The impact of genetic factors and cognitive reserve on brain network organizations of healthy elderly adults

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Declaration of Originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in this thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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Statement of Ethical Conduct

The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator, and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University. Ethics Approval No H0016317

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Abstract

Resting-state functional magnetic resonance imaging (fMRI) has the potential to identify abnormalities in brain networks. Neuroimaging techniques have exhibited that structural and functional connectivity are impaired in the course of Alzheimer's disease. However, this condition happens on the background of aging, and it is less clear what may be the important functional changes that occur during aging that are associated with aging-related risk of conditions such as Alzheimer's disease. Therefore, it might be possible that genes play an important role in functional brain connectivity. Studies of older healthy individuals may provide a better understanding of how brain connectivity may be affected. In particular, healthy older individuals carrying specific genotypes related to dementia risk or activity-dependent plasticity may be variably associated with aging-related alterations or disruption to connectivity. This work explored the common genetic polymorphisms of *APOE* and *BDNF* Val66Met, and the lifecourse factor, cognitive reserve, in relation to age-related changes in functional and structural connectivity brain networks. Investigations in blood oxygenation level dependent (BOLD) signals found in resting-state data, maps of brain activity can be created to represent and assess structural and functional connections between different brain regions. Resting-state networks such as the default mode network (DMN), dorsal attention network (DAN), and Salience Network (SA), may provide possible explanations for differences between healthy aging and neurodegenerative diseases.

Seventy-eight healthy older adults (average age 63.3) from the Tasmanian Healthy Brain Project, a longitudinal study investigating whether education later in life increases cognitive reserve and/or provides resilience to cognitive decline, were invited to participate in this project. FMRIB Software Library (FSL) was used to pre-process and clean the datasets. Functional datasets were analysed using whole-brain and seed-based analyses via FSL applying Multivariate Exploratory Linear Optimised Decomposition of Independent

Components (MELODIC) and dual regression. Structural images were analysed using Statistical Parametric Mapping (SPM12) and Matlab to examine the covariance patterns of grey and white matter volume. Additionally, Freesurfer software was used to investigate region-specific alterations in grey matter volume and cortical thickness.

The first aim was to investigate functional edge strength using graph theoretical measures within the DMN, DAN, and SA. Findings showed no significant differences in functional edge strength related to polymorphisms in *APOE* and *BDNF* within all three networks. Cognitive reserve was not significantly associated with edge strength.

The second aim was to examine functional organization related to variations of the *APOE* and *BDNF* Val66Met polymorphisms in the DMN, DAN, and SA. Genetic interactions and the effects of cognitive reserve were also assessed. *APOE* ε3 homozygotes showed stronger functional connectivity than *APOE* ε4 carriers. No significant differences were found in functional connectivity in DMN, DAN, and SA between *BDNF* Met carriers and *BDNF* Val homozygotes. We observed a significant interaction, measuring stronger functional connectivity in Val/ε3 homozygotes than in Met/ε4 between the DMN and grey matter primary auditory cortex, as well as stronger connectivity in Met/ε3 carriers than in Met/ε4 carriers and Val/ε4 carriers respectively, between the dorsal attention networks and default mode regions, as well as between the dorsal ventral stream and visual cortex. This analysis also revealed positive functional correlations with cognitive reserve within the DAN, and negative functional correlations with cognitive reserve within the DMN.

Previous colleagues examining the THBP cohort found that increasing cognitive activities, such as later life education at university, enhanced cognitive reserve (Lenehan et al., 2016). Furthermore, this improvement within the THBP was then examined in relation to different cognitive domains, such as episodic memory, working memory, executive function, and language processing (Thow et al., 2018). Results showed that later life education enhanced

language processing within the THBP cohort. Therefore, the third aim was to examine functional organization of the language network (Broca's and Wernicke's areas) using seed-based analyses within variations of the *APOE* and *BDNF* Val66Met polymorphisms and genetic interactions. The key findings were that Met/ε3 showed stronger functional connectivity than Met/ε4 and Val/ε3, respectively, between the left Broca's area and right parahippocampal gyrus and left parahippocampal gyrus. Further, it was found that Val/ε3 had stronger functional connectivity between left Broca's area and clusters of the poscentral gyrus and precuneus. Another interesting finding was that age correlated positively with functional connectivity within left Broca's area, while cognitive reserve correlated negatively with functional connectivity between left Broca's area and the left hippocampal regions. One of the most important findings to emerge from this aim was a positive correlation between age and functional connectivity within the language network, suggesting that healthy older adults are recruiting frontal lobe regions to support their language processing through neural compensation and maintaining the language function.

The objective of the fourth aim was to examine differences between *APOE*, *BDNF*, and cognitive reserve relative to covariance patterns in grey and white matter. Additionally, specific brain structures, such as grey matter volume of the hippocampus and amygdala, and cortical thickness of the entorhinal cortex and parahippocampal cortex, were investigated in relation to *APOE* and *BDNF* genotypes. Further, correlations between cognitive function and whole brain structure metrics were examined. Covariance within whole brain grey matter structures were not significantly different with respect to polymorphisms. Grey matter of the hippocampal and amygdala volume, as well as cortical thickness of the parahippcampus and entorhinal cortex, showed no significant differences relative to both, *APOE* and *BDNF* genotypes. Age was significantly associated with hippocampal and amygdala volume, indicating a decrease of volume with increasing age. Episodic memory and language function

did not show significant associations with grey matter volume and cortical thickness. Working memory and executive function were associated with volume and thickness of some brain regions.

Overall, the results of this thesis demonstrated that the *BDNF* Val66Met polymorphism did not markedly affect the brain organization of healthy older adults. *APOE* status, however, may have an effect on functional connectivity and may decrease brain connectivity when the individual is carrying an ε4 allele. The findings of this project suggest that genetic interactions and the aging process influence functional connectivity in the absences of clinical signs of disease. Structurally, the brain is losing volume with increasing age, which predicted cognitive function status in healthy older participants.

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Chapter 1

Introduction

The influence of genetic factors and cognitive reserve on structural and functional resting-state brain networks in aging and Alzheimer's disease

Recently, it was estimated that more than 47 million elderly people are affected by dementia globally (Alzheimer's Disease International, 2009; Prince, Comas-Herrera, Knapp, Guerchet, & Karagiannidou, 2016) and that an additional 131 million people will develop this health-challenging syndrome by 2050 (Prince et al., 2016). Alzheimer's disease (AD), a progressive condition causing behavioural changes, memory loss, and decline in learning capacity (Anand, Gill, & Mahdi, 2014), is the most common cause of dementia worldwide (Hardy, 1997). Most cases of AD occur in individuals over the age of 75, but, relatively younger individuals, including those carrying certain genetic mutations (Loy, Schofield, Turner, & Kwok, 2014), may develop the disease before 65 years of age (Alzheimer's Association, 2015).

Knowledge of the brain changes that occur in AD has increased remarkably from the late 20th century due to extensive research on a range of related neurodegenerative processes. Particular progress has been made with regard to what has been termed the pathological 'hallmarks' of AD – the presence of amyloid plaques and neurofibrillary tangles (NFTs) – which detrimentally affect axons, dendrites, and synapses (Vickers et al., 2000; Vickers et al., 2016). Plaques are the result of accumulations of an abnormal form of the beta amyloid (Aβ) protein in the brain. NFTs are formed by the aggregation of aberrant tau protein (Savva et al., 2009; Vickers et al., 2000) and are more directly related to the death of neurons (Jacobs, Van Boxtel, Jolles, Verhey, & Uylings, 2012). Within the cerebral cortex, the earliest plaques are usually found in the neocortex, whilst initial formation of tangles occurs in medial temporal lobe (MTL) structures, such as the entorhinal cortex and hippocampus (Price & Morris,

1999). The MTL is an important region responsible for memory formation and long-term memory (Squire & Zola-Morgan, 1991). Throughout the cerebral cortex, neurons that provide long corticocortical connections are the most prone to NFT-induced deterioration (Morrison & Hof, 1997), which may then underlie the pattern of synaptic loss seen in AD. Entorhinal-hippocampal circuits are compromised early in AD, followed by the gradual disconnection of the MTL, and then the loss of connectivity between association neocortices (Morrison & Hof, 1997). This pattern of progressive and degenerative pathology may underlie the deterioration of certain cognitive functions during aging, leading eventually to frank AD. The early pathological accumulation of Aβ has been linked to cognitive impairment and could also affect functional connectivity between spatially distant brain regions (Delbeuck, Van der Linden, & Collette, 2003). A summary table of studies examining functional connectivity and Aβ in healthy aging and AD can be found in Table 1.1. Neuroimaging is a vital component of research into AD internationally (Hendrix et al., 2015) and has been used to investigate mechanisms of interrupted structural and functional connectivity underlying the course of AD (Dennis & Thompson, 2014). A better understanding of how the pathological changes in AD affect the organization of brain networks, or how these networks may respond or adapt to accumulating pathology, might offer further insights into the potential scope of functional resilience. The term resilience is described as the capability of a tissue to be resistant to damage (Cosco, Howse, & Brayne, 2017) and is particularly relevant to AD as we know that the amount of brain pathology does not correlate well with cognitive function. In this respect, factors such as education and lifestyle could increase resilience by heightened connectional redundancy and/or preserving functional connections in the brain, and may ultimately delay the clinical expression of AD pathology. Indeed, studies investigating the association of education and cognitive decline in AD have found that more highly educated individuals are able to tolerate more neuropathology before the clinical expression of AD (Bennett et al.,

2003), potentially because education moderates the relationship between brain pathological load and cognitive impairments (Brayne et al., 2010; Valenzuela et al., 2011), as well as functional connections (Marques et al., 2016).

Studies have shown that functional connectivity is damaged or interrupted in AD (Stam et al., 2008; Stam, Jones, Nolte, Breakspear, & Scheltens, 2006), and, conversely, investigating the impact of AD on structural and functional networks may also provide more accurate information regarding brain connectivity and how brain regions communicate with each other (Sheline & Raichle, 2013). This review of the literature focuses on the methods with which brain connectivity is analysed, the changes in structural and functional networks found in AD, and the role of cognitive reserve and specific genetic factors in partially determining functional brain connectivity. In this regard, potential changes in functional connectivity and resistance to pathology involve both non-modifiable and modifiable factors that will impact on how brain systems respond to accumulating pathological burden. Hence, I discuss features of structural and functional brain networks in relation to genetic biomarkers and environmental factors linked to AD risk, progression and resilience.

Table 1.1. Studies examining functional connectivity and amyloid-beta in healthy aging and Alzheimer's disease

Study	Samples	Imaging measures	Main findings
Fischer et al. (2015)	CN preclinical AD (*n*=12), Age-matched controls (*n*=31)	DTI using tractography, measuring fludeoxyglucose-PET	CN preclinical AD (with Aβ positivity) exhibited similar white matter network changes to clinical AD as compared to controls; for instance, CN preclinical AD had more shorter paths and reduced global efficiency compared to controls.
Grandjean et al. (2014)	Transgenic mice (*n*=38) Wild-type mice (*n*=36)	Structural MRI, Rs-fMRI DTI	The progression of functional connectivity was disrupted in somatosensory and motor cortex in ArcAβ transgenic mice compared to wild-type mice. This decrease was noticeable even before amyloidosis in transgenic mice.
Mormino et al. (2011)	CN older (*n*=44), AD (*n*=22)	Structural MRI, Rs-fMRI, PIB-PET imaging	Increased Aβ in CN older individuals was associated with decreased default mode network functional connectivity in multiple posteromedial regions suggesting that the accumulation of Aβ and related brain changes occurs before overt cognitive impairment.
Sheline, Raichle et al. (2010)	35 AD, 68 CN older PIB- (*n*=24) PIB+ (*n*=20)	Structural MRI Rs-fMRI, & Dynamic PET scan	CN people with Aβ deposition exhibited impairments in functional connectivity, particularly default mode network disruptions.
Bero et al. (2012)	Young APP/PS1 transgenic mice (*n*=7) Old APP/PS1 transgenic mice (*n*=7) Young wild type mice (*n*=13) Old wild type mice (*n*=10)	Functional connectivity optical intrinsic signal imaging	Aβ accumulation was related to decreased functional connectivity in older APP/PS1 mice compared to young APP/PS1 mice and wild-type mice. Brain regions that had more Aβ showed the most conspicuous age-related decreases in connectivity.

Hedden et al. (2009)	38 CN older adults, PIB- (*n*=17), PIB+ (*n*=21)	Structural MRI, FMRI, Dynamic PET	Functional connectivity was disrupted in CN older adults with Aβ positivity. Connectivity impairments related to Aβ deposition were evident between the hippocampus and posterior cingulate (default mode network regions) and associated with memory deficit.
Drzezga et al. (2011)	CN PiB - (*n*=12) CN PiB+ (*n*=12) MCI PiB+ (*n*=13)	Structural MRI, Rs-fMRI, fluorodeoxyglucose-PET, PiB-PET	MCI with Aβ burden exhibited hypometabolism, decrease of neuronal activity and disruption of functional connectivity in posterior brain regions (precuneus/posterior cingulate) compared to CN older adults.
Lim et al. (2013)	165 CN PiB- (*n*=116) PiB+ (*n*=49) BDNF Met carriers (*n*=58) BDNF Val/Val (*n*=107) APOE ε4 (*n*=70)	Structural MRI, PET PiB imaging, Neuropsychological assessments at baseline, 18 & 36 months	BDNF Met carriers with Aβ burden positivity demonstrated an accelerated decline in memory function as well as a reduction of hippocampal volume compared to BDNF Val homozygotes.
Franzmeier et al. (2017b)	CN Aβ+ (*n*=24) amnestic MCI Aβ (*n*=44)	Structural MRI, Rs-fMRI, FDG-PET	Individuals with amnestic MCI with Aβ positivity and more years of education demonstrated attenuation of precuneus hypometabolism and relatively increased global frontal cortex functional connectivity.

AD Alzheimer's disease, *Aβ* Amyloid-beta, *APP*/*PS1* Amyloid precursor protein presenilin, *APOE* Apolipoprotein E, *BDNF* Brain-derived neurotrophic factor, *CN* Cognitively normal, *DTI* Diffusion tensor imaging, *FDG* Fluodeoxyglucose, *MCI* Mild cognitive impairment, *MRI* Magnetic resonance imaging, *PET* Positron-Emissions-Tomography, *PiB* Pittsburgh Compound B, *Rs-fMRI* Resting-state functional magnetic resonance imaging.

Methods to analyse connectivity

Neuroimaging techniques (Figure 1.2), such as magnetic resonance imaging (MRI), have long been used to investigate anatomical connections, detect pathological alterations, and monitor the progression of neurodegenerative diseases, including AD (Figure 1.2.A). MRI involves the generation of a strong static magnetic field to create images and to map fluctuating signals related to brain activity (Heeger & Ress, 2002). MRI also allows the quantification of brain atrophy, which can be used to distinguish normal brain aging from AD (Frankó, Joly, & Alzheimer's Disease Neuroimaging Initiative, 2013). For example, a recent study found that MRI and cognitive testing in cognitively healthy individuals are useful tools for predicting the development of AD, particularly when investigating the progress from healthy cognition to the appearance of mild cognitive impairment (MCIs) after 5 years (Albert et al., 2018). Mild cognitive impairment refers to reduced cognitive function, which appears to be greater than anticipated for a cognitively normal individual. The delayed presence of clinical symptoms makes it challenging to diagnose individuals in preclinical stages and therefore animal models are often used to provide an opportunity to identify biomarkers of early disease (Sabbagh, Kinney, & Cummings, 2013), which include insights from neuroimaging, such as grey and white matter alterations measured by diffusion tensor imaging (DTI) (Weston, Simpson, Ryan, Ourselin, & Fox, 2015).

Magnetic resonance imaging (MRI) is a non-invasive technique that examines brain anatomy of different types of brain tissues, such as grey matter, white matter, cerebro-spinal fluid, which are of high resolution. Each MRI has three essential components: the magnet generating a static and homogeneous magnetic field (B0), a gradient coil, which varies the B field in three dimensions creating smaller magnetic fields in directions x,y, and z for spin

localization, and lastly the radiofrequency coils, which send the radiofrequency into the tissue to identify the signal echoes from the brain tissue (Collins & Wang, 2011).

MRI uses an extremely powerful magnetic field to produce a magnetization in the nuclei of atoms within the body. The most prevalent element consisting of one single proton is hydrogen. Every water molecule in the body carries two hydrogens, therefore, 70 % of the human body is made up of water (Steen, 1988). Structural images are obtained through the hydrogen nucleus. These hydrogen protons spin on its axis and have a magnetic north and south pole similar to a magnet, which rotates within the body (Berger, 2002). These spins have irregular orientations, however, when an external stimulus (radio frequency wave) is applied, the protons spin in accordance with the magnetic field. Thus, placing the human body into an MRI scanner lines up all the protons in the body with that field creating a magnetic vector. A radiofrequency pulse disturbs the alignment of the nuclei activating the protons to spin out of equilibrium. The MRI scanners can identify this kind of released energy of the proton (Berger, 2002). There are two approaches to obtain the time it takes for the proton to completely relax. T1 relaxation is referred to longitudinal relaxation, in which the magnetic vector returns back to equilibrium. While T2 relaxation is the dephasing of the axial spin (spin-spin relaxation) returning to its state of rest (Berger, 2002). Mechanisms have to be applied to firstly, differentiate various contrasts of brain tissues from one another, such as grey matter from white matter tissue, and secondly to estimate this contrast for each individual volume, which is defined as voxel. One voxel can comprise millions of brain cells. These contrasts of brain tissue are produced through spin density, water diffusion, or relaxation times (Logothetis, 2008). Subsequently, the raw data images called DICOM files must be converted into a format that neuroimaging packages can work with, such as a NifTi file. Fundamentally, the registration and segmentation of MR images is applied by the

neuroimaging software packages, for example FSL (https://fsl.fmri.ox.ac.uk/fsl/fslwiki) or SPM (SPM12, Welcome Department of Cognitive Neurology).

Diffusion tensor imaging is an MRI-based neuroimaging method that measures the diffusion of water molecules, enabling the assessment of the fibre-tract structures of white matter (Jones, Knösche, & Turner, 2013; Teipel et al., 2016). This technique allows the strengths and differences of white matter tract connections in specific population groups to be compared (Jones et al., 2013) before a reduction of cognition is evident (López-Gil et al., 2014), for example between older individuals with and without AD (Figure 1.2.C). Other structural imaging parameters that are currently used to gain further insight into the integrity of the brain over the life include intracranial volume and the presence and number of white matter hyper-intensities (Bartrés-Faz & Arenaza-Urquijo, 2011).

Functional MRI (fMRI) is another technique of neuroimaging that uses magnetic resonance imaging to produce 3-dimensional images of brain activity reflected by neural activity. Functional images typically have lower spatial resolution than structural images, but higher temporal resolution. The spatial resolution is limited by signal to noise ratio, implying that as the voxel size gets smaller, the signal to noise ratio decreases. One limiting factor of acquiring higher resolution images is that the acquisition requires time to obtain the higher resolution data, in which the signal decays. Thus, even if more data is collected for an extended period of time, the signal disappears. Temporal resolution on the other hand can be increased by collecting several slices at the same time allowing to gain twice as many slices at the same time (Kim et al., 1997). Temporal resolution means that the lowest frequency waves that can be measured are <0.1 Hz and this is important because slow neuronal activity fluctuations can be differentiated (Pfurtscheller et al., 2018). Further, because the scan is

voxel-by-voxel through the whole brain it takes 2 seconds for each volume (Lindquist, 2008), so it is the signal for each voxel at a different time to a signal in the next volume that is measured. As aforementioned, MRI comprises of multiple magnetic fields, in which protons of the nuclei of hydrogen atoms react to the magnetic fields and produce a detectable electromagnetic signal (Poldrack et al., 2017). Functional MRI (fMRI) measures the alteration in blood oxygenation patterns and blood flow across time and permits simultaneous monitoring of the activity of different brain regions while a subject is at rest or performing a task (Binder et al., 1999). In fMRI, oxygen in blood is measured through blood-oxygen-level-dependent (BOLD) signals (Heeger & Ress, 2002; Ogawa, Lee, Kay, & Tank, 1990). These oxygenation levels in the blood flow can be detected by MRI scanners. In particular, BOLD emerges from alterations in blood concentrations of oxyhemoglobin and deoxyhemoglobin. Oxyhemoglobin is the hemoglobin molecule that is loaded with oxygen (diamagnetic), while deoxyhemoglobin refers to hemoglobin that releases oxygen (paramagnetic) (Pauling & Coryell, 1936). The brain is showing activity when regional blood flow is enhanced expanding the capillaries with oxygenated blood to meet the metabolic demands. Therefore, functional MRI signal increases when the concentration of deoxyhemoglobin diminishes. Specifically, when a region of the brain is processing information, blood flow of that particular area is increased, which is also defined as the demand of active neurons in the brain and correlated to neural activity.

Although, the amount of firing nerve cells is linked to BOLD signal, it is the extracellular local field potential (electrical field potential) that is measured (Ekstrom, 2010; Logothetis, Pauls, Augath, Trinath, & Oeltermann, 2001). Local field potentials are referred to as altered voltages that are recorded from a vast substantial group of brain cells compromising the entire activity of the neural network. Alterations in the MR signal are caused via neuronal activity, also known as hemodynamic response function (Friston et al., 2000). The

hemodynamic response function depicts how neural activity is reflected by the BOLD signal changes. Approximately 4 to 6 seconds after commencement of neuronal activity, BOLD signal peaks, followed by a post-undershoot and return to baseline (Uludag et al., 2004). It is assumed that the peak is related to neuronal activity and increase in local blood flow, and the post-undershoot is induced by continuous neuronal metabolism, which depletes the voxel's initial oxygen supply (Figure 1.1) (Stocco, 2014).

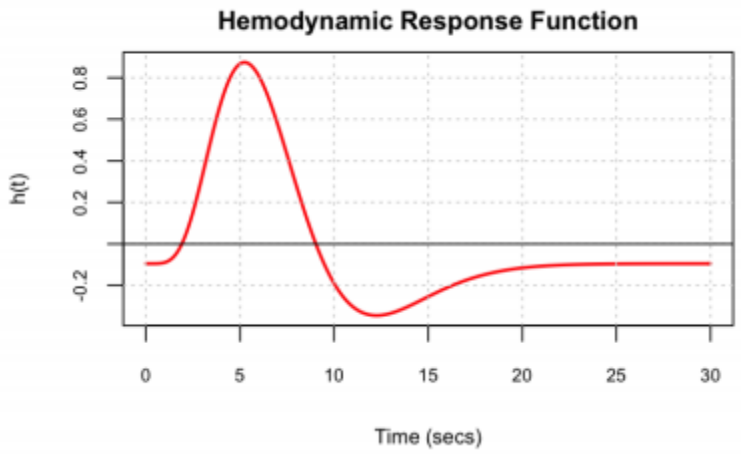


Figure 1.1 Reprint of Stocco (2014) demonstrating the shape of the Hemodynamic Response function.

When oxygen is removed from the bloodstream, hemoglobin develops into a paramagnetic phase causing a rapid decline in signal enhancing deoxyhemoglobin and decreasing the BOLD signal (Ekstrom, 2010). However, an increase of blood flow compensation reduces the strength of the concentration of deoxyhemoglobin and shifts the equilibrium to oxyhemoglobin resulting in a peak in BOLD signal. Thus, the local field potential represents the postsynaptic activity or postsynaptic potentials among the number of neurons which makes it possible to measure changes in oxygen concentration, cerebral blood flow (CBF) and volume (CBV) that are delayed by 1–2 s after MRI excitation. This excitations is produced by a strong radio-frequency pulse generating a magnetization, which can then be detected by the receiver coils of the MRI (hemodynamic response) (Buxton, Uludağ,

Dubowitz, & Liu, 2004). Elevated brain activity is improved by greater field strength. However, alterations in neural activity are not always characterised as BOLD signal. Studies have shown that metabolic or vascular substructures may work independently and create electrical brain activity similar to BOLD signal in the absence of actual neuronal spiking (Sirotin & Das, 2009). Nonetheless, there is strong evidence demonstrating that the local field potential depicts BOLD signal (Logothetis et al., 2001). BOLD signal is often investigated in studies using resting-state fMRI, in which there is a shared assumption: Two brain regions are assumed to be functionally connected if their BOLD time-series are correlated displaying similar patterns of activity (For example firing at the same frequencies) (Smith et al., 2013). As a result, functional connectivity is defined as a statistical association among the time series measurements of different brain regions. Thus, functional connectivity refers to calculations of correlations between the timeseries of a seed-voxels and the timeseries of every other voxel in the brain (Raichle, 1998). Although, various brain regions may exhibit cortical synchronization, demonstrated by local field potential or action potentials, they may not always necessarily be connected functionally, but could have been triggered by the same stimuli (Logothetis, Kayser, & Oeltermann, 2007).

Functional connections, defined as temporal correlations between spatially distant cortical brain regions, are revealed through spontaneous fluctuations in low-frequency (<0.1 Hz) portions of BOLD signals (Ogawa et al., 1990). Taken together, with BOLD signal, hemodynamic changes in blood flow are measured, which over time have the tendency to develop slowly. Studies have shown that with age, functional connectivity networks gradually decrease (Dennis & Thompson, 2014), which may be important for understanding early AD or the series of brain changes that make the older brain more or less susceptible to additional disease processes.

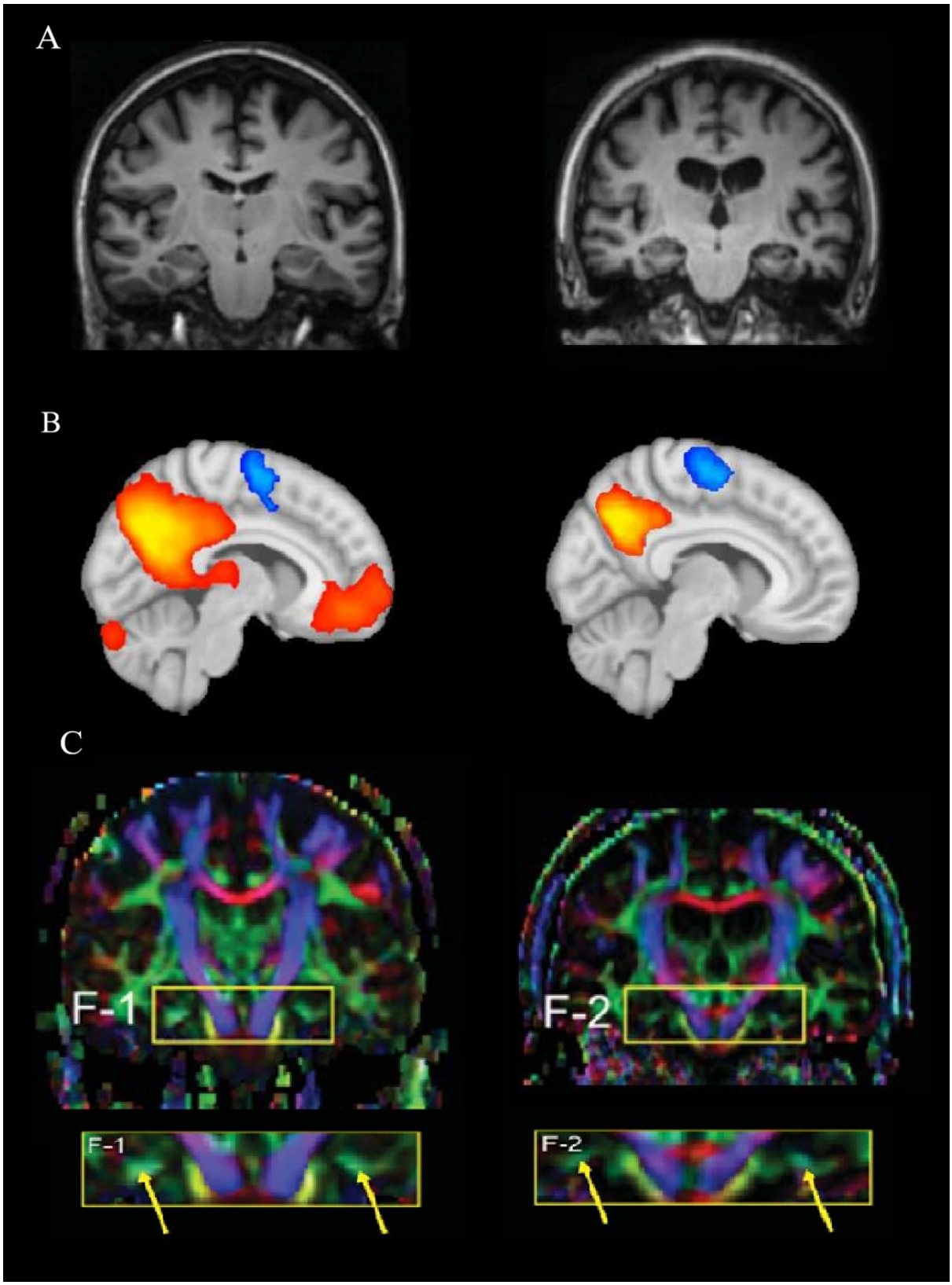


Figure 1.2. Differences among the imaging techniques, MRI, fMRI, and DTI. (A) A structural MRI comparison between a healthy human brain (left) compared to pathological changes in Alzheimer's disease (AD, right; (Oishi, Mielke, Albert, Lyketsos, & Mori, 2011).

(B) A functional MRI representing brain activation of a resting-state network in a healthy brain (left) compared to a hypothetical AD brain activation (right). The representation of the connectivity map shows how brain activity decreases with pathology within the default mode network (DMN); red/orange represents higher connectivity, while blue represents inversely correlated activity. (C) A comparison between a cognitively healthy woman (72 years old, left) and a woman with AD (70 years old; Oishi et al., 2011). The yellow arrows show differences in hues, which are used to represent differences in signal strength of the cingulum hippocampal area after DTI analysis. For example, the signal strength is greater in healthy (A) compared to AD (C).Reprinted from Oishi et al. (2011) with permission from IOS Press. The publication is available at IOS Press through https://content.iospress.com/articles/journal-of-alzheimers-disease/jad0007.

Resting-state fMRI is an increasingly frequent method employed to study differences between various cohorts and involves the investigation of the activity of the brain while the individual is at rest and not performing a task. Resting-state fMRI can be used to determine how different brain regions operate and process information in functional space. Additional advantages are that resting-state fMRI is less demanding on the individual and easier to apply than task-related fMRI (Sheline & Raichle, 2013). The individual is instructed to not fall asleep while keeping their eyes closed in a lying position. A great feature of investigating resting-state fMRI is that the data is replicable, for instance, when examining several diverse populations and performing the same analysis, in this case independent components analysis (ICA), the same brain networks can be found, demonstrating how robust and reproducible this approach is (Damoiseaux et al., 2006). Major resting-state networks that have been found in different populations after using ICA include the default mode network (DMN), dorsal-attention network (DAN), visual network, sensory motor network, executive control network, auditory network, and the salience network (SN), which also have been depicted in Chapter 4, Figure 4.1.

One very well-known resting state network is the DMN(Buckner, Andrews-Hanna, & Schacter, 2008). It is a mysterious but attractive network because it has been linked with

many abstract brain functions that distinguishes the humankind from other primates or animals. One theory is that the DMN is associated with reasoning and introspection including other people's mental states and self-centered processing (Buckner et al., 2008). This includes the ability to recall events from the past and predict the future. Taken together, the DMN is associated with any part of our thinking or perceptions, and daydreaming based on memories or recollections of our past experiences. Scientifically, it is interesting because particular areas of the brain show activity when we are not focusing on any tasks. These unconscious activations reflect how the brain consolidates experiences, as well as prepares us how to react to the environment. Brain regions comprising the DMN are the medial prefrontal cortex, medial parietal cortex and the medial temporal lobe (Buckner et al., 2008). Research has shown that the DMN is one of the most connected networks in the brain, and therefore is a crucial mediator for other interacting networks. In the field of neurodegenerative diseases, for example AD, the DMN exhibits abnormal functioning (Greicius, Srivastava, Reiss, & Menon, 2004).

The SN is another large-scale network has been proposed to be responsible for two important functions. One function is that it combines signals from our body, for example behavioral emotions with our thinking and reasoning (Menon & Uddin, 2010). The other function is that it labels situations in our extrinsic environment as deserving of our attention. As a whole, the SN mediates the automatic shift in attention, which is known as stimulus-driven attention (Menon & Uddin, 2010). The SN includes the anterior insula and the dorsal anterior cingulate cortex. There is evidence that the SN has functional connectivity impairments not only in AD and amnestic MCI, but are also developing in healthy aging (He et al., 2014).

Complementary to the SN is the DAN, actively directing our attention to the extrinsic environment (Corbetta & Shulman, 2002). It is also known as the task-positive network and is in charge of goal-directed behavior and voluntarily attention distribution. Brain regions such as the parietal, occipital and some frontal areas belong to the DAN making it possible to shift our attention and to facilitate the processing of information. The DAN appears to be in direct competition with the DMN (Seeley et al., 2007). Whenever one of the networks is active the counterpart is deactivated, mediating the rivalry between internal and external attention. Resting-state fMRI studies have demonstrated that this attention network is disrupted in AD, suggesting that the DAN could be used as a potential biomarker differentiating AD from healthy (Li et al., 2012).

There are a variety of approaches for analyzing resting-state fMRI. For instance, seed-based analysis (Beckmann, DeLuca, Devlin, & Smith, 2005a) investigates the BOLD signals between the selected region of interest (seed region) and the rest of the brain (Biswal, Zerrin Yetkin, Haughton, & Hyde, 1995). In AD, the precuneus has showed decreased functional connectivity to other brain regions, such as the left hippocampus, left parahippocampus, anterior cingulate cortex and gyrus rectus, as compared to non-dementia controls (Sheline & Raichle, 2013).

Independent component analysis (ICA) is an exploratory technique for identifying hidden patterns in fMRI signals. It is a tool detecting and separating signals with no preceding information of the experiment or hemodynamic response function. For example, ICA can differentiate BOLD signals of important brain regions, which may have been linearly mixed together, from other signals that are related to noise (Griffanti et al., 2017). In more details, ICA is summarizing data by decomposing 4D images (space x time) into a collection of 3-D

spatial maps, also called components. Each component reflects one independent signal linked to a time course (Griffanti et al., 2017). Some of these components represent BOLD signal, while others may represent noise (motion, physiological effects) or other artefactual processes, which are extracted in the cleaning process (Salimi-Khorshidi et al., 2014, Griffanti et al., 2017). As a result, ICA is defined as a model-free approach that works by examining the distributions of a mixtures of signals. These distributions have each a distinctive shape, however, they all share the same feature of being non-gaussian (non normal distributions) and are independent, which means that the components are not correlated (i.e., one component is from a different neural process than another). is one significant but safe assumptions that is done by ICA (Hyvärinen & Oja, 1998). The ICA is performed with MELODIC (Multivariate Exploratory Linear Optimised Decomposition of Independence Components; Beckmann & Smith, 2004), which is a within-subject spatial ICA with automatic dimensionality estimation, explained in more details in Chapter 2. The group-level analysis is a group-ICA based on temporal concatenations, in which the time series of each subject is concatenated to one data file. The outcome are time courses and spatial map components representing resting-state networks. Aforementioned, these group-level components or networks have been investigated and are highly replicable across a variety of populations (Damoiseaux et al., 2006).

Taken together, ICA is a technique used to process functional neuronal connections across the brain by separating signals into concatenated components, turning data from a multitude of signal channels into fewer components without losing too much information and reconstructing the original signal, which is a wholly data-drive form of analysis (Beckmann et al., 2005b). A figure was created from own data for illustrative purposes (Figure 1.3). Using ICA-based analysis, Greicius et al. (2004) reported a decline of resting-state functional

connectivity between hippocampus and posterior cingulate cortex (PCC) in a group of people diagnosed with AD compared to healthy older individuals.

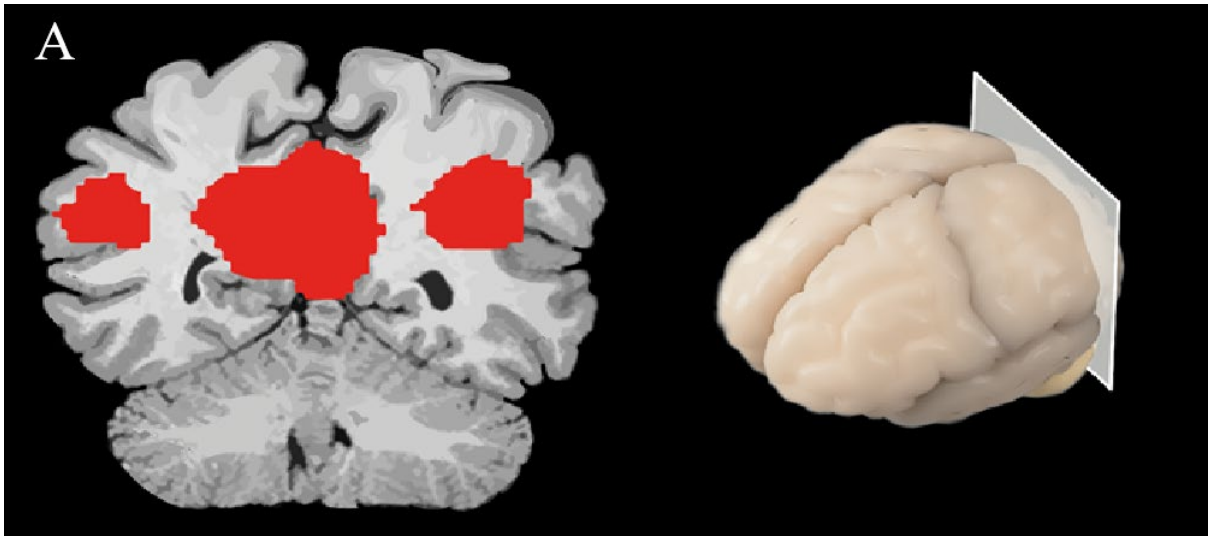


Figure 1.3. Illustration showing a representation of a spatial map of a brain slice demonstrating brain activity in the DMN; red represents regions that are most active while the individual is at rest. This figure was created in cartoon mode from one ICA component.

Another technique used to examine resting-state functional connectivity is graph analysis. Graphs are simple representations reducing extremely complex phenomena, for example the brain, into an architecture of dots and lines. The underlying concept of graph theory is to quantify, visualize and understand how the brain is organized in a simple model. This approach can reveal new information on the structure and function of brain hubs, as well as their constructions, development and clinical manifestations (Sporn 2018). Graph theory uses a mathematical and computational applications that investigates the associations between nodes in a network. This is a powerful way to quantitatively define the topological organization of brain connectivity by using higher-order statistics in the form of multivariate analyses (Mijalkov et al., 2017). The number of pairwise connections create these networks, which are encapsulated in an adjacency matrix. Matrices are the equivalent to graphs, in particular, graphs are reflected by the columns and rows of the matrix. Graphs can be binary,

in which the value of the edge is either zero or one, or they can be weighted, in which the value of the edges is continuous, ranging from strong (high) or weak (low) numbered values (Farahani, Karwowski & Lighthall, 2019). In addition, depending on their directionality, graphs can be directed or undirected (Figure 1.4).Functional connectivity is represented as a series of 'nodes' (voxels) and 'edges' (correlated activity between nodes) (Stam, Jones, Nolte, Breakspear, & Scheltens, 2007; Watts & Strogatz, 1998). In fMRI studies, statistical dependencies are calculated across neuronal time series to create a matrix. However, in the end, the measurement methods decides whether a single node (single neuron) or a complete brain region is investigated. Taken together, with graph theory disorganization can be transformed into neat networks.

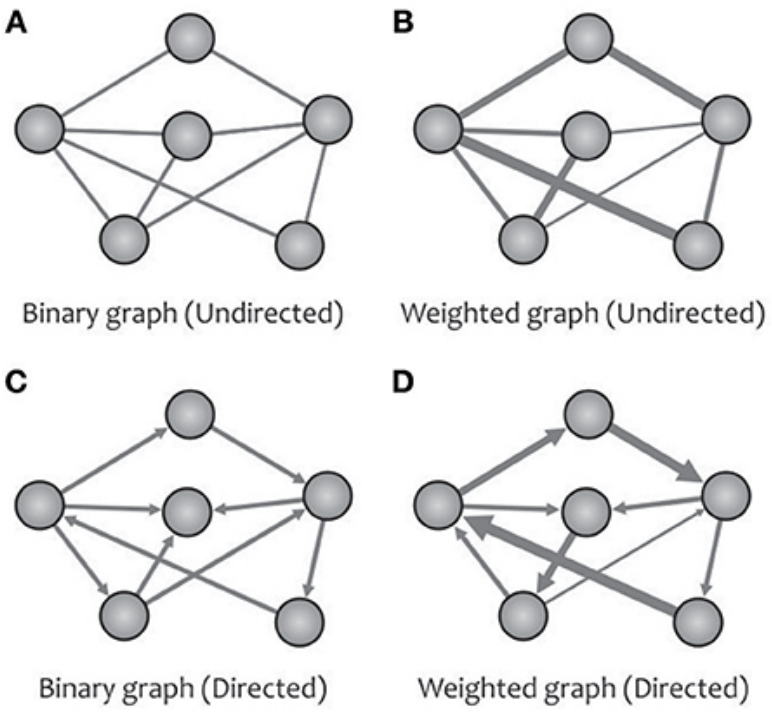


Figure 1.4. Illustration of different graph variants. Reprinted from Farahanir et al. (2019). (https://www.frontiersin.org/articles/10.3389/fnins.2019.00585/full).

Graph theory comprises of several different network measures. For example, nodal degree is a measure that examines the connections of a node and their relationships to other nodes. As a

result, the number of connections is equal to the number of neighbor nodes (Rubinov & Sporns, 2010). Because a node can connect to multiple nodes within the network, as well as its ability to disperse information to various brain regions faster, the node with the most connections or edges, also known as a hub, is identified as more important in a network. Modularity on the other hand measures the degree to which the network can be subdivided into clearly defined groups (Rubinov & Sporns, 2010). A schematic figure is used to demonstrate both measures (Figure 1.5) (Sporns, 2013).

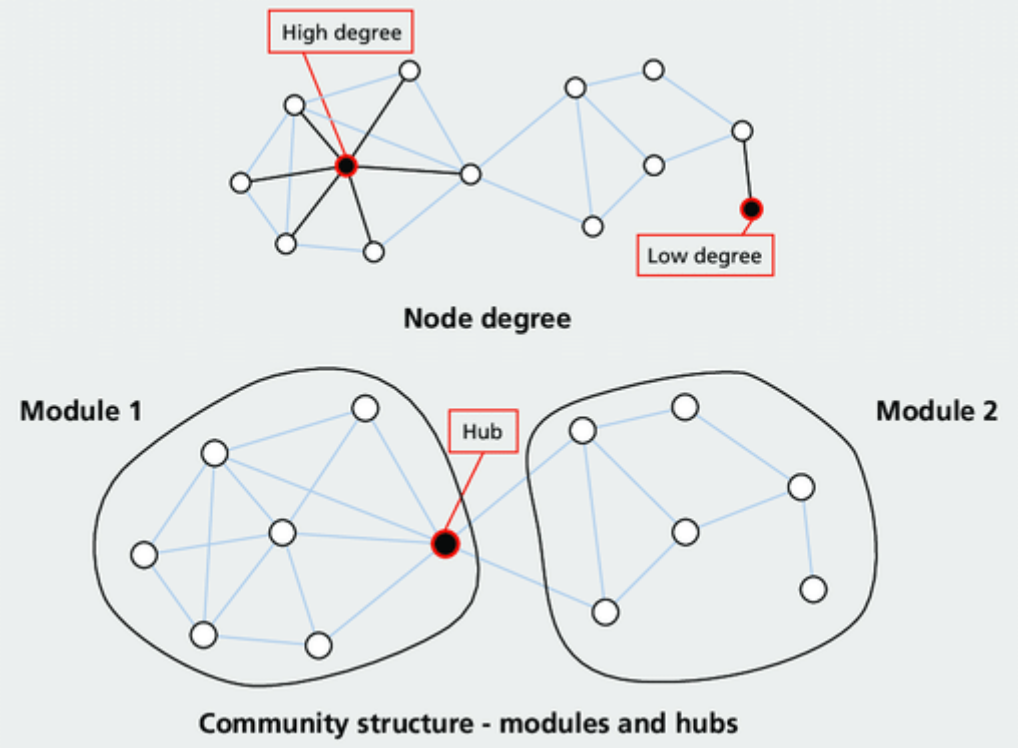


Figure 1.5. Illustration of graph theoretical measurement, nodal degree and modularity. Figure reprinted from Sporn (2013). (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3811098/)

Another valuable measurement is the clustering coefficient, which is described as a measurement of nodes that are locally interconnected (Kaminski & Blinowska, 2018). Clustering coefficient represents the proportion of a node's neighbor's possible interconnections that are connected neighbors of each other, capturing the probability that two nodes that are connected to each other are also part of a larger connected node forming

clusters in the neighboring regions (measuring of how many nodes have a tendency to create clusters, thus a measure of segregation, Figure 1.6, reprinted from Sporn, 2013). This approach is particularly useful when measuring and comparing differences in structural and functional connectivity (Bullmore & Sporns, 2009),

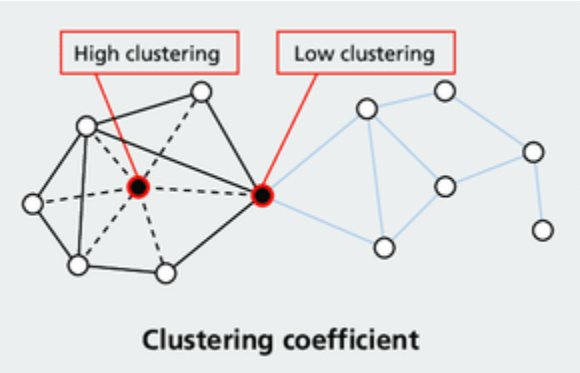


Figure 1.6. Illustration of clustering coefficient. Figure reprinted from Sporn (2013). (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3811098/)

The proximity of a node to another node within a network, especially the interactions in which information is connected from dispersed brain regions, is measured by centrality (Rubinov & Sporns, 2010). It is crucial to understand how information flows across the network, and to what degree the distribution of nodes may affect how information in the brain is transmitted and retrieved.

A network's efficiency is a measure of how well it shares information. In a network, the principle of efficiency can be implemented to both the local and global scales (Brier et al., 2014). The degree of nodes in a network is measured by density. It is determined by dividing the total number of edges in the network by the maximum number of edges, also known as the measure of the network's total "wiring cost" (Rubinov & Sporns, 2010).

Current research is investigating small world networks, which is characterised by a few long distance connections with a great number of shorter paths between pairs of nodes, generally developed by hubs (Figure 1.7) (Bullmore & Sporns 2009). It is noteworthy that the majority of real-world networks appears to have these small world properties. In human fMRI studies, it has been predicted that small-world networks with low-frequency oscillation might reveal connectivity of the brain structure. A specific focus of this form of analysis in network organization is the average minimum number of edges that must be traversed between any two nodes in a brain network, referred to as 'effective path length.' The characteristics of small-world networks are clustering coefficient, high integration and their typical feature is shorter effective path length (Kaminski & Blinowska, 2018; Rubinov & Sporns, 2010; Travers & Milgram, 1967).

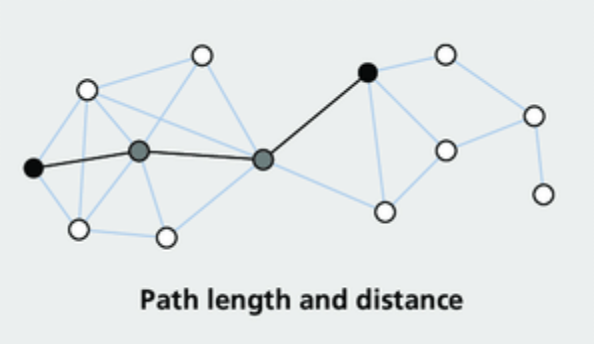


Figure 1.7. Illustration of how path length in a small-world network is measured. Figure reprinted from (Sporn 2013). (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3811098/)

The features of graph theory in brain networks have been found to correspond with differences in cognitive function, indicating that small-world networks are involved (Li et al., 2009). Studies have found correlations between intelligence scores and small-world properties, indicating that more intelligent individuals had shorter effective path lengths, as well as global efficiency (Li et al., 2009), indicating that transmission of information in the brain was more efficient in individuals with greater intelligence scores. Even in healthy aging the network architecture is progressing and it is possible that cognitive decline with age is

affecting these networks (Zhu et al., 2012). For example, reduced network efficiency was found (Gong et al., 2009), as well as longer path length was found in healthy older individuals compared to younger (Wang, Li, Metzak, He, & Woodward, 2010), suggesting that with aging the integration of information may be restricted on a global scale because of the shift to more local communication.

As previously discussed, graph theoretical measures are used to simplify complex networks. These simplified measurements can then be used to investigate differences between groups to an equivalent parameter of the population. It is these clear graphical measures that advance our understanding of the pathology of neurodegenerative diseases. For example, individuals affected by neurodegenerative diseases may demonstrate disrupted graph theoretical networks by possessing less connections or missing major hubs. The immensely complex, yet highly structured network topologies are modified by disease progression and influence brain disorders. These measures not only advance our understanding of potential pathological processes, but also give us permission to develop a predictive model. Therefore, I created a figure (Figure 1.8) to illustrate how the brain networks may be affected within my cohort. This figure was adapted from Stam et al. (2007). Here, path length is affected, in particular the path length in "AD" is significantly longer compared to "Healthy" (A) because people with AD may lose nodes and edges defined by activity patterns earlier than cognitively. This can also be applied in genotypes, such as the *BDNF* Val66Met, in my case, path length in the Val group (B) shows more paths than in the Met carrier group. Similarly, the Val/ non-ε4 group has more paths than the Met/ ε4 group (C), again indicating that the Met/ε4 group will have an overall longer path length.

Neuroscientific research involving graph theory is a effective technique that assists in mapping, tracking and predicting brain networks that are affected by disease. An advantage is that it allows for more precise explanations of how different progression is influencing the brain (Fornito et al., 2015). By representing very high dimensional data in a lower-dimensional network space and comparing differences in network topology, many fewer statistical comparisons are made, reducing the number of false discoveries.

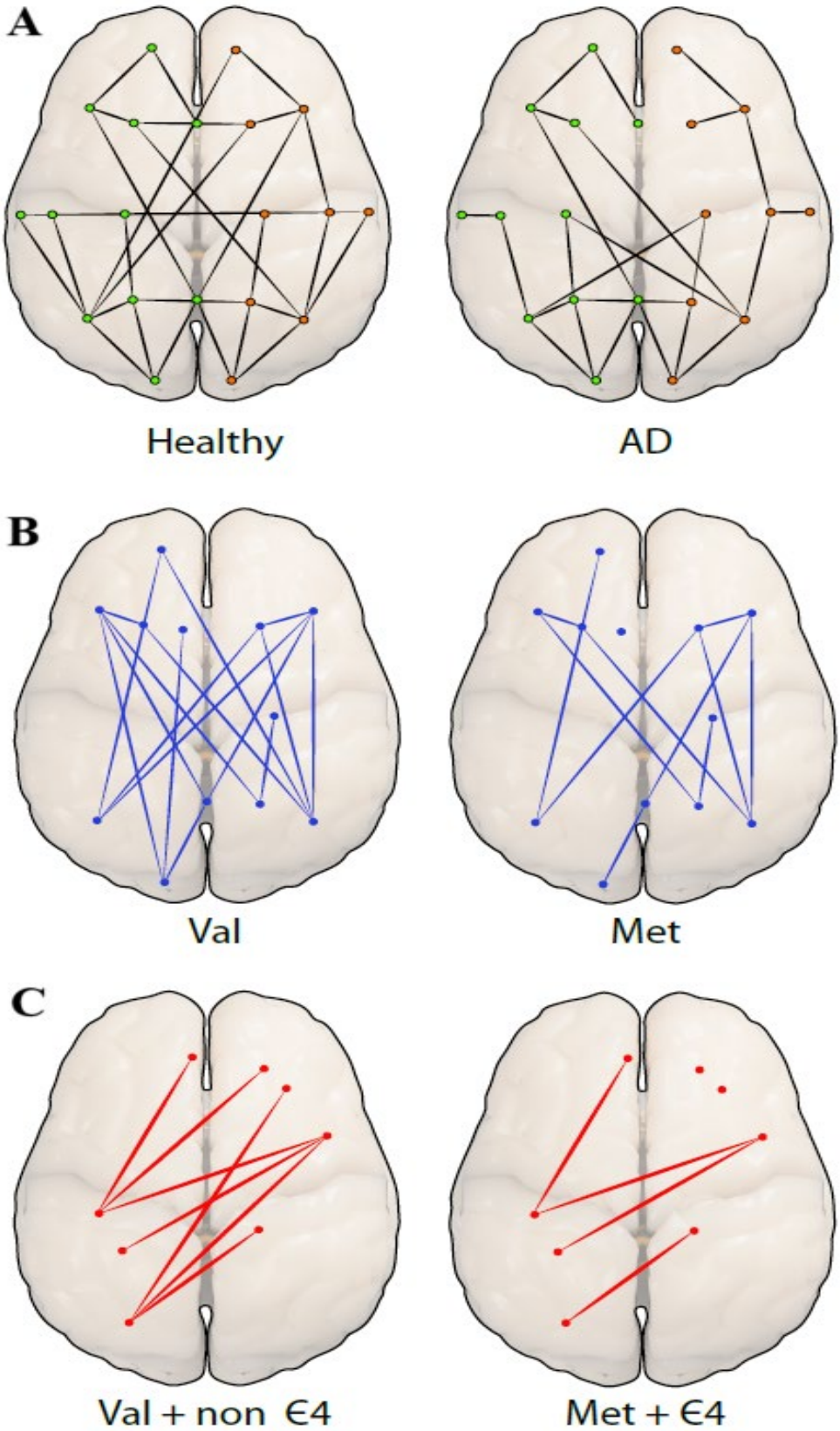


Figure 1.8. Illustration showing differences among network organizations using a graph theoretical approach adapted from Stam et al. 2007. (A) A graph of a healthy person (left) is compared to a person with Alzheimer's disease (AD; right), showing fewer connections (edges) between the spatially distant brain regions (nodes or dots) in AD. The green (left) and

orange (right) dots represent hemispheres. The next two figures are also hypothetical figures of the BDNF Val66Met polymorphism (B), in which the connections are noticeably decreased in Met carriers. The last figure represents the carriage of both (C), BDNF and APOE displaying a distinct reduction of edges and nodes in individuals. Part B and C were created without the intention of displaying different intrahemispheric connections.

Changes in structural and functional connectivity in AD

Structural connectivity

In AD, the loss of connections between neurons can result in other structural alterations, such as atrophy, hypometabolism, and NFT accumulation (Zhang et al., 2009). Significant atrophy in AD, identified through MRI, occurs in the posterior hippocampus and the temporal and parietal cortices, which are three of the structures that are involved in the DMN (Greicius, Krasnow, Reiss, & Menon, 2003). The default mode is a network in the brain that is activated when individuals are not engaged in a task, but are spontaneously thinking of past or future events (Buckner et al., 2008). The DMN is a highly interconnected set of cortical regions that demonstrate substantial correlated activity, particularly when the attentional network is inactive (Buckner et al., 2008; Shulman et al., 1997).

Healthy aging is linked to cognitive impairments and local brain shrinkage. Grey matter volume is particularly decreased in the hippocampus, temporal lobe and prefrontal lobe regions, as well as the cingulate cortex (Good et al., 2001; Resnick, Pham, Kraut, Zonderman, & Davatzikos, 2003). A more recent investigation found that with age the whole brain covariance of grey matter volume declined in subcortical networks, such as the sensorimotor network, as well as in posterior and anterior cingulate cortices suggesting that healthy elderly may struggle with cognitive challenging tasks(Hafkemeijer et al., 2014). The presence of positively correlated inter-individual differences of regional brain regions is characterized as structural covariance (Alexander-Bloch, Giedd, & Bullmore, 2013). In

particular, anatomical brain regions that have strong correlation in brain size illustrate structural correlations. Studies have demonstrated that the application of structural covariance pattern analysis has the ability to detect the cause of neuropathology because these patterns are able to depict neuronal activity that underpins the assessment of cognitive function more accurately (Bullmore & Sporns, 2009; He, Chen, Gong, & Evans, 2009). For example, a study investigated covariance of grey and white matter patterns and found correlations between covariance patterns in both tissue types and age, suggesting that structural networks decrease in the aging process, which is associated with the impairments of cognitive performance (Brickman, Habeck, Zarahn, Flynn, & Stern, 2007).Collectively, alterations in grey matter volume are used as a biomarker to classify diseases such as AD (Guo et al., 2014).

Diffusion tensor imaging studies investigating white matter changes in individuals with AD have demonstrated that the disease causes a deterioration of white matter fibre bundles in the MTL (Zhang et al., 2007), which may be present years before overt episodic memory deficits (Sexton et al., 2010), impaired executive function (Reijmer et al., 2014), and other symptoms of cognitive impairment (Fischer, Wolf, Scheurich, Fellgiebel, & Alzheimer's Disease Neuroimaging Initiative, 2015; Zhang et al., 2007). Similarly, in an animal model, López-Gil et al. (2014) reported neuronal differences in structural networks of chronically hypertensive rats before the manifestation of disrupted executive functioning occurred, which may provide insights into early stages of dementia. Moreover, Grandjean et al. (2014) discovered reduced fractional anisotropy values in transgenic mice with cerebral Aβ. In cognitively healthy individuals with elevated Aβ in the brain, potentially the pathological correlate of early AD, structural changes appear similar to individuals with MCI in terms of the topology of structural network connectivity (Fischer et al., 2015). Interestingly, these individuals with

high brain Aβ load despite no overt cognitive symptomatology, demonstrated increased shortest path length in white matter networks in the absence of major neurodegenerative features such as atrophy or reduction of cortical glucose (Fischer et al., 2015).

Finally, the structural networks (or nodes) of individuals with AD who possessed fewer connections (or edges) were more susceptible to global disruption of white matter tracts than individuals with more connections (Daianu et al., 2015). In addition, a rat transgenic model bearing mutant human amyloid precursor protein (APP) and presenilin genes also demonstrated a reduction of local and global efficiency, as well as less clustering as compared to non-transgenic rats (Muñoz-Moreno, Tudela, López-Gil, & Soria, 2018). Moreover, Muñoz-Moreno et al. (2018) found alterations in the right medial PFC in these transgenic rats, while in human studies, the right medial frontal cortical areas in AD indicated a decline in nodal efficiency compared to healthy controls (Lo et al., 2010). In summary, changes in structural connectivity could be useful in predicting the degradation of white matter bundles, as well as the strength of functional connectivity networks (Greicius, Supekar, Menon, & Dougherty, 2009).

Functional connectivity

Performance within many domains of cognitive function decreases slowly with age, but, importantly, higher cognitive performance has been correlated with increased functional connectivity in older adults (Arenaza-Urquijo et al., 2013). In animal studies, transgenic AD rat models require longer cognitive training to achieve the same performance as non-transgenic rats (Muñoz-Moreno et al., 2018). In this study, the authors showed that although the structural network was changed, these alteration did not result in functional network differences suggesting associations between the capability to learn and the reorganization of

functional networks in the brain (Muñoz-Moreno et al., 2018). Nevertheless, a gradual decrease in functional connectivity among the hippocampus and medial prefrontal cortex (PFC) is expected with age (Damoiseaux, Viviano, Yuan, & Raz, 2016)

It has been proposed that disconnection of functional networks in the brain, such as those observed in AD, could serve as critical markers for the presence of early stages of neurodegenerative diseases, particularly with regard to the abnormal accumulation of Aβ in the brain (Stam et al., 2007; Zhang & Raichle, 2010). Resting-state studies have reported a decrease in functional connectivity in healthy older individuals with Aβ burden in the posteromedial regions, ventral medial PFC, right angular gyrus, and the left middle and superior frontal gyri (Mormino et al., 2011), as well as between the precuneus and left hippocampus, parahippocampus, anterior cingulate, gyrus rectus and dorsal cingulate (Sheline, Price, Yan, & Mintun, 2010; Sheline, Raichle, et al., 2010). Early accumulation of Aβ in older healthy individuals, particularly in the precuneus, has been suggested to result in impairment in hippocampal function (Sheline, Price, et al., 2010; Sheline, Raichle, et al., 2010). In contrast, Mormino et al. (2011) reported that DMN connectivity responds in a varied manner to the presence of higher Aβ deposition in older non-demented people with Aβ accumulation. Specifically, the authors found that there was increased connectivity in regions of the right dorsal PFC, left anterior medial PFC and left temporal cortices, as well as decreased DMN connectivity in several posteromedial regions, the ventral medial PFC, right angular gyrus, and the left frontal gyri (Mormino et al., 2011). Disruption within the DMN has also been found in healthy older individuals with high amyloid burden (Hedden et al., 2009). Interestingly, these healthy individuals (n = 38) exhibited the same amount of Aβ burden compared to half of the individuals with MCI (n = 46) and all individuals with AD (n = 35).

Such associations have also been investigated in animal models. Bero et al. (2012) demonstrated an aging-related reduction of bilateral functional connectivity in the retrosplenial cortex in wild-type mice, which could be a pre-existing biomarker for neural dysfunction due to its significant association with memory performance (Corcoran et al., 2011). Interestingly, in a transgenic AD mouse model involving cortical amyloidosis, it has been shown that an age-related decrease in functional connectivity in specific brain regions is more severe in the presence of higher Aβ deposition (Bero et al., 2012). Grandjean et al. (2014) also reported reduced functional connectivity in transgenic mice, however, this reduction appeared in the early months before the accumulation of Aβ in the somatosensory and motor cortex.

A study investigating whole-brain connectivity found abnormalities in cortical hubs of the temporo-parietal cortex and precuneus/PCC in healthy mild cognitive impaired subjects with Aβ burden (Drzezga et al., 2011). In general, greater atrophy has been related to less brain connectivity (Hoffstaedter et al., 2015), but not all studies have found support for this association. For example, a study by Gili et al. (2011), reported that functional connectivity decline was not related to the amount of grey matter atrophy in the PCC in individual with MCI.

Disconnection between functional networks could be an essential biomarker for AD. For instance, individuals with AD exhibit disruption of functional connectivity between the inferior lateral temporal cortex (ITC), precuneus, right thalamus and the PCC (Zhang et al., 2009), between the left hippocampus and PCC (Sorg et al., 2007), as well as between the right hippocampus and the right and left cuneus, precuneus, and right ITC (Wang et al.,

2006). This pattern of disconnection is likely associated with impairments in memory, processing speed and executive function (Damoiseaux et al., 2016). Another proposed early biomarker for AD could lie in the disruptions that have been identified within the visual cortices, specifically the impairments in connectivity between the PCC and the dorsal and ventral visual pathways (Zhang et al., 2009). These changes have been suggested to lead to deteriorating visual function in AD (Zhang et al., 2009).

Small-world network analysis in AD has shown longer path length in the central, temporal, and frontal brain regions as compared to age-matched, non-demented individuals (Stam et al., 2007). Decreased local connectedness within networks, also called clustering, has also been reported in individuals with AD, and correlated with lower cognitive performance (Stam et al., 2007). This finding led Stam et al. (2007) to speculate that individuals in the early stages of AD may show relatively diminished topology of small-world networks. A recent study found support for this notion by demonstrating that individuals with MCI and AD had a longer characteristic path length compared to healthy controls (Mijalkov et al., 2017). Moreover, AD appeared to be associated with a greater number of edges connecting to a node regionally, as well as increases and decreases in the efficiency of local nodes when compared to the controls (Mijalkov et al., 2017).

To understand how pathology could potentially alter network topology, it may be important to account for genetic variations that might affect the organization of the brain. There is strong evidence that alterations in the functional architecture of the aging brain may be affected by genetic influences (Fornito et al., 2011). Previous studies have also shown that architecture of intrinsic functional networks are heritable (Sinclair et al., 2015). Therefore, this study investigated genetic polymorphisms that are also linked to neurodegeneration in

AD and aging-related condition. One AD-related gene is Apolipoprotein E (APOE) allele 4, which is associated with higher risk of developing AD (Morris et al., 2010), decreased (Goveas et al., 2013) and increased functional connectivity impairments (Zheng et al., 2018). While another gene is brain derived neurotrophic factor (BDNF), which has been observed to have a relationship with brain function through aging within the THBP population (Ward et al.,2015a).

Figure 1.8 illustrates a model of what brain network organization. Part A is an example where the healthy brain exhibits more nodes and edges defined by their activity patterns than the AD brain. Part B is an example, in which Met carriers may have less edges and nodes, while Part C demonstrates an example between ε4/Met carriers and ε3 and Val homozygotes. These illustrations are examples, there was no intention of omitting intrahemispheric connections.

Role of genetic factors related to AD in functional connectivity

Apolipoprotein E (APOE)

The inheritance of gene-related factors such as apolipoprotein E (*APOE*), in particular the *APOE* ε4 allele, is associated with an increased risk of AD (Mahley, Weisgraber, & Huang, 2006). This genetic polymorphism is associated with increased Aβ deposition in the brain (Mahley, Weisgraber, & Huang, 2009; Morris et al., 2010; Sheline, Raichle, et al., 2010), possibly influencing brain functional connectivity (Mahley et al., 2009), as well as affecting cognitive functioning in older age (Wisdom, Callahan, & Hawkins, 2011).

Resting-state fMRI studies have reported diverging associations of *APOE* polymorphisms and functional connectivity in healthy individuals that may relate to the age of the sample groups (Goveas et al., 2013; Wu et al., 2016). For example, *APOE* ε4 alleles have been associated with both increased and decreased DMN functional connectivity in cognitively

healthy individuals (Fleisher et al., 2009). Comparing non-demented middle-aged (50–65 years) individuals carrying the *APOE*ε4 with non-carriers, ε4 carriers showed elevated functional connectivity in the middle frontal gyrus, whilst non-ε4 carriers had greater functional connectivity in the right medial frontal gyrus (Wu et al., 2016).

Conversely, Goveas et al. (2013), demonstrated decreased functional connectivity within the DMN in cognitively healthy *APOE* ε4 carriers (44–65 years of age) in the bilateral dorsomedial PFC, superior frontal gyri, and in the left hippocampus, as well as increased functional connectivity in the left lentiform nucleus and bilateral caudate. Additionally, a decrease in interhemispheric functional connectivity within the DMN was found in healthy elderly *APOE*ε4 carriers (65–80 years of age) (Lu et al., 2017). Notably, most of these regions are also affected in AD, which emphasises the significance of the involvement of the DMN in the preclinical phase of AD (Sheline, Morris, et al., 2010). More recently, Zheng et al. (2018) investigated functional connectivity in young adults who were APPs/presenilin-1/2 mutation carriers or *APOE*ε4 positive carriers relative to adults without these AD-linked genetic factors (18–35 years). Interestingly, greater functional connectivity was observed in both the *APOE*ε4 carriers and in the APP/presenilin-1/2 group as compared to healthy controls. This increased connectivity was found between the left hippocampus and the bilateral medial PFC/precuneus. Only *APOE*ε4 carriers displayed increased connectivity between the right hippocampus and the left middle temporal gyrus. Here, the authors have suggested that the 'beneficial' effect of *APOE* ε4 in functional connectivity in younger individuals may be due to mechanisms of compensation of cognitive disruption, which may be detrimental as the individual ages.

Due to inconsistencies in published evidence, it is important to consider how *APOE* polymorphisms may be associated with other measures of functional connectivity. Studies that have investigated *APOE* effects in small-world networks have reported higher susceptibility of fewer functional hubs and reduced centrality in healthy older ε4 carriers compared to non-ε4 carriers (Seo et al., 2013). Regional cerebral glucose metabolism, clustering of whole-brain functional networks, and path length have all been reported to be decreased in ε4 carriers (Seo et al., 2013). However, in a study with a greater sample size of 147 cognitively normal individuals, more clustering and longer path lengths were identified in ε4 carriers when compared to non-carriers (Goryawala et al., 2015). Non-demented ε4-carriers also had more long-distance connections in the parietal and temporal lobes, whilst non-ε4 carriers exhibited more short-distance connections in the parietal and occipital lobe. Healthy older individuals with the ε4 allele also had less short-distance connections in the frontal lobe connections, while both groups showed more long-distance connections in the frontal lobe (Goryawala et al., 2015). In summary, this study found the brain networks of those carrying *APOE*ε4 to be organised into an abnormal structure when compared to non-carriers, with fewer connections in the frontal lobe and more structural long length connections, which could partially explain the negative *APOE* ε4 cognitive phenotype.

Brain-derived neurotrophic factor (BDNF)

Another genetic factor related to AD is the *BDNF* gene (Brown et al., 2014). The BDNF protein belongs to the family of nerve growth factors, which affect neurogenesis (Erickson et al., 2010) as well as long-term potentiation (LTP) and activity-dependent synaptic plasticity (Egan et al., 2003). Post-mortem studies of AD have shown that BDNF protein levels are decreased in the hippocampus, entorhinal cortex, temporal, frontal, and parietal cortex when compared to cognitively intact age-matched controls (Connor et al., 1997; Garzon, Yu, &

Fahnestock, 2002). Lower BDNF levels may be related to volume loss in the hippocampus (Erickson et al., 2010), but this may be secondary to other pathological changes that occur in AD (Buchman et al., 2016). BDNF concentration is highly variable between individuals and is relative to physiological state; for example, after physical exercise, peripheral blood BDNF concentration is increased (Dinoff et al., 2016). A recent review supported this finding by reporting increased neurogenesis and plasticity in the hippocampus in rats and mice after treadmill exercise, which led to improved short- and long-term memory functions (Jahangiri, Gholamnezhad, & Hosseini, 2018).

A common single nucleotide polymorphism in the *BDNF* gene, specifically a valine-to-methionine substitution at codon 66 (Val66Met), has an influence on LTP as well as activity-dependent BDNF secretion (Egan et al., 2003). *BDNF* Val66Met has been associated with cognitive performance as well as with AD brain morphology. In particular, the *BDNF* Met gene carriers (aged 60 and older), which were in preclinical stages of AD, demonstrated reduced memory function and smaller hippocampal and temporal lobe volume as compared to Val homozygotes (Brown et al., 2014; Lim et al., 2013). Authors also observed that more physical exercise was related to larger hippocampal and temporal lobe volumes in Val homozygotes but not in Met carriers (Brown et al., 2014). Notably, in Met carriers, physical activity was linked to reduced volumes of the temporal lobe, which is likely due to more apoptotic alterations (Brown et al., 2014). Likewise, Egan et al. (2003) demonstrated that the *BDNF* Met allele is related to qualitative changes of the hippocampus, which might cause insufficient memory functioning. Studies have proposed that there might be a relationship between Aβ and *BDNF* Val66Met, in which the *BDNF* polymorphism might mediate the effects on Aβ neurotoxicity on the brain (Fahnestock, 2011). Lim et al. (2013) reported not only a faster rate of atrophy in hippocampal volume, but also a faster decline in episodic

memory performance in *BDNF* Met carriers who had a high Aβ load over a 36-month period compared to healthy individuals with *BDNF* Met but low levels of Aβ. Relative to Val homozygotes with a low Aβ load, Val homozygotes with a high Aβ load also experienced reduced cognitive performance, indicating that being a Val homozygote would not necessary protect against cognitive decline (Lim et al., 2013).

In older adults with late-onset depression, *BDNF* Met carriage was associated with reduced resting-state functional connectivity between the bilateral hippocampus and cerebellum (Yin, Hou, Wang, Sui, & Yuan, 2015). *BDNF* Met carriers with late-onset depression also had reduced strong (positive) functional connectivity between the hippocampus and the temporal cortex; however, there was also evidence of increased anti-correlated (negative) functional connectivity between the hippocampus and the dorsal anterior cingulate cortex, dorsal-lateral PFC, and angular gyrus (Yin et al., 2015). Similarly, Wang et al. (2014) observed elevated functional connectivity between the dorsal lateral PFC and the anterior insula in cognitively healthy *BDNF* Met carriers. Finally, Park et al. (2017) investigated the influence of *BDNF* Val66Met polymorphism on structural networks of middle-aged healthy individuals. The authors targeted nodes and edges in their analysis and simulated manipulation of the white matter networks. They demonstrated that Val homozygotes were more robust and resistant to gray matter damage compared to Met carriers (Park et al., 2017). Studies of white matter networks determined that *BDNF* Met carriers were more susceptible to node disruptions than Val homozygotes (Park et al., 2017).

The interaction of the *BDNF* Met and *APOE*ε4 polymorphisms was investigated by Gomar, Conejero-Goldberg, Huey, Davies, and Goldberg (2016) in healthy older adults, as well as in individuals with MCI and AD. Here, the authors found that *BDNF* Met alleles were

associated with poorer cognitive performance, predominantly in memory and semantic fluency. In support, Ward et al. (2014) found decreased performance in episodic memory function in *BDNF* Met carriers, however, only in combination with carriage of the *APOE* ε4 allele, the latter perhaps representing a cumulative effect of carriage of both risk alleles. This cumulative effect may be influencing the functional brain networks and reduce connections between different brain regions. *BDNF* Met carriers may have fewer connections compared to *BDNF* Val homozygotes (Figure 1.8.B) and *APOE* ε4/*BDNF* Met carriers may have even fewer connections compared to non ε4/*BDNF* Val homozygote carriers, which may decrease connectivity (Figure 1.8.C).

In a separate study, *BDNF* Met*/APOE*ε4 carriers with high brain Aβ levels demonstrated a faster rate of decline over a 54-month period in verbal and visual episodic memory and language processing when compared to *BDNF* Met/non-*APOE* ε4 carriers (Lim et al., 2015). In comparison, *BDNF Val/*ε4 carriers with a high Aβ burden demonstrated a relatively mild reduction in cognitive functioning. In *BDNF* Met*/APOE*ε4 carriers with high Aβ load, memory deficits are detectable after 3 years, whereas it takes 10 years in *APOE*ε4-*/BDNF* Val homozygotes with a high Aβ load to reach the same clinical threshold (Lim et al., 2015). A recent meta-analysis investigated the relationship between *APOE* and *BDNF Val66Met* and concluded that there were more women with AD carrying the *BDNF Met* polymorphism (Zhao et al., 2018). However, no significant relationships between *APOE* ε4 carriers and *BDNF* Met carriers were identified in the overall analysis that included both men and women with AD.

APOE and *BDNF* polymorphisms may interact with each other and possibly influence functional connectivity. *BDNF* Met carriers with the *APOE* ε4 allele exhibited decreased

brain activation in the MTL (Kauppi, Nilsson, Persson, & Nyberg, 2014). Atrophy, particularly in the entorhinal cortex, and acceleration of AD pathology, has been linked to poor compensation mechanisms of the brain in individuals with *BDNF* Met carrying the *APOE* ε4 (Gomar et al., 2016). Ward, Summers, et al. (2015a) investigated the effect of *BDNF* and *APOE* on cognitive function and cognitive reserve, the latter which is a theoretical construct where neural networks compensate for lost neurons and connections (Stern, 2002). The authors observed that the *BDNF* Val66Met polymorphism, but not *APOE* variants, moderated the relationship between executive function and cognitive reserve, in which exposure to a more cognitively enriched environment was associated with better executive functioning in Val homozygotes but not in Met carriers (Ward, Summers, et al., 2015a). In another study, Ward et al. (2017) investigated the same healthy older adult sample and found that differences in executive functioning between cognitive reserve tertile groups became smaller over time in *BDNF* Val homozygotes, but cognitive reserve-related differences became more pronounced in *BDNF* Met carriers. An explanation for these results is that cognitive reserve could have varying cognitive effects depending on the *BDNF* Val66Met polymorphism (Ward et al., 2017). Altogether, experimental studies indicate that the *BDNF* polymorphism influences key neurobiological processes associated with development and activity-dependent learning (Egan et al., 2003).

Cognitive reserve and brain connectivity

It is possible that common variation in the *BDNF* gene may result in differences in the development and maintenance of structural and functional networks throughout the life course, which ultimately may be associated with either better or worse brain resilience to neurodegenerative disease processes, such as in AD. Given the role of *BDNF* in development and adult brain plasticity, it is also possible that this gene variation may have an influence on

the construction of patterns of connectivity that underlie resistance to pathology, perhaps related to the theoretical construct of cognitive reserve (Stern, 2002, 2006), in which neurons are compensating for impaired and lost neurons.

Stern (2002, 2009) proposed two different kinds of reserve in relation to a brain challenged by insult and/or neurodegeneration. Brain or neural reserve, which is often referred to as the 'passive' model of reserve, focuses on anatomical brain structures, especially brain size and the number and architecture of neurons and synapses (Katzman, 1993). This model, later revised by Satz (1993), proposed that individuals with higher synaptic count, dendritic branching and larger brain volume should be able to withstand the loss of more neurons without functional consequence, providing compensation for the pathological changes of AD (Stern, 2009). The brain reserve model suggests that most of its capacity is established in the early years of life, usually by the age of five (Reiss, Abrams, Singer, Ross, & Denckla, 1996). Nevertheless, investigations have demonstrated that brain reserve may be modifiable. For example, the brains of adult monkeys are able to form and renew cells throughout life (Eriksson et al., 1998), and human brains have also been proposed to have neurogenic capacity, particularly in the dentate gyrus (Kempermann, Song, & Gage, 2015).

The 'active' model of reserve is often referred to as 'cognitive reserve,' which is a hypothetical construct that relates to the functional resilience of the brain against accumulating pathological changes (Stern, Albert, Tang, & Tsai, 1999). According to the theory of cognitive reserve, brains with more complex neural networks have a higher level of inbuilt redundancy, which are subsequently able to compensate for degenerative or lost neurons (Stern, 2002, 2006). Factors such as lifetime experience, educational and occupational attainment, and socioeconomic status are posited to play a significant role in the

development of cognitive reserve (Stern, 2009, 2012). For example, individuals with AD and higher cognitive reserve (education levels) had greater DMN connectivity compared to individuals with AD and lower education levels (Bozzali et al., 2015). Bastin et al. (2012) determined that there was more cerebral pathology and reduced activity of metabolism in the temporoparietal cortex in healthy individuals with higher education. Furthermore, although Brayne et al. (2010) found that the amount of accumulation of pathological burden in the brain was not affected by the number of years of education that an individual had completed, higher levels of educational attainment was found to be associated with a lower risk of demonstrating dementia on the background of the burden of pathology.

Lifelong engagement in cognitively stimulating activities may reduce the risk of developing dementia by 40% (Scarmeas & Stern, 2003; Valenzuela et al., 2011). In support, Jahangiri et al. (2018) noted that exercise was associated with improved memory function, as well as reduced risk of developing neurodegenerative disease in different animal models. In human studies, Larsson et al. (2017) reported that individuals with higher educational attainment had a lower risk of developing AD. Similarly, in healthy participants (50–79 years), education later in life (university study for at least 12 months) was positively associated with cognitive reserve (as estimated by current psychological assessment scores) compared to those who did not complete any further university education (Lenehan et al., 2016). Associations between education and age are evident particularly in the attention and speed processing domains (Perry et al., 2017). In line with these findings, Summers et al. (2017) found that 92.5% of individuals 50 years and older who had attended university for at least 12 months showed increased cognitive performance in domains that may be a proxy for cognitive reserve.

Stern (2009) hypothesised that individuals with AD who have higher cognitive reserve possess more flexible neural networks and will retain a higher level of cognitive performance with an increasing neuropathological load. This notion of neural flexibility could potentially be demonstrated in re-organizable functional networks of the brain observed in cognitively healthy individuals (Bosch et al., 2010). In this study of healthy older individuals, higher cognitive reserve was associated with increased brain activity in the DMN, but it was also associated with decreased brain activity in regions associated with speech comprehension. In contrast, in individuals with MCI or AD, decreased activation in the DMN and more activation in language processing in subjects was associated with higher cognitive reserve (Bosch et al., 2010).

Education and cognitive reserve have a positive effect on functional connectivity networks (Marques et al., 2016) and cognitive functioning (Bozzali et al., 2015). There is evidence that high cognitive reserve levels were related to working memory, while age had a negative effect on cognition (Ward, Summers, Saunders, & Vickers, 2015). High cognitive reserve has been associated with greater functional connectivity in healthy elderly individuals (Marques et al., 2016). Arenaza-Urquijo et al. (2013) examined a cognitively healthy older population (60–80 years) and described better brain metabolism, higher grey matter volume as well as enhanced functional connectivity in individuals who had more years of early-life formal education. In particular, the authors found higher functional connectivity in regions such as the anterior cingulate cortex, right hippocampus, right PCC, left inferior frontal lobe and left angular gyrus in those with more education.

Marques, Soares, Magalhães, Santos, and Sousa (2015) likewise examined the relationship between education and functional connectivity and found that individuals with more

education had larger networks. These enlarged networks were connected to all lobes in each hemisphere and influenced functional connections in a positive way, which was predicted to moderate the effects of age on brain connectivity (Marques et al., 2015). Moreover, Marques et al. (2016) investigated whether sex and the number of years of education [used as demographic characteristics (DEM)], in 120 healthy older individuals influenced functional networks in the brain. The authors demonstrated that the DEM had a positive effect locally (in the neighborhood areas), on the strength of nodes, efficiency and on clustering coefficient, exhibiting greater communication within the networks of the occipital and parietal lobe areas. There was also a relationship found between the DEM and network transitivity indicating that individuals with more education use different neural processing (Marques et al., 2016). Network transitivity is defined as the connection between two nodes that are linked to each other via an edge in a network.

In addition, Marques et al. (2016) examined how cognitive reserve measured by educational attainment affected functional connectivity in resting state fMRI. They demonstrated that larger networks with more functional connections in the brain were related to higher cognitive reserve. Greater local efficiency and higher local clustering in the cuneus, as well as in the areas of the superior and middle occipital lobe were related to higher levels of cognitive reserve (Marques et al., 2016). The inferior temporal gyrus is predicted to have a significant role for cognitive reserve, because of its betweenness centrality and nodal strength, which demonstrated a positive correlation with cognitive reserve. The fraction of all shortest paths in the network that pass through a given node is called betweenness centrality (Rubinov & Sporns, 2010). The inferior temporal gyrus is a significant hub responsible for recognition and visualization of words and numbers (Grotheer, Herrmann, & Kovács, 2016), which are important functions involved in cognitive reserve networks (Marques et al., 2016).

Finally, global efficiency, which is “a measure of functional integration” (Marques et al., 2016), was greater in individuals displaying higher cognitive reserve compared to individuals with lesser cognitive reserve.

Colangeli et al. (2016) conducted a meta-analysis of whether functional brain networks were associated with cognitive reserve in healthy older adults, as well as in amnestic MCI (aMCI) and AD. Findings in all subgroups showed greater functional brain activation in the anterior cingulate in the left hemisphere while performing a cognitively stimulating task (e.g., recognition memory task). However, the cognitively healthy older adult group demonstrated greater activation in several brain regions as compared to the aMCI and AD groups. These activated brain regions included the left anterior cingulate and left precuneus, the right cingulate gyrus, and the superior frontal gyrus of the dorso-lateral PFC, all of which are susceptible to degenerative changes in individuals diagnosed with AD and aMCI (Colangeli et al., 2016).

Bozzali et al. (2015) investigated whether cognitive reserve modifies resting-state functional connectivity in healthy, aMCI, and AD individuals (mean age 74.6 years). Functional connectivity was associated with the cognitive reserve proxy, education, within the DMN. Higher functional connectivity within the PCC was associated with higher education in individuals with AD, in which education possibly initiated mechanism of compensation. Education may also have led to brain plasticity and supported the PCC from atrophying. Some of the aMCI group exhibited similar connectivity strength, however, there was no strong functional connectivity found in the healthy group (Bozzali et al., 2015).

Franzmeier et al. (2016) also demonstrated that higher global functional connectivity was present in individuals with MCI with relatively higher levels of education. Individuals with more years of education and prodromal AD were able to compensate for fluorodeoxyglucose (FDG)-PET hypometabolism in the precuneus and had greater connectivity in the left frontal lobe, as well as better performance in memory (Franzmeier et al., 2017a; Franzmeier et al., 2017b). Moreover, Franzmeier et al. (2017b) demonstrated that individuals with MCI who had higher educational attainment and high Aβ levels had a more global left frontal cortex connectivity when controlled for age and sex, whereas, in healthy individuals, global left frontal cortex connectivity was not related with metabolism in the precuneus. Negative connectivity between the left lateral frontal cortex and the DMN was also found in people with MCI who had achieved higher education (Franzmeier et al., 2017a). Perry et al. (2017) demonstrated a positive correlation between years of education and cognitive functioning (e.g., visuospatial, executive function, language) but a weak relationship between education and brain networks, especially when the brain already showed evidence of age-related changes in healthy individuals. The greatest impact in age-related alterations later in life was found in the sensorimotor networks, especially those underlying processing speed and attention (Perry et al., 2017).

Education early in life and other life-long cognitively stimulating activities could be possible protectors against neurodegenerative diseases, and might bolster cognitive reserve later in life (Ward, Summers, Saunders, & Vickers, 2015). For example, Thow et al. (2018) discovered that cognitive reserve was associated with improved performance of language processing in healthy middle-aged to older adults.

Language function

Language is a basic but fundamental skill that does not show impairment until later stages in healthy aging (Murman, 2015). Brain regions related to language processing are the superior temporal area, also known as Wernicke's area, and the inferior frontal, or Broca's area (Price, 2010). Wernicke's area is in control of speech comprehension, while Broca's area is closely linked with speech production (Hagoort, 2014). With new advances of fMRI, it was shown that additional brain regions were recruited in older individuals compared to younger when investigating into language processing, suggesting that older individuals use neural elasticity to compensate for age-related impairments and maintaining language function (Diaz, Rizio, & Zhuang, 2016).

Conclusion

The brain is a large set of complex networks that are connected structurally and functionally. Different areas of the brain share and communicate information in functional space, creating these networks. The networks can be adversely or positively influenced by various genetic and environmental factors. For instance, studies reported that *APOE* ε4 was associated with decreased functional connectivity (Lu et al., 2017) and longer path length in functional networks (Goryawala et al., 2015). However, there was also decreased path length (Seo et al., 2013) and increased functional connectivity found in healthy *APOE* ε4 carriers (Wu et al., 2016). Similarly, healthy older *BDNF* Met carriers were associated with reduced functional connectivity (Rodríguez-Rojo et al., 2018) and a more vulnerable structural network, while Val homozygotes showed a more robust white matter structural network in the brain (Park et al., 2017). Cognitive activities and environmental enrichment have favorable effects on *BDNF* Val homozygotes, and over time also on *BDNF* Met carriers (Ward et al., 2017), which possibly may promote maintaining healthy cognitive functioning and reduce the detrimental effects of progressing age. In general, studies provide evidence that education and

cognitive reserve are associated with an increase of functional connectivity in the brain networks (Marques et al., 2016). This could potentially affect brain networks in a positive way and may mitigate and protect against cognitive impairments later in life, and delay or even prevent the onset of AD (Prince et al., 2013). Education later in life increases cognitive reserve and could provide more resistance and resilience to brain pathology, which has been observed particularly in language processing function. Overall, these findings indicate that the functional networks of the brain are influenced by a combination of genetic and environmental factors. An improved understanding of these relationships is vital in order to fully grasp how neurodegenerative changes affect brain function, but also to determine how cognitive resilience to neurodegenerative changes may be promoted.

Thesis aims and hypotheses

Genetic factors and cognitive reserve influence the organization of the functional and structural brain. How the *APOE* and *BDNF* Val66Met polymorphisms, as well as cognitive reserve as a third component, influence the organization of the brain is still debatable. Understanding how these may change the functional and structural organization of the brain could lead to empirical improvements relative to detecting healthy older individuals at risk of developing neurodegenerative diseases, such as the Alzheimer's dementia type. Cognitive reserve may have a protective effect on connectivity in the brain to potentially prevent or delay the onset of AD and to delay progression (Prince et al., 2013). Genetic variations influence age-related cognitive decline, however, the possible interaction with cognitive reserve may moderate the effects. As such, the goal was to investigate functional connectivity within the *APOE* and *BDNF* polymorphisms, as well as in correlation to cognitive reserve. Although, there is limited reported literature, I also investigated into genetic interactions with

cognitive reserve. It is imperative to understand how the brain is influenced by a combination of genetic factors and cognitive reserve.

The Tasmanian Healthy Brain Project (THBP) is a longitudinal study investigating whether later-life education may affect age-related cognitive decline in healthy older adults and potentially reduce the risk for developing dementia (Summers et al., 2013). Supplementary, the THBP explored whether later life education increased cognitive reserve, as well as whether AD-related genes and polymorphisms associated with cognitive performance modified age-related cognitive decline. The THBP project began in 2011 (Summers et al., 2013), and by 2015, approximately 467 participants underwent an intervention of at least 12 months of part-time university studies (the experimental group), while the control group, which did not undertake any educational intervention had approximately 100 participants (Ward, Summers, et al., 2015a). Participants were assessed with a comprehensive neuropsychological test battery every year for the first four years. Subsequently, participants were assessed every two years.

The present investigation used a cross-sectional design to examine the aims listed below. In cognitively unimpaired older individuals that participated in the longitudinal THBP (Summers et al., 2013), the aims were:

1. To examine potential group differences in edge strength between different polymorphisms (*APOE* and *BDNF* Val66Met) within the DMN, DAN, and SA in healthy older adults, as well as how cognitive reserve may affect edge strengths in functional networks.

2. To explore resting-state functional connectivity related to the *BDNF* and *APOE* gene variants, and how cognitive reserve may influence this connectivity within the DMN, DAN, and SA.
3. To investigate whether cognitive reserve influences functional connectivity within the language network (Broca's and Wernicke's area), as well as whether there are differences within the genetic variations of *APOE* and *BDNF* Val66Met within the language network.
4. To examine differences between *APOE* and *BDNF* Val66Met within brain structure metrics (grey and white matter covariance patterns of the whole brain, grey matter volume and cortical thickness in temporal lobe structures).
5. To investigate whether cortical brain structures correlate with cognitive function on different functional domains.

Related to these aims, 12 hypotheses were proposed:

1. Functional edge strength is expected to be stronger in *APOE* ε3 and *BDNF* Val homozygotes compared to *APOE* ε4 and *BDNF* Met carriers, respectively.
2. Cognitive reserve influences functional edge strength positively.
3. Functional connectivity will differ between *APOE* (ε3 & ε4) and between the *BDNF* Val66Met polymorphisms within the DMN, DAN, and SA.
4. Cognitive reserve will influence functional connectivity within the DMN, DAN and SA.
5. *BDNF* Val66Met and *APOE* (ε3 & ε4) polymorphisms independently will show age-related differences in functional connectivity within the language network.

6. Cognitive reserve is associated with increased functional connectivity within the language networks (Broca's and Wernicke's area).
7. *APOE* ε3 homozygotes are predicted to have more grey matter volume pattern compared to *APOE* ε4 carriers possessing.
8. *BDNF* Met carriers will show less grey matter volume patterns compared to *BDNF* Val homozygotes.
9. *APOE* ε3 homozygotes are associated with increased cortical thickness volumes within the parahippocampus and enthorhinal cortex, and increased grey matter volumes within the hippocampus and amygdala compared to *APOE* ε4 carriers.
10. *BDNF* Val homozygotes are associated with increased cortical thickness volumes within the parahippocampus and enthorhinal cortex, and increased grey matter volumes within the hippocampus and amygdala compared to *BDNF* Met carriers.
11. Whole brain structures of grey matter volume and cortical thickness will positively correlate with cognitive function status (episodic memory, working memory, executive function, and language processing).

Chapter 2

Materials and methodology

Preamble

Magnetic resonance imaging (MRI)-based imaging methods are the most frequently applied technique to investigate resting-state brain network differences. For the development of therapies and improving treatment strategies, it is essential to understand how the environment, education, and different genes influence the brain, anatomically and functionally. MRI provides a great range of diverse methods to study the organization of a cognitively healthy brain. Therefore, a functional analysis model was created, which is supported by a structural analysis model. This chapter will describe study recruitment, assessments, and analysis procedures in more detail.

Study population

The participants were recruited from the Tasmanian Healthy Brain Project (THBP) (Summers et al., 2013). The THBP had 467 active participants after four years within the study (Ward, Summers, Saunders, & Vickers, 2015). For the purpose of this thesis and due to high cost of imaging studies, a total sample of 78 participants (53 females, 27 males) were recruited from this cohort, the average age at time of entry was 60 years; the average age of recruited subjects for this thesis project was 63.3 years. The THBP is a unique interventional study that has been running for 10 years (2010-2020) investigating whether education in later life could reduce the risk of developing dementia. The THBP consists of 'experimental' and 'control' groups. Both groups were included in this study. Participants in the experimental group completed a minimum of 12 months (undergraduate or postgraduate) part-time or full-time university study, while controls did not participate in university study.

At the time of entry into the study (2011), the participants showed no psychological, psychiatric, or medical disorders as described in Summers et al. (2013). Individuals

displaying any significant conditions of mental illness and /or impairment of cognitive function, neurological disorders, (such as dementia, multiple sclerosis, epilepsy, cerebrovascular disease, aneurysm) and medical health problems, (such as diabetes, hypertension, hypotension) were excluded at baseline. Genetic profiles of the *APOE* and *BDNF* genotypes of all consenting participants was collected at baseline. A newsletter was sent out to all THBP participants for recruitment to this thesis project. Volunteers interested in participating were specifically selected for a balanced sample of *APOE* ε3/ε3 and ε3/ε4 variants and *BDNF Val66Met* Val/Val and Val/Met variants.

All participants that voluntarily participated were provided with information sheets. Informed consent was obtained from all participants involved in this project. This research project has been approved by the Tasmania Health and Medical Human Research Ethics Committee (Ref No: H0016317) and conducted in accordance with *National Statement on Ethical Conduct in Human Research* (National Health and Medical Research Council of Australia).

Health Screening and Neuropsychological test battery

All participants completed a comprehensive test battery, which assessed neuropsychological, cognitive, health, and psychosocial factors at baseline. The purpose of the test battery was to obtain a detailed measurement of neuropsychological, psychological, and health estimates, as well as values related to cognitive reserve. All participants provided written consent before undertaking the assessments.

Screening

Multiple screenings were used to detect significant mental illness or a neurological disorder:

- Mattis Dementia Rating Scale-2 (DRS-2) (Jurica, Leitten, & Mattis, 2001) is a screening tool to measure cognitive functions and detect signs of dementia, normal general cognitive functioning (DRS−2 AEMSS score ≥9).
- Hospital Anxiety and Depression Scale (HADS) (Zigmond & Snaith, 1983) evaluates any symptoms of mental health problems related to anxiety or depression.
- Medical Health Status questionnaire (Summers et al., 2013) examines health and medical conditions, as well as the use of medication, alcohol and drug.

Test battery

A neuropsychological test battery was used to assess domains of cognitive functions related to learning, episodic memory, working memory, executive functioning, and language processing. Participants were assessed every year for four years, afterwards, participants were assessed every two years.

The test battery included the Rey Auditory Verbal Learning Test (RAVLT) (Strauss, Sherman, & Spreen, 2006), the Logical Memory test (LM) (Wechsler, 1997a), as well as the visual Paired Associated Learning Test (PAL) (Cambridge Cognition Ltd., 2004) from the Cambridge Automated Neuropsychological Assessment Battery (CANTAB), which was used to measure episodic memory. The WAIS-III Digit Span (DSP) (Wechsler, 1997b), WAIS-III Letter-Number Sequencing (LNS) (Wechsler, 1997b), CANTAB Spatial Span (SSP; Cambridge Cognition Ltd, 2004), and CANTAB Spatial Working Memory (SWM; Cambridge Cognition Ltd, 2004) were used to obtain working memory scores. Executive functioning was measured with the CANTAB Rapid Visual Processing (RVP; Cambridge Cognition Ltd, 2004), 24-item Victoria version Stroop test (Lezak, Howieson, Loring, & Fischer, 2004), and Trail-Making Test (TMT) (Lezak et al., 2004). The WAIS-III Vocabulary

(VOC) (Wechsler, 1997a), WAIS-III Comprehension (COM) (Wechsler, 1997b), and Boston Naming Test (BNT) (Kaplan, Goodglass, & Weintraub, 1983) were used to measure language processing.

Cognitive reserve

Cognitive reserve is a theoretical construct, in which brain function is protected in relation to lesions and degeneration because some individuals endure brain pathology better than others. Three standardised tests were used to obtain a composite score related to cognitive reserve (Ward, Summers, Saunders, & Vickers, 2015), including the Wechsler Test of Adult Reading (WTAR) (The Psychological Corporation, 2001), the Lifetime of Experience Questionnaire (LEQ) (Valenzuela & Sachdev, 2007), and the number of years of prior formal education recorded on the Medical Health Questionnaire (Summers et al., 2013). The raw data of each test was transformed into baseline z-scores, standardised, and a factor analysis was performed (Ward, Summers, Saunders, & Vickers, 2015).

Wechsler Test of Adult Reading (WTAR; The Psychological Corporation, 2001) provides information about premorbid intelligence of individuals ranging from 16 to 89 years and can be administered to healthy and individuals diagnosed with disease. This test takes up to 10 minutes, in which the participant must read aloud 50 words with atypical anomalous spelling (Wechsler, 1997b).

Lifetime of Experience Questionnaire (LEQ) (Valenzuela & Sachdev, 2007) is a self-report questionnaire designed for participants over the age of 65 years and measures lifespan mental activity. The questionnaire examines an individual's complete lifetime starting at young adulthood (13-30 years), middle ages (30-65

years), and later life (65 and forward). Every stage of life has different questions, one stage for example questions about their mental activity, such as educational and occupational activities. Other stages ask for more general information, such as sport and hobbies. Participants must reflect on past experiences to recall information for each lifetime stage. The total score of the LEQ is calculated for each stage and exhibits how the individual's mental activity has been over their lifetime. Low scores indicate little complex mental activity, and vice versa (Valenzuela, Sachdev, Wen, Chen, & Brodaty, 2008).

Medical Health Status Questionnaire (MHSQ, Summers et al., 2013) is a self-report questionnaire obtaining demographic information about age, gender, height, weight, handedness, and marital status, as well as medical condition, health, medication, drug- and alcohol use for the preceding 12 months of the participants. It established for the THBP. The Medical Health Status Questionnaire was handed out every year until their third annual assessment, then, a short-form version was used.

Genotyping

DNA samples were collected from participants to genotype *APOE* and *BDNF* polymorphisms. Genotypes for common *BDNF* and *APOE* polymorphisms were obtained from previous studies (Ward et al., 2014).

Materials

Collection of DNA samples was undertaken with Oragene DNA self-collection saliva kits provided by Genotek. Instruments and reagents used for DNA extraction and purification

were a microcentrifuge (Eppendorf), 1.5 mL microcentrifuge tubes (Astral Scientific: 11210-00), PrepIT.L2P reagent (Genotek: QUO-09611-KZNX), DNA TE storage buffer (pH 8.0), ethanol, and an air incubator.

With a one-step reinforced refractory mutation system polymerase chain reaction (ARMS-PCR) (Little, 2001) and following gel electrophoresis the genotypes *BDNF* Val66Met and *APOE* were specified. Tools for genotyping were a vortex, a Mastercycle gradient thermal-cycler (Eppendorf), Red-extract-NAmp (Sigma: R4775), Diethyl Pyrocarbonate (DEPC) treated water (150903), Sybr-safe DNA gel stain (Invitrogen: 533102), a Chemi-do UV light source (Biorad), wide-mini gel electrophoresis trays (Biorad), Tris-acetate-EDTA (TAE) buffer, agarose powder (Bioline Australia: Bio-41025), and Hyperladder IV (Bioline: Bio-33031).

For *APOE* genotyping, polymerase chain reaction (PCR) primers were applied following the assay from Donohoe, Salomäki, Lehtimäki, Pulkki, and Kairisto (1999). For *APOE*, rs429358 and rs7412 were determined by a method reported in Donohoe et al. (1999).
For *BDNF* genotyping, PCR primers were applied following the assay from Sheikh, Hayden, Kryski, Smith, and Singh (2010).

Magnetic resonance imaging (MRI)

A General Electric (GE) Signa 3-Telsa scanner in the Royal Hobart Hospital, Tasmania was used to obtain all brain scans. Functional and structural MRI scans were obtained under the supervision of a senior specialist radiographer from the Royal Hobart Hospital. Informed consent was handed out before the MRI acquisition to make sure that the participant

understood the danger of the high-frequency field. All MRI protocols of functional and structural image acquisition are described in more detail within the following chapters.

Functional data analyses

Voxel-based analyses were performed investigating functional connectivity within resting-state networks, such as the DMN, DAN, SN, as well as the language network.

The processes (pre-processing, data cleaning) used to analyse the functional dataset are described in more details in Chapter 3,4, and 5.

Whole brain analyses

Group Independent Component Analysis (ICA)

A group-ICA (GICA) registered all subjects to standard space and concatenated the temporally registered data from all subjects together. A GICA decomposition was performed to extract 70 (Chapter 3) and 25 (Chapter 4 & 5) independent components using MELODIC. The output of the GICA comprises of a set of timeseries for each component, as well as a spatial group map for each component. The output files of the GICA were used for further analyses. The components of the template were manually classified as resting-state networks or noise artefacts (Beckmann, DeLuca, Devlin, & Smith, 2005b; Salimi-Khorshidi et al., 2014).

Dual regression

Dual regression analyses were performed to investigate group differences. Dual regression has multiple regression stages (Smith et al., 2014). The first stage completes a multiple regression analysis, in which the pre-processed BOLD signal of each single-subject

(dependent variable) is extracted from the model input of the GICA (independent variable) resulting in a set of timecourses characterising the temporal structure of each component for that subject. The second stage performs another multiple regression analysis using the pre-processed BOLD signal of each single-subject (dependent variable) and the output of the first stage (timeseries) to create subject specific spatial maps. The result of both stages are subject maps that best fit the GICA maps, which are composed of Z-statistics or beta-values at every voxel. In the third stage the output of the second stage of the dual regression is used for cross-subject group level analyses. In particular, differences in functional connectivity were investigated between different genetic groups, *BDNF* Met vs *BDNF* Val, *APOE* ε4 vs *APOE* non-ε4; and the interactions between Met/ε3, Met/ε4, Val/ ε3, Val/ε4.

Region of interest (seed-based general linear model)

The purpose of a seed-based analysis is to investigate how strength of functional connectivity of each voxel of the whole brain relates to the seed (region of interest). A seed-based approach was used to explore correlations between time series from the region of interest (seed) and the timeseries across all other voxels in the brain. The seed was defined by using voxel corrdinates in MNI space, subjects were already registered to common space before performing the seed-based analyses. Major language processing regions were chosen: Broca's area and Wernicke's area.

Node-based analysis

Modelling functional networks matrices

Network modelling was performed to examine functional edge strength within resting-state networks, such as the DMN, DAN, and SN.

The node-based technique is different from the voxel-based methods; it does not investigate connectivity maps, but connections described as 'edges' and brain regions described as 'nodes'. This method used partial correlation and analyses the edge strength of connections within the resting-state networks between the two groups of interests. The first step of a node-based analysis is to determine the nodes. To determine nodes, a group-ICA was performed using MELODIC. Components or 'nodes' were chosen based on the temporal spectra of the resting-state networks, spatial maps, timecourses and frequency spectrum. All maps were manually examined and components comprising the DMN, DAN, and SA were selected.
A dual regression was performed to obtain the BOLD signal timeseries of each component. The output of dual regression stage 1 (timeseries) was loaded into Matlab via FSLNets (a program provided from FSL) (Smith et al., 2001). Networks were estimated with *nets_load*. After cleaning the data with the *net_tsclean* function in Matlab, a network matrix was created to examine the strength of the connections with *netsmats*. Correlations between all pairs of time series were created using partial correlation to create a network matrix (matrix of correlation strength). Partial correlation estimated the similarity between two times series after regressing out all other time series and indicated direct functional network connections (Smith et al., 2011). The output of the partial correlation is a node-by-node network matrix. Functional network modelling analysis was done with a mass univariate approach in FSLNets, which also used General Linear Models, however, instead of investigating voxels, edges were examined and compared. A simple groups-level analysis was performed to investigate the average network matrix across all subjects. Finally, a cross-subject comparison was performed using a two-sample t-test (univariate) to investigate differences between different polymorphisms. Statistical significance was evaluated using Monte Carlo permutation-based statistical testing with 5000 permutations with alpha = 0.05 (Nichols &

Holmes, 2002). More details about functional network analysis can be found elsewhere (Smith et al., 2013).

Structural data analyses

Structural datasets were used to investigate brain structures, such as grey matter volume and cortical thickness in relation to *APOE* and *BDNF* genotypes.

All raw files of the T1 3D BRAVO acquisitions were transformed into 3D NIfTI (SPM 8) files to obtain volumetric and surface-based data for each subject. For structural pre-processing, Statistical Parametric Mapping (SPM12; Welcome Department of Cognitive Neurology) was used in Matlab R2018 (Mathworks, Natick, MA). Covariance pattern analyses were performed in Matlab.

A principal component analysis (PCA) on target image array was performed to remove task-independent effects from all images resulting in Eigen values and Eigen images. Sets of principal components and their related subject-specific pattern values were obtained and grouped by deducting the mean of each image from each voxel. These specific pattern scores indicated the degree to which a subject exhibits a particular pattern. Using the PCA Eigen images, grey matter covariance patterns related to genetic polymorphisms (*APOE* & *BDNF* Val66Met) were computed using the best-fit linear combination of the subjects scaling factors (independent variable) and the linear combination of covariates (dependent variable). This was performed with the Akaike information criteria (AIC) (Burnham & Anderson, 2002). From the sequential inclusion of predictors, the smallest of three values of the AIC (log-likelihood penalised for number of variables) and their respective predictors were chosen.

These group level analyses allow the detection of key nodes in grey matter volume covariance networks, as well as in white matter volume covariance networks (Habeck, Krakauer, et al., 2005; Habeck & Stern, 2007; Steffener, Brickman, Habeck, Salthouse, & Stern, 2013).

To investigate structural brain changes region-specific, such as hippocampal and amygdala volume, and entorhinal and parahippocamal cortex thickness, FreeSurfer software (https://surfer.nmr.mgh.harvard.edu/) version 6.0.0 and RStudio 3.1.1 (Team, 2015; Team, 2018) was used.

Chapter 3

Functional connection strength is not associated with allelic variations of *APOE* and *BDNF* polymorphisms in healthy older adults

Abstract

Functional connectivity may be used as a potential biomarker of early pathological alterations in the brain of otherwise healthy older adults. Resting-state functional magnetic resonance imaging (fMRI) provides useful information and is commonly used to investigate the organization of the brain. Genes may potentially influence brain organization and aging-related functional connectivity changes. These may include Apolipoprotein E (*APOE*) and brain-derived neurotrophic factor (*BDNF*). *APOE* epsilon4 (ε4) is a major risk factor for late onset Alzheimer's disease and the Met allele of the *BDNF* Val66Met polymorphism has been associated with reduced memory function. Both polymorphisms have been previously associated with disruption in functional connectivity. Cognitive reserve, a theoretical construct related to functional resilience, has been associated with greater functional connectivity. The aim of this chapter was to investigate functional edge strength differences within the DMN, DAN, and SA between individuals with the common *BDNF* and *APOE* polymorphisms in healthy older adults (mean age = 60.5 years). Further, it was investigated whether cognitive reserve might have modified edge strength connections. Independent Components Analysis was used to decompose the datasets into time series and spatial maps to perform dual regression. Timeseries from the dual regression was used to further perform network modelling analyses within Matlab. Results showed no significant differences between *APOE* ε3 homozygotes and *APOE* ε4 carriers, and between *BDNF* Val homozygotes and Met carriers, within all networks. Cognitive reserve did not modify functional edge strength. The results suggest that genetic polymorphisms and cognitive reserve did not associate with functional edge strength.

Introduction

Literature has shown that the aging brain changes throughout life, including cognitive function (Peters, Morrison, Rosene, & Hyman, 1998), which may be linked to alterations in functional connectivity in healthy older adults. One early biomarker for detecting brain changes is resting-state functional magnetic resonance imaging (fMRI), as discussed in chapter 1, which is a non-invasive method used to map functional brain connections (Dennis & Thompson, 2014). In a review, Dennis and Thompson (2014) evaluated three different techniques measuring functional connectivity: Seed-based analysis, Independent Component Analysis (ICA), and graph theory. The difference between these techniques is that seed-based and ICA are voxel-based methods (Beckmann et al., 2005a), while graph theory is node-based (Dennis & Thompson, 2014). The current chapter focused on graph-theoretical measures to provide insight into the architecture of functionally connected brain networks relative to common human genetic polymorphisms. In structure, graphs are depicted as nodes (voxels) and edges (functional activity between nodes)(Stam et al., 2007).

Metrics of structure and functionality have disclosed network alterations in the brain through aging (Achard, Salvador, Whitcher, Suckling, & Bullmore, 2006; Wang et al., 2010). Resting-state studies have described a decline of global functional connectivity in healthy older adults involving a reduction in nodal efficiency and modularity, which resulted in unbalanced integration and segregation (Brier et al., 2014). Understanding and exploring differences in network topology may be a potential biomarker in healthy aging to identify abnormalities related to cognitive impairment. The investigation of resting-state networks including the DMN, DAN, and SN may offer information to better understand brain changes that are associated with neurodegeneration.

The DMN is of particular interest because it is active while the person is not occupied with a task or activity (Buckner, Andrews-Hanna, & Schacter, 2008). This network activity has been found to be insufficient in AD compared to healthy elderly individuals (Greicius et al., 2004). Previous studies also showed that DMN integration declined in healthy elderly compared to young adults, and clustering values were higher in younger adults, indicating that connections between networks diminished already with the aging process (Toussaint et al., 2014). Research in *APOE* and *BDNF* polymorphisms have discovered that the DMN may be more susceptible to reduced connectivity when carrying either the ε4 allele (Machulda et al., 2011) or Met allele (Thomason et al., 2009).

The DAN is involved in mechanisms that require attention, such as sensory orientation tasks, also known as top-down processes and is anticorrelated to the DMN. Particularly, it is activated when the individual has to pay attention to specific cues or tasks (Fox et al., 2006). Aging also had an impact on the DAN, where functional connectivity density (important functional brain hubs) was reduced, suggesting that mechanisms of attention, in particular, sustained attention, decrease with aging (Tomasi & Volkow, 2012a). Functional connectivity was even more affected impaired in AD than in healthy aging (Li et al., 2012) proposing that the DAN might be a responsive and precise biomarker in neurodegeneration for separating AD from healthy ageing. These functional connectivity alterations might be detected in different genotypes. One study investigating sustained and covert attention observed that task-related recruitment of parietal areas was lower in mid-aged ε4 carrier (55-65 years) compared to non- ε4 carriers (Evans et al., 2014). However, findings also showed that ε4 carriers recruited more frontal lobe regions suggesting some kind of compensation mechanism in ε4 carriers supporting the weakened activity in the parietal region to maintain cognitive function (Evans et al., 2014). Investigation in

the *BDNF* Val66Met polymorphisms showed that there is limited information about the effects of *BDNF* Val66Met on the DAN.

Similar to the DAN, the SN is also anticorrelated to the DMN consisting of the anterior cingulate and dorsal anterior cingulate cortex (Seeley et al., 2007). In healthy aging within the SN has also been associated with disruptions that have correlated with cognitive decline (Onoda, Ishihara, & Yamaguchi, 2012). Interestingly, research found a study has demonstrated more functional connectivity in ε4 carriers within the salience network than in non ε4 carriers (Machulda et al., 2011). Authors suggested that this could be due to balance disruption between the DMN and SA and the decline of the posterior DMN causing enhanced SA activity. Congruent to these findings, Thomason et al. (2009) also found more connectivity in cortical regions of the amygdala, insula and caudate in *BDNF* Met allele carriers than in Val homozygotes, suggesting that the Met allele could be associated with protective effects, as well as with higher chances of developing neurodegenerative diseases.

Variations of AD-related genes may affect brain organization in healthy aging (Kauppi et al., 2014) and may alter functionality of a network prior to clinical symptoms. As described in chapter 1, one of these AD-related genes is Apolipoprotein E (APOE), of which the ε4 allele is related to increased risk to develop AD (Liu, Kanekiyo, Xu, & Bu, 2013). Different graph theoretical measures of the whole-brain functional network were investigated and findings revealed a decline in functional hubs and disorganization of network topology in ε4 carriers compared to non-ε4 carriers, suggesting less efficient information flow within the brain of an ε4 carrier (Seo et al., 2013). In addition, fewer short connections within frontal lobe regions and aberrant small-world networks in cortical networks creating an impairment in large scale

anatomical cortical networks were observed in ε4 carriers compared to non-ε4 carriers (Goryawala et al., 2015).

Another AD-related gene, as previously discussed in chapter 1, is brain-derived neurotrophic factor (BDNF) Val66Met polymorphism (Brown et al., 2014). Carriage of the *BDNF* Met allele has been associated with brain volume reduction (Brown et al., 2014), reduced resting-state functional connectivity (Yin et al., 2015), and node disruption in white matter networks (Park et al., 2017). Using gamma-band functional connectivity, reduced functional connectivity was reported in Met carriers relative to Val homozygotes suggesting that the Met polymorphisms may be linked with a higher susceptibility to connectivity failure, and therefore could be related to dementia (Rodríguez-Rojo et al., 2018).

Conversely, cognitive reserve and brain stimulation may beneficially influence functional connectivity within healthy elderly individuals (Valenzuela et al., 2011). In particular, cognitive reserve, discussed in chapter 1 & 2 in more details, is known as a theoretical construct in which the brain compensates for degeneration by recruiting more brain regions to maintain cognitive functions (Stern, 2002), with years of education commonly used as a proxy (Bozzali et al., 2015). Positive correlations between cognitive reserve and functional brain networks have been described, indicating that higher cognitive reserve affected graph theoretical measures by increasing local and global efficiency, clustering, and centrality of the functional networks in healthy elderly possibly activating various neural processes to assist the brain to manufacture segregated functional networks (Marques et al., 2016).

Given that both polymorphisms, *APOE* (Seo et al., 2013) and *BDNF* (Park et al., 2017), as well as cognitive reserve (Marques et al., 2016), may be associated with differences in

functional brain networks, it was of great interest to examine functional brain network differences between genotype variants (*APOE* & *BDNF*) of healthy elderly within the DMN, DAN, and SA. This chapter addresses hypotheses 1 and 2. Graph theoretical modelling is an approach to visually represent brain changes that may assist in comprehension of how alterations in the brain lead to neurodegenerative diseases, such as AD (Brier et al., 2014). Therefore, the goal of this chapter was to identify whether healthy older individuals (mean age = 60.5 years) with a particular polymorphism (*APOE* and *BDNF* Val66Met) would show differences in functional edge strength within the DMN, DAN, and SN. Further, this thesis was also interested in whether cognitive reserve may modify functional edge strength.

Methods

Participants

For this study, a total sample of 78 healthy participants (53 females, 27 males) aged between 53 and 81 years (63.33 average age) were recruited from the THBP, a prospective cohort study investigating the effect of later-life education on cognitive aging. A letter was sent out to all THBP participants inviting them to participate in the study. The information about the genetic variants of each consenting participants was known from previous studies and was examined. Volunteers who were invited to participate in this study were purposively selected for a balanced sample of *APOE* ε3/ε3 and ε3/ε4 variants and *BDNF Val66Met* Val/Val and Val/Met variants.

At the time of entry into the THBP (from 2011), the participants were healthy and reported no serious psychological, psychiatric, or medical disorders. Participants with pre-existing conditions were excluded, including cerebrovascular complications, poorly controlled diabetes, poorly controlled hypertension or hypotension, and neurological disorders. A total of 383 adults had commenced in the THBP by December 2012(Summers et al., 2013). The

current fMRI study participants were recruited between September 2017 and April 2018. Participants have a follow up every two years. The questionnaires examine current background situations such as neurological conditions, psychological conditions, heart-diseases, cancer, colour-blindness, eye vision, blood pressure, cholesterol, head injury, diabetes, kidney- and liver function, and vitamin intake. Within the time of recruitment and scanning (four years in), there was six participants reporting mild to moderate depression, four participants reporting treated cancer, 12 participants reporting higher cholesterol levels of which three are not controlled, six participants reported visual impairments and treatments, and one having a possible stroke.

Study protocols for the THBP are described in Summers et al. (2013). A flow chart representing all participants can be found within Figure 3.1.

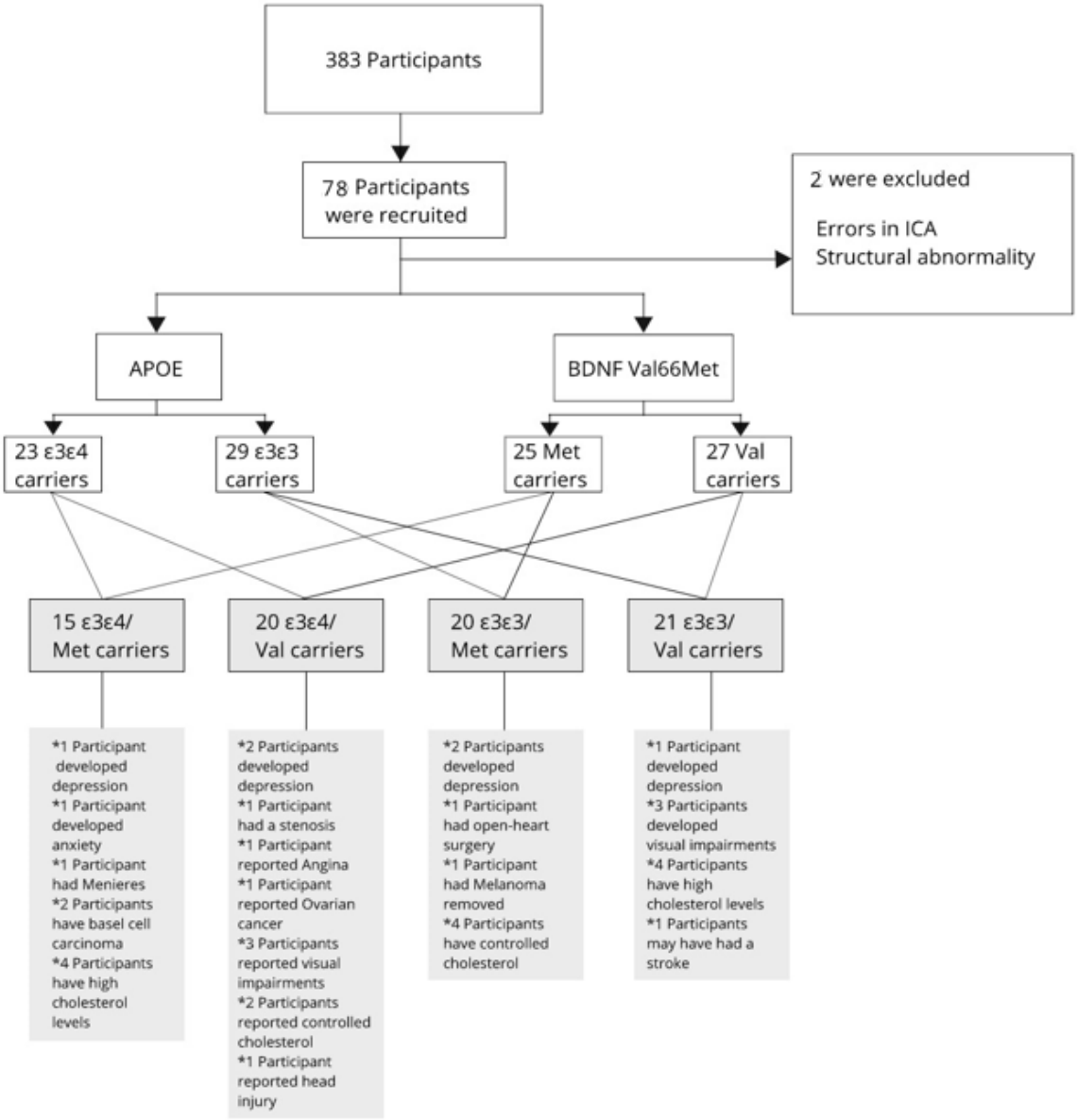


Figure 3.1. Tasmanian Healthy Brain Project participants, recruitment, inclusion, exclusion, genotypes, and developed medical conditions.

At the time of recruitment, the participants sample increased from 383 (in 2012) to 460 (in Ward et al., 2015). For the current study 76 participants included in the analysis. Recruitment occurred based on genotypes. Every two years participants are followed up and a medical questionnaire reports all medical conditions the participants experienced.

As part of the larger THBP study, participants that decided to undertake full-time university studies while participating were classified as 'experimental', while participants not undertaking full time studies were defined as 'control' groups. More, precisely, participants in the experimental group completed a minimum of 12 months (undergraduate or postgraduate) part-time or full-time university study as part of the THBP, while control subjects did not participate in the education intervention. More specific information about the study load of the experimental group can be found in Summers et al. 2013. At the start of recruitment for the THBP, participants were community-residing adults between the age range of 50-79 years (Summers et al., 2013). A previous study investigated whether there were differences between experimental and controls within the *BDNF* Val66Met polymorphism and found that there were no significant differences between those two groups (Ward et al., 2017). However, significant differences were found between baseline cognitive reserve x time interaction for working memory indicating that the performance was influenced by cognitive reserve between Met carriers and Val homozygotes (Ward et al., 2017). Within the cohort selected for this thesis, there were no significant differences in cognitive reserve between *BDNF* Met carriers and Val homozygotes, or between *APOE* ε4 carriers and ε3 homozygotes. The THBP participants proportion for this chapter of experimental and control participants within the ε3 homozygotes (experimental = 33, controls = 9) and ε4 carriers (experimental = 26, controls = 8), and within Val homozygotes (experimental = 31, controls = 10) and Met carriers (experimental = 28, controls = 7) had a balanced proportion. I purposively sampled to obtain a balanced proportion of ε3 homozygotes, ε4 carriers, Val homozygotes, Met carriers to avoid class imbalance, since recurring aim of the study was to investigate the interaction between APOE and BDNF genotype. Most of the time, covariates, such as age, gender, cognitive reserve were included in the analyses, while experimental and controls were not additionally included because most

of the analyses took extensive computation time, therefore, it was not feasible to control for confounding variables in every analysis, which is one of the limitations of this thesis.

Procedure

At baseline, THBP participants undertook a comprehensive clinical test battery measuring neuropsychological, cognitive, health, and psychosocial factors (Summers et al., 2013), with clinical outcomes overseen by a neuropsychologist. Individuals with significant mental illness and/or impairment of cognitive function were excluded at baseline. This research study has been approved by the Tasmanian Health and Medical Human Research Ethics Committee (Ref No: H0016317) and conducted in accordance with *National Statement on Ethical Conduct in Human Research* (National Health and Medical Research Council of Australia). All subjects provided written informed consent.

Cognitive reserve

Cognitive reserve, as described in chapter 1 and 2, is a hypothetical construct that relates to properties of brain function that are protective in relation to neurodegeneration due to pathological burden. To measure cognitive reserve at baseline, a composite score was derived by factor analysis from the Wechsler Test of Adult Reading (WTAR; The Psychological Corporation, 2001)(Wechsler, 2001), the Lifetime of Experience Questionnaire ((LEQ; Valenzuela & Sachdev, 2007); and the number of years of prior formal education (Summers et al., 2013),(Ward, Summers, Saunders, & Vickers, 2015).

Cognitive reserve was estimated at baseline using a PCA-derived (Principal components analysis) weighted composite score, which were obtained from Ward, Summers, Saunders, and Vickers (2015).

Genotyping

DNA samples from all 78 participants were available for the current study. The collection of DNA samples was carried out with Oragene DNA self-collection kits supplied by Genotek (DNA Genotek Inc., n.d.). *BDNF* Val66Met and *APOE* genotypes were determined through one-step amplified refractory mutation system polymeare chain reaction (ARMS-PCR)(Little, 2001) and subsequent gel electrophoresis. For *APOE*, rs429358 and rs7412 were determined by a method reported in Donohoe et al. (1999). For *BDNF*, Val66Met was determined by the method outlined in Sheikh et al. (2010). For more information, please review Ward et al. (2014). Participants were selected for the current study based on their genetic profile: *APOE* ε3ε3 & ε3ε4 and *BDNF* Val/Met & Val/Val. More information about genotyping is described in chapter 2.

Magnetic resonance imaging (MRI)

All brain scans were acquired using a General Electric (GE) Signa 3-Telsa scanner in the Royal Hobart Hospital. Foam pads and headphones were provided to minimize head movement and scanner noise. A Senior Specialist Radiographer supervised the MRI and was responsible for maintaining a safe working environment throughout the MRI session. Before entering the scanner, participants were instructed to keep their eyes closed, to be relaxed and not to move, and not to fall asleep during data acquisition. The scan took approximately 30min for each individual.

The imaging sequence was as follows: structural images were acquired using a T1-weighted 3D BRAVO sequence (TR=1000, TE= 2.53 ms, 256 x 256 x 176 matrix, 1 x 1 x 1 mm voxels). T2-weighted FLAIR (Fluid attenuated inversion recovery) were acquired with 2mm Iso, TR=100ms, TE=10000ms, TI=2250ms, bandwidth =83.33Hz/pixel. Gradient field maps were also acquired (TR= 1000 TE= 2.35 ms, 256 x 256 x 48 matrix, TR = 1000ms, flip angle

60degrees, 0.86 x 0.86 x 3.4 mm^3 voxels). Resting state functional MRI scan was acquired using echo planar imaging with a 64 x 64 matrix, 3.438 x 3.438mm in-plane resolution; slice thickness = 3.4 mm, TR=2500ms, TE=30ms, Field of view (FOV)=22cm.

Functional magnetic resonance imaging (fMRI) data analysis

Resting-state fMRI Data Pre-processing

Resting-state images were pre-processed using FMRIB Software Library (FSL), as discussed in Chapter 2 (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012; Smith et al., 2004). Each resting-state fMRI dataset consisted of 164 volumes. The first two volumes of each resting-state fMRI image were deleted to avoid potential field inhomogeneities at the beginning of image acquisition. For each dataset MCFLIRT (Motion Correction FMRIB's Linear Image Registration Tool)(Jenkinson, Bannister, Brady, & Smith, 2002) was used to correct for head motion. Brain Extraction Tool (BET) (Smith, 2002) was used to digitally remove the skull and other non-brain tissue. Additionally, residual non brain tissue was manually removed from T1 structural images using FEAT (FMRIB's Easy Analysis Tool, Woolrich, Ripley, Brady, & Smith, 2001). This ensured appropriate registration from subject-to standard-space (Montreal Neurosciences Institutes (MNI152); (BBR; Greve & Fischl, 2009). Each resting-state image was registered to the corresponding structural T1 image (FLIRT) first, and then registered to MNI152 standard space using non-linear FNIRT tool(Andersson, Jenkinson, & Smith, 2007). Each dataset was resampled to 2x2x2 mm^3 resolution in the final MNI152 space. Single datasets of the resting-state functional MRI were temporally high-pass filtered to eliminate slow drifts (cutoff period ~ 100.0 seconds). In order to reduce noise and preserve spatial information, spatial smoothing with full width at half maximum (FWHM) of 5mm Gaussian kernel was used to obtain resting-state networks for each participant.

The pre-processed data was de-noised and analysed using MELODIC (Multivariate Exploratory Linear Optimised Decomposition of Independent Components; Beckmann & Smith, 2004) Version v6.00 within FSL to decompose the data into independent components for each subject. Automatic dimensionality estimation was chosen to avoid overfitting. Each individual dataset was manually classified as signal and noise by looking at thresholded spatial maps and temporal power spectra, as well as time courses(Salimi-Khorshidi et al., 2014). No specific software was used to clean the data. Each component labelled as noise was regressed out of the signal using *fsl_regfilt*. All clean data sets were transformed from subject to standard space.

Group-level analyses

Resting-state networks were identified via a group-level ICA decomposition. Seventy components were extracted using MELODIC. The components of the template were manually classified as resting-state networks or noise artifacts (Beckmann et al., 2005a; Salimi-Khorshidi et al., 2014). For analysis, these components of interest were retained: DMN, SN, DAN.

Dual regression

To investigate functional connectivity among different genotypes, the group-ICA templates were used in a dual regression analysis. The dual regression analysis estimates sensitivity to amplitude network activity and alterations in spatially distributed correlation patterns (Beckmann, Mackay, Filippini, & Smith, 2009; Nickerson, Smith, Öngür, & Beckmann, 2017) of resting-state networks. The three outputs are subject-specific data time courses,

subject-specific spatial maps, and statistical maps results of the cross-subject analysis (Smith et al., 2014). Only, the first output stage (time series) were used for further analyses.

Network modelling

Resting-state networks were classified into DMN, DAN, and SA after the group-ICA. These networks were used as nodes. For network modelling, as described in chapter 2, output stage 1 (time courses) were used and loaded into Matlab R2018a (Mathworks, Natrick, MA) via a toolbox called FSLNets (v0.6; (Smith et al., 2011). Spatial maps templates of each resting-state network were created and applied on a MNI152 standard space to determine which network (after group-ICA) are acceptable for further analyses (Figure 3.2). Artifacts and noise nodes (components) were manually removed based on temporal spectra, spatial maps, and time courses. Network matrices were computed for each dataset, which show the correlation strengths. In particular, partial correlation was performed to estimate direct network connections. A simple group-level analysis was performed to investigate the mean network matrix from all datasets. Subsequently, cross-subject analyses were performed to investigate differences in 'netmats' between different genotypes. A univariate test examined edges (or connections) of each network matrix individually. Multiple comparisons were corrected for all edges.

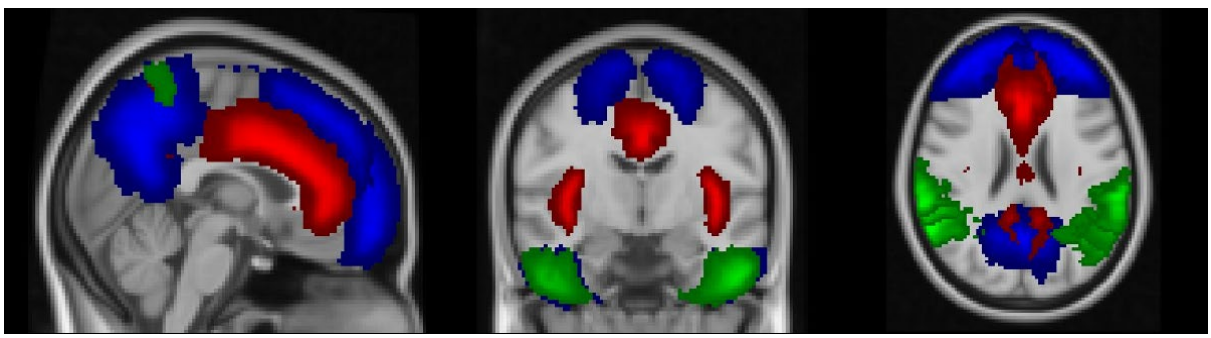


Figure 3.2. Spatial map template displaying the DMN (blue), DAN (green), and SN (red). The template of the DMN and SN have been generated with the Harvard-Oxford cortical atlas, while the DAN has been created with the Juelich Histological Atlas, overlaid on an MNI152 space image.

Statistical analyses in SPSS

Statistical analyses for descriptive and demographics were performed using Statistical Package for Social Sciences (SPSS) Version 24.0.0.1 from Windows (Chicago, IL, USA).

Results

Eighty individuals mean age (63 years) were recruited for this project, with no conflicting mental health conditions, such as depression or Alzheimer's disease. Only 77 participants were included in the analysis. Only *APOE* ε3 homozygotes and ε3/ε4 carriers (non-ε4 carriers) were included for analysis. Hence, two subjects were excluded due to *APOE* genotypes (*APOE* ε2ε3 & *APOE* ε4ε4) inconsistent with the analytical framework. One additional subject was excluded due to reoccurring errors within the single-subject independent component analysis. Demographic details are shown in Table 3.2. Independent *t*-tests were performed to investigate differences between experimental and controls. There were no significant differences in cognitive reserve between *BDNF* Met carriers an Val homozygotes, $t(1,75) = .34$; $p = .735$, or between *APOE* ε4 carriers an ε3 homozygotes, $t(1,75) = 1.22$; $p = .226$.

Table 3.2. Participants demographics expressed as mean (M) ± standard deviation (SD) unless otherwise noted

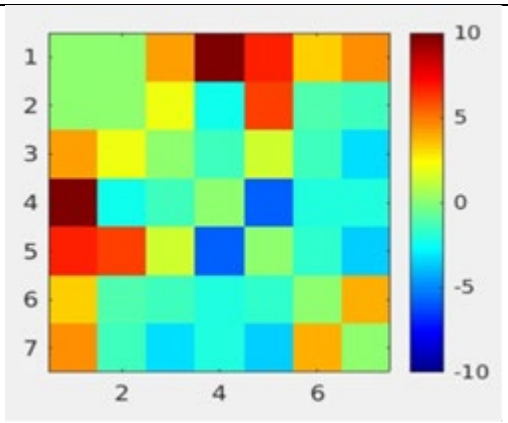
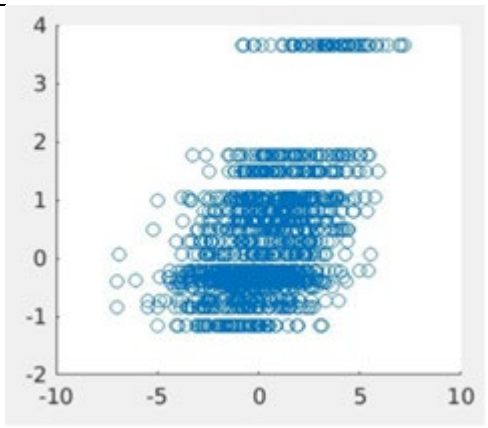
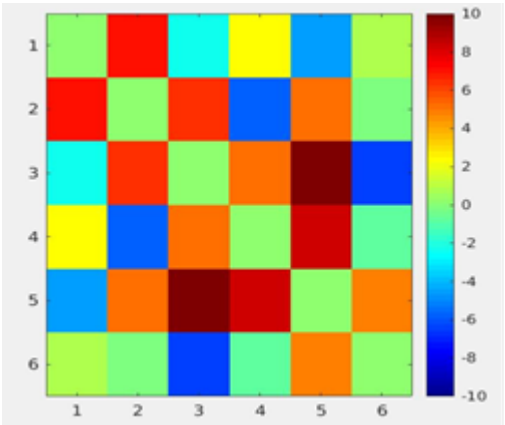
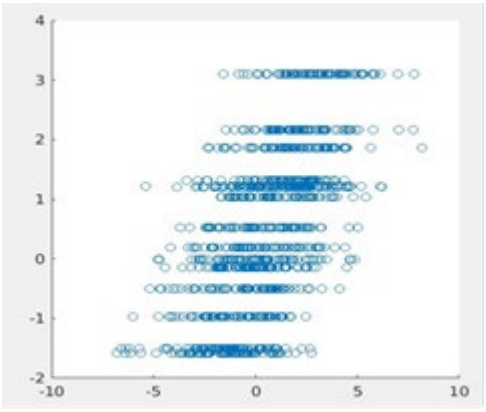
	APOE		*BDNF*	
	ε3	ε4	Met	Val
N	43	34	36	41
Gender F / M	30 / 13	23 / 11	26 / 10	27 / 14
Age M ± SD	63.4 ± 7.1	63.3 ± 5.2	63.1 ± 5.6	63.6 ± 6.8
Cognitive reserve	.28 ± .76	.06 ± .95	.20 ±.82	.16 ± .88

Functional edge strength within the DMN, DAN, & SA

For each resting-state network, components or 'nodes' were chosen. A total of 70 components were chosen for these analyses because there is a trade-off between computational complexity and information, such that the more nodes are included the longer it takes to compute the analysis, however, the more variance was captured. According to literature (Ray et al., 2013) as well as FSL documentation, 70 components were chosen. Resting-state network components or nodes were defined using MELODIC (ICA), which was based on timeseries, spatial map, and frequency spectra. Because MELODIC was used to obtain the nodes, the nodes are non-contiguous and are weighted(Bijsterbosch & Beckmann, 2017).

All other artefacts or noise components were removed with a code called ts = nets_tsclean. Networks of interest were chosen by visually viewing their activation and comparing them to the spatial map (Figure 3.2.). After careful inspection, the DMN was found in seven component (out of 70) or 'nodes', the DAN was found in six nodes, and the SA was found in four nodes. A simple group-levels analysis was performed for each resting-state network individually to investigate the mean network matrix for each network respectively, across all subjects (Figure 3.3.). Cognitive reserve was included as a covariate in the analyses and showed no correlation with network connectivity. Separate univariate two-sample *t*-tests were performed within each resting-state network to investigate the edge strength for each

polymorphism. There were no significant main effects found for either polymorphism independently, in all networks (Figure 3.4, 3.5, 3.6). There were no significant correlations between functional edge strength and cognitive reserve. After adjusting for each *APOE* and *BDNF*, there were also no correlations found between cognitive reserve and functional edge strength (Table 3.3.).

Group mean matrix summaries of all interested networks		
	Group average network matrix across all subjects	Consistency scatter plot across all subjects
A. DMN		
B. DAN		
C. SA		

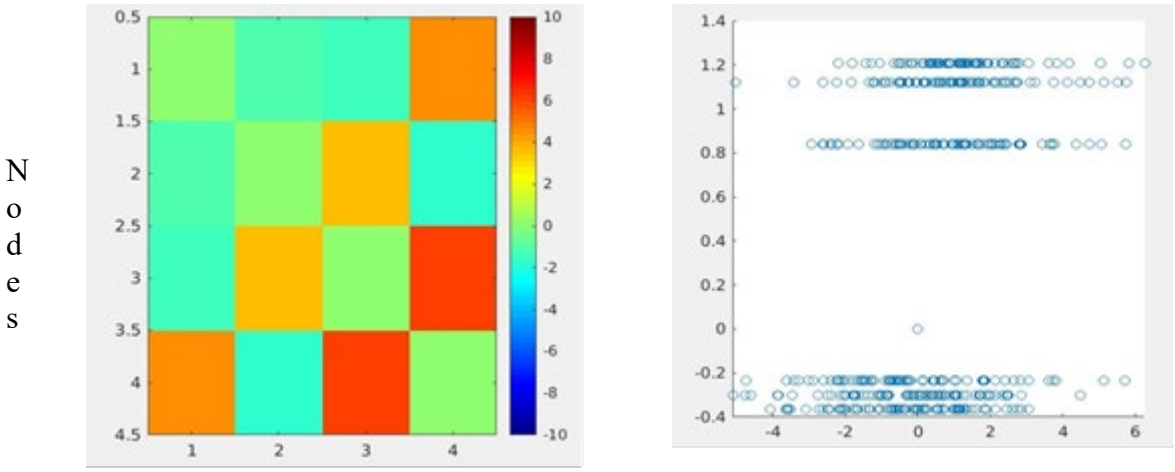


Figure 3.3. Left: Group *t*-test at each edge transformed to *Z*-stats. The X-axis and Y-axis represent the selected nodes within each network. The intensity of colours in the correlation matrices indicate the averaged z-scores. Right: Consistency scatter plots. Each subject edge is plotted against the group mean.

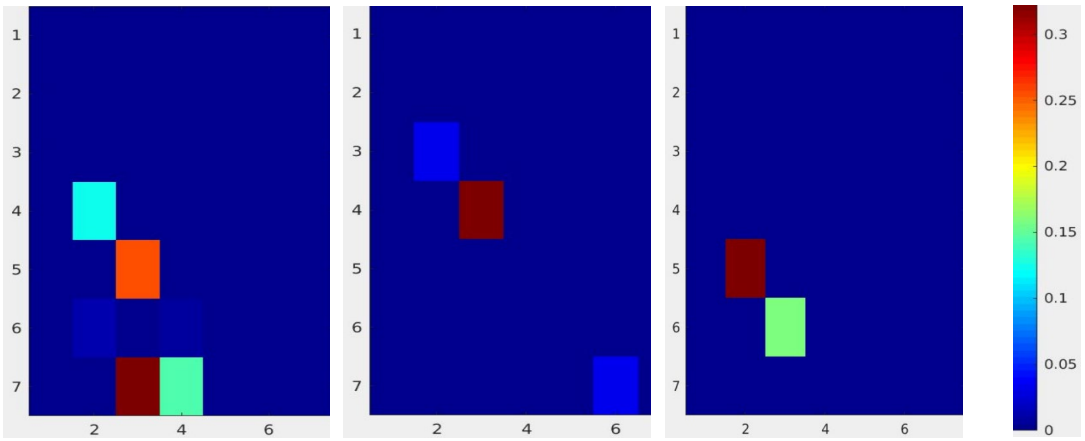
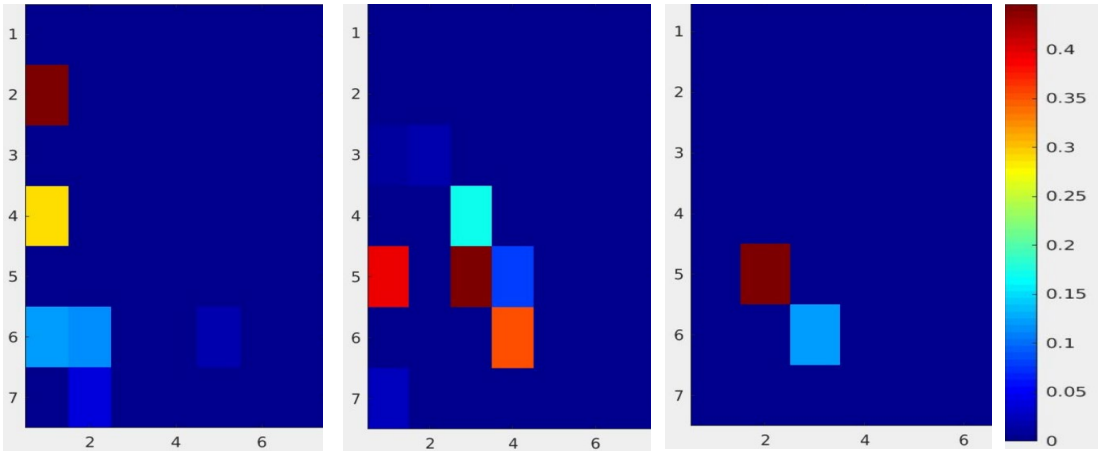
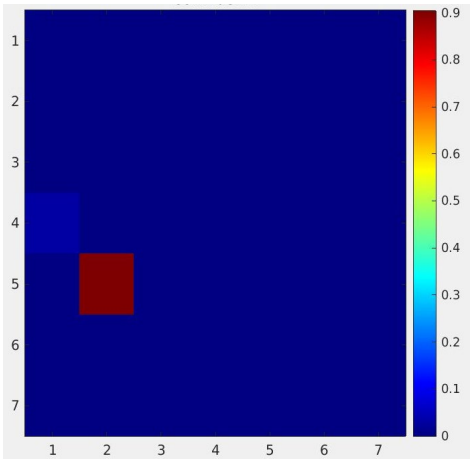
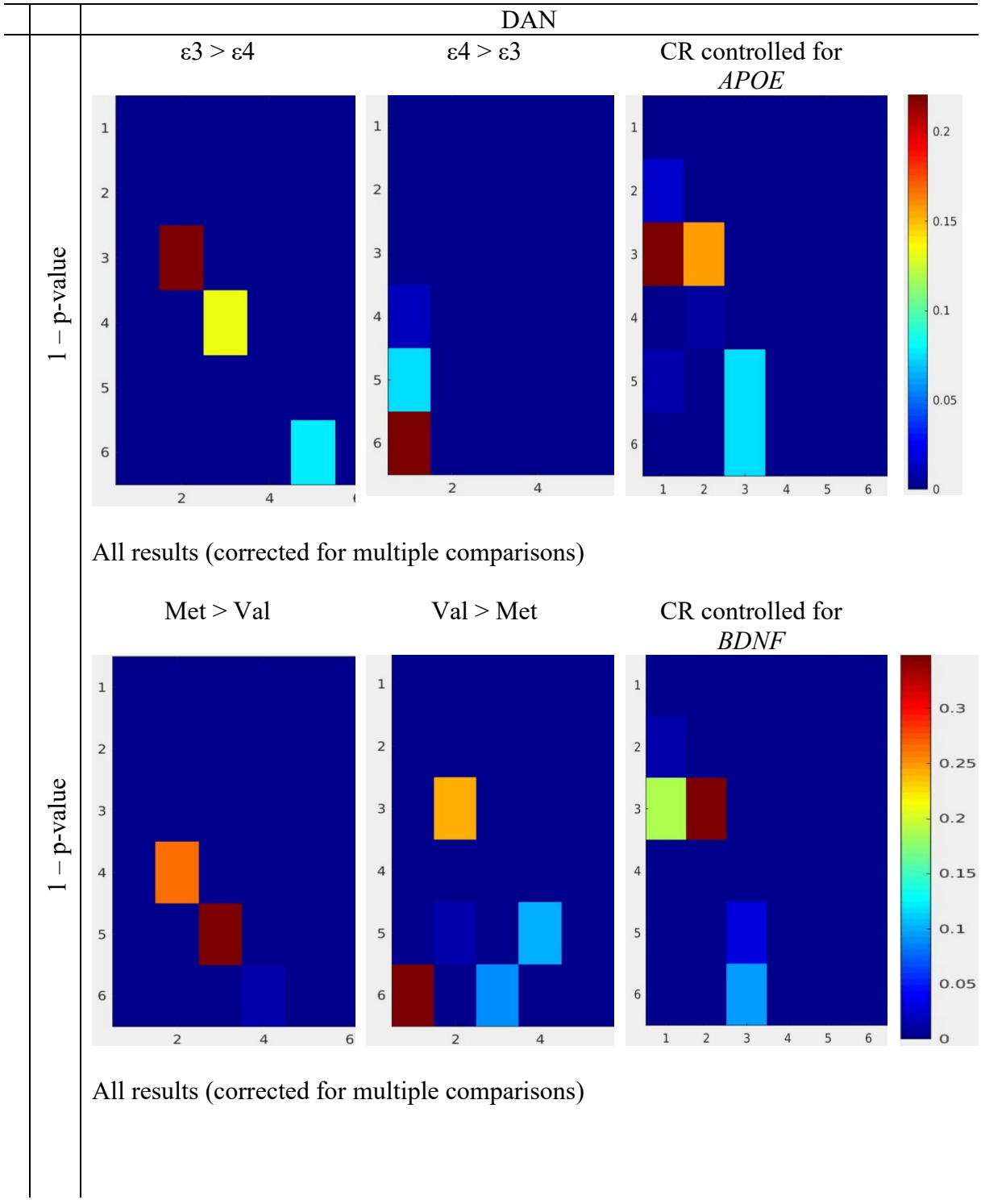
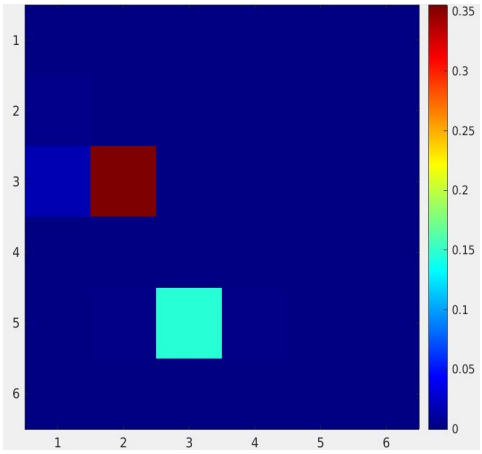
		DMN
	1 – p-value	ε3 > ε4 / ε4 > ε3 / CR controlled for *APOE*  All results (corrected for multiple comparisons)
	1 – p-value	Met > Val / Val > Met / CR controlled for *BDNF*  All results (corrected for multiple comparisons)
	1 – p-value	Correlation between CR and functional edge strength within the DMN  All results (corrected for multiple comparisons)

Figure 3.4. The statistical result (Netmats) demonstrating group differences between genotypes within the DMN. The X-axis and Y-axis represent the selected nodes within each network. A regularized Pearson correlation (Bijsterbosch et al., 2017) was computed and no significant differences were found between ε3 & ε4, and between Met & Val after performing a two-sample *t*-test. After running cross-subject comparison with netmats to perform comparison (5000 permutations for each contrast), there were no correlations found between cognitive reserve and functional connectivity within the DMN.



1 – p-value

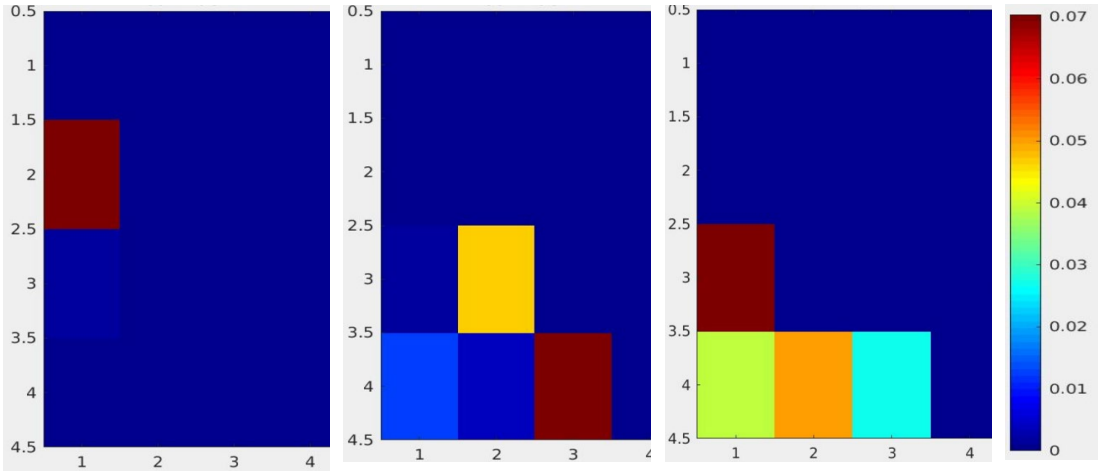
Correlation between CR and functional edge strength within the DMN



All results (corrected for multiple comparisons)

Figure 3.5. Netmats demonstrating group differences between genotypes within the DAN. No significant differences were found between ε3 & ε4, and between Met & Val after performing a two-sample *t*-test. There were no correlations found between cognitive reserve and functional connectivity within the DAN.

	SN		
1 – p-value	ε3 > ε4	ε4 > ε3	CR controlled for *APOE*



All results (corrected for multiple comparisons)

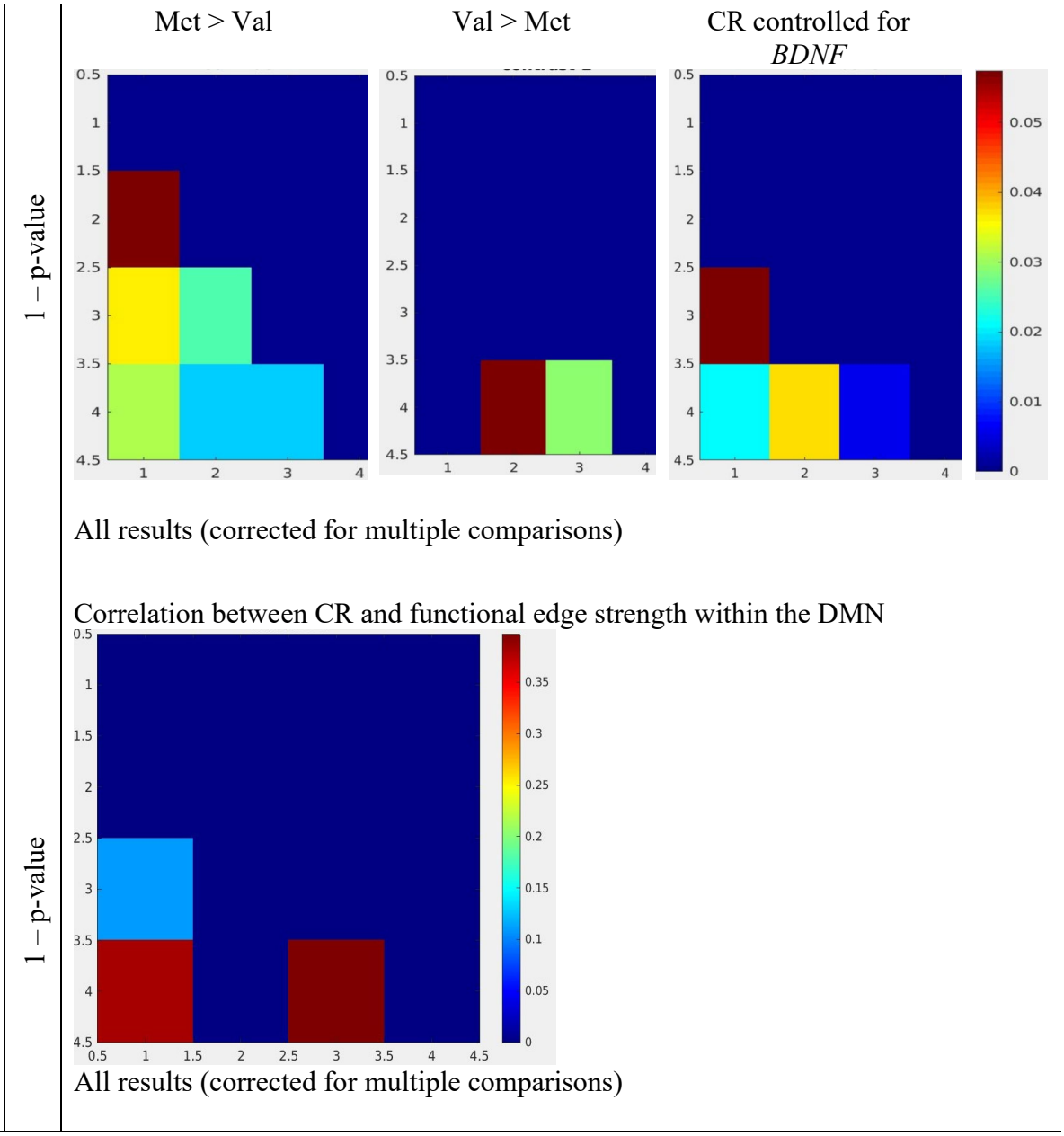


Figure 3.6. Netmats demonstrating group differences between genotypes within the SN. No significant differences were found between ε3 & ε4, and between Met & Val after performing a two-sample *t*-test. There were no correlations found between cognitive reserve and functional connectivity within the SN.

Table 3.3. Edge strength results between nodes of all networks

	Nodes	Uncorrected *p*-values	FWE corrected *p*-values	Rho
Default Mode Network (DMN)				
ε3 > ε4	7 & 3	0.1	0.9	-0.64
ε4 > ε3	4 & 3	0.01	0.19	-0.24
APOE * CR	5 & 2	0.05	0.68	0.91
Met>Val	2 & 1	0.09	0.89	0.06
Val>Met	5 & 3	0.05	0.73	0.29
BDNF * CR	5 & 2	0.04	0.55	0.91
CR	5 & 2	0.01	0.09	0.91
Dorsal-Attention Network (DAN)				
ε3 > ε4	3 & 2	0.14	0.92	0.87
ε4 > ε3	6 & 1	0.03	0.36	0.2
APOE * CR	3 & 1	0.09	0.78	-0.47
Met>Val	5 & 3	0.16	0.96	0.996
Val>Met	6 & 1	0.03	0.32	0.2
BDNF * CR	3 & 2	0.06	0.65	0.87
CR	3 & 2	0.03	0.64	0.87
Salience Network (SA)				
ε3 > ε4	2 & 1	0.22	0.79	-0.23
ε4 > ε3	4 & 3	0.05	0.29	0.84
APOE * CR	3 & 1	0.33	0.93	-0.29
Met>Val	2 & 1	0.15	0.65	-0.23
Val>Met	4 & 2	0.71	1	-0.35
BDNF * CR	3 & 1	0.35	0.94	-0.29
CR	4 & 3	0.1	0.6	0.84

Note: FEW = family-wise error; *APOE* = apolipoprotein E; *BDNF* = brain-derived neurotrophic factor; CR = cognitive reserve.

Discussion

The goal of this chapter was to investigate any differences in functional connection strength within both allelic variations of the *APOE* (ε3 and ε4) and *BDNF* Val66Met polymorphism within a recruited sample of the THBP. To my knowledge, this is the first project that explored differences in resting-state functional networks of the DMN, DAN, and SA between

healthy, older individuals with the *APOE* and *BDNF* Val66Met polymorphisms and relative to cognitive reserve. Higher functional edge strength was anticipated in *APOE* ε3 homozygotes and *BDNF* Val homozygotes, respectively, compared to ε4 carriers and Met carriers, respectively. In all analyses, no significant functional differences in edge strength were found. There were no significant associations found between cognitive reserve and edge strength in these networks, nor did cognitive reserve moderate the associations between the *APOE* and *BDNF* polymorphisms and edge strength.

Previous studies have shown both a decrease and increase in functional connectivity brain networks in healthy older adults (Toussaint et al., 2014). Frontal and temporal brain regions of the DMN exhibited functional reduction between the frontal gyri and precuneus-posterior cingulate, as well as between the temporal areas and precuneus-posterior cingulate with age in healthy older individuals (mean age 61) compared to younger adults (mean age 20) suggesting lower global efficiency, which was even more pronounced in AD (Toussaint et al., 2014). The increase of functional interactions of the frontal and parietal sub-networks of the DMN (intra-DMN) demonstrated enhanced higher local clustering (higher integration) in older adults than in the young group, suggesting that healthy elderly may be using compensatory mechanisms (Toussaint et al., 2014). Recently, enhanced functional connection strength was observed in AD within the frontal and temporal lobe regions compared to MCI (Li et al., 2016), suggesting compensatory mechanisms may in an attempt balance out impairments in brain function (Wang et al., 2015). Further, reduced connectivity within the DMN was found when investigating functional connectivity strength in AD, MCI, and controls (Li et al., 2016). There was a decrease of connection strength in MCI and AD compared to healthy controls, as well as a decline in functional connectivity in healthy individuals that converted to MCI after a 24 month follow-up, suggesting that functional

impairments can be observed in older individuals most at risk of conversion to MCI and AD (Li et al., 2016). Taking into consideration the clinical predictability of healthy individuals converting to preclinical AD (Li et al., 2016), probable biomarkers within the *APOE* and *BDNF* Val66Met polymorphisms were expected to be found, however, no differences in functional edge strength were detected. This suggests that carriership of the *APOE* ε4 allele or *BDNF* Met allele, respectively, did not influence functional brain organization in this group of older adults. Similarly, Mentink et al. (2020) investigated within a large sample size (approx. 1200) whether healthy younger *APOE* ε4 carriers would show functional co-activation within the DMN. Results revealed that carrying the ε4 allele did not affect the brain function, which could be due to a 'regression towards the mean effect' because greater cohort samples could contribute to higher heterogeneity (Mentink et al., 2020).

In the current chapter, this thesis was interested as to whether the *APOE* and *BDNF* Val66Met polymorphisms showed differences in network organizations within the DMN, DAN, and SN. No differences were found, which is inconsistent with Chen et al. (2015) who observed that healthy older ε4 carriers ($N = 35$) presented topological changes and reduced global efficiency within the parahippocampal gyrus in functional and structural white matter networks than ε4-non carriers ($N = 40$). The authors proposed that *APOE* ε4 carriers may be more vulnerable to AD-related impairments than ε4 non-carriers (Chen et al., 2015). Few studies have been published investigating the effect of the *BDNF* Val66Met polymorphisms on functional brain network organizations using graph theoretical measures within a healthy aging population. Therefore, it was examined whether there are any functional significant differences between the Met allele and Val allele in healthy elderly. In support, recently, Qi et al. (2020) reported no significant findings on resting-state degree centrality (number of connection of a node) between the *BDNF* genotypes in a Chinese cohort with posttraumatic

stress disorder (Qi et al., 2020). One simulation study explored white matter structural network connectivity and found that central nodes of white matter networks were more robust in Val homozygotes than Met carriers, also causing a reduction in global efficiency in Met carriers (Park et al., 2017). Structural connectivity (Park et al., 2017), as well as functional connectivity within healthy individuals in long-distance networks, was reduced in Met allele carriers compared to Val homozygotes (Rodríguez-Rojo et al., 2018) Potential explanations why the Met allele carriers may be broadly more susceptible to brain disorders is due to loss in antero-posterior gamma band functional connectivity caused by disruption of the excitatory-inhibition connection, which subsequently decreased synchronization with other relevant brain regions in Met allele carriers (Rodríguez-Rojo et al., 2018). Taken together, inconsistent with previous findings, the current chapter results suggest that healthy older adults did not show differences related to the *APOE* and *BDNF* Val66Met polymorphisms, respectively, within the DMN, DAN, and SN.

It is important to highlight that cognitive reserve was not associated with higher functional connection strength within the DMN, DAN, and SN, and also did not modify differences between polymorphisms (*APOE* and *BDNF* Val66Met). This is in contrast to data with graph network measures (Marques et al. (2016) that showed that greater functional connectivity, local efficiency and clustering of occipital regions, nodal strength, and betweenness centrality (many shortest paths connections) were linked to cognitive reserve, particularly higher levels of education. In support, it was found that education enhanced network reliability during healthy aging (Yoo et al., 2015). Further, it was suggested that cognitive reserve may be ensuring the flow of information through neural compensation using substitute connections within a network. Relatedly, it has been proposed that neural processing is triggered by cognitive reserve balancing integration and segregation within a network (Marques et al.

(2016). While it has been established that cognitive reserve is associated with greater functional connectivity and better network measures, it is less clear why cognitive reserve is neither influencing functional connections strength nor modifying the difference between the *APOE* and *BDNF* Val66Met polymorphisms in the current chapter.

There may be several explanations for these inconsistent findings. One possibility is that the sample size (*N*=76) of the current project was too small to detect any significant findings. Increasing sample size would enhance statistical power in this project, however fMRI studies have relatively high cost and therefore this would have been challenging. In future studies, the extraction of more than 70 components from MELODIC should be considered to potentially obtain higher numbers of nodes. This is a highly intensive computational process and a computer with greater power would potentially have been advantageous.

In conclusion, the current results suggest that functional edge strength was not significantly different between healthy older adults carrying variants of the *APOE* and *BDNF* genotypes. No correlation between cognitive reserve and functional edge strength was observed. This outcome was hypothesised to reflect alterations between genotypes, in which ε3 homozygotes and Val homozygotes may have stronger edge strength than ε4 carriers and Met carriers. Overall, functional graph measures have shown high reliability in previous studies and may play an important role as a biomarker to identify individuals at risk for neurodegenerative diseases. Future studies should consider measuring biomarkers of AD-related pathologies such as amyloid-beta and tau in the design of statistical analyses.

Note: This chapter was exploratory investigating resting-state fMRI. After re-examination the data, an inclusion of a subject with a brain tumour was identified. Subsequently, this subject

was removed from further analyses within the following chapters, reducing the sample size from 77 to 76. Reducing the sample size by one participant would have not changed the results of the following presented chapters. Furthermore, because of high computational and time-consuming analyses, the components were reduced from 70 (network modelling approach) to 25 components (voxel-based approach).

Chapter 4

Interactive effects of the *APOE* and *BDNF* polymorphisms on functional brain connectivity

Abstract

Resting-state functional magnetic resonance imaging (fMRI) can measure the consequences of pathological alterations in neurodegenerative diseases, including Alzheimer's disease (AD). Disruption in functional connectivity may be a potential biomarker of aging and early brain changes associated with AD-related genes, such as APOE and BDNF. The objective of this study was to identify group differences in resting-state networks between individuals with *BDNF* Val66Met and *APOE* polymorphisms in cognitively healthy older persons. Dual regression following Independent Components Analysis were performed to examine differences associated with these polymorphisms. *APOE* ε3 homozygotes showed stronger functional connectivity than *APOE* ε4 carriers. Males showed stronger functional connectivity between the Default Mode Network (DMN) and grey matter premotor cortex, while females showed stronger functional connectivity between the executive network and lateral occipital cortex and parahippocampal gyrus cingulate gyrus. Additionally, it was found that with increasing cognitive reserve, functional connectivity increased within the Dorsal Attention Network (DAN), but decreased within the DMN. Interaction effects indicated stronger functional connectivity in Val/ε3 homozygotes and Met/ε3 carriers than in Met/ε4 and Val/ε4 within both the DMN and DAN. Stronger functional connectivity was also found in Met/ε3 compared to Val/ε4 carriers between the dorso-ventral stream and occipital cortex. APOE/BDNF interactions may therefore influence the integrity of functional brain connections in older adults, and may underlie a vulnerable phenotype for subsequent Alzheimer's-type dementia.

Introduction

Alzheimer's disease (AD) is the most common form of aging-related dementia, as described in Chapter 1, accounting for 60 to 80% of all cases(Gaugler, James, Johnson, Marin, & Weuve, 2019). The primary functional manifestations of AD include memory loss, impairment in executive functioning, difficulties with language, and changes in personality and behaviour, with brain pathology characterised by neurofibrillary tangles and amyloid-beta deposition (Gaugler et al., 2019; Mattson, 2004). Neuroimaging techniques investigating activity within and between resting-state networks, such as the default mode network (DMN)(Raichle et al., 2001), the dorsal-attention network (DAN)(Corbetta & Shulman, 2002), and salience network (SN)(He et al., 2014), may help provide an understanding of the elementary brain changes that are associated with aging and subsequent risk of AD. In this regard, significant aging-related changes in functional connectivity have been observed within the DMN (Binnewijzend et al., 2012; Greicius et al., 2004). Many studies have focused on the role of the DMN in AD, with some studies showing increased functional connectivity (Pasquini et al., 2015), while others showed decreased functional connectivity (Binnewijzend et al., 2012; Greicius et al., 2004; Zhang et al., 2009). A further interesting network is the DAN, which is activated during goal-directed behaviour (Corbetta & Shulman, 2002). The DAN showed significant decline in functional connectivity in the amnestic form of mild cognitive impairment (MCI) and in AD (Zhang et al., 2015) compared to neurologically healthy individuals. This disturbance in connectivity increases with disease progression. Similar results were found within the SN, in which reduced grey matter volume and functional connectivity were found in patients with AD. Moreover, it was found that healthy older individuals had intra-network functional connectivity impairments between crucial nodes, such as the DMN, indicating that the SN is affected by normal aging before manifestation of AD (He et al., 2014). The SN is involved with processing and filtering of

incoming information (Peters, Iyer, Itti, & Koch, 2005), and is active in higher-order processing (Menon, 2015).

Both life-course and genetic factors may influence susceptibility to aging and AD-related changes in functional connectivity. Cognitive reserve, which is a theoretical construct (Stern, 2006) as discussed in Chapter 1, is an example of a potentially modifiable risk factor for cognitive decline (Stern & Munn, 2010) and may influence functional brain organization. Other possible factors that may influence functional connectivity are gender difference. When comparing males and females' economic position, men have a superior status in the socio-economic world with higher income and better education, however, women have a higher life expectancy (Carmel, 2019). Nowadays, more women take up tertiary educations. These different lifestyles may influence the connectivity in the brain, therefore, exploratory I wanted to investigate whether there are differences in resting-state functional connectivity in healthy older adults. Further, well-known factors that can influence functional connectivity are variations in genes associated with risk of aging and AD-related cognitive decline.

For example, as described in previous chapters, the apolipoprotein E gene (*APOE*) ε4 allelic variant is known to be a major risk factor for late-onset AD as compared to the ε3 and ε2 alleles (de-Almada et al., 2012). With respect to functional connectivity, it is unclear if healthy older adult ε4 carriers show changes in DMN functional connectivity compared to ε3 homozygotes. Studies variably show decreased DMN connectivity (Goveas et al., 2013; Lu et al., 2017) or increased connectivity (Wu et al., 2016); whereas others found no difference between ε4 carriers and ε3 homozygotes (Staffaroni et al., 2018).

A common variation is the gene encoding is brain-derived neurotrophic factor (*BDNF* Val66Met). As characterised in earlier chapters, BDNF is a protein important for neurogenesis and synaptic plasticity, has also been investigated for its potential role in brain aging and AD-related decline. Decreased memory function and reduced hippocampal volume

have been described in Met allele carriers compared to Val homozygotes (Brown et al., 2014; Lim et al., 2016). Resting-state functional connectivity has been reported to be relatively decreased in the hippocampus in middle-aged (Yin et al., 2015) and older (Rodríguez-Rojo et al., 2018) individuals who are Met carriers. Such studies have also demonstrated increased functional connectivity in the dorsal lateral prefrontal cortex and anterior insula of Met carriers in a younger healthy population compared to Val homozygotes (Wang et al., 2014). Interactions between the APOE and BDNF gene variants may also be possible, as *APOE* ε4 and *BDNF* Met carriers may show relatively increased aging-related episodic memory impairment (Ward et al., 2014).

The current chapter examined the potential individual and interactive roles of the BDNF and APOE gene variants relative to functional connectivity of resting-state networks in subjects purposively sampled from the cohort study, the Tasmanian Healthy Brain Project (THBP). Subject-specific maps were used to examine age-related differences in functional connectivity within the DMN, DAN and SN. This chapter addresses the hypotheses five and six of this thesis; namely, it was hypothesised that the connectivity of the selected resting-state networks will differ between the *APOE* (ε3 & ε4) and between the *BDNF* Val66Met polymorphisms, and it was also predicted that interaction effects will be found between these polymorphisms based on the findings of Ward et al. (2014) in this cohort. In particular, lower functional connectivity was expected in Met carriers and ε4 carriers compared to Val homozygotes and ε3 homozygotes. All analyses were controlled for age, cognitive reserve, and GM maps. Finally, this thesis investigated whether cognitive reserve influenced functional connectivity in the three aforementioned networks, controlled individually for each analysis for BDNF and APOE. Afterall, gender differences were exploratory investigated within the DMN, DAN, SN.

Materials and methods

Study population

Participants

The cohort and recruitment strategies for this chapter is described in chapter 3 (page 77). The proportion of experimental and control participants within the ε3 homozygotes (experimental = 33, controls = 9) and ε4 carriers (experimental = 26, controls = 8), and within Val homozygotes (experimental = 31, controls = 10) and Met carriers (experimental = 28, controls = 7) was relatively balanced and therefore the proportion of experimental and controls were not included as a covariate. Demographic and clinical data for the final sample at baseline are presented in Table 4.1.

Procedure

As described in Chapter 3, THBP participants undertook a comprehensive clinical test battery measuring neuropsychological, cognitive, health, and psychosocial factors (Summers et al., 2013).

Cognitive reserve

The composite score of cognitive reserve, as described in Chapter 3, has been used within this chapter.

Genotyping

DNA samples from all 78 participants were available through previous work. Details about the collection of DNA samples is described in Chapter 2 (page 66).

Magnetic resonance imaging (MRI)

As described in Chapter 2 & 3, all brain scans were acquired using a General Electric (GE) Signa 3-Telsa scanner in the Royal Hobart Hospital. All acquisition information is outlined in more detail in Chapter 2 & 3 (page 66 & page 83).

Subcortical and cortical volumetric processing

Subcortical and cortical volumetric processing was performed with FreeSurfer software (https://surfer.nmr.mgh.harvard.edu/) version 6.0.0. Freesurfer was used to obtain total intracranial volume, which was used as a covariate in the analyses of this chapter. Briefly, the automated FreeSurfer pipeline involved motion correction (Reuter, Rosas, & Fischl, 2010), non-uniform intensity normalisation (N3) at 500 iterations to correct for intensity non-uniformity artifacts (Sled, Zijdenbos, & Evans, 1998), automated Talairach transformation, normalization (Sled et al., 1998), removal of non-brain tissue (Segonne et al., 2004), linear and non-linear registration, cortical parcellation (Desikan et al., 2006), and segmentation of white and grey matter volume (Fischl et al., 2002; Fischl et al., 2004). Cortical thickness is calculated as the closest distance from grey/white boundary to grey/CSF boundary at each vertex on the tessellated surface (Fischl & Dale, 2000). For further statistical analysis, grey matter volumes of hippocampus and amygdala, and cortical thickness values of entorhinal cortex and parahippocampus were extracted from FreeSurfer's automated parcellation procedure (Desikan et al., 2006; Reuter, Schmansky, Rosas, & Fischl, 2012). Registered data that was extracted for each subject was visually inspected for accurate representation of the cortical surface. No images were excluded.

Functional magnetic resonance imaging (fMRI) data analysis

Resting-state fMRI Data Pre-processing

Resting-state images were pre-processed using FMRIB Software Library (FSL) (Jenkinson et al., 2012; Smith et al., 2004). All pre-processing steps information are outlined in Chapter 2 & 3 (page 67 and page 83). After pre-processing and cleaning the dataset, the same datasets as in Chapter 3 were used for further analyses.

Group independent components analysis

To identify resting-state networks, a group-level ICA decomposition was used, which has been discussed in earlier chapters. Following Feis et al. (2015) and Damoiseaux et al. (2006), 25 independent components were extracted using MELODIC. This number of components was chosen to avoid "over-fitting", which implies that too many components could have caused fragmentation of signal across multiple component maps decreasing the ability to identify the signals of interest. The group-average maps were used as a template for dual regression (see below). According to Griffanti et al. (2017), the components of the template were manually classified as resting-state networks or noise artefacts (Beckmann et al., 2005a; Salimi-Khorshidi et al., 2014). Examples of noise artefacts are motion, cardiac pulsation, or respiration, which are also referred to structured noise because these artefacts are gaussian distributed. As previously discussed, with ICA, these structural noise components can be separated from components with neuronal signal. Differentiating these independent components can be completed using FSLeyes, which includes a MELODIC mode layout. Within this layout, there is an axial slice's view of the spatial map for each component (Figure 4.1). On the right side of the FSLeyes layout is a list displayed that consists of rows for each single component, which I used to label the component either for signal or noise. At the bottom is a time course, which is related to that component and a power spectrum, which is its Fourier transform. Thus, after melodic, I had to view each individual components spatial

map, time course, and power spectrum and decide and label it either as noise or neuronal signal.

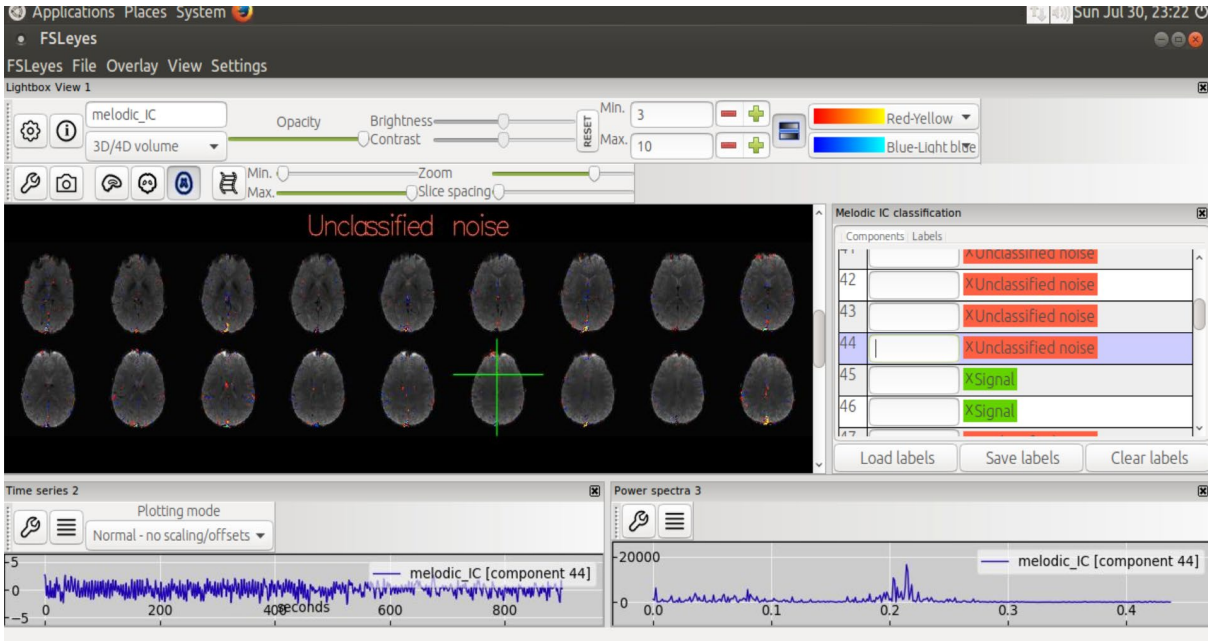


Figure 4.1. FSLeyes layout of one single subject, displaying the main view, the list on the right to label components, time course and power spectrum at the bottom.

Dual regression

The template from the group-ICA was used in a dual regression analysis to estimate differences in connectivity between different genetic groups. An additional dual regression was performed to investigate whether cognitive reserve alone influenced functional connectivity. The dual regression analysis estimates sensitivity to amplitude network activity and to alterations in spatially distributed correlation patterns (Beckmann et al., 2009; Nickerson et al., 2017) of resting-state networks. The output of the dual regression produces subject-specific spatial maps and subject-specific data time courses (Smith et al., 2014). The FSL tool, randomise, was used for nonparametric permutation inference (Winkler, Ridgway, Webster, Smith, & Nichols, 2014). General Linear Models (GLM) were used to identify group differences, using the GLM tool in FSL. Statistical significance was evaluated using Monte Carlo permutation-based statistical testing with 5000 permutations at alpha = 0.05

(Nichols & Holmes, 2002). Family-wise error corrections for multiple comparisons using threshold-free cluster enhancement (Smith & Nichols, 2009) was used to control Type 1 error rate when investigating differences between *BDNF* Met vs *BDNF* Val, *APOE* ε4 vs *APOE* non-ε4; and the interactions between Met/ε3, Met/ε4, Val/ ε3, Val/ε4. For analyses of genotype, cognitive reserve, age, and GM maps were included as covariates to control for individual differences in these parameters. Using the Harvard-Oxford Cortical and Subcortical atlases (Zilles & Amunts, 2010), probabilistic anatomical labels for local maxima were obtained. All coordinates are reported in MNI space.

Statistical analysis of demographics

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) Version 24 from Windows (Chicago, IL, USA). For demographics and clinical characteristics of the different polymorphisms, descriptive statistics were applied. A chi-squared test was performed to compare group differences in gender and education intervention groups across genotypes. A composite score for cognitive function on the domain of language was computed using factor loadings published in earlier work (Ward et al., 2014). The cognitive tests included were Wechsler adult intelligence scale vocabulary and comprehension subtests, and the Boston naming test. To aid interpretability, z-scores were computed by mean-centering and scaling to unit standard deviation .

Results

The current study included only ε3 homozygotes and ε3ε4 carriers. Hence, two subjects were excluded due to the *APOE* genotypes, *APOE* ε2ε3 and *APOE* ε4ε4. One further subject was excluded due to recurring errors within the single-subject independent component analysis,

and one subject was excluded due to structural abnormalities in the brain. Participant demographics and characteristics of the 76 THBP subjects comprising this study population are provided in Table 4.1. *APOE* and *BDNF* polymorphisms were balanced between genders. A chi-square test was performed to explore the relationship between *APOE* and *BDNF* genotypes and gender. The results showed that the cohort proportions between *APOE* ε3 homozygotes and ε4 carriers, and between *BDNF* Val homozygotes and Met carriers were fairly balanced with no significant differences in proportion. Family-wise error corrections for multiple comparisons using threshold-free cluster enhancement (Smith & Nichols, 2009) was used to control Type 1 error rate when investigating differences between *BDNF* Met vs *BDNF* Val, *APOE* ε4 vs *APOE* non-ε4; and the interactions between Met/ε3, Met/ε4, Val/ ε3, Val/ε4.

Table 4.1. Seventy-six participants characteristics expressed as mean (M) ± standard deviation (SD) unless otherwise noted

	Met	Val	*p*-value	ε4	ε3	*p*-value
Gender (F : M)	25:10	27:14	0.60	23:11	29:13	0.90
Intervention (*N*) (Ex : Con)	28:7	31:10	0.65	26:8	33:9	0.83
Age (years)	63 ± 5.69	63.6 ± 6.82		63.3 ± 5.15	63.3 ± 7.14	
Education (years)	16.7 ± 1.32	16.8 ± 1.04		16.5 ± 1.11	16.9 ± 1.2	
Cognitive reserve (*z*)	0.20 ± 0.82	0.20 ± 0.88		0.06 ± 0.95	0.28 ± 0.76	
Language (*z*)	0.01 ± 0.93	.16 ± 0.92		0.11 ± 1.01	0.08 ± 0.87	

Note: Data represented are mean values (*SD*) for continuous variables and proportions for categorical variables. Met and Val relate to participants with specific *BDNF* Val66Met polymorphisms. ε4+ and ε4- refer to participants of the *APOE* polymorphism.
F = female; M = male; Ex = Experimental; Con = Controls; *z* = z score.

Resting-state network identification

The group independent component analysis (ICA) output allowed me to identify networks from 25 independent components. Within all independent components, 12 networks were detected that have been defined in healthy individuals (Becerra et al., 2014; Beckmann et al.,

2005a; Damoiseaux et al., 2006; De Luca, Beckmann, De Stefano, Matthews, & Smith, 2006). Identified networks comprised the precuneus together with the DMN, SN, DAN including the left & right dorsal-/ lateral ventral stream, as well as the visual networks (medial, lateral), sensory-motor network, executive control network, auditory network, and cerebellum network (Figure 4.1.).

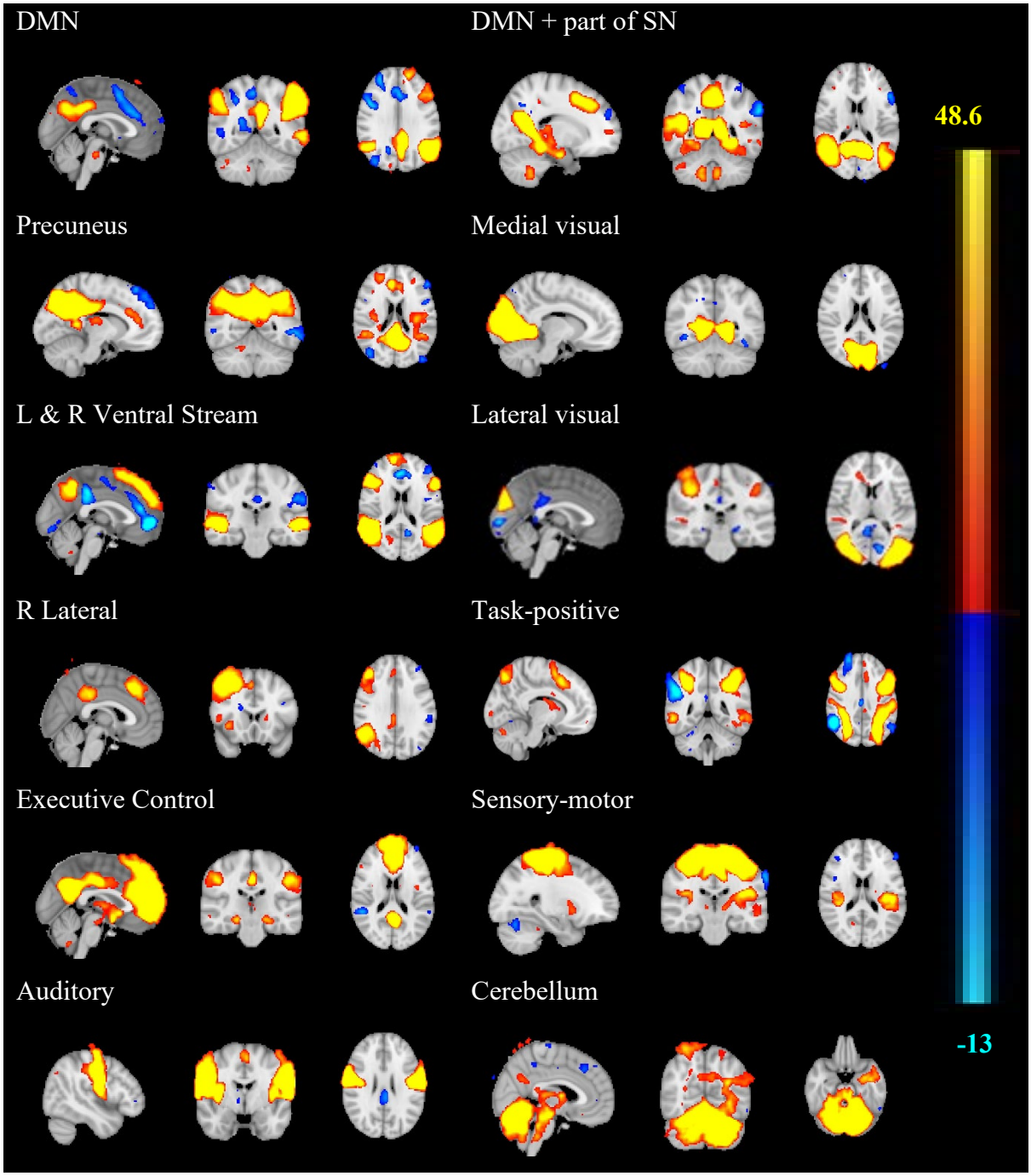


Figure 4.1. Thirteen resting-state networks from healthy older participants of the Tasmanian Healthy Brain Project. Images depict the percentage of BOLD signal change resulting from the Group-ICA, overlaid on an average high-resolution image transformed into standard

space (MNI152), thresholded of min brightness of 3 and max contrast of 8. Red to yellow indicates the percentage signal change showing positive values with this component. Blue shows the negative values indicating anti-correlation with this component.

Multivariate voxel-based analyses within resting-state networks of the polymorphisms

After performing a dual regression for each *APOE* and *BDNF* polymorphisms, there was a significant difference in functional connectivity between DAN and occipital cortex, superior division, where *APOE* ε3 homozygotes showed stronger functional connectivity compared to ε4 carriers, p = .02, (p =.04 after controlling for age & grey matter (GM) maps, p =.03 after controlling for gender & GM maps, p =.04 after controlling for cognitive reserve & GM maps,, Figure 4.2.,Table 4.2.). The dual regression for the *BDNF* Val66Met polymorphisms revealed no significant differences between Met carriers and Val homozygotes.

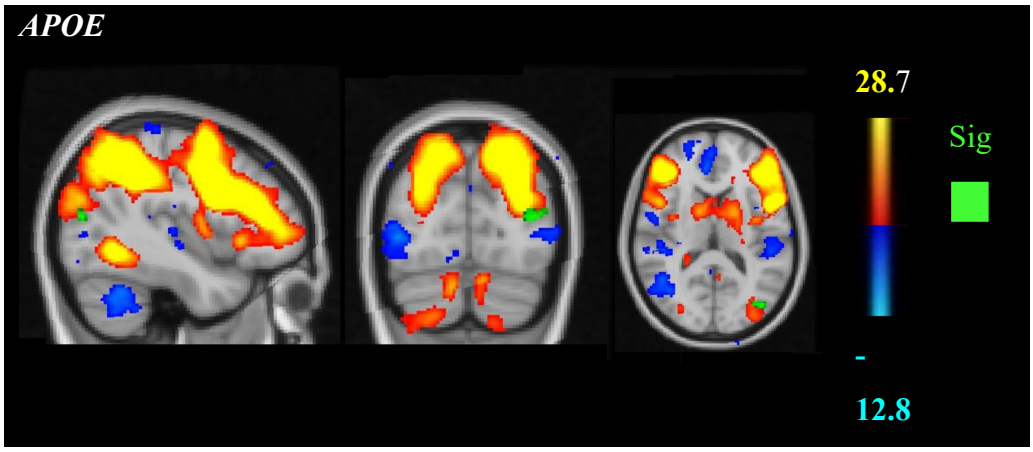


Figure 4.2. Red-yellow represent the dorsal attention network (DAN) after performing a group-ICA. Blue shows the negative values indicating anti-correlation with this component. Green voxels show increased functional connectivity in ε3 homozygotes compared to ε4 carriers in the occipital cortex ($p < 0.05$ family-wise error [FWE]-corrected). Montreal Neurological Institute (MNI) coordinates x = -42, y = -80, z = 16. Results were controlled for age, gender, and cognitive reserve.

Table 4.2 | Apolipoprotein E (*APOE*) results of analyses after running dual regressions using GLMs

Cluster Index	Voxels	X (vox)	Y (vox)	Z (vox)	*1-p*	*p*-value	Location
APOE (controlled for GM maps only)							
DAN (ε3 > ε4)							
1	63	66	23	44	0.98	.02	Lateral occipital cortex (superior and inferior division)
APOE (controlled for age and GM maps)							
1	40	65	24	44	0.962	.038	Lateral occipital cortex (superior and inferior division)
APOE (controlled for gender and GM maps)							
1	64	65	24	44	0.974	.026	Lateral occipital cortex (superior and inferior division)
APOE (controlled for cognitive reserve and GM maps)							
1	36	66	23	44	0.963	.037	Lateral occipital cortex (superior and inferior division)
APOE (controlled for gender, age, cognitive reserve, & GM maps)							
1	9	65	24	44	0.952	.048	Lateral occipital cortex (superior and inferior division)

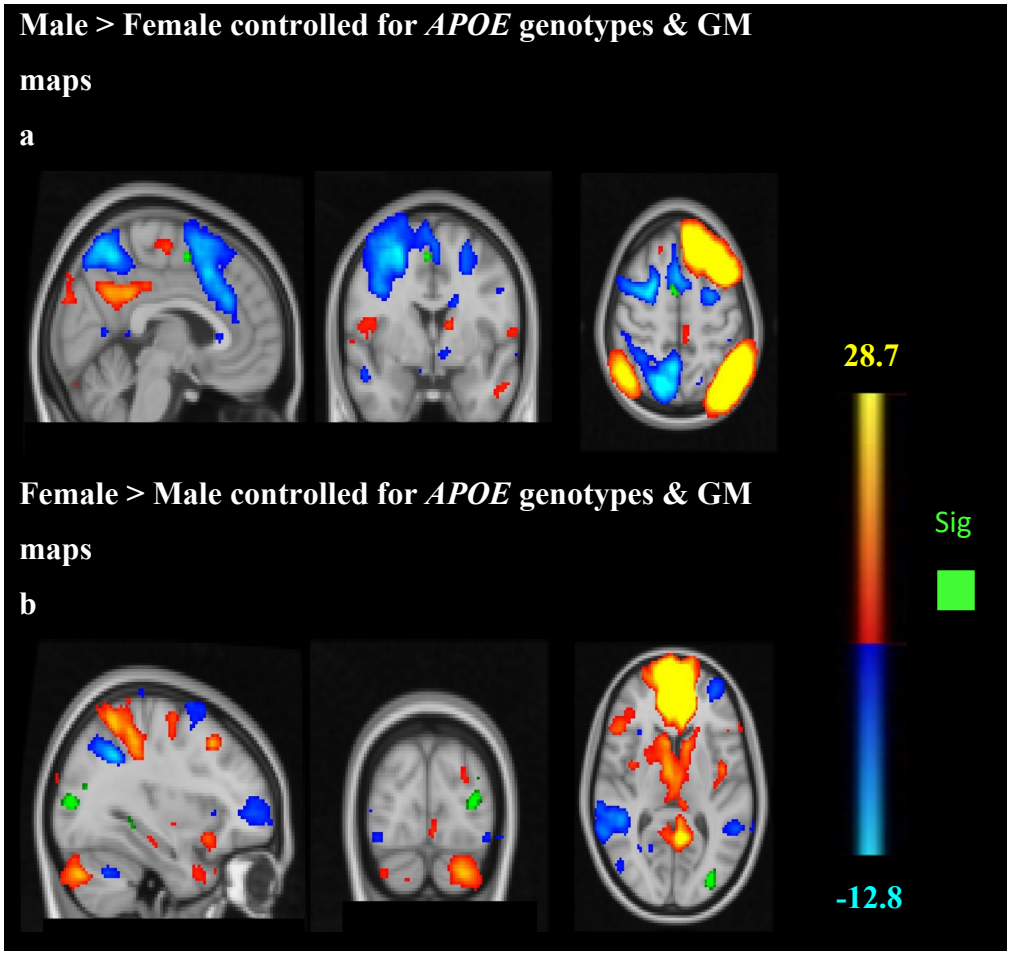
Note: GLMs = General Linear Models; DAN = Dorsal Attention Network; APOE = Apolipoprotein E; ε3 = ε3ε3 homozygotes; ε4 = ε3ε4 carriers; GM = Grey matter.

Gender differences

This analysis demonstrated that there were gender differences, in which males showed stronger average functional connectivity between the DMN and the Juxtapositional Lobule cortex, p = .018, controlled for *APOE* polymorphisms and GM maps. Female subjects showed stronger functional connectivity between the executive network and six different clusters, of which the lateral occipital cortex (inferior & superior divisions) p = .005 and the parahippocampal gyrus/lingual gyrus, p = .014 showed the strongest differences, controlled

for *APOE* genotypes and GM maps, p = .03 controlled for *APOE* genotypes, age, cognitive reserve and GM maps

Similarly, males showed stronger functional connectivity between the DMN and the Juxtapositional Lobule cortex, p = .017, controlled for *BDNF* genotypes and GM maps. While, females showed stronger functional connectivity between the executive network and seven different clusters, of which the lateral occipital cortex (inferior & superior divisions) p = .005 and the parahippocampal gyrus/lingual gyrus, p = .011 showed the strongest differences, controlled for *BDNF* genotypes and GM maps, p = .03 controlled for *BDNF* genotypes, age, gender, cognitive reserve and GM maps.



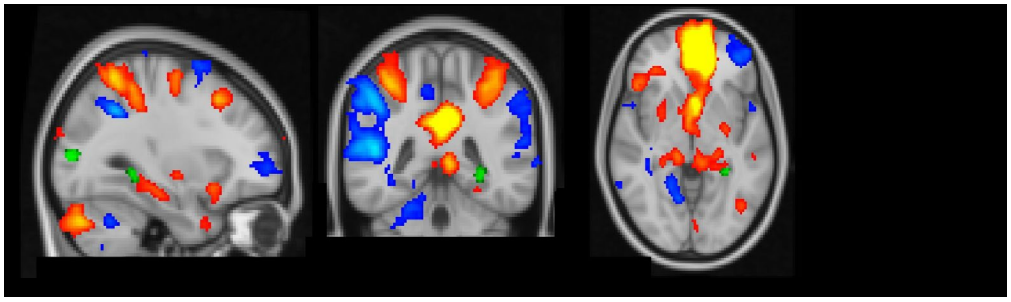


Figure 4.2. Group-ICA components of the DMN/dorsal-ventral stream and DMN/executive function network. Males demonstrating stronger functional connectivity than females between the DMN/dorsal ventral stream (red-yellow/blue) and Juxtapositional lobule cortex (green) ($p < 0.05$ family-wise error [FWE]-corrected). Montreal Neurological Institute (MNI) coordinates x = 4, y = 0, z = 54. (b). Females showed stronger functional connectivity between the DMN/executive network (red-yellow/blue) and the lateral occipital cortex (superior & inferior divisions) (x = -32, y = -86, z = 8) and the parahippocampal region (x = -30, y = -42, z = -4).

APOE x BDNF interactions

An interaction between the *BDNF* Val66Met and *APOE* polymorphisms was found, with slightly stronger functional connectivity between the DAN and the posterior default mode region in Met/ε3 carriers compared to Met/ε4, p = .04. Stronger functional connectivity was found between the DMN/dorsal-ventral stream and occipital pole in Met/ε3 relative to Val/ε4 carriers, p = .016 (Figure 4.4.a, Table 4.3.).

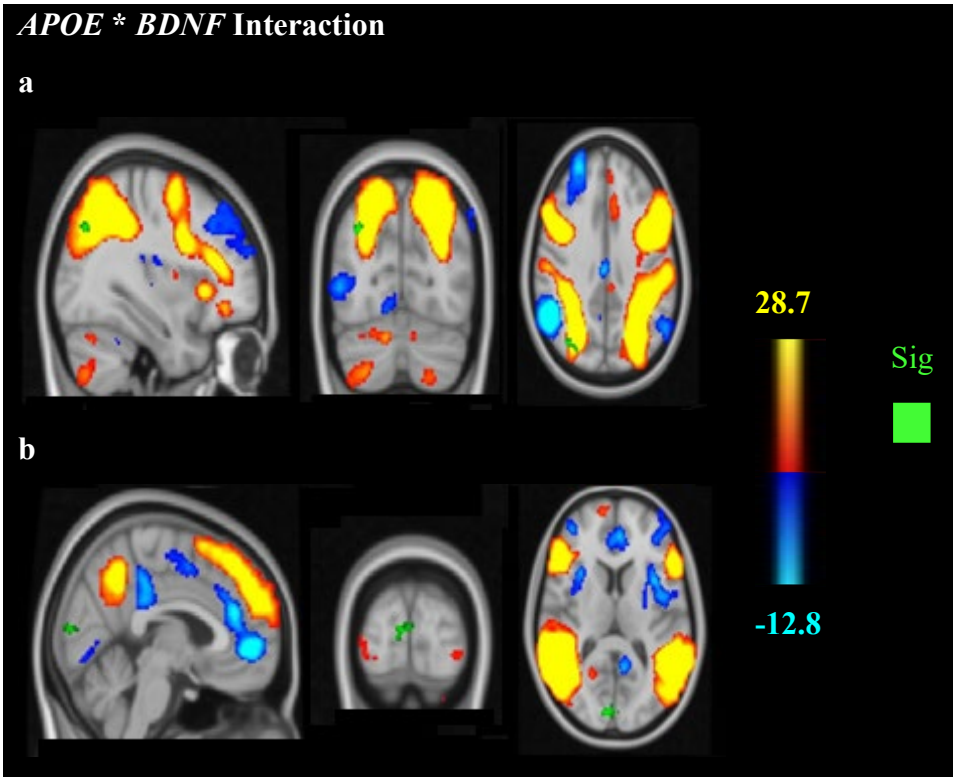


Figure 4.4. A & B. Group-ICA spatial maps of the DAN (A) and the dorso-ventral stream/DMN (B). **(a)** The findings demonstrate increased connectivity between the DAN and posterior default mode regions (green) in Met/ε3 compared to Met/ε4 (x = 48, y = -18, z = 2). **(b)** Stronger connectivity was found between the dorsal-ventral stream/DMN and the visual cortex (green) in Met/ε3 carriers than in Val/ε4 carriers (x = 8, y = -90, z = 8) ($p < 0.05$ family-wise error [FWE]-corrected).

Table 4.3 | Apolipoprotein E *(APOE)* * Brain-derived neurotrophic factor (*BDNF)* Interaction

DAN (Met/ε3 > Met/ε4)							
Cluster Index	Voxels	X (vox)	Y (vox)	Z (vox)	*1-p*	*p*-value	Location
1	3	30	25	54	0.953	.047	Lateral Occipital Cortex, superior divison
2	11	27	27	54	0.963	.037	Lateral Occipital Cortex, superior divison
DMN/dorsal-ventral stream (Met/ε3 > Val/ε4)							
1	110	41	18	40	0.984	.016	Occipital pole, Intracalcarine Cortex

Note: APOE = Apolipoprotein E; BDNF = brain-derived neurotrophic factor; DMN = Default mode network; DAN = Dorsal Attention Network; GM = grey matter; Met/ε3 = participants not carrying the *APOE* ε4, but at least one copy of the *BDNF* Met alleles; Met/ε4 = participants carrying at least one copy of the *APOE* ε4/*BDNF* Met alleles; Val/ε4 = participants being a Val homozygote and carrying at least one of the *APOE* ε4 alleles; Val/ε3 = participants not carrying the *APOE* ε4 allele and being a Val homozygote.

Cognitive Reserve

Cognitive reserve was associated with increased functional connectivity between the DAN and two clusters, left grey matter hippocampal and amygdala regions, p = .038, and subcallosal cortex, p = .015 as well as between the DAN and white matter callosal cortex controlled for *APOE*, p = .03 & p = .046, controlled for *BDNF*, p = .016; and between the central executive network and postcentral gyrus, controlled for *APOE* genotypes, p = .04. Interestingly, functional connectivity decreased with increasing cognitive reserve within the DMN, controlled for *APOE* genotypes, p = .02, controlled for *BDNF* genotypes, p = .017 (Table 4.4, Supplementary Figure 4.5).

Table 4.4 | Cognitive reserve results of analyses after running dual regressions using GLMs

Positive correlation between cognitive reserve and functional connectivity, controlled for GM maps							
DAN							
Cluster Index	Voxels	X (vox)	Y (vox)	Z (vox)	*1-p*	*p*-value	Location
1	8	59	58	27	0.962	.038	Left GM hippocampus regions, Left GM amygdala regions
2	24	44	76	34	0.985	.015	Subcallosal Cortex
Negative correlation between cognitive reserve & functional connectivity, controlled for *APOE* genotypes & GM maps							
DMN							
1	2	33	26	46	0.954	.046	Lateral Occipital cortex, superior division
2	6	50	28	44	0.958	.042	Intracalcarine Cortex, Supracalcarine Cortex, Cuneal Cortex, Precuneus
3	80	56	28	44	0.98	.02	Cuneal Cortex, Supracalcarine Cortex, Precuneus,
4	142	42	33	41	0.98	.02	Precuneus, Intracalcarine Cortex
Positive correlation between cognitive reserve & functional connectivity, controlled for *APOE* genotypes & GM maps							

DAN							
1	3	46	75	38	0.954	.046	WM Callosal Cortex
2	9	44	76	38	0.974	.026	WM Callosal Cortex
Central Executive Network							
1	3	12	59	51	0.96	.04	Postcentral gyrus
Negative correlation between cognitive reserve and functional connectivity, controlled for *BDNF* genotypes & GM maps							
DMN							
1	2	32	31	43	0.952	.048	Supracalcalinne Cortex, Intracalcarine Cortex, Cuneal Cortex,
2	12	50	28	44	0.963	0.37	Intracalcarine Cortex, Supracalcalinne Cortex, Cuneal Cortex, Precuneus Cortex
3	41	33	26	46	0.971	.029	Lateral Occipital Cortex (superior division)
4	83	56	28	44	0.976	.024	Cuneal Cortex, Supracalcarine Cortex
5	196	35	35	44	0.983	.017	Precuneus, Supracalcarine Cortex
Positive correlation between cognitive reserve & functional connectivity controlled for *BDNF* genotypes & GM maps							
DAN							
1	21	43	75	35	0.984	.016	Subcallosal Cortex

Note: GLMs = General Linear Models; DMN = Default Mode Network; DAN = Dorsal Attention Network; APOE = Apolipoprotein E; GM = grey matter; BDNF = Brain-derived neurotrophic factor.

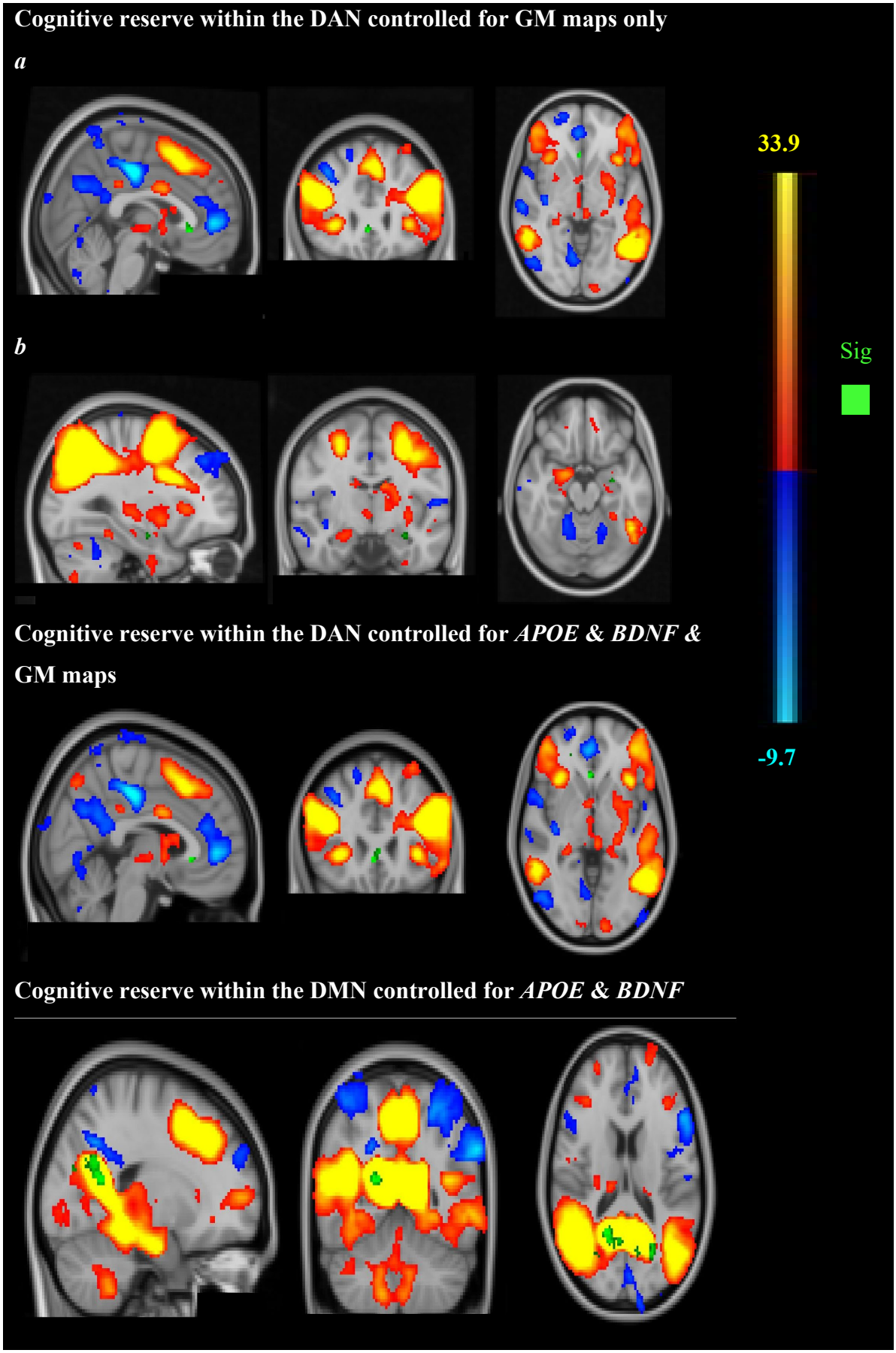

Cognitive reserve within the DAN controlled for GM maps only
a
b
33.9
Sig
-9.7
Cognitive reserve within the DAN controlled for APOE & BDNF & GM maps
Cognitive reserve within the DMN controlled for APOE & BDNF

Figure 4.5. Cognitive reserve influences on functional resting networks. Group-level ICA spatial maps of the DAN (top) and DMN (bottom; in red-yellow) overlaid with clusters showing significant influences of cognitive reserve on functional connectivity. Increased functional connectivity associated with increased cognitive reserve in the DAN. MNI coordinates x = -26, y = -8, z = -4 (a; left hippocampus/left amygdala). MNI coordinates x = 2, y = 26, z = -4 (b; Subcallosal Cortex). Increased functional connectivity associated with increased cognitive reserve within the DAN. MNI coordinates x = -2, y = 26, z = 4 (WM Callosal Cortex). Decreased functional connectivity was associated with increased cognitive reserve in the DMN. MNI coordinates x = 20, y = -56, z = 16 (Precuneus). These results are reported after controlling for *APOE* & *BDNF* genotypes, and GM maps. (All results were $p < 0.05$ family-wise error [FWE]-corrected).

Discussion

In this thesis, cross-sectional differences in resting-state networks were investigated between variants of the *BDNF* Val66Met and *APOE* (ε4 & ε3) polymorphisms using resting-state fMRI in an older adult population. In this chapter, we identified stronger functional connectivity in APOE ε3 homozygotes than in APOE ε4 carriers, as well as interacting associations between the *APOE* and *BDNF* polymorphisms with respect to connectivity in the DAN and dorsal-ventral stream/DMN. However, there was no significant differences in functional connectivity between *BDNF* Met carriers and Val homozygotes after controlling for GM maps. Last, it was found that cognitive reserve was positively associated with functional connectivity within the DAN and executive network but was negatively associated with increasing connectivity within the DMN.

Previous research found decreased functional connectivity within the DMN in healthy *APOE* ε4 carriers (Dowell et al., 2016; Goveas et al., 2013; Wu et al., 2016). In this study significant differences were found between ε3 homozygotes and ε4 carriers within the DAN after controlling for GM maps, as well as after controlling for age, gender, and cognitive reserve. In support Goveas et al. (2013) also reported disrupted functional connectivity in healthy older ε4 carriers. On the other hand, Dowell et al. (2016) investigated 37 healthy mid-aged

individuals (average age 50) and did not find any *APOE* effects within any resting-state networks, suggesting that alterations within the DMN are not found before the age of 55 years. Furthermore, Dowell et al. (2016) also reported stronger functional connectivity in the medial visual network in ε3 homozygotes, however, only in younger healthy ε3 homozygotes (average age 21) compared to ε4 carriers. Compared to our study, Goveas et al. (2013) had a similar methodological approach, with almost identical fMRI acquisitions. Goveas et al. (2013) controlled for age, gender, education (in years), and grey matter volumes, while this chapter controlled for age, cognitive reserve, and GM maps. There were differences in total sample sizes; while Goveas et al. (2013) had a sample size of 46 healthy individuals recruited in Wisconsin (United States), this chapter recruited 76 healthy elderly in Tasmania (Australia). Furthermore, Goveas et al. (2013) included 20 ε4 carriers (mean age 52.4), as well as 26 non-ε4 carriers that included both, ε2 and ε3 carriers (mean age 54.5), while the current study comprised of 34 ε4 carriers (mean age 63) and 42 ε3 homozygotes (mean age 63) . The *APOE* ε2 allele is known to have protective effects against AD pathology (Suri, Heise, Trachtenberg, & Mackay, 2013). Both studies, Goveas et al. (2013) and our study indicated that carriage of an *APOE* ε4 allele could cause potential disruption in functional resting-state networks.

In this study, there were no significant differences between *BDNF* Met carriers and *BDNF* Val homozygotes for functional connectivity within the DMN, DAN, and SN. In a previous study, Rodríguez-Rojo et al. (2018) found that 36 female *BDNF* Met carriers showed poor functional connectivity and reduced memory performance compared to Val homozygotes, using gamma band resting-state functional connectivity. Together, these results suggest that functional connectivity may be influenced by the *APOE* polymorphisms, which possibly is related to AD-pathology, for instance amyloid-beta accumulation in the brain,

while the *BDNF* polymorphisms may not have a strong influence on aging-related functional connectivity alterations in brain networks.

An additional finding of the current study was that interaction effects were found between the *APOE* and the *BDNF* polymorphisms. Kauppi et al. (2014) previously described that *APOE* ε3/ε4 alleles and *BDNF* Met/Met alleles individually, were less likely to recruit regions of the hippocampus during an encoding task (memory processing). This gene combination was related to a reduction of brain activation in the parahippocampal gyrus and hippocampus, possibly triggering poor memory performance, while non-carriers of both *APOE* ε4 and *BDNF* Met, demonstrated greater activation (Kauppi et al., 2014). The current results from whole brain resting-state functional connectivity analyses revealed slightly stronger functional connectivity within the DAN in Met carriers/ε3 homozygotes relative to Met/ε4 carriers. In addition, it was found that Met/ε3 carriers had greater functional connectivity than Val/ε4 carriers between lateral dorsal-ventral stream/DMN and the visual cortex. These findings support the theoretical approach shown in Figure 1.8, in which it was predicted that connectivity in ε4 carriers and Met carriers would be decreased.

Older adults without dementia but with the *APOE* ε4 allele have a relatively high chance of demonstrating amyloid-beta deposits in the brain (Morris et al., 2010). Hence, the current observations of ε4 carries demonstrating functional connectivity decrease could potentially be related to variations in amyloid-beta burden levels that individuals with the *APOE* ε4 allele in our cohort may carry, however, this is speculative and needs to be further investigated. Chiesa et al. (2019) observed in longitudinal studies that neither amyloid burden, nor the interaction of *APOE* and amyloid accumulation, altered resting-state functional connectivity within the DMN, proposing that other mechanisms may be involved in resting state

alterations in ε4 carriers within the DMN. How the *APOE* status may influence functional connectivity within the *BDNF* Val66Met polymorphisms is also not clear. It has been reported that the expression of the Met allele within ε4 carriers was associated with more amyloid beta load as compared to Val homozygotes, particularly in the precuneus, orbitofrontal cortex, gyrus rectus, and lateral prefrontal cortex (Adamczuk et al., 2013). Previous literature has indicated that cognitive performance significantly deteriorates over 3 years in healthy older adult Met/ε4 carriers with higher amyloid-beta load in comparison to 10 years in Val/non-ε4 carriers (Lim et al., 2014). Conversely, low amyloid-beta levels in healthy elderly individuals was not associated with significant differences in cognitive performance in Met/ε4 carriers, Met/non-ε4 carriers, and Val/ε4 carriers, suggesting that neither the status of *APOE* nor *BDNF* polymorphisms mediated the performance in cognition (Lim et al., 2014). Indeed, amyloid beta burden can be detected before any alterations in cognition or behaviour, and commences and accumulates in DMN regions, such as precuneus, medial orbitofrontal, and posterior cingulate areas, potentially disrupting functional connectivity in cognitively healthy individuals (Sheline, Raichle, et al., 2010) and in AD (Greicius et al., 2004). Therefore, individuals with amyloid accumulation and mild cognitive impairments (MCI) are more susceptible to develop AD (Palmqvist et al., 2017).

Another key aspect of this study was to investigate the relationship between cognitive reserve and functional connectivity. Here, it was shown that increased cognitive reserve was positively associated with functional connectivity between the executive network and white matter callosal body, and between the executive network and postcentral gyrus when controlled for both *APOE* and *BDNF* genotypes. There was a further positive correlation between the DAN and the subcallosal cortex. The subcallosal cingulate brain regions have been found to be involved in respiration control, blood pressure (Barbas, 1997), and in

emotional behaviour(Devinsky, Morrell, & Vogt, 1995), and is related to mood disorders such as depression (Drevets et al., 1997). A further positive correlation between functional connectivity and cognitive reserve showed functional connectivity between the central executive network and postcentral and precentral gyrus (primary somatosensory cortex). Cognitive reserve is a theoretical construct used to describe a mechanisms, as described in Chapter 1 (page 48) in which the brain compensates or differentially recruits brain networks to maintain cognitive performance despite pathological disturbances (Stern, 2009), however the exact constructs of cognitive reserve are not clear. In a recent review, cognitive reserve is described as being adaptable and flexible, which was related to improved efficiency and capacity of the brain (Stern et al., 2018). Cabeza et al. (2018) on the other hand defined reserve as development of structural processes, in which neural processes enhance cognitive processes more efficiently.

The question why cognitive reserve increased functional connectivity between the DAN and subcallosal cingulate area is difficult to interpret. Previous studies have shown that individuals with more years of education (used as a proxy for cognitive reserve) had enhanced functional connectivity implying that education may promote neural processing, reorganise the brain, and preserve cognitive functions in the process of healthy aging (Arenaza-Urquijo et al., 2013; Marques et al., 2016). More recently, Franzmeier et al. (2016) found that higher cognitive reserve in individuals with amnestic MCI was associated with protection of the functional networks of the anterior DMN-DAN anti-correlation, and also was associated with relatively preserved memory performance. Further, it was observed that people with MCI and high cognitive reserve showed enhanced global functional connectivity within the cognitive control network compared to people with MCI with low cognitive reserve. It was suggested that the brain may compensate in individuals with higher cognitive

reserve recruiting other brain regions to accomplish the cognitive functions. Another explanation was that functional connectivity may be enhanced in MCI before pathological alterations of AD manifest and reduce this connectivity (Franzmeier et al., 2017). Although, cognitive reserve may influence functional connectivity positively and may have a protective effect on the brain, a decrease in functional connectivity with increasing cognitive reserve was also observed within the DMN and the intracalcarine cortex, supracalcarine cortex, cuneal cortex, and precuneus cortex (controlled for *APOE* and *BDNF*), which was surprising. In contrast, Bosch et al. (2010) found increased DMN activity in healthy older individuals with higher cognitive reserve. These findings might reflect the different cognitive reserve measurements and different sample sizes. Utevsky, Smith, and Huettel (2014) also reported increased connectivity between the precuneus and the DMN in individuals at rest, however, when individuals were performing a task, enhanced connectivity was found between the precuneus and the fronto-parietal network suggesting high functional flexibility of the precuneus. In patients with MCI or AD, Bozzali et al. (2015) also described increased DMN connectivity with higher education. Conversely, healthy controls did not show any significant increases in DMN connectivity indicating that education may have moderated functional connectivity in the context of pathological impacts on the brain through compensatory mechanisms and recruiting other brain regions.

A limitation of this study is the small sample size, at 76 participants. A much larger sample size, as reported in Cacciaglia et al. (2018), may have increased the detection power relative to the *BDNF* and *APOE* polymorphisms. The study also focussed on an island cohort, hence replication in more diverse populations would be required. As noted above, it is also possible that this data may be influenced by the presence of sub-clinical pathological processes in the brains of older adults, such as alterations in amyloid-beta and tau, as well as any potential

contributions from cerebrovascular disease. The exclusion of *APOE* ε2 allele carriers is another limitation. Investigations have shown protective effects of the *APOE* ε2, which could have provided further useful information. However, there were not sufficient numbers of *APOE* ε2 allele carriers to be meaningfully included in the analysis. Because 160 volumes (time-points) for resting-state fMRI studies is limited and higher numbers of time-points provide a more accurate estimation, this is a further limitation of this study. Additionally, fieldmaps were acquired but due to technical errors in their acquisition they were unable to be used in the analysis, therefore I used a combination of manual and ICA-based denoising in the preprocessing stage (see Methods). Lastly, the resolution of the fMRI images was slightly lower than comparable studies (3.4mm).

Conclusion

To my knowledge, this is the first study to investigate functional connectivity with respect to combinations of *APOE* and *BDNF* variations and relative to cognitive reserve. The carriage of the *APOE* ε4 allele may influence functional connectivity in older adults without dementia. The *APOE* x *BDNF* interaction may be influenced by the *APOE* status and therefore alter functional brain connections, which subsequently may promote development of degenerative diseases, such as the AD. Finally, this study showed that increased cognitive reserve is associated with enhanced or decreased functional connectivity in a network-specific fashion in older adults without dementia.

Chapter 5

Language networks recruit frontal lobe regions to support functional connectivity in heathy older adults

Abstract

Language is a cognitive function that shows relatively little deficit through healthy aging. Important brain regions related to language function are Broca's and Wernicke's area, broadly associated with speech production and comprehension, respectively. The objective of this chapter was to investigate age-related differences of functional connectivity in the language networks (Broca's and Wernicke's area) in cognitively healthy people aged 50+ years with a focus on the potential role of Apolipoprotein (APOE) and brain-derived neurotrophic factor (BDNF)Val66Met polymorphisms, gene variations linked to neurodegeneration and brain plasticity, respectively. FMRIB Software Library (FSL) MELODIC was used to pre-process, clean, and analyse the dataset. Seed-based analysis was performed to investigate correlations in connectivity between the region of interest and other brain areas. No significant differences were found in functional connectivity linked to *APOE* or *BDNF* polymorphisms individually. Age correlated positively with functional connectivity between the left Broca's area and frontal lobe regions indicating possible recruitment mechanisms to support language processes. Significant genetic interactions were evident in functional connectivity between the right and left Broca's area respectively, and parietal and medial temporal lobe regions. These finding suggest that the degree to which the language network is interconnected and influenced by aging may be associated with specific genetic interactions. The current data shows that healthy older adults may be able to recruit further brain regions to maintain language function.

Introduction

A fundamental cognitive function that is one of the least affected by healthy aging is language (Murman, 2015; Wingfield & Grossman, 2006). Important brain regions for language function include Broca's and Wernicke's areas (Hagoort, 2014). Broca's area is associated with speech production and located in the inferior frontal regions consisting of the pars opercularis, pars orbitalis and pars triangularis. Wernicke's area is responsible for speech comprehension and is localised to the left superior temporal lobe (Hagoort, 2014). Language function is a form of crystallised intelligence, and a key question is what brain mechanisms support the relative preservation of language processing through aging. Studies examining language processes reported that age-related alterations are more substantial in language production than in comprehension. It is important to note that both younger and older individuals recruit similar brain regions, in particular the left inferior frontal gyrus, when challenged with a comprehension task (Diaz et al., 2016). Functional magnetic resonance imaging (fMRI) revealed that older individuals have additional bilateral activation of the right inferior frontal gyrus when challenged with grammatical tasks, suggesting neural reorganization of the frontal dorsal stream and compensation mechanisms through aging (Diaz et al., 2016). It has been suggested that the brain has sufficient neural elasticity and cognitive resources to compensate and overcome age-related alterations to preserve language function (Wingfield & Grossman, 2006).

Two well-known genes that may influence brain networks because of their relationship with aging-related neurodegeneration and neuronal plasticity are Apolipoprotein E (*APOE*) and brain-derived neurotrophic factor (*BDNF*). As described in Chapter1, the *APOE* ε4

polymorphism has been identified as the major genetic risk factors for sporadic late-onset AD and is associated with more amyloid beta in healthy older individuals (Morris et al., 2010). Although *BDNF* is crucial for synaptic plasticity and neuronal growth (Mattson, Maudsley, & Martin, 2004), the *BDNF* Met variant at codon 66 is related to relatively reduced impairment of synaptic plasticity and decline in memory performance (Egan et al., 2003; Pattwell et al., 2012).

A life-course factor that may influence the role of genetic variations in moderating brain connectivity and cognitive decline is cognitive reserve (Colangeli et al., 2016; Stern, 2002). Cognitive reserve, discussed in previous chapters, is known as theoretical construct adapted by environment and life experiences, in which the brain engages through compensation and/or recruitment of other brain networks to maintain cognitive functions when challenged by injury (Stern, 2002). Recently, Stern et al. (2018) described cognitive reserve as a theoretical construct that includes efficiency, capacity, flexibility, and compensation which is reinforced through flexible functional brain processes. From a functional point of view, previous studies have demonstrated the positive correlation between education (used as a proxy for cognitive reserve) and functional connectivity in healthy elderly (Arenaza-Urquijo et al., 2013) and in AD (Bozzali et al., 2015).

The Tasmanian Healthy Brain Project (THBP) is a longitudinal study investigating whether later-life education improves cognitive reserve and reduces the risk for developing AD (Summers et al., 2013). One unique benefit working with participants of the THBP is that most individuals have an enriched environment. For example, one study of the THBP demonstrated that higher cognitive stimulation boosted cognitive reserve in healthy elderly (Lenehan et al., 2016). Subsequently, Thow et al. (2018) investigated whether this

improvement in cognitive reserve may be associated with several cognitive domains over a period of time. Observed was that increased cognitive reserve was linked with enhanced language processing performance within the THBP cohort (Thow et al., 2018).

In previous studies, *APOE* and *BDNF* polymorphisms have been shown to interact to influence memory function (Kauppi et al., 2013; Ward et al., 2014; Wisdom et al., 2011). Recent investigations have observed that engaging in later life education (university studies) was associated with language processing in the *BDNF* Val66Met polymorphisms in older adults of the THBP (Ward et al., 2020). In particular, it was found that Met carriers had enhanced language processing performance after education intervention, compared to Val homozygotes, who did not show any associations between educational load and language processing after 24 months. Therefore, the current study was interested in the role of these individual gene polymorphisms and their possible interplay, as well as the role of cognitive reserve, relative to language processing and functional changes in brain connectivity in a cohort of older adults. The current study utilised functional brain imaging measures with a subset of the THBP cohort to examine three hypotheses related to language processing: first, that the common *BDNF* Val66Met and *APOE* (ε3 & ε4) polymorphisms independently influence age-related differences in functional connectivity within the language network. Second, that *APOE* and *BDNF* polymorphisms interact to alter functional connectivity within the language network. Third, that cognitive reserve is associated with increased functional connectivity within the language network. To examine the language network, blood-oxygen-level-dependent (BOLD) signal was measured between Broca's area (inferior frontal gyrus involving the pars opercularis, pars orbitalis, pars triangularis), as well as Wernicke's area (left superior temporal gyrus) relative to the rest of the brain. All analyses were controlled for age, gender, and cognitive reserve.

Materials and methods

Study population

Participants

Similar to Chapter 4, this chapter has used the identical cohort with recruitment details described elsewhere (Chapter 3). Demographic and clinical data of the final sample for this chapter are represented in Table 4.1.

Information about procedure, cognitive reserve, genotyping, MRI acquisition and protocol, as well as resting-state fMRI pre-processing are described in Chapter 3 and Chapter 4.

Seed-based general linear model

A seed-based approach was used to calculate a whole-brain correlation map. In particular, two major language processing regions were chosen: Broca's area and Wernicke's area. Regions were masked using the Harvard-Oxford cortical atlas. The mask of each region, Broca left (Broca L), Broca right (Broca R), and Wernicke left were created using Harvard-Oxford cortical and subcortical structural atlases. In particular, pars opercularis and pars triangularis were chosen for Broca's area (Hagoort, 2014), and Brodmann Area 22 located in the left superior temporal gyrus was chosen from Wernicke's area (Demonet et al., 1992). Each image was registered to in MNI 152 space. Within each of these masks, a seed was placed in the middle. From this seed, coordinates were used to generate and create spheres (area; radius 5 mm) on Broca L (MNI coordinates 68, 73, 45), on Broca R (21, 73, 44), and on Wernicke's area (73, 43, 42). An example illustration of how the seed was placed in Broca's area (A) and Wernicke's area (B) is represented in Figure 5.1. The average time-

series extracted from Broca's and Wernicke's area were correlated with all other brain voxels to derive subject-level seed-based correlation maps for each seed.

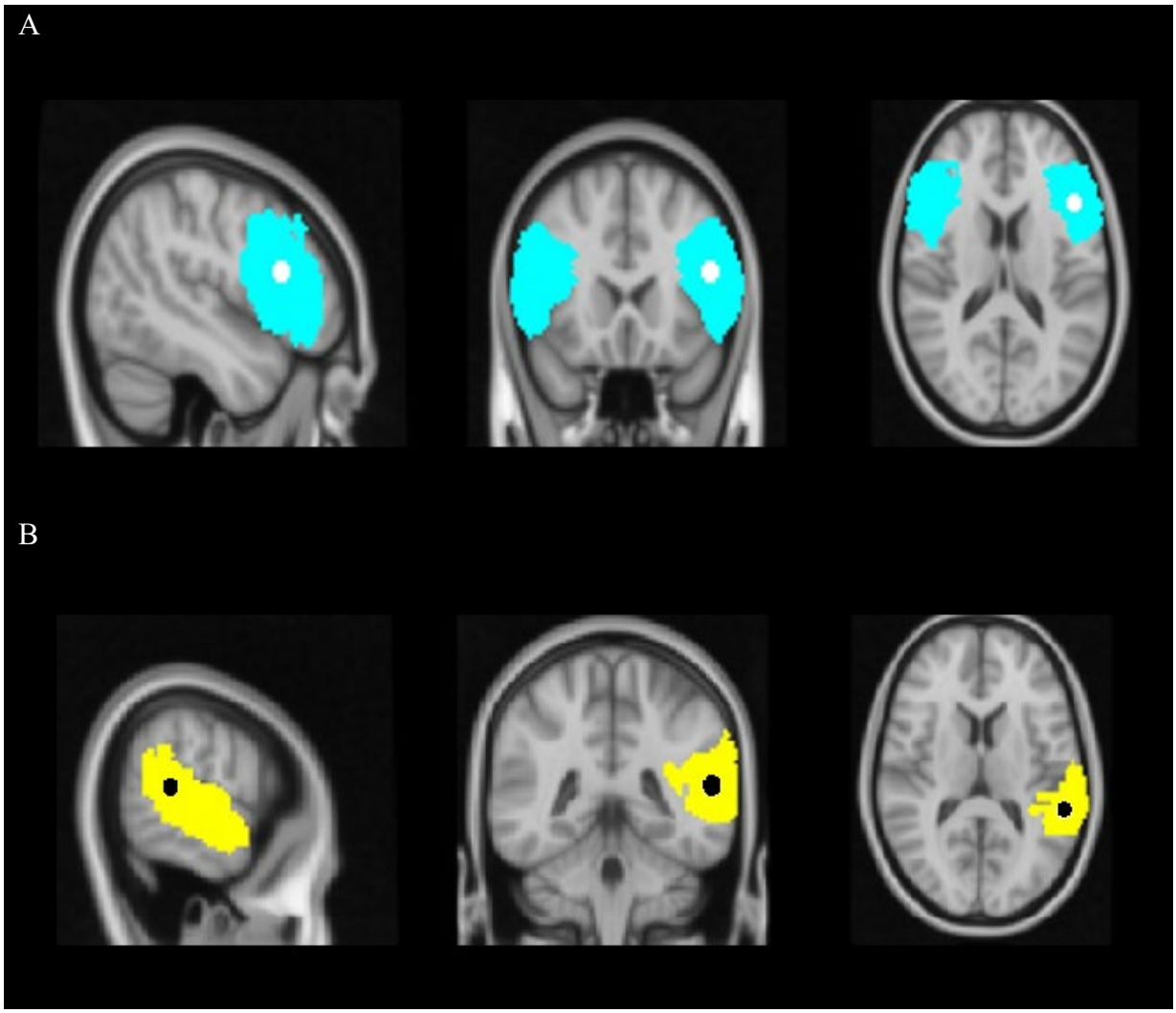


Figure 5.1. Illustration of how the seed was placed in Broca's area (A) and Wernicke's areas (B).

First-level analysis with FEAT was used to analyse time series of each dataset of the raw fMRI data. This first step identified voxels within the brain indicative of significant correlation (*Z* threshold > 2.3) with the seed time series, and to derive a subject-level seed-based correlation map for each seed. The output of the first-level analysis was then used for higher-level analysis (Woolrich et al., 2001). The higher analysis within FEAT was used to fit a GLM across subjects. FMRIB's Local Analysis of Mixed Effects (FLAME) was used to estimate by-subject random effects component of the mixed effects variance using

Metropolis-Hastings Markov Chain Monte Carlo (MCMC) sampling to obtain a precise estimation of the true random effects variance and degrees of freedom at each voxel, to which a general *t*-distribution is then fit (Woolrich, Behrens, Beckmann, Jenkinson, & Smith, 2004). FLAME is a two-stage process using Bayesian modelling and estimation. A single group average model was chosen for the model setup. *Z*-(Gaussianised *t*-maps) statistic images were thresholded using clusters determined by $Z>2.3$ and a (corrected) cluster significance threshold of P=0.05 (Worsley, 2001). Cluster of voxels that are activated at a particular significance level of >2.3 are shown in transparent blobs. Multiple comparisons were performed after z maps were generated which specify a z-value at each voxel in the brain using a cluster threshold with a cluster *p*-threshold of 0.05. Post-hoc contrasts (age, gender, and cognitive reserve) were used to calculate *p*-values.

Results

The sample consisted of 76 healthy older adults, average age 63.3 years (Table 5.1). The current study included only *APOE* ε3 homozygotes and ε3ε4 carriers. Out of the initial 78 recruited participants, two subjects were excluded due to *APOE* genotypes (*APOE* ε2ε3 & *APOE* ε4ε4). One subject was excluded due to recurring errors within the single-subject independent component analysis. Radiological abnormalities were also detected in the brain of one individual, leaving a total of 76 subjects for analysis.

Table 5.1. Participants characteristics expressed as mean (M) ± standard deviation (SD) unless otherwise noted

	Met	Val	*p*-value	ε4	ε3	*p*-value
Gender F : M (%F)	25:10 (71.4%)	27:14 (65.9%)	0.60	23:11 (67.6%)	29:13 (69.0%)	0.90
Intervention Exp : Con (%Exp)	28:7 (80%)	31:10 (75.6%)	0.65	26:8 (76.5%)	33:9 (78.6%)	0.83
Age (years)	63 ± 5.69	63.6 ± 6.82		63.3 ± 5.15	63.3 ± 7.14	
Education (years)	16.7 ± 1.32	16.8 ± 1.04		16.5 ± 1.11	16.9 ± 1.2	

Cognitive reserve (*z*)	0.20 ± 0.82	0.20 ± 0.88	0.06 ± 0.95	0.28 ± 0.76
Language (*z*)	0.01 ± 0.93	.16 ± 0.92	0.11 ± 1.01	0.08 ± 0.87

Note: F = female, M = male, Exp = experimental, Con = Controls.

Relationship of APOE and BDNF polymorphisms to the language network

For both *APOE* and *BDNF* polymorphisms, separate seed-based correlation analyses (in total 6 analyses) were computed for the left and right Broca's areas, as well as left Wernicke's area. There were no significant main effects found for either polymorphism independently between seed regions and the rest of the brain. After performing an analysis on the *APOE* polymorphism and controlling for age, gender, cognitive reserve, there was a positive correlation between age and functional connectivity between left Broca's area and left middle and superior frontal gyrus ($p < .0001$, Figure 5.1.A), and between Wernicke's area and two clusters involving the right putamen and pallidum ($p < .0001$), as well as the supramarginal gyrus (anterior and posterior division) and parietal operculum ($p = .015$, Figure 5.1.B). After controlling for *BDNF*, there was also a positive correlation between age and functional connectivity, which was identical to the *APOE* analysis, between left Broca's area and left middle and superior frontal gyrus ($p = .003$, Figure 5.2.A), and between Wernicke's area and two clusters, one cluster involved the right putamen and pallidum ($p < .0001$), the second cluster consisted of right supramarginal gyrus (anterior and posterior division) and parietal operculum ($p = .015$, Figure 5.2.B). All results can be found in Table 5.2.

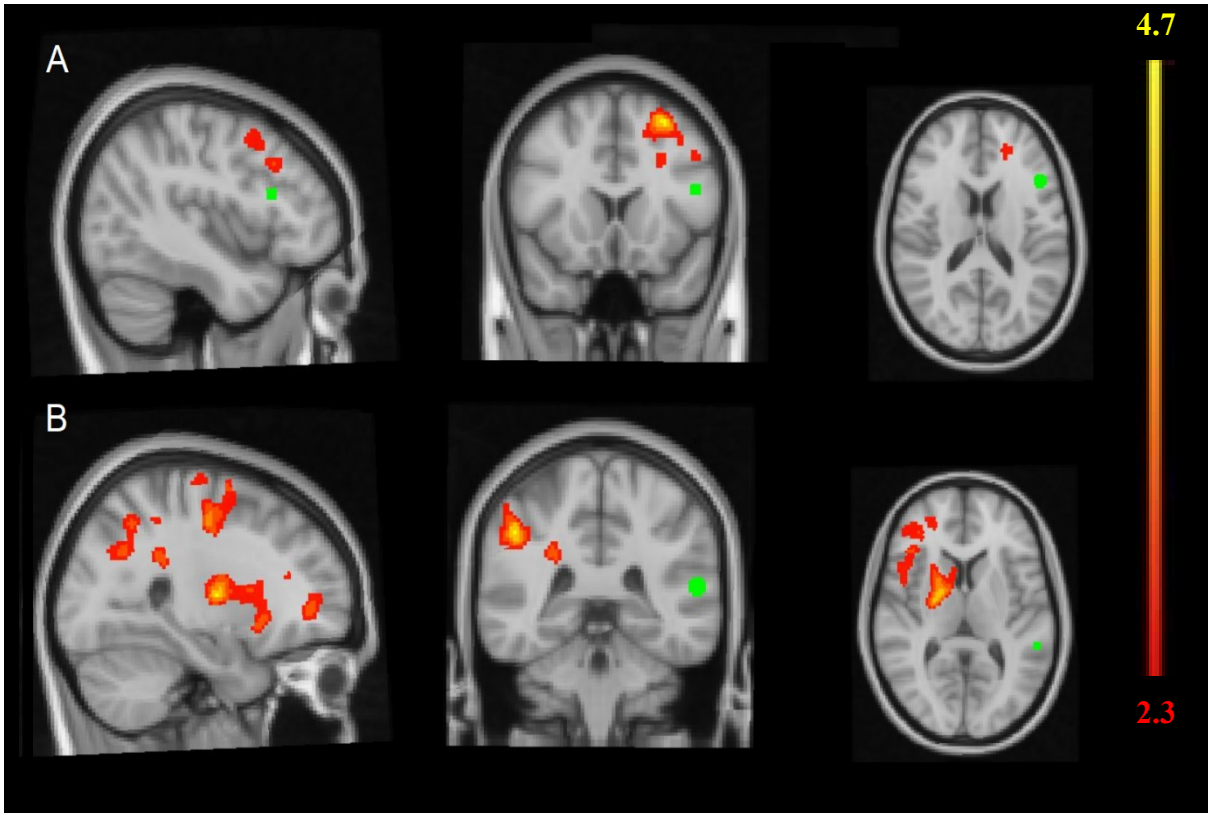


Figure 5.2. (A) Spatial maps representing significant functional connectivity after performing seed-based analysis. Age correlated positively with functional connectivity between the left Broca's area (region of interest – green dot) and the left middle frontal gyrus and superior frontal gyrus (yellow-red). Montreal Neurological Institute (MNI) coordinated x = -28, y = 16, z = 54. (B) Another correlation showed functional connectivity between Wernicke and two clusters, one in the putamen and pallidum (MNI coordinates, x = 22, y = -6, z =10), and the second cluster was found within the parietal lobe (MNI, x = 50, y = 36, z = 40). These analyses were controlled for *APOE* genotypes. All results were corrected using the cluster threshold method with a cluster *p*-threshold of 0.05. Red is the minimum z-statistic after thresholding, yellow is the maximum Z statistic.

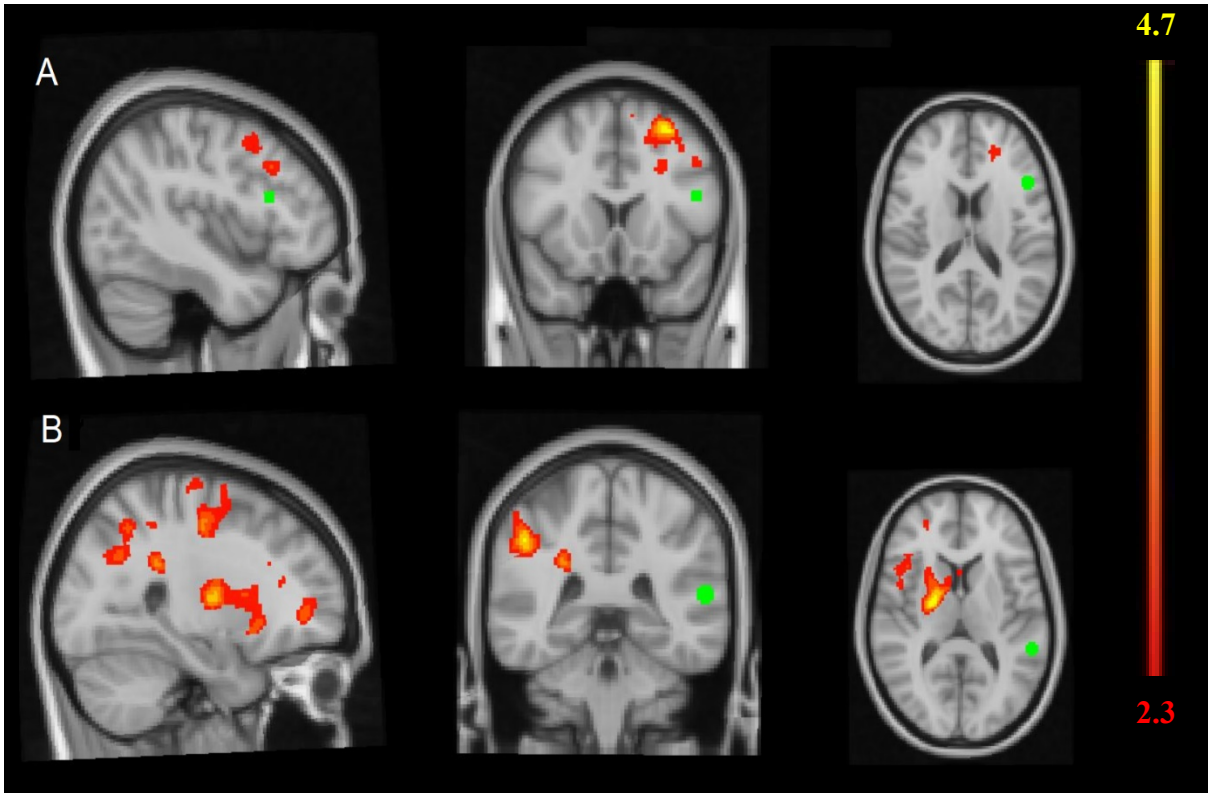


Figure 5.3. (A) Spatial maps after performing seed-based analysis. After controlling for *BDNF* genotype, age correlated positively with functional connectivity between the left Broca's area (green dot) and the left middle frontal gyrus and superior frontal gyrus (yellow-red). MNI coordinates x = -26, y = 14, z = 56. (B) Another correlation showed functional connectivity between Wernicke and two clusters, one cluster was found in the putamen, pallidum and thalamus, MNI coordinates x = 22, y = -6, z = 10, and the second cluster was found within the parietal lobe, MNI coordinates x = 50, y = -36, z = 40. Red- yellow indicates positive values. Green shows the significance, $p > 0.95$ (1 - *p*-value). All results were corrected using the cluster threshold method with a cluster *p*-threshold of 0.05.

Table 5.2. Main effects of *APOE* and *BDNF* polymorphisms

Cluster Index	Voxels	X (mm)	Y (mm)	Z (mm)	*p*-value	Location
Age correlated positively with functional connectivity in left Broca's area (controlled for *APOE* genotypes)						
1	1882	-28	16	54	< .0001	Left Middle frontal gyrus, superior frontal gyrus
Age correlated positively with functional connectivity in Wernicke's area (controlled for *APOE* genotypes)						
2	4366	22	-6	10	< .0001	Right putamen, right pallidum
1	1443	50	-36	40	.015	Right supramarginal gyrus (posterior and anterior division), parietal operculum, postcentral gyrus

Cluster Index	Voxels	X (mm)	Y (mm)	Z (mm)	*p*-value	Location
Age correlated positively with functional connectivity in left Broca's area (controlled for *BDNF* genotypes)						
1	1958	-26	14	56	.003	Left Middle frontal gyrus, superior frontal gyrus,
Age correlated positively with functional connectivity in Wernicke's area (controlled for *BDNF* genotypes)						
2	4410	22	-6	10	< .0001	Right putamen, right pallidum, thalamus
1	1447	50	-36	40	.015	Right supramarginal gyrus (posterior and anterior division), parietal operculum, postcentral gyrus

Abbreviations: APOE, Apolipoprotein E; BDNF, brain-derived neurotrophic factor.

Interaction between APOE and BDNF polymorphisms

All interactions were thresholded using clusters determined by $Z>2.3$ and a (corrected) cluster significance threshold of $p=0.05$. Post-hoc contrasts (age, gender, and cognitive reserve) were used to calculate *p*-values. There was a significant *APOE* x *BDNF* interaction on functional connections between right Broca's area and one cluster consisting of the post- and precentral gyrus, precuneus and superior parietal lobule, representing stronger connectivity for Val/ ε3 carriers as compared to Val/ ε4 genotypes, $p < .0001$ (Figure 5.3. & Table 5.3.).

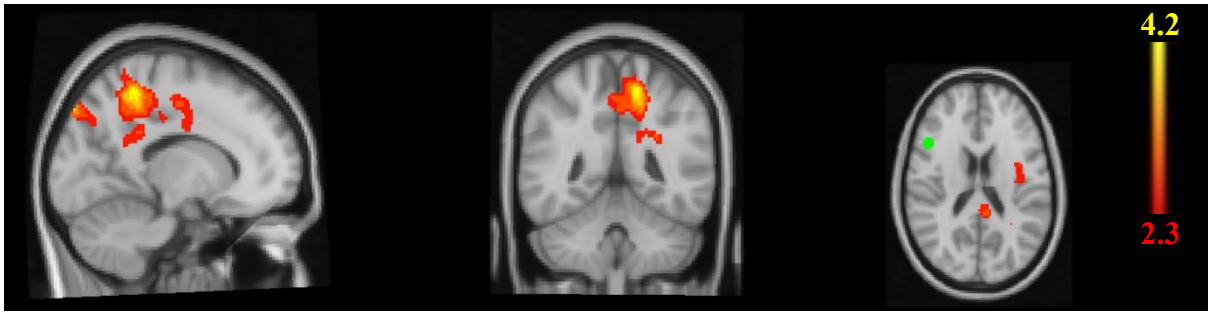


Figure 5.4. Interaction effects within the right Broca's area and parietal lobe regions (including the post- and precentral gyrus, precuneus, and superior parietal lobule) showing more functional connectivity in Val/ ε3 carriers than for Val/ ε4. MNI coordinates x = -14, y = -44, z = 56. All results were corrected using the cluster threshold method with a cluster *p*-threshold of 0.05.

There was a significant interaction between the *APOE* and *BDNF* polymorphisms on functional connections between the left Broca's area and two clusters, one cluster located in the parahippocampal gyrus (anterior and posterior division), ($p < .0001$), a second cluster located in the precentral and postcentral gyrus, in which Met/ ε3 genotypes had stronger connectivity than Met/ ε4 genotypes ($p = .007$, Figure 5.4.A). Another significant *APOE* x *BDNF* interaction was found between left Broca's area and the left hippocampus, where Met/ ε3 genotypes had greater connectivity than Val/ ε3 genotypes ($p < .0001$, Figure 5.4.B). After controlling for *APOE* x *BDNF* interactions and their main effects, there was a significant negative correlation between CR and functional connections between left Broca's area and right hippocampal regions ($p < .0001$, Figure 5.4.C). Age was positively correlated with functional connections between left Broca's area and left superior and middle frontal gyrus ($p < .0001$), after controlling for the *APOE* x *BDNF* interactions and their main effects (Figure 5.4.D). Results have been corrected for multiple comparison via the clustering-based thresholding (Friston, Worsley, Frackowiak, Mazziotta, & Evans, 1994). All analyses were controlled for age, gender, and cognitive reserve.

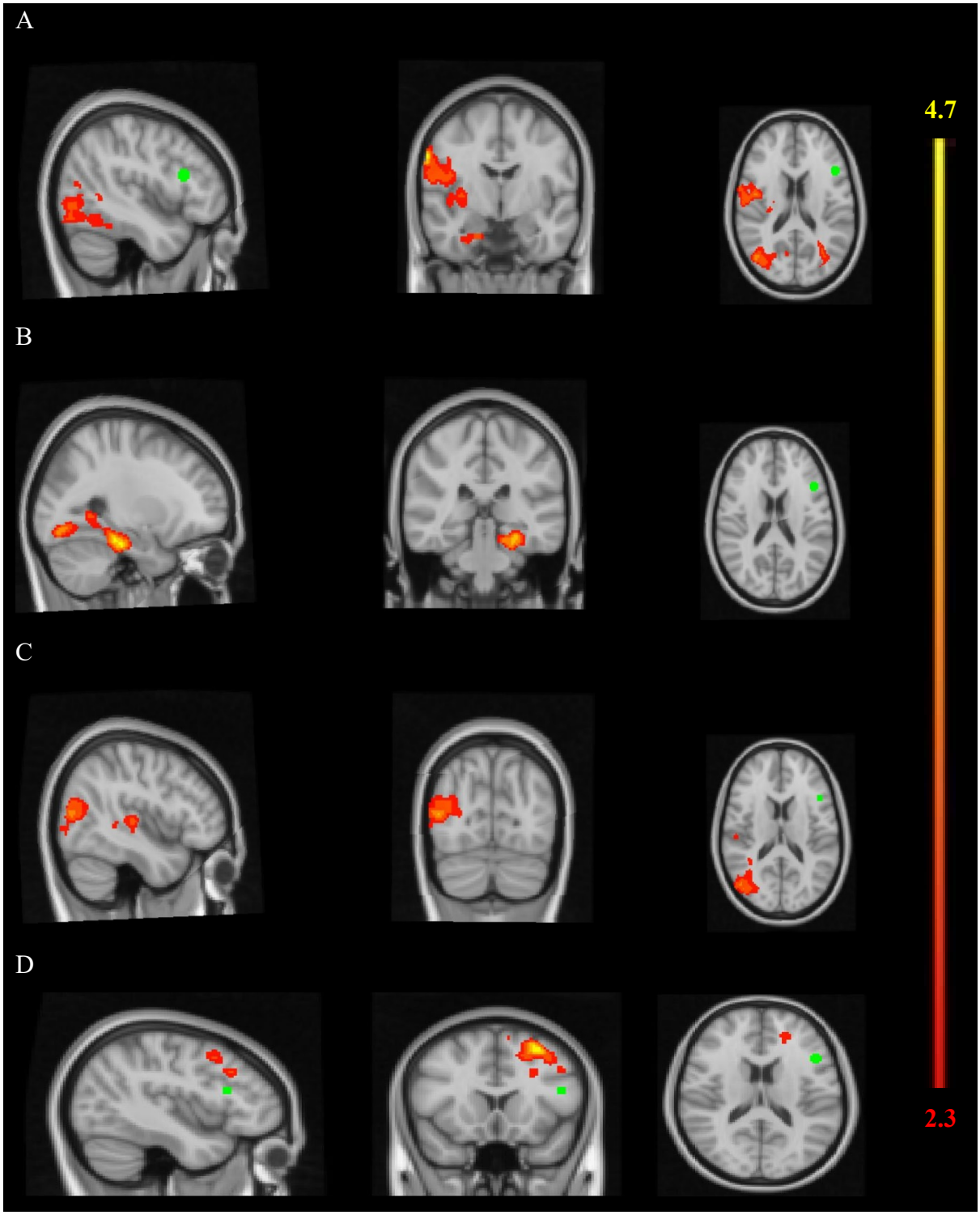


Figure 5.5. A – D. Interaction effects were found within left Broca's area. (A) Met/ ε3 had stronger connectivity than Met/ ε4 between the left Broca's area and the two clusters, the parahippocampal gyrus (MNI coordinates x = -24, y = -26, z = -24) and the precentral & postcentral gyrus (MNI coordinates x = 64, y = 0, z = 30). (B) Met/ ε3 carriers showed stronger functional connectivity than Val/ ε3 carriers between the left Broca's area and left hippocampal regions (MNI coordinates x = -26, y = -32, z = -18). (C) Negative correlations were found between cognitive reserve and functional connectivity between the left Broca's area and right hippocampal regions (MNI coordinates x = 28, y = -18, z = -24). (D) Positive correlations were found between age and functional connectivity between left Broca's area

and left superior and middle frontal gyrus (MNI coordinates x = -26, y = 14, z = 56). All results were corrected using the cluster threshold method with a cluster *p*-threshold of 0.05.

Table 5.3. Interaction effects on the language networks

Interaction left Broca's area						
Met/ ε3 > Met/ ε4						
Cluster Index	Voxels	X (mm)	Y (mm)	Z (mm)	*p*-value	Location
2	11941	-24	-26	-24	< .0001	Parahippocampal gyrus (anterior and posterior divisions)
1	1651	64	0	30	.007	Precentral & postcentral gyrus,
Met/ ε3 > Val/ ε3						
1	1666	-26	-32	-18	< .0001	Parahippocampa gyrus (L hippocampus), posterior division; temporal fusiform posterior division
Cognitive reserve correlated negatively with functional connectivity						
1	1995	28	-18	-24	< .0001	Parahippocampal gyrus (R hippocampus), anterior and posterior division, temporal fusiform
Age correlated positively with functional connectivity						
1	2009	-26	14	56	< .0001	Left Superior frontal gyrus, middle frontal gyrus
Interaction right Broca's area						
Val/ ε3 > Val/ ε4						
Cluster Index	Voxels	X (mm)	Y (mm)	Z (mm)	*p*-value	Location
1	4613	-14	-44	56	< .0001	Postcentral gyrus, precuneous cortex, superior parietal lobule, precetral gyrus
Interaction Wernicke's area						
Age correlated positively with functional connectivity						
Cluster Index	Voxels	X (mm)	Y (mm)	Z (mm)	*p*-value	Location
2	3205	22	-6	10	< .0001	Right pallidum, right putamen, thalamus
1	1310	50	-36	40	0.03	Supramarginal gyrus (anterior & posterior division), parietal operculum, postcentral gyrus

In a model which included *APOE* x *BDNF* interactions and their main effects, there was a significant effect of age on functional connections between Wernicke's areas and two clusters; one cluster consisted of the right pallidum and putamen, and thalamus ($p < .0001$); and the second cluster involved the supramarginal gyrus (posterior and anterior division), parietal operculum, postcentral gyrus ($p = .025$, Figure 5.5).

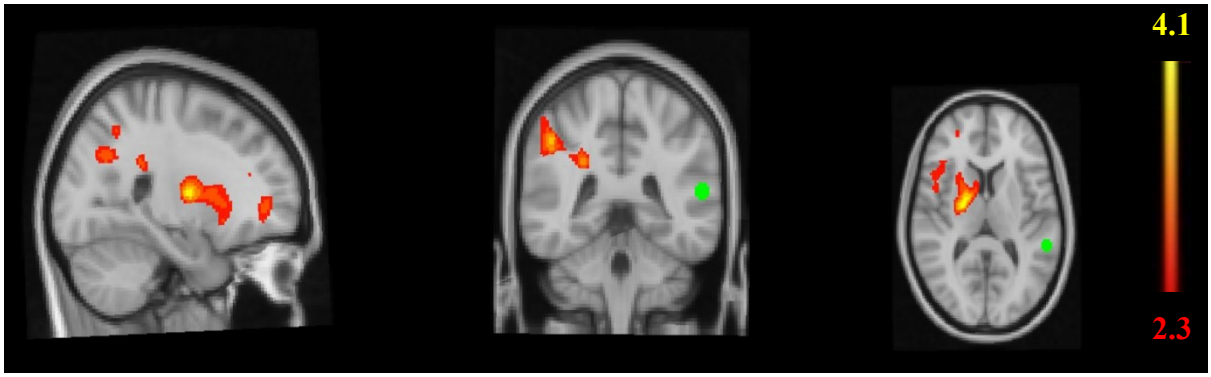


Figure 5.6. (A) After performing an interaction seed-based analysis, Wernicke's area (green dot) showed positive correlation with two clusters shown in red-yellow (right pallidum, right putamen, thalamus, MNI coordinates x = 22, y = -6, z = 10, & supramarginal gyrus, MNI coordinates x = 50, y = -36, z = 40), controlled for *APOE* x *BDNF* interactions. All results were corrected using the cluster threshold method with a cluster *p*-threshold of 0.05.

Cognitive reserve and the functional language network

Cognitive reserve was not significantly associated with functional connectivity involving Broca's and Wernicke's regions and other brain regions. After controlling for *APOE* x *BDNF* interactions, cognitive reserve showed negative correlation with functional connectivity between left Broca's area and parahippocampal gyrus.

Discussion

In the current cross-sectional study, differences in resting-state language networks related to the *BDNF* Val66Met and *APOE* (ε4 & ε3) polymorphisms were investigated in a healthy older adult population. It was also examined whether the language network, connectivity with Broca's and Wernicke's areas, correlated with individual or interacting patterns of these polymorphisms linked to aging-related neurodegeneration and brain plasticity.

In the present study, a positive correlation between age and functional connectivity was found indicating more connectivity in this sample of healthy older adult cohort between left Broca's are and left middle and superior frontal gyrus, and between Wernicke's area and two clusters

involving the right putamen and pallidum, and the right supramarginal gyrus (posterior and anterior division) with increased age.

Aging has been previously associated with impairment within and between functional networks of the brain (Geerligs, Renken, Saliasi, Maurits, & Lorist, 2015), as well as within networks that are important for higher cognitive functions, such as the default-mode network (Salimi-Khorshidi et al., 2014). In contrast, findings of this study demonstrated a positive impact of age on functional connectivity within the language network. In particular, a positive correlation between age and connectivity was found between left Broca's area and the left middle and superior frontal gyrus after controlling for *APOE* and *BDNF*. These findings may either underlie some compensation mechanisms of the brain showing a greater amount of functional connectivity. One investigation showed significant improvement of language processing after 4 years following a 12-month university level education intervention in healthy older adults suggesting that crystallised knowledge is improving with age (Thow et al., 2018). Geerligs et al. (2015) observed decreased intra-network connectivity, but also increased inter-network connectivity, in healthy older adults (mean age 64.8), suggesting overactivation of brain regions due to reduction of functional pattern distinction among brain regions.

In support of findings of this study, Tomasi and Volkow (2012b) also found correlation between Broca's and Wernicke's area and the middle frontal gyrus, as well as putamen and pallidus, and multiple other regions, suggesting that these brain regions are functionally involved in language processing. The middle and superior frontal gyrus are both located in the frontal lobe (Boisgueheneuc et al., 2006), whereas the putamen and pallidus are part of the basal ganglia (Lanciego, Luquin, & Obeso, 2012). While the middle frontal gyrus has

been implicated in perceptual processing of language production (Wen et al., 2017), the superior frontal gyrus is known to play an essential role in working memory processing (Boisgueheneuc et al., 2006). The current data showed increased functional connectivity with normal aging between Broca's area and the frontal lobe, which may be pertinent for understanding why language performance shows relatively more stability with advancing years. As aforementioned, Broca's area has been associated with speech production (Hagoort, 2014), which is an important process in language processing, therefore, we speculated that increased functional connectivity between language –related regions and other brain areas work together to compensate for each other. Positive correlations between functional connectivity and aging within the frontal lobe may also suggest that people may be recruiting executive function networks to support their language processing in a compensatory way. However, this assumption should be interpreted with caution, as it is identified as one of the limitations in this chapter; due to a limited timeframe, I was not able to perform analysis on the associations between performance and functional connectivity, which would have shed light into whether or not increased functional connectivity would have been compensatory. Nevertheless, previous studies reported that when central networks are altered due to age-related atrophy in older compared to younger people, healthy older individuals may recruit substitute networks as neural compensation to sustain cognitive performance (Martins, Joanette, & Monchi, 2015).Hence, the claim that frontal lobe regions were recruited as compensatory mechanisms for language processing is should be tested in future studies.

A further possible interpretation is that my cohort may be showing overactivation of functional connectivity between Wernicke's area and the parietal lobe regions, the putamen and pallidum, in response to increasing aging-related, but as yet subclinical, pathological burden in these brain regions, which also could be related to a decline of management

efficiency of neural resources (Stevens, Hasher, Chiew, & Grady, 2008). Similar to earlier inferences, this explanation should also be interpreted with caution because this was not tested and is a further limitation of this chapter. In this context, it is important to note that the pathological changes of AD, such as amyloid-beta deposits, occur many years before frank dementia, and are linked to inheritance of *APOE* ε4 polymorphism (reviewed in Vickers et al. 2016) and could potentially have an impact on neural network connectivity (Salimi-Khorshidi et al., 2014).

The *APOE* ε4 allele is a major risk factor for developing AD. Carriership of an ε4 allele is also associated with reduced cognitive function and increased amyloid-beta deposition, which is a crucial early brain change in AD (Liu et al., 2013). Another potential biomarker for AD is BDNF, which plays a major role in neuronal growth as well as plasticity deficits (Egan et al., 2003). In particular, the *BDNF* Met allele showed to have damaging effects on cognitive function and was associated with decreased brain activation in the medial temporal lobe regions in older individuals, which could contribute to increased risk of developing dementia (Brown et al., 2020). The combination of *APOE* and *BDNF* polymorphisms, as well as amyloid-beta burden, was investigated in healthy individuals within the language network and in associate-semantic processing demonstrating no significant effects on activity pattern within the language networks of both the *APOE* and *BDNF* polymorphisms (Adamczuk et al., 2016). However, healthy individuals with evident amyloid-beta burden in the brain (potentially a preclinical stage of AD) demonstrated an increased activation in left posterior middle temporal gyrus as well as increased semantic-processing, suggesting that the brain may be showing increased activity in preclinical stages, but decreased activity with more advanced staging of AD pathology (Adamczuk et al., 2016). These data also suggested that

cortical brain regions implicated in semantic processes may be early compensatory biomarkers, perhaps a hallmark of increased amyloid-beta deposition in the brain.

In the present study, functional connectivity correlations were found relative to interaction effects of the *APOE* and *BDNF* polymorphisms. In particular, the Val/ε3 combination was associated with stronger connectivity between right Broca's area and the cluster of postcentral gyri and precuneus relative to Val/ε4 carriers, both important brain regions related to somatosensory functions (Kropf, Syan, Minuzzi, & Frey, 2019) and various higher-order functions including episodic memory retrieval and visuo-spatial imagery (Cavanna & Trimble, 2006). Ward et al. (2014) investigated the combination of *APOE* ε4 and *BDNF* Met within the THBP cohort and observed a relatively lower performance on episodic memory tasks in this combination than in non-carriers of these risk alleles (Ward et al., 2014). Relatedly, *APOE* ε4 + *BDNF* Met carriers exhibited more amyloid beta accumulation than *BDNF* Val homozygotes + *APOE* ε4 carriers (Adamczuk et al., 2013). This has been subsequently supported by a study, in which *APOE* ε4 / *BDNF* Met carriers with amyloid-beta accumulation exhibited an accelerated deterioration of working memory and language after 54 months compared to *APOE* ε3 *BDNF* Val homozygotes exhibiting decay after 10 years (Lim et al., 2015). Yet, individuals with low amyloid-beta levels presented no significant difference in cognitive performance relative to Met/ε4 carriers, Met/non-ε4 carriers, and Val/ε4 carriers indicating that amyloid burden triggers accelerated cognitive performance decrease. This outcome motivated me to investigate how these polymorphisms and their interactions may play a role in functional connectivity.

Conversely, a recent longitudinal study also observed no impact of amyloid beta on connectivity dynamics in ε4 carriers, suggesting that other mechanisms may play a role in

alterations within ε4 carriers (Chiesa et al., 2019). Furthermore, healthy older ε4 carriers with no amyloid beta burden had decreased functional connectivity within the default mode network, indicating that ε4 carriers may be affected before clinical diagnosis of disease (Machulda et al., 2011; Sheline, Morris, et al., 2010). This is what may be seen within the next interaction effect, in which Met/ε3 carriers exhibited stronger functional connectivity than Met/ε4 carriers (Figure 1.8) between the left Broca's area and two separate clusters, the parahippocampal gyrus (posterior & anterior division), and the precentral and postcentral gyrus. The parahippocampal gyrus is a significant interconnected hub linked to temporal, parietal, and frontal lobes, all of which are involved in higher cognitive processes (Aminoff, Kveraga, & Bar, 2013), while the precentral and postcentral gyrus have been related to voluntary motor movement (Schott, 1993) and somatosensory function (Kropf et al., 2019). As previously noted, carriers of the ε4 allele have higher risks of amyloid beta accumulation in the brain (Morris et al., 2010). Thus, the results may be associated with different levels of amyloid beta burden in ε4 carriers within our cohort. This assumption, however, should be interpreted with precaution, as amyloid-beta accumulation was not included within the analyses imposing another limitation of this chapter. However, the results of the current and previous studies are inconsistent with regard to amyloid-beta burden and it is suggested that future research may implement amyloid-beta levels within the *APOE* studies.

The final interaction effect involved stronger functional connectivity between left Broca's area and the left parahippocampal gyrus (posterior division) in Met/ε3 carriers compared to the Val/ε3 combination. This finding could be a result of a possible intervention effect of cognitive reserve (later-life university education) in Met carriers than Val homozygotes. It should be taken into account that this is a presumption, and an interaction effect was not tested. However, there are studies reporting that the *BDNF* Met allele carriage has been

associated with impairments in memory performance (Erickson et al.,2013). More recent investigations, have shown that cognitive reserve has had an effect on cognitive functioning (executive function) over time in Met carriers (Ward et al., 2017). This may signify that Met carriers of the THBP could potentially preserve their functional connectivity in language-related brain regions, therefore, I speculate that increasing connectivity or recruiting brain regions as a compensating mechanism in functional connectivity.

In this study, cognitive reserve did not correlate positively with functional connectivity, yet, cognitive reserve correlated negatively with increasing functional connectivity between the left Broca's area and the right parahippocampal gyrus (anterior and posterior division), controlled for all the genetic interactions. This finding was unexpected, as our studies have previously showed that education was not only related to greater language processing capacity at baseline, but that additional education also improved language processing over a period of four years, potentially through the enrichment of crystallised cognitive functions (Thow et al., 2018). A seed-based analysis showed positive correlations between baseline years of education and functional connectivity indicating stronger functional connectivity between the anterior cingulate cortex and the right hippocampus supporting the hypothesis of cognitive reserve improving cognitive performance and functional connectivity in healthy elderly (Arenaza-Urquijo et al., 2013). However, why functional connectivity correlated negatively with cognitive reserve between the parahippocampal gyrus and left Broca's area in this study is less clear. Positive correlations were expected here, especially because the parahippocampal gyrus is associated with several key regions implicated in a range of various cognitive functions (Aminoff et al., 2013). Although, Bozzali et al. (2015) also did not find any significant correlation between education levels and functional connectivity in healthy elderly, individuals with AD on the other hand, showed that increased education levels were

related to greater functional connectivity in posterior cingulate regions (Bozzali et al., 2015). This suggests that patients with higher cognitive reserve are able to recruit brain regions on a neuronal level and cope with pathological alterations. A further explanation and a potential strength of this study is that there may have been no effect of cognitive reserve on functional connectivity because the current cohort had relatively higher cognitive reserve levels. Cognitive reserve was not measured from the commencing study (Summers et al., 2013). However, given the previous research, it is postulated that building up cognitive reserve could potentially stimulate cognitive performance and protect the brain from destructive effects in functional connectivity (Matyas et al., 2019; Rodríguez-Rojo et al., 2018).

Some limitations of the present study should be considered. (1) Although, the sample size of 76 participants was comparable to previous studies, there is still a need for larger studies to be performed to increase the confidence intervals in the estimates and to understand how functional network abnormalities evolve. Data about brain amyloid-beta pathological burden was not obtained, which is thought to be associated with ε4 carriers (Morris et al., 2010), and possibly weakened functional connectivity. There is evidence that healthy individuals with amyloid beta burden had disruptions in resting-state functional connectivity prior to exhibiting any cognitive deterioration suggesting that there is a clear impact of amyloid beta on functional connectivity within the DMN (Sheline et al., 2010) (Hedden et al., 2009). Moreover, it was found that amyloid-beta load correlated with intrinsic DMN functional connectivity in preclinical stages (cognitively healthy individuals with amyloid beta burden). This correlation was found to come to a clear plateau in clinical stages of early MCI (Pasquini et al., 2017), indicating that the negative effects of amyloid beta are more stable in MCI and that the intensity of functional connectivity determined the degenerative impact on the brain instead of amyloid-beta. Nevertheless, voxels in networks with greater intrinsic

functional connectivity had more amyloid-beta accumulation than functional networks with reduced connectivity. This finding implied that important networks, such as the DMN with greater connectivity, are impacted by amyloid-beta burden in preclinical AD stages (Pasquini et al., 2017). Despite the fact that amyloid-beta was found in crucial networks (Sheline et al., 2010) (Hedden et al., 2009; Pasquini et al., 2017), recent research has disputed this theory observing increased DMN activity in women harbouring amyloid-beta that are at risk for developing AD and in early stages of AD (Caldwell et al., 2019). This inconsistency may be related to the different loading of amyloid-beta, as well as other compensation mechanisms that occur in the brain subsequent to hyper- and hypoconnectivity (Hasani, Mayeli, Salehi, & Parizi, 2021). It remains to be determined whether or not amyloid-beta was associated with functional connectivity impairments within our cohort.

Conclusion

Together, this study investigated the language functional network relative to combinations of the *APOE* & *BDNF* genotypes, and cognitive reserve. Both increases and decreases of functional connectivity were found within the language networks. The question whether the increase of functional connectivity within the language network is related to a positive or a negative effect is not clear, however, it was assumed that a decrease may be related to disconnection and loss to speech and comprehension. Furthermore, it is hypothesized that the increase of functional connectivity within the frontal lobe may be a compensatory recruitment mechanism reinforcing language processes in healthy older adults. On the other hand, increased connectivity may also reflect reduced efficiency or increased noise. There is a possibility that connectivity may be associated with noise variance, however, studies found that after performing ICA and cleaning and regressing out the

independent components, the identification of functional networks through temporal BOLD signal is very robust(Bright & Murphy, 2015).

While most studies observed enhanced cognitive reserve associated with great functional connectivity (Bozzali et al., 2015; Franzmeier et al., 2017a; Marques et al., 2016), anticorrelation between cognitive reserve and functional connectivity have been found in this study. This finding could be linked to the neural efficiency hypothesis, which states that performance can be accomplished without the need of more functional brain activity (Haier et al., 1988). In particular, the neural efficiency hypothesis refers to the findings that clever people have lower (however more efficient) brain activity when performing a task (Haier et al., 1992; Haier et al., 1988). In particular, Raven's Advanced Progressive Matrices test (nonverbal test for measuring general intelligence) was performed while the individuals were scanned using PET. The outcome was that the correlation between regional metabolic rate and test scores fluctuated, suggesting that smarter individuals with better cognitive performance may exhibit less energy waste because their brain networks are more efficient(Haier et al., 1988). In contrast, individuals with decreased intelligence may use different/or less efficient brain circuits that may need more cortical activity to accomplish a task, which subsequently need more neuronal energy and are therefore inefficient (Haier et al., 1992). Relatedly, a study reported that fluid intelligence may be influenced and improved by training (Jaeggi et al., 2008), indicating that cognitive exercise resulted in less effort and greater neural efficiency in the brain (Neubauer et al., 2009). In the end, cognitive exercise altered patterns of the brain, suggesting that individuals with more knowledge recruited hardly any neural brain resources than those individuals with reduced knowledge(Kelly & Garavan, 2005). As a result, the network of the brain is the neural foundation for intelligence, defined through the interactions of several brain regions. Resting-state fMRI studies reported

significant correlations between functional connectivity and intelligence (Song et al., 2008). In particular, tests scores of cognitive domains, such as executive functioning, correlated with frontal lobe and posterior brain regions, suggesting that the intrinsic functional interaction of differing areas contribute to intelligence. Above evidence in literature showed that intelligence may have evolved in higher educated people, therefore, it may be possible that higher IQ levels in the THBP cohort were reflected in intrinsic brain connectivity in a way that maximised neural efficiency.

One investigation strengthened the theory of neural efficiency that greater performance, in this case semantic function, was related to lower brain activation in the posterior middle temporal gyrus (Jung, Kim, Cho, & Nam, 2017). Hence, individuals with less semantic efficiency may necessitate more brain activity in the middle temporal gyrus while performing a semantic task. On the contrary, studies showed that greater activity of the left posterior middle temporal gyrus was associated with enhanced performance in semantic processing (Wei et al., 2012), suggesting that efficiency of semantic processing is represented by functional brain activity. The explanation of this variance is that the left posterior middle temporal gyrus may have different state-dependent brain activity (Jung et al., 2017). Examining both articles, it is possible that sample size and cohort population may have influenced the outcome; while Jung et al. (2017) included 16 Koreans (both gender), the Wei et al. (2012) study comprised of 34 female Chinese. It would have been interesting to see how efficiency within my cohort might have contributed to this debate. However, due to time constraints of this thesis, I did not investigate the association between performance and functional connectivity, which has to be acknowledged as a further limitation of this chapter. Therefore, my assumption that healthy older adults are able to recruit frontal lobe regions to preserve language function desires caution with interpretation.

Nonetheless, i believe that reduced connectivity in individuals carrying the ε4 allele may be related to higher amyloid-beta pathology accumulation impacting functional connectivity within the language networks. Future longitudinal studies are vital to investigate whether specific gene combinations, as well as cognitive reserve may further influence the language network, which could potentially be applied in neurogenerative disease prevention programs.

Chapter 6

Brain structure metrics related to *APOE* and *BDNF* polymorphisms, and cognitive function in healthy older adults

Abstract

Cortical thickness and grey matter volume are altered with age. This alteration may potentially be influenced by common human genotypes, such as *APOE* ε4 which has been associated with reduced cortical thickness as well as increased risk of Alzheimer's disease, and the *BDNF* Met polymorphism which has been associated with grey matter volume and cognitive function in healthy older adults. Dementia occurs on the background of brain aging, and the pathology of diseases that cause dementia accumulates many years before frank cognitive impairment. Therefore, the aim of this study was to investigate the associations between *APOE* and *BDNF* polymorphisms and domains of cognitive function with respect to brain structural morphometry (grey and white matter covariance patterns of the whole brain, grey matter volume and cortical thickness in temporal lobe structures) in cognitively unimpaired older adults. We obtained T1-weighted brain scans from 75 participants (average age 63 years) to examine brain structure. Cognitive test scores were obtained to construct composite scores of episodic memory, working memory, executive function, and language processing.

Following cross-validation to determine an elastic-net penalty that minimised bias and bootstrapping to estimate confidence intervals, we identified several brain regions significantly associated with scores on executive function and working memory domains (after adjustment for age). Cognitively intact older adults showed thinning and volume loss in relation to decreased cognitive function performance.

While a link between structure and cognitive function in cognitively normal volunteers was evident in this sample, a corresponding link between common *APOE* and *BDNF* polymorphisms and structure (grey and white matter covariance patterns, cortical thickness and grey matter volume) was not found.

Introduction

Grey matter reduction may reflect active neurodegeneration related to conditions that cause dementia in certain cases, for example, when grey matter decrease occurs in the medial temporal lobe (MTL) and related brain regions such as the hippocampus (Teipel et al., 2014). Magnetic resonance imaging (MRI) is used to identify cerebral atrophy (reduction of grey and white matter volume) in the aging brain (Lemaître et al., 2005; Peters et al., 1998). Research to date has investigated grey matter volume (Lemaître et al., 2005) and cortical thickness in healthy individuals (Storsve et al., 2014) longitudinally and reported that atrophy in the MTL regions predicted the development of dementia and was subsequently related to cognitive decline (Den Heijer et al., 2002; Guderian et al., 2015).

Studies have shown that a degree of cognitive decline is a normal part of aging (Murman, 2015). Changes in structure correlate with normal cognitive decline as we age affecting cognitive domains such as working memory and executive function (Murman, 2015). Genetic factors potentially associated with cognitive decline in aging are apolipoprotein E (*APOE*) and brain-derived neurotrophic factor (*BDNF). APOE* is important for lipid transportation and controlling homeostasis (Mahley et al., 2006), and consists of isoforms, APOE ε2, APOE ε3, and APOE ε4. The *APOE* ε4 allele is a major risk factor for late onset AD and has been linked to the accumulation of extracellular amyloid-beta deposits and inflammation in the brain (Hudry et al., 2013; Liu et al., 2013; Mahley et al., 2006). The BDNF protein has roles in synaptic plasticity and neurogenesis (Binder & Scharfman, 2004; Erickson et al., 2010), and the encoding gene has single nucleotide polymorphisms coded as valine (Val) to methionine (Met) substitution at position 66 (Brown, Vickers, Stuart, Cechova, & Ward, 2020). The Met allele of the *BDNF* Val66Met polymorphisms has been linked with reduced BDNF secretion and associated with relative impairment in hippocampal function and episodic memory deficiency (Egan et al., 2003).

Historically, studies of the of the *APOE* ε4 variant indicated age-related cognitive decline and reduced cortical thickness in hippocampal regions (Brown et al., 2014; Burggren et al., 2008; Lim et al., 2016; Liu et al., 2013). Previous research has also reported that *BDNF* Met carriers have smaller volumes of the hippocampus and other temporal lobe regions compared to *BDNF* Val homozygotes in healthy older (Brown et al., 2014) and younger (Montag, Weber, Fliessbach, Elger, & Reuter, 2009) people, as well as reduced cortical thickness in Met carriers in contrast to Val homozygotes (Voineskos et al., 2011). Studies investigating the *APOE* and *BDNF* Val66Met polymorphisms in healthy individuals have observed age-related volume reduction within the hippocampus in *APOE* ε4 carriers compared to *APOE* ε3 homozygotes (Burggren et al., 2008), as well as in *BDNF* Met carriers compared to *BDNF* Val homozygotes in healthy individuals (Bueller et al., 2006; Hajek, Kopecek, & Höschl, 2012). Not only, hippocampal volume, but amygdala volume baseline measures in healthy individuals predict risk of progression to preclinical AD stages (den Heijer et al., 2006; Guderian et al., 2015) and alterations can be detected before clinical diagnosis (Clerx et al., 2013; Querbes et al., 2009).

Polymorphisms in the *APOE* and *BDNF* genes have been associated with cognitive function and brain structure, which can influence the risk of AD. Figure 6.1. illustrated the conceptual associations between genes, brain structure, cognitive function, and AD risk.

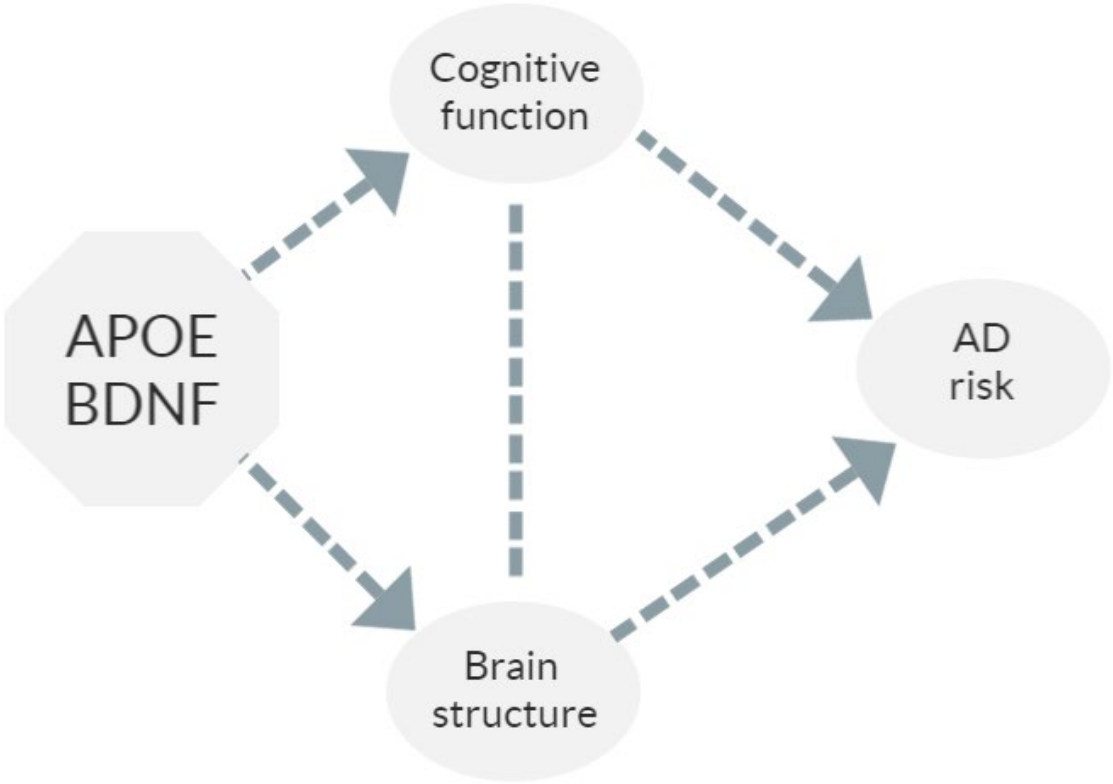


Figure 6.1. Conceptual diagram of the putative associations between genotypes (*APOE* & *BDNF*), brain structure, cognitive function, and risk of Alzheimer's disease diagnosis.

Voxel-based morphometric (VBM) approaches from the analysis of structural magnetic resonance images (MRI) allow for group comparison of grey and white matter volume. In this regard, regions that are anatomically connected could express structural covariances in their growth dynamics (Mechelli, Friston, Frackowiak, & Price, 2005). In particular, two different spatially distant brain regions are part of the same covariance network if inter-pattern correlations were found, for example, correlating each pattern with the other pattern between two groups (on group level), also known as covariance pattern analysis. Positive and negative loadings are associated with increased and decreased grey matter volume. Investigating differences in covariance patterns within the *APOE* (ε3 & ε4) and *BDNF* (Met

& Val) polymorphisms in cognitively unimpaired older adults may provide evidence of genetic-structural links associated with AD risk genotypes. For example, the polymorphisms that are more susceptible to develop AD, such as the ε4 carriers or Met carriers, would show a decrease grey matter pattern compared than ε3 homozygotes and Val homozygotes. However, previous studies also illustrated the importance of investigating region-specific differences, for example, the MTL. A study involving healthy individuals ranged between 26 and 82 years indicated aging-related volume loss in the hippocampus (Raz, Rodrigue, Head, Kennedy, & Acker, 2004). As a result of these observation that *APOE* and *BDNF* Val66Met polymorphisms have been associated with aging-related brain degeneration and the path of pathological progression of AD (Gomar et al., 2016), as well as aging-related changes in cognitive function (Lim et al., 2015), it is important to investigate whether alterations to specific regions within the healthy brain differ in grey matter volume between genetic polymorphisms earlier in individuals with different genotypes, before the potential manifestation of AD.

The first aim of this study was to investigate whether variants of the *APOE* and *BDNF* polymorphisms differ in covariance patterns in grey matter and white matter within healthy, older adults to characterise links to aging and perhaps to identify aging-related brain changes that increase susceptibility to conditions such as dementia and/or to highlight measures that may detect older adults in pre-clinical stages. The second aim was to examine the differences between *APOE*, *BDNF*, and cortical thickness (parahippocampus and entorhinal cortex), and grey matter volume (hippocampus and amygdala) in a healthy older adult cohort. Based on other studies (Liu et al., 2010; Sublette et al., 2008), it is hypothesised that *APOE* ε4 carriers and *BDNF* Met carriers will have lower cortical thickness compared to *APOE* ε3 homozygotes and *BDNF* Val homozygotes respectively. Previous work by Ward et al. (2014)

within the same cohort as this study reported interactions between *APOE* and *BDNF* polymorphisms predicting episodic memory with lowest scores found in ε4/Met carriers (Ward et al., 2014), hence, an *APOE* x *BDNF* interaction is also expected between *APOE* and *BDNF* within the same cohort. In particular, it may be predicted that ε3/Val carriers would show higher grey matter volume and thickness values than ε4/Met carriers. Additionally, it is predicted that relative cortical thinning will be found in the entorhinal cortex and hippocampus, as highly vulnerable sites for AD pathology, in *APOE* ε4 and *BDNF* Met carriers (Voineskos et al., 2011).

An additional aim of this study is to investigate whether brain morphometrics, such as volume and cortical thickness of the whole brain were associated with domain-specific cognitive status within this cohort (episodic memory, working memory, executive function, and language processes). Previous studies have shown that age-related alterations in brain structure have been associated with cognitive performance. In particular, memory function is associated with atrophy in the medial temporal lobe and other brain regions in elderly individuals (Visser, Verhey, Hofman, Scheltens, & Jolles, 2002). Further, it has been observed that age-related cortical thinning (Swierkot & Rajah, 2018) and brain atrophy (Pelletier et al., 2017) were associated with episodic memory decline.

Methods

Participants

This chapter has used the same cohort as Chapter 3, Chapter 4, and Chapter 5. Recruitment details can be found within Chapter 3. Demographic and clinical data of this chapter are represented in Table 6.1.

Information about procedure, neuropsychological assessments, cognitive reserve, genotyping, MRI acquisition and protocol are described in Chapter 3 and Chapter 4.

Magnetic Resonance Imaging (MRI)

Structural images were acquired using a T1-weighted 3D BRAVO sequence (echo time (TE) = 2.53 ms, 256 x 256 x 176 matrix, giving 1mm^3 voxels).

MRI Data pre-processing

Covariance Pattern Analysis

In Matlab R2018a (Mathworks, Natick, MA), Statistical Parametric Mapping (SPM12; Welcome Department of Cognitive Neurology) was used to ensure accurate normalization of all coordinates. T1-images were manually reoriented to the anterior commissure – posterior commissure line. Voxel-Based-Morphometry (VBM) was used to segment each image into grey matter, white matter, and cerebrospinal fluid (Ashburner & Friston, 2005). A more accurate inter-subject alignment was achieved via Diffeomorphic Anatomical Registration Through Exponentiated Line Algebra (DARTEL) to shape each brain, applying three parameters for each voxel. Specific crisp average templates were created by simultaneously orienting and aligning grey and white matter in DARTEL so that the data was iteratively aligned (Ashburner, 2007). Grey matter and white matter maps are produced in the same space as the original T1-weighted image, in which each voxel is assigned a probability of grey matter volume. We manually checked each probability map to assure appropriate segmentation, so we could normalise each map spatially into Montreal Neurologic Institute (MNI) standard space (Friston et al., 1995). Probability maps were spatially smoothed with an isotropic Gaussian kernel, full-width-at-half-maximum = 8mm.

Group-level covariance analyses

Grey and white matter covariance patterns (networks) were identified using multivariate analyses. Patterns of the *APOE* and *BDNF* genotypes were independently investigated. Covariates included were age, sex, and education. The same analyses were performed including cognitive reserve as an additional variable to investigate whether patterns were systematically associated with cognitive reserve. Principal components analysis suite (http://www.nitrc.org/projects/gcva_pca (Habeck, Rakitin, et al., 2005; Habeck & Stern, 2007) was implemented in Matlab. In SPM, we used Image Calculator (ImCal) to create a grey matter binary mask with the same dimensions of each T1 image. This mask was used on grey matter probability maps to only comprise >20% grey matter probability. A PCA was performed to transform a number of possible correlated images into smaller components to examine the covariance structure via linear combinations, in other words, a set of principal components and their related subject-specific pattern values were obtained and grouped by deducting the mean of each image from each voxel.

Participant-specific pattern scores reflect the degree to which a participant displays a particular component or pattern. A total of 6 analyses were performed to investigate grey and white matter separately within the *APOE* and *BDNF* genotypes and cognitive reserve. *APOE* and *BDNF* genotypes, and cognitive reserve associated with the grey and white matter volume covariance patterns were then computed by performing a linear regression that used a best-fit linear combination of subject-specific factor scores from the best linear combination Blumen et al. (2019)of principal components (PCs) selected using the Akaike information criteria as have, against genotype, which was provided by the program (Burnham & Anderson, 2002).

Bootstrap resampling (1000) (Efron & Tibshirani, 1994) was performed to test the stability of the voxels in each GM volume covariance pattern associated with different genotype. Each voxel in a pattern has a corresponding z-score. Significant voxels with bootstrap samples were located between [Z] > + 1.96 or < −1.96, $p < .05$ (.025 in each tail). These group-level covariance analyses allow identifying key 'nodes' in the grey matter volume covariance 'networks' (Habeck, Krakauer, et al., 2005; Habeck & Stern, 2007; Steffener et al., 2013) associated with genotype. Positive and negative weightings (or loadings) to each voxel were predicted by the multivariate analysis (Habeck et al., 2008). In this study, positive loadings are related to more grey matter volume in ε3 homozygotes and Val homozygotes compared to brain regions displaying negative loadings (less volume) in ε4 carriers and Met carriers, respectively. Brain regions displaying negative loadings are associated with less grey matter volume. Both loadings, positive and negative are important and form grey matter covariance patterns that have been related to the *APOE* and *BDNF* genotypes (Habeck et al., 2008; Spetsieris & Eidelberg, 2011; Steffener et al., 2013).

Subcortical and cortical volumetric processing

Subcortical and cortical volumetric processing was performed with FreeSurfer software (https://surfer.nmr.mgh.harvard.edu/) version 6.0.0. Cortical thickness analysis procedures have been validated against histological analysis and manual measurements and have good test-retest reliability across scanner manufacturers and field strengths (Han et al., 2006; Reuter et al., 2012). Technical details have been defined in previous publications (Fischl, 2012) or see the Feesurfer website (https://surfer.nmr.mgh.harvard.edu/). Briefly, the automated FreeSurfer pipeline involved motion correction (Reuter et al., 2010), non-uniform intensity normalisation (N3) at 500 iterations to correct for intensity non-uniformity artifacts (Sled et al., 1998), automated Talairach transformation, normalization (Sled et al., 1998),

removal of non-brain tissue (Segonne et al., 2004), linear and non-linear registration, cortical parcellation (Desikan et al., 2006), and segmentation of white and grey matter volume (Fischl et al., 2002; Fischl et al., 2004). Cortical thickness is calculated as the closest distance from grey/white boundary to grey/CSF boundary at each vertex on the tessellated surface (Fischl & Dale, 2000). Procedures for the measurement of cortical thickness have been validated against histological analysis (Rosas et al., 2002). For further statistical analysis, grey matter volumes of hippocampus and amygdala, and cortical thickness values of entorhinal cortex and parahippocampus were extracted from FreeSurfer's automated parcellation procedure (Desikan et al., 2006; Reuter et al., 2012).

Statistical analyses

Left and right hemisphere structures were summed to obtain a total cortical thickness of the entorhinal cortex and paraphippocampus and grey matter volume of hippocampus and amygdala, after plotting structures from the left hemisphere and right hemisphere against each other and checking visually for reasonable concordance between contra-lateral brain structures. Entorhinal cortex thickness and parahippocampus thickness, as well as hippocampus volume and amygdala volume were investigated for differences between the *APOE* and *BDNF* polymorphisms.

Genotypes

Univariate multiple linear regression models were fitted to estimate mean amygdala and hippocampus volumes, and mean parahippocampus and entorhinal cortex thicknesses which were hypothesised to be associated with the *APOE* and *BDNF* Val66Met polymorphisms, and their interaction. We adjusted for total intracranial volume (TIV) and age in years.

Cognitive Status

To investigate the relationship between brain structure and cognitive status in healthy, older adult participants in the THBP, we used penalised regression methods ('elastic net') to predict cognitive status from cortical/sub-cortical morphometry. Penalised regression aims to minimise bias by finding an optimally complex model by penalizing coefficients. A grid-search algorithm was used to minimise the root mean-squared error (RMSE) over a grid of tunable parameters using 30-fold cross-validation. We fitted univariate models for each cognitive domain using the *glmnet* package in the R statistical computing environment (v4.0) (Team, 2018). The varying parameters were $0 \leq \alpha \leq 1$ and $10^{-3} \leq \lambda \leq 10^{5}$ making a 20 (α) x 200 (λ) grid. The α parameter controls the type of penalty, with $\alpha = 0$ being a ridge-regression model (L2 penalty), $\alpha = 1$ being the LASSO (L1 penalty), and values in-between being elastic net regression. The λ parameter is the penalty term applied. Since coefficients were shrunk towards zero by penalization, further penalty to control the false discovery rate was deemed too conservative in this context so we have reported all significant coefficients without further adjustment for false discovery.

A wide matrix of structural variables was used which included all cortical/sub-cortical volumes and cortical/sub-cortical thicknesses measured. All grey matter volume and cortical thickness brain structures of left and right hemisphere structures were summed. FreeSurfer was used to obtain structural variables of cortical thickness and cortical grey matter volumes. We adjusted for total intracranial volume and age. All predictor variables were mean-centered and standardised to a unit standard deviation, which is required of the penalised regression algorithm so that the penalty is applied equally across all predictors. The dependent variables were factor-analysis derived composite scores computed using equations in Ward et al. (2014 and); Ward, Summers, et al. (2015b). Univariate models were fitted for

each of the four cognitive domains, being episodic memory, language function, working memory, and executive function. As the number of predictors was large relative to the number of observations, the potential for over-fitting was high even with appropriate penalties. We used non-parametric bootstrapping to estimate uncertainty, and report bootstrapped 95% CIs for all test statistics and coefficients. Coefficients were considered significant if the 95% confidence interval (the range of parameter estimates compatible with the data) did not overlap zero.

Statistical assumptions for both sets of analyses were checked using graphical methods including residual and Q-Q plots.

Results

Key demographic characteristics of participants of the *APOE* groups and *BDNF* groups are presented in Table 6.1. *APOE* and *BDNF* polymorphisms were balanced between genders. A chi-square test demonstrated no significant differences in gender between *APOE* ε4 carriers and ε3 homozygotes $p = .81$, as well as *BDNF* Met carriers and Val homozygotes, $p = .92$. Figure 6.2. represents the standardised voxels between genotypes in parahippocampus thickness, hippocampus thickness, hippocampus volume and amygdala volume.

Table 6.1. Demographic profile of *APOE* & *BDNF* genotypes in the study sample

	ε3 **homozygote (n=39)**	ε4 **carrier (n=36)**
Age (years)		
Mean (SD)	63.1 (6.78)	63.1 (5.39)
Median [Min, Max]	63.0 [53.0, 79.0]	63.0 [53.0, 72.0]
Gender		
Female	27 (69.2%)	24 (66.7%)
Male	12 (30.8%)	12 (33.3%)
BDNF Val66Met		

	ε3 **homozygote (n=39)**	ε4 **carrier (n=36)**
Val homozygote	19 (48.7%)	21 (58.3%)
Met carrier	20 (51.3%)	15 (41.7%)

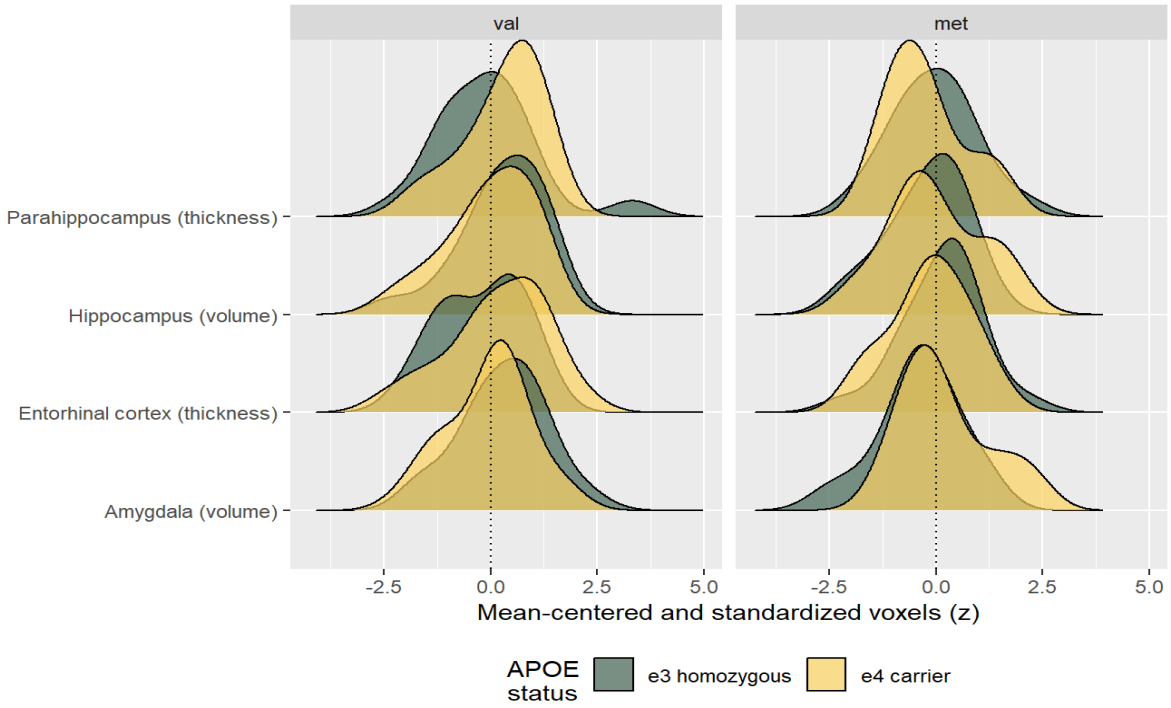


Figure 6.2. Density of mean-centered and standardised voxels of parahippocampus thickness, hippocampus volume, entorhinal thickness, and amygdala volume between *APOE* ε4 carriers, *APOE* ε3 homozygotes, *BDNF* Met carriers, and *BDNF* Val homozygotes.

Covariance Pattern Analysis (whole brain)

Whole-brain grey matter volume covariance pattern analyses were performed to determine whether *APOE* ε4 carriers and ε3 homozygotes, and *BDNF* Met carriers and Val homozygotes had differences in covarying grey and white matter patterns, as well as whether there were any interactions between *APOE* and *BDNF* genotypes. No significant differences in patterns were found across these analyses. Grey matter covariance patterns showed no significant differences between *APOE* ε3 homozygotes and ε4 carriers (p = .55) and between *BDNF* Val homozygotes and Met carriers (p = .43), respectively, adjusted for age, gender,

and cognitive reserve. There were also no significant differences in white matter covariance patterns between the *APOE* ε3 homozygotes and ε4 carriers ($p = .84$) and between *BDNF* Val homozygotes and Met carriers ($p = .22$) polymorphisms respectively, again adjusted for age, gender, and cognitive reserve. Cognitive reserve was not associated with grey matter patterns ($p = .97$) and white matter patterns ($p = .57$) within this cohort.

Region of interest analyses for APOE and BDNF genotypes

The covariates, age and TIV, were significantly associated with hippocampus and amgydala volume (Table 6.2). Adjusting for these covariates, there were no significant main effects or interactions between *APOE* and *BDNF* genotypes (Figure 6.3). Mean volumes and cortical thickness values are represented in Table 6.3., depicting that ε3/Val have the largest hippocampus volume, ε4/Met have the greatest amygdala volume, and ε4/Val have the greatest entorhinal and parahippocampus thickness.

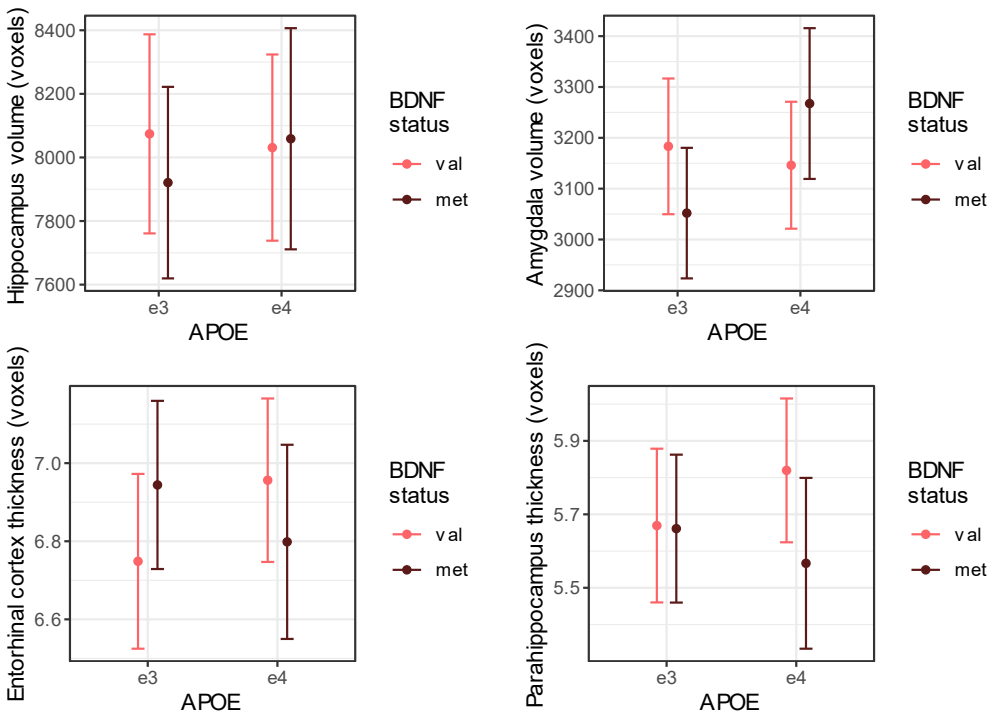


Figure 6.3. Interaction between *APOE* and *BDNF* genotypes related to hippocampus and amygdala volumes, and parahippocampus and entorhinal thicknesses. Estimated marginal means of summed (left + right) voxels with 95% Confidence Intervals (CIs).

	total hippocampus volume			total amygdala volume			total entorhinal thickness			total parahippocampus thickness		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	5805.52	3428.63 – 8182.41	**<0.001**	1902.46	889.18 – 2915.74	**<0.001**	7.38	5.68 – 9.08	**<0.001**	6.39	4.80 – 7.98	**<0.001**
TIV	0.00	0.00 – 0.00	**<0.001**	0.00	0.00 – 0.00	**<0.001**	0.00	-0.00 – 0.00	0.715	-0.00	-0.00 – 0.00	0.300
Age (years)	-34.40	-60.43 – -8.37	**0.010**	-16.00	-27.10 – -4.90	**0.005**	-0.01	-0.03 – 0.01	0.154	-0.00	-0.02 – 0.02	0.812
APOE (ε4+)	-43.16	-480.28 – 393.96	0.844	-37.14	-223.49 – 149.20	0.692	0.21	-0.10 – 0.52	0.189	0.15	-0.14 – 0.44	0.309
BDNF (Met+)	-153.37	-599.59 – 292.84	0.495	-131.36	-321.59 – 58.86	0.173	0.20	-0.12 – 0.51	0.225	-0.01	-0.31 – 0.29	0.956
APOE (ε4+) x BDNF (met+)	181.15	-458.81 – 821.12	0.574	252.70	-20.12 – 525.52	0.069	-0.35	-0.81 – 0.10	0.128	-0.24	-0.67 – 0.18	0.258

Observations	75	75	75	75
R^2 / R^2 adjusted	0.352 / 0.305	0.454 / 0.414	0.059 / -0.009	0.054 / -0.015

Table 6.2. Estimated means (and 95% CI) show *p*-values of total hippocampus volume, total amygdala volume, total entorhinal thickness, total parahippocampus thickness between *APOE* and *BDNF* Val66Met polymorphisms. There were significant correlations between age and hippocampus volume and amygdala volume.

Table 6.3. | Estimated marginal means with 95% CI for interaction between *APOE* & *BDNF* genotypes

	ε3/Met	*CI*	ε4/Met	*CI*	ε3/Val	*CI*	ε4/Val	*CI*
Hippocampus volume	7920.79	7619.6-8222	8058.79	7711-8406.6	8074.16	7761-8387.3	8031.01	7738-8323.9
Amygdala volume	3051.88	2923.5-3180	3267.43	3119-3415.7	3183.25	3049.8-3316.7	3146.1	3021.2-3271
Entorhinal thickness	6.94	6.73-7.16	6.8	6.55-7.05	6.75	6.52-6.97	6.96	6.75-7.17
Parahippocampus. thickness	5.66	5.46-5.86	5.57	5.33-5.8	5.67	5.46-5.88	5.82	5.6-6.02

Note: CI = Confidence Intervals, APOE = Apolipoprotein E; BDNF = brain-derived neurotrophic factor; ε3/Met = participants not carrying the *APOE* ε4, but at least one copy of the *BDNF* Met alleles; ε4/Met = participants carrying at least one copy of the *APOE* ε4/*BDNF* Met alleles; ε3/Val = participants not carrying the *APOE* ε4 allele and being a Val homozygote; ε4/Val = participants being a Val homozygote and carrying at least one of the *APOE* ε4 alleles.

Correlations between brain structure metrics and cognitive status

Brain areas were identified via parcellation used in Freesurfer; Left and right hemisphere, as explained in methods, were summed to a total cortical volume (in total 8 volume structures) and to a total cortical thickness (in total 34 cortical thickness structures).

Table 6.4. Correlation between brain structure and cognitive status coefficients

	Alpha	Lambda	R^2
Episodic memory	0.16	0.59	43.40%
Working memory	0	0.45	73.70%
Language	0.05	0.71	53.60%
Executive function	0	0.45	76.60%

Lambda is the amount of penalty, alpha is the type of penalty where 0 = L2-norm, 1 = L1-norm, values in-between are a mix of L2 ad L1. R^2 is the variance explained (with95 % confidence interval obtained from bootstrap

There were no significant coefficients for episodic memory or language function. The cross-validated alpha and lambda were .16 and .59 for episodic memory and .05 and .71 for language function (all results can also be found in Table 6.4). Lambda is the amount of penalty applied, the bigger lambda is, the more the coefficient is shrunk towards zero. Alpha is the "type" of penalty, L1, L2, or a mix between L1 and L2. If alpha = 0, the L2 penalty is applied which only shrinks coefficients towards zero. If alpha = 1, the L1 penalty is applied which will set some proportion of coefficients to exactly 0. The bootstrap-estimated variance explained (R^2) by structural morphometrics for episodic memory was 43.4% [95%CI 29.1, 58.9] and 53.6% [95%CI 37.1, 68.8] for language function, however no morphometric variables had significant coefficients for these domains. Inferior-temporal, post-central, and frontal pole thickness, and accumbens area all had significant coefficients predicting working memory scores (represented in Figure 6.4 (a). Bootstrap-estimated variance explained (R^2) was 73.7% [95%CI 61.1, 83.4]. Similarly, there were significant coefficients predicting

executive function for caudal middle frontal, superior temporal, supramarginal, temporal pole thicknesses, accumbens area, and putamen and pallidum volumes (Figure 6.4 (b)). Bootstrap-estimated variance explained (R^2) was 76.6% [95%CI 66.1, 85.4]. Cross-validated alpha was 0 and lambda was .45 for both working memory and executive function penalised regression models. Multiple comparison was done by using penalized regression which shrinks coefficients towards zero (more details can be found within methods).

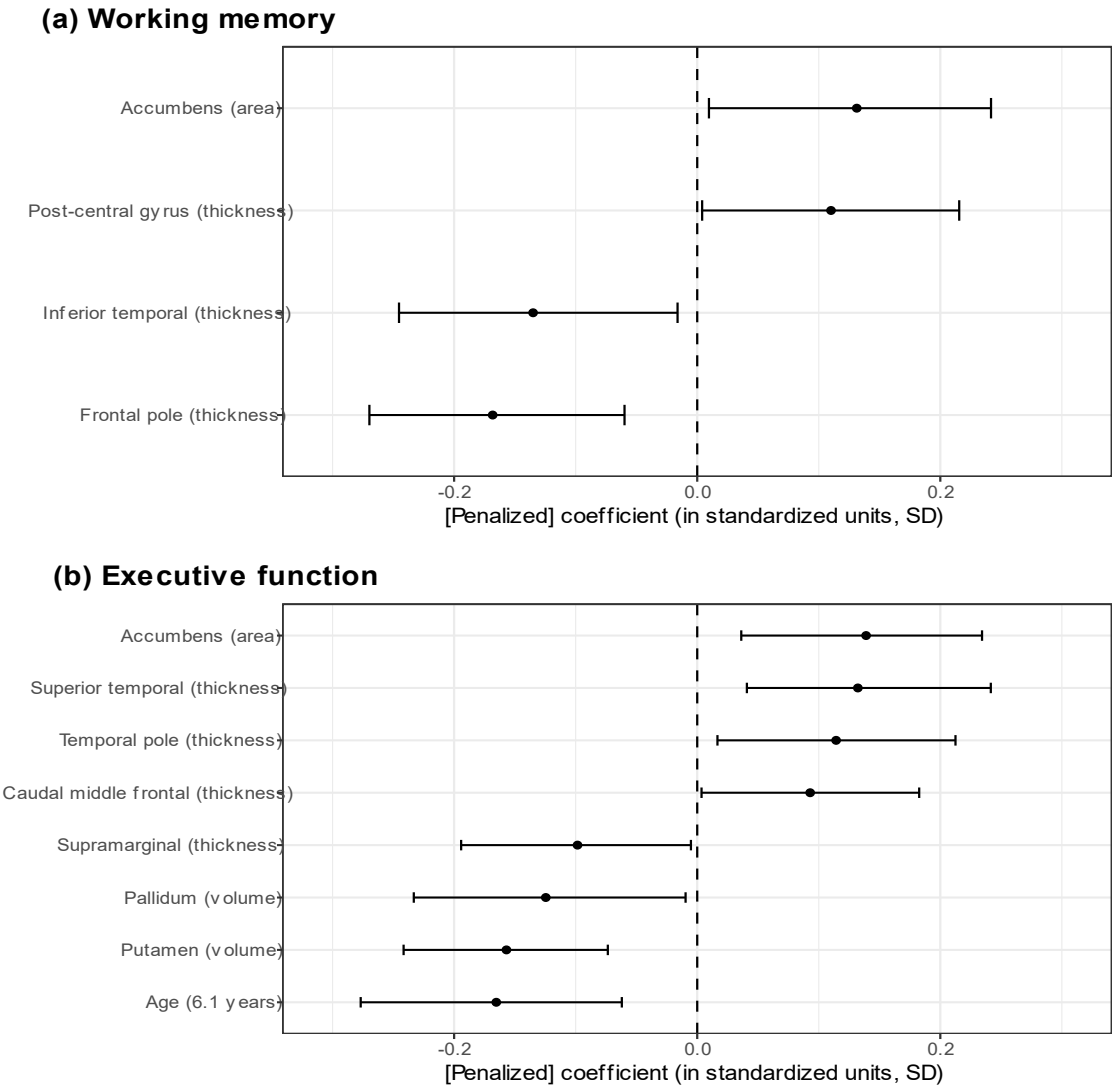


Figure 6.4. Significant correlation coefficients for penalised regression models predicting (a) working memory and (b) executive function scores from brain morphometrics (adjusted for age). Whiskers show 95% confidence intervals estimated using bootstrap resampling. Coefficients are in standardised units (*i.e.* one standard deviation change in structural

measurement is associated with β*SD change in cognitive domain score, where β is the coefficient).

Discussion

In the present study, the aim was to investigate cross-sectional grey and white matter differences between variants of the *BDNF* Val66Met polymorphism, and *APOE* (ε3 and ε4) in a healthy older participant sample of the Tasmanian Healthy Brain Project (THBP). We expected different loadings (positive and negative) to be linked with polymorphisms. Loadings are assigned to each voxel. For example, brain regions with positive loadings show more grey matter volumes and were assumed to be found within *APOE* ε3 homozygotes and *BDNF* Val homozygotes, respectively. While negative loadings show less grey matter volume and were predicted to be found within *BDNF* Met carriers and *APOE* ε4 carriers. In all covariance pattern analyses, we did not find any evidence to support the assumptions that Met carriers and ε4 carriers would show different grey and white matter volume patterns compared to ε3 homozygotes and Val homozygotes.

A recent study, with a greater sample size, demonstrated more voxels in the posterior cingulate cortex and a larger structural covariance in the executive network in *BDNF* Met homozygotes than in Val/Met carriers and Val homozygotes, however, this was found in participants with AD (Lin et al., 2016). This finding suggests that differences in structural covariance pattern may not be visible in healthy older adults, but effects could be apparent in established disease. Hence, the *BDNF* genotype may modulate the grey matter covariance patterns of medial temporal lobe regions only in adults with extensive AD pathology. As opposed to Lin et al.'s (2016) outcome, my results showed no significant differences in covariance grey matter patterns between Met carriers and Val homozygotes. This is not surprising because other studies found no correlations between genes and cortical surface/

thickness, as these structures are distinct regions that work independent of each other (Panizzon et al., 2009). Moreover, heritability may affect both, grey matter patterns from gene-brain relationship and cortical brain structures. Additionally, the genetic variance also alters throughout the development and maturating phase indicating that patterns in the brain may develop diversly throughout time (Jasinska et al., 2017).

With respect to *APOE*, structural covariance patterns in healthy young ε4 carriers (average age 24.3 years) demonstrated covarying volume patterns between anterior hippocampus and a few frontal regions, as well as negative covariance patterns between the precuneus and anterior hippocampus in female ε4 carriers compared to female non-ε4 carriers, and male ε4 carriers and non-ε4 carriers (Stening et al., 2017). It was proposed that the brain has a different organization between gender, ε4 carriers and non-ε4 carriers, which may also be related to reduced functional resting-state connectivity in healthy older individuals between the hippocampus and precuneus. Conversely, I investigated in healthy elderly individuals and did not find any differences in grey matter covariance patterns between ε4 carriers and ε3 homozygotes. In line with this study, Bergfield et al. (2010) did also not find any age-related grey matter network differences between ε4 carriers and non-ε4 carriers suggesting that any reduction of grey matter pattern alterations in healthy older individuals compared to young individuals may not be interpreted by an enhanced risk for developing dementia as a result of the *APOE* ε4 allele. Rather, it might be possible that other factors related to disease could alter grey matter patterns. One investigation examining the *APOE* x *BDNF* interaction has observed stronger structural covariance in Val/Val homozygotes compared to Val/Met carriers and Met/Met homozygotes (Lin et al., 2016).Taken together, there is a possibility that this cohort did not reach the age range or incipient (preclinical) disease profile to show structural alterations between these polymorphisms, or it may be possible that genetic

variations are expressed in different ways during the aging process (Jasinsak et al., 2018). and therefore, this cohort may have not shown any differences between genotypes after all. Additionally, it is likely that even if there are covariance differences across polymorphisms, one limitation of this study is that there was not enough power to detect those difference in our cohort.

Existing evidence validated that healthy elderly participants (59-90 years of age), free of dementia and other neurological and psychological conditions, demonstrated atrophy in several specific brain regions which are related to memory impairments, such as frontal-, and temporal lobe brain regions, as well as the hippocampus, after only 1 year of imaging (Fjell et al., 2009). This indicates that preclinical changes may happen before actual diagnosis of AD. Therefore, further analyses were performed using a regions of interest approach to investigate brain structure metrics associated with memory function, such as thickness of the entorhinal cortex and parahippocampus cortex, and volume of the hippocampus and amygdala. The current results demonstrated that hippocampus and amygdala volume were reduced with increasing age, which is indicating that grey matter volume reductions in limbic areas may predict cognitive decline and identify individuals in preclinical AD stages (Zanchi, Giannakopoulos, Borgwardt, Rodriguez, & Haller, 2017). Previous studies observed hippocampus volume reductions with age in healthy individuals (aged 50 to 95 years) prior to amyloid-beta pathology, indicating that amyloid-beta burden did not trigger brain volume loss in this region in healthy older adults (Jack et al., 2015). This is in line with a more recent investigation reporting grey matter volume reduction in both the hippocampus and amygdala in healthy elderly individuals (Zanchi, Giannakopoulos, Borgwardt, Rodriguez, & Haller, 2017). Overall, my results add to the pre-existing findings that reduced hippocampal and amygdala volume could be used as future biomarkers to detect individuals at potential risk for

neurodegenerative diseases. As a result, aging has been recognised to be the strongest source of deteriorating brain structures and cognitive performance in healthy aging (Breteler et al., 1994), which could explain why we found relatively reduced size of these brain structures and cognitive performance.

Grey matter volume of hippocampus and amygdala showed no significant differences in my healthy cohort between *APOE* and *BDNF* Val66Met polymorphisms. Likewise, cortical thickness of entorhinal and parahippocampus cortex showed also no significant differences between the polymorphisms respectively. This may be due to not enough statistical power and lower sample size. Previous evidence demonstrated reduced cortical thickness in healthy middle-aged (Burggren et al., 2008) and adolescent *APOE* ε4 carriers (Shaw et al., 2007) compared to non-ε4 carriers. Similarly, healthy older *BDNF* Met carriers demonstrated reduced grey matter volume (Brown et al., 2014) and healthy younger Met carriers showed reduced cortical thickness (Yang et al., 2012) within temporal regions compared to Val homozygotes. In AD, the progression of cortical thickness and grey matter volume decrease is more accelerated (Teipel et al., 2014; Younes, 2014). We were not successful in replicating previous findings demonstrating the impact of the *APOE* ε4 allele (Burggren et al., 2008; Cacciaglia et al., 2018) and *BDNF* Met allele on volume and cortical thickness (Gomar et al., 2016). Though, it is interesting to see that ε4 carriers mirror their brain structure metrics in showing more volume and thickness in *BDNF* Val homozygotes than *BDNF* Met carriers, which did not reach statistical significance in this study. This pattern is more variable in ε3 homozygotes, showing more hippocampus and amygdala volume, as well as more entorhinal cortex thickness in Val homozygotes than in Met carriers (Figure 6.2.). Possible explanations for these patterns could be that the BDNF Val66Met polymorphisms impairs the transport of *BDNF* protein (Egan et al., 2003). More surprising is that there was not a significant

interaction effect between the *APOE* x *BDNF* genotypes. An interaction effect was expected, in which ε3 and Val homozygotes were assumed to have more grey matter volume and increased cortical thickness because data of the functional connectivity studies in previous chapters showed differences. However, this finding could indicate that the brain structure is not solely influenced by the genotypes in humans. McKay, Moreau, Henare, and Kirk (2019) for example, did not find any significant differences in white matter analyses between Met and Val homozygotes in a healthy younger population (sample size 61, average age 23 years) suggesting that correlation between the hippocampus and the *BDNF* Val66Met polymorphisms may not be as strong as anticipated. Moreover, carrying one or two copies of the *BDNF* Met allele may also play a significant role in different results; similar to the majority of studies (Bueller et al. 2006; McKay et al.), within this study participants with a copy of the Val allele + Met allele were defined as 'Met carriers'. Forde et al. (2014) investigated possible differences in homozygosity and heterozygosity in brain structure morphometrics and failed to support the hypothesis of a dose-dependent effect of the Met allele. Furthermore, it was discovered that the individual with the Val/Met heterozygote showed the greatest different grey matter and white matter structures than Val/Val and Met/Met homozygotes (Forde et al., 2014). It is feasible that by combining Met carriers together, I may have missed out on possible interaction effects between Met and Val. To clarify how the *BDNF* genotype may affect brain structures, further research is needed, however, this is beyond the current study. Several other studies did also not find significant alterations in hippocampus volume within the *BDNF* Val66Met and the *APOE* genotypes (Harrisberger et al., 2014; Richter-Schmidinger et al., 2011), as well as interaction effects between *BDNF* and *APOE* in healthy young individuals (Richter-Schmidinger et al., 2011). In the end, the substantive question whether genotypes alter brain structure prior to pathological alteration remains open (Figure 6.1).

This chapter did not find any relationship between *APOE* polymorphisms and cognition, as well as between *BDNF* polymorphisms and cognitive status. However, researchers examining the relationship between polymorphisms and aging identified a linear effect, in particular a detrimental outcome in *APOE* ε4 carriers with rising age (Wisdom et al., 2011). According to most current literature, the mean size effect is often minimal when examining associations of ε4 carriers and ε4 non-carriers on cognition across the average ages (Henson et al., 2020). Other studies also observed a greater deleterious impact on in older ε4 carriers than in younger ε4 carriers (Lyall et al., 2019; Marioni et al., 2016). As reported in Lyall et al. (2019), I also did not find any interaction effects between age and polymorphisms, as well as cognitive function status and polymorphisms. An explanation for the absence of interactions is the bias that the cohort population may comprise of healthier ε4 carriers. Previous investigations of the whole THBP cohort did also not find associations between single gene polymorphisms and cognitive functions (Ward et al., 2014), as well as between *APOE* polymorphism and cognitive reserve on cognitive function (Ward, Summers, et al., 2015a). Lyall et al. (2019) also investigated the *APOE* ε4 genotype in relation to lifestyle factors and was not able to identify any interactions, suggesting that the cohort sample was either too healthy or too young (56 years at baseline) to detect any significant effects. Similar to Lyall et al. (2019), population bias could have potentially influenced the investigation between the polymorphisms, age, and cognitive reserve in this chapter, as the THBP cohort may be healthier due to better socio-economic status, may have higher IQ and are more educated. On the other hand, Liu et al. (2010) found a significant interaction between ε4 carriers and age on memory performance in older (50+ years) but not in younger individuals suggesting that cognitive performance in ε4 carriers is age-dependent.

Detrimental effects were also found in episodic memory and executive function performance in *BDNF* Met allele carriers in older individuals (Papenberg et al., 2015). In particular, a *BDNF* x age interaction was found indicating that age affected memory performance in *BDNF* Met carriers stronger than in *BDNF* Val homozygotes (Kennedy et al., 2015). In contrast, the study in this chapter was not able to show significant differences of the *BDNF* Val66Met polymorphisms on brain morphometric structures, however previously colleagues demonstrated within the whole dataset of the THBP that an *APOE* x *BDNF* interaction associated with episodic memory, adjusted for age and education. In particular *BDNF* Met carriers carrying the *APOE* ε4 allele indicated poorer performance than Val homozygotes (Ward et al., 2014), suggesting additive effects of the AD-related polymorphism may have been the source of statistical significance. Moreover, Ward et al (2015a) also observed that the *BDNF* Val66Met polymorphisms moderated the effects of cognitive reserve on cognitions. A cognitive reserve x *BDNF* interaction was found, in which executive performance was improved in Val homozygotes, adjusted for age. This suggests that Met carriers struggle to acquire the beneficial effects after exposure to cognitive stimulating activities contributing to cognitive reserve compared to Val homozygotes (Ward, Summers, et al., 2015a). Although, these findings exhibited that the Met allele had detrimental effects on cognitive function, the study of this chapter was unable to show significant differences of the *BDNF* Val66Met polymorphism on brain morphometrics, which may be due to higher educated individuals and better IQ status within the THBP population. In particular, Ward et al. (2017) highlighted *BDNF* Met carriers had "more years of education than *BDNF* Val homozygotes", concluding that after 35 months cognitive reserve is improving performance in executive functioning in *BDNF* Met carriers. The nil findings of this chapter could also be possibly related to the small sample size in this thesis, as all of the aforementioned studies included a wider cohort population.

Studies observed that performance of different cognitive domains correlated with grey matter volume of the temporal lobe and hippocampus regions (Zhang et al., 2011) and cortical thickness of the frontal cortex, parietal cortex, posterior cingulate cortex and temporal cortex (Burzynska et al., 2012). Within this study, brain structure was associated with cognitive function status within this cohort. In particular, working memory and executive function showed significant associations with grey matter volume and cortical thickness, while episodic memory and language function showed no significant associations with any brain structure. Working memory was significantly predicted by accumbens area, post-central gyrus thickness, inferior temporal thickness, and frontal pole thickness. A decrease of the accumbens area and the post-central gyrus thickness was associated with a decline in working memory within this cohort indicating that the limbic system may be more susceptible to degeneration prior to manifestation of dementia (de Jong et al., 2012). The accumbens area is a network implicated in motivation and emotions (Russo & Nestler, 2013), while the postcentral gyrus is associated with the somatosensory cortex (Kropf et al., 2019). The accumbens is part of the basal ganglia and previous research found that information that was relevant was stored, while insignificant information was previously filtered by the basal ganglia (McNab & Klingberg, 2008). This may suggest that older members of this cohort may have problems with the attentional control system and therefore show a decline in working memory storage.

Interestingly, negative correlations were found between working memory and frontal pole thickness, inferior temporal thickness. The increase of the frontal pole thickness and inferior temporal thickness was associated with the decrease of working memory. Similarly, research has found that greater brain volumes were linked to poor task performance implying that a

reduction of cognition could be caused by retained volume of certain brain regions in comparison to other areas in aging (Salat, Kaye, & Janowsky, 2002). The frontal pole has many interconnections and it is presumed that it plays a role as supervisory attentional control system (Koechlin, 2011) and the inferior temporal gyrus is related to visual stimuli processing (Gross, 1994), while working memory is defined as short-term memory or temporary storage and has been associated with fronto-parietal brain regions (Diamond, 2013). This connection signifies that working memory function is not explicitly associated with prefrontal cortex areas but may have an inter-connected functional network throughout the brain. The findings of this chapter fail to support hypothesis 11, in which it was expected that brain volume and thickness would correlate positively with cognitive function. A possible explanation of this anticorrelation could be that with decreasing brain volume, cognitive performance increased because other brain regions were able to take over certain functions or different brain regions are recruited to accomplish the cognitive task, which would be characterized as compensations mechanisms (Heuninckx et al., 2008). Fox et al. (2005) articulated the difference of interpretation of data between correlation and anticorrelation that "While correlation may serve an integrative role in combining neuronal activity subserving similar goals or representations, anticorrelations may serve a differentiating role segregating neuronal processes subserving opposite goals or competing representations". Yet, Hampson, Driesen, Roth, Gore, and Constable (2010) implied that anticorrelation could also play a role in integration, in which brain regions are supposed to work in a cooperative way sharing data. One investigation for example found that greater anticorrelations were related to less fluctuation in cognition indicating enhanced cognitive performance was association with functional connectivity (Kelly, Uddin, Biswal, Castellanos, & Milham, 2008). Significant anticorrelations could be associated with activity of the DMN, as is has been shown that important resting-state networks are interconnected to structural

connectivity (Van Den Heuvel, Mandl, Kahn, & Hulshoff Pol, 2009). One study found age-related increased connectivity in modular structures of the DMN and the sensorimotor/visual/spatial network, including brain structures such as the precuneus, superior occipital gyrus, and superior parietal gyrus (Chen, He, Rosa-Neto, Gong, & Evans, 2011). Explanations are that the aging brain may have higher regional connectivity, as well as improved recruitment mechanisms of inter-modul brain areas, which previously have been related to overactivation. Subsequently, this could reflect the recruitment of functional brain regions in healthy aging (Chen et al., 2011). A tentative explanation for the results of this chapter is that because age and structural changes are on the same causal path to cognitive decline, that we have conditioned on a collider (Elwert & Winship, 2014) and induced a sign-reversal in some coefficients. There is evidence that age causes structural changes (Good et al., 2001; Resnick et al., 2003), so by including age in the regression model we may obtain a higher R2, but at the expense of valid interpretation of the coefficients. A similar concern would apply to adjusting for intracranial volume or total grey matter. Future work should include mapping the causal pathways using directed acyclic graphs (DAGs) to explain modeling choices, which may include taking age, intracranial volume, and total grey matter out of the penalised regression model.

In the current study, positive association were observed between executive function and temporal pole thickness, superior temporal thickness, caudal middle frontal thickness, and the volume of the accumbens area. Particularly, lower executive function performance was correlated with thinning and smaller volumes of these brain structures. The temporal pole is associated with many key functions, such as semantic processing, socio-affective behaviour, and memory (Córcoles-Parada et al., 2019) and the superior temporal sulcus has been associated with speech perception and communication, and is therefore vital for social

interactions, which is an important component of executive functions (Hickok & Poeppel, 2007). Studies have observed that the superior temporal gyri, as well as the superior and inferior frontal gyri showed the largest cortical thinning in relation to worse executive function within the healthy aging process (Burzynska et al., 2012). These findings indicate that age-related effects on executive functions are not mainly related to the frontal areas (Burzynska et al., 2012). Yet, it is well-known that the prefrontal cortex is associated with executive function, characterised by higher cognitive processes such as reasoning, planning and problem-solving (Diamond, 2013). The result of this chapter is in line with previous studies demonstrating that executive function was linked to frontal lobe regions, here specifically with the caudal middle frontal gyrus (Burzynska et al., 2012).

Executive function also exhibited significant associations with the accumbens area. A decrease in volume of the accumbens area was associated with reduced executive function within this cohort. The nucleus accumbens has many interconnections and is involved in cognitive processes such as emotions, motivation, and pleasure (Russo & Nestler, 2013), and is related to the reward system. This reward system is activated by dopamine neurons in the ventral tegmental area when some specific behaviour activates the reward pathways.
In aging, a significant deterioration of the dopamine receptors and transporters have been found (Dreher, Meyer-Lindenberg, Kohn, & Berman, 2008). In particular, dopamine synthesis and reward-associated prefrontal cortex activity are altered in healthy elderly. Other studies have shown that the nucleus accumbens is closely connected to the ventral hippocampus, which is known to accumulate early life experience ,as well as controlling behavior that is related to anxiety (Padilla-Coreano et al., 2016). This may indicate that the nucelus accumbens is more vulnerable to stress situations and therefore decreases brain volume, which could be associated with a decline in executive function in stressful situations.

Most recent findings reported that the pathway between the ventral hippocampus and nucleus accumbens is predicting behavior related to anxiety and social interaction, suggesting that certain life situations may regulate changes in neural activity pathways (Muir et al., 2020) Therefore, with increasing age, certain brain regions, such as the hippocampus and frontal lobe regions, are more susceptible to oxidative stress (Venkateshappa et al., 2012). According to one longitudinal study, the volume of the nucleus accumbens declined in a linear progression over time, suggesting that the nucleus accumbens is a brain region that develops earlier(Narvacan, Treit, Camicioli, Martin, & Beaulieu, 2017), and therefore may deteriorate earlier. The nucleus accumbens could potentially play a significant role in clinical settings, as a substantial volume degeneration in correlation with cognitive decline (Mini Mental State Examination, MMSE; Montreal Cognitive Assessment, MoCa) was found in AD and MCI compared to healthy controls(Nie et al., 2017). The finding of my study adds to pre-existing knowledge that measures of the nucleus accumbens volume could be used as a biomarker for identifying people at risk developing AD.

Remarkable associations were found between executive function and individual structures of the brain. Negative correlations were found between executive function and the putamen, pallidum and supramarginal thickness, in which increased volume of the putamen and pallidum, as well as supramarginal thickness, was correlated with relatively reduced executive function performance. As aforementioned, some greater brain structures volume associated with cognitive performance may cause weaker cognitive performance in aging (Salat et al., 2002). An alternative theory could be that enhanced neural efficiency is improving cognitive performance without the necessity of increased brain volume and thickness. While it has been established that the putamen is part of the dorsal striatum and basal nuclei related to motor functioning (Alexander & Crutcher, 1990) and plays a

significant role in neurodegenerative diseases, such as AD (de Jong et al., 2008), it is less clear why the results displayed negative correlations between the putamen and executive function. Age-related longitudinal studies have demonstrated that the volume of the putamen decreases moderately in healthy adults (ranged between 20 and 77 years). This suggests that the putamen may explain age-related alterations in cognitive functioning and behaviour (Raz et al., 2003). In support to this notion, strong functional connectivity has been found between the putamen and the prefrontal regions, suggesting that the putamen may play a prominent role in cognition (Marchand et al., 2008).

Several limitations should be considered for this study. One of these is that the current data is cross-sectional, a longitudinal study may allow the exploration of the two polymorphisms on size of brain structures over time. The sample size (N =75), which could have restricted the possibility to detect significant associations, therefore the interpretation of null results has to be cautious. The size of my sample is similar to many other MRI studies, but a larger sample would be preferable. The analysis of this study was a penalised regression model, which were internally well validated (by cross-validation and bootstrapping), however, they are not externally validated (out-of-sample). It is recommended to replicate this study with a larger sample size to confirm the results. Regardless of these limitations, these results might be beneficial for future research in the field of neuroimaging and human brain aging.

In conclusion, the current study presented that *BDNF* Val66Met and *APOE* (ε3 and ε4) polymorphisms, and interaction between *APOE* and *BDNF* genotypes, did not influence brain structure metrics within this cohort. Age was associated with decline in hippocampus and amygdala volume. Volume reductions of the nucleus accumbens and decreased cortical thickness of postcentral gyrus, temporal and frontal regions were associated with weaker

performance of working memory and executive functions (Nie et al., 2017; (Burzynska et al., 2012) suggesting that with the aging process, certain brain regions are more vulnerable to deteriorate quicker. While increased structures of the inferior temporal thickness, frontal pole thickness, supramarginal thickness, pallidum volume and putamen volume were associated decreased cognitive performance. This suggests that loss of certain cognitive function could be the consequence of a conserved brain structure in comparison to other brain areas with advanced age. Overall, brain morphometric structures of frontal and temporal thickness, as well as pallidum and putamen volume predicted cognitive measurements of working memory and executive function.

Chapter 7

Discussion

Functional and structural MRI techniques have been used extensively as a tool for diagnosis and monitoring progression of pathological changes (Damoiseaux & Greicius, 2009), as well as to measure human brain connectivity (Hendrix et al., 2015; Dennis and Thompson, 2014). In particular, structural MRI methods are often used in clinical work-up for suspected dementia.

The investigation of brain connectivity disruption in relation to genetic biomarkers and cognitive reserve may help in understanding how the brain networks function in healthy older individuals and why they deteriorate in relation to cognitive changes associated with healthy aging and in neurodegenerative diseases. Aging-related brain changes may predispose healthy older individuals to neurodegenerative conditions such as dementia, and genetic factors such as the allele ε4 of the *APOE* genotype (Licher et al., 2019) or the Met allele of the *BDNF* Val66Met polymorphism (Brown et al., 2020) may contribute further susceptibility. Therefore, this work focused on both brain structural differences and functional connectivity related to common genetic polymorphisms in the *APOE* and *BDNF* genes, as well as in relation to cognitive reserve.

Thesis aims

Overall, the present thesis had five primary aims. First, to explore functional edge strength related to the *APOE* and *BDNF* Val66Met polymorphisms, and how cognitive reserve influences functional edge strength within the DMN, DAN, and SN, using graph theoretical measures. Second, to investigate differences in functional connectivity between the *APOE* and *BDNF* Val66Met polymorphisms, as well as how cognitive reserve may influence connectivity within the DMN, DAN, and SN, using dual regression. Third, to examine the functional organization of the language network (Broca's and Wernicke's areas) with respect to variations of the *APOE* and *BDNF* genes, using a seed-based approach. Fourth, to

elucidate differences in brain structure (cortical thickness & grey matter volume) related to *APOE* and *BDNF* Val66Met polymorphisms. Fifth, to examine whether there was any association between whole brain structures, such as volume and thickness, and cognitive domains (episodic memory, working memory, executive function, and language processing).

Functional connection strength is not associated with allelic variations of *APOE* and *BDNF* polymorphisms in healthy older adults (chapter 3)

The aim of the first results chapter was to explore the functional organization of the brain, in particular functional edge strength within the DMN, DAN, and SN, related to *APOE* and *BDNF* genotypes using graph theoretical measures. A further aim was to explore whether cognitive reserve would correlate positively with functional edge strength within the aforementioned resting-state networks. Here, it was hypothesised that variants of the *APOE* and *BDNF* Val66Met polymorphisms would demonstrate differences and that cognitive reserve would correlate positively with functional edge strength. To achieve this, each dataset was cleaned by performing a single-subject ICA. This was an important step, as following chapters relied on the clean datasets. Subsequently, group-ICA, or Melodic with 70 components and a dual regression within the FSL software, was applied. Resting-state networks were examined and chosen based on the group-ICA. Network modelling was performed using FSLNets within Matlab. Partial correlation analyses were performed to estimate direct network connections, cross-subject analyses were completed to examine differences in matrices, and separate univariate tests were performed to analyse edges of each network.

Key findings

The first hypothesis, that both genotypes would show differences in functional edge strength within the DMN, DAN, and SN, was not supported. The second hypothesis that cognitive reserve may modify functional edge strength within each resting-state network was also not supported. Due to previous inconsistencies of in the literature on increased and decreased functional connectivity in healthy elderly (Arenaza-Urquijo et al., 2013; Hafkemeijer et al., 2013; Toussaint et al., 2014), this thesis contributed with further information about functional edge strength within these networks. However, recent studies likewise presented no effect of *APOE* ε4 carriership on brain functions in healthy younger adults (Mentink et al., 2020), indicating that the ε4 allele may not influence brain function in healthy individuals, rather may be more implicated in dementia risk alone.

Note: One participant was removed (due to tumour or noise) bringing the sample size down from 77 to 76 subjects after re-screening data.. Additionally, due to computational and time constraints of performing a group-ICA and dual regression with ≥ 70 components, a voxel-based approach (n 76, 25 components) was utilised.

Interactive effects of the *APOE* and *BDNF* polymorphisms on functional brain connectivity: The Tasmanian Healthy Brain Project (chapter 4)

The second results chapter aimed to determine if there were whole-brain group differences in resting-state networks (DMN, DAN, SN) related to *BDNF* Val66Met and *APOE* polymorphisms in cognitively healthy older persons. Additionally, cognitive reserve was investigated. Clean single-subject ICA datasets from chapter 4 were used to perform a group-ICA with 25 components. Resting-state networks were selected, which was a major step in the investigation, as future examination (i.e. chapter 5) relied upon the timeseries that were

obtained from the single-subject ICA. Dual regression was performed for cross-subject statistics.

Subject data is as described in chapter 3; all participants consented to the fMRI acquisition. General linear models were fitted to each polymorphism, respectively, including age, gender, and cognitive reserve as covariates.

Key findings

The results of this chapter demonstrated that ε4 carriers exhibited reduced functional connectivity between the DAN and occipital cortex compared with ε3 homozygotes. It is hypothesized that these findings could potentially be related to AD pathology, however, this result needs to be interpreted with care, as this has not been evidenced by our data. However, other studies (Goveas et al., 2013; Lu et al., 2017), suggested that older adults who are *APOE* ε4 allele carriers may have AD-related pathology in the brain possibly influencing functional connectivity. One such pathology is amyloid-beta burden in the brain (Morris et al., 2010), which has been associated with *APOE* ε4 carriership in cognitively healthy adults. The *BDNF* Val66Met polymorphism did not show significant differences. Interaction effect analysis showed stronger functional connectivity in Met/ε3 relative to Met/ε4 carriers between the DAN and posterior regions of the default mode, as well as stronger functional connectivity in Met/ε3 compared to Val/ε4 carriers between the dorso-ventral stream /DMN and lateral occipital cortex. Gender differences were identified, wherein males demonstrated stronger functional connectivity than females between the DMN and three different regions, the juxtapositional lobule, the cingulate gyrus and the precuneus cortex. Conversely, females evidenced stronger functional connectivity between the executive function network and cingulate gyrus. Analysis of cognitive reserve revealed positive and negative correlations

with functional connectivity. Positive correlations were found between the central executive network and white matter callosal body, as well as between the central executive network and postcentral gyrus. Further positive correlations were found between the DAN and subcallosal cortex. Negative correlations were found between the DMN and a few cluster regions, such as intracalcarine cortex, occipital cortex and precuneus (controlled for *APOE* & *BDNF* genotypes).

Taken together, these results indicate that the *APOE* ε4 allele may be influencing functional connectivity via genetic interactions. *APOE* ε4 carriership is a strong correlate of AD-related pathology (Morris et al., 2010), therefore, these findings suggest that *APOE* ε4 carriers may affect functional connectivity in older adults without dementia but who may demonstrate central amyloid pathology. Cognitive reserve was expected to show positive correlation increasing functional connectivity within healthy older individuals; however, it is unclear why cognitive reserve also showed a negative correlation, controlled for *APOE* and *BDNF* genotypes. With this anticorrelation, it is likely that cognitive performance is maintained or improved even though the brain atrophied, which could be related to reorganization of the brain and/or recruitment of different networks to preserve cognitive performance, which, has been described as compensation (Heuninckx et al., 2008). However, whether this system of neuroplasticity is occurring within our healthy elderly cohort in some pathways, which requires further investigations. The ideal experiment would include behavioural assessments and fMRI scanning at two different timepoints, at baseline and after a certain amount of time learning a different language for example, as described in Bubbico et al. (2019).

Language networks recruit frontal lobe regions to support functional connectivity in heathy older adults (chapter 5)

The third research chapter aimed to determine whether the language networks (Broca's and Wernicke's areas) presented differences in functional connectivity within variants of the *APOE* and *BDNF* Val66Met polymorphisms. Further, it was investigated whether cognitive reserve showed positive correlations with functional connectivity. Earlier studies reported that educational intervention improved language processing over time within the THBP (Thow et al., 2018). Three hypotheses were proposed. First, stronger functional connectivity would be associated with *BDNF* Val homozygotes and *APOE* ε3 homozygotes compared to Met carriers and ε4 carriers, respectively. Second, interaction effects would alter functional connectivity. Third, cognitive reserve would be associated with increased functional connectivity within the language network. To achieve this aim, each pre-processed and cleaned functional dataset from chapter 3 was used to create functional Broca's and Wernicke's spheres, which were then used for higher-level analyses (Bayesian modelling within FEAT).

This study used the same functional dataset of the participants that were involved in chapter 3. A total of 76 subjects of the THBP were included. General linear models were fitted to each polymorphism, respectively, including age, gender, and cognitive reserve as covariates.

Key findings

Given the findings of chapter 4, that *APOE* status may influence whole-brain functional connectivity networks within genetic interactions between *APOE* and *BDNF* Val66Met polymorphisms in participants of the THBP, a seed-based approach was applied to investigate the language network. This was particularly important to clarify given that education and cognitive reserve may be linked with enhanced language processing within this cohort (Thow et al., 2018). The analysis first explored whether there were significant differences in functional connectivity between the seed and the rest of the brain within

variants of *APOE* and *BDNF* Val66Met polymorphisms, independently. The results indicated that both polymorphisms respectively did not differ in functional connectivity between the language networks and the rest of the brain. Furthermore, my results showed that with increasing age, functional connectivity increased between Broca's area and the left middle and superior frontal gyrus, as well as between Wernicke's area and the right putamen and right supramarginal gyrus, adjusted for *BDNF* and *APOE* genotypes. These results suggest that with increasing age, language function improves by recruiting frontal brain regions. In literature, this recruitment process has been referred to compensatory in healthy elderly (Diaz et al., 2016), in which the brain is recruiting other parts of the brain to maintain cognitive functioning, in this case language processes. It remains unclear whether or not this recruitment is compensatory within our study because we were not able to check whether participants were maintaining performance to the same degree, therefore it can only be speculated that this process is a compensatory process because we were not able to check whether participants were maintaining performance to the same degree, therefore it can only be speculated that this process is a compensatory process.

Although, there were no independent effects of both polymorphisms, *APOE* x *BDNF* interaction effects were found. Val/ε3 homozygotes showed stronger functional connectivity than Val homozygotes/ε4 carriers between right Broca's area and pre- & postcentral gyrus/precuneus cortex. Met carriers/ε3 homozygotes demonstrated greater functional connectivity than Met/ε4 carriers between left Broca's area and pre- & postcentral gyrus/ parahippocampal gyrus. This data adds to the growing body of evidence suggesting that the ε4 allele may influence genetic interactions decreasing functional connectivity in healthy older individuals, which is possibly linked to higher amyloid-beta burden (Liu et al., 2013). Another interaction effect revealed stronger functional connectivity in Met carriers/ε3 homozygotes than in Val/ε3 homozygotes between left Broca's area and parahippocampal

gyrus. A previous investigation found an interaction between cognitive reserve-related differences in executive function and the *BDNF* Val66Met polymorphisms, demonstrating that executive functioning differences between three cognitive reserve groups decreased in Val homozygotes, however, were more noticeable in Met carriers (Ward et al., 2017). Hence, cognitive reserve may influence a range of sophisticated cognitive processes differently within the *BDNF* Val66Met variation in healthy individuals.

Another finding of this chapter was that cognitive reserve correlated negatively with functional connectivity between left Broca's area and parahippocampal regions, indicating that with increasing cognitive reserve, functional connectivity was decreasing or vice versa. Previous studies observed associations of greater functional connectivity and higher cognitive reserve (Marques et al., 2016; Arenaza-Urquijo et al., 2013), proposing that higher education levels may stimulate neural processing and increase efficiency of information processing (Marques et al., 2016). On the other hand, negative correlations were reported in task-related fMRI studies between cognitive reserve and metabolism in the intraparietal sulcus and temporopolar cortex, brain regions that comprise to the DMN and DAN (Bastin et al., 2012). This outcome indicated that individuals with greater vocabulary levels and education may have enhanced neural efficiency and not need as much activity in certain functional brain network, which permits mental processes normally directed to the self and attention, to direct to external stimuli while being at rest (Bastin et al., 2012).

Although, cognitive reserve has been increasingly positively correlated with improved functional connectivity (Bozzali et al., 2014; Franzmeier et al., 2017; Marques et al., 2016 Wei et al., 2012), unexpectedly, the current study showed that increased cognitive reserve was associated with decreased functional connectivity. This discovery could also be linked to the neural efficiency hypothesis, in which the cognitive function may still be performed without the necessity of increased functional brain connectivity (Jung et al., 2017). The

neural efficiency hypothesis of intelligence is referred to the association that more intelligent individuals exhibit lower (but more efficient) activity in the brain while executing as task (Haier et al., 1992; Haier et al., 1988). A test measuring general intelligence was correlated with metabolic rate showing high variability, indicating that intelligent individuals with advance cognitive functioning used more efficient brain networks and therefore less energy in the brain (Haier et al., 1988). Studies have shown that the interactions of various brain regions are the neural groundwork for intelligence, both in task- and resting-state fMRI (Song et al., 2008; Neubauer et al., 2009), suggesting that intelligence can be improved and that people with more education need less brain resources because their networks are more efficient. Together, our results may suggest that healthy elderly individuals with greater cognitive reserve may have a higher IQ and therefore, may not need increased functional connectivity to maintain cognitive functions.

Brain structure metrics related to *APOE* and *BDNF* polymorphisms, and cognitive function in healthy older adults (chapter 6)

The aim of the fourth study was to investigate possible differences in brain structure related to genetic polymorphisms and cognitive reserve. In particular, this thesis was interested in whether *APOE* and *BDNF* Val66Met polymorphisms showed differences in grey and white matter volume covariance patterns. This thesis also examined whether cognitive reserve would correlate positively with covariance grey matter patterns. The third aim was to explore genetic interactions. A further aim of this study was to investigate differences in cortical thickness (parahippocampus and entorhinal cortex) and grey matter volume (hippocampus and amygdala) between *APOE* and *BDNF* genotypes. The final aim was to examine correlations between cortical brain structures and cognitive status (episodic memory, working

memory, executive function, and language processing) throughout the whole brain. Here, SPM12 in Matlab was applied for pre-processing and analysed the data using PCA within Matlab. Additionally, Freesurfer software was utilised to obtain measures of cortical thickness and grey matter volume of regions of interest (ROIs). Data analysis was performed using RStudio using a penalised regression method called elastic net to predict cognitive status from cortical features.

Key findings

No significant differences between *APOE* and *BDNF* genotypes independently were found in either cortical thickness or grey matter volume. Cognitive reserve did not modify covariance patterns of the brain. Investigating cognitive status and volume and thickness of the whole brain showed that cortical thickness and volume of specific brain regions were associated with performance in working memory and executive function. In particular, reduced size of particular brain structures was associated with relatively reduced cognitive function.

General discussion & integration of findings

Recent studies have shown that functional and structural connectivity in relation to genetic and environmental factors can be used as a biomarker identifying individuals at risk for AD (Stam et al., 2006, 2008; Burggen et al., 2008; Brown et al., 2014; Wang et al., 2014; Lu et al., 2017). Cognitive stimulation and genetic make-up may be beneficial and preserve cognitive functions, however, genetic factors may also be associated with risk of rapid degeneration of cognition in older adults (Nyberg, Lövdén, Riklund, Lindenberger, & Bäckman, 2012). The overarching aim of this thesis was to explore differences in patterns of brain connectivity of healthy older individuals with *APOE* and *BDNF* genotypes, including the influence of cognitive reserve on underlying connectivity.

One approach to visualise functional connectivity is graph analysis, in which voxels are described as 'nodes' and connections between nodes are characterised as 'edges' (Watts and Strogatz, 1998; Stam et al., 2007). Functional networks of brain connectivity may demonstrate disruption in vulnerable individuals with specific genotypes, such as *APOE* or *BDNF* Val66Met. Previous studies utilising graph theoretical approaches indicated that *APOE* ε4 carriers have a different functional structure than *APOE* ε3 homozygotes (Seo et al., 2013; Goryawala et al., 2015) and were less efficient. For example, *APOE* ε4 carriers exhibited abnormal small-world networks and longer path lengths than *APOE* ε3 homozygotes (Goryawala et al., 2015; Seo et al., 2013). A *BDNF* Val66Met study also showed node disruption in Met carriers compared to Val homozygotes in white matter networks (Park et al., 2017). However, there is limited research about the combination of both genotypes, as well as cognitive reserve, in relation to resting-state networks, such as the DMN, DAN, and SN. Given the sparse publications on genotypes and functional brain networks, the overarching aim was to investigate functional edge strength within these resting-state networks. Despite research showing disrupted brain networks within the DMN in AD (Hahn et al., 2013), this study did not show genotypic differences in functional edge strength in all selected network in healthy older adults (Chapter 3). This may have been due to analysis of 70 independent components detecting only a handful of networks representing the DMN, DAN, and SN, or 'nodes' / networks that have been the focus of this chapter. However, it may be possible that my healthy cohort did not have significant differences in functional edge strength between genotypes. No significant differences in small-world properties, cluster coefficients, or hub identification was found between amnestic MCI and mild dementia (Frantzidis et al., 2014). However, significant differences between healthy controls and MCI and mild dementia were found in terms of small-world properties, cluster

coefficients, and path length, suggesting that network disruption is more pronounced in individuals with pathological alterations in functional connectivity resulting from risk genotypes versus healthy individuals. Overall, graph theoretical analysis could be a valuable technique for identifying age-related pathology.

Considering the evidence of the *APOE* ε4 allele affecting functional connectivity (Goveas et al., 2013; Wu et al., 2016; Lu et al., 2017), the findings presented in this thesis provide further support for the use of ε4 carriership as a potential correlate of functional connectivity disruption in healthy older individuals. Both approaches, data-driven (Chapter 4) and seed-based correlations (Chapter 5) showed that the ε4 allele was influencing the interaction of *APOE* x *BDNF*. Stronger functional connectivity was found in ε3 homozygotes than ε4 carriers between the DAN and occipital cortex (Chapter 4). The fact that the ε4 allele is associated with AD pathology (Morris et al., 2010) suggests that ε4 carriership may lead to detrimental brain connectivity. A study investigated in amyloid-beta PET (positron emission tomography) imaging and found that the load of cortical amyloid beta was associated with functional connectivity impairments in medial temporal lobe in cognitively healthy elderly individuals (Song et al., 2015). In opposition to the amyloid-beta hypothesis, studies found that the spread of plaques in the brain in healthy cognitively normal elderly people is frequently found to be as heavy as in patients with dementia (Chételat et al., 2013; Price et al., 2009), implying that the accumulation of amyloid-beta is a natural part of aging and is not directly associated with the commencement of AD. These discrepancies prompted researchers to investigate more deeply into the pathological mechanisms. In particular, animal studies reported that mice with amyloid beta accumulation and senile plaques in the brain did not show neuronal loss, deterioration of nerve cells, and disruption in cognition (Kim et al., 2013; Kim et al., 2007). Research presented that AD-related pathological

mutations, such as the APP mutation, may be the cause for synaptic defects and cognitive deterioration (Tamayev, Matsuda, Arancio, & D'Adamio, 2012). Mutations of the APP results in APP C-terminal fragments, which subsequently triggers neuronal dysfunction. One resting-state investigation also found no significant correlation between functional connectivity and amyloid beta load after adjusting for multiple comparison, proposing that a possible non-linear association between functional connectivity and amyloid burden (Teipel et al., 2018). These inconsistencies show that it is difficult to base our findings of the *APOE* ε4 allele disrupting functional connectivity completely on the amyloid hypothesis. Therefore, my assumptions require caution when interpreting them, as we have not obtained any further biomarker data to test this. Earlier investigations have shown that ε4 and Met carriers were more susceptible to reduced brain activation in hippocampal regions, leading to low cognitive performance, compared to non-carriers of the *APOE* ε4 and *BDNF* Met alleles (Kauppi et al., 2014). In line with these studies, the present examination of *APOE* x *BDNF* combinations revealed that Met/ε3 had stronger functional connectivity than Met/ε4 and/or Val/ε4 carriers. Assumptions are that healthy older *APOE* ε4 carriers are more susceptible to functional disruption potentially due to accumulating amyloid-beta in the brain (Kropf et al., 2019; Lim et al., 2014; Lim et al., 2015; Adamczuk et al., 2013).

In the present investigation, no evidence was found to support the effects of the *BDNF* Met allele on resting-state functional connectivity (chapter 3, 4, & 5) nor within the morphometry of brain structures (chapter 6). Prior studies found more brain volume of the hippocampus and temporal lobe regions (Brown et al., 2014). Enhanced resting-state functional connectivity within the hippocampal region was also found in Val homozygotes than in Met carriers (Yin et al., 2015), therefore positive correlations were expected between *BDNF* Met carriage and brain volume, and between the Met allele and functional connectivity.

Reversely, non-linear relationship has been found between brain structure and genotypic effect, elucidating that the effect of *BDNF* on brain structure is still relatively unknown, however, by classifying all Met carriers into one group, it is clear that studies might miss significant interactions between Met and Val (Forde et al., 2014).

The *BDNF* Val66Met polymorphism was not associated with reduced functional connectivity, cortical thickness, and grey matter volume. Recent data have indicated no reduction in functional connectivity and cognitive performance in healthy female *BDNF* Met carriers compared to Val homozygotes (Rodriguez et al.2018). On the other hand, a recent review analysing *BDNF* Val66Met polymorphisms documented detrimental effects of the Met allele on cognition, brain activation, and brain structures (Brown et al., 2020). While studies demonstrated that the *BDNF* Met allele has harmful effects (Yin et al., 2015), it is less clear why the results showed null findings. Ultimately, it is possible that the sample size was too small to obtain significant differences. However, there is still inconsistencies with respect to brain volume and genotypes, as several studies with greater sample size also did not find found significant correlations (Harrisberger et al., 2014; Richter-Schmidinger et al., 2011).

While the aging process has been related to impaired functional connectivity within the DMN (Geerlig et al., 2015), the findings of the current thesis discovered positive correlations. With increasing age, functional connectivity increased within the executive control/ default mode network regions (Chapter 4) and within the language networks (Broca L & Wernicke's area, Chapter 5). This outcome suggests that healthy older individuals possibly recruit frontal regions to support both their endogenous attentional and language processes. In support of this notion, research has reported recruitment in older individuals, especially when central

networks are interrupted, thus, neural compensation appears to be present to preserve cognitive performance (Martins et al., 2015).

Evidence has demonstrated that boosting cognitive reserve may promote more dendritic connections and preserve the brain from harmful effects (Matyas et al., 2019) and increase functional connectivity (Marques et al., 2016). The finding that cognitive reserve increased functional connectivity between the central executive network and postcentral gyrus, as well as within the DAN (Chapter 4) was particularly important because it signifies that increased cognitive reserve is enhancing sensory and attentional processes in healthy elderly. Two investigations found improved functional connectivity and increased grey matter volume in healthy older individuals with higher education levels, a commonly used proxy for cognitive reserve (Arenaza et al., 2013; Marques et al., 2016). To support this notion, one investigation observed protective effects of cognitive reserve, in which the brain recruited other brain regions to compensate for cognitive functioning in individuals with AD-pathology (Franzmeier et al., 2017).

In the present thesis, cognitive reserve also showed negative correlations with functional connectivity within the DMN regions (Chapter 4) and within left Broca's area (Chapter 5). While previous studies exhibited greater DMN connectivity in correlation with higher education in MCI and AD (Bozzali et al., 2015), it is less clear why functional connectivity was decreased in my healthy cohort in association with higher cognitive reserve. An older investigation reported that excessive cognitive load may trigger stress and anxiety (Callicott et al., 1999) possibly reducing functional connectivity. However, a later study observed no functional connectivity decline in prefrontal lobe areas linked to cognitive load (Jaeggi et al., 2003). More recently, a study in healthy elderly participants did not identify functional

connectivity increases within the DMN (Bozzali et al., 2015), proposing that potential effect may be visible once pathology has progressed.

One study observed that capacity of working memory is restricted and that greater working memory load interrupted the relationship between activation of the dorsal lateral PFC and performance (Callicott et al., 1999). This may potentially cause a mental overload and lead to stress decreasing functional connectivity (Callicott et al., 1999). Yet, a study investigating memory load discovered no reduction in functional connectivity within the prefrontal regions (Jaeggi et al., 2003).

In one investigation, a significant age by education interaction was found indicating that older individuals with more education had less reduction of grey matter volume than individuals with lower education levels, implying that education might play a compensatory and protective role on cortical volume especially in older age (Boller, Mellah, Ducharme-Laliberté, & Belleville, 2017). Another investigation into educational attainment and hippocampal volume identified that educational level had an impact on hippocampal volume in healthy aging men, but not in women (Jiang et al., 2019). It may be possible that no significant correlations between cognitive reserve and brain structure metrics in the present work is due to a gender bias (51 females : 24 males). In other investigations, cognitive reserve was shown to not significantly improve cognitive functions (Christensen et al., 2001; Van Dijk et al., 2008; Tucker-Drob et al., 2009; Zahodne et al., 2011), suggesting that cognitive reserve may play a compensatory rather than a protective role.

Previous reports have detected a decline in brain structure metrics, for example, cortical thickness and grey matter volume, within the MTL regions, such as the hippocampus, entorhinal cortex, and parahippocampus, in cognitively healthy carriers of the *APOE* ε4 allele

and/ or *BDNF* Met allele (Brown et al., 2014; Lim et al., 2016; Liu et al., 2013; Lin et al., 2016). This was not evident in the present thesis (Chapter 6). Cognitive reserve did also not associate with covariance patterns of grey matter and white matter volume of the whole brain and did not modify *APOE* and *BDNF* genotypes. A possible explanation may be that my cohort may have not reached the age (mean age 63), in which structural changes occur, compared to a study demonstrating significantly more grey matter volume in Val homozygotes than in Met carriers (mean age 73) (Lin et al., 2016). However, more recent investigations found no significant differences in white matter structures (McKay et al. 2019) and hippocampal volume (Harrisberger et al., 2014; Richter-Schmidinger et al., 2011) between *APOE* and *BDNF* genotypes, indicating that those genotypes need to be treated with more care when using brain structures as biomarker for AD.

Previous studies have observed cognitive functions can be linked to medial temporal lobe atrophy in individuals with MCI (Visser et al., 2002). In particular, cortical thinning (Pelletier et al. 2017) and volume decrease (Zhang et al., 2011) have been correlated with episodic memory decline. The present project also investigated whether cognitive function status would associate with grey matter volume and cortical thickness within the whole brain of healthy older adults. Results from the current study indicated positive and negative correlations. Specifically, it was found that inferior temporal thickness, frontal pole thickness, supramarginal thickness, pallidum volume and putamen volume correlated negatively with working memory and executive function. This is result is unclear because previous studies observed that the volume of the putamen decreases with age (Raz et al. 2003). Positive correlations were found between brain structures, such as the accumbens area, post-central gyrus thickness, superior temporal thickness, temporal pole thickness, caudal middle frontal thickness and cognitive function status, for example working memory and executive function.

This result indicates that atrophy of these brain regions in healthy older adults is linked to declining memory and executive function.

Taken together, some of the data presented in this thesis are consistent with the idea that carriership of the *APOE* ε4 allele is associated with a disruption in functional connectivity and may influence functional outcomes linked to interaction of the *APOE* x *BDNF* genotypes, potentially due to higher AD-pathology, such as amyloid-beta in ε4 carriers. Although, this hypothesis has not been tested within this cohort, the results in combination with previous literature propose that this may be the case. Overall, it may be that no main effects of the *BDNF* polymorphisms on structural and functional connectivity, as well as no main effects of the *APOE* polymorphisms on brain structure were found in healthy elderly individuals probably because this cohort is either still very healthy or may have not reached the stage yet, where pathological alterations may negatively impact the structure of the brain. Based on existing literature, it is speculated that pathological alterations within the aforementioned polymorphisms may be visible between the ages of 70 and 75 years (Brown et al., 2014; Crivello et al., 2010).

Limitations

There are several limitations to this thesis. One central limitation of this thesis is that my model was cross-sectional, therefore, this thesis could not ascertain whether cognitive reserve may have modified differences in structural and functional connectivity between the *APOE* and *BDNF* genotypes from the commencing study (Summers et al., 2013). For example, previous studies demonstrated that cognitive reserve-related differences became more pronounced over time in *BDNF* Met carriers than in *BDNF* Val homozygotes (Ward et al., 2017). Additionally, because this thesis used a cross-sectional approach, the direction of

causality between cognitive reserve and functional and structural connectivity could not be analysed.

Sample size may influence the results in MRI studies, which represents another potential limitation within this study. A sample size of about 80 participants is fairly comparable with previous studies, however, a larger sample size may have given more power, particularly when investigating *APOE* and *BDNF* genotypes individually. For instance, an investigation into brain structure metrics examining approximately 533 individuals across *APOE* genotypes observed that the ε4 allele had an additive effect and was linked to reduced grey matter volume in healthy middle-aged individuals (Cacciaglia et al., 2018). A longitudinal study analysed about 400 healthy individuals of three different groups and noted cortical thickness and grey matter volume decline in Met carriers/ ε4 carriers compared to Val homozygotes (Gomar et al., 2016).

This data presented may have been affected by potential sub-clinical pathological processes, such as amyloid-beta and/or tau occurring in the brain of older individuals. Amyloid-beta and tau levels were not obtained through imaging or biomarkers, and, therefore, could not be included in correlation analyses within these studies, which imposes another limitation of this thesis. Amyloid-beta deposition in the brain has been linked with *APOE* ε4 carriership (Morris et al., 2010), occurring many years before frank dementia, and could have potentially influenced functional connectivity within this cohort. Additionally, it is widely considered that the ε4 allele may have a dose effect on AD risk (Corder et al., 1993), while the *BDNF* Val66Met polymorphisms may have a dose-dependent effect on memory function (Egan et al., 2003) and the DMN-medial temporal lobe network (Lin et al., 2016). However, with only one excluded ε4/ε4 carrier and only two Met/Met carriers, an analysis inspecting dose effect

of each polymorphism was not possible. There is controversial evidence about the amyloid-beta hypothesis indicating that amyloid-beta alone is not cytotoxic and that amyloid-beta did not cause the aggregation of tau (Kametani & Hasegawa, 2018). Instead, it has been suggested that deficiencies in amyloid precursor protein (APP) metabolism and the accumulation of APP-C terminal fragments stimulate tau accumulation, causing axonal and synaptic dysfunction, and are closely linked to primary causes of AD(Kametani & Hasegawa, 2018). Studies have observed that the accumulation of amyloid was not directly associated with functional connectivity (Lin et al., 2020) in the DMN (Adiaanse et al., 2014) proposing that sample size, as well as the heterogeneity of amyloid-beta impact on connectivity may be linked to spatially varied sizes between and within the neural networks. The accumulation of amyloid was not directly associated with functional connectivity in the DMN (Lin et al., 2020), suggesting that sample size, as well as the heterogeneity of amyloid beta may be linked to spatially varied sizes between and within the neural networks (Adiaanse et al., 2014). Taken together, pathological processes such as the defect of the APP metabolism may excite tau pathology subsequently leading to AD. How underlying pathology may have affected my cohort is uncertain, as we did not obtain any pathological biomarkers for this study, therefore the interpretation of my results have to be considered with caution.

Participants for this investigation were invited and recruited from the THBP (Summers et al., 2013). In particular, individuals with the *APOE* ε3/ε3 and ε3/ε4 variants and *BDNF* Val66Met Val/Val, Val/Met, and Met/Met variants were selected, therefore, the distribution between females and males was not considered in the recruiting phase, however, gender was always included as a covariate. In the current investigation, more females ($N = 53$) than males ($N = 27$) were recruited. This could also potentially present another limitation, as a recent study reported that education correlated positively with grey matter volume in men, but not in

women (Jiang et al., 2019). Potential explanations may be related to sex differences in volume, in which females had smaller brain volumes than men (Crivello, Tzourio-Mazoyer, Tzourio, & Mazoyer, 2014; Krogsrud et al., 2014). On the other hand, Jancke, Liem, & Hanggi (2015) demonstrated no or a very small effect of sex on brain compartmental volume. Specifically, cerebral WM volume, cerebellar GM volume, CSF, and amygdala were very slightly determined by gender. Hence, as a result, sex is just a marginal predictor when investigating in compartmental volume (Jancke et al., 2015). This is in line with previous studies also observing small or no influences of sex on brain volume (Fjell et al., 2010; Lemaitre et al., 2005). After all, gender may not necessarily influence brain volume and brain size plays a more relevant role in clarifying inter-individual variation when investigating brain volume (Jancke et al., 2015).

An additional major limitation of this thesis is that the THBP cohort participants have a higher IQ, are more educated and have a higher socio-economic status. Studies have demonstrated that individuals with greater IQ are more likely to acquire more knowledge through further schooling, which subsequently enhanced IQ (Brinch & Galloway, 2012). Participants that felt better equipped to undertake study (more time, more study, less cognitive decline) would be more likely to enrol at university than someone who was struggling with any of these aspects. However, because participants were not assigned to intervention groups at random, it is difficult to say that the intervention group may have caused any differences, therefore, an association was found, which could have been caused by the intervention, or healthier and wealthier people have been observed. This provides a limitation of non-random sampling, in which it is not possible to know that my cohort is effectively reflecting the population. Resting-state fMRI BOLD signal is simply corrupted through artifacts that originate from the slightest movement of the body or head while

acquiring the data (Maknojia, Churchill, Schweizer, & Graham, 2019). Other artifacts influencing the resting-state images are orthodontic appliances within the body. To correct for these problems, fieldmaps are used when pre-processing the data. However, due to technical errors, the acquired fieldmaps were unable to be used. Therefore, this is a further limitation of this thesis. Nevertheless, guidance was sought from FSL Experts at Oxford and attempted to re-register the data via manually extracting the skull, running FEAT, denoising, excluding signal within artifacts and non-brain tissues before transforming to standard space, and finally performing the group-ICA.

A further limitation is the exclusion of task-based imaging. The understanding of how the brain operates originated from experiments that included tasks while being scanned (Daliri & Behroozi, 2013). Task-based imaging is a great source to identify structural networks that represent functional networks in various brain regions. Moreover, resting-state fMRI allowed us to examine a larger number of participants.

Performing multi-modal analyses is an additional limitation of this thesis, in which structural analyses are combined to functional analyses to obtain additional insights into how the brain functions in structural and spatiotemporal space. Learning how to acquire fMRI and MRI scans, as well as examining how all these images have to be pre-processed/cleaned and analysed was a time consuming learning curve. It would have been interesting to integrate multimodal analyses, however, a re-analysis is beyond the scope of this PhD project. Although this analysis would have offered many insights, my team and I did not have the technology and funding to perform this kind of analysis.

Implications

One implication of this study is that the impact of a single *APOE* ε4 allele influences functional connectivity (Yang et al., 2014, Wu et al., 2016). However, the replication of previous *APOE* studies on structural brain changes was not successful (Dowell et al., 2016; Taylor et al., 2014), as well as other *BDNF* Val66Met studies on functional (Yin et al., 2015; Wang et al., 2014) and structural connectivity (Egan, et al., 2003; Brown et al., 2014), even with a comparatively similar or higher sample size. There is some uncertainty that an analysis of one single gene may lead to translatable results, yet, these can be examined as exploratory observations to detect possible risk factors for future research. Regardless, *APOE* x *BDNF* interactions were observed, in which ε4 carriership was associated with deficient functional connectivity. This finding may be due to the accumulation of AD-like pathology in ε4 carriers (Morris et al., 2010). Overall, these results imply that healthy elderly adults who inherit the *APOE* ε4 allele are at higher risk for disruption of functional connectivity.

An additional implication of this thesis is that cognitive reserve variably influenced functional connectivity. The increase of functional connectivity with increasing cognitive reserve has been demonstrated in previous research (Franzmeier et al., 2017a; Franzmeier et al., 2017b). The decrease of functional connectivity with increasing cognitive reserve within the DMN regions is less understood. Previously, it was found that working memory load disrupted the association between activation of the dorsal lateral PFC and performance (Callicott et al., 1999), which potentially may have caused a decrease in functional connectivity. Yet, a study investigating memory load discovered no reduction in functional connectivity within the prefrontal regions (Jaeggi et al., 2003).

Findings of this thesis revealed positive correlations between age and functional connectivity in the frontal lobe regions. This outcome raises implications as to the importance of recruitment strategies and compensatory mechanisms the brain is using to maintain and perform cognitive functions as we age. Moreover, recruitment and compensatory mechanisms may offer a beneficial strategy for delaying pathological alterations in the brain.

A further important finding from the present thesis was the correlation between brain structure metrics and cognitive performance. Relative atrophy of the accumbens, caudal middle frontal gyrus, and superior temporal gyrus were associated with decreased working memory and executive function performance. The correlation between accumbens and cognitive function has been observed in MCI and AD, suggesting that clinical progression was predicted by atrophy of the accumbens (Yi et al., 2016). Similarly, executive functioning performance decreased with reduced frontal thickness in healthy adults (Burzynska et al., 2012). This implies that in healthy older adults, the aging process on brain structure is influencing functional outcomes.

Future research

This thesis is based on an island cohort, therefore, replicating this study with another cohort would provide further underlying information about alterations in the brain associated with aging. It would be beneficial if future studies include pathological biomarkers, such as for tau and amyloid-beta, as well as potential contributions from cerebrovascular disease.

Longitudinal studies are required to examine how *APOE* x *BDNF* interactions and age may affect functional connectivity over time and whether specific gene variations that are known, to negatively influence brain connectivity are consequently responsible for AD. Future

studies in combination with *APOE* x *BDNF* x cognitive reserve would be beneficial and may offer further understanding of whether cognitive reserve can be classified as a modifier for genotypes in relation to functional and structural connectivity, which could be used in preventive programs. In future, studies could also investigate whether cognitive reserve and environmental enrichment work as compensatory mechanisms to influence and alter the networks of people with genetic polymorphisms associated with susceptibility to AD, such as *APOE* ε4 and *BDNF* Met carriers. After all, imaging studies are high-cost investigations, which may pose a barrier to future investigations when recruiting larger sample sizes, which would make this very challenging.

In future, it would be worth to perform multi-modal analyses, in which structural analyses are combined to functional analyses to obtain additional insights into how the brain functions in structural and spatiotemporal space(Franke & Gaser, 2012).

Conclusion

In conclusion, *APOE* ε4 carriers had significant differences from ε3 homozygotes in functional connectivity within the dorsal attention network characterised as lower connectivity compared to ε3 homozygotes within healthy elderly in a sample population in Tasmania. The carriage of the *APOE* ε4 allele within the interaction of *APOE* x *BDNF* genotypes is associated with reduction in functional connectivity. The findings of this thesis suggest that *APOE* ε4 carriage in general is associated with decreased brain connectivity. Moreover, Val/ ε3 homozygotes and Met carriers/ ε3 homozygotes have a protective effect with respect to functional brain connectivity in healthy aging within our cohort. It is debatable whether ε4 carriers have this disruption due to amyloid-beta or other pathologies

(Kametani & Hasegawa, 2018), however, testing this was beyond the scope of this thesis and can therefore only be speculated.

Moreover, it is theorized that healthy elderly adults have the potential to recruit other brain regions as a compensatory brain mechanism to maintain and perform cognitive functions. Studies have reported that healthy elderly recruited other brain regions to compensate for age-related atrophy and preserved their cognitive performance (Martins, Joanette, & Monchi, 2015). There was a negative correlation between functional connectivity and cognitive reserve. It remains unclear whether this correlation is related to noise, however, other possible explanations are that these anticorrelation may reflect increased neural efficiency within our cohort. As most of the THBP cohort was involved in later life education, it may be possible that my cohort was more intelligent with higher IQ levels using more efficient networks therefore not requiring more functional connectivity. Studies have shown that individuals with higher education have more efficient networks (Haier et al., 1988; Kelly & Garavan, 2005).

Throughout the whole thesis, there was an interaction with *APOE* but no additive effect of the *BDNF* Val66Met polymorphisms alone, suggesting that functional connectivity and brain structure is not exclusively influenced by this genotype but by the interaction with other genes.

Aging was also associated with a degree of atrophy in the hippocampus and amygdala, supporting previous literature that these brain regions could be used as a potential biomarker for AD. Cognitive performance was associated with both, increased and decreased volume and cortical thickness in different structures, and some of these associations were unexpected.

The unexpected associations may be caused by a collider bias because age, intracranial volume and total grey matter volume were included in the penalised regression model, and these are correlated with structural morphometry. Directed acyclic graphs, a technique used in causal inference, could be used in future works to reduce or avoid collider bias.

Magnetic resonance imaging and functional magnetic resonance imaging are non-invasive neuroimaging techniques that can be used to understand the links between structure, function, and risk alleles for dementia and cognitive decline.

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Appendix A – Ethics Approval Letter

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HUMAN RESEARCH ETHICS COMMITTEE (TASMANIA) NETWORK

UTAS

31 July 2017

Professor James Vickers
C/- University of Tasmania

Sent via email

Dear Professor Vickers

REF NO: H0016317
TITLE: Markers of stress, brain plasticity and brain connectivity in a cohort of older adults

Document	*Version*	*Date*
General Medical - NEAF	Version 1	
Clinical Safe Work Procedure Phlebotomy		
Finance and Administration		
MRI Safety, Policies and Procedures manual		
Questionnaire I Before MRI	Version 1	
Stress Brain Plasticity Info Sheet and Consent Form	Version 3	13 June 2017
Study Protocol Pro Forma	Version 2	19 April 2017

The Tasmanian Health and Medical Human Research Ethics Committee considered and approved the above documentation on **06 July 2017** to be conducted at the following site(s):

Wicking Dementia Research and Education Centre

Please ensure that all investigators involved with this project have cited the approved versions of the documents listed within this letter and use only these versions in conducting this research project.

This approval constitutes ethical clearance by the Health and Medical HREC. The decision and authority to commence the associated research may be dependent on factors beyond the remit of the ethics review process. For example, your research may need ethics clearance from other organisations or review by your research governance coordinator or Head of Department. It is your responsibility to find out if the approvals of other bodies or authorities are required. It is recommended that the proposed research should not commence until you have satisfied these requirements.

All committees operating under the Human Research Ethics Committee (Tasmania) Network are registered and required to comply with the *National Statement on the Ethical Conduct in Human Research* (NHMRC 2007 updated 2014).

Therefore, the Chief Investigator's responsibility is to ensure that:

(1) The individual researcher's protocol complies with the HREC approved protocol.

(2) Modifications to the protocol do not proceed until **approval** is obtained in writing from the HREC. Please note that all requests for changes to approved documents must include a version number and date when submitted for review by the HREC.

(3) Section 5.5.3 of the National Statement states:

Researchers have a significant responsibility in monitoring approved research as they are in the best position to observe any adverse events or unexpected outcomes. They should report such events or outcomes promptly to the relevant institution/s and ethical review body/ies and take prompt steps to deal with any unexpected risks.

The appropriate forms for reporting such events in relation to clinical and non-clinical trials and innovations can be located at the website below. All adverse events must be reported regardless of whether or not the event, in your opinion, is a direct effect of the therapeutic goods being tested. http://www.utas.edu.au/research-admin/research-integrity-and-ethics-unit-rieu/human-ethics/human-research-ethics-review-process/health-and-medical-hrec/managing-your-approved-project

(4) All research participants must be provided with the current Patient Information Sheet and Consent Form, unless otherwise approved by the Committee.

(5) The Committee is notified if any investigators are added to, or cease involvement with, the project.

(6) This study has approval for four years contingent upon annual review. A *Progress Report* is to be provided on the anniversary date of your approval. Your first report is due 6 July 2017**.** You will be sent a courtesy reminder closer to this due date.

(7) A *Final Report* and a copy of the published material, either in full or abstract, must be provided at the end of the project.

Should you have any queries please do not hesitate to contact me on (03) 6226 6254.

Yours sincerely

Jude Vienna-Hallam
Ethics Administration Officer

Appendix B – Information Sheet

UNIVERSITY of TASMANIA | WICKING Dementia Research & Education Centre

PARTICIPANT INFORMATION & CONSENT FORM

Version 3 – Dated 13 June 2017

Full Project Title: Markers of stress, brain plasticity and brain connectivity in a cohort of older adults

Principal Researchers: Professor James Vickers, Associate Professor Mathew Summers, Associate Professor Anna King, Professor Velandai Srikanth, Professor Michael Valenzuela, Dr David Ward and Kimberley Stuart.

This Participant Information and Consent Form is 10 pages long. Please make sure you have all the pages.

1. Introduction

You are invited to take part in this research project because you have previously participated in the Tasmanian Healthy Brain Project (THBP). This THBP sub-study aims to investigate and understand markers of stress, brain plasticity and brain connectivity in older adults.

This Participant Information and Consent Form contains detailed information about the research project. Its purpose is to explain to you, as openly and clearly as possible, all the procedures involved in this project to help you decide whether or not to take part in the research.

Please read this information carefully. Feel free to ask questions about any information in the document that you do not understand or want to know more about. You may also wish to discuss the project with a relative, friend or healthcare worker. Feel free to do this.

Participation in this research is voluntary. This will not affect your participation in the main studies of the THBP.

Once you understand what the project is about and if you decide to take part in it, you will be asked to sign the consent section. By signing it, you are telling us that you:

- Understand what you have read;
- Consent to take part in the research project;
- Consent to participate in the research processes that are described;
- Consent to the use of your personal and health information as described.

You will be given a copy of this Participant Information and Consent Form to keep.

2. Purpose and Background

The following three studies are the purpose of this project:

1) Examine patterns of connectivity in the brain using Magnetic Resonance Imaging (MRI). These scans will look at the structure of the brain as well as patterns of blood flow to particular areas. This will provide an indication of 'functional connectivity' of the brain. Functional connectivity is a measure of how brain regions interact with each other, and can be investigated relative to variations in genes involved in brain as well as cognitive function.

2) Determine whether levels of brain proteins, such as brain-derived neurotrophic factor, in blood are related to current cognitive function, or may also be associated with eventual risk of significant cognitive decline and/or dementia.

3) Examine whether accumulated levels of stress hormone, measured in hair samples, have any correlation with risk of significant cognitive decline and/or dementia. We will also examine gene variations that may related to how people respond to stress.

Background:

You are invited to participate in this research project because you have previously participated in the Tasmanian Healthy Brain Project (THBP). This new research study has three main components and you may opt in or out to any of these.

Study 1

The first study using scanning technology to determine which brain areas are activated together, and are therefore likely to have substantial functional connections. It is possible to examine the connectivity between brain regions using Magnetic Resonance Imaging (MRI) and software tools to detect coincidence in subtle variations in blood flow, as blood flow is increased when a brain area is active.

Recent brain imaging studies suggest that the connectivity between individual brain regions is disrupted in Alzheimer's disease (AD), reflecting the progressive degeneration of connections in this condition. Individuals will vary in the extent of functional connectivity between brain regions, and we are interested in whether this may be modifiable and potentially linked to brain resilience when challenged by a disease process such as AD. Furthermore, we have obtained data already from many THBP participants on normal variations in genes potentially associated with dementia risk (for example, Apolipoprotein E (APOE)) and brain plasticity (brain-derived neurotrophic factor (BDNF)) and have examined these in relation to cognitive function and the effects of the education intervention of the THBP. We plan to now investigate whether these genes influence how the brain regions interact with each other, or their functional connectivity, especially in relation to measures of cognitive reserve and cognitive changes linked to the education intervention.

Study 2

The second study involves a focus on circulating levels of the BDNF protein, a protein important for brain plasticity, that may be detectable in blood samples. Previous animal studies have indicated that blood levels of BDNF reflect brain levels of this protein, which indicates that BDNF produced in the brain may be released into the bloodstream. This investigation will focus on measuring blood levels of BDNF and determining if this is linked to BDNF gene variations, cognitive measures and the potential effects of the education intervention. Furthermore, we will establish whether BDNF levels in blood may be associated with future risk of cognitive decline and/or dementia.

Study 3

The third study will focus on how exposure to stress may affect brain function and resilience to cognitive changes associated with ageing. Circulating levels of the stress hormone cortisol are incorporated into the growing hair shaft of humans. Measuring cortisol levels in hair provides a marker of exposure to this stress hormone over several weeks. This measure can then be compared to your inheritance of particular variants of the 'FKBP5' gene, which has been linked to stress responsiveness, and we can compare cortisol levels to other genetic and cognitive measures obtained through the THBP.This research has been initiated by the investigators (Professor James Vickers, Associate Professor Mathew Summers, Associate Professor Anna King, Professor Velandai Srikanth, Professor Michael Valenzuela, Dr David Ward and Kimberly Stuart).

3. Procedures, and what is involved

Should you agree to participate, you will be asked to provide a small sample of blood (Study 2) and a sample of your hair (Study 3). If you meet specific selection criteria, you may also be asked to

participate in Study 1, involving a Magnetic Resonance Imaging (MRI) examination on two occasions (baseline and two years later). You can decide which procedures you would like to consent to and which you do not want to consent to.

Functional Magnetic Resonance Imaging (fMRI): This aspect of the study involves examining the structure of your brain and measuring your brain activity using fMRI. The fMRI examination will be conducted at the Royal Hobart Hospital. We estimate that the total time commitment required of you for the fMRI would not exceed 60 minutes. The examination involves lying on a bed while a frame (magnetic coil) is placed around your head. The bed will then slide into the cylindrical opening of the MRI machine.

During the examination, you will be asked to lie silently in the scanner. You will hear knocking sounds which result from the electrical switching operations of the magnetic fields. In order to reduce the noise level and to prevent damage to your hearing, you will be provided with ear protection. You will be able to speak to the radiologist and researcher at any time via an intercom system. In addition, an alarm button (pressure ball) will be placed in your hand. You can ask the examination to be stopped at any time.

Magnetic Resonance Imaging is widely used in hospitals and clinics for medical diagnosis. This technique is based on the principle of the magnetisability of hydrogen molecules. The magnetic resonance signals from water or other naturally occurring molecules are measured using strong magnetic fields to obtain imaging of body structure. fMRI is a variation of this imaging technique which involves using software on images obtained from a MRI to derive a measure of blood flow to particular parts of the brain.

People with some medical implants or other non-removable metal inside the body (such as heart pacemakers, artificial heart valves, vascular clips, hearing aids, metal splinters) may be unable to safely undergo an fMRI examination.

The Magnetic Resonance Images will be assigned a code number to protect your confidentiality and the images will then be stored on a password protected computer at the Wicking Centre. All data obtained from your participation will be stored in a de-identified manner. Electronic data will be stored on one computer under password protection. The data will only be accessible to the investigators named on this Patient Information Sheet.

By signing this consent form, you consent to the study investigators and affiliated staff of this research project to cross-reference medical and research information obtained from the THBP. You will be asked about an updated medical history at the time of sample collection. Demographic details such as your name, date of birth, and contact details will be asked and verified at the time of sample collection and prior to undergoing fMRI.

The data collected in this study can be accessed by members of the Human Research Ethics Committee to audit the study as it is being undertaken. Any publications that arise from this study will have information from the study participants as a group and no information that can identify any individual will be included.

Blood Sample: You will be asked to provide a small sample of blood (3x tubes 10ml). The blood sample will be collected by a phlebotomist (someone who is trained to draw bood) from a vein in your arm with a needle. You do not need to fast for this blood test. You will be asked to avoid exercise in the 24 hours prior to collection, as acute exercise may result in transiently elevated peripheral BDNF and other proteins. We will determine whether BDNF levels in the blood sample are related to variations in the BDNF gene, and also whether the intervention results in sustained increased levels of BDNF. We will also examine the blood samples for the presence of other proteins that are relevant to cognitive function, plasticity and ageing-related brain change.

Hair Sample: The hair sample is taken from the back of the head (posterior vertex of the skull). Sample collection involves securing the entire length of the hair to be sampled (up to a pencil-width in diameter) with a rubber band or clip. The hair is then cut as close to the scalp as possible with clean scissors. The main focus of this study is to measure the amount of the stress hormone, cortisol. We will also be examining the sample for other indicators of metabolic function, as well as proteins related to stress exposure.

4. Possible Benefits

In the short term, there are unlikely to be any direct benefits to you personally. The proposed study will build directly on the THBP research by the investigator team to determine whether engagement in complex cognitive activity may protect from significant deterioration in cognitive performance and dementia, and whether specific genetic factors modify our capacity to gain benefit from an enhanced cognitive lifestyle. In the future, it may be that the THBP informs strategies to reduce risk of significant ageing-related cognitive decline, and we envisage that publications arising from this study will assist in a greater understanding of brain changes associated with ageing-related cognitive decline and dementia.

The MRI examination is not an examination with a diagnostic question. Therefore, the results cannot be used to rule out brain diseases. Nevertheless, sometimes abnormalities can be detected and these will be assessed by Professor Velandai Srikanth, who is a medically-qualified geriatrician. Professor Srikanth will then inform you whether further clarification of the random findings is recommended.

5. Possible Risks

Blood Sample

Blood samples will be obtained by a trained phlebotomist at the Clinical Research facility of the Medical Sciences Precinct. The risks associated with providing a blood sample are minimal, however, these may include bleeding after the procedure and infection of the injection site. The risk of bleeding will be minimized through careful technique and the use of aseptic measures will minimize the risk of infection. If the injection site becomes swollen, red, warm or painful please telephone the Wicking Centre on 1800 982 600, or, depending on severity, attend your general practitioner or local emergency department. You will also be provided with contact details of the researcher should any untoward discomfort, excessive bleeding or infection arise after the procedure.

Functional Magnetic Resonance Imaging

Magnetic Resonance Imaging will be conducted by trained staff in the the Radiology Department of the Royal Hobart Hospital. Based on current knowledge, no side effects are to be expected. There may be possible physical discomfort of lying still continuously while the fMRI is taking place, and you may find the external noise of the operation of the imaging scanner to be bothersome. However, in extremely rare cases, chronic cardiac dysfunction may be caused. Although painless,

fMRI scans can be unpleasant for those who are anxious in enclosed spaces. People with even mild claustrophobia are sometimes unable to tolerate an fMRI scan. You will be provided with contact details of the research team should any untoward discomfort arise after the procedure.

As a participant, you can suspend or even end your participation in this project at any time. Should this occur please contact either one of the study investigators or the Secretary of the HREC.

6. Participation is Voluntary

Participation in any research project is voluntary. If you do not wish to take part, you are not obliged to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your participation in the THBP or your relationship with any medical or psychiatric service.

Before you make your decision, a member of the research team will be available to answer any questions you have about the research project. Please sign the Consent Form only after you have had a chance to ask your questions and have received satisfactory answers.

If you decide to withdraw from this project, please notify a member of the research team at your convenience.

7. Results of Project

We envisage that the results from this work will be presented at scientific meetings and be pubslihed in journals relevant to this research field. You will not be able to be directly identified from these publications or presentations.

As participants of the THBP, you will receive a general newsletter updating you with the findings and any significant research outcomes.

8. Privacy, Confidentiality and Disclosure of Information

All data will be de-identified and all specimens coded with a unique code, which is only known by the Principal Study Investigator. All electronic data will be stored on a password-protected computer. Only researchers involved with this work will have access to this de-identified data.

Given that genetic techniques and technology are continuing to advance, we do not currently plan to dispose of the stored material and we request your approval to examine for other genetic markers, as they arise. Likewise, proteins will be purified from the blood and hair samples, so we plan to retain these to examine for future protein markers, as they arise.

Any information obtained in connection with this project that can identify you will remain confidential and securely stored. It will only be disclosed with your permission, except as required by law.

We currently have no active plans for any commercial development of the research results. Nonetheless, it is important to note that patenting scientific discoveries developing commercial tests and therapies could be possible, with no sharing of royalties with donors or participants.

In any publication and/or presentation, information will be provided in such a way that you cannot be identified. In accordance with relevant Australian privacy and other relevant laws, you have the right to access the information collected and stored by the researchers about you. You also have the right to request that any information with which you disagree be corrected. Please contact one of the researchers named in this document if you would like to access your information.

9. Injury

In the event that you suffer an injury as a result of participating in this research project, hospital care and treatment will be provided by the public health service at no extra cost to you if you elect to be treated as a public patient.

10. Reimbursement for your costs

You will not be paid for your participation in this research. We will reimburse you for any reasonable expenses incurred as part of your participation (eg parking).

11. Ethical Guidelines

The ethical aspects of this research project have been approved by the Tasmanian Health and Medical Human Research Ethics Committee of the University of Tasmania.

This project will be carried out according to the *National Statement on Ethical Conduct in Human Research* (2007) produced by the National Health and Medical Research Council of Australia. This statement has been developed to protect the interests of people who agree to participate in human research studies.

12. Who can I contact?

The person you may need to contact will depend on the nature of your query. Therefore, please note the following:

For further information or appointments

If you require further information concerning this project or if you have any medical problems which may be related to your involvement in the project (for example, any side effects), you can contact the principal investigator Professor James Vickers on **1800 982 600.**

Reviewing HREC:

This study has been approved by the Tasmanian Health and Medical Human Research Ethics Committee. If you have concerns or complaints about the conduct of this study, you should contact the Executive Officer of the HREC (Tasmania) Network on:
(03) 6226 7479 or email **human.ethics@utas.edu.au**

The Executive Officer is the person nominated to receive complaints from research participants.

You will need to quote:

REVOCATION OF CONSENT FORM

(To be used for participants who wish to withdraw from the project.)

Full Project Title: Markers of stress, brain plasticity and brain connectivity in a cohort of older adults

Principal Researchers: Professor James Vickers, Associate Professor Mathew Summers, Associate Professor Anna King, Professor Velandai Srikanth, Professor Michael Valenzuela, Dr David Ward and Kimberly Stuart.

I hereby wish to WITHDRAW my consent to participate in the research proposal described above and understand that such withdrawal WILL NOT jeopardize my relationship with any other research projects.

Participant's Name (printed) .....

Signature Date

UNIVERSITY of TASMANIA | WICKING Dementia Research & Education Centre

CONSENT FORM

Full Project Title: Markers of stress, brain plasticity and brain connectivity in a cohort of older adults

Principal Researchers: Professor James Vickers, Associate Professor Anna King, Professor Velandai Srikanth, Associate Professor Mathew Summers, Dr David Ward and Kimberley Stuart.

Study ID: …… Sex: M / F

DOB: ……

- I have read, or have had read to me in a language that I understand, this document and I understand the purposes, procedures and risks of this research project as described within it.
- I have had an opportunity to ask questions and I am satisfied with the answers I have received.
- I freely agree to participate in this project according to the conditions in this document.
- I will be given a copy of the Participant Information and Consent Form to keep
- The researcher has agreed not to reveal my identity and personal details if information about this project is published or presented in any public form.

Consent Options:

☐ I do ☐ I do not consent to providing a sample of my hair as described in this document.

☐ I do ☐ I do not consent to providing a sample of my blood as described in this document.

☐ I do ☐ I do not consent to the use of my hair sample as described in this document.

☐ I do ☐ I do not consent to the use of my blood samples as described in this document.

☐ I do ☐ I do not consent to a Functional Magnetic Resonance Imaging (MRI) examination as described in this document.

☐ I do ☐ I do not consent to the use of my Magnetic Resonance Images (MRI) as described in this document.

☐ I do ☐ I do not consent to the storage and use of my clinical and research data and samples as indicated in the document for future, currently unforeseen ethically approved studies.

☐ I do ☐ I do not consent to sharing of de-identified data derived from the analysis of blood and hair samples, as well as MRI images, with other national and international researchers, with appropriate confidentiality protections. NB. If we are approached by potential external collaborators to share data that does not contain any personally identifying information.

Participant's Name (printed) ……

Signature Date

Witness (Required when participant cannot read this document for him/herself except where an interpreter is used.)

Name of Witness to Participant's Signature (printed) .....

Signature Date

Declaration by researcher*: I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Researcher's Name (printed) .....

Signature Date

* A senior member of the research team must provide the explanation and provision of information concerning the research project.

Note: All parties signing the Consent Form must date their own signature.

UNIVERSITY of TASMANIA | WICKING Dementia Research & Education Centre

EXPERIMENTAL PARTICIPANT'S STATEMENT OF RIGHTS

It important that you know:

Any person who is asked to participate in a research study involving a medical experiment, or who is requested to consent on behalf of another, has the right to:

1. Be informed of the nature and purpose of the experiment.
2. Be given an explanation of the procedures to be followed and any drugs used in the medical experiment.
3. Be given a description of discomforts and risks reasonably expected from the experiment, if applicable.
4. Be given an explanation of any benefits to the participant reasonably to be expected from the experiment, if applicable.
5. Be advised of appropriate, alternative procedures, drugs, or devices that might be advantageous to the participant, and their relative risks and benefits.
6. Be informed of the avenue of medical treatment, if any, available to the participant after the experiment if complications should arise.
7. Be given an opportunity to ask questions concerning the experiment or the procedures involved.
8. Know that consent to participate in the medical experiment may be withdrawn at any time, and that the participant may discontinue participation in the medical experiment without prejudice.
9. Be given a copy of the signed and dated written consent form when one is required.
10. Be given the opportunity to decide to consent or not to consent to a medical experiment without the intervention of any element of force, fraud, deceit, duress, coercion or undue influence.

Appendix C – Declaration of consent

I was thoroughly enlightened about the nature, significance, and risks of the study. I have read and understood the information as well as the data protection statement printed here. I had the opportunity to talk with the examiner of this study. All my questions were answered satisfactory.

Name of the candidate

_____ _____

Born Participant number

Appendix D – Pietzuch, King, Ward, & Vickers (2019) reprint

frontiers in Aging Neuroscience

REVIEW
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The Influence of Genetic Factors and Cognitive Reserve on Structural and Functional Resting-State Brain Networks in Aging and Alzheimer's Disease

Manuela Pietzuch[1], Anna E. King[1], David D. Ward[2] and James C. Vickers[1]*

[1] Wicking Dementia Research and Education Centre, College of Health and Medicine, University of Tasmania, Hobart, TAS, Australia, [2] Population Health Sciences, German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany

Magnetic resonance imaging (MRI) offers significant insight into the complex organization of neural networks within the human brain. Using resting-state functional MRI data, topological maps can be created to visualize changes in brain activity, as well as to represent and assess the structural and functional connections between different brain regions. Crucially, Alzheimer's disease (AD) is associated with progressive loss in this connectivity, which is particularly evident within the default mode network. In this paper, we review the recent literature on how factors that are associated with risk of dementia may influence the organization of the brain network structures. In particular, we focus on cognitive reserve and the common genetic polymorphisms of *APOE* and *BDNF* Val66Met.

Keywords: fMRI, Alzheimer's disease, default mode network, cognitive reserve, BDNF, APOE

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INTRODUCTION

Recently, it was estimated that more than 47 million elderly people are affected by dementia globally (Alzheimer's Disease International, 2009; Prince et al., 2016) and that an additional 131 million people will develop this health-challenging syndrome by 2050 (Prince et al., 2016). Alzheimer's disease (AD), a progressive condition causing behavioral changes, memory loss, and decline in learning capacity (Anand et al., 2014), is the most common cause of dementia worldwide (Hardy, 1997). Most cases of AD occur in individuals over the age of 75, but, relatively younger individuals, including those carrying certain genetic mutations (Loy et al., 2014), may develop the disease before 65 years of age (Alzheimer's Association, 2015).

Knowledge of the brain changes that occur in AD has increased remarkably from the late 20th century due to extensive research on a range of related neurodegenerative processes. Particular progress has been made with regard to what has been termed the pathological 'hallmarks' of AD – the presence of amyloid plaques and neurofibrillary tangles (NFTs) – which detrimentally affect axons, dendrites, and synapses (Vickers et al., 2000, 2016). Plaques are the result of accumulations of an abnormal form of the beta amyloid (Aβ) protein in the brain. NFTs are formed by the aggregation of aberrant tau protein (Vickers et al., 2000; Savva et al., 2009) and are more directly related to the

death of neurons (Jacobs et al., 2012). Within the cerebral cortex, the earliest plaques are usually found in the neocortex, whilst initial formation of tangles occurs in medial temporal lobe (MTL) structures, such as the entorhinal cortex and hippocampus (Price and Morris, 1999). The MTL is a very important region responsible for memory formation and long-term memory (Squire and Zola-Morgan, 1991). Throughout the cerebral cortex, neurons that provide long corticocortical connections are the most prone to NFT-induced deterioration (Morrison and Hof, 1997), which may then underlie the pattern of synaptic loss seen in AD. Entorhinal-hippocampal circuits are compromised early in AD, followed by the gradual disconnection of the MTL, and then the loss of connectivity between association neocortices (Morrison and Hof, 1997). This pattern of progressive and degenerative pathology may underlie the deterioration of certain cognitive functions during aging, leading eventually to frank AD. The early pathological accumulation of Aβ has been linked to cognitive impairment and could also affect functional connectivity between spatially distant brain regions (Delbeuck et al., 2003). A summary table of studies examining functional connectivity and Aβ in healthy aging and AD can be found in **Table 1**. Neuroimaging is a vital component of international research collaborations (Hendrix et al., 2015) and has been used to investigate mechanisms of interrupted structural and functional connectivity underlying the course of AD (Dennis and Thompson, 2014). A better understanding of how the pathological changes in AD affect the organization of brain networks, or how these networks may respond or adapt to accumulating pathology, might offer further insights into the potential scope of functional resilience. The term resilience is described as the capability of a tissue to be resistant to damage (Cosco et al., 2017). In this respect, factors such as education and lifestyle could increase resilience by heightened connectional redundancy and/or preserving functional connections in the brain, and may ultimately delay the clinical expression of AD pathology. Indeed, studies investigating the association of education and cognitive decline in AD have found that more highly educated individuals are able to tolerate more neuropathology before the clinical expression of AD (Bennett et al., 2003), potentially because education moderates the relationship between brain pathological load and cognitive impairments (Brayne et al., 2010; Valenzuela et al., 2011), as well as functional connections (Marques et al., 2016).

Studies have shown that functional connectivity is damaged or interrupted in AD (Stam et al., 2006, 2008), and, conversely, investigating the impact of AD on structural and functional networks may also provide more accurate information regarding brain connectivity and how brain regions communicate with each other (Sheline and Raichle, 2013). This review focuses on the methods with which brain connectivity is analyzed, the changes in structural and functional networks found in AD, and the role of cognitive reserve and specific genetic factors in partially determining functional brain connectivity. In this regard, potential changes in functional connectivity and resistance to pathology will involve both non-modifiable and modifiable factors that will impact on how brain systems respond to accumulating pathological burden. Hence, we discuss features of structural and functional brain networks in relation to genetic biomarkers and environmental factors linked to AD risk, progression and resilience.

METHODS TO ANALYZE CONNECTIVITY

Neuroimaging techniques (**Figure 1**), such as magnetic resonance imaging (MRI), have long been used to investigate anatomical connections, detect pathological alterations, and monitor the progression of neurodegenerative diseases, including AD (**Figure 1A**). MRI involves the generation of a strong static magnetic field to create images and to map fluctuation signals related to brain activity (Heeger and Ress, 2002). MRI also allows the quantification of brain atrophy, which can be used to distinguish normal brain aging from AD (Frankó et al., 2013). For example, a recent study found that MRI and cognitive testing in cognitively healthy individuals are useful tools for predicting the development of AD, particularly when investigating the progress from healthy cognition to the appearance of mild cognitive impairments (MCIs) after 5 years (Albert et al., 2018). The delayed presence of clinical symptoms makes it challenging to diagnose individuals in preclinical stages. Therefore, animal models could provide an opportunity to identify biomarkers of early disease (Sabbagh et al., 2013), which include insights from neuroimaging, such as gray and white matter alterations measured by diffusion tensor imaging (DTI; Weston et al., 2015).

Diffusion tensor imaging is an MRI-based neuroimaging method that measures the diffusion of water molecules, enabling the assessment of the fiber-tract structures of white matter (Jones et al., 2013; Teipel et al., 2016). This technique allows the strengths and differences of white matter tract connections in specific population groups to be compared (Jones et al., 2013) before a reduction of cognition is evident (López-Gil et al., 2014), for example between older individuals with and without AD (**Figure 1C**). Other structural imaging parameters that are currently used to gain further insight into the integrity of the brain over the life include intracranial volume and the presence and number of white matter hyper-intensities (Bartrés-Faz and Arenaza-Urquijo, 2011).

Functional MRI (fMRI) permits simultaneous monitoring of the activity of different brain regions while a subject is at rest or performing a task (Binder et al., 1999). In fMRI, oxygen in blood is measured through blood-oxygen-level-dependent (BOLD) signals (Ogawa et al., 1990; Heeger and Ress, 2002). Specifically, the underlying premise is that more oxygen is required for greater neuronal activity, thereby creating a signal that can be detected using fMRI (**Figure 1B**). Thus, it is possible to measure changes in oxygen concentration, cerebral blood flow (CBF) and volume (CBV) that are delayed by 1–2 s after MRI excitation. This is referred to as the hemodynamic response (Buxton et al., 2004). If the BOLD signal from different areas of the brain show similar and synchronized activity, it is assumed that these regions communicate with each other and transfer information, which is defined as functional connectivity (Raichle, 1998). Functional

TABLE 1 | Studies examining functional connectivity and amyloid-beta in healthy aging and Alzheimer's disease.

Study	Samples	Imaging measures	Main findings
Fischer et al. (2015)	CN preclinical AD (n = 12), Age-matched controls (n = 31)	DTI using tractography, measuring fludeoxyglucose-PET	CN preclinical AD (with Aβ positivity) exhibited similar white matter network changes to clinical AD as compared to controls; for instance, CN preclinical AD had more shorter paths and reduced global efficiency compared to controls.
Grandjean et al. (2014)	Transgenic mice (n = 38) Wild-type mice (n = 36)	Structural MRI, Rs-fMRI DTI	The progression of functional connectivity was disrupted in somatosensory and motor cortex in ArcAβ transgenic mice compared to wild-type mice. This decrease was noticeable even before amyloidosis in transgenic mice.
Mormino et al. (2011)	CN older (n = 44), AD (n=22)	Structural MRI, Rs-fMRI, PIB-PET Imaging	Increased Aβ in CN older individuals was associated with decreased default mode network functional connectivity in multiple posteromedial regions suggesting that the accumulation of Aβ and related brain changes occurs before overt cognitive impairment.
Sheline et al. (2010b)	35 AD, 68 CN older PIB− (n = 24) PIB+ (n=20)	Structural MRI, Rs-fMRI, and Dynamic PET scan	CN people with Aβ deposition exhibited impairments in functional connectivity, particularly default mode network disruptions.
Bero et al. (2012)	Young APP/PS1 transgenic mice (n = 7) Old APP/PS1 transgenic mice (n = 7) Young wild type mice (n = 13) Old wild type mice (n = 10)	Functional connectivity optical intrinsic signal imaging	Aβ accumulation was related to decreased functional connectivity in older APP/PS1 mice compared to young APP/PS1 mice and wild-type mice. Brain regions that had more Aβ showed the most conspicuous age-related decreases in connectivity.
Hedden et al. (2009)	38 CN older adults, PIB− (n = 17), PIB+ (n = 21)	Structural MRI, fMRI, Dynamic PET	Functional connectivity was disrupted in CN older adults with Aβ positivity. Connectivity impairments related to Aβ deposition were evident between the hippocampus and posterior cingulate (default mode network regions) and associated with memory deficit.
Drzezga et al. (2011)	CN PIB− (n=12) CN PIB+ (n = 12) MCI PIB+ (n = 13)	Structural MRI, Rs-fMRI, fluorodeoxyglucose-PET, PIB-PET	MCI with Aβ burden exhibited hypometabolism, decrease of neuronal activity and disruption of functional connectivity in posterior brain regions (precuneus/posterior cingulate) compared to CN older adults.
Lim et al. (2013)	165 CN PIB− (n = 116) PIB+ (n = 49) BDNF Met carriers (n = 58) BDNF Val/Val (n = 107) APOE e4 (n=70)	Structural MRI, PET PIB Imaging, Neuropsychological assessments at baseline, 18 and 36 months	BDNF Met carriers with Aβ burden positivity demonstrated an accelerated decline in memory function as well as a reduction of hippocampal volume compared to BDNF Val homozygotes.
Franzmeier et al. (2017b)	CN Aβ+ (n = 24) amnestic MCI Aβ (n = 44)	Structural MRI, Rs-fMRI, FDG-PET	Individuals with amnestic MCI with Aβ positivity and more years of education demonstrated attenuation of precuneus hypometabolism and relatively increased global frontal cortex functional connectivity.

AD Alzheimer's disease, Aβ Amyloid-beta, APP/PS1 Amyloid precursor protein presenilin, APOE Apolipoprotein E, BDNF Brain-derived neurotrophic factor, CN Cognitively normal, DTI Diffusion tensor imaging, FDG Fluodeoxyglucose, MCI Mild cognitive impairment, MRI Magnetic resonance imaging, PET Positron-Emissions-Tomography, PIB Pittsburgh Compound B, Rs-fMRI Resting-state functional magnetic resonance imaging.

connections, defined as temporal correlations between spatially distant cortical brain regions, are revealed through fluctuations in low-frequency portions of BOLD signals (Ogawa et al., 1990). With age, functional connectivity networks gradually decrease (Dennis and Thompson, 2014), which may be important for understanding early AD or the series of brain changes that make the older brain more or less susceptible to additional disease processes.

Resting-state fMRI is an increasingly frequent method employed to study differences between various cohorts and involves the investigation of the activity of the brain while the individual is at rest and not performing a task. Resting-state fMRI can be used to determine how different brain regions operate and process information in functional space. Additional advantages are that resting-state fMRI is less demanding on the individual and easier to apply than task-related fMRI (Sheline and Raichle, 2013). The individual is instructed to not fall asleep while keeping their eyes closed in a lying position.

There are a variety of approaches for analyzing resting-state fMRI. For instance, seed-based analysis (Beckmann et al., 2005) investigates the BOLD signals between the selected region of interest (seed region) and the rest of the brain (Biswal et al., 1995). In AD, the precuneus has showed decreased functional connectivity to other brain regions, such as the left hippocampus, left parahippocampus, anterior cingulate cortex and gyrus rectus, as compared to non-dementia controls (Sheline and Raichle, 2013). The investigation of simultaneous neuronal connections across the brain is called independent component analysis (ICA),

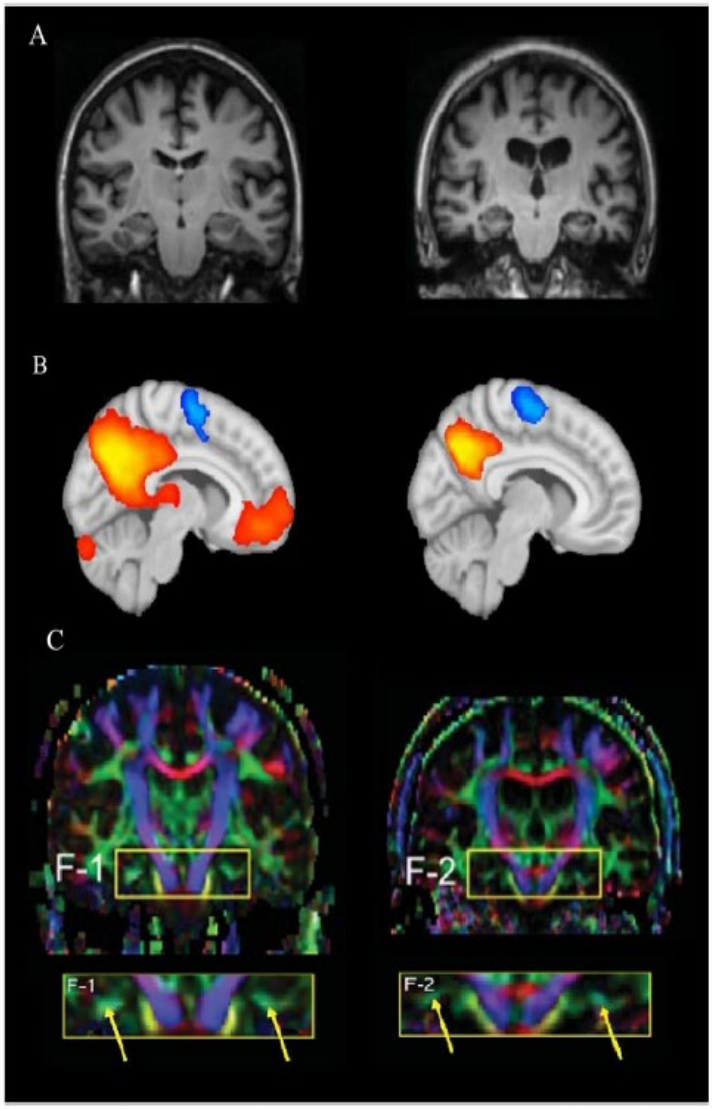

FIGURE 1 | Differences among the imaging techniques, MRI, fMRI, and DTI. **(A)** A structural MRI comparison between a healthy human brain (left) compared to pathological changes in Alzheimer's disease (AD, right; Olshi et al., 2011). **(B)** A functional MRI representing brain activation of a resting-state network in a healthy brain (left) compared to a hypothetical AD brain activation (right). The representation of the connectivity map shows how brain activity decreases with pathology within the default mode network (DMN); red/orange represents higher connectivity, while blue represents inversely correlated activity. **(C)** A comparison between a cognitively healthy woman (72 years old, left) and a woman with AD (70 years old; Olshi et al., 2011). The yellow arrows show the different color strength of the cingulum hippocampal area after DTI analysis. **(A,C)** Reprinted from Olshi et al. (2011) with permission from IOS Press. The publication is available at IOS Press through https://content.iospress.com/articles/journal-of-alzheimers-disease/jad00007.

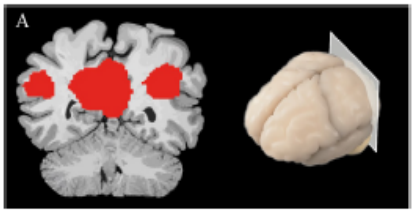

FIGURE 2 | A spatial map of a brain slice is represented, demonstrating brain activity in the DMN; red represents regions that are most active while the individual is at rest.

and is a wholly data-driven form of analysis (Beckmann et al., 2005) (**Figure 2**). Using ICA-based analysis, Greicius et al. (2004) reported a decline of resting-state functional connectivity between hippocampus and posterior cingulate cortex (PCC) in the AD group compared to healthy older individuals.

Another technique used to examine resting-state functional connectivity is graph analysis, which employs a way of specifically visualizing the complex interactions in the brain (Mijalkov et al., 2017). Using graph theory, functional connectivity is represented as a series of 'nodes' (voxels) and 'edges' (correlated activity between nodes) (Watts and Strogatz, 1998; Stam et al., 2007). It has been predicted that small-world networks in human fMRI studies with low-frequency oscillation might reveal connectivity of the brain structure. A specific focus of this form of analysis in network organization is the average minimum number of edges that must be traversed between any two nodes in a brain network, referred to as 'effective path length.' The characteristics of small-world networks are clustering coefficient, high integration and their typical feature is shorter effective path length (Travers and Milgram, 1967; Rubinov and Sporns, 2010; Kaminski and Blinowska, 2018). Cluster coefficient is described as a measurement of nodes that are locally interconnected (Kaminski and Blinowska, 2018). This approach is particularly useful when measuring and comparing differences in structural and functional connectivity (Bullmore and Sporns, 2009), and could be used to advance our understanding of the pathology of neurodegenerative diseases (**Figure 3A**). A further advantage of graph theory analysis is that it makes no assumptions about how close any two nodes are in space.

CHANGES IN STRUCTURAL AND FUNCTIONAL CONNECTIVITY IN AD

Structural Connectivity

In AD, the loss of connections between neurons can result in other structural alterations, such as atrophy, hypometabolism, and NFT accumulation (Zhang et al., 2009). Significant atrophy in AD, identified through MRI, occurs in the posterior hippocampus and the temporal and parietal cortices, which are three of the structures that are involved in the default mode network (DMN; Greicius et al., 2003). The default mode is a network in the brain that is activated when individuals are not engaged in a task, but are spontaneously thinking of past or future events (Buckner et al., 2008). The DMN is a highly interconnected set of cortical regions that demonstrate substantial correlated activity, particularly when the attentional network is inactive (Shulman et al., 1997; Buckner et al., 2008).

Diffusion tensor imaging studies investigating white matter changes in individuals with AD have demonstrated that the disease causes a deterioration of white matter fiber bundles in the MTL (Zhang et al., 2007), which may be present years before overt episodic memory deficits (Sexton et al., 2010), impaired executive function (Reijmer et al., 2014), and other symptoms of cognitive impairment (Zhang et al., 2007; Fischer et al., 2015). Similarly, in an animal model, López-Gil et al. (2014) reported neuronal differences in structural networks of chronically hypertensive rats before the manifestation of disrupted executive functioning occurred, which may provide insights into early stages of dementia. Moreover, Grandjean et al. (2014) discovered reduced fractional anisotropy values in transgenic mice with cerebral Aβ. In cognitively healthy individuals with elevated Aβ in the brain, potentially the pathological correlate of early AD, structural changes appear similar to individuals with MCI in terms of the topology of structural network connectivity (Fischer et al., 2015). Interestingly, these individuals with high brain Aβ load despite no overt cognitive symptomatology, demonstrated increased shortest path length in white matter networks in the absence of major neurodegenerative features such as atrophy or reduction of cortical glucose (Fischer et al., 2015).

Finally, the structural networks (or nodes) of individuals with AD who possessed fewer connections (or edges) were more susceptible to global disruption of white matter tracts than individuals with more connections (Daianu et al., 2015). In addition, a rat transgenic model bearing mutant human amyloid precursor protein (APP) and presenilin genes also demonstrated a reduction of local and global efficiency, as well as less clustering as compared to non-transgenic rats (Muñoz-Moreno et al., 2018). Moreover, Muñoz-Moreno et al. (2018) found alterations in the right medial PFC in these transgenic rats, while in human studies, the right medial frontal cortical areas in AD indicated a decline in nodal efficiency compared to healthy controls (Lo et al., 2010). In summary, changes in structural connectivity could be useful in predicting the degradation of white matter bundles, as well as the strength of functional connectivity networks (Greicius et al., 2009).

Functional Connectivity

Performance within many domains of cognitive function decreases slowly with age, but, importantly, higher cognitive performance has been correlated with increased functional connectivity in older adults (Arenaza-Urquijo et al., 2013). Compared to animal studies, transgenic AD rat models require longer cognitive training to achieve the same performance as non-transgenic rats. Although the structural network was changed, these alteration did not result in functional network differences proposing associations between the capability to learn and the reorganization of functional networks in the

brain (Muñoz-Moreno et al., 2018). Nevertheless, a gradual decrease in functional connectivity among the hippocampus and medial prefrontal cortex (PFC) is expected with age (Damoiseaux et al., 2016).

It has been proposed that disconnection of functional networks in the brain, such as those observed in AD, could serve as critical markers for the presence of early stages of neurodegenerative diseases, particularly with regard to the abnormal accumulation of Aβ in the brain (Stam et al., 2007; Zhang and Raichle, 2010). Resting-state studies have reported a decrease in functional connectivity in healthy older individuals with Aβ burden in the posteromedial regions, ventral medial PFC, right angular gyrus, and the left middle and superior frontal gyri (Mormino et al., 2011), as well as between the precuneus and left hippocampus, parahippocampus, anterior cingulate, gyrus rectus and dorsal cingulate (Sheline et al., 2010a,b). Early accumulation of Aβ in older healthy individuals, particularly in the precuneus, has been suggested to result in impairment in hippocampal function (Sheline et al., 2010a,b). In contrast, Mormino et al. (2011) reported that DMN connectivity responds in a varied manner to the presence of higher Aβ deposition in older non-demented people with Aβ accumulation. Specifically, the authors found that there was increased connectivity in regions of the right dorsal PFC, left anterior medial PFC and left temporal cortices, as well as decreased DMN connectivity in several posteromedial regions, the ventral medial PFC, right angular gyrus, and the left frontal gyri (Mormino et al., 2011). Disruption within the DMN has also been found in healthy older individuals with high amyloid burden (Hedden et al., 2009). Interestingly, these healthy individuals (n = 38) exhibited the same amount of Aβ burden compared to half of the individuals with MCI (n = 46) and all individuals with AD (n = 35).

Such associations have also been investigated in animal models. Bero et al. (2012) demonstrated an aging-related reduction of bilateral functional connectivity in the retrosplenial cortex in wild-type mice, which could be a pre-existing biomarker for neural dysfunction due to its significant association with memory performance (Corcoran et al., 2011). Interestingly, in transgenic AD mouse model involving cortical amyloidosis, it has been shown that an age-related decrease in functional connectivity in specific brain regions is more severe in the presence of higher Aβ deposition (Bero et al., 2012). Grandjean et al. (2014) also reported reduced functional connectivity in transgenic mice, however, this reduction appeared in the early months before the accumulation of Aβ in the somatosensory and motor cortex.

A study investigating whole-brain connectivity found abnormalities in cortical hubs of the temporo-parietal cortex and precuneus/PCC in healthy mild cognitive impaired subjects with Aβ burden (Drzezga et al., 2011). In general, greater atrophy has been related to less brain connectivity (Hoffstaedter et al., 2015), but not all studies have found support for this association. For example, a study by Gili et al. (2011), reported that functional connectivity decline was not related to the amount of gray matter atrophy in the PCC in individual with MCI.

Disconnection between functional networks could be an essential biomarker for AD. For instance, individuals with AD exhibit disruption of functional connectivity between the inferior lateral temporal cortex (ITC), precuneus, right thalamus and the PCC (Zhang et al., 2009), between the left hippocampus and PCC (Sorg et al., 2007), as well as between the right hippocampus and the right and left cuneus, precuneus, and right ITC (Wang et al., 2006). This pattern of disconnection is likely associated with impairments in memory, processing speed and executive function (Damoiseaux et al., 2016). Another proposed early biomarker for AD could lie in the disruptions that have been identified within the visual cortices, specifically the impairments in connectivity between the PCC and the dorsal and ventral visual pathways (Zhang et al., 2009). These changes have been suggested to lead to deteriorating visual function in AD (Zhang et al., 2009).

Small-world network analysis in AD has shown longer path length in the central, temporal, and frontal brain regions as compared to age-matched, non-demented individuals (Stam et al., 2007). Decreased local connectedness within networks, also called clustering, has also been reported in individuals with AD, and correlated with lower cognitive performance (Stam et al., 2007). This finding led Stam et al. (2007) to speculate that individuals in the early stages of AD may show relatively diminished topology of small-world networks. A recent study found support for this notion by demonstrating that individuals with MCI and AD had a longer characteristic path length compared to healthy controls (Mijalkov et al., 2017). Moreover, AD appeared to be associated with a greater number of edges connecting to a node regionally, as well as increases and decreases in the efficiency of local nodes when compared to the controls (Mijalkov et al., 2017). To understand these differences in network topology, it is necessary to account for genetic variations that might affect the organization of the brain and which may also be linked to neurodegeneration in AD (**Figure 3A**).

ROLE OF GENETIC FACTORS RELATED TO AD IN FUNCTIONAL CONNECTIVITY

Apolipoprotein E (APOE)

The inheritance of gene-related factors such as apolipoprotein E (*APOE*), in particular the *APOE* ε4 allele, is associated with an increased risk of AD (Mahley et al., 2006, This genetic polymorphism is associated with increased Aβ deposition in the brain (Mahley et al., 2009; Morris et al., 2010; Sheline et al., 2010a), possibly influencing brain functional connectivity (Mahley et al., 2009), as well as affecting cognitive functioning in older age (Wisdom et al., 2011).

Resting-state fMRI studies have reported diverging associations of *APOE* polymorphisms and functional connectivity in healthy individuals that may relate to the age of the sample groups (Goveas et al., 2013; Wu et al., 2016). For example, *APOE* ε4 alleles have been associated with both increased and decreased DMN functional connectivity in cognitively healthy individuals (Fleisher et al., 2009). Comparing non-demented middle-aged (50–65 years) individuals carrying the *APOE*ε4 with non-carriers, ε4 carriers showed elevated functional connectivity in the middle frontal gyrus, whilst non-ε4 carriers had greater functional connectivity in the right medial

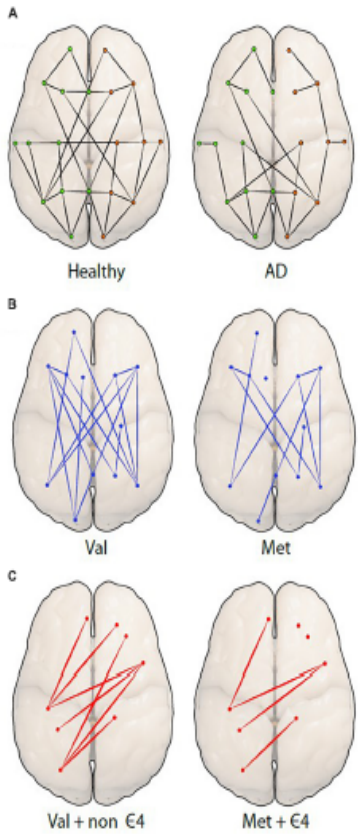

FIGURE 3 | Differences among network organizations are shown using a graph theoretical approach. **(A)** A graph of a healthy person (left) is compared to a person with Alzheimer's disease (AD; right), showing fewer connections (edges) between the spatially distant brain regions (nodes or dots) in AD. The green (left) and orange (right) dots represent hemispheres. The next two figures are hypothetical figures of the *BDNF* Val66Met polymorphism **(B)**, in which the connections are noticeably decreased in Met carriers. The last figure represents the carriage of both **(C)**, *BDNF* and *APOE* displaying a distinct reduction of edges and nodes in individuals.

frontal gyrus (Wu et al., 2016). Conversely, Goveas et al. (2013), demonstrated decreased functional connectivity within the DMN in cognitively healthy *APOE* ε4 carriers (44–65 years of age) in the bilateral dorsomedial PFC, superior frontal gyri, and in the left hippocampus, as well as increased functional connectivity in the left lentiform nucleus and bilateral caudate. Additionally, a decrease in interhemispheric functional connectivity within the DMN was found in healthy elderly *APOE*ε4 carriers (65–80 years of age; Lu et al., 2017). Notably, most of these regions are also affected in AD, which emphasizes the significance of the involvement of the DMN in the preclinical phase of AD (Sheline et al., 2010a). More recently, Zheng et al. (2018) investigated functional connectivity in young adults who were APPs/presenilin-1/2 mutation carriers or *APOE*ε4 positive carriers relative to adults without these AD-linked genetic factors (18–35 years). Interestingly, greater functional connectivity was observed in both the *APOE*ε4 carriers and in the APP/presenilin-1/2 group as compared to healthy controls. This increased connectivity was found between the left hippocampus and the bilateral medial PFC/precuneus. Only *APOE*ε4 carriers displayed increased connectivity between the right hippocampus and the left middle temporal gyrus. Here, the authors have suggested that the 'beneficial' effect of *APOE* ε4 in functional connectivity in younger individuals may be due to mechanisms of compensation of cognitive disruptions, which may be detrimental as the individual ages.

Due to inconsistencies in published evidence, it is important to consider how *APOE* polymorphisms may be associated with other measures of functional connectivity. Studies that have investigated *APOE* effects in small-world networks have reported higher susceptibility of fewer functional hubs and reduced centrality in healthy older ε4 carriers compared to non-ε4 carriers (Seo et al., 2013). Regional cerebral glucose metabolism, clustering of whole-brain functional networks, and path length have all been reported to be decreased in ε4 carriers (Seo et al., 2013). However, in a study with a greater sample size of 147 cognitively normal individuals, more clustering and longer path lengths were identified in ε4 carriers when compared to non-carriers (Goryawala et al., 2015). Non-demented ε4-carriers also had more long-distance connections in the parietal and temporal lobes, whilst non-ε4 carriers exhibited more short-distance connections in the parietal and occipital lobe. Healthy older individuals with the ε4 allele also had less short-distance connections in the frontal lobe connections, while both groups showed more long-distance connections in the frontal lobe (Goryawala et al., 2015). In summary, this study found the brain networks of those carrying *APOE*ε4 to be organized into an abnormal structure when compared to non-carriers, with fewer connections in the frontal lobe and more structural long length connections, which could partially explain the negative *APOE* ε4 cognitive phenotype.

Brain-Derived Neurotrophic Factor (BDNF)

Another genetic factor related to AD is the *BDNF* gene (Brown et al., 2014). The BDNF protein belongs to the family of nerve growth factors, which affect neurogenesis (Erickson et al., 2010) as well as long-term potentiation (LTP) and activity-dependent synaptic plasticity (Egan et al., 2003). Post-mortem studies of AD have shown that BDNF protein levels are decreased in the hippocampus, entorhinal cortex, temporal, frontal, and parietal cortex when compared to cognitively intact age-matched controls (Connor et al., 1997; Garzon et al., 2002). Lower BDNF levels may be related to volume loss in the hippocampus (Erickson et al., 2010), but this may be secondary to other pathological changes that occur in AD (Buchman et al., 2016). BDNF concentration is highly variable between individuals and is relative to physiological state; for example, after physical exercise,

peripheral blood BDNF concentration is increased (Dinoff et al., 2016). A recent review supported this finding by reporting increased neurogenesis and plasticity in the hippocampus in rats and mice after treadmill exercise, which led to improved short- and long-term memory functions (Jahangiri et al., 2018).

A common single nucleotide polymorphism in the *BDNF* gene, specifically a valine-to-methionine substitution at codon 66 (Val66Met), has an influence on LTP as well as activity-dependent BDNF secretion (Egan et al., 2003). *BDNF* Val66Met has been associated with cognitive performance as well as with AD brain morphology. In particular, the *BDNF* Met gene carriers (aged 60 and older), which were in preclinical stages of AD, demonstrated reduced memory function and smaller hippocampal and temporal lobe volume as compared to Val homozygotes (Lim et al., 2013; Brown et al., 2014). Authors also observed that more physical exercise was related to larger hippocampal and temporal lobe volumes in Val homozygotes but not in Met carriers (Brown et al., 2014). Notably, in Met carriers, physical activity was linked to reduced volumes of the temporal lobe, which is likely due to more apoptotic alterations (Brown et al., 2014). Likewise, Egan et al. (2003) demonstrated that the *BDNF* Met allele is related to qualitative changes of the hippocampus, which might cause insufficient memory functioning. Studies have proposed that there might be a relationship between Aβ and *BDNF* Val66Met, in which the *BDNF* polymorphism might mediate the effects on Aβ neurotoxicity on the brain (Fahnestock, 2011). Lim et al. (2013) reported not only a faster rate of atrophy in hippocampal volume, but also a faster decline in episodic memory performance in *BDNF* Met carriers who had a high Aβ load over a 36-month period compared to healthy individuals with *BDNF* Met but low levels of Aβ. Relative to Val homozygotes with a low Aβ load, Val homozygotes with a high Aβ load also experienced reduced cognitive performance, indicating that being a Val homozygote would not necessary protect against cognitive decline (Lim et al., 2013).

In older adults with late-onset depression, *BDNF* Met carriage was associated with reduced resting-state functional connectivity between the bilateral hippocampus and cerebellum (Yin et al., 2015). *BDNF* Met carriers with late-onset depression also had reduced strong (positive) functional connectivity between the hippocampus and the temporal cortex; however, there was also evidence of increased anti-correlated (negative) functional connectivity between the hippocampus and the dorsal anterior cingulate cortex, dorsal-lateral PFC, and angular gyrus (Yin et al., 2015). Similarly, Wang et al. (2014) observed elevated functional connectivity between the dorsal lateral PFC and the anterior insula in cognitively healthy *BDNF* Met carriers. Finally, Park et al. (2017) investigated the influence of *BDNF* Val66Met polymorphism on structural networks of middle-aged healthy individuals. The authors targeted nodes and edges in their analysis and simulated manipulation of the white matter networks. They demonstrated that Val homozygotes were more robust and resistant to gray matter damage compared to Met carriers (Park et al., 2017). Studies of white matter networks determined that *BDNF* Met carriers were more susceptible to node disruptions than Val homozygotes (Park et al., 2017).

The interaction of the *BDNF* Met and *APOE*ε4 polymorphisms was investigated by Gomar et al. (2016) in healthy older adults, as well as in individuals with MCI and AD. Here, the authors found that *BDNF* Met alleles were associated with poorer cognitive performance, predominantly in memory and semantic fluency. In support, Ward et al. (2014) found decreased performance in episodic memory function in *BDNF* Met carriers, however, only in combination with carriage of the *APOE* ε4 allele, the latter perhaps representing a cumulative effect of carriage of both risk alleles. This cumulative effect may be influencing the functional brain networks and reduce connections between different brain regions. *BDNF* Met carriers may have fewer connections compared to *BDNF* Val homozygotes (**Figure 3B**) and *APOE* ε4/*BDNF* Met carriers may have even fewer connections compared to non ε4/*BDNF* Val homozygote carriers, which may decrease connectivity (**Figure 3C**).

In a separate study, *BDNF* Met/*APOE*ε4 carriers with high brain Aβ levels demonstrated a faster rate of decline over a 54-month period in verbal and visual episodic memory and language processing when compared to *BDNF* Met/non-*APOE* ε4 carriers (Lim et al., 2015). In comparison, *BDNF Val*/ε4 carriers with a high Aβ burden demonstrated a relatively mild reduction in cognitive functioning. In *BDNF* Met/*APOE*ε4 carriers with high Aβ load, memory deficits are detectable after 3 years, whereas it takes 10 years in *APOE*ε4-/*BDNF* Val homozygotes with a high Aβ load to reach the same clinical threshold (Lim et al., 2015). A recent meta-analysis investigated the relationship between *APOE* and *BDNF Val66Met* and concluded that there were more women with AD carrying the *BDNF Met* polymorphism (Zhao et al., 2018). However, no significant relationships between *APOE*ε4 carriers and *BDNF* Met carriers were identified in the overall analysis that included both men and women with AD.

APOE and *BDNF* polymorphisms may interact with each other and possibly influence functional connectivity. *BDNF* Met carriers with the *APOE* ε4 allele exhibited decreased brain activation in the MTL (Kauppi et al., 2014). Atrophy, particularly in the entorhinal cortex, and acceleration of AD pathology, has been linked to poor compensation mechanisms of the brain in individuals with *BDNF* Met carrying the *APOE*ε4 (Gomar et al., 2016). Ward et al. (2015b) investigated the effect of *BDNF* and *APOE* on cognitive function and cognitive reserve, the latter which is a theoretical construct where neural networks compensate for lost neurons and connections (Stern, 2002). The authors observed that the *BDNF* Val66Met polymorphism, but not *APOE* variants, moderated the relationship between executive function and cognitive reserve, in which exposure to a more cognitively enriched environment was associated with better executive functioning in Val homozygotes but not in Met carriers (Ward et al., 2015a). In another study, Ward et al. (2017) investigated the same healthy older adult sample and found that differences in executive functioning between cognitive reserve tertile groups became smaller over time in *BDNF* Val homozygotes, but cognitive reserve-related differences became more pronounced in *BDNF* Met carriers. An explanation for these results is that cognitive reserve could have varying cognitive effects depending on the *BDNF* Val66Met polymorphism (Ward

et al., 2017). Altogether, experimental studies indicate that the *BDNF* polymorphism influences key neurobiological processes associated with development and activity-dependent learning (Egan et al., 2003).

COGNITIVE RESERVE AND BRAIN CONNECTIVITY

It is possible that common variation in the *BDNF* gene may result in differences in the development and maintenance of structural and functional networks throughout the life course, which ultimately may be associated with either better or worse brain resilience to neurodegenerative disease processes, such as in AD. Given the role of *BDNF* in development and adult brain plasticity, it is also possible that this gene variation may have an influence on the construction of patterns of connectivity that underlie resistance to pathology, perhaps related to the theoretical construct of cognitive reserve (Stern, 2002, 2006), in which neurons are compensating for impaired and lost neurons.

Stern (2002, 2009) proposed two different kinds of reserve in relation to a brain challenged by insult and/or neurodegeneration. Brain or neural reserve, which is often referred to as the 'passive' model of reserve, focuses on anatomical brain structures, especially brain size and the number and architecture of neurons and synapses (Katzman, 1993). This model, later revised by Satz (1993), proposed that individuals with higher synaptic count, dendritic branching and larger brain volume should be able to withstand the loss of more neurons without functional consequence, providing compensation for the pathological changes of AD (Stern, 2009). The brain reserve model suggests that most of its capacity is established in the early years of life, usually by the age of five (Reiss et al., 1996). Nevertheless, investigations have demonstrated that brain reserve may be modifiable. For example, the brains of adult monkeys are able to form and renew cells throughout life (Eriksson et al., 1998), and human brains have also been proposed to have neurogenic capacity, particularly in the dentate gyrus (Kempermann et al., 2015).

The 'active' model of reserve is often referred to as 'cognitive reserve,' which is a hypothetical construct that relates to the functional resilience of the brain against accumulating pathological changes (Stern et al., 1999). According to the theory of cognitive reserve, brains with more complex neural networks have a higher level of inbuilt redundancy, which are subsequently able to compensate for degenerative or lost neurons (Stern, 2002, 2006). Factors such as lifetime experience, educational and occupational attainment, and socioeconomic status are posited to play a significant role in the development of cognitive reserve (Stern, 2009, 2012). For example, individuals with AD and higher cognitive reserve (education levels) had greater DMN connectivity compared to individuals with AD and lower education levels (Bozzali et al., 2015). Bastin et al. (2012) on the other hand, determined that there was more cerebral pathology and reduced activity of metabolism in the temporoparietal cortex in healthy individuals with higher education. Furthermore, although Brayne et al. (2010) found that the amount of accumulation of pathological burden in the brain was not affected by the number of years of education that an individual had completed, higher levels of educational attainment was found to be associated with a lower risk of demonstrating dementia on the background of the burden of pathology.

Lifelong engagement in cognitively stimulating activities may reduce the risk of developing dementia by 40% (Scarmeas and Stern, 2003; Valenzuela et al., 2011). In support, Jahangiri et al. (2018) noted that exercise was associated with improved memory function, as well as reduced risk of developing neurodegenerative disease in different animal models. In human studies, Larsson et al. (2017) reported that individuals with higher educational attainment had a lower risk of developing AD. Similarly, in healthy participants (50–79 years), education later in life (university study for at least 12 months) was positively associated with cognitive reserve (as estimated by current psychological assessment scores) compared to those who did not complete any further university education (Lenehan et al., 2016). Associations between education and age are evident particularly in the attention and speed processing domains (Perry et al., 2017). In line with these findings, Summers et al. (2017) found that 92.5% of individuals 50 years and older who had attended university for at least 12 months showed increased cognitive performance in domains that may be a proxy for cognitive reserve.

Stern (2009) hypothesized that individuals with AD who have higher cognitive reserve possess more flexible neural networks and will retain a higher level of cognitive performance with an increasing neuropathological load. This notion of neural flexibility could potentially be demonstrated in re-organizable functional networks of the brain observed in cognitively healthy individuals (Bosch et al., 2010). In this study of healthy older individuals, higher cognitive reserve was associated with increased brain activity in the DMN, but it was also associated with decreased brain activity in regions associated with speech comprehension. In contrast, in individuals with MCI or AD, decreased activation in the DMN and more activation in language processing in subjects was associated with higher cognitive reserve (Bosch et al., 2010).

Education and cognitive reserve have a positive effect on functional connectivity networks (Marques et al., 2016) and cognitive functioning (Bozzali et al., 2015). There is evidence that high cognitive reserve levels were related to working memory, while age had a negative effect on cognition (Ward et al., 2015a). High cognitive reserve has been associated with greater functional connectivity in healthy elderly individuals (Marques et al., 2016). Arenaza-Urquijo et al. (2013) examined a cognitively healthy older population (60–80 years) and described better brain metabolism, higher gray matter volume as well as enhanced functional connectivity in individuals who had more years of early-life formal education. In particular, the authors found higher functional connectivity in regions such as the anterior cingulate cortex, right hippocampus, right PCC, left inferior frontal lobe and left angular gyrus in people with those with more education.

Marques et al. (2015) likewise examined the relationship between education and functional connectivity and found that individuals with more education had larger networks.

These enlarged networks were connected to all lobes in each hemisphere and influenced functional connections in a positive way, which was predicted to moderate the effects of age on brain connectivity (Marques et al., 2015). Moreover, Marques et al. (2016) investigated whether sex and the number of years of education [used as demographic characteristics (DEM)], in 120 healthy older individuals influenced functional networks in the brain. The authors demonstrated that the DEM had a positive effect locally (in the neighborhood areas), on the strength of nodes, efficiency and on clustering coefficient, exhibiting greater communication within the networks of the occipital and parietal lobe areas. There was also a relationship found between the DEM and network transitivity indicating that individuals with more education use different neural processing (Marques et al., 2016). Network transitivity is defined as the connection between two nodes that are linked to each other via an edge in a network.

In addition, Marques et al. (2016) examined how cognitive reserve measured by educational attainment affected functional connectivity in resting state fMRI. They demonstrated that larger networks with more functional connections in the brain were related to higher cognitive reserve. Greater local efficiency and higher local clustering in the cuneus, as well as in the areas of the superior and middle occipital lobe were related to higher levels of cognitive reserve (Marques et al., 2016). The inferior temporal gyrus is predicted to have a significant role for cognitive reserve, because of its betweenness centrality and nodal strength, which demonstrated a positive correlation with cognitive reserve. The fraction of all shortest paths in the network that pass through a given node is called betweenness centrality (Rubinov and Sporns, 2010). The inferior temporal gyrus is a significant hub responsible for recognition and visualization of words and numbers (Grotheer et al., 2016), which are important functions involved in cognitive reserve networks (Marques et al., 2016). Finally, global efficiency, which is "a measure of functional integration" (Marques et al., 2016), was greater in individuals displaying higher cognitive reserve compared to individuals with lesser cognitive reserve.

Colangeli et al. (2016) conducted a meta-analysis of whether functional brain networks were associated with cognitive reserve in healthy older adults, as well as in amnestic MCI (aMCI) and AD. Findings in all subgroups showed greater functional brain activation in the anterior cingulate in the left hemisphere while performing a cognitively stimulating task (e.g., recognition memory task). However, the cognitively healthy older adult group demonstrated greater activation in several brain regions as compared to the aMCI and AD groups. These activated brain regions included the left anterior cingulate and left precuneus, the right cingulate gyrus, and the superior frontal gyrus of the dorsolateral PFC, all of which are susceptible to degenerative changes in individuals diagnosed with AD and aMCI (Colangeli et al., 2016).

Bozzali et al. (2015) investigated whether cognitive reserve modifies resting-state functional connectivity in healthy, aMCI, and AD individuals (mean age 74.6 years). Functional connectivity was associated with the cognitive reserve proxy, education, within the DMN. Higher functional connectivity within the PCC was associated with higher education in individuals with AD, in which education possibly initiated mechanism of compensation. Education may also have led to brain plasticity and supported the PCC from atrophying. Some of the aMCI group exhibited similar connectivity strength, however, there was no strong functional connectivity found in the healthy group (Bozzali et al., 2015).

Franzmeier et al. (2016) also demonstrated that higher global functional connectivity was present in individuals with MCI with relatively higher levels of education. Individuals with more years of education and prodromal AD were able to compensate for fluorodeoxyglucose (FDG)-PET hypometabolism in the precuneus and had greater connectivity in the left frontal lobe, as well as better performance in memory (Franzmeier et al., 2017a,b). Moreover, Franzmeier et al. (2017b) demonstrated that individuals with MCI who had higher educational attainment and high Aβ levels had a more global left frontal cortex connectivity when controlled for age and sex, whereas, in healthy individuals, global left frontal cortex connectivity was not related with metabolism in the precuneus. Negative connectivity between the left lateral frontal cortex and the DMN was also found in people with MCI who had achieved higher education (Franzmeier et al., 2017a). Perry et al. (2017) demonstrated a positive correlation between years of education and cognitive functioning (e.g., visuospatial, executive function, language) but a weak relationship between education and brain networks, especially when the brain already showed evidence of age-related changes in healthy individuals. The greatest impact in age-related alterations later in life was found in the sensorimotor networks, especially those underlying processing speed and attention (Perry et al., 2017).

In summary, education early in life and other life-long cognitively stimulating activities could be possible protectors against neurodegenerative diseases, and might bolster cognitive reserve later in life (Ward et al., 2015b).

CONCLUSION

The brain is a large set of complex networks that are connected structurally and functionally. Different areas of the brain share and communicate information in functional space, creating networks. These networks can be adversely or positively influenced by various genetic and environmental factors. For instance, studies reported that *APOE* ε4 was associated with decreased functional connectivity (Lu et al., 2017) and longer path length in functional networks (Goryawala et al., 2015). However, there was also decreased path length (Seo et al., 2013) and increased functional connectivity found in healthy *APOE* ε4 carriers (Wu et al., 2016). Similarly, healthy older *BDNF* Met carriers were associated with reduced functional connectivity, while Val homozygotes showed a more robust network in the brain structure (Park et al., 2017). Cognitive activities and environmental enrichment have favorable effects on *BDNF* Val homozygotes, and over time also on *BDNF* Met carriers (Ward et al., 2017), which possibly may promote maintaining healthy cognitive functioning and reduce the detrimental effects progressing age. In general, studies provided evidence that education

and cognitive reserve are associated with an increase of functional connectivity in the brain networks (Marques et al., 2016). This could potentially affect brain networks in a positive way and may mitigate and protect against cognitive impairments later in life, and hopefully delay or even prevent the onset of AD (Prince et al., 2013). Future studies should investigate whether cognitive reserve and environmental enrichment work as compensatory mechanisms to influence and alter the networks of more susceptible genetic polymorphisms to AD, such as *APOE* ε4 and *BDNF* Met carriers. Education later in life increases cognitive reserve and could provide more resistance and resilience to brain pathology. Overall, these findings indicate that the functional networks of the brain are influenced by a combination of genetic and environmental factors. An improved understanding of these relationships is vital in order to fully grasp how neurodegenerative changes affect brain function, but also to determine how cognitive resilience to neurodegenerative changes may be promoted.

AUTHOR CONTRIBUTIONS

The manuscript of this review was prepared, formalized, and developed by MP. AK and DW assisted with refinement. JV contributed to the development and refinement of the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix E – Pietzuch, Bindoff, Jamadar, &Vickers (2021) reprint

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Interactive effects of the *APOE* and *BDNF* polymorphisms on functional brain connectivity: the Tasmanian Healthy Brain Project

Manuela Pietzuch[1✉], Aidan Bindoff[1], Sharna Jamadar[2] & James C. Vickers[1✉]

Resting-state functional magnetic resonance imaging measures pathological alterations in neurodegenerative diseases, including Alzheimer's disease. Disruption in functional connectivity may be a potential biomarker of ageing and early brain changes associated with AD-related genes, such as APOE and BDNF. The objective of this study was to identify group differences in resting-state networks between individuals with *BDNF* Val66Met and *APOE* polymorphisms in cognitively healthy older persons. Dual regression following Independent Components Analysis were performed to examine differences associated with these polymorphisms. *APOE* ε3 homozygotes showed stronger functional connectivity than *APOE* ε4 carriers. Males showed stronger functional connectivity between the Default Mode Network (DMN) and grey matter premotor cortex, while females showed stronger functional connectivity between the executive network and lateral occipital cortex and parahippocampal gyrus. Additionally, we found that with increasing cognitive reserve, functional connectivity increased within the Dorsal Attention Network (DAN), but decreased within the DMN. Interaction effects indicated stronger functional connectivity in Met/ε3 carriers than in Met/ε4 and Val/ε4 within both the DMN and DAN. *APOE*/*BDNF* interactions may therefore influence the integrity of functional brain connections in older adults, and may underlie a vulnerable phenotype for subsequent Alzheimer's-type dementia.

Alzheimer's disease (AD) is the most common form of ageing-related dementia, accounting for 60 to 80% of all cases[1]. The primary functional manifestations of AD include memory loss, impairment in executive functioning, difficulties with language, and changes in personality and behaviour, with brain pathology characterized by neurofibrillary tangles and amyloid-beta deposition[1,2]. Neuroimaging techniques investigating activity within and between resting-state networks, such as the default mode network (DMN)[3], the dorsal-attention network (DAN)[4], and salience network (SN)[5], may help provide an understanding of the elementary brain changes that are associated with ageing and subsequent risk of AD. In this regard, significant ageing-related changes in functional connectivity have been observed within the DMN[6,7]. Many studies have focused on the role of the DMN in AD, with some studies showing increased functional connectivity[8], while others showed decreased functional connectivity[6,7,9]. A further interesting network is the DAN, which is activated during goal-directed behaviour[4]. The DAN showed significant decline in functional connectivity in the amnestic form of mild cognitive impairment (MCI) and in AD[10] compared to neurologically healthy individuals. This disturbance in connectivity increases with disease progression. Similar results were found within the SN, in which reduced grey matter volume and disrupted functional connectivity were found in patients with AD. Moreover, it was found that healthy older individuals had intra-network functional connectivity impairments between crucial nodes, such as the DMN, indicating that the SN is affected by normal ageing before manifestation of AD[5]. The SN is involved in incoming information processing and filtering information[11], and is active in higher-order processing such as selecting specific stimuli[12].

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Appendix F – Manuscript Submission for Chapter 5

Language networks recruit frontal lobe regions to support functional connectivity in heathy older adults

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ABSTRACT = 224 words

INTRODUCTION = 747

ARTICLE = 4702

TABLES = 3

FIGURES = 5

Appendix G – Manuscript Submission for Chapter 6

Brain structure metrics related to *APOE* and *BDNF* polymorphisms, and cognitive function in healthy older adults

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ABSTRACT = 256 words

INTRODUCTION = 1199

ARTICLE = 6296

TABLES = 3

FIGURES = 4