA values and negatively correlated to MD values. It should be noted, however that a phenomenon known as crossed or kissing fibers can occur during DTI analysis in which multiple white matter bundles in the same voxel have different orientations. The resultant diffusion vector may not be representative of the actual flow of diffusion within the voxel and may lead to an improper account of the white matter integrity or neurodegeneration within the voxel (Jbabdi et al., 2010; Le Bihan et al., 2006).

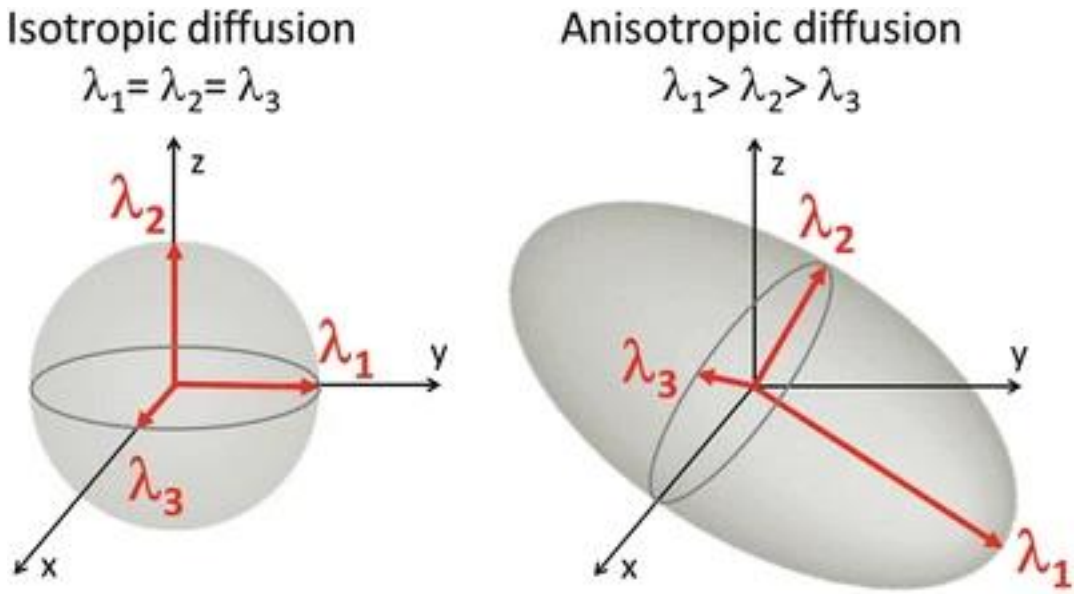

Figure 3. An example of a spheroid (left) and an ellipsoid (right). In DTI analysis, a spheroid shape is the result of isotropic diffusion where vectors λ_1, λ_2, and λ_3 are equal in magnitude, while an ellipsoid shape results from anisotropic diffusion where vectors λ_1, λ_2, and λ_3 are unequal in magnitude. Figure adapted from Lope-Piedrafita, S. (2018).

Table 1
Diffusion Metrics

	Metric	
	Fractional Anisotropy (FA)	Mean Diffusivity (MD)
Calculation	$\sqrt{\frac{(\lambda_1-\lambda_2)^2+(\lambda_1-\lambda_3)^2+(\lambda_2-\lambda_3)^2}{2(\lambda_1^2+\lambda_2^2+\lambda_3^2)}}$	$\frac{\lambda_1^2+\lambda_2^2+\lambda_3^2}{3}$
Description	The degree of anisotropic diffusion within a voxel based on the shape of the diffusion tensor ellipsoid	The magnitude of non-directional diffusion within a voxel
Directionality with neurodegeneration	Decreased in white matter	Increased in white matter
Sensitivity	Sensitive to a wide range of pathologies	Sensitive to cellularity, edema, and necrosis
Limitations	Voxels containing crossing fibers with high integrity may register as having low FA	Measurement based on presence of crossing fibers

Note. The values λ_1, λ_2, and λ_3 correspond to the vectors with the greatest, middle, and least magnitude, respectively. Information adapted from Alexander et al., 2007; Budde et al., 2009; Song et al., 2002; Vos et al., 2012.

Aging research using structural MRI and DTI

Studies that examine volumetric changes primarily focus on grey matter. Cell somas, better known as cell bodies, of neurons within the central nervous system comprise grey matter and contain most of the organelles of neurons within them. The axon, on the other hand, is a protrusion from the neuronal soma and is heavily involved in the transmission of signals between neurons. Within the brain, axons can be wrapped by glial cells known as oligodendrocytes, in a process known as myelination which creates an insulating barrier called a myelin sheath which increases the transmission speed of the signal sent along the axon. These myelinated axons make up what is known as white matter within the central nervous system. On a volumetric basis, white matter comprises roughly half of all brain tissue, so changes in structural connectivity should also be examined regarding changes within the brain, namely the aging process. Notable DTI studies on aging have been compiled and summarized in Table 2 at the end of this section. Notable studies for the sake of this paper are those with similarities in design or measures examined.

In a unique study conducted by Ziegler et al. (2010), both grey and white matter in 36 young adults (aged 18-28) and 38 older adults (aged 61-86) were compared using whole-brain statistical analyses. The older adults were found to have decreased amounts of grey matter compared to the younger adults in the region of interest (ROI) analyses of cortical thickness. While using the DTI metrics of FA and MD to examine the white matter of these participants, Ziegler et al. (2010) found decreased FA when comparing the older adult group to the younger adult group. Decreased cortical thickness could not be conclusively linked to decreases in any of the measured domains of cognition.

Decreases in white matter integrity, however, were associated with decreases in cognitive control and episodic memory at a significant level (Ziegler et al., 2010).

Table 2
Summary of Notable Diffusion Tensor Imaging (DTI) Aging Studies

Study	Sample (F:M)	Age Range (years)	FA	MD	Notes
Beck et al., 2021	702 (401:301)	18 - 94	Decreased	Increased	MD decreased initially then had an upward trend
Kantarci et al., 2017	21 (7:14) Low NFT 25 (8:17) High NFT	N/A	Decreased	Increased	No differences in DTI metrics found when comparing groups with different NP scores
Kodiweera et al., 2016	47 (24:23)	18 – 55	Decreased	Not significant	AD decreased and RD increased as a function of age
Mayo et al., 2017	31 (16:17) HC 34 (10:24) Alzheimer's	N/A	Decreased	Increased	Participants pooled from ADNI database with elderly participant age
Moscufo et al., 2018	41 (22:19)	N/A	Decreased	N/A	AD decreased and RD increased as a function of age
Ugwu et al., 2015	46 (27:19) HC 46 (28:18) MDD	18 - 65	Decreased	N/A	LD decreased and RD increased for many ROIs as a function of age

Note. FA = Fractional Anisotropy, MD = Mean Diffusivity, NFT = Neurofibrillary Tangles, NP = Neuropsychological, AD = Axial Diffusivity, RD = Radial Diffusivity, HC = Healthy Controls, MDD = Major Depressive Disorder, LD = Longitudinal Diffusivity = Axial Diffusivity

Cognitive Changes Due to Aging

According to the National Institute on Aging, common cognitive changes may include slower recall of names, diminished ability to multitask, and decreased attentional span (National Institute on Aging, 2020). The extent to which cognition declines through the aging process is still being heavily investigated and a relatively new concept known as super agers has emerged (National Institute on Aging, 2020). These individuals show a far less marked decline in cognitive abilities than other individuals their age although

what differentiates these adults from their peers, whether it be an overall lower level of neural degeneration as they age or greater employment of compensatory mechanisms, is still unknown. The Scaffolding Theory of Cognitive Aging (STAC) postulates that through the aging process, as neuronal degeneration occurs, competing compensatory processes are deployed to combat the overall decrease in cognition. This mix of compensation and degeneration-induced cognitive decline results in the overall level of cognitive function of an individual (Reuter-Lorenz & Park, 2014).

Some aspects of memory performance decline with age, while some types of memory are stable (Murman, 2015). In particular, retrieval and the ability to learn new information are thought to decline with age. It is also notable that memory for historical information is relatively stable with aging, as are procedural memories (e.g., riding a bike).

In terms of executive function, decision making, problem solving, planning and sequencing of responses, and multitasking are known to decline with age (Murman, 2015). In particular, novel tasks can become more difficult with age.

Declines in processing speed are also expected with aging and may have an interaction with other domains of function. For example, studies exploring the association between memory and processing speed have shown that some of the decline in memory is due to an age-related decrease in cognitive processing speed (Park & Festini, 2017).

Declines have been reported many times throughout the study of cognition, as with a study by Vlahou et al. (2014) on the association between resting neural activity and age. 53 healthy adults (aged 18-89) had their processing speed and executive functioning assessed with both the A and B versions of the trail making test (Trails-A &

Trails-B). Participants then had their Alpha and Theta waves recorded in a magnetoencephalogram (MEG) scanner. Vlahou et al. (2014) found that increased age was associated with decreased processing speed and executive function as well as decreased neural activity throughout the sensory regions of the brain. This shows that, depending on the task performed, domains of executive function may also draw on sensory regions associated with the task and is not a solely frontal lobe-associated process. A review conducted by Harada et al. (2013) examined the literature on changes to cognition in healthy aging and found similar results in the domains of processing speed and executive functioning as well as noting a decline in attention and working memory. Interestingly enough, both Vlahou et al. and Harada et al. (2014; 2013) noted that the decline was not consistent throughout the entirety of life but, rather, remains fairly consistent until later adulthood and then begins a gradual decline.

Relationship Between DTI Findings and Cognition

As stated earlier, with the process of aging comes an expected level of cognitive decline. Even in healthy aging, several cognitive abilities that rely on processing speed and executive functioning have shown notable decreases (Madden et al., 2012). These abilities are known as fluid cognitive abilities and are less stable over time than crystallized abilities associated with knowledge and expertise. Reduced white matter integrity associated with aging has also been linked with significantly decreased accuracy in episodic memory (Lockhart et al., 2012).

Several DTI studies have looked at clinical populations with brain injuries or neurodegenerative disorders and examined the relationship between white matter integrity and cognition for those participants. For instance, a study by Mayo et al., (2019)

examined the cognitive domains of memory and executive function in individuals with Alzheimer's disease gathered from a national database of participants in the United States known as the Alzheimer's Disease Neuroimaging Initiative (ADNI) and compared them to healthy controls. This examination found that participants with Alzheimer's had lower FA than their healthy controls in white matter tracts within the corpus callosum, left internal capsule, corona radiata, posterior thalamic radiations, inferior longitudinal fasciculi, and tapetum. Alzheimer's participants also had significantly higher MD compared to healthy controls in white matter tracts within the corpus callosum, internal capsule, corona radiata, left posterior thalamic radiation, left inferior longitudinal fasciculi, inferior fronto-occipital fasciculi, cingulate gyri, right hippocampal cingulum, fornix, superior longitudinal fasciculi, and tapetum (Mayo et al., 2019). Along with these changes in the white matter microstructure, Mayo et al., (2019) found that memory scores were positively correlated with FA and negatively correlated with MD in widespread regions. As for executive function, similar results denoting a positive correlation with FA and negative correlation with MD were found. Another study by Shim et al., (2017) compared hospital patients with Alzheimer's disease to those with mild cognitive impairment (MCI) and healthy controls to see if there was an association with their DTI metrics and cognitive domains of depression, memory, and executive function as assessed by the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) assessment package. Their analysis found that whole-brain FA and MD values were good predictors of performance on the cognitive assessment for patients with Alzheimer's and MCI. A meta-analysis performed by Wallace et al. (2018) examined the connection between the DTI metrics of FA and MD with cognitive functioning after traumatic brain

injury (TBI). After examining 20 studies for this meta-analysis, Wallace et al. (2018) found that in 72% of the analyses performed based on FA there was a positive correlation with cognitive performance while 62% of the analyses performed based on MD found a negative correlation with cognitive performance. These investigations into the link between white matter and cognition are in line with the current theory of structural changes in white matter influencing various cognitive processes within the brain. There is still room for investigation, however, as these associations can sometimes disappear when controlling for variables such as sex, age, and level of education.

Previous DTI Studies on Healthy Aging and Cognition

Table 3 at the end of this section looks at several notable DTI studies on aging and cognition and summarizes their findings. Notable studies here refer to papers with similarities in measures examined. A DTI study performed by Boekel and Hsieh (2018) aimed to determine if changes in FA and MD were associated with trait mindfulness and age. They looked at 97 healthy participants (aged 40-77) and uncovered through whole-brain analysis that, overall, their older participants had lower FA than younger participants throughout the fornix, the body and genu of the corpus callosum, left anterior corona radiate, bilateral posterior limbs of the internal capsule, left anterior limb of the internal capsule, bilateral fornix stria terminalis, bilateral external capsules, bilateral posterior thalamic radiations, bilateral superior corona radiates, left sagittal stratum, left uncinate fasciculus, and right cerebral peduncle (Boekel & Hsieh, 2018). As for MD, a whole brain analysis was performed and found that age was positively correlated with MD (Boekel & Hsieh, 2018).

Another cross-sectional DTI study by Bendlin et al. (2010) examined 120 healthy participants (aged 18-83) with the aim of finding links between age and white matter microstructure as well as cognition. They found a significant negative correlation between age and FA in the corpus callosum, inferior fronto-occipital fasciculus, inferior longitudinal fasciculus, posterior thalamic radiations, superior longitudinal fasciculus, cingulum internal capsule, anterior coronal radiata, superior coronal radiata, and posterior corona radiata (Bendlin et al., 2010). These correlations were found to be strongest in the frontal lobe white matter tracts, as well as anterior and posterior temporal white matter tracts, which are regions that are heavily involved in executive function and memory. For MD, Bendlin et al. (2010) found a significant positive correlation with age in the superior fronto-occipital fasciculus, cingulum, fornix, cerebral peduncles, thalamus, and the genu of the corpus callosum. Using Trails-A, MD was positively correlated with response time. Performance on Trails-B, as a measure of executive function, however, was not significantly correlated to MD but was negatively correlated with FA. Bendlin et al. (2010) also tested participants on their visual and verbal memory through the Brief Visuospatial Memory Test (BVMT) and Rey Auditory Verbal Learning Test (RAVLT), respectively and found that visuospatial memory was positively correlated with FA but not correlated with MD. Verbal memory was found to not be correlated to either FA or MD through their analyses.

Table 3
Summary of Notable Diffusion Tensor Imaging Studies of Cognition and Aging

Study	Sample (F:M)	FA	MD	Cognitive Domain(s): Association	Notes
Bendlin et al., 2010	120 (71:49)	Decreased	Increased	Processing Speed: Negative with FA; Positive with MD	FA decreases were strongest in temporal and frontal white matter tracts
Boekel & Hsieh, 2018	97 (48:49)	Decreased	Increased	Trait Mindfulness: No association with FA or MD	Trait mindfulness may be a mediating factor for FA and age
Halliday et al., 2019	44 (25:19)	Decreased	Increased	Memory and Executive Function: Positive with FA; Negative with MD	Participants pooled from ADNI database with elderly participant age
Li et al., 2022	348 (173:155)	Decreased	Increased	Working Memory: Positive with FA; Negative with MD	FA may be a mediating factor for age and working memory
Lockhart et al., 2012	15 (6:9) HC 13 (11:2) Low WMH 15 (9:6) High WMH	Decreased	N/A	Episodic Memory: Positive with FA	Associations strongest in frontal, temporal, and parietal connections
Mayo et al., 2019	49 (19:30) Alzheimer's 48 (26:22) HC	Decreased	Increased	Memory and Executive Function: Positive with FA; Negative with MD	Associations strongest in frontal and temporal regions
Ohlhauser et al., 2019	30 (20:10) SCD 44 (25:18) HC	Decreased	Increased	Memory: No association Executive Function: Positive with FA; Negative with MD	Associations only detected in SCD group
Shim et al., 2017	49 (49:0) AD 66 (66:0) MCI 33 (33:0) HC	Decreased	Increased	Memory and Executive Function: Positive with FA; Negative with MD	Metrics only significant predictors of MCI
Ryan et al., 2011	88 (68:20) Noncarriers 32 (25:7) Heterozygotes 6 (5:1) Homozygotes	Decreased	Increased	Memory: No association with FA; Negative with MD	Associations increase in the presence of ε4 allele
Ystad et al., 2011	100 (64:36)	Decreased	N/A	Executive Function and Processing Speed: Positive with FA	Associations strongest in the connections between the putamen and the Default Mode Network and the dorsal attention network

Note. FA = Fractional Anisotropy, MD = Mean Diffusivity, HC = Healthy Controls, WMH = White matter Hyperintensities, SCD = Subjective Cognitive Decline, MCI = Mild Cognitive Impairment.

Future Directions

Previous studies on aging primarily focus on pathological aging involving a neurodegenerative disorder like Alzheimer's Disease and, as such, are not the most apt tools to describe the processes and changes to expect with healthy aging. Previous studies focusing on structural changes within the brain have mostly used structural MRI to look at changes in grey matter within the brain (Mak et al., 2017). Since white matter constitutes roughly 50% of the brain and represents the connections within the brain, to fully understand the changes within the brain due to the aging process, white matter should also be examined. As such, a hole in the literature exists for studies that incorporate both healthy aging and a focus on white matter within the brain to create a more robust examination of the aging process both structurally and, by use of neurocognitive assessments, functionally.

[1]Chapter 2

Introduction

White matter and cognition have been shown to be tightly intertwined various times throughout the history of neuropsychology (Filley & Fields, 2016). Initially, lesion studies on aphasias led to emphasis on the importance of white matter tracts, such as the arcuate fasciculus, for receptive and expressive language function (O'Muircheartaigh et al., 2013). Histopathological studies have also demonstrated a loss of myelinated nerve fibres with age that has been suggested to partially account for age related cognitive decline (Marner et al., 2003). More recently, neuroimaging techniques, such as magnetic resonance imaging (MRI) and, with it, diffusion tensor imaging (DTI), have made possible the ability to examine white matter on a previously inaccessible microstructural level *in vivo* (Assaf & Pasternak, 2008). Age related neurodegenerative disorders such as Alzheimer's disease (AD), have been linked to decreased measures of the white matter microstructure of the brain, as well as decreases in cognition (Mayo et al., 2017; Ziegler et al., 2010). Such studies often compare individuals with neurodegenerative disorders to those undergoing healthy aging. However, it is also imperative to understand changes in white matter and cognition that occur during the healthy aging process. The objective of the current study is to better understand the healthy aging brain.

The current study derived DTI metrics of white matter microstructural integrity and compared them between a group of older adults and a group of younger adults to

[1] This chapter is written as a manuscript to be submitted to Journal of the International Neuropsychological Society (word counts are in line with the author guidelines for this journal).

mimic the healthy aging process. The relationship between DTI metrics and cognitive performance on neuropsychological assessment for these participants was also examined. This study comprehensively examined differences in white matter microstructure between healthy older and younger adults and how observed differences relate to cognitive performance in key domains, including memory and executive function.

Based on previous literature that has revealed an inverse relationship between age and processing speed, memory, and executive function (Boekel & Hsieh, 2018; Shim et al., 2017; Ystad et al., 2011; Zelinski et al., 2011), it is hypothesized that the older adult group will show widespread lower white matter integrity, particularly in the frontal and temporal lobes, as well as lower scores in tests of cognition than the younger adult group.

Methods

Participants

Participants included 34 healthy adults, equally divided into one of two categories: 17 younger adults (YA; F = 9) and 17 older adults (OA; F = 9). The YA group had an age range of 25-35 years (Mean = 28.1 years, SD = 2.8 years) and an education range of 12-23 years (Mean = 17.7 years, SD = 3.2 years). The OA group had an age range of 65-82 years (Mean = 70.9 years, SD = 5.4 years) and an education range of 9-23 years (Mean = 17.2 years, SD = 3.0 years) (Table 4). All participants were native-English speakers, with normal or corrected-to-normal vision. Inclusion criteria specified no history of major neurological (e.g. neurodegenerative disorders, stroke, moderate to severe traumatic brain injury) or psychiatric disorders (e.g. schizophrenia, bipolar disorder). Individuals were excluded based on contraindications for MRI (e.g. metal

implants). There were no significant differences between the groups in sex or level of education (Table 4).

The study protocol was approved by the Human Research Ethics Board at the University of Victoria.

Table 4
Participant Demographics

	Younger Adults	Older Adults	*p*-value
Mean Age and SD	28.1 ± 2.8	70.9 ± 5.4	$p = 0.0099 \times 10^{-12}$
Age Range	25 – 35 (10)	65 – 82 (17)	-
Mean Education and SD	17.7 ± 3.2	17.2 ± 3.0	$p = 0.6177$
Education Range	12 – 23 (11)	9 – 23 (14)	-
Number of Males	8	8	-
Number of Females	9	9	-

Note. Age and education calculated in years.

DTI Acquisition

DTI data were collected at West Coast Medical Imaging (Victoria, BC) on a 3T GE Signa Pioneer MRI scanner. The images were acquired with a SE-EPI sequence, axially, with the following parameters: TR = 8000 ms, TE = 101 ms, flip angle = 90°, 52 slices, voxel size = 1.4 x 1.4 x 2.0 mm. There were 48 images acquired for each scan: 45 diffusion-weighted images (b = 1000 s/mm^2) and 3 non-diffusion-weighted images (b = 0 s/mm^2). The acquisition took approximately 6 minutes per participant.

Neuropsychological Assessment

The neuropsychological assessment included 3 tests which measured the cognitive domains of short-term memory and executive function (more specifically alternating attention): Trail making test (Trails-A & Trails-B) and the California Verbal Learning Test 2nd Edition (CVLT-II). Trails-A and Trails-B are visual attention and task switching tests used to evaluate processing speed and the ability to switch between various sets of rules, while the CVLT-II is a list learning task with a delayed portion to test recall or recognition abilities. The *z*-scores were used for these measures, as opposed to the raw scores, to control for several confounds such as age, sex, and level of education. Lower scores on the Trails-B assessment and higher scores on the CVLT-II indicate better performance on the tasks.

DTI Analyses

All analyses were performed using Functional MRI of the Brain Software Library (FSL) version 5.0.10 (FSL, 2021; Jenkinson et al., 2012; Smith, et al., 2004).

Preprocessing

Images were converted from DICOM to NIfTI format. To correct for any present head motion and eddy current distortions, diffusion weighted images (DWI) were aligned to the initial "b_0" image using the "eddy correction tool". Brain tissue was then differentiated from the skull, spinal cord, and CSF using the "brain extraction tool", with the resulting images being visually inspected for accuracy.

Tract Based Spatial Statistics

To project the participant groups' DTI metrics onto a mean white matter representation, the "DTIFit" tool was used to create FA images which are used in an analysis technique known as Tract-Based Spatial Statistics (TBSS). TBSS creates an "FA skeleton" on which the Fractional Anisotropy (FA) and Mean Diffusivity (MD) data for an entire group of participants is overlayed onto. An FA threshold of 0.2 was used for the FA skeleton in this study, as is common for DTI analyses. The mean FA skeleton for the participant pool is then superimposed over a standard brain skeleton in standard space (FMRIB_FA_58). To then determine the difference between groups, for both FA and MD, the "randomise" tool was used to perform voxel-wise statistical analyses of the white matter FA skeleton. This tool computed an independent sample t-test, undergoing 5000 permutations and corrected for multiple-comparisons, sex, and years of education ($p < 0.05$) with threshold free cluster enhancement. Each group was then examined separately to investigate the correlation between domains of cognition (memory and executive function) and white matter microstructure (FA and MD). In these analyses, age was also corrected for in addition to sex, years of education, and multiple comparisons ($p < 0.05$). This allowed for an examination of the changes in white matter integrity throughout the entire white matter FA skeleton. Regions of significance were identified using the John Hopkin's University ICBM DTI-81 white matter atlas.

Results

Descriptive Statistics

Microstructural white matter differences between healthy older and younger adults

A statistical comparison of FA and MD values between participants in the older adult group and the younger adult group did not reveal any areas within the white matter

microstructure where older adults had significantly higher FA than younger adults. Furthermore, no areas were revealed that showed younger adults with significantly higher MD than older adults. However, younger adults did have significantly higher FA within the left cerebral peduncle, posterior limb of the internal capsule, right anterior limb of the internal capsule, bilateral posterior thalamic radiations, superior longitudinal fasciculi, anterior corona radiata, cingulum, superior coronal radiata, posterior corona radiata, external capsule, optic radiations, stria terminalis, retrolenticular parts of the internal capsule, crura of the fornix, sagittal strata, inferior fronto-occipital fasciculi, inferior longitudinal fasciculi, as well as the body of the fornix and the splenium, genu, and body of the corpus callosum (*Figure 4*).

It was also shown that older adults had significantly higher MD within the bilateral anterior corona radiata, external capsules, retrolenticular parts of the internal capsule, post thalamic radiations, optic radiations, sagittal strata, inferior fronto-occipital fasciculi, inferior longitudinal fasciculi, superior longitudinal fasciculi, superior corona radiata, posterior corona radiata, anterior limbs of the internal capsule, crura of the fornix, stria terminalis, as well as the body of the fornix, and the genu and body of the corpus callosum (*Figure 5*).

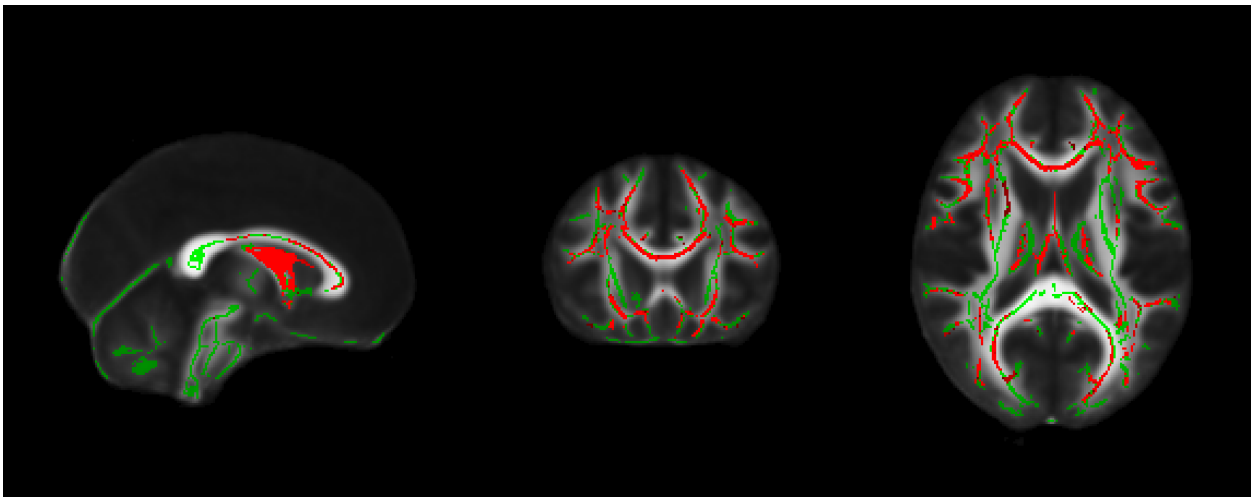

Figure 4. From left to right: sagittal, coronal, and axial slices of the standard FMRIB_FA_58 brain displaying results of TBSS analyses showing regions that have significantly higher FA (red) overlaid on the white matter skeleton (green) in younger adults compared to older adults ($p < 0.05$, corrected for multiple comparisons as well as sex and years of education, radiological view).

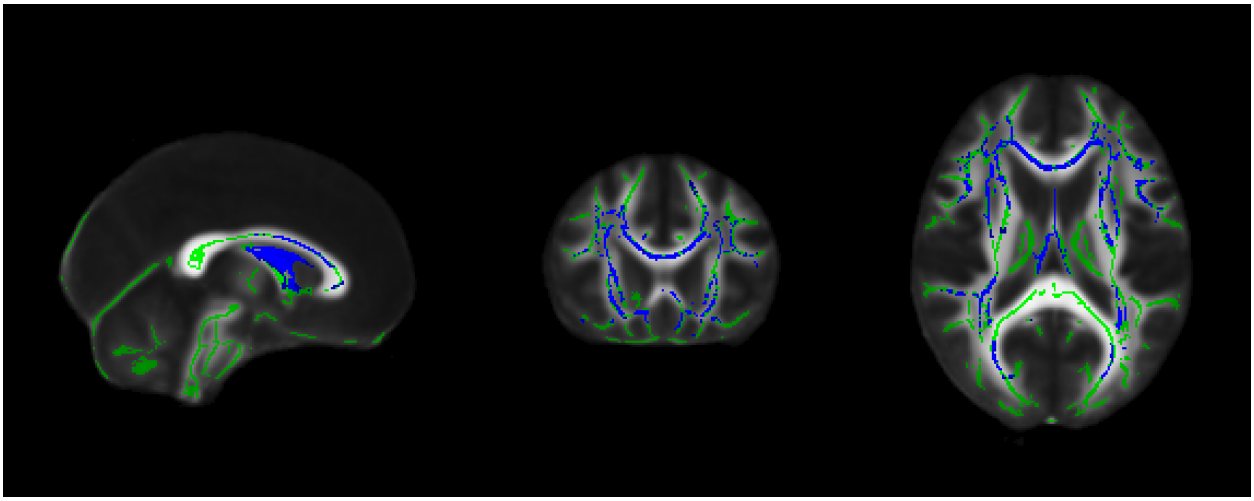

Figure 5. From left to right: sagittal, coronal, and axial slices of the standard FMRIB_FA_58 brain displaying results of TBSS analyses showing regions that have significantly higher MD (blue) overlaid on the white matter skeleton (green) in older adults compared to younger adults ($p < 0.05$, corrected for multiple comparisons as well as sex and years of education, radiological view).

Correlations

Relationship between microstructural white matter and cognitive performance

Bivariate correlations were computed to examine the relationship between cognitive scores and age within each group separately (i.e. younger adults vs. older adults) (Table 5).

Analyses investigating the relationship between DTI metrics and memory performance, (as measured by scores on the California Verbal Learning Test 2nd Edition) did not detect significant relationships in healthy older or younger adults.

No significant correlations were found between executive functioning (as measured by scores on the Trails-B) and FA in either the older adult group or the younger adult group. However, a comparison of executive function (as measured by Trails-B) and MD found a significant negative correlation in the older adult group in the right superior and anterior corona radiata and the body of the corpus callosum (*Figure 6*). In order to control for processing speed, Trails-A scores were also compared to MD. No significant correlation was found in either direction between Trails-A and MD when performing this analysis.

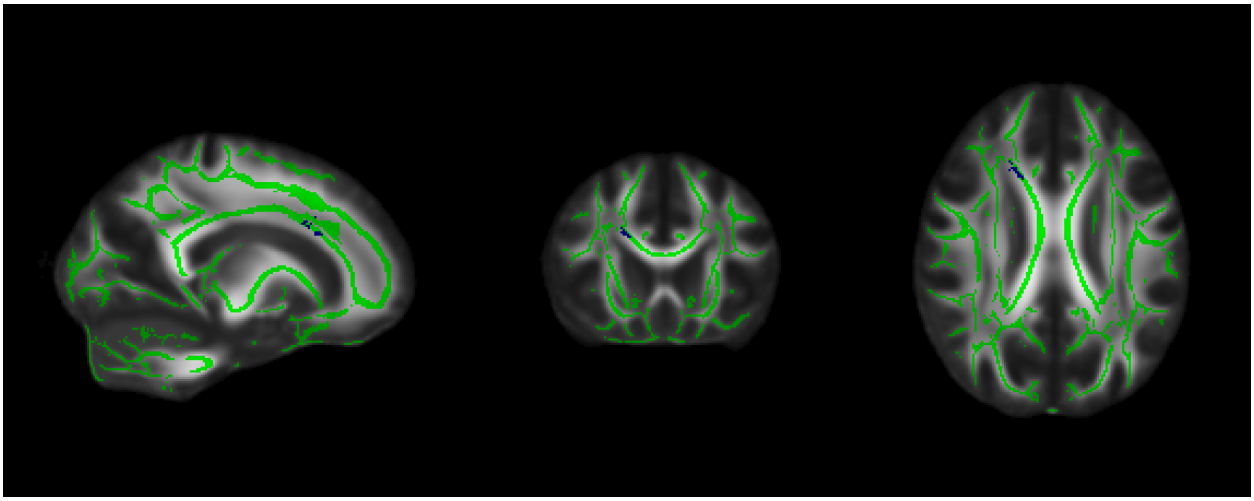

Figure 6. From left to right: sagittal, coronal, and axial slices of the standard FMRIB_FA_58 brain displaying results of TBSS analyses showing regions that with a statistically significant negative correlation between MD and executive function (blue) overlaid on the white matter skeleton (green) in older adults (MNI coordinates: 71, 141, 104 right superior corona radiata; 72, 145, 99 right anterior corona radiata; 75, 142, 99

body of the corpus callosum) ($p < 0.05$, corrected for multiple comparisons as well as age, sex, and years of education. Radiological view).

Table 5
Z-scores for the Trail Making Test and California Verbal Learning Test

ID #	Trails-A	Trails-B	CVLT-II LD Free-recall
YA_001	0.849	0.379	1
YA_002	0.849	-0.43	-2
YA_003	1.538	1.43	1
YA_004	-1.791	0.298	0
YA_005	0.046	0.136	1.5
YA_006	0.849	0.864	1
YA_007	0.39	-0.754	-0.5
YA_008	0.276	-0.43	-2
YA_009	0.735	-0.107	1
YA_010	0.505	-0.269	1.5
YA_011	0.161	0.298	0
YA_012	0.046	-0.592	-1
YA_013	1.424	1.673	0
YA_014	0.85	1.754	0.5
YA_015	1.243	-0.094	-1.5
YA_016	0.62	0.783	0
YA_017	-2.25	-3.181	-1.5
OA_001	2.218	2.801	0.5
OA_002	1.295	1.199	1.5
OA_003	0.078	-0.653	0.5
OA_004	-0.92	1.946	-1.5
OA_005	0.769	0.385	1
OA_006	-1.07	0.872	0.5
OA_007	1.919	1.624	1
OA_008	2.069	2.16	0
OA_009	0.626	0.888	0
OA_010	-0.079	1.307	-1.5
OA_011	-0.323	0.872	0
OA_012	0.354	0.219	0.5
OA_013	0.7	0.26	1
OA_014	2.069	3.665	0.5
OA_015	1.252	3.418	1
OA_016	0.63	0.468	0.5
OA_017	1.172	0.872	1.5

Note. The trail making test used both A and B versions (Trails-A & Trails-B) and the California Verbal Learning Test 2nd Edition used the Long Delay Free-recall portion (CVLT-II LD Free-recall) for each participant.

Discussion

This current study aimed to understand differences in white matter microstructure as a function of age, and how the integrity of white matter relates to cognitive performance on measures of memory and executive function.

It was hypothesized that older adults would show decreased white matter integrity in diffuse regions including the temporal and frontal lobes, compared to younger adults. As expected, analyses revealed that older adults had significantly lower FA and higher MD than younger adults, in widespread regions, including the temporal and frontal lobes. Specifically, the following tracts showed significantly lower integrity in older adults: the left cerebral peduncle and posterior limb of the internal capsule, bilateral anterior corona radiata, anterior limbs of the internal capsule, cingulum, crura of the fornix, external capsules, inferior fronto-occipital fasciculi, inferior longitudinal fasciculi, optic radiations, posterior corona radiata, posterior thalamic radiations, retrolenticular parts of the internal capsule, sagittal strata, stria terminalis, superior corona radiata, superior longitudinal fasciculi, and the body of the fornix, as well as the splenium, genu, and body of the corpus callosum. These findings are highly consistent with those of Boekel and Hsieh (2018) that compared older and younger adults to examine associations between white matter microstructure and age as well as trait mindfulness. They also found that the negative associations between FA and age were found throughout the brain, but mainly in the temporal and frontal lobes (Boekel & Hsieh, 2018). Longitudinal studies, such as the one conducted by Moscufo et al. (2018) have also found a negative correlation between age and FA and Radial Diffusivity (RD), as well as a positive correlation between age and Axial Diffusivity (AD) over a 4-year interval (AD and RD, together, comprise MD).

Neurogenesis throughout much of the brain ceases fairly early in the developmental process, and we can also expect to see evidence of neurodegeneration, or neuronal cell death, as people age. From early fetal life, both grey matter and white matter increase in volume at a rapid rate as neurogenesis occurs at a greater rate than neurodegeneration. These increases taper off between late childhood and young adulthood, after which they begin to decline in volume as neurodegeneration begins to outpace neurogenesis (Bethlehem et al., 2022). As also seen throughout the aging process, over time a certain level of cognitive decline is to be expected. As both these structural and cognitive changes have been linked to the aging process, it is important to understand the relationship between age-related structural changes and cognitive performance.

Expecting a relationship between structural and cognitive changes in the brain, it was hypothesized that performance on measures of executive function and memory would be associated with white matter microstructure in the frontal and temporal lobes, respectively for both younger and older adults. Although executive function refers to a broad array of cognitive abilities, the current study used a measure of alternating attention, the trail making test, as a proxy.

The trail making test tests both processing speed and executive function using different sets (Trails-A & Trails-B). In Trails-A, participants are to follow one set of rules, such as draw a line in ascending number order to see how fast they can complete the task, as a measure of processing speed only. In Trails-B, however, the participants must keep track of two rules, such as draw a line switching from ascending number order to ascending letter order to see how fast they can complete the task. This measures both

their processing speed, as well as their ability to keep track of multiple sets of rules and their ability to switch between these sets. The findings of the current study revealed that poorer performance on the Trails-B task was associated with decreased white matter integrity in the older adult group, specifically in the right superior and anterior corona radiata as well as the body of the corpus callosum. Previous literature has established an important role for the frontal lobe in executive function (e.g. Cristofori et al., 2019; Fiske & Holmboe, 2019; Müller & Kerns, 2015; Stuss ,2011; Stuss & Benson, 1987). The right lateralization of the findings may be due to the Trails-B task being a visuospatial task which are generally lateralized to the right hemisphere (Springer & Deutsch, 1989). In order to investigate the relative contribution of divided attention versus motor processing speed to these findings, the relationship between DTI metrics and Trails-A scores were also examined, with no significant findings. The correlation seen with Trails-B, while using the Trails-A results in order to disentangle divided attention (EF) from processing speed, is consistent with the view that white matter integrity is particularly crucial for efficient executive function in aging or, more specifically, that white matter integrity is directly proportional to executive function and that decreases in white matter microstructure are associated with decreases in executive function (Kerchner et al., 2012; Ryan et al., 2011; Sorg et al., 2015).

It is possible that the correlation found in the older adult group may be due to protective effects found in younger adults that keep their MD from increasing. This effect would keep MD artificially low, even as executive function scores decreased, leading to the lack of an association between MD and executive function. If such an effect would wane through the aging process, this would result in older adults showing more

substantial increases in MD, while their executive functioning scores decreased, leading to an association between these variables that could then be detected. This could be examined in future studies using a longitudinal design. Bethlehem et al. (2022) charted neurodevelopmental milestones and found that white matter volumes increase from natal development until young adulthood, after which the white matter volume begins to decline, and ventricular volume increases at a steep rate. This may indicate the presence of the aforementioned protective effect and could explain why the association was only found in older adults.

As an index of memory, we used the long-delay free recall portion of the California Verbal Learning Test 2nd Edition (CVLT-II), which requires participants to memorize a list of words and recall that list after a period of time has passed. The long-delay free recall portion was used to best encapsulate short-term memory out of the sections gathered by the CVLT-II. In analyses that were corrected for age, sex and education level, there was no significant relationship between memory and DTI metrics in either younger or older adults.

Contrary to the findings in this study, Li et al. (2022) found a positive association between working memory and FA. That study, however, used a much larger sample size with many more age groupings. It is possible that these differences in design could have led to different results (Li et al., 2022). It is also possible that memory scores could be due to changes in grey matter, rather than white matter tracts, as hippocampal volume has been associated with memory scores (Van Petten, 2004).

Limitations

Although the current study took a thorough approach to examining both brain structure and cognitive performance in two age groups, there are several limitations to this work that can be addressed in future research. First, the current study used a cross-sectional approach and offers a comparative analysis between different groups of people rather than a longitudinal aging study, which limits the applicability of these results to the actual aging process. Future approaches will use one group of participants and compare their DTI metrics over time.

Additionally, the participant groups were pooled from a sample with high education, which may indicate some protective effects on white matter integrity. Sampling from a more diverse population, in terms of education and socioeconomic status will lead to the results being more generalizable to the public at large.

Due to the nature of the study being one of recruitment by word of mouth and referrals, the resulting sample size was fairly small. Having a larger sample would increase the statistical power to detect the effects of age on DTI metrics.

Conclusions

Aging is a process of which our understanding is incomplete. Various changes, both structural and functional are known to occur but, the extent of changes expected is still elusive. In this study, we found correlations between performance on the Trails-B test and the MRI correlates of neurodegeneration. These associations found in the frontal regions remained significant even after accounting for sex and level of education, in agreeance with previous literature that microstructural changes in these tracts impact cognition and executive function. In contrast, memory was not associated with white

matter integrity in any of the white matter tracts within the brain. Through this study and other aging studies of its kind, the hope is to garner a greater understanding of the aging process. Based on this understanding, new approaches to care and diagnoses of age-related diseases can be determined. Going forward, longitudinal studies that have a larger, more robust sample size from a more diverse participant pool in terms of education and socioeconomic status would help control for the limitations found in this study.

Chapter 3

This master's thesis represents an important examination of the process of healthy aging and its resultant effects on white matter microstructure and cognition. The results of this study revealed decreased FA and increased MD in older adults relative to younger adults, which implies significant neurodegeneration due to the aging process, even outside of the context of a diseased state. This lowered microstructural integrity was observed in diffuse regions in both hemispheres of the brain. A significant negative association between MD and alternating attention was shown in the right superior and anterior corona radiata and the body of the corpus callosum for older adults. No association was found between MD and executive function for younger adults or between FA or MD and memory for either older or younger adults. Future studies into these associations are necessary as this current study found significant results, despite several limitations, which are discussed below.

Limitations

As previously mentioned, this current study has limitations and shortcomings that, although they do not discredit the legitimacy or validity of the findings, should be acknowledged, and addressed for the sake of transparency. First, the current study used a cross-sectional approach and offers a comparative analysis between different groups of people. Cross-sectional aging studies focus on between-group differences at a single point in time and are subject to confounding variables that are present between the participants of the study. Longitudinal aging studies, on the other hand, compare the participants at baseline to themselves at a later time. This measure of within-group

change controls for the confounds of cross-sectional studies as much of the variability that would arise from comparing two different groups of people is cancelled out. Any changes exhibited throughout a longitudinal study can largely be attributed to changes that occur due to time, if no interventions (or large environmental changes) are applied to the participants. Thus, longitudinal aging studies have the potential to examine the changes that result from the aging within each participant. Longitudinal studies can also examine trajectories of individual change over time. As such, the results of this current cross-sectional study are less applicable to the actual aging process and actually offers a comparative analysis of the white matter microstructure between the brains of older adults and younger adults. It should be noted, however, that when performing longitudinal studies, a considerable amount of time, specifically the amount of time the study is interested in examining, must be allotted to the study itself, as the participants would be undergoing those changes in real time. Over time, as technology advances and testing parameters change, it can be difficult to maintain consistency in image acquisition for longitudinal aging studies. A great benefit to cross-sectional studies such as these is that, depending on the age range of the study sample, a long period of aging can be mimicked. Although the results would not be directly attributable to the aging process, any significance such as the results found in this study would signal that further research would be necessary to determine if those changes are present during actual aging. In order to help account for confounding variables in cross-sectional analyses, the effects of age, sex, and level of education can be covaried out, as was the case with this study.

Second, the participant groups were pooled from a sample with high education, which may indicate some protective effects on cognition. High levels of education have

been shown to provide a protective effect against decreases in memory and other cognitive domains such as emotional intelligence, that are attributed to the aging process (Cabello et al., 2014; National Institute on Aging, 2020). Since the level of education for participants in this current study was high, with the average years of education acquired for both groups being equivalent to completing a bachelor's degree, the results of this study may not be applicable to the population at large and may not be representative of the actual changes in cognition that occur due to healthy aging. Level of education was controlled for in the individual analyses run for this current study but without a comparative sample of lower education individuals, an overarching protective effect on cognition cannot be ruled out.

Third, due to the nature of this study being one of recruitment by word of mouth and referrals, through the University of Victoria, the recruitment methods increased the likelihood of obtaining participants of university level education, as mentioned earlier, since the participant pool was mainly people affiliated with the University directly or people with some connection to someone affiliated with the University. Also, given that the study involved collecting MRI data, the participants were limited to Victoria and participants were not recruited from rural areas. As compensation was not provided for this study, participants are also those who could afford to take the time to participate and would, therefore, be of a higher socioeconomic status than people who may not have been able to take time off from work to participate. On the other hand, studies that obtain participants through organizations or databases are able to collect data from many participants from a wider area which results in larger samples.

Finally, due in part to the high cost of MRI data collection, the resulting sample size was fairly small. A relatively small sample size means that the results of this study suffer from a lack of statistical power to detect any effects should they actually exist. It should also be noted that additional recruitment attempts were not possible due to the onset of the COVID-19 pandemic. Despite being a cross-sectional study with the potential for a larger sample size than a longitudinal study in the same vein, all of these intervening factors culminated in the decreased size that was used. Although small in number overall, this study contains a sample in line with those of other imaging studies and, as such, does not constitute as a severe limitation but rather highlights the need for future studies that can corroborate or refute the results and findings of this current study.

Regardless of the aforementioned limitations, this current study still represents a significant contribution to the existing literature by exploring the associations between changes in white matter microstructure and cognition and has paved the way for future studies that will further our understanding of healthy aging and its underlying neurobiological and neuropsychological mechanisms.

Can DTI Detect Links Between Microstructure and Cognition in Healthy Aging?

This current study found no association between FA or MD and memory for either older or younger adults. Additionally, through the analyses performed for this study, an association was only found for MD and executive function for older adults. Within the previous literature, most of the studies examining executive function in association with DTI looked at clinical populations such as Alzheimer's Disease, MCI, and SCD, or looked at a sample of older participants rather than an association in healthy aging spanning younger adulthood to older adulthood (Halliday et al., 2019; Mayo et al.,

2019; Ohlhauser et al., 2019; Shim et al., 2017). Previous studies that examined the microstructural changes in white matter over the lifespan show that even though there may be a linear trend for increased MD or decreased white matter volume, the actual values may maintain at steady levels or even improve before making a steep change later in life (Beck et al., 2021; Bethlehem et al., 2022). As such, it is possible that the older adult group happens to be within the age range where participants are experiencing a steep decline in white matter volume and a steep increase in MD. This would explain the presence of a significant association with the DTI metric and cognition for only this older adult group. The younger adult group would have more steady values for MD and white matter volume, which would result in a weaker association between the DTI metrics and cognition and may result in the lack of a detected association as seen in this current study. Looking forward, it will be important to investigate relationships between DTI metrics and cognitive performance in larger groups that would have enough power to detect such relationships.

Future Directions

Further studies hoping to expand upon the findings of this study and explore the aging process in a more direct manner should undergo steps to perform a longitudinal analysis. Although more time intensive and sensitive to participant drop-out, the benefits of such an approach would be the ability to measure changes in each participant over time. This would show what is happening to people as a result of the aging process. In keeping with this approach, compiling, or making use of a database of participants will also help with the lack of diversity of the participant pool. By reaching out to various areas over time, in a multi-site study for instance, a participant pool of varying education

and socioeconomic status could be obtained and would help to properly control for possible protective effects on cognition that these factors may provide. As such, this will lead to the results of follow-up studies being more generalizable to the public at large. Taking the time to comprise a larger and more robust sample would increase the statistical power within the study to detect the effects of the aging process on white matter microstructure and cognition. This analysis, having more validity, would then be able to draw more accurate conclusions as to what associations found between cognition and microstructural changes may mean for aging at large.

Conclusion

The complex mechanisms underlying the aging process are important to understand; this remains true even under conditions of healthy aging. The current study explored the potential associations between the microstructural differences found between older adults and younger adults with the cognitive domains of memory and executive function in order to mimic the effects of healthy aging on the brain. Findings showed significantly lower FA and higher MD in older adults compared to younger adults widespread throughout the brain, which is consistent with the current literature on microstructural changes due to aging. This study also found a significant negative association between MD and executive function in the right frontal lobe of older adults. This finding is an indication of the importance of further exploring changes in cognition, namely executive function, in healthy aging as well as potential protective effects that may have prevented such an association or its inverse from being present in either participant group. If a thorough understanding of the aging process is to be obtained and thus a more generalizable trajectory of white matter microstructural changes in healthy

aging is to be determined, as per the goal of this line of research, more studies using DTI to track these changes should be performed.

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