

Genetic risk and resilience for Alzheimer's disease:

**The effect of the three *APOE* alleles on brain
function across life**



Sana Suri

Keble College

University of Oxford

A thesis submitted for the degree of

Doctor of Philosophy

Michaelmas Term 2015

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Abstract

The human apolipoprotein E (*APOE*) gene has three alleles ($\epsilon 2$, $\epsilon 3$ and $\epsilon 4$). Relative to the most common $\epsilon 3$ allele, $\epsilon 4$ is associated with a higher risk of developing sporadic late-onset Alzheimer's disease (AD) whereas $\epsilon 2$ is believed to confer a protection to AD. Because of $\epsilon 4$'s close association with cognitive decline, the majority of *APOE*-research has focused on this allele, with $\epsilon 2$ receiving little attention. This DPhil thesis used advanced multi-modal magnetic resonance imaging techniques in cognitively healthy young (20-40 years), middle-age (35-55 years) and older (60-85 years) adults to fulfil two objectives: (1) to present the first detailed examination of the effects of $\epsilon 2$ on brain structure and function across life, and (2) to study the early-life effects of *APOE* on cerebrovascular and neurochemical health, both of which are characteristically impaired in AD but have not been investigated in relation to *APOE*. We found that while *APOE* did not exert strong effects on brain structure or neurochemistry across the lifespan, there were significant allele-specific changes in brain function and vascular health. *APOE*'s effects on functional connectivity of the posterior cingulate cortex varied with age (connectivity in young adults: $\epsilon 4 > (\epsilon 2 \text{ and } \epsilon 3)$; older adults: $(\epsilon 2 \text{ and } \epsilon 3) > \epsilon 4$). *APOE* $\epsilon 2$ carriers displayed an upward and $\epsilon 4$ carriers a declining trend in functional connectivity from young to older ages, with the middle-age range serving as a crossing point for the two opposing trajectories. Notably, similar increases in brain activity were observed within the hippocampus during a memory-encoding task in young $\epsilon 4$ carriers relative to non-carriers, suggesting a link between $\epsilon 4$ and early-life functional hyperactivity. We also found that young $\epsilon 4$ carriers had the worst and $\epsilon 2$ carriers the most robust measures of cerebrovascular reactivity (measured as the compensatory blood flow to the brain in response to breathing carbon dioxide, *i.e.* CO₂-CVR). Early-life changes in cerebrovascular function may explain why $\epsilon 4$ carriers are more vulnerable to amyloid deposition and by contrast, why $\epsilon 2$ carriers remain relatively protected from AD-related neuropathology. Evidence suggests that impairments in CO₂-CVR can be pharmacologically restored, making this measure a particularly useful preclinical marker of AD risk. Overall, this thesis provides valuable insights into the effects of *APOE* in the brain, and lends support to our growing understanding of AD as a vascular disorder.

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List of Abbreviations

AAT	arterial arrival time
AD	Alzheimer's disease
ANOVA	analysis of variance
ApoE	apolipoprotein
<i>APOE</i>	apolipoprotein gene
ε2 carriers	<i>APOE</i> ε2ε2 or ε2ε3 carriers
ε3 carriers	<i>APOE</i> ε3ε3 carriers
ε4 carriers	<i>APOE</i> ε3ε4 or ε4ε4 carriers
APP	amyloid precursor protein
ASL	arterial spin labeling
Aβ	amyloid β
BDNF	brain-derived neurotrophic factor
BOLD	blood-oxygen-level dependent
CBF	cerebral blood flow
CBF₀	cerebral blood flow at rest
CBV	cerebral blood volume
CES-D	Centre for Epidemiological Studies depression scale
CMRO₂	cerebral metabolic rate of oxygen consumption
CO₂	carbon dioxide
CO₂-CVR	cerebrovascular reactivity to carbon dioxide
COPE	contrast of parameter estimate
Cr	creatinine
CSF	cerebrospinal fluid
CVR	cerebrovascular reactivity
DMN	default mode network
DTI	diffusion tensor imaging
FA	fractional anisotropy
FEAT	FMRI expert analysis tool
FIRST	FMRI's automated model-based segmentation/registration tool
FIX	FMRI's ICA-based X-noiseifier
FLIRT	FMRI's linear image registration tool
fMRI	functional magnetic resonance imaging
FNIRT	FMRI's non-linear image registration tool
FSL	FMRI software library
FWHM	full-width at half-maximum
GLM	general linear model
GM	grey matter
¹H-MRS	proton magnetic resonance spectroscopy
HIV	human immune-deficiency virus
HRF	hemodynamic response function

ICA	independent component analysis
LCModel	linear combination of model
LTP	long-term potentiation
MANCOVA	multivariate analysis of covariance
MCI	mild cognitive impairment
MD	mean diffusivity
ME-MPRAGE	multi-echo MPRAGE
MELODIC	multivariate exploratory linear optimized decomposition into independent components
MoCA	Montreal Cognitive Assessment
MPRAGE	magnetization-prepared rapid acquisition with gradient echo
MRI	magnetic resonance imaging
NAA	N-acetyl aspartate
NFT	neurofibrillary tangles
O₂	oxygen
pCASL	pseudo-continuous arterial spin labeling
PCC	posterior cingulate cortex
PE	parameter estimate
PET	positron emission technology
PSD-95	postsynaptic density-95
RF	radiofrequency
ROI	region of interest
RSN	resting-state network
SCA	seed-based correlation analysis
SCID-I	Structured Clinical Interview for DSM-IV-TR Axis I Disorders
SD	standard deviation
SE	standard error
SNR	signal-to-noise ratio
SPECIAL	short echo time full intensity acquired localized
TBSS	tract-based spatial statistics
TE	echo time
TR	repetition time
VAPOR	variable power radio-frequency pulses with optimized relaxation delays
VBM	voxel-based-morphometry
WM	white matter

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List of Publications and Presentations

Publications arising from this thesis

Suri S, Mackay CE, Kelly ME, Germuska M, Tunbridge L, Frisoni GB, Matthews PM, Ebmeier KP, Bulte DP, Filippini N. (2014). Reduced cerebrovascular reactivity in young adults carrying the *APOE* ϵ 4 allele. *Alzheimers Dement.*; S1552-5260(14)02467-4.

Suri S, Topiwala A, Mackay CE, Ebmeier KP, Filippini N. (2014). Using structural and diffusion magnetic resonance imaging to differentiate the dementias. *Curr Neurol Neurosci Rep*; 14(9):475.

Suri S, Heise V, Trachtenberg AJ, Mackay CE. (2013). The forgotten *APOE* allele: a review of the evidence and suggested mechanisms for the protective effect of *APOE*2. *Neurosci Biobehav Rev*, 37(10 Pt2): 2878-86. **Top Author Award** by Elsevier.

Suri S, Emir U, Stagg C, Near J, Mekle R, Schubert F, Zsoldos E, Mahmood, Singh-Manoux A, Kivimaki M, Mackay CE, Ebmeier KP, Filippini N. The effect of age and the *APOE* gene on metabolite concentrations in the posterior cingulate cortex. (*submitted*)

Other publications during the DPhil period

Abdi A, Mallet N, Mohamed F, Sharott A, Dodson P, Nakamura K, **Suri S**, Avery S, Larvin J, Garas F, Garas S, Vinciati F, Morin S, Bezard E, Baufreton J, Magill P. (2015). Prototypic and Arkypallidal Neurons in the Dopamine-Intact External Globus Pallidus. *J Neuroscience*; 35(17):6667-88.

Heise V, Filippini N, Trachtenberg AJ, **Suri S**, Ebmeier KP, Mackay CE. (2014). Apolipoprotein E genotype, gender and age modulate connectivity of the hippocampus in healthy adults. *Neuroimage*; 98:23-30.

Rajkumar R, **Suri S**, Deng HM, Dawe GS. (2013). Nicotine and clozapine cross-prime the locus coeruleus noradrenergic system to induce a long-lasting potentiation in the rat perforant path-dentate gyrus synapse. *Hippocampus*, 23(7): 616-24.

Book chapters

Ebmeier, KP, Filippini, N, Mackay, CE, **Suri, S**, Valkanova, V. Chapter 11: Functional brain imaging and connectivity in dementia. In: Ames/O'Brien/Burns (ed.) *Dementia* 5th ed. (*in press*).

Conference Presentations

Suri S, Filippini N, Heise V, Ebmeier KP, Mackay, C (2015). The effects of the *APOE* gene on resting-state functional connectivity vary with age. OxDARE Dementia Research Day, Oxford.

Suri S, Mackay CE *et al.* (2015). Poor brain health in young people at a genetic risk for Alzheimer's disease.

- Set for Britain Poster Competition, **House of Commons, UK Parliament.**
(Bronze Award for Best Presentation)
- Poster presentation at Oxford Females in Engineering, Science, and Technology (OxFEST) annual conference, Oxford. **(Best Poster Award)**

Suri S, Mackay CE *et al.* (2014) Reduced cerebrovascular reactivity in young adults carrying the *APOE* ϵ 4 allele.

- Poster presentation at the Organisation for Human Brain Mapping, Germany.
- Oral and poster presentation at Alzheimer's Research U.K. Conference, Oxford.
- Poster presentation at 5th Annual Oxford Neuroscience Symposium, Oxford.
(Best Poster Award)
- Poster presentation at Department of Psychiatry Strategic Away Day, Oxford.
(Best Poster Award)

Chapter 1

Overview and Objectives

Ongoing efforts to improve survival and enhance quality of life have led biomedical research to focus on disease and the mechanisms that increase risk for disease. The other side of that coin may be as important, *i.e.* examining the protective factors that allow some individuals to enjoy long, healthy lives. One of the best examples of a gene that positively influences cognitive health is the apolipoprotein (*APOE*) $\epsilon 2$ allele. The *APOE* gene has three alleles ($\epsilon 2$, $\epsilon 3$, $\epsilon 4$), which differently influence survival to old age. In contrast to the neuroprotective $\epsilon 2$ allele, the $\epsilon 4$ variant is a well-established risk factor for sporadic late-onset Alzheimer's disease (AD), and the common $\epsilon 3$ allele presents an “average” risk of cognitive decline. Given its close association with AD, a substantial proportion of *APOE* research has extensively focused on examining $\epsilon 4$, and the putative protective role of $\epsilon 2$ has received little attention. However, in order to curb the widespread personal, societal, and financial impacts of diseases like AD, it is also crucial to gain an understanding of the genetic variations that provide protection and promote healthy ageing. With a focus on $\epsilon 2$, my DPhil thesis uses multi-modal magnetic resonance imaging (MRI) techniques to characterize the effects of all three *APOE* alleles on brain structure, function and chemistry in cognitively healthy individuals across a wide age-range.

There are two principal objectives of this thesis. The first is to ***complete*** our current understanding of the *APOE* gene and plug the gaps in the literature on the $\epsilon 2$ allele. I do

this by studying MRI markers of AD that have thus far been comprehensively examined in relation to $\epsilon 4$ but not $\epsilon 2$, *viz.* brain structure and resting-state function. The second objective is to ***expand*** our understanding of the effects of *APOE* by studying imaging markers of AD that have not yet been investigated in relation to *APOE*; *viz.* cerebrovascular health and neurochemical levels.

The rationale for this is that over the last two decades, *APOE* $\epsilon 4$'s effects on brain structure and function have been studied in detail, and any differences between $\epsilon 4$ carriers and $\epsilon 3$ homozygotes have always been interpreted in the context of risk for AD. However, a few recent studies that have included $\epsilon 2$ carriers have contradicted this interpretation and suggested that both the low-risk and high-risk groups may present similar changes in brain function relative to $\epsilon 3$ homozygotes. It is important for us to know if $\epsilon 2$ and $\epsilon 4$ exert apparently similar effects in the brain because without this knowledge, we cannot use *APOE*-associated risk as a stepping-stone towards the use of advanced MRI techniques for predicting conversion to AD. The ultimate aim of this work, therefore, is to identify neuroimaging markers that most accurately reflect one's genetic status of risk or resilience for late-onset AD. In the following chapters, I meet these objectives by asking five questions:

1.1 What do we know about the *APOE* $\epsilon 2$ allele?

In **Chapter 2** of this thesis, I review the existing literature on $\epsilon 2$ and outline empirical and evolutionary evidence for the allele's protective effects within the brain. Using the influential amyloid cascade hypothesis as a reference, I explore mechanisms through which this protection may be conferred. I conclude by discussing a few inconsistent

findings and presenting my arguments for including $\epsilon 2$ in future examinations of *APOE* genotype-dependent effects on behaviour. This chapter sets the stage for the experimental work in my thesis and has been published as a review article in *Neuroscience and Biobehavioural Reviews* (Suri et al., 2013), for which I was recognized as a “**Top Author**” by Elsevier.

I provide an overview of the participants and MRI techniques used in my thesis in **Chapter 3**. Evidence suggests that the effects of *APOE* $\epsilon 4$ in the brain may vary with age, but we know little about when and how $\epsilon 2$ begins to modulate brain function. To address this, I have examined relatively large datasets spanning a wide-age range. Briefly, there are three cross-sectional age groups of cognitively healthy individuals: 58 young (20-40 years), 84 middle (35-55 years) and 242 older (60-85 years). The young group was newly recruited for my DPhil project; previous students in our lab acquired data for the middle group, and the older individuals are part of the Whitehall II imaging cohort.

1.2 Do *APOE* $\epsilon 2$ and $\epsilon 4$ differently influence structural integrity of the brain across adulthood?

During my DPhil, I published a literature review on the diagnostic and prognostic potential of the MRI tools used to evaluate brain structure (Suri et al., 2014b). While there is strong evidence for structural atrophy in the brain of AD patients, these changes are not always observed in cognitively intact $\epsilon 4$ carriers, and far less studied in $\epsilon 2$ carriers. In fact, one recent study points towards comparable changes in white matter microstructure in $\epsilon 2$ and $\epsilon 4$ carriers, which is surprising given their opposite effects on

lifetime risk for AD. In **Chapter 4** I examine the effects of the three *APOE* alleles on the structural integrity of grey and white matter in the young, middle and older age groups.

1.3 Do *APOE* ϵ 2 and ϵ 4 differently influence functional connectivity of the resting brain across adulthood?

Even when we are not engaged in a specific task, there are correlations in activity between different parts of the brain. Alterations in the strength of these “baseline” or “resting-state” correlations have been implicated in AD (more so in patients with the ϵ 4 allele) and also observed in healthy ϵ 4 carriers. However, like with white matter structure, the only two investigations of resting activity to include the ϵ 2 genotype have found similarities in ϵ 2 and ϵ 4 carriers relative to ϵ 3 homozygotes during mid-late adulthood. In **Chapter 5**, I discuss the effects of *APOE* on resting-state connectivity in the three age groups. I ask if these similarities are also present in early life, and if they persist across the lifespan. I focus on regions that are first affected by AD pathology, *i.e.* the hippocampus and posterior cingulate cortex.

1.4 Does *APOE* modulate cerebrovascular health in early adulthood?

I concentrate on the young group in **Chapter 6**, where I discuss how *APOE* exerts early influences on cerebrovascular health. The *APOE* gene, in addition to influencing risk for dementia is also a mediator of cardiovascular and cerebrovascular disorders. There is a well-established overlap between the vascular risk factors that contribute to cardiovascular disorders and those that influence lifetime risk for AD. In this chapter, I

present a model of vascular dysfunction in AD and discuss how *APOE* may fit into this model. I then assess if *APOE* modulates vascular health in the brain during early adulthood in a pattern that is reflective of one's risk for developing AD-related vascular pathology. I also examine how *APOE* influences hippocampal activity during a memory-encoding task in young adults, with the aim of replicating past findings from our group (Filippini et al., 2009) and extending them to include $\epsilon 2$ carriers. I have published this chapter as an article in *Alzheimer's & Dementia* (Suri et al., 2014a). These results have been presented at national and international conferences and won “**Best Poster**” awards on four occasions, including at Set for Britain, 2015 where I presented these findings to Members of Parliament at the *House of Commons*.

1.5 Does *APOE* modulate the neurochemical profile of the brain across life?

In the last experimental chapter of my thesis, **Chapter 7**, I use magnetic resonance spectroscopy to explore how *APOE* may influence the levels of neurochemicals and metabolites in the brain, and in particular those that are significantly altered in AD. There have only been four independent examinations of the effects of *APOE* on metabolite concentrations, all producing conflicting results. We therefore know very little about whether the characteristic metabolite signatures of AD precede its clinical onset, *i.e.* if they are present in $\epsilon 4$ carriers. There are also no studies to date that include the $\epsilon 2$ allele. In this chapter, I examine the effects of *APOE* on the neurochemical profile of the posterior cingulate cortex, a region that has been extensively studied in spectroscopic

examinations of AD. This chapter has been prepared as a manuscript for publication (**Suri et al., submitted**).

1.6 Summary of objectives

Objective 1: To plug the gaps in the current *APOE* literature

1. What do we know about the *APOE* $\epsilon 2$ allele? Chapter 2
2. Do *APOE* $\epsilon 2$ and $\epsilon 4$ differently influence structural integrity of the brain across adulthood? Chapter 4
3. Do *APOE* $\epsilon 2$ and $\epsilon 4$ differently influence functional connectivity of the resting brain across adulthood? Chapter 5

Objective 2: To extend our understanding of *APOE* by studying novel MRI techniques

4. Does *APOE* modulate cerebrovascular health in early adulthood? Chapter 6
5. Does *APOE* modulate the neurochemical profile of the brain across life? Chapter 7

Chapter 2

The forgotten *APOE* allele

In this chapter I review the evidence for *APOE* $\epsilon 2$'s protective effects within the brain and outline possible mechanisms through which this protection may be conferred. I conclude by addressing a few inconsistencies in the literature and setting the stage for my thesis.

A modified version of this chapter has been published as a review article titled “*The forgotten APOE allele: a review of the evidence and suggested mechanisms for the protective effects of APOE $\epsilon 2$* ” in *Neuroscience and Biobehavioural Reviews* (Suri et al., 2013).

2.1 Introduction

AD is the leading cause of dependency in older people and its devastating personal, societal and financial impacts have recently brought it to the top of the scientific and political agenda in the United Kingdom (G8 Dementia Summit, 2013; Prime Minister's Challenge on Dementia, 2012). Despite on-going efforts over the last 20 years, there is still no effective treatment for AD and by the time we are able to clinically diagnose the disease, the brain is already severely damaged beyond repair. AD-related neuropathology and its downstream effects on the structure and function of the brain start decades before cognitive symptoms begin to manifest (Hardy and Selkoe, 2002). An effective strategy

for early treatment and prevention, therefore, would be to develop ways to detect these AD-related changes *before* any significant neuronal loss has occurred. This would allow us to predict who is likely to develop the disease before their symptoms appear. One such approach is to investigate how genes that influence risk for AD affect the brain in early life.

In the last few decades several genes that increase susceptibility to AD have been identified, of which the *APOE* $\epsilon 4$ allele stands out as the best-established risk factor for sporadic late-onset AD (Corder et al., 1993; Farrer et al., 1997). The human *APOE* gene has three allelic variants, $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$, which differently influence survival and cognitive health. While $\epsilon 4$ is a strong risk factor for AD (as well as cardiovascular disorders), the $\epsilon 2$ allele is believed to confer a protection to these diseases. Because of its strong association with an increased risk for AD, a substantial proportion of *APOE* research has extensively focused on examining the $\epsilon 4$ allele (Reinvang et al., 2013) and the putative protective role of $\epsilon 2$ has received little attention (Suri et al., 2013) (Figure 2.1).

In this chapter, I stress the importance of including $\epsilon 2$ while examining the role of *APOE* in the brain. I describe the effects of $\epsilon 2$ on the structure and function of the brain and present empirical and evolutionary evidence for the allele's status as a neuroprotective factor. I then explore potential mechanisms through which this protection may be conferred by using the influential "amyloid cascade hypothesis" of AD-related neurodegeneration as a point of reference. I conclude by addressing a few inconsistencies and gaps in the literature, particularly in the context of the experimental work of my thesis. Hereafter, I will refer to individuals with the *APOE* $\epsilon 2\epsilon 2$ and $\epsilon 2\epsilon 3$ genotypes as

‘*APOE* $\epsilon 2$ carriers’, since the combined effects of the two differing alleles in individuals with the $\epsilon 2\epsilon 4$ genotype are yet to be fully understood.

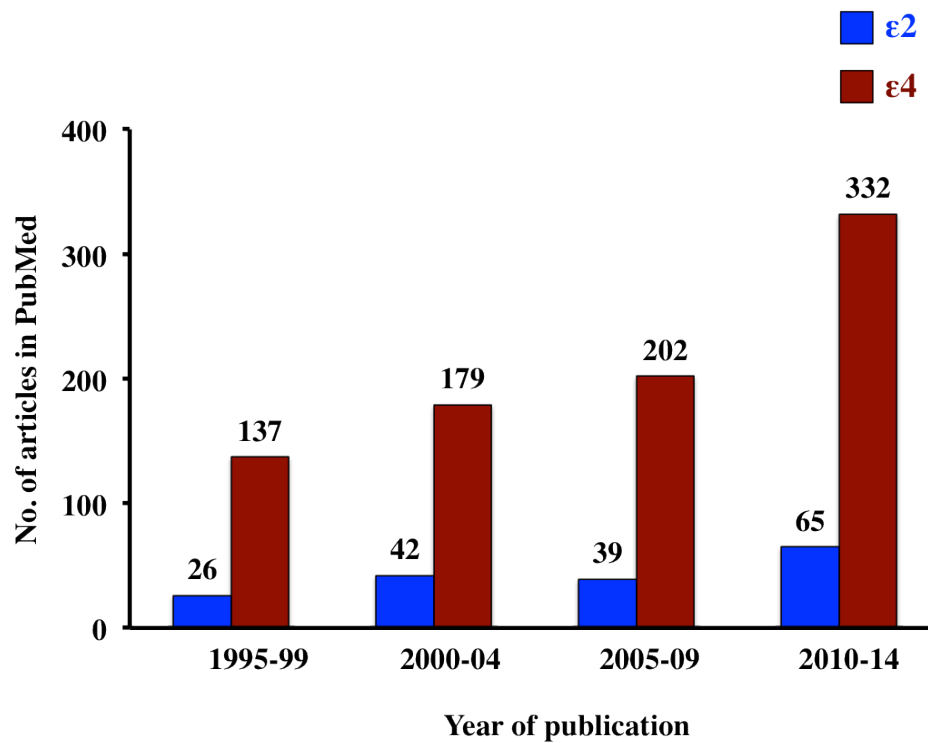


Figure 2.1: The forgotten *APOE* allele.

The number of published articles with the keywords “*APOE2*” AND “Alzheimer’s disease” (blue) and “*APOE4*” AND “Alzheimer’s disease” (red) within their title/abstract from 1994-2014. Data retrieved from the PubMed database in August 2015.

2.2 Apolipoprotein E

Apolipoprotein E (apoE, protein; *APOE*, gene) is a polymorphic apolipoprotein synthesized primarily by the astrocytes within the central nervous system and by the liver in the periphery. Within the brain, it coordinates the mobilization and redistribution of

lipids and cholesterol and plays a key role in neuronal growth, synaptic plasticity and membrane repair (Mahley, 1988).

The three major variants ($\epsilon 2$, $\epsilon 3$ and $\epsilon 4$) code for the production of the three isoforms of apoE (E2, E3 and E4) which differ from each other by single amino acid substitutions at residues 112 and 158; apoE3 contains a cysteine and arginine at these positions, whereas apoE2 has two cysteines and apoE4 two arginines (Weisgraber et al., 1981; Zannis et al., 1981).

A protein's structure determines its function and the neuroprotective effects of apoE2 may have their roots in its unique protein structure. The differences in cysteine content give rise to variability in domain interactions, protein stability and protein folding, which influence the activity of apoE in an allele-specific manner in both the healthy and pathological states (Hatters et al., 2006; Mahley and Huang, 2012a). The cysteine-to-arginine substitution in apoE2 makes it more stable and resistant to thermal and chemical denaturation than apoE3 and apoE4 (Zhong and Weisgraber, 2009). The negative cysteine residue at position 112 in both apoE2 and apoE3 makes them less likely to exhibit domain interactions than apoE4, which has a positive arginine residue at that position (Xu et al., 2004). Domain interactions, *i.e.* ionic interactions between the N and C terminal domains, are believed to contribute to the neurodegenerative effects of apoE4 and it has been suggested that the development of drugs that can convert apoE4 to a more apoE3-like or apoE2-like structure may be beneficial for individuals with neurodegenerative disorders (Mahley and Huang, 2012b; Xu et al., 2004).

2.3 Evidence for the protective effects of the *APOE* $\epsilon 2$ allele

2.3.1 *APOE* $\epsilon 2$ and lower risk for AD

The three *APOE* alleles are present in varying frequencies according to ethnicity and disease state. In a healthy Caucasian population, $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$ have approximate allele frequencies of ~ 7%, 80% and 13% respectively (Myers et al., 1996; Zannis et al., 1981). In patients with late-onset AD, the frequency of $\epsilon 2$ is significantly lowered to 2.8%-4.5%, even after allowing for the increased frequency of $\epsilon 4$ in affected individuals (Corder et al., 1993) (Figure 2.2). *APOE* $\epsilon 2$ carriers typically have a lower risk and a delayed age of onset of AD compared to $\epsilon 4$ carriers who can develop the disorder up to 10-15 years earlier than $\epsilon 3$ homozygotes. Relative to $\epsilon 3$, occurrence of the $\epsilon 2$ allele has been reported to decrease risk for AD by a factor of four, while each occurrence of an $\epsilon 4$ allele increases risk by a factor of more than four (Corder et al., 1994; Farrer et al., 1997).

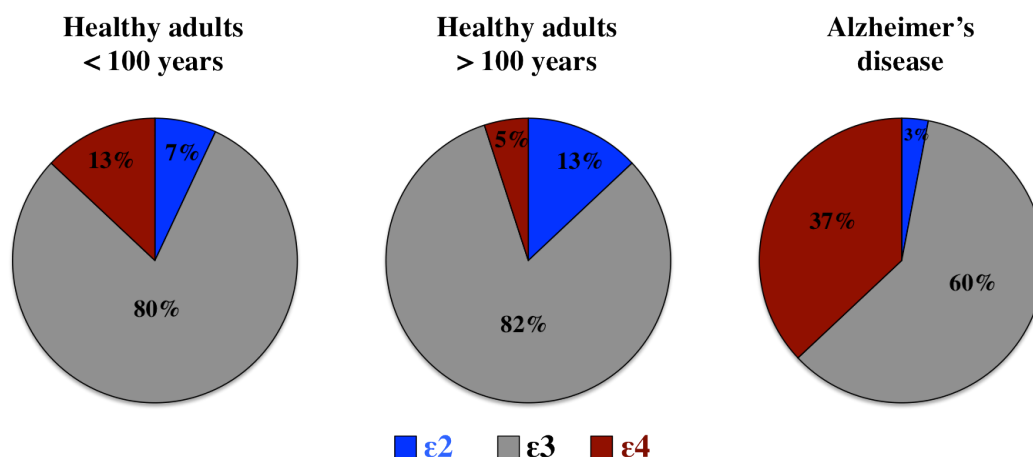


Figure 2.2: *APOE* allele frequencies.

APOE $\epsilon 2$ carriers are more likely to live longer and less likely to develop AD than $\epsilon 4$ carriers. Figure created using data from Corder et al., 1994 and Schachter et al., 1994.

2.3.2 APOE ε2 and longevity: an evolutionary perspective

Human longevity is, to some extent, heritable, and *APOE* is one of the best examples of a gene that contributes to survival in old age (Drenos and Kirkwood, 2010). Specifically, the ε2 allele is associated with a greater likelihood of survival to advanced age than ε3 and ε4 (Blanché et al., 2001; Frisoni et al., 2001; Lewis and Brunner, 2004; Louhija et al., 1994; Rea et al., 2001; Schächter et al., 1994). In people aged over 85 years, the risk of death during the next six years is fourfold lower in ε2 than ε4 carriers (Corder et al., 1996) and ε2 carriers are four times as likely as ε4 carriers to become centenarians (Schächter et al., 1994).

Studies that have compared *APOE* allele frequencies in age-stratified groups have consistently reported a significantly higher frequency of ε2 in elderly individuals and centenarians than in younger populations, and this marked increase in allele frequency has been reported in several different ethnicities (Corder et al., 1996; Frisoni et al., 2001; Louhija et al., 1994; Rea et al., 2001). For example, Schachter and colleagues report significant increases in ε2 allele frequency from 6.8% in young adults (aged 20-70 years) to 12.8% in centenarians, whereas the ε4 allele frequency decreases from 11.2% to 5.2% in the same groups (Schächter et al., 1994) (Figure 2.2). There are significant increases in the frequency of ε2 carriers even within centenarians, from 9% in individuals aged 100-101, to 21% in individuals aged 102-103 and 25% in persons 104 years and older (Frisoni et al., 2001). While the epidemiological evidence appears to be strong and reproducible, the underlying reasons for why ε2 carriers seem to age more successfully than ε3 and ε4 carriers remain unclear.

In order to better understand the allele-specific effects on longevity, we must consider the evolutionary history of the *APOE* gene (Figure 2.3). Analyses of the DNA sequence haplotypes in humans, and non human-primates have revealed that *APOE* $\epsilon 4$ is the ancestral gene, which existed alone until 200,000 years ago when the *APOE* $\epsilon 3$ allele appeared (Fullerton et al., 2000; Hanlon and Rubinsztein, 1995; Hixson et al., 1988; Mahley and Rall, 1999). It is estimated that the $\epsilon 2$ allele then mutated from the $\epsilon 3$ allele 60-80,000 years ago (Fullerton et al., 2000). Traditional evolutionary theories of ageing suggest that if a gene exerts an effect after the reproductive phase of the lifespan (as would seem to be the case for *APOE*'s effect on late-life events like AD and cardiovascular disease) it is likely that the gene will escape the force of natural selection (Hamilton, 1966). However, since *APOE* $\epsilon 3$, and not $\epsilon 4$, is currently the most abundant allele in the human population, there is an indication of selection on the alleles, with some models predicting a gradual increase of the $\epsilon 2$ and $\epsilon 3$ alleles at the expense of $\epsilon 4$ with each successive generation (Drenos and Kirkwood, 2010).

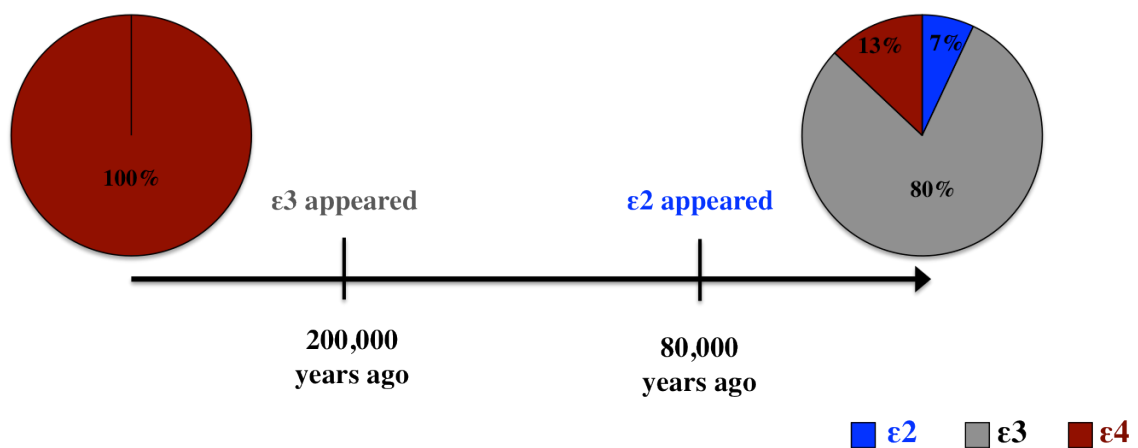


Figure 2.3: Evolutionary history of the *APOE* gene.

The timeline shows a gradual increase in allele frequency of the $\epsilon 2$ and $\epsilon 3$ alleles at the expense of $\epsilon 4$. Figure created using data from Fullerton et al., 2000.

The exact environmental pressures on *APOE*'s evolution are unknown, and the genetic drift from $\epsilon 4$ to $\epsilon 3$ to $\epsilon 2$ has been discussed in detail in a review by Finch and Sapolsky who address both late-life and developmental events that may have influenced this shift (Finch and Sapolsky, 1999). One argument, the “*grandmother hypothesis*”, seeks to explain how a long post-reproductive lifespan can provide a selective advantage by enhancing the survival and fitness of younger generations (Hawkes, 2004). Briefly, in some hunter-gatherer populations, elderly individuals (typically females) are known contribute as many calories to the diet of their grandchildren via hunting as fathers, a phenomenon termed “*grandmothering*” (Hawkes et al., 1998). To do so would have demanded intact cardiovascular fitness and cognitive health, and the beneficial *APOE* $\epsilon 2$ and $\epsilon 3$ alleles would therefore have favoured effective “*grandmothering*” in early humans (Finch and Sapolsky, 1999). Some have suggested that longer lifespans are tied to an interaction between diet and genotype, and that *APOE*'s status as a “*meat adaptive gene*” would have allowed for the safe consumption of animal meat during the course of evolution (Finch and Stanford, 2004; Sapolsky and Finch, 2000). *APOE* $\epsilon 2$ and $\epsilon 3$ would likely have conferred a resistance to the cholesterol and vascular disorders associated with a meat-based diet, whereas $\epsilon 4$'s association with high cholesterol and accumulation of atherosclerotic plaques would have been detrimental. Consequently, possession of the former would have lengthened the life span of early meat-eaters who could reap the benefits of better nutrition, more energy and larger body sizes.

Alternatively, the gradual removal of the $\epsilon 4$ allele from the population may also have been influenced by relatively rarer allele-specific effects on earlier-life susceptibility to infectious diseases (Mahley et al., 2009). For example, cultured cells are more susceptible to human immune-deficiency virus (HIV) fusion and entry in the presence of apoE4 than

apoE3; and HIV-positive patients with the $\epsilon 4\epsilon 4$ genotype demonstrate an accelerated progression to AIDS relative to $\epsilon 3$ homozygotes (Burt et al., 2008). However, the evidence for *APOE*'s association with early-life deleterious events is weak and the mechanisms by which infectious diseases interact with, and contribute to the evolution of, *APOE* remain to be explored. Overall, while it may be difficult to differentiate between these competing explanations, one thing is clear: the drift towards $\epsilon 2$ and $\epsilon 3$ cannot be attributed to chance alone.

2.3.3 APOE $\epsilon 2$ and lower AD-related neuropathology

AD is pathologically characterized by the accumulation of extracellular senile plaques containing amyloid β ($A\beta$) deposits and intracellular neurofibrillary tangles (NFTs) containing hyperphosphorylated tau protein aggregates within the brain. Most post-mortem and amyloid-PET (positron emission technology) analyses have revealed that people with the *APOE* $\epsilon 2\epsilon 3$ genotype develop fewer $A\beta$ plaques and amyloid angiopathy in widespread cortical areas relative to those with the more common $\epsilon 3\epsilon 3$ genotype (Lippa et al., 1997; Morris et al., 2010; Polvikoski et al., 1995; Tiraboschi et al., 2004). Some studies report a protective effect of the $\epsilon 2$ allele against both amyloid deposition and NFT formation (Nagy et al., 1995; Oyama et al., 1995), and others point towards a reduction in NFTs, but not $A\beta$ plaques in $\epsilon 2$ carriers (Morris et al., 1995).

There are also reports to the contrary. Berlau et al. (2009) found that, in a population aged 90 years and older, the $\epsilon 2$ carriers had a reduced risk of clinical dementia, but paradoxically possessed *increased* plaque neuropathology relative to the $\epsilon 3$ homozygotes, suggesting that the relationship between AD pathology and *APOE* status may vary with

age (Berlau et al., 2009). In support of this, another recent study in people over 85 years has found an increased frequency of the $\epsilon 2$ allele in “asymptomatic AD” subjects relative to patients with mild cognitive impairment (MCI) or AD (Iacono et al., 2015). “Asymptomatic AD” is the term for individuals who are symptomatically silent for AD but have comparable levels of AD pathology to MCI and AD patients at autopsy. Thus, while possession of an $\epsilon 2$ allele was insufficient to reduce pathological build-up in these individuals, it served to lessen the consequences on cognitive decline.

Notably, all the studies that have found an association between possession of $\epsilon 2$ and lower AD-related neuropathology have largely involved participants with a mean age of approximately 85 years. However, Berlau and colleagues’ “90+ study” focused on the “oldest-old”, averaging 97 years. Indeed, there is prior evidence for an age-related effect; compared to $\epsilon 3$ homozygotes, $\epsilon 2$ carriers below 80 years are reported to have lower levels of neurofibrillary pathology, but this pattern is reversed with increasing age (Berlau et al., 2007; Ohm et al., 1999). The absence of linearity between cognitive decline and advanced AD pathology in old $\epsilon 2$ carriers is perhaps indicative of a compensatory mechanism that functions independently of and downstream to the hallmark pathological lesions (Berlau et al., 2009). Thus, even though the oldest-old *APOE* $\epsilon 2$ carriers may have equivalent or higher amounts of AD pathology than non-carriers they still have a comparatively lower risk of developing dementia.

2.3.4 APOE $\epsilon 2$ and brain structural integrity

The hippocampus and entorhinal cortex, lying within the medial temporal lobe, are among the first brain regions to develop the hallmark AD-related neuropathological

lesions, perhaps as early as 20 years before the onset of clinical symptoms (Braak and Braak, 1991). Lower hippocampal and entorhinal volumes are therefore sensitive predictors of the development of AD (Jack et al., 2011). There have been isolated reports of lower rates of hippocampal atrophy (Chiang et al., 2010) and larger hippocampal volumes in $\epsilon 2$ carriers relative to non-carriers in both healthy elderly individuals (Alexopoulos et al., 2011) and AD patients (Liu et al., 2010), but most studies have reported no *APOE* genotype-dependent differences in hippocampal volumes in healthy subjects (Bunce et al., 2012; Cherbuin et al., 2008; Khan et al., 2014; Liu et al., 2010; Trachtenberg et al., 2012c).

By contrast, there is stronger evidence for an effect of $\epsilon 2$ on entorhinal cortical thickness. Although not all studies report differences (Bunce et al., 2012), Shaw et al. noted a stepwise decrease moving from the thickest cortex in $\epsilon 2$ carriers, to an intermediate thickness for $\epsilon 3$ homozygotes, and the thinnest cortex in adolescent $\epsilon 4$ carriers (Shaw et al., 2007). Similar genotype effects on entorhinal volume have been observed in cognitively healthy middle-aged (Fennema-Notestine et al., 2011) and elderly subjects (Fan et al., 2010) as well as MCI and AD populations (Liu et al., 2010) with $\epsilon 2$ carriers having significantly thicker regional cortices than $\epsilon 3$ homozygotes and $\epsilon 4$ carriers respectively. It has been suggested that greater regional cortical thickness in key areas of the brain both in healthy and demented $\epsilon 2$ carriers could represent a neural endophenotype that may contribute, in part, to delayed age of onset of AD in these individuals. Thus, $\epsilon 2$ carriers may need to lose more cortical thickness than non-carriers in order to pass the critical anatomical threshold that results in cognitive impairments (Shaw et al., 2007).

2.3.5 APOE ε2 and slower cognitive decline

Rapid cognitive decline is a characteristic feature of AD, but the ε4 allele is also associated with impaired cognitive function and a decline in episodic memory in normal ageing (Caselli et al., 2009; Haan et al., 1999; Henderson et al., 1995; Mayeux et al., 2001; Rusted and Carare, 2015). Several studies with large cohorts of healthy older individuals report an association between the ε2 and reduced cognitive decline (Helkala et al., 1996, 1995; Hyman et al., 1996; Staehelin et al., 1999; Wilson et al., 2002). *APOE* ε2 carriers display superior verbal learning abilities in that they remember significantly more words during both immediate and long-term recall than their non-ε2 counterparts (approximately 10% and 17% more words recalled respectively) (Helkala et al., 1995). They also maintain performance over a three-year follow-up period during which the learning ability of subjects with other *APOE* genotypes deteriorates significantly faster (Helkala et al., 1996). Further, ε2 carriers also show slower decline in episodic memory (Helkala et al., 1996), faster processing of information, as well as better performance on tests of free recall than non-carriers (Staehelin et al., 1999). Possession of at least one copy of the *APOE* ε2 allele has also been associated with an improvement in performance on tests of episodic memory over a five-year period during which ε3 homozygotes showed a slight decline and ε4 carriers a sharp decline in performance, suggesting that the ε2 allele may in fact be protective against episodic memory impairments in normal ageing (Wilson et al., 2002).

Like with AD-pathology, not all studies report a cognitive advantage for non-demented ε2 carriers and recent investigations seem to question the link between *APOE* and cognitive function in healthy elderly populations. One longitudinal study reported no

differences between healthy $\epsilon 2$ carriers and $\epsilon 3$ homozygotes in rates of cognitive or episodic memory decline over a period of two years, although this might be explained by minimal rates of change of cognitive scores in either group (Chiang et al., 2010). Other larger and more current reports have also found no effects of any of the three *APOE* alleles on cognitive performance in healthy elderly populations (Batterham et al., 2013; Bunce et al., 2014; Jorm et al., 2007; Quintas et al., 2014; Salo et al., 2001).

The inconsistent reports on the relationship between *APOE* and cognitive function may be confounded by three factors. First, the standard cognitive tests that have been used to characterise the effects of *APOE* in healthy individuals are, in most cases, the same as those used to distinguish AD patients from controls. Accordingly, they may not be sensitive enough to detect subtle genotype differences that may exist in healthy people. Second, cross-sectional studies of cognitive performance can be limited by population biases, which, particularly in studies of $\epsilon 4$, could confound our interpretations. Without longitudinal follow-ups it is impossible to ascertain if the elderly groups comprise “survivors” who have bypassed AD-associated cognitive decline, or “preclinical AD” subjects for whom the observed cognitive decline may be a consequence of preclinical dementia. Since both groups would likely perform differently on tests of memory function, this would explain some of the variability between studies of *APOE* in healthy ageing. Lastly, *APOE* may not be the solitary mediator of cognitive performance in ageing. Brain-derived neurotrophic factor (*BDNF*), a protein involved in neuronal growth, synaptic plasticity and cell survival is also known to account for variance in cognitive function in healthy adults (Bath and Lee, 2006). A common single-nucleotide polymorphism in the *BDNF* gene resulting in a valine (Val) to methionine (Met) substitution is associated with memory impairments and reduced hippocampal volume

(Egan et al., 2003). Ward and colleagues recently explored the possibility of a gene-gene interaction between the *APOE* alleles and the *BDNF* polymorphisms on cognitive performance in 433 participants aged 50-79 years (Ward et al., 2014). They found no main effects of either gene on measures of episodic memory, working memory, executive function and language processing. However, they identified a significant *APOE* x *BDNF* interaction on episodic memory performance within the *BDNF* Met+ group in a pattern predictive of *APOE*-related risk for AD; *APOE* ϵ 2/*BDNF* Met+ carriers had the best and *APOE* ϵ 4/*BDNF* Met+ carriers the worst performance. Thus, the respective beneficial/detrimental effects of ϵ 2 and ϵ 4 were ***only apparent*** in individuals with *BDNF* Met+ polymorphisms, demonstrating the need for considering multiple genes in studies of cognition.

Taken together, there is strong evidence for a protective effect of ϵ 2 on lifetime risk for AD and survival to old age. The allele is also associated with favourable measures of AD-related neuropathology, brain structure and cognitive performance, and where observed, a lack of consistent support may be influenced by an interaction of *APOE* with other genetic mediators of cognitive decline. In the following section I refer to the amyloid cascade hypothesis to discuss the mechanisms that may drive these protective effects.

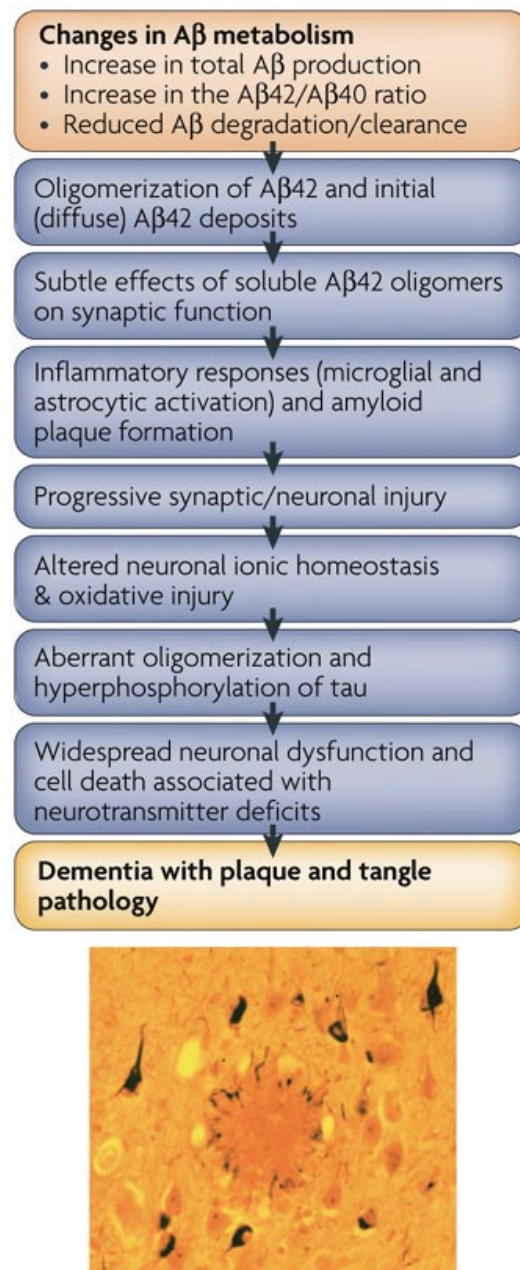
2.4 Mechanisms that underlie the effects of *APOE* ϵ 2 in the brain

According to the highly influential “*amyloid cascade hypothesis*”, AD pathogenesis is primarily driven by an imbalance of the production and clearance of amyloid β from the neuropil, resulting in an aggregation of A β into fibrillar amyloid deposits called senile plaques (Hardy and Selkoe, 2002). A β is a peptide of between 36 and 43 amino acids that

is produced by cleavage of the amyloid precursor protein (APP). All forms of A β have a tendency to aggregate, but the longer peptides are more likely to do so, making them comparatively more pathogenic. Most early-onset forms of familial AD are caused by a mutation in the APP gene, leading to an overproduction of A β or an increase in the ratio of the longer A β_{42} peptide to A β_{40} . This imbalance triggers a complex pathogenic cascade of inflammatory responses, synaptic injury and oxidative damage that culminates in the symptoms of AD (Haass and Selkoe, 2007; Hardy and Higgins, 1992) (Figure 2.4).

By contrast, we know comparatively little about how A β accumulation is initiated in the more common sporadic late-onset form of AD for which *APOE* $\epsilon 4$ is the best-established genetic risk factor. Although the amyloid cascade hypothesis has given rise to new unanswered questions in recent years, particularly in the context of the relationship between amyloid and tau (Armstrong, 2014; Reitz, 2012), it has generally braved the test of time since its inception in 1992 (Hardy and Higgins, 1992). It is therefore reasonable to interpret the relationship between *APOE* and AD in the context of this central framework. As it stands, *APOE* has been slotted into the cascade at almost every stage and there is strong and reproducible evidence to suggest that *APOE* $\epsilon 4$ plays a catalytic role in the amyloid cascade (Lovestone, 2000; Potter and Wisniewski, 2012).

I focus instead on the role of $\epsilon 2$, which has been relatively less studied, but can provide valuable insights into the link between *APOE* and AD. In the following sections I discuss the effects of $\epsilon 2$ on A β accumulation and clearance, and downstream inflammatory responses, oxidative damage and synaptic injury.



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Figure 2.4: The amyloid cascade hypothesis.

Figure from Haas and Selkoe, 2007

2.4.1 *APOE* $\epsilon 2$ and $A\beta$ deposition/clearance

ApoE plays a critical role in regulating $A\beta$ homeostasis, and all apoE isoforms are involved, to some extent, in the conversion of $A\beta$ to pathogenic oligomers with ensuing neuritic plaque formation (Fagan and Holtzman, 2000; Holtzman, 2004; Ma et al., 1994). In fact, in the complete absence of apoE, the mutant APP gene and the resultant $A\beta$ peptide produce neither amyloid deposits nor cognitive decline, suggesting that apoE is essential for the formation of amyloid aggregates (Bales et al., 1999). However, the extent to which apoE initiates $A\beta$ polymerization is dependent on the specific isoform. Ageing APP transgenic mice expressing the human apoE2 isoform have a sustained ability to prevent $A\beta$ from converting to these oligomeric forms (Fagan et al., 2002), and have lower levels of fibrillar $A\beta$ burden (Bales et al., 2009; Castellano et al., 2011) in comparison to their E3 and E4 expressing counterparts. *In vitro* studies provide further corroboration; among the three apoE proteins, purified apoE2 demonstrates the slowest rates of $A\beta$ polymerization and is least effective in forming oligomers (Ma et al., 1994). Further, an imbalance in the brain and cerebrospinal fluid (CSF) pools of $A\beta$ can be an indicator of amyloid pathology. Low levels of $A\beta_{42}$ in the CSF suggest high levels of $A\beta$ aggregation within the brain, and are correlated with brain atrophy in preclinical AD (Fagan et al., 2009). Cognitively healthy elderly *APOE* $\epsilon 2\epsilon 3$ and $\epsilon 3\epsilon 3$ carriers have higher levels of CSF $A\beta$ compared to $\epsilon 4$, indicating lower levels of cerebral amyloid deposition in these individuals (Castellano et al., 2011).

In addition to influencing the $A\beta$ deposition, all isoforms of apoE have been shown to modulate $A\beta$ clearance. $A\beta$ is cleared either by cellular uptake and degradation, or by being transported out of the brain and into the periphery, where circulating proteins bind,

sequester, and prevent it from exerting its deleterious effects. A β complexes that are formed with apoE2 and apoE3 are cleared across the mouse blood-brain barrier at substantially faster rates than A β bound to apoE4 (Deane et al., 2008). Recently, Castellano and colleagues reported that the half-life of A β in the interstitial fluid within the brain was significantly lower in apoE2 transgenic mice compared to apoE3 and apoE4 mice, meaning that E2 mice display the most efficient clearance of endogenous A β from the brain (Castellano et al., 2011). According to the “*peripheral sink hypothesis*”, A β transported to the periphery then has to be cleared from the plasma to prevent its accumulation (DeMattos et al., 2001; Selkoe, 2001). *APOE* ϵ 2 mice are also most effective at clearing A β from their bloodstream (Sharman et al., 2010) and degrading A β aggregates (Jiang et al., 2008), suggesting that both the peripheral clearance and degradation of A β is altered by *APOE* genotype in a pattern that is reflective of AD risk.

The three apoE isoforms are known to bind A β with different strengths, and the binding efficiency correlates inversely with the risk of developing late-onset AD. The fact that apoE forms complexes with A β in AD brains led to the idea that apoE may be a chaperone protein that controls the metabolism and clearance of A β via the apoE receptor (Wisniewski and Frangione, 1992). This process is believed to restrict A β -mediated neurotoxicity. ApoE2 and E3, but not E4 are able to form these stable complexes with A β *in vitro* (Aleshkov et al., 1997; LaDu et al., 1994). E2 and E3 may therefore protect the neurons by binding tightly to and clearing A β more efficiently than E4 (Jordán et al., 1998; Yang et al., 1997). In fact, the ability of apoE2 to form tight complexes with A β not only helps its clearance, but also prevents A β from spontaneously polymerizing and aggregating into deposits, therefore limiting A β -mediated neurotoxicity (Drouet et al., 2001).

Hyperphosphorylation of the tau protein and the resultant formation of NFTs is also a hallmark of AD pathology. I have described reports of a lower NFT burden in individuals with the $\epsilon 2$ allele in Section 2.3.3, but the underlying mechanisms for this reduction have not yet been thoroughly investigated. *In vitro* studies have shown that apoE3 binds unphosphorylated tau more efficiently than apoE4 (Strittmatter et al., 1994), but there is still no conclusive evidence investigating interactions between apoE2 and tau.

2.4.2 APOE $\epsilon 2$ and anti-oxidant/anti-inflammatory activity

Oxidative damage, caused by free radicals and reactive oxygen species, is a part of normal ageing, but is greater in the brain of AD patients than healthy controls (Benzi and Moretti, 1995). ApoE protects cells from oxidative stress-induced cell death *in vitro* by exhibiting allele-specific antioxidant activity, with E2 being the most effective, E3 moderately effective and E4 least effective in doing so (Miyata and Smith, 1996). One explanation for the antioxidant properties of apoE is its ability to bind metals and act as an inhibitor of ion-mediated oxidation of lipoproteins (Miyata and Smith, 1996; Pham et al., 2005). How the allele-specific effects on antioxidant activity relate to the intrinsic differences in protein structure is yet to be determined, but it may have to do with the differential cysteine content of the three isoforms at residue 112 since cysteine itself binds metal ions (Hatters et al., 2006).

Chronic inflammation and glial activation is typical in AD, and promotes the accumulation of plaques and tangles as well as subsequent cell loss (Griffin et al., 1998; Mrak and Griffin, 2005). ApoE is believed to play a role in modulating neuroinflammation and gliosis. Neurons induce the synthesis of apoE in response to

neural injury, so as to initiate membrane repair by redistributing and transporting lipids. However, because of its unique structure and its tendency to exhibit domain interactions, apoE4 can be recognized as abnormal by neurons and may subsequently be targeted for proteolytic cleavage, generating neurotoxic fragments that cause mitochondrial dysfunction, cytoskeletal alterations, tau phosphorylation and NFT formation (Huang, 2011; Mahley and Huang, 2012a; Mahley et al., 2006). The extent of such proteolytic cleavage is lower for apoE3 and least for apoE2 (Mahley and Huang, 2012a). *APOE* also differentially affects glial activation in mice, such that those expressing $\epsilon 2$ or $\epsilon 3$ respond to an acute, brain inflammatory challenge more efficiently than those expressing $\epsilon 4$ (Zhu et al., 2012).

2.4.3 APOE $\epsilon 2$ and synaptic integrity

In addition to A β plaques and tau tangles, AD is characterized by gliosis and brain atrophy secondary to synaptic and neuronal loss (DeKosky and Scheff, 1990). One of the few studies to analyse spine morphology across all *APOE* genotypes found *APOE* $\epsilon 2$ mice that lacked AD pathology to have increased dendritic complexity, longer dendritic spines and significantly greater dendritic arborization in their cortical neurons compared to their $\epsilon 3$ and $\epsilon 4$ counterparts (Dumanis et al., 2009). Further, expression of human $\epsilon 2$ was reported to decrease plaque deposition and dendritic spine loss in transgenic mice that had AD pathology (Lanz et al., 2003). The apoE2-mediated increase in dendritic outgrowth enhances the formation of new synapses and can therefore protect against the synaptic deterioration that is characteristic of AD (Dumanis et al., 2009). Additionally, since these isoform-dependent differential effects exist both in the presence and absence

of AD pathology, apoE is believed to modulate normal brain functions in addition to affecting AD risk.

APOE is known to affect synaptogenesis (Love et al., 2006) and synaptic plasticity (Trommer et al., 2004). Markers of synaptic integrity such as the presynaptic proteins synaptophysin and syntaxin 1, and the postsynaptic protein postsynaptic density-95 (PSD-95) are markedly reduced in AD (Love et al., 2006; Masliah et al., 1989). There are similar *APOE*-dependent differences in the levels of these three proteins in the normal human brain, in that $\epsilon 2$ carriers have highest levels of PSD-95 in the superior temporal cortex and $\epsilon 4$ carriers have the lowest concentrations of synaptophysin and syntaxin 1 (Love et al., 2006). These findings are in accordance with other studies of synaptophysin immunoreactivity that demonstrate significantly higher levels of synaptophysin in the frontal cortex of non-demented $\epsilon 2$ carriers compared to $\epsilon 3$ homozygotes (Berlau et al., 2009), which together suggest that the maintenance of synaptic integrity in $\epsilon 2$ carriers may contribute to their lower risk of dementia. In fact this was the mechanism through which Berlau and colleagues explained the paradoxical increase in AD-pathology but lack of cognitive decline in the “oldest-old” *APOE* $\epsilon 2$ carriers in their “90+ study” discussed in Section 2.3.3 (Berlau et al., 2009).

2.5 Inconsistencies in the neuroimaging literature

I have presented evidence for the neuroprotective effects of the $\epsilon 2$ allele and have interpreted them in the context of the amyloid cascade hypothesis. Most findings discussed so far have followed an allele-specific pattern that is reflective of genetic risk for AD, *i.e.* for all measures, the $\epsilon 2$ and $\epsilon 4$ alleles show opposite patterns within the brain. However, this is not always the case, particularly in MRI studies of brain structure and function. Most MRI studies to date have typically compared $\epsilon 4$ carriers with non-carriers, and attributed any differences in MRI measures to differences in AD risk (Trachtenberg et al., 2012c). The few studies that have examined the $\epsilon 2$ allele have produced unexpected results with respect to the link between *APOE* genotype, brain function, and the risk for developing AD (Figure 2.5).

2.5.1 Evidence from fMRI studies

Functional magnetic resonance imaging (fMRI) has proven to be a useful tool to detect early changes in brain function that are associated with AD. The primary metric is the blood-oxygen-level dependent (BOLD) signal, the physiological basis of which is outlined in detail in Chapter 3. If the level of BOLD activity were related to lifetime risk for AD, one would predict that $\epsilon 2$ and $\epsilon 4$ carriers should show opposite patterns of activation. However, our group has previously shown that cognitively healthy $\epsilon 2$ and $\epsilon 4$ carriers both have similar changes in the BOLD signal relative to $\epsilon 3$ homozygotes at rest (Trachtenberg et al., 2012b), as well as during memory-encoding and attention tasks (Trachtenberg et al., 2012a). Moreover, these changes in activation occur in the same brain regions in both $\epsilon 2$ and $\epsilon 4$ carriers. Similar observations have been reported by Shu

and colleagues who also found comparable changes in resting-state functional connectivity in cognitively healthy $\epsilon 2$ and $\epsilon 4$ carriers relative to $\epsilon 3$ homozygotes (Shu et al., 2014)

The only previous fMRI study to investigate the $\epsilon 2$ allele contradicts, to some extent, the findings of Trachtenberg et al. (2012a,b) and Shu et al. (2014) (Mondadori et al., 2007). Over successive runs of a memory task, Mondadori and colleagues. reported altered and opposite patterns of BOLD activation in $\epsilon 2$ and $\epsilon 4$ carriers when compared to $\epsilon 3$ homozygotes, a finding that is seemingly in line with the well-established association between *APOE* and AD risk. However, when comparing $\epsilon 2$ and $\epsilon 4$ carriers on only the first run of their memory task, a comparison that Trachtenberg and colleagues found to be more similar to their own task, Mondadori et al. found no significant differences between the $\epsilon 2$ and $\epsilon 4$ groups (Mondadori et al., 2007).

It is evident that *APOE* does seem to exert an effect on brain function by altering the activity in $\epsilon 2$ and $\epsilon 4$ carriers relative to $\epsilon 3$ homozygotes, but this effect does not seem to be reflective of AD risk since the direction of this altered activity is the same for both low-risk and high-risk groups. It is worth noting that had these studies simply compared $\epsilon 4$ carriers with $\epsilon 3$ homozygotes, their results may have been mistakenly interpreted as an association between increased AD risk and altered BOLD response, when they may instead be indicative of an more general intrinsic effect of the gene on the brain.

2.5.2 Evidence from DTI studies

Diffusion Tensor Imaging (DTI) is an imaging technique sensitive to the directional diffusion of water within tissues and is used to characterize white matter microstructure *in vivo*. DTI-derived measures like fractional anisotropy (FA) and mean diffusivity (MD), which reflect the directionality and magnitude of water diffusion respectively, are considered sensitive measures for the detection of disease-related changes in white matter (details in Chapter 3). DTI studies have found widespread white matter degeneration (*i.e.*, decreases in FA and increases in MD) in AD subjects (Sexton et al., 2011), as well as asymptomatic $\epsilon 4$ carriers (Heise et al., 2011). To date, only two studies have examined white matter integrity in healthy elderly $\epsilon 2$ carriers (Chiang et al., 2012; Westlye et al., 2012), both reporting opposite results.

The findings of Westlye *et al.* are generally in agreement with the recent reports of similar functional connectivity in healthy $\epsilon 2$ and $\epsilon 4$ carriers, (Shu et al., 2014; Trachtenberg et al., 2012a, 2012b) discussed earlier. They found that relative to $\epsilon 3$ homozygotes, both healthy $\epsilon 2$ and $\epsilon 4$ carriers had poorer measures of white matter structural integrity (lower FA and higher MD) in widespread brain regions, with no differences in these measures between $\epsilon 2$ and $\epsilon 4$ carriers. Conversely, Chiang *et al.* report that relative to *APOE* $\epsilon 3$ homozygotes, $\epsilon 2$ carriers had higher FA and lower MD (*i.e.* higher white matter integrity) in several white matter tracts. However, since Chiang and colleagues did not include $\epsilon 4$ carriers in their analysis, we must exercise caution before attributing their reported differences between $\epsilon 2$ and $\epsilon 3$ carriers to differences in future risk for AD.

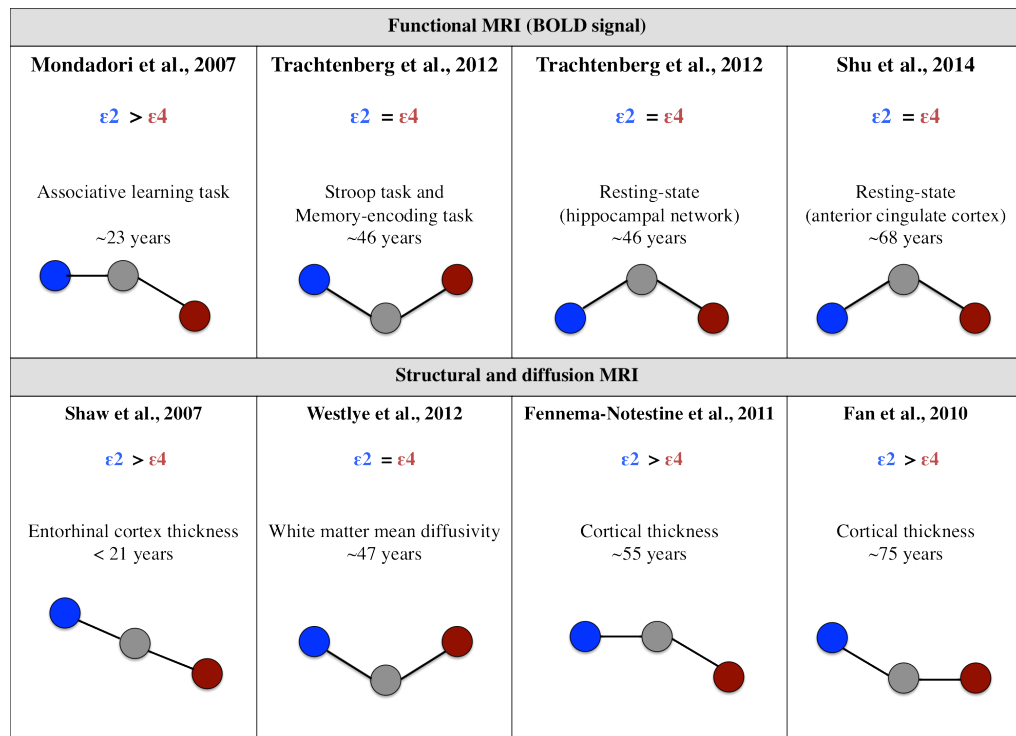


Figure 2.5: A simplistic overview of the inconsistencies in the neuroimaging literature.

The figure shows showing trends in *APOE* allele-specific effects within the brain. The blue, grey and red circles represent $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$ carriers respectively. Although there is a difference in the direction, fMRI studies find $\epsilon 2$ and $\epsilon 4$ similarities in resting-state and task-based BOLD activity in middle-older aged healthy subjects (Trachtenberg et al., 2012; Shu et al., 2014). However, $\epsilon 2$ and $\epsilon 4$ carriers are reported to show opposite patterns of activation during associative learning task in younger subjects (Mondadori et al., 2007). Similarly, structural MRI studies find $\epsilon 2$ and $\epsilon 4$ similarities in brain white matter structure in middle-aged individuals (Westlye et al., 2012), but opposite patterns for cortical thickness in younger and older individuals (Shaw et al., 2007; Fennema-Notestine et al., 2011; Fan et al., 2010). There are methodological differences between these studies, but these changing patterns suggest that the effects of $\epsilon 2$ and $\epsilon 4$ in the brain may vary with age, imaging modality and perhaps region of interest. Clearly, not enough is known about $\epsilon 2$ in order to justify grouping them together with $\epsilon 3$ homozygotes as “non- $\epsilon 4$ carriers”. All figures are simplistic representations of *APOE* trends and have been reconstructed from bar graphs present in the original papers.

2.6 Reconciling the inconsistencies: directions for future research

Because of its low allele frequency, studies with the $\epsilon 2$ allele are often limited by small sample sizes and may therefore suffer from insufficient power to reliably detect subtle neuroanatomical or cognitive differences. Beyond methodological considerations, there are a number of biological factors that should be considered when trying to explain the inconsistencies between neuroimaging studies.

2.6.1 Reconciling $\epsilon 2$ and $\epsilon 4$ similarities at a neural systems level

The majority of the evidence from cellular and molecular biology outlined in Section 2.3 cannot explain the findings of Trachtenberg et al. (2012a, b), Shu et al. (2014) and Westlye et al. (2012), all of whom report similar trends in brain structure and function in $\epsilon 2$ and $\epsilon 4$ carriers. There is, however, one study on long-term potentiation (LTP) that reports similar effects of *APOE* $\epsilon 2$ and $\epsilon 4$ at a neural system level. LTP is a form of synaptic plasticity that is widely accepted as a model for understanding the cellular basis of learning and memory. Briefly, it is a long-lasting enhancement in synaptic efficacy that results from experimentally induced high-frequency stimulations of a chemical synapse (Bliss and Collingridge, 1993), and studies of hippocampal LTP are considered useful tools to investigate mechanisms of susceptibility to memory impairment. One study examining the effects of all three *APOE* alleles on hippocampal LTP in mice has reported significantly lower magnitudes of LTP in both *APOE* $\epsilon 2$ and $\epsilon 4$ target-replacement mice relative to mice carrying the *APOE* $\epsilon 3$ allele (Trommer et al., 2004). Given their opposing effects on lifetime risk for AD, the fact that hippocampal LTP in *APOE* $\epsilon 2$ and $\epsilon 4$ mice was comparable is surprising, but seems to mirror the findings outlined in Section 2.5. It

is unclear if the reduction in LTP in mice with the two different alleles is driven by similar mechanisms, and how, if at all, it relates to the maintenance of cortical networks and white matter integrity. Studies of allele-specific effects on signal transduction pathways, receptor affinities, and neurotransmitter function seem to be promising avenues for future research.

2.6.2 Exploring the relationship between APOE and age

I have outlined inconsistencies in several of the domains that have investigated the effects of *APOE* ϵ 2. An important factor to take into account when comparing these studies is age. Several studies have highlighted the importance of age in examinations of *APOE* within the brain (for review, see (Trachtenberg et al., 2012c)). Our lab has previously shown a differential effect of ϵ 4 on brain function in younger (Filippini et al., 2009) and older (Filippini et al., 2011) and these findings have been replicated independently (Dennis et al., 2010). *APOE* ϵ 4 has been associated with enhanced cognitive performances in younger adults (Puttonen et al., 2003; Rusted et al., 2013; Yu et al., 2000) and increased risk of dementia in older individuals. Interactions between *APOE* and age have sparked the idea that the varying effect of *APOE* on cognition throughout the lifespan can be reconciled through the antagonistic pleiotropy concept, which suggests that the ϵ 4 allele may indeed be beneficial in early ages, and exert deleterious effects on cognition in later life (Tuminello and Han, 2011). It may be that aforementioned similarities between ϵ 2 and ϵ 4 carriers are simply reflecting an interaction between *APOE* and age in mid-life and this notion, although speculative, is attractive enough to warrant further examination. However, most of the studies that

investigate the relationship between *APOE* and age are largely limited to the effects of $\epsilon 4$ and little is known about the equivalent for the $\epsilon 2$ allele.

2.6.3 Looking beyond the BOLD signal to understand the effects of APOE

The BOLD signal is an indirect measure of neuronal activity, and is dependent on several vascular measures like cerebral blood flow, blood volume and blood oxygenation (discussed in Chapter 3). Given that there are reports of $\epsilon 2$ and $\epsilon 4$ similarities in BOLD activity, it would be valuable to look beyond the BOLD signal and specifically studying markers of neuronal and vascular health in the brain in order gain a better understanding of the complexities of *APOE*'s effects.

AD-related alterations in brain metabolite and neurotransmitter levels have been studied using proton magnetic resonance spectroscopy (^1H -MRS). The AD brain is associated with characteristic metabolite signatures like an increase in the concentration of myo-inositol, and a decrease in N-acetyl aspartate (Graff-Radford and Kantarci, 2013; Kantarci et al., 2007, 2000) These metabolites serve as reliable markers of glial proliferation and neuronal health respectively. Only four studies have examined the effect of *APOE* on brain metabolites; all have produced conflicting results and none have examined $\epsilon 2$ (Gomar et al., 2014; Kantarci et al., 2002a, 2000; Riese et al., 2015).

In addition to its neuropathological hallmarks, AD is also associated with impairments in cerebral vasculature. In fact, there is a substantial overlap between risk factors for AD and those associated with developing cerebrovascular disorders, viz. stroke, hypertension, diabetes and obesity. A valuable marker of vascular health in the brain is cerebrovascular

reactivity (CVR). CVR is the ability of small vessels in the brain to dilate in response to a vasodilatory stimulus and is an important mechanism for maintaining constant levels of blood supply to the brain. Impairments in CVR are observed in both cardiovascular and neurodegenerative disorders, including stroke (Yonas et al., 1993), hypertension (Settakris et al., 2003), diabetes mellitus type 1 and 2 (Fülesdi et al., 1997), mild cognitive impairment and AD (Glodzik et al., 2013). The mechanisms for these reductions are still unknown, but CVR can provide useful prognostic information on the risk of subsequent cerebral events since the impairments in this measure are known to contribute to cerebrovascular disease if they persist over a long period of time (Schwertfeger et al., 2006). There is also a close link between *APOE* and vascular health in that $\epsilon 4$, in addition to being a risk factor for AD, is also associated with a high lifetime risk of developing cardiovascular disorders whereas the opposite is true for $\epsilon 2$ carriers (Davignon et al., 1998). It is therefore of interest to study *APOE* in the context of cerebrovascular health in an effort to identify early impairments that may reflect genetic risk for AD.

2.7 Summary

This review emphasizes the importance of studying the *APOE* $\epsilon 2$ allele to understand individual differences in healthy ageing and also provides directions for future research. The neuroprotective effects of the $\epsilon 2$ allele range from lower risk for AD, reduced AD-related pathological burden, greater cortical thickness, and lower age-related cognitive decline. These effects can stem from $\epsilon 2$'s role in the amyloid cascade, *viz.* its efficient clearance of $A\beta$, enhanced synaptic integrity, and its antioxidant properties. However, *APOE* $\epsilon 2$'s marked under-representation in the literature means that many questions are left unanswered. We need a greater understanding of its effect on receptor affinities, lipid

binding, synaptic integrity, and interactions with amyloid and tau. Likewise, there are inconsistencies in the neuroimaging literature. It is important that we unpick the reasons why *APOE* $\epsilon 2$ and $\epsilon 4$ exert apparently similar effects on white matter integrity and the BOLD signal; particularly given the strong evidence for age-specific effects of *APOE* $\epsilon 4$. Going forward, I will address three key issues highlighted in this review:

1. Interpretations of *APOE*-related effects in the brain are incomplete, and perhaps erroneous, without including the $\epsilon 2$ allele.
2. The effects of *APOE* in the brain may vary with age.
3. We may need to look beyond “conventional” MRI techniques to obtain more insight into the relationship between *APOE*, brain function and risk for AD.

Chapter 3

Magnetic Resonance Imaging

With the emergence of advanced neuroimaging techniques, we are now able to non-invasively study the living brain and identify markers that are unique to specific disease states like AD. There has been an explosion of interest in MRI since its development in the early 1990s. MRI-derived measurements are being recognized as crucial surrogate markers of treatment response in AD clinical trials, and are widely being used in diagnosing and tracking disease progress (Suri et al., 2014). In this chapter, I describe the multi-modal MRI techniques used in my thesis, and define some key terms that are used in subsequent chapters. I also provide an overview of the participants and MRI protocol for the young, middle and older age groups.

3.1 Principles underlying MRI

MRI measures signals from the hydrogen atoms present in water within the tissue. The nucleus of a hydrogen atom consists of a single proton wobbling or “precessing” on its own axis. This movement of the proton, combined with its positive charge, results in the hydrogen atom having a net magnetic field, called a magnetic moment or “spin”. In biological tissues, the hydrogen atoms are randomly oriented. This causes their individual magnetic moments to cancel out and results in a net magnetic moment of zero for the tissue. When placed in an MRI scanner with an external magnetic field (B_0), the protons begin to orient themselves along the direction of the scanner’s field. The rate of

precession of the proton around B_0 is called Larmor frequency, and it is directly proportional to the strength of B_0 (in this thesis, it was 3 Tesla).

To obtain an MR image, a second magnetic field must be applied to the sample, in a direction perpendicular to B_0 : this is called the radiofrequency (RF) field. The RF field oscillates at a frequency equal to the Larmor frequency and causes the magnetic moment of the proton to tilt away from B_0 . Once this RF signal is removed, the protons will realign themselves such that their net magnetic moment is again parallel to B_0 . This return to equilibrium is called “*relaxation*” and during this transition, the protons lose energy by emitting their own RF signal. This is the signal that is reconstructed to produce an MR image. Three relaxation time constants are essential to produce structural and functional images, *viz.* T1, T2 and T2* (Jezzard and Clare, 2001; Matthews and Jezzard, 2004).

3.1.1 Structural MRI

How do we differentiate between grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) on a structural MRI image? Very simply, the differences in their relaxation times are exploited to obtain detailed information about the tissue types within the brain. The time it takes for 63% of the net magnetization to be restored along the original B_0 direction after the RF pulse has been removed is called T1 relaxation time. T1 relaxation varies depending on the type of tissue in the brain and how freely protons can move within the tissue. (T1 (ms): CSF > GM > WM). The difference in T1 relaxation between tissues means that when the RF pulse is removed, magnetization is restored fastest in WM and slowest in CSF. If a second RF pulse is then applied before net magnetization along B_0 is fully restored, it leads to a significant discrepancy in the signal

intensity for each tissue. The time in between RF pulses is called the *repetition time (TR)*. A long TR means that there is enough time between pulses for all tissue types to fully restore magnetization, making the strength of the signal detected in each tissue type roughly similar. A typical T1-weighted image therefore has a short TR in order to differentiate between WM, which has a high signal intensity and consequently appears bright on the image, CSF (low signal intensity = dark) and GM (intermediate intensity = grey) (Figure 3.1).

In this thesis, the structural T1-weighted sequence was used to study GM structure in both cortical and subcortical brain regions. T1-weighted images for the middle age group were acquired using a standard magnetization-prepared rapid acquisition with gradient echo (MPRAGE) sequence. The young and old groups received a newer multi-echo MPRAGE (ME-MPRAGE) sequence, which allows for better visualisation of subcortical structures (van der Kouwe et al., 2008).

3.1.2 Functional MRI

Functional MRI images rely heavily on another relaxation constant, *viz.* *T2* relaxation*. While T1 relaxation describes the *restoration* of magnetization along B_0 after the RF pulse is removed, T2 and T2* relaxation relate to the *decay* of the transverse magnetization produced by the RF pulse. Specifically, T2 is the time it takes for the transverse magnetization to decay to 37% of its initial size.

As discussed, when an RF pulse is applied, the protons are excited and their magnetization is flipped away from B_0 and into the transverse plane. At first, these

excited protons precess at the Larmor frequency, producing a strong MR signal. However, over time, the protons begin to precess at slightly different frequencies (*i.e.* they begin to “dephase”) because of variations or inhomogeneity in the local magnetic field. This dephasing leads to a decay of the net magnetic field along the transverse plane and a resultant decrease in MR signal. Both T2 and T2* result from this dephasing, but they differ based on the *cause* of the variations in local magnetic field. Similar to T1, T2 relaxation time varies based on microscopic tissue variations (T2 (ms): CSF > GM > WM). Thus WM, which has a shorter T2 loses signal faster than CSF. T2-weighted images were not used in this thesis and so will not be discussed further.

T2* relaxation on the other hand, is caused by more macroscopic variations in how a brain region responds to B_0 (*e.g.* at tissue-air boundaries, or tissue-blood boundaries). For instance, regions close to air-filled sinuses experience rapid dephasing of spins and more rapid T2* decay. Similarly, varying levels of oxygen in blood can also alter its magnetic susceptibility. Oxyhemoglobin is diamagnetic (essentially non-magnetic) whereas deoxyhemoglobin is paramagnetic. The presence of deoxyhemoglobin in the blood vessels creates a difference in magnetic susceptibility between the vessels and tissue, causing more rapid T2* decay than oxygenated blood. Therefore, the intensity of a T2*-weighted image depends largely on the local level of blood oxygenation: higher deoxyhemoglobin results in increased magnetic susceptibility, shorter T2* and consequently lower signal intensity. This is the principle underlying the blood-oxygen-level dependent (BOLD) contrast using in functional MRI (Ogawa et al., 1990).

In order to acquire T2* weighted images, a long TR is used so that all tissue types fully restore magnetization along B_0 when the RF pulse is removed (therefore eliminating T1

contrast). The signal is then read at a longer period after the RF pulse, so that enough signal decay may occur to differentiate tissue types based on their $T2^*$ relaxation times. The time between signal generation and detection is called the *echo time (TE)*. Thus an acquisition with a long TR and long TE produces a $T2^*$ -weighted image with contrast based on local variation in magnetic susceptibility (Jezzard and Clare, 2001) (Figure 3.1).

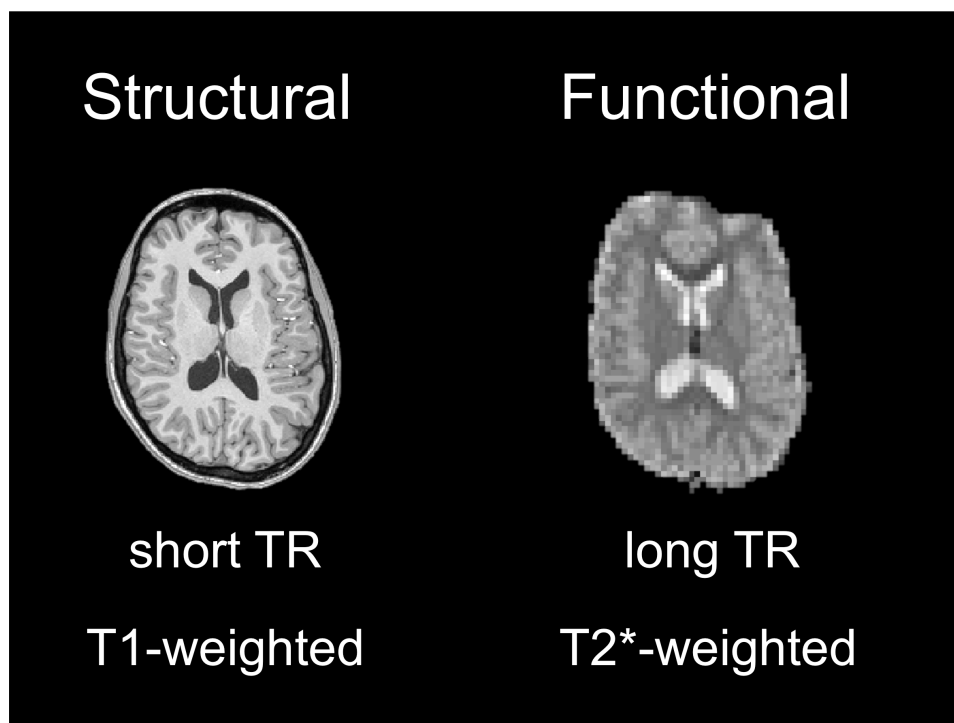


Figure 3.1: Examples of structural and functional MRI images.

The differences in relaxation times between the tissues in the brain are exploited to produce MRI image contrast. Structural images (left) are obtained using a short repetition time resulting in a $T1$ -weighted contrast with WM in white, GM in grey and CSF in dark. Functional images (right) are obtained using a long repetition time resulting in a $T2^*$ -weighted contrast with WM in dark, GM in grey and CSF in white. The MRI images show data of a participant in this thesis.

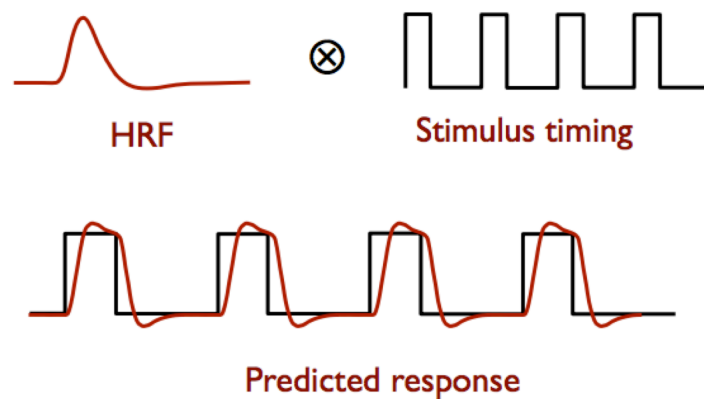
3.1.3 The BOLD signal: neurovascular coupling

Neuronal activity requires energy in the form of glucose and oxygen (O_2). This energy is supplied to the neurons via oxygen in blood vessels. During neuronal activation (*e.g.* while doing a particular task), there is an increase in the extraction of oxygen from the blood vessels into the neuron, and a heightened cerebral metabolic rate of oxygen consumption ($CMRO_2$). One might expect therefore, that if more oxygen is extracted from the blood, that the blood vessels would contain more deoxygenated (paramagnetic) blood, resulting in a decrease in MR signal of active tissue. However, this is not the case because the BOLD signal is sensitive not only to changes in $CMRO_2$ but also to the cerebral blood flow (CBF) and cerebral blood volume (CBV), which together form the basis of the hemodynamic response to neural activity. Neural activation triggers an increase in CBF, which far exceeds the increase in $CMRO_2$ within the active tissue (*i.e.* supply of O_2 to the neuron >>> demand for O_2 within the neuron). Heightened CBF delivers an abundance of oxyhemoglobin, which effectively overrides the increases in deoxyhemoglobin caused by local oxygen consumption. This results in an increase in MR signal of the T2*-weighted image in regions corresponding to neural activity. Although the specifics of neurovascular coupling are not entirely understood (for instance, why the increase in CBF is much greater than the increase in oxygen metabolism), BOLD fMRI is a well-established technique for studying brain function (Matthews and Jezzard, 2004).

In this thesis, we measured brain function both during a task (Chapter 6) and in the absence of a task, *i.e.* at “rest” (Chapter 5). During the task fMRI acquisition, we used a block design paradigm to compare brain activity during the presentation of a stimulus (active state) with that during the presentation of a blank screen (baseline state). A series

of T2*-weighted images are acquired during each state and compared using the general linear model (GLM). Briefly, the GLM compares the expected response to the stimulus with the actual BOLD response observed for each participant, and if there is a fit between the two, then it is likely that the BOLD response was caused by the stimulus (Poldrack and Nichols, 2011) (Figure 3.2).

(A) Modeling a predicted response



(B) Comparing the model with the measured response

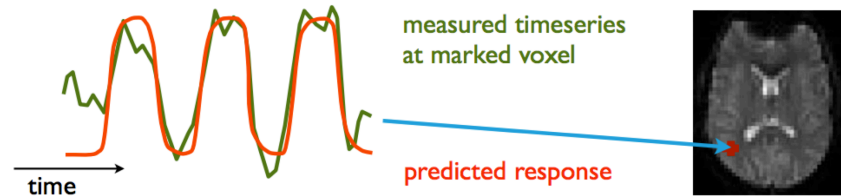


Figure 3.2: Measuring the BOLD signal during a task.

(A) A model for the predicted BOLD response is created based on the timing of stimulus presentation and the hemodynamic response function (HRF). A typical HRF consists of a 1-2 second delay after the stimulus is presented, following which there is an increase in the BOLD signal, which peaks ~4-8 seconds after stimulus onset, and starts to decline after the stimulus is withdrawn. (B) This predicted response is then compared with the data for each voxel in the fMRI image across the entire brain. Voxels whose data fits the model are considered “active” (marked red on the fMRI image). The figure is adapted with permission from the FMRI Graduate Program.

Even in the absence of a task, there are spontaneous changes in the BOLD signal that are of interest, and are studied using resting-state fMRI. This is discussed in further detail in Chapter 5.

3.1.4 Cerebral blood flow and cerebrovascular reactivity

Our body has several mechanisms to ensure that there is a constant supply of blood to the brain. As discussed above, *neurovascular coupling* is one such regulatory mechanism whereby an increase in neuronal activity creates a demand for oxygen that is met by an increase in CBF to the active regions (Ogawa et al., 1990). Another regulatory mechanism is *cerebral autoregulation*, where constant CBF is maintained in the face of changing arterial pressure. Changes in blood pressure cause the blood vessels to dilate and boost blood flow to the brain (van Beek et al., 2008). The ability of the blood vessels to dilate in response to a vasoactive stimulus is called *cerebrovascular reactivity* (CVR), which serves as a measure of the brain's vascular health (Claassen et al., 2007). CVR has been measured using a variety of vasodilatory stimuli, including the induction of systemic changes in blood pressure (*e.g.* through voluntary hyperventilation, breath-holding and rebreathing tasks), the use of intravenous administrations chemical stressors like acetazolamide, and exposure to respiratory dilators like carbon dioxide (CO₂). A recent review of these techniques concluded that CO₂ inhalation was the safest, least invasive, most reproducible, and therefore most clinically feasible stimulus to measure CVR (Fierstra et al., 2013).

Impairments in the ability to regulate cerebral perfusion are associated with several cerebrovascular, cardiovascular and neurodegenerative disorders including AD. Direct

measures of CBF and CVR are therefore useful markers of disease states. As discussed above, the BOLD signal provides an indirect measure of brain function by relating the level of oxygenation in the blood of a given brain region to neural activity within that region. While it assumes that neural activity demands an increase in local blood flow, the BOLD signal does not provide a *quantifiable* measure of this increased blood flow. In Chapter 6, we use two advanced MRI techniques to measure CBF during (1) neuronal stimulation, *i.e.* a task and (2) physiological stimulation, *i.e.* carbon dioxide (CO₂) inhalation.

CBF during neuronal stimulation: CBF is measured using a technique called arterial spin labeling (ASL). In this method, arterial blood flowing up to the brain is magnetically tagged with a brief pulse while it is still in the neck. Thus the protons in blood destined for the brain are now inverted, making it possible to trace their path through the cerebral vasculature. An image of the brain is then acquired after a brief time delay during which the labeled blood flows from the neck into the brain. This image, called the tag image, has a low T1-weighted signal because the inverted protons perfusing the region of interest reduce the net magnetic moment of that region. A second image (control image) is then acquired without magnetically labeling the incoming blood. Subtracting the tag image from the control image allows us to directly measure the signal caused by the cerebral blood flow to the brain.

In this thesis, we used a simultaneous BOLD and ASL sequence in order to study the CBF *during* the task in young participants (Chapter 6). This was achieved by using a dual-echo sequence, which provides two images per repetition. The short echo time provides ASL-weighted images, while the longer provides BOLD-weighted images. A

similar dual-echo sequence was used to study CBF during resting-state fMRI, and CO₂ inhalation in the young subjects.

CBF during CO₂ inhalation: Measuring CBF during CO₂ inhalation serves as a useful complement to the measurement of CBF during neuronal stimulation, because it allows us to assess if the cerebral arterioles are functioning adequately enough to maintain a constant supply of blood (and therefore O₂) to the brain. Breathing in CO₂ increases the arterial partial pressure of CO₂ and decreases the arterial partial pressure of O₂. This imbalance initiates cascade of cellular changes that result in a boost of blood flow to the brain in order to restore the oxygen tension. I elaborate on this cascade in Chapter 6.

In this thesis, cerebrovascular reactivity to CO₂ (or CO₂-CVR) was calculated by using ASL to measure the CBF in response to CO₂. Briefly, respiratory gases were delivered to participants via a nasal cannula while they were lying in the MRI scanner. Similar to the task fMRI paradigm, the gases were supplied in a block design fashion, with blocks of hyperoxia (100% oxygen supply), hypercapnia (10% CO₂, 21% oxygen and balance nitrogen), and rest (medical air supply). The level of CO₂ was specifically selected in order to provide a robust CBF response with minimal discomfort for the subject (Bulte et al., 2012). During this respiratory paradigm, simultaneous BOLD and ASL data were acquired. End-tidal values (the concentration of CO₂ and O₂ at the end of an exhaled breath) were obtained from a respiratory trace produced during the scan. CO₂-CVR was calculated for each voxel by measuring the fractional change in CBF per mm change in end-tidal CO₂ (specific details of image analysis are provided in Chapter 6).

3.1.5 Diffusion Tensor Imaging

Diffusion tensor imaging (DTI) is a useful tool for assessing subtle changes in WM microstructure within the brain (Beaulieu, 2002; Mori and Zhang, 2006). DTI is sensitive to the directional diffusion of water within tissues. Water molecules diffuse differently depending on the type and structural integrity of the tissue, and on their interaction with barriers like cell membranes, which restrict diffusion. For instance, diffusion in CSF is unrestricted in all directions (isotropic diffusion), whereas that in WM is unrestricted in directions parallel to the axon but hindered orthogonally due to the presence of the myelin sheath (anisotropic diffusion). In diffusion-weighted imaging, the magnitude and direction of water diffusion in each voxel is measured in the x-, y- and z-dimensions. A diffusion tensor model is then fitted to the measurements as an ellipsoid with three axes: a principal long axis with length λ_1 , and two small axes that describe the width and depth (λ_2 and λ_3) of the ellipsoid (Figure 3.3). For WM, λ_1 measures the diffusion of water along the axon (*axial diffusivity*), and λ_2 and λ_3 are usually averaged to produce a measure of diffusivity perpendicular to the axon (*radial diffusivity*). The *magnitude* of overall diffusion is called *mean diffusivity* (MD), which is the average of all three axes. The *direction* of diffusion is called *fractional anisotropy* (FA), which is the ratio of axial to radial diffusivity. FA and MD are considered sensitive measures of disease-related changes in WM. Axon degeneration and myelin damage are typically characterised by an increase in MD and a decrease in FA. These changes in WM microstructure can precede GM atrophy, so DTI can potentially detect subtle changes that may be missed by volumetric methods. When used in combination with conventional structural MRI, DTI is believed to increase the accuracy of early and differential diagnosis of the AD (Ringman et al., 2007).

To acquire a diffusion-weighted image, two gradient pulses are applied: the first pulse dephases the magnetization of the protons in water, and the second pulse (same magnitude but opposite direction to the first pulse) brings the protons back in phase. Had the protons been stationary in the time in between the two pulses, the phases produced by both the gradient pulses would have completely cancelled out and there would have been no resultant signal. However, we are measuring signals from diffusing water molecules, and each proton has a different rate of movement along the direction of the diffusion gradient in the time between pulses. The protons are therefore not perfectly brought back in phase after the second pulse. This results in a loss in MR signal, which is proportional to the amount or magnitude of diffusion. The signal loss is measured by comparing the diffusion-weighted image with an additional image that is acquired without diffusion weighting (B_0 image). In order to compute the direction of diffusion, multiple diffusion-weighted scans are acquired, each with the diffusion gradient applied in a different directions.

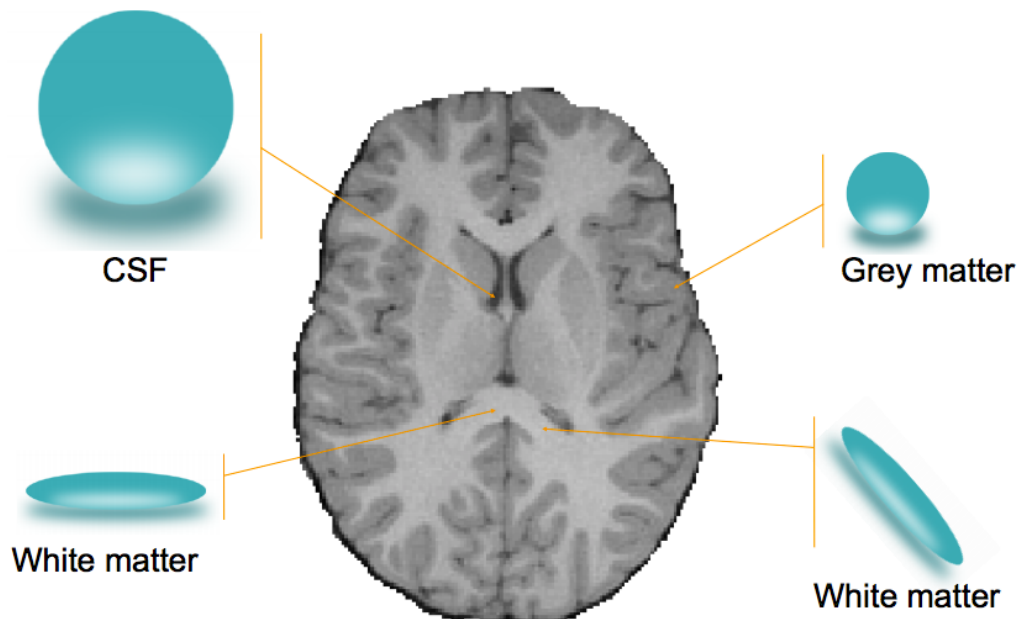


Figure 3.3: Diffusion tensor imaging.

Diffusion ellipsoids are modeled for each voxel to produce a measure of the magnitude and directionality of diffusion. For CSF, $\lambda_1 = \lambda_2 = \lambda_3$, and the resultant model is in the shape of sphere. For WM, $\lambda_1 \gg \lambda_2 \sim \lambda_3$, and this produces an elongated ellipsoid. The image is reproduced with permission from the FMRIB Graduate Program.

3.1.6 Magnetic Resonance Spectroscopy

Proton magnetic resonance spectroscopy (^1H -MRS) is used to determine the relative concentrations of brain metabolites, and can be used together with structural MRI to diagnose and track disease progress. For instance, while structural MRI can provide anatomical information about the size and location of a tumour, ^1H -MRS helps us understand its underlying biochemical profile. The output of an ^1H -MRS acquisition is a

spectrum, *i.e.* a frequency analysis (Fourier-transform) of the signal that is detected during an MR acquisition. The spectrum is a collection of peaks, each corresponding to a specific metabolite, and it serves as a biochemical fingerprint of the tissue. It varies depending on the region of the brain studied, and if there is any structural damage to that region. In this thesis, ^1H -MRS signals were extracted from a region of interest within the posterior cingulate cortex (Figure 3.4a).

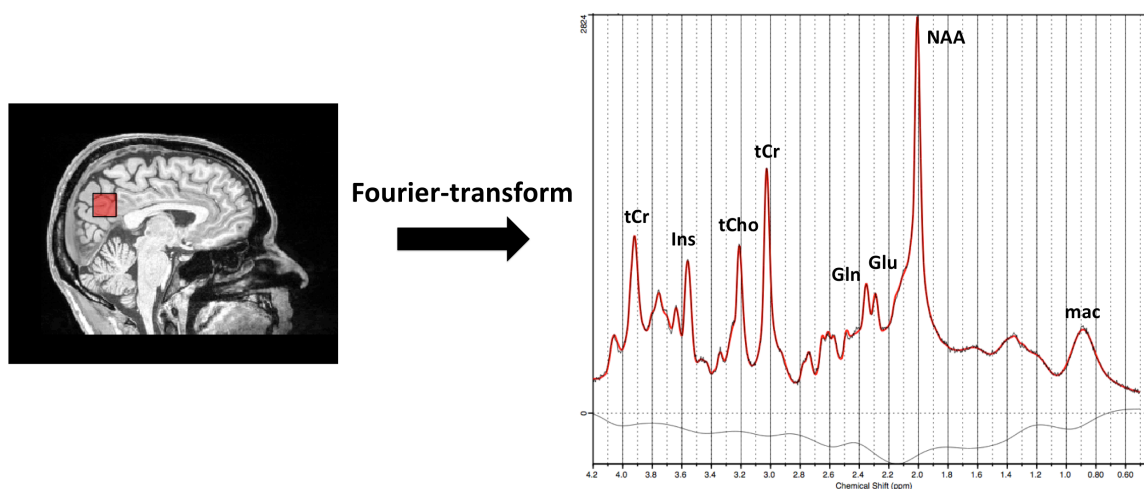
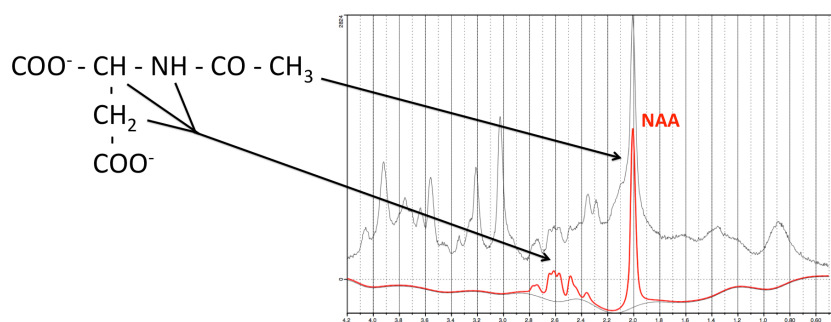
A ^1H -MRS acquisition typically starts with the acquisition of a structural MRI image, which is used as a guide to select a region of interest for spectroscopic analysis. The ^1H -MRS signal is then only acquired from this region by applying a pulse sequence that suppresses the signal from other regions. A ^1H -MRS sequence is acquired similarly to a T1-weighted image, however it requires a few additional steps. First, MRS measurements are especially sensitive to inhomogeneities in the magnetic field of the scanner (B_0 field) and the RF pulse. These inhomogeneities stem from variety of factors, including eddy currents, tissue-air boundaries, metallic implants or any imperfection that influences the ability of a substance to be magnetised. An additional step, or shim, is therefore needed to minimize the drift in B_0 field prior to the scan. Second, because water is the most abundant hydrogen-containing molecule within tissues, its signal in the ^1H -MRS spectrum is several orders of magnitude higher than that of other metabolites (ratio of water to metabolite: 10,000 : 1). In order to be able to resolve weaker metabolite signals, the signal from water must be suppressed when the metabolite spectra are being acquired. This is achieved by using a water-suppressing pulse sequence.

The MR spectrum is produced by a Fourier-transform of the MR image and is a two dimensional plot of metabolite frequency in parts per million (horizontal axis) versus

signal intensity (vertical axis) (details of reconstruction of the spectrum are beyond the scope of this thesis and are available elsewhere (Lei et al., 2014)). The exact frequency of a particular metabolite depends on its molecular structure, *i.e.* the number and positioning of the protons within the molecule. An example of how a metabolite's structure relates to its spectrum is depicted in (Figure 3.4b).

The spectrum for each participant is compared with a “model spectrum” which is obtained with similar scan parameters but using individual metabolite solutions. This model spectrum is usually supplied with the software package (in this case, LCModel). LCModel produces a “best fit” for the analysis of the acquired MR spectrum with overlapping peaks from the model spectrum (Provencher, 2001) and provides an estimate of the concentration of each metabolite in the measured tissue. Estimates of uncertainty arising from noise are presented as *Cramer-Rao lower bounds* or CRLBs (% standard deviation of estimated concentrations), which are multiplied by two to get a rough 95% confidence interval. Most studies use CRLBs < 20% as a rough criterion for estimates of acceptable reliability. Finally, spectra are visually inspected to ensure good signal to noise ratio, symmetrical, well-resolved and distinct peaks, and narrow linewidths to avoid overlap of adjacent peaks.

The metabolites that are most commonly detected by MRS are small mobile molecules present within the tissue in millimolar concentrations. Some key neurochemicals like dopamine that are present in the micromolar range and larger molecules like proteins therefore cannot be quantified by MRS. In this thesis, a recently developed short-TE MRS sequence (SPECIAL) was used which allows for the simultaneous quantification of several metabolites.

(A) MR spectrum from a single 8 cm³ voxel in the posterior cingulate cortex**(B) Molecular structure of NAA produces its characteristic peak****Figure 3.4: ¹H-MR spectrum produced by LCModel.**

(A) The MR spectrum obtained from an 8 cm³ voxel in the posterior cingulate cortex of a sample participant. Each peak in the spectrum corresponds to a particular metabolite. (B) The model spectrum (black) and the spectral peak for N acetyl aspartate (NAA) (red) are shown. NAA is the most reliably measured metabolite and has the largest peak. The NAA molecule has protons at different positions. The three protons of the –CH₃ group are equivalent and their individual signals add up to produce to prominent peak at 2.0 ppm. The protons of the –NH, –CH and –CH₂ groups interact with each other because of their close proximity and produce a more complex pattern of multiple peaks which also contributes to the overall concentration of NAA in this sample. Abbreviations in spectrum: tCr: total creatine, Ins: myo-inositol, tCho: total choline, Glu: glutamate, Gln: glutamine, mac: macromolecules.

3.2 Participants

Three cross-sectional age groups were used in this study: young, middle-aged and older (Figure 3.5, Figure 3.6). The young group was newly recruited for this DPhil project. Participants in the middle age group were recruited by previous DPhil students in the lab, and those for the older group belonged to the Whitehall II imaging sub-study. Details of the recruitment, genotyping and imaging protocols are described below. In this thesis, I refer to individuals with the $\epsilon 2\epsilon 2$ and $\epsilon 2\epsilon 3$ genotypes as “ $\epsilon 2$ carriers”, those homozygous for the $\epsilon 3$ allele as “ $\epsilon 3$ carriers” and those with $\epsilon 3\epsilon 4$ and $\epsilon 4\epsilon 4$ genotypes as “ $\epsilon 4$ carriers”. Participants with $\epsilon 2\epsilon 4$ were excluded from this study because of the rare frequency of this genotype and the lack of understanding of the combined effects of the two differing alleles.

3.2.1 Young group (20-40 years)

A total of 191 right-handed subjects were recruited in Oxfordshire, UK. Exclusion criteria were current/past neurologic or psychiatric disorders, head injury, substance abuse, smoking, corticosteroid, or diabetic treatment. DNA was extracted from buccal swab samples and genotyped for *APOE*. Genotyping was successful for 184 of 191 subjects. The *APOE* distribution reflected that expected in a healthy Caucasian population ($\chi^2=2.23$, $df = 5$, $P = 1$) (Menzel et al., 1983). *APOE* $\epsilon 2$ ($n=19$), $\epsilon 3$ ($n=20$) and $\epsilon 4$ carriers ($n=20$) were matched for age, gender, education level, and family history of dementia, and received an MRI scan. Family history of dementia was based on self-report and included either a parent or grandparent with dementia. One subject ($\epsilon 3$ homozygote) was excluded from the analysis because of an incidental finding and was subsequently

referred to a neurologist. To study the effects of age and *APOE* on brain metabolites in Chapter 7, 30 subjects (4 ϵ 2 carriers, 18 ϵ 3 homozygotes, 8 ϵ 4 carriers) were randomly selected from the remaining 58 so that their *APOE* allele distribution was similar to that in the old group. This was a single-blind study approved by the local Ethics Committee (10/H606/ 34), and informed consent was obtained from all subjects.

3.2.2 Middle group (35-55 years)

Participants from this group were selected from the Oxford Biobank, an age-stratified random sample of 1800 healthy adults from Oxfordshire. Biobank subjects attend a screening visit at the Clinical Research Unit, Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford. Exclusion criteria for this study included mental or physical illness, and substance abuse. DNA was isolated from blood samples, and consent was obtained to allow subsequent genotyping and recall for future projects. *APOE* ϵ 2 ($n=27$), ϵ 3 ($n=28$) and ϵ 4 carriers ($n=37$) were matched for age, gender, education, family history of dementia and received an MRI scan. Family history of dementia was based on self-report and included either a parent or grandparent with dementia. The study was approved by the local ethics committee and informed consent was obtained.

3.2.3 Old group (60-85 years)

A detailed protocol for the Whitehall II imaging sub-study is available elsewhere (Filippini et al., 2014). Briefly, 10,308 civil servants from the U.K were part of the initial Whitehall II study established in 1985 at University College London. In 2011, 800 of

these participants (then 60-85 years old) were randomly selected to participate in the neuroimaging sub-study held at the FMRIB center, Oxford. By February 2015, 552 participants had received an MRI scan and were included in this DPhil study. They were screened for depression using the Centre for Epidemiological Studies Depression scale (CES-D) (Radloff, 1977) and the Structured Clinical Interview for DSM-IV-TR Axis I Disorders (SCID-I) (First et al., 2002) and cognitive impairment, using the Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005). Exclusion criteria similar to those in the young and middle cohort were applied, and after excluding the data for subjects with incidental findings on the MRI scan, current neurological disorders, CES-D scores > 16 and/or a diagnosis of current depression as indicated by the SCID-I, and MoCA scores < 26, a final sample of 242 cognitive healthy subjects was used in Chapters 4 and 5.

Of the initial 552 Whitehall II participants, 221 received an ^1H -MRS scan. After screening for depression, cognitive impairment, incidental findings and poor spectral quality, a final sample of 117 older participants was used to study the effect of age on PCC metabolite concentrations. *APOE* information was only available for 101 of the 117 subjects, of which one subject with the $\epsilon 2\epsilon 4$ genotype was excluded. Accordingly, 100 older participants (10 $\epsilon 2$ carriers, 68 $\epsilon 3$ homozygotes and 22 $\epsilon 4$ carriers) were used to study the effect of *APOE* on brain metabolites in Chapter 7.

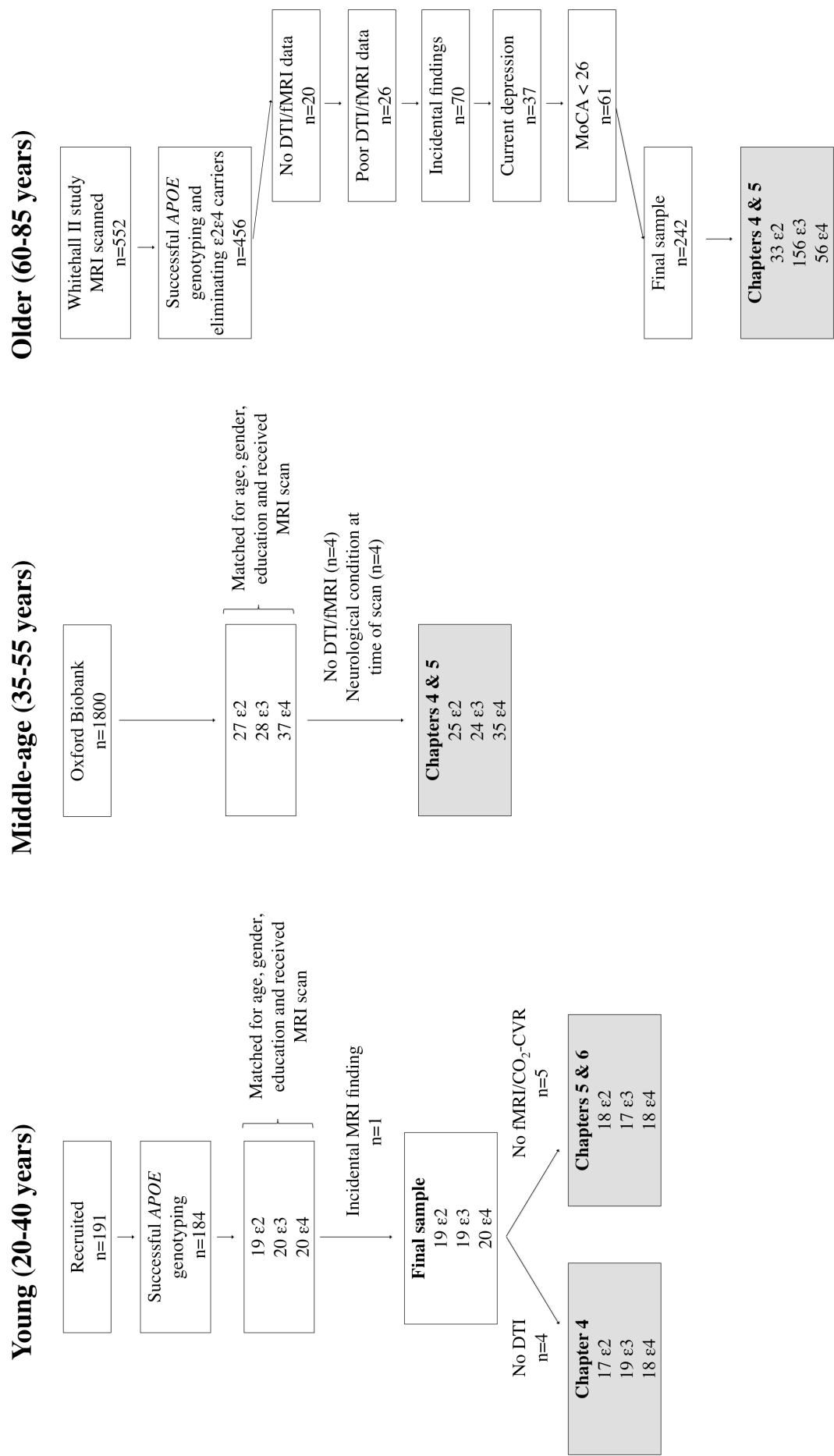
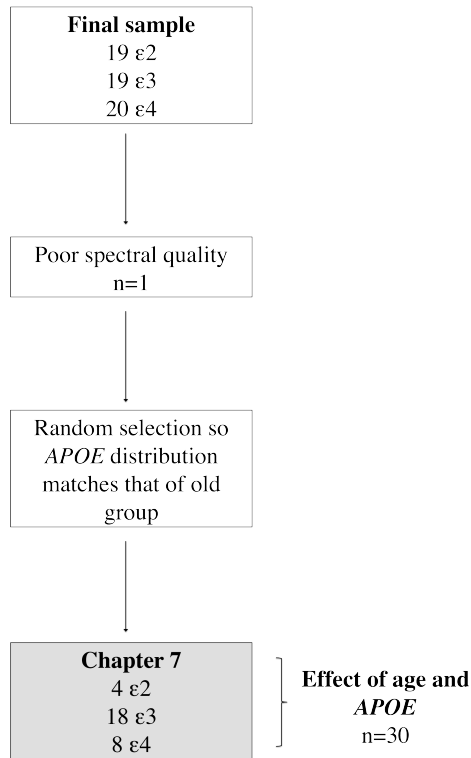
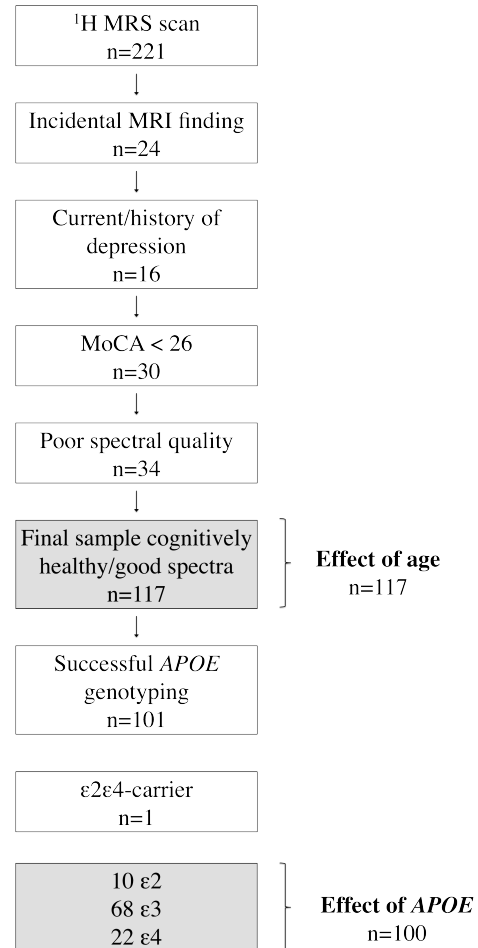


Figure 3.5: Selection of participants for Chapters 4, 5 and 6

Young (20-40 years)**Old (60-85 years)****Figure 3.6: Selection of participants for Chapter 7.**

Only a subset of the Whitehall group receive an ¹H-MRS scan. These participants underwent the same exclusion protocol and a final sample of 117 older adults was used to study the effect of age on metabolites concentrations. Data from 100 of the 117 subjects were used to study the effects of *APOE*. For the young group, 30 participants were randomly selected from the final 58 so that the *APOE* genotype distribution of the young participants matched that in the older group.

3.3 Neuroimaging protocol

The young and old groups were scanned at the Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB), Oxford using a 3T Siemens Verio scanner with a 32-channel head coil. The middle group was scanned at the University of Oxford Centre for Clinical Magnetic Resonance Research (OCMR) on a 3T Siemens Trio scanner with a 12-channel head coil. Details of the MRI sequences for each scan are provided in Table 3.1.

Table 3.1 MRI parameters for the young, middle and older age groups

	Young	Middle	Old
Structural MRI (Chapter 4)			
Sequence	ME-MPRAGE	MPRAGE	
TR (ms)	2530	2040	
TE (ms)	1.79/3.65/5.51/7.37	4.7	
TI (ms)	1380	900	Same as young-
Flip angle	7°	8°	
Voxel dimension (mm ³)	1 x 1 x 1	1 x 1 x 1	
Field of View (mm)	256	192	
Acquisition time	6 min 12 sec	5 min 56 sec	
Diffusion Tensor Imaging (Chapter 4)			
Sequence	GRAPPA	EPI	
TR (ms)	8900	9300	
TE (ms)	91.2	94	
Voxel dimension (mm ³)	2 x 2 x 2	2 x 2 x 2	Same as young
Field of View (mm)	192	192	
Diffusion directions	60	60	
B0 value (s/mm ²)	1500	1000	
Acquisition time	9 min 56 sec	10 min 31 sec	

	Young	Middle	Old
Resting-state fMRI (Chapter 5)			
Sequence	Dual echo EPI	Gradient echo EPI	Multiband
TR (ms)	4370	2000	1300
TE (ms)	TE ₁ =13/TE ₂ =30	28	40
Flip angle	90°	89°	66°
Voxel dimension (mm ³)	3.3 x 3.3 x 4	3 x 3 x 3.5	2 x 2 x 2
Field of View (mm)	210	224	212
No. of volumes	120	180	460
Acquisition time	8 min 58 sec	6 min 4 sec	10 min 10 sec
Task-based fMRI & Cerebrovascular Reactivity (Chapter 6)			
Sequence	Dual echo EPI		
TR (ms)	4170		
TE (ms)	TE ₁ =13/TE ₂ =30		
Flip angle	90°		
Voxel dimension (mm ³)	3.3 x 3.3 x 4	-----	-----
Field of View	210		
Acquisition time (task)	10 min 30 sec		
Acquisition time (CVR)	16 min 53 sec		
Magnetic Resonance Spectroscopy (Chapter 7)			
Sequence	SPECIAL		
TR (ms)	4000		
TE (ms)	8.5		
Flip angle	90°	-----	Same as young
Voxel dimension (cm ³)	2 x 2 x 2 (PCC)		
No. of averages	128		
Acquisition time	9 min 6 sec		

Chapter 4

APOE and brain structure

Structural and diffusion MRI-based markers have provided us with an evolving model of how the AD brain differs from that of a healthy individual (Suri et al., 2014b), however AD-related changes have not consistently been observed in cognitively intact *APOE* $\epsilon 4$ carriers. Further, the evidence for $\epsilon 2$ carriers is limited by small sample sizes and fewer studies (Suri et al., 2013). In this chapter, I examine the effects of all three *APOE* alleles on brain grey and white matter structure across adulthood.

4.1 Introduction

Grey matter atrophy is increasingly being used as a disease-specific marker of AD to the point where structural MRI is now a mandatory part of the diagnostic work-up (McKhann et al., 2011). The most robust finding in AD is progressive regional atrophy of the medial temporal lobe, particularly within the hippocampus and entorhinal cortex (Frisoni et al., 2010). Such changes are pronounced in patients with the $\epsilon 4$ allele (Agosta et al., 2009; Bigler et al., 2000; Wang et al., 2015) and have also been observed, although less consistently, in healthy *APOE* $\epsilon 4$ carriers. Some studies report smaller hippocampi (Barboriak et al., 2000; Burggren et al., 2008; Honea et al., 2009; Lind et al., 2006), thinner entorhinal cortices (Shaw et al., 2007), lower medial temporal lobe grey matter density (Wishart et al., 2006) and greater hippocampal atrophy rates (den Heijer et al., 2002; Risacher et al., 2010) in cognitively intact $\epsilon 4$ carriers relative to $\epsilon 3$ homozygotes.

Most investigations of brain structure have included elderly participants, however two studies in younger populations are worth noting. One recent study of 272 neonates found significantly lower hippocampal volumes in $\epsilon 4$ babies compared to $\epsilon 3$ homozygotes (Knickmeyer et al., 2014) and an earlier examination of entorhinal thickness in adolescents found thinner left cortices in $\epsilon 4$ carriers relative to non-carriers (Shaw et al., 2007), suggesting that the effects of *APOE* $\epsilon 4$ on brain structure may already be present in early life. As discussed in Chapter 2, there are also reports on the protective effects of $\epsilon 2$ on entorhinal cortical thickness in healthy (Alexopoulos et al., 2011; Chiang et al., 2010; Fennema-Notestine et al., 2011; Shaw et al., 2007) and patient populations (Fan et al., 2010; Liu et al., 2010).

On the other hand, there is considerable evidence to suggest that *APOE* does not mediate hippocampal or whole-brain grey matter volumes in healthy adults (Alexander et al., 2012; Bigler et al., 2003; Cherbuin et al., 2008; Liu et al., 2010; Okonkwo et al., 2012; Reiman et al., 1998; Reiter et al., 2012; Schmidt et al., 1996; Trachtenberg et al., 2012a). In fact, two of the largest studies, one in 1400 healthy adolescents (Khan et al., 2014) and one in over 600 healthy older adults (40-68 years) (Bunce et al., 2012) found no effect of the three alleles on medial temporal lobe and hippocampal volumes. Thus, while regional GM atrophy may be a useful tool for diagnosing AD (Suri et al., 2014b), the evidence for its use as an early marker of risk for developing AD is not as convincing.

Although AD has traditionally been thought of as a disorder of the GM, recent reports suggest that WM damage may occur independently of and perhaps even before, GM atrophy in AD (Amlie and Fjell, 2014). Impairments in WM microstructure can be measured using DTI and it is possible that DTI can detect subtle changes in structure that

may be missed by volumetric methods. In fact, changes in DTI-derived metrics of the hippocampus are considered better predictors of conversion from MCI to AD than hippocampal atrophy measured with conventional structural MRI (Hong et al., 2013; Kantarci et al., 2005; Müller et al., 2005). DTI studies in healthy subjects mainly report lower fractional anisotropy (FA) (Heise et al., 2011; Honea et al., 2009; Nierenberg et al., 2005; Persson et al., 2006) and accelerated age-related decline in structural connectivity (Brown et al., 2011) in $\epsilon 4$ carriers compared to $\epsilon 3$ homozygotes. These studies largely display effects in the corpus callosum, parahippocampal WM, cingulum and precuneus tracts and point towards axonal damage within these tracts. However, the largest DTI study to compare $\epsilon 4$ carriers (n=56) and $\epsilon 3$ homozygotes (n=80) found no effect of the $\epsilon 4$ allele on any DTI parameter in healthy middle-aged individuals (Bendlin et al., 2010). Moreover, when $\epsilon 2$ carriers are thrown into the mix, the results are slightly unexpected. While one study has reported more robust WM microstructure in $\epsilon 2$ relative to $\epsilon 3$ carriers (Chiang et al., 2012), another observed that $\epsilon 2$ and $\epsilon 4$ carriers may show similar declines in WM structure relative to healthy $\epsilon 3$ homozygotes (Westlye et al., 2012). Lastly, we know little about the relative timing of the onset of any *APOE* mediated changes in brain structure; some aforementioned studies suggest these differences may already exist early in development, while others point towards late-life onset of allele-specific effects.

4.1.1 Objectives

Given the conflicting information on the effect of *APOE* on brain structure, the objectives for this study were to determine if:

1. (a) Whole-brain grey matter and hippocampal volumes and (b) whole-brain white matter and hippocampal cingulum microstructure vary according to *APOE* genotype,
2. If these variations are reflective of AD risk (*i.e.*, if $\epsilon 2$ and $\epsilon 4$ show opposite effects),
3. If these variations are influenced by age.

We focused on the hippocampus and the white matter tract connecting the hippocampus to the posterior cingulate cortex, as these are the earliest and most consistently reported regions to show AD-related structural atrophy.

4.2 Methods

4.2.1 Participants

Structural and DTI data from 54 young, 84 middle and 242 older participants were analysed in this study. Details of participant recruitment, exclusion criteria and *APOE* genotyping are presented in Chapter 3.

4.2.2 MRI acquisition

The structural and DTI scans for the young and old groups were acquired on a 3T Siemens Verio scanner at the FMRIB Centre, Oxford. The scan sequence parameters for the two groups were identical. The middle group was scanned using a different protocol at the OCMR Centre on a 3T Siemens Trio scanner (Chapter 3).

4.2.3 MRI analysis

All image analysis was performed using FMRIB Software Library (FSL) tools (Smith et al., 2004). The imaging analysis protocol was identical for the three age groups. However, because the middle group was scanned on a different MRI scanner, *APOE* differences in brain structure were separately assessed for each age group. The young and old groups were subsequently combined to study interactions between *APOE* genotype and age.

Structural MRI: Brain tissues were segmented using FMRIB's Automated Segmentation Tool to extract measures of total brain volume, GM, WM, and CSF. An automated model-based segmentation/registration tool (FIRST) was used to extract measures of hippocampal volumes (Patenaude et al., 2011). A whole-brain investigation of *APOE*-related changes in GM volume was carried out using a voxel-based-morphometry analysis (FSL-VBM) (Douaud et al., 2007). Brain extraction and tissue-type segmentation was performed, and resulting GM volume images were aligned to standard space using first linear (FLIRT) and then nonlinear (FNIRT) registration tools. The resulting images were averaged, modulated and smoothed with an isotropic Gaussian kernel of 3 mm (~7 mm full-width at half-maximum, FWHM) to create a study-specific template. GM images were re-registered to the template, including modulation by the warp field Jacobian. Finally, voxelwise GLM was applied using permutation-based non-parametric testing (5000 permutations) and a significance threshold of $p < 0.05$ correcting for multiple comparisons across space. *APOE* groups were used as variables of interest in the GLM.

DTI: Raw diffusion-weighted images were pre-processed using eddy current correction in order to correct for distortions due to the applied gradient directions. FA and MD images were generated using DTIFit, part of FMRIB's Diffusion Toolbox, that fits a diffusion tensor model at each voxel. Voxelwise statistical analysis of the FA data was carried out using tract-based spatial statistics (TBSS) (Smith et al., 2006). All subjects' FA data were aligned into a common space using FNIRT (Andersson et al., 2007a, 2007b). Next, the mean FA image was generated and thinned to create a mean FA skeleton, which represents the centres of all tracts common to the group. Each subject's aligned FA data was then projected onto this skeleton and the resulting data fed into

cross-subject statistics. A voxelwise nonparametric test with 5000 permutations was used to assess group-related differences on a whole-brain level. Additionally, a region of interest (ROI) analysis of the mean FA skeleton was performed on the hippocampal branch of the cingulum. This white matter tract connects the hippocampus and posterior cingulate cortex (PCC). The resulting parameter estimates from the ROI analysis were z scored within each age group and plotted as graphical representations of the voxelwise results. In addition to FA data, MD, DA and DR were also compared using TBSS in an analogous fashion. All results are shown at $p < 0.05$ corrected for multiple comparisons across space.

4.2.4 Statistics

Non-imaging variables were analysed using SPSS (SPSS, Chicago IL). Analysis of variance (ANOVA) and Bonferroni correction for post-hoc tests were used to compare sociodemographic variables, brain volumes and regions of interest. Exact Fisher's test was used for categorical variables (gender and family history of dementia). $P < 0.05$ was considered significant for all analyses.

4.3 Results

4.3.1 Sociodemographics

There were no significant *APOE*-related differences in age, education, gender, or family history of dementia in any age group. The older group also had no genotype differences in MOCA scores (Table 4.1).

4.3.2 Effect of *APOE* on grey matter

Whole-brain VBM analysis revealed no effect of *APOE* on GM density in the young, middle and older groups. There were also no *APOE* differences in whole-brain volume, segmented GM, WM, CSF and hippocampal volumes in each age group (Table 4.1). The young and old groups were subsequently combined to study interactions between *APOE* genotype and age. The older group had significantly more males ($\chi^2_{(1,294)}=37.93$, $p<0.001$) and fewer years of education ($t_{(1,294)} = 4.66$, $p<0.001$) than the young group, and accordingly these two variables were included as covariates in the analysis. Both whole-brain VBM and ROI analyses of the hippocampi showed no effect of *APOE* or any *APOE* x age interaction.

4.3.3 Effect of *APOE* on white matter

There were no significant *APOE* differences in global FA, MD, and axial or radial diffusivity in any age group using a whole-brain TBSS approach. A voxelwise ROI

analysis of the hippocampal cingulum revealed significant effects of *APOE* in the young and middle-age groups. Young $\epsilon 2$ carriers had significantly higher FA and axial diffusivity and significantly lower radial diffusivity in the cingulum than $\epsilon 3$ homozygotes (Figure 4.1). There were no significant differences between $\epsilon 4$ carriers and $\epsilon 2$ carriers or $\epsilon 3$ homozygotes in the voxelwise analysis, however a post-hoc analysis of the z-scored axial diffusivity values extracted from the significant clusters showed differences between $\epsilon 4$ and $\epsilon 2$ carriers ($p < 0.05$). Conversely, in the middle age group, the largest differences in WM integrity were observed between the $\epsilon 4$ and $\epsilon 3$ genotypes, with $\epsilon 2$ carriers falling in between the two extremes (Figure 4.2). Using both whole-brain TBSS and region of interest analyses there was no significant effect of *APOE* in the older age group, or any *APOE* x age interaction when the young and old groups were combined.

Table 4.1 Effect of *APOE* on brain structure

Socio-demographics and brain volumes for the three *APOE* genotypes in the young, middle and old age groups. Values represent means $\pm$ standard deviations. P values are results of chi-square tests (for gender and family history of AD) and one-way ANOVA (for all other variables). GM, WM, CSF and hippocampal volumes are expressed as percentages of whole-brain volumes. L and R: left and right respectively

	Young				Middle				Old			
	ϵ^2 N=17	ϵ^3 N=19	ϵ^4 N=18	P	ϵ^2 N=25	ϵ^3 N=24	ϵ^4 N=35	P	ϵ^2 N=33	ϵ^3 N=156	ϵ^4 N=53	P
Socio-demographics												
Age (yrs)	24.42 $\pm$ 5.32	23.74 $\pm$ 4.82	24.00 $\pm$ 4.70	0.92	43.92 $\pm$ 5.35	46.33 $\pm$ 3.40	46.20 $\pm$ 5.90	0.17	68.45 $\pm$ 4.76	69.24 $\pm$ 5.12	68.44 $\pm$ 4.56	0.49
Education (yrs)	17.47 $\pm$ 1.70	17.29 $\pm$ 2.81	17.22 $\pm$ 2.13	0.95	14.66 $\pm$ 2.28	15.42 $\pm$ 2.77	14.40 $\pm$ 2.95	0.36	15.88 $\pm$ 3.46	15.45 $\pm$ 3.17	15.88 $\pm$ 3.43	0.61
Gender (% males)	29.40%	47.40%	44.40%	0.51	48%	58.30%	51.40%	0.76	87.90%	78.80%	84.90%	0.51
Family history of AD	2	1	1	0.71	4	8	10	0.33	2	2	4	0.08
MOCA	-	-	-	-	-	-	-	-	28.33 $\pm$ 1.11	28.10 $\pm$ 1.28	28.18 $\pm$ 1.24	0.39
Brain volumes												
Whole brain (mm ³)	1423 $\pm$ 93	1449 $\pm$ 136	1456 $\pm$ 175	0.77	1466 $\pm$ 115	1476 $\pm$ 147	1472 $\pm$ 136	0.96	1448 $\pm$ 130	1439 $\pm$ 123	1452 $\pm$ 96	0.78
GM (%)	43.56 $\pm$ 1.70	43.85 $\pm$ 1.57	44.42 $\pm$ 2.38	0.41	42.17 $\pm$ 1.49	41.83 $\pm$ 1.29	42.41 $\pm$ 1.35	0.29	36.92 $\pm$ 2.42	37.15 $\pm$ 2.06	37.25 $\pm$ 1.85	0.77
WM (%)	36.68 $\pm$ 1.62	36.89 $\pm$ 1.23	37.20 $\pm$ 1.42	0.55	36.19 $\pm$ 1.11	36.09 $\pm$ 1.11	36.43 $\pm$ 1.01	0.46	36.96 $\pm$ 1.62	37.15 $\pm$ 1.64	37.54 $\pm$ 1.59	0.2
CSF (%)	19.76 $\pm$ 1.38	19.26 $\pm$ 1.98	18.38 $\pm$ 2.48	0.13	21.64 $\pm$ 1.66	22.08 $\pm$ 1.46	21.16 $\pm$ 1.90	0.13	26.12 $\pm$ 3.46	25.71 $\pm$ 2.74	25.21 $\pm$ 2.40	0.31
L hippocampus (%)	0.25 $\pm$ 0.03	0.27 $\pm$ 0.02	0.27 $\pm$ 0.03	0.06	0.29 $\pm$ 0.02	0.28 $\pm$ 0.02	0.28 $\pm$ 0.02	0.81	0.24 $\pm$ 0.04	0.24 $\pm$ 0.03	0.24 $\pm$ 0.03	0.54
R hippocampus (%)	0.25 $\pm$ 0.02	0.27 $\pm$ 0.03	0.27 $\pm$ 0.02	0.25	0.29 $\pm$ 0.02	0.28 $\pm$ 0.03	0.29 $\pm$ 0.02	0.71	0.25 $\pm$ 0.03	0.24 $\pm$ 0.03	0.26 $\pm$ 0.03	0.09

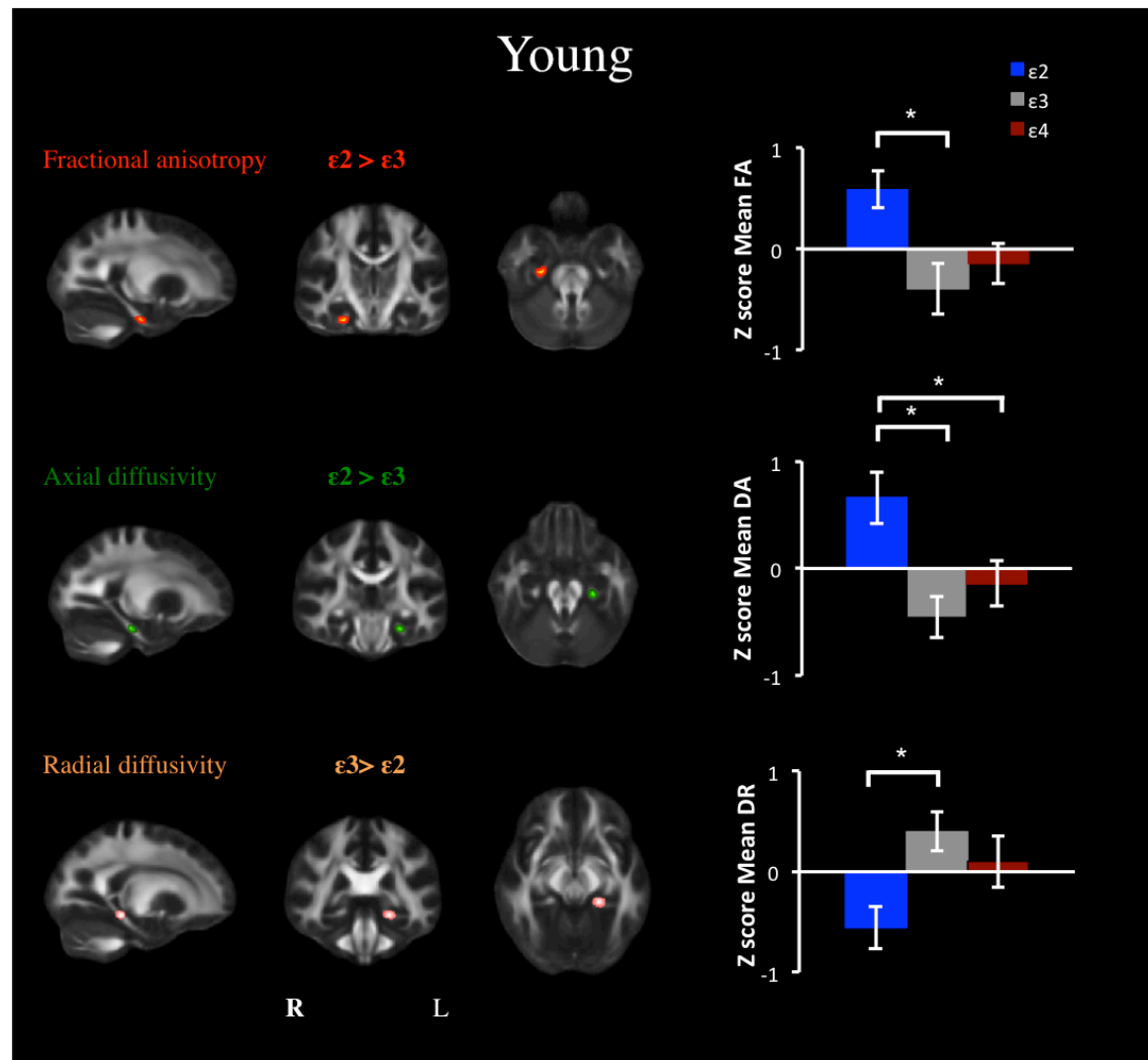


Figure 4.1: ROI analysis of the hippocampal cingulum in the young group.

Regions of significance are depicted in red (FA), green (axial diffusivity, DA) and orange (radial diffusivity, DR). Images were thresholded using $z > 2.3$ and corrected for multiple comparisons using a corrected cluster significance threshold of $p < 0.05$. Z scores (normalised by age group) of mean values extracted from the significant regions are plotted on the right. Error bars represent standard errors of the mean. * denotes significant differences after post-hoc Bonferroni correction ($p < 0.05$). R and L: right and left hemisphere respectively.

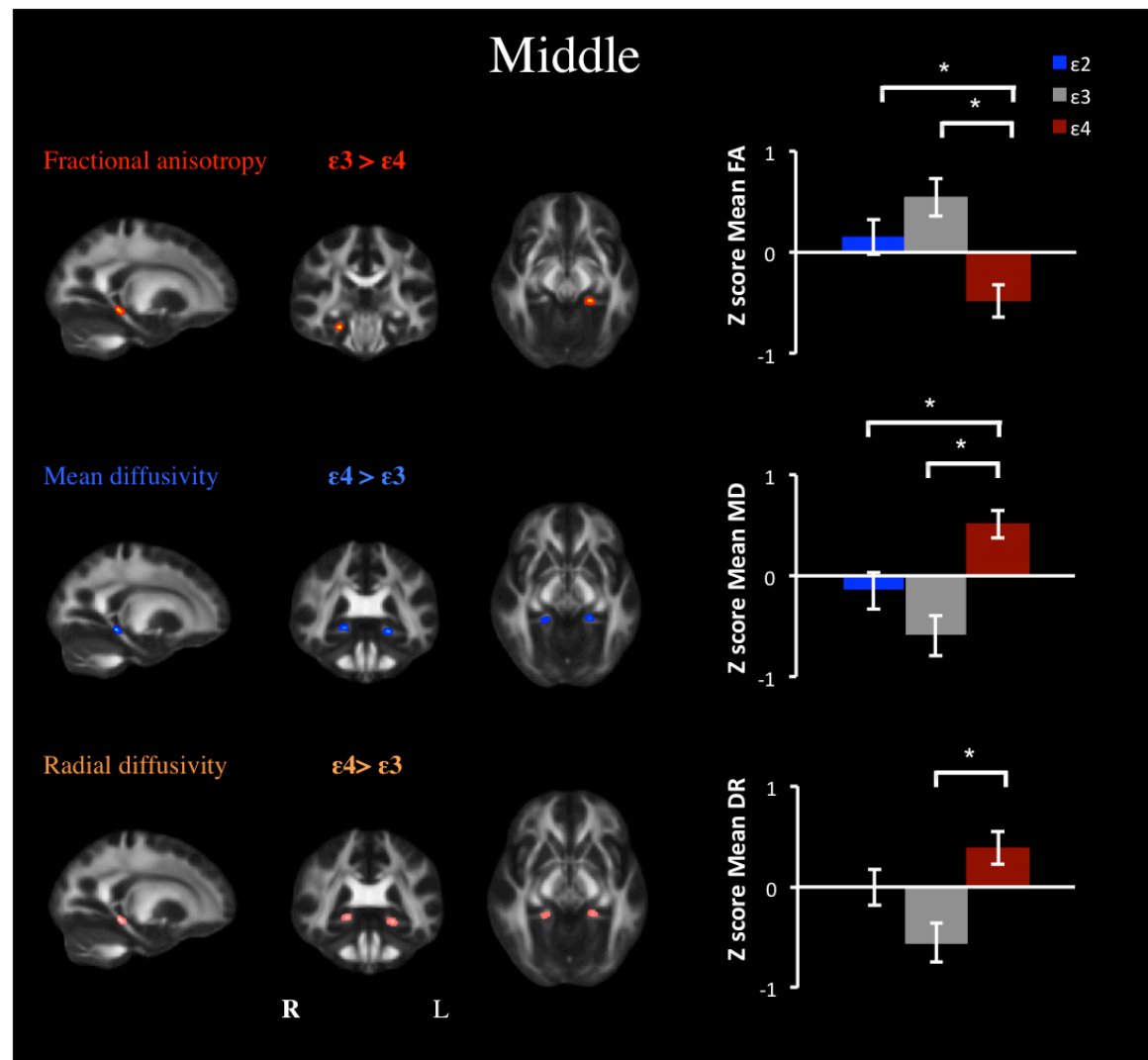


Figure 4.2: ROI analysis of the hippocampal cingulum in the middle group.

Regions of significance are depicted in red (FA), blue (mean diffusivity, MD) and orange (radial diffusivity, DR). Images were thresholded using $z > 2.3$ and corrected for multiple comparisons using a corrected cluster significance threshold of $p < 0.05$. Z scores (normalised by age group) of mean values extracted from the significant regions are plotted on the right. Error bars represent standard errors of the mean. * denotes significant differences after post-hoc Bonferroni correction ($p < 0.05$). R and L: right and left hemisphere respectively.

4.4 Discussion

This is the first investigation of the effects of all three *APOE* alleles on brain grey and white matter across adulthood (20-85 years). Our study has its strengths in its relatively large sample size, its inclusion of the $\epsilon 2$ allele, and wide age-range of participants. However, we were limited by the fact that the middle age group received a different structural scan sequence and we therefore could not combine the three age groups to study the effects of *APOE* across the entire 20-85 year age-range. Upon analysing the individual age groups separately, we found no *APOE*-related effects on whole-brain GM density, global WM integrity or hippocampal volumes in young, middle-aged and older people. There was also no interaction of *APOE* with age on any of these measures when the young and old groups (who received the same scan) were combined. An ROI analysis of WM revealed allele-specific effects on the hippocampal branch of the cingulum in the young and middle age groups but not in the older population.

4.4.1 No effects of APOE on grey matter

The absence of significant *APOE* group differences in hippocampal and global GM volumes is in agreement with the majority of the studies discussed in Section 4.1, which taken together suggest that *APOE* may not influence the structural integrity of GM during healthy ageing.

However, there may be subtle effects in individuals who are homozygous for the $\epsilon 4$ allele. One of the largest *APOE* studies to use a VBM-based approach, conducted in 750 healthy elderly individuals (63-75 years), found that $\epsilon 4$ homozygotes had significant

decreases in grey matter bilaterally within the medial temporal lobes, including in the hippocampus compared to $\epsilon 4$ heterozygotes and non-carriers (Lemaître et al., 2005). However, they found no effects of *APOE* on GM density or hippocampal volumes between the other genotypes, and therefore concluded that in a normal elderly population, *APOE* effects on cortical atrophy may be limited to $\epsilon 4$ homozygotes. Our study did not have sufficient $\epsilon 4$ homozygotes in the three age groups to perform a gene-dose analysis (two young, seven middle-aged and three older $\epsilon 4\epsilon 4$ carriers) and future examinations of structural decline should consider the focusing on this genotype.

Another avenue for future research would be to study the relationship between $\epsilon 4$ and family history of dementia, the latter being an established risk factor for AD, particularly in combination with the presence of an $\epsilon 4$ allele (Green et al., 2002). A recent VBM study examined individuals with the $\epsilon 4$ allele ($n=74$), family history of dementia ($n=228$), and those with both ($n=62$) (ten Kate et al., 2015). While they found no effect of either *APOE* or family history of dementia on grey matter, they reported a modest combined effect of the two risk factors (only observed at a lower statistical significance threshold). *APOE* $\epsilon 4$ carriers with a first-degree family history of dementia had significantly more atrophy in the bilateral insula than non-carriers with no family history. Although subtle, their results are suggestive of a possible additive effect of *APOE* and family history of AD, which we could not test here given the low numbers of individuals with family history in this study.

We note that we did not find evidence for a protective effect of $\epsilon 2$ on hippocampal volumes during healthy ageing, which is in contrast to some (Alexopoulos et al., 2011) but not all studies (Bunce et al., 2012; Khan et al., 2014). As discussed in Chapter 2, there is stronger support for an effect of $\epsilon 2$ on regional cortical thickness than on volumes

(Fennema-Notestine et al., 2011; Shaw et al., 2007). Measurement of cortical thickness, curvature and surface area using advanced surface reconstruction and segmentation tools like Freesurfer would serve as a useful complement to the volumetric approach employed in this study.

Several authors have also suggested that the rates of change of GM volumes may be more useful than cross-sectional values in predicting lifetime risk for AD (Bookheimer and Burggren, 2009). Measurements of longitudinal change in hippocampal and entorhinal cortical volumes that are used to predict conversion from MCI to AD are particularly pronounced in patients with the $\epsilon 4$ allele (Jack et al., 2008; Rusinek et al., 2003). Similar atrophy rates have not been thoroughly investigated in cognitively intact individuals and it may be that a single, static measure of volume is not sensitive enough to differentiate between the *APOE* groups in healthy populations (Bookheimer and Burggren, 2009).

In a recent meta-analysis of genome-wide association studies that examined genetic signatures of hippocampal and whole-brain volumes in over 5700 cognitively healthy participants, *APOE* did not emerge as being associated with any structural measures (Stein et al., 2012). In considering our results together with the available literature, it seems that while volumetric hippocampal measurements may be useful markers of AD progression, they may not accurately reflect a healthy individual's genetic risk for developing AD.

4.4.2 Effect of APOE on cingulum microstructure

There were no significant effects of *APOE* on whole-brain WM microstructure in any age group, although an ROI analysis of the hippocampal branch of the cingulum was significant in the young and middle-age groups. In line with previous results, we found that $\epsilon 2$ carriers had significantly higher FA and axial diffusivity and lower radial diffusivity than non-carriers (Figure 4.1) (Chiang et al., 2012) and that $\epsilon 4$ carriers had the significantly lower FA and higher MD and radial diffusivity than non-carriers (Figure 4.2) (Heise et al., 2011; Nierenberg et al., 2005; Persson et al., 2006). There were no regions where $\epsilon 4$ carriers had significantly higher FA or lower MD than non-carriers in any age group. Reductions in FA and increases in mean and radial diffusivity are suggestive of underlying axonal degeneration or demyelination (Beaulieu, 2002). It seems therefore that at least within the cingulum, the $\epsilon 2$ carriers have an early-life structural advantage compared to the other two *APOE* groups, whereas the $\epsilon 4$ carriers start to decline more noticeably in mid-life.

Neither the old group, nor a combination of the young and old groups showed any *APOE*-related changes in the cingulum. The lack of observable $\epsilon 4$ effects in the older group may not strictly reflect an absence of *APOE*-related changes, but may be a result of the limitations of our population. All the participants in this age group were screened for cognitive impairment, and there is a possibility that our sample contains individuals who have survived or at least delayed any potential detrimental effects of $\epsilon 4$. Another consideration is that the older group was largely male (~80-90% male in each *APOE* group), and this imbalance in gender could potentially have masked *APOE*-mediated differences. Indeed there is evidence to suggest that the effects of *APOE* in the brain are

most pronounced in females, with female $\epsilon 4$ carriers showing the greatest lifetime risk for AD (Farrer et al., 1997), the lowest entorhinal cortical volumes (Juottonen et al., 1998), and the worst WM metrics globally (Smith et al., 2010) and within the cingulum (Heise et al., 2014). However, we lacked the statistical power to sufficiently test this in our study because of the small number of females in the old group (4 out of 33 $\epsilon 2$ females and 8 out of 53 $\epsilon 4$ females).

The hippocampal branch of the cingulum is the white matter tract linking the hippocampus to the posterior cingulate cortex, both of which are amongst the first brain regions to be affected by AD pathology. Disruptions to this tract have been noted in MCI and AD patients, and may contribute to the structural and functional disconnection of these central memory-regulating structures from the rest of the brain (Bozoki et al., 2012; Bozzali et al., 2012; Choo et al., 2010). It may therefore be tempting to suggest that the robust cingulum structure observed in the young $\epsilon 2$ carriers and the decline in structural integrity in middle-aged $\epsilon 4$ carriers may contribute to their opposing effects on risk for AD. However, although we observed genotype differences in the cingulum in our study, we stress that the effects were small, and were only apparent when we conducted a more localised region-based analysis. This is in contrast with past findings of more widespread *APOE* changes in WM microstructure that were not solely restricted to small regions within the cingulum (Heise et al., 2011), but are in line with a relatively large study finding no strong effect of *APOE* on global WM (Bendlin et al., 2010).

In conclusion, we cannot say with confidence that cross-sectional structural MRI and DTI-derived measurements used in this study are sensitive predictors of lifetime risk for developing AD. We found no effects of *APOE* on hippocampal volumes, or whole-brain

GM and WM structure. An analysis of the hippocampal branch of the cingulum revealed significant *APOE*-related changes in this tract in the young and middle age ranges. Our findings point towards a significant, but localised decline in WM structure in cognitively healthy middle-aged $\epsilon 4$ carriers, and we suggest that the effects of *APOE* in older ages ranges should be further investigated using a gender-balanced sample. We recommend that future studies use more detailed imaging analysis tools to assess *APOE*'s effect on brain structure. In particular, Freesurfer and fibre tractography would give us valuable information on cortical thickness and white matter projections respectively. Additionally, advanced multi-modal imaging analysis techniques, *i.e.* those that combine complementary imaging modalities like structural MRI and DTI in the same analysis, could provide a more comprehensive understanding of whether or not *APOE* influences brain structure during healthy ageing (Groves et al., 2012).

Given that we observed changes in structural connectivity in a white matter tract between the hippocampus and posterior cingulate cortex, we subsequently investigated the functional connectivity between these two regions in the following chapter.

Chapter 5

APOE and the resting brain

In the last 15 years, there has been a growing interest in brain regions in which neuronal activity is greater in the absence than in the presence of a task. Studies measuring this baseline or “resting-state” activity have found that *APOE* ϵ 4 carriers exhibit altered levels of activity when compared to ϵ 3 homozygotes, and that the direction of these changes may be age-specific. By contrast, there is very little evidence describing how ϵ 2 influences activity within resting brain. The only two studies to investigate ϵ 2 report somewhat unexpected similarities in function between ϵ 2 and ϵ 4 carriers. In this chapter, I present the effects of all three *APOE* alleles on functional connectivity within the resting brain in young, middle-aged and older individuals, with a specific focus on activity within the posterior cingulate cortex and hippocampus.

5.1 Introduction

5.1.1 Ageing, AD and the resting brain

Functional MRI studies usually investigate brain activity by comparing the BOLD signal during experimental and control conditions of a task. However, even in the absence of a task, there are spontaneous fluctuations in the BOLD signal, which give rise to “resting-state” activity within the brain. The posterior cingulate cortex (PCC) is one such region that consistently shows on-going activity in the absence of a task, and by contrast is

deactivated during cognitive tests (Raichle et al., 2001). Spontaneous resting-state activity within the PCC is highly correlated with that of other brain regions, *viz.* the anterior cingulate cortex, precuneus, hippocampal formation, medial prefrontal cortex, lateral temporal cortex, and inferior parietal lobule. Together these regions are organized into a coherent resting-state network (RSN) known as the default mode network (DMN) (Buckner et al., 2008). The DMN is one of around eight or ten consistently identified RSNs; others are involved in sensory, motor and executive function (Beckmann et al., 2005). While the exact significance of the DMN is still unknown, this network is believed to play a role in mind wandering and internally-directed thought processes, *i.e.* when we think about past events or future plans (Andrews-Hanna et al., 2010). This network is of particular interest in the context of AD as it was the first RSN reported to be impaired in AD, it includes brain regions first targeted by plaque pathology, and it is associated with episodic memory, a cognitive domain affected in AD (Buckner et al., 2005; Greicius et al., 2004).

Techniques that measure resting-state activity serve as a useful complement to task-based fMRI studies (Cole et al., 2010). Two of the most widely used methods that explore how remote brain areas work together within the resting brain are independent component analysis (ICA), which examines whole networks, and seed-based correlation analysis (SCA), which estimates the functional connectivity of a specific region (or seed) with the rest of the brain. Thus while ICA allows us to study the DMN as a whole, SCA is a useful approach to study the individual structures that make up the DMN.

Considerable attention has been given to the DMN in relation to ageing and dementia (Hafkemeijer et al., 2012; Liu et al., 2008). With advancing age, studies generally find

decreases in functional connectivity within the DMN particularly between the anterior and posterior DMN hubs (Andrews-Hanna et al., 2007; Biswal et al., 2010; Koch et al., 2010). These changes persist even after accounting for age-related grey matter atrophy (Damoiseaux et al., 2008) and are most consistently observed in the PCC. In AD patients, studies using the PCC as a seed have found decreases in functional connectivity between this region and other parts of the DMN like the anterior cingulate, precuneus and medial prefrontal cortex (Wang et al., 2007; Zhang et al., 2009). Hippocampal seed analyses also point towards decreases in connectivity to the PCC/precuneus in AD patients relative to healthy controls (Allen et al., 2007; Wang et al., 2006). These findings reinforce ICA reports of reductions in DMN-hippocampal connectivity in AD that, notably, are correlated with disease severity (Allen et al., 2007; Greicius et al., 2004; Wu et al., 2011; Zhou et al., 2010, 2008). Neuropathological, electrophysiological and neuroimaging studies have demonstrated the structural and functional isolation of the hippocampal formation and posterior cingulate cortex from the rest of the brain in AD patients (Delbeuck et al., 2003; Leech and Sharp, 2014). Consistent observations of disruptions in resting-state functional connectivity in AD therefore lend support to this prevailing idea of AD as a “*disconnection syndrome*”, characterized by a functional disconnect between the hippocampus and PCC, among other brain regions (Allen et al., 2006).

Taken together, there are age-related reductions in functional connectivity of key regions within the DMN during normal ageing, with significantly more pronounced reductions in patients with AD (Hafkemeijer et al., 2012). Impaired resting-state activity within the DMN and its constituent structures (most notably the PCC and hippocampus) is therefore considered a sensitive marker of AD onset.

5.1.2 *APOE* and the resting brain

The findings from studies examining healthy $\epsilon 4$ carriers are not as consistent as those in AD patients. These studies differ in their analysis techniques (ICA vs seed-based) and region of interest (PCC, precuneus, DMN), and Table 5.1 highlights how this together with the age of participants may contribute to the current variability in results. Studies in young adults show higher DMN-hippocampal (Filippini et al., 2009) and PCC-medial temporal lobe connectivity (Dennis et al., 2010) in $\epsilon 4$ carriers than $\epsilon 3$ homozygotes. This patterns is reversed in older adults, with older $\epsilon 4$ carriers undergoing a disproportionately rapid decline in functional connectivity within the PCC, precuneus and posterior DMN compared to $\epsilon 3$ homozygotes (Damoiseaux et al., 2012; Fleisher et al., 2009; Machulda et al., 2011; Sheline et al., 2010; Shu et al., 2014). Interestingly, in the middle age-range, $\epsilon 4$ carriers and $\epsilon 3$ homozygotes reportedly show similar levels of functional connectivity within the DMN perhaps because the $\epsilon 4$ carriers are in the midst of a transition from high to low resting-state connectivity at this age (Trachtenberg et al., 2012b). Two studies are in disagreement with the idea that older *APOE* $\epsilon 4$ carriers have significantly lower resting-state activity than $\epsilon 3$ homozygotes (Westlye et al., 2011; Yang et al., 2014). Both have suggested that the relatively higher activity in older $\epsilon 4$ carriers reflects their need for additional compensatory neural recruitment mechanisms to maintain similar levels of cognitive performance. However, while this is a plausible explanation for task-based function where there is usually a directed cognitive demand, there is a need for further investigation to understand how it may apply to changes in activity within the resting brain.

Table 5.1: Inconsistencies in resting-state fMRI literature.

A review of the seed-based and ICA studies examining the effect of APOE on resting-state functional connectivity in cognitively healthy adults

Abbreviations: MTL: medial temporal lobe, DMN: default mode network, PCC: posterior cingulate cortex, ACC: anterior cingulate cortex
Contrasts where ϵ_4 -carriers have relatively higher and lower functional connectivity than non-carriers are depicted in red and blue respectively

Age group	Mean age	Age-range (Mean $\pm$ SD where range not present)	Publication	Analysis technique	Region	Participants	Result	E2 separately analysed?
Young	~20 years	ϵ_4 : 21.8 ± 3.3 non- ϵ_4 : 20.8 ± 2.9	Dennis et al. 2010	Seed	MTL	12 ϵ_4 , 12 non- ϵ_4	$\epsilon_4 > \epsilon_3$ (PCC) $\epsilon_3 > \epsilon_4$ (Anterior cortex)	No
	~27 years	20-35 years	Filippini et al. 2009	ICA	DMN	18 ϵ_4 , 18 non- ϵ_4	$\epsilon_4 > \epsilon_3$ (hippocampus)	No
	~27 years	20-39 years	Yang et al. 2014	Seed	PCC	16 ϵ_4 , 84 non- ϵ_4	No effect of APOE	No
Middle	~45 years	32-55 years	Trachtenberg et al. 2012	ICA	DMN	23 ϵ_2 , 20 $\epsilon_3\epsilon_3$, 34 ϵ_4	No effect of APOE	Yes
	~57 years	50-65 years	Fleisher et al. 2009	Seed	PCC	17 ϵ_4 , 12 non- ϵ_4	$\epsilon_4 > \epsilon_3$ (frontal cortex) $\epsilon_3 > \epsilon_4$ (precuneus)	No
Older	~62 years	61.8 ± 8.1 years	Sheline et al. 2010	Seed	Precuneus	38 ϵ_4 , 62 non- ϵ_4	$\epsilon_3 > \epsilon_4$ (posterior DMN, hippocampus)	No
	~62 years	50-80 years	Westlye et al. 2011	ICA	DMN	33 ϵ_4 , 63 non- ϵ_4	$\epsilon_4 > \epsilon_3$ (hippocampus)	No
	~68 years	60-79 years	Yang et al. 2014	Seed	PCC	17 ϵ_4 , 95 non- ϵ_4	$\epsilon_4 > \epsilon_3$ (frontal cortex)	No
	~68 years	54-80 years	Shu et al. 2014	Seed	PCC	36 ϵ_2 , 44 $\epsilon_3\epsilon_3$, 49 ϵ_4	$\epsilon_3 > (\epsilon_4 = \epsilon_2)$ (ACC) Note: ϵ_2 and ϵ_4 had opposite ageing trajectories	Yes
	~70 years	ϵ_4 : 70.2 ± 6.9 $\epsilon_3\epsilon_3$: 70.8 ± 6.9	Damoiseaux et al. 2012	ICA	DMN	43 ϵ_4 , 88 non- ϵ_4	$\epsilon_3 > \epsilon_4$ (ACC and PCC)	No
	~78 years *(median)	63-91 years	Machulda et al. 2011	Seed	PCC	56 ϵ_4 , 56 non- ϵ_4	$\epsilon_3 > \epsilon_4$ (posterior DMN)	No

If we considered an age-related decline in resting-state connectivity to be a marker of AD-risk, we would expect individuals with the protective $\epsilon 2$ allele to show opposite patterns of connectivity relative to those with $\epsilon 4$. It is surprising, therefore, that the only two studies to have included $\epsilon 2$ carriers have found that $\epsilon 2$ and $\epsilon 4$ carriers show similar levels of functional connectivity (Shu et al., 2014; Trachtenberg et al., 2012b). Trachtenberg et al. (2012) performed an ICA study of 10 RSNs and although they found no *APOE* differences within the DMN (Table 5.1), they found genotype effects within the two hippocampal, auditory, lateral visual and left frontal-parietal networks. In all these networks, $\epsilon 2$ and $\epsilon 4$ carriers showed similar alterations in functional connectivity relative to $\epsilon 3$ homozygotes. Shu et al. (2014) carried out a seed-based analysis of the PCC in a slightly older population and also found similar reductions in functional connectivity to the anterior cingulate cortex in $\epsilon 2$ and $\epsilon 4$ carriers compared to $\epsilon 3$ homozygotes. However, Shu and colleagues stress that the effects of genetic risk and resilience for AD may be better reflected in the ageing trajectories of functional connectivity within the DMN. With increasing age, $\epsilon 2$ carriers showed elevated connectivity whereas the $\epsilon 4$ carriers exhibited a decline in connectivity.

5.1.3 Objectives and hypotheses

Three things stand out from the literature: first, it is evident that there are changes in functional connectivity in *APOE* $\epsilon 4$ carriers even in the absence of any cognitive impairment. All participants included in Table 5.1 were cognitively healthy and in one instance were even screened for the absence of amyloid plaques (Sheline et al., 2010). Second, it may be that the effect of $\epsilon 4$ on resting-state functional connectivity may vary with age; after an initial phase of increased resting-state connectivity $\epsilon 4$ carriers seem to

decline faster than $\epsilon 3$ homozygotes. Third, it is clear that we need to study the $\epsilon 2$ allele, as the limited evidence to date prevents us from attributing any differences between $\epsilon 4$ carriers and “non-carriers” to lifetime risk for AD.

We therefore examined the effects of all three *APOE* alleles on resting-state functional connectivity in three distinct age groups (young, middle-aged and older) using both seed-based and ICA approaches. We focussed on the PCC and hippocampus for the seed analyses as these are key DMN structures and are among the first regions to be affected by AD pathology (Buckner et al., 2005; Frisoni et al., 2010; Minoshima et al., 1997). These regions are therefore of interest in relation to *APOE* and have been examined in *APOE* studies (Table 5.1). Based on the literature, we predicted that:

- a) $\epsilon 4$ carriers would show the highest resting-state connectivity in young individuals and the lowest in the old, with the middle age-range potentially representing a transition or turning point.
- b) if resting-state connectivity can in fact be used as a marker of genetic risk for AD then $\epsilon 2$ carriers should show opposite patterns of connectivity to $\epsilon 4$ carriers, at least in young and old groups, with the middle age group potentially serving as a crossing point for the two opposing trajectories.

5.2 Methods

5.2.1 Participants

Resting-state fMRI data from 53 young, 84 middle-aged and 242 older participants were analysed in this study. Details of participant recruitment, exclusion criteria and *APOE* genotyping are presented in Chapter 3.

5.2.2 MRI acquisition

Different resting-state scan sequences were used for each of the three age groups (Table 3.1). The young group received a simultaneous perfusion and BOLD imaging sequence, the middle group received a gradient echo planar imaging scan, and the older group received a multiband MRI scan (detailed parameters described in Chapter 3). The young and old groups were scanned at the FMRI Centre, Oxford while the middle group was scanned at OCMR Centre, Oxford. In all cases subjects were instructed to lie awake in dimmed light with their eyes open, blink normally, but not to fall asleep.

5.2.3 MRI analysis

Because the three age groups were scanned using different sequences, the effects of *APOE* on resting-state function were separately assessed for each age category. All image analysis was performed using FSL tools (Smith et al., 2004), and data for the three groups were analysed using a similar pipeline.

Pre-processing: Pre-processing was performed using MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components) and consisted of motion correction, brain extraction, high-pass temporal filtering with a cut off of 150s, and field-map correction (Beckmann et al., 2005; Jenkinson, 2003). ICA attempts to split the functional image into a set of independent components, or spatial maps, each with an associated time course. The result of single-subject MELODIC ICA is a set of components, some of which are related to activation, and others that are related to noise (*e.g.* other physiological processes respiration, or imaging artefacts due to motion). In order to reduce the presence of noisy components, a data-cleaning approach was applied. Single-subject ICA was followed by an automatic component classification with FMRIB's ICA-based X-noiseifier (FIX) to identify and regress out the “signal” of the artefactual components that reflect non-neuronal fluctuations (Griffanti et al., 2014; Salimi-Khorshidi et al., 2014). FIX attempts to auto-classify ICA components into “good” (signal) vs “bad” (noise) components, and needs to be trained from multiple datasets that have already had the ICA components classified into “good” and “bad” by hand. For FIX to work well, it is crucial to have well trained data that is also matched to the scan parameters of the data to be analysed. The young and middle age groups received an EPI scan and were analysed using a matched training dataset from the FSL FIX package (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FIX>; “Standard Data”; TR = 3 s, Resolution = 3.5 x 3.5 x 3.5 mm). This training dataset had been spatially smoothed during the pre-processing stage. Accordingly the scans from the young and middle age groups were also spatially smoothed using a Gaussian kernel of 6mm FWHM *before* pre-processing with FIX. Because the older group received an accelerated EPI scan with very different scan parameters than the other two age groups, this group was cleaned using a more study-specific training dataset derived from the Whitehall imaging study

(<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FIX>; “WH_II MB6 Data”, TR = 1.3 s, Resolution = 2 x 2 x 2 mm). As the “WH_II MB6” training dataset consisted of unsmoothed images, the scans from the older group were only smoothed *after* pre-processing with FIX, using a Gaussian kernel of 6mm FWHM.

Independent Component Analysis: The pre-processed smoothed and “fixed” functional data were affine registered to the individual's structural scan using FLIRT (Jenkinson and Smith, 2001; Jenkinson et al., 2002) and then optimized using boundary-based-registration (Greve and Fischl, 2009). Registration from structural to standard (MNI) space was carried out using FNIRT. Usually after performing a single-session ICA, the next step is to run a group-level ICA to describe large-scale patterns of functional connectivity across participants. This creates a study-specific group ICA template that is used to identify the resting-state networks of interest. However, because the young, middle and older participants in this study received different scans, their data could not be combined to create a study-specific ICA template. Therefore, an unbiased template of resting-state networks was created from an external dataset comprising 45 healthy participants (15 each in the young, middle and older age-ranges) who were scanned at the OCMR Centre (TR = 2 s, voxel dimension=3 x 3 x 3.5 mm³, acquisition time = 6 mins). This external dataset was specifically chosen to be as similar as possible in age, gender distribution and years of education with the participants in this group. Each of the resultant networks in this group ICA template was correlated with the original maps of the eight resting-state networks identified by Beckmann et al. (2005) to identify which map in the current data corresponded to the DMN in the Beckmann data. Two separate DMN components were identified by visual inspection of the data (Figure 5.1). Similar fractionation of the DMN into sub-components has been commonly reported, and has

been attributed to age-related changes in network organisation and cognitive function rather than methodological artefacts (Damoiseaux et al., 2012; Westlye et al., 2011). Specifically, in this study, the DMN split may have been driven by the wide age-range of participants included in the study-specific template. Therefore, for completeness we analysed both the DMN components to study the effects of *APOE*. As a control, the effects of *APOE* were also compared within the sensory motor network.

Dual regression was used to test the effects of *APOE* genotype on the DMN (Beckmann et al., 2005; Filippini et al., 2009). First, the average time course for each component was generated for each participant, by regressing the group-ICA spatial maps into each subject's preprocessed functional data (spatial regression). This created a set of subject-specific timeseries, which best represented the spatial pattern of the group-level network in the individual participant data. Next, those timeseries were regressed into the same preprocessed functional data, resulting in a set of subject-specific spatial maps (temporal regression). The result of this dual regression was a statistical map of parameter estimates (PEs) for each network describing the strength of each voxel's connection to that network for each participant. The individual participant maps of the DMN were then concatenated into a 4D file that was used to determine differences in DMN connectivity between *APOE* genotype groups. A GLM was applied using FSL's *randomise* non-parametric permutation-testing tool with 5000 permutations. To ensure that differences in functional connectivity were not driven by structural differences, grey matter maps prepared using the FSL *feat_gm_prepare* script were included as voxelwise covariates of no interest in the group analysis. The resulting statistical maps were thresholded using $z > 2.3$ and a family wise error corrected cluster significance threshold of $p < 0.05$ correcting for multiple comparisons across space.

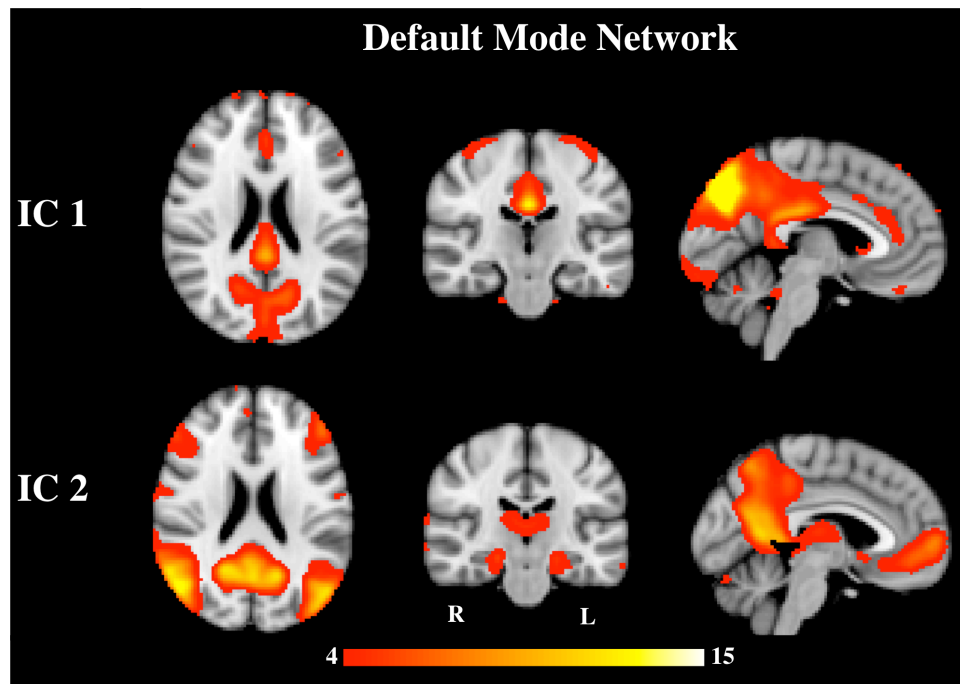


Figure 5.1: The default mode network

Group-ICA performed on the template derived from the external dataset revealed that the DMN was split into two independent components (ICs). Both components were examined for effects of *APOE*. Images are thresholded at $z > 4$ for visualization. R & L: right and left hemisphere.

Seed-based correlation analysis: To determine functional connectivity of the posterior cingulate cortex, and bilateral hippocampi, a seed-based voxel correlation was performed using FMRI Expert Analysis Tool (FEAT) Version 6.00. Here, “connectivity” refers to positive timeseries correlations between the seed region and other brain areas. The PCC seed was an 8 mm sphere centred at MNI coordinates $x, y, z = 0, -56, 20$ as described previously (Bluhm et al., 2009). The hippocampal seeds were created from MNI standard space atlases. The seeds were registered to each subject’s functional image using FNIRT. The registered seeds were visually inspected to ensure that only voxels within the ROI were included in the analysis. For each seed, an average hemodynamic timeseries was extracted from the pre-processed and “fixed” functional image of each subject. The

timeseries was used to compute positive and negative correlation maps, with WM and CSF signals as nuisance covariates. Specifically, for each seed and for each subject, the GLM included the timeseries for the seed, WM and CSF. Voxelwise group-level analyses were carried out in MNI standard space using FMRIB's Local Analysis of Mixed Effects (FLAME) with automatic outlier detection (Beckmann et al., 2003; Woolrich, 2008; Woolrich et al., 2004). This created thresholded z statistic maps of voxels positively and negatively correlated with each seed. The z statistic images were thresholded using $z > 2.3$ and a family wise error corrected cluster significance threshold of $p < 0.05$. Like with the ICA analysis, structural images were added as voxelwise covariates of no interest in the group analysis to isolate only those functional differences that could not be explained by structural changes. To extract the contrast of parameter estimate (COPE) value for each participant, significant clusters were used as binary masks. The resulting COPEs were z scored within each age group and plotted as graphical representations of the voxelwise results. For each seed, the mean COPEs were extracted using a whole-brain mask. This represents the mean connectivity of that seed to the brain for that participant. The extracted COPEs were z scored within each age group to allow for comparison across the lifespan.

5.2.4 Statistical analysis

Non-imaging variables were analysed using SPSS. ANOVA and post-hoc Bonferroni corrections were used for socio-demographic variables, head motion, regional brain volumes, and mean COPEs extracted from significant clusters. Exact Fisher's test was used for categorical variables. Mean COPEs were correlated with age using Pearson's correlation and Fisher r-to-z transformation was used to assess the significance of the differences between correlation coefficients of the *APOE* groups.

5.3 Results

5.3.1 Socio-demographics and brain structure

There were no significant *APOE*-related differences in age, education, gender, or family history of dementia in any age group. The older group had no genotype differences in MOCA scores. The *APOE* groups were also similar for whole-brain volumes, segmented GM, WM, CSF and hippocampal volumes within each age range (Table 5.2).

Table 5.2: Effect of *APOE* on socio-demographics and brain structure

Socio-demographics and brain volumes for the three *APOE* genotypes in the young, middle and old age groups. Values represent means $\pm$ standard deviations. P values are results of chi-square tests (for gender and family history of AD) and one-way ANOVA (for all other variables). GM, WM, CSF and hippocampal volumes are expressed as percentages of whole-brain volumes. L and R: left and right respectively.

	Young				Middle				Old			
	$\epsilon 2$ N=18	$\epsilon 3$ N=17	$\epsilon 4$ N=18	P	$\epsilon 2$ N=25	$\epsilon 3$ N=24	$\epsilon 4$ N=35	P	$\epsilon 2$ N=33	$\epsilon 3$ N=156	$\epsilon 4$ N=53	P
Socio-demographics												
Age (yrs)	24.11 $\pm$ 5.31	24.11 $\pm$ 4.96	23.88 $\pm$ 4.75	0.99	43.92 $\pm$ 5.35	46.33 $\pm$ 3.40	46.20 $\pm$ 5.90	0.17	68.45 $\pm$ 4.76	69.24 $\pm$ 5.12	68.44 $\pm$ 4.56	0.49
Education (yrs)	17.38 $\pm$ 1.75	17.50 $\pm$ 2.89	17.05 $\pm$ 2.18	0.84	14.66 $\pm$ 2.28	15.42 $\pm$ 2.77	14.40 $\pm$ 2.95	0.36	15.88 $\pm$ 3.46	15.45 $\pm$ 3.17	15.88 $\pm$ 3.43	0.61
Gender (% males)	38.89%	47.05%	44.44%	0.74	48%	58.30%	51.40%	0.76	87.90%	78.80%	84.90%	0.51
Family history of AD	2	1	0	0.53	4	8	10	0.33	2	2	4	0.08
MOCA	-	-	-	-	-	-	-	-	28.33 $\pm$ 1.11	28.10 $\pm$ 1.28	28.18 $\pm$ 1.24	0.39
Brain volumes												
Whole brain (mm ³)	1439 $\pm$ 101	1465 $\pm$ 122	1449 $\pm$ 178	0.85	1466 $\pm$ 115	1476 $\pm$ 147	1472 $\pm$ 136	0.96	1448 $\pm$ 130	1439 $\pm$ 123	1452 $\pm$ 96	0.78
GM (%)	43.60 $\pm$ 1.71	43.90 $\pm$ 1.60	44.30 $\pm$ 2.20	0.57	42.17 $\pm$ 1.49	41.83 $\pm$ 1.29	42.41 $\pm$ 1.35	0.29	36.92 $\pm$ 2.42	37.15 $\pm$ 2.06	37.25 $\pm$ 1.85	0.77
WM (%)	36.80 $\pm$ 1.58	36.90 $\pm$ 1.35	37.10 $\pm$ 1.51	0.82	36.19 $\pm$ 1.11	36.09 $\pm$ 1.11	36.43 $\pm$ 1.01	0.46	36.96 $\pm$ 1.62	37.15 $\pm$ 1.64	37.54 $\pm$ 1.59	0.2
CSF (%)	19.60 $\pm$ 1.33	19.20 $\pm$ 2.09	18.60 $\pm$ 2.39	0.35	21.64 $\pm$ 1.66	22.08 $\pm$ 1.46	21.16 $\pm$ 1.90	0.13	26.12 $\pm$ 3.46	25.71 $\pm$ 2.74	25.21 $\pm$ 2.40	0.31
L hippocampus(%)	0.26 $\pm$ 0.02	0.26 $\pm$ 0.03	0.27 $\pm$ 0.02	0.36	0.29 $\pm$ 0.02	0.28 $\pm$ 0.02	0.28 $\pm$ 0.02	0.81	0.24 $\pm$ 0.04	0.24 $\pm$ 0.03	0.24 $\pm$ 0.03	0.54
R hippocampus(%)	0.26 $\pm$ 0.02	0.26 $\pm$ 0.03	0.26 $\pm$ 0.02	0.5	0.29 $\pm$ 0.02	0.28 $\pm$ 0.03	0.29 $\pm$ 0.02	0.71	0.25 $\pm$ 0.03	0.24 $\pm$ 0.03	0.26 $\pm$ 0.03	0.09

5.3.2 Seeds

A seed-based analysis of hippocampal functional connectivity was first performed using separate seeds for the left and right hippocampi. The results were identical for both seeds (data not shown) and a subsequent analysis was carried out using a single seed of the bilateral hippocampi. Both the PCC and hippocampal seeds correctly identified the DMN in all participants (Figure 5.2). Specifically, activity within the anterior and posterior cingulate cortices, precuneus, hippocampal formation, dorsal and ventral medial prefrontal cortices, inferior parietal lobule and lateral temporal cortex was significantly correlated with both seeds. A visual inspection revealed more localized and intense correlation maps with increasing numbers of participants in the age groups.

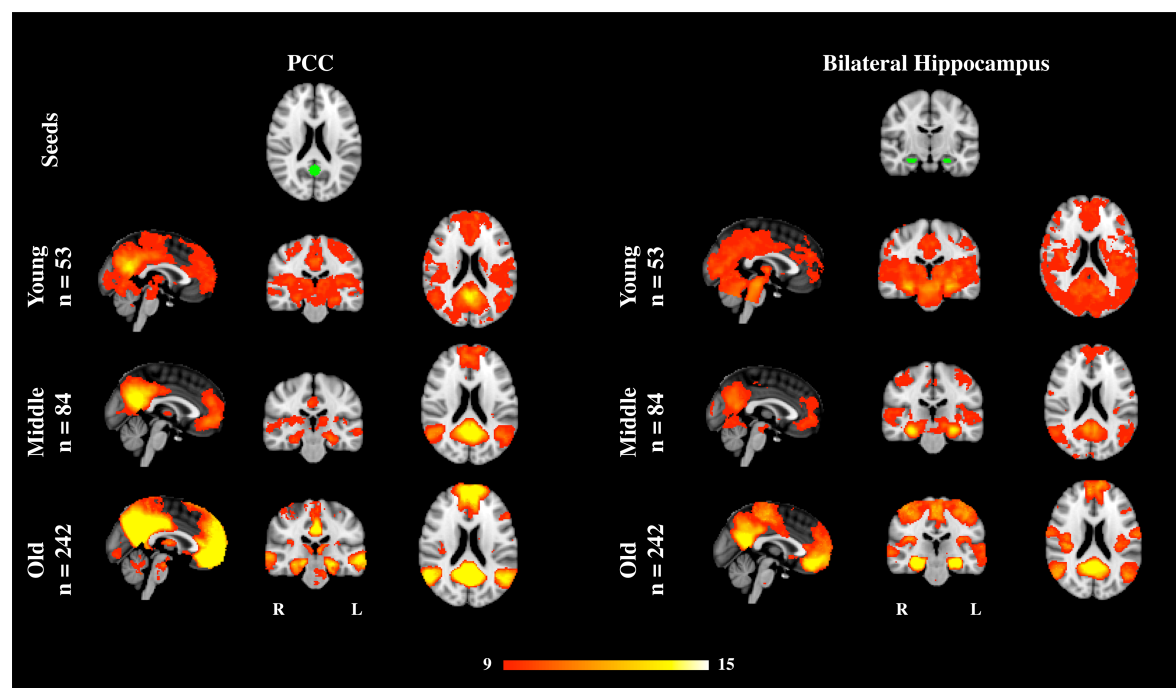


Figure 5.2: Functional connectivity maps of the PCC and hippocampal seeds.

The seeds (top row) and the corresponding connectivity maps (below) are displayed for the 53 young, 84 middle and 242 older individuals. Images are thresholded at $z > 9$ for visualization.

5.3.3 Functional connectivity of the PCC

APOE modulated the functional connectivity of the PCC in an allele-specific manner and the effects of both the $\epsilon 2$ and $\epsilon 4$ alleles varied with age. Detailed results of the voxelwise seed-based analyses and post-hoc ROI analysis of significant clusters are presented in Figure 5.3 and Figure 5.4

Within the young age group, $\epsilon 4$ carriers had the highest PCC connectivity measures within the hippocampus and basal ganglia when compared to $\epsilon 3$ homozygotes and $\epsilon 2$ carriers respectively. This pattern was reversed in the older group, where older $\epsilon 4$ carriers displayed the lowest and $\epsilon 2$ carriers the highest connectivity from the PCC to the precuneus. In the middle age range, both $\epsilon 3$ and $\epsilon 4$ carriers displayed higher connectivity from the PCC to the parietal operculum than $\epsilon 2$ carriers. Post-hoc tests of the significant clusters revealed the following patterns of PCC connectivity across the age groups: *young: $\epsilon 4 > (\epsilon 2 \text{ and } \epsilon 3)$; middle: $(\epsilon 3 \text{ and } \epsilon 4) > \epsilon 2$; old: $(\epsilon 2 \text{ and } \epsilon 3) > \epsilon 4$.*

The mean COPE values (representing the connectivity of the PCC to the whole brain) were extracted for all participants and z scored within each age group. Only $\epsilon 4$ carriers showed a significant negative correlation between age and global PCC connectivity ($\epsilon 2$: $r = 0.10$, $p = 0.38$, $n = 78$; $\epsilon 3$: $r = 0.01$, $p = 0.86$, $n = 198$, $\epsilon 4$: $r = -0.20$, $p < 0.05$, $n = 107$; Figure 5.4) and the correlation coefficients of $\epsilon 2$ and $\epsilon 4$ carriers were significantly different ($z = 2$, two-tailed $p < 0.05$).

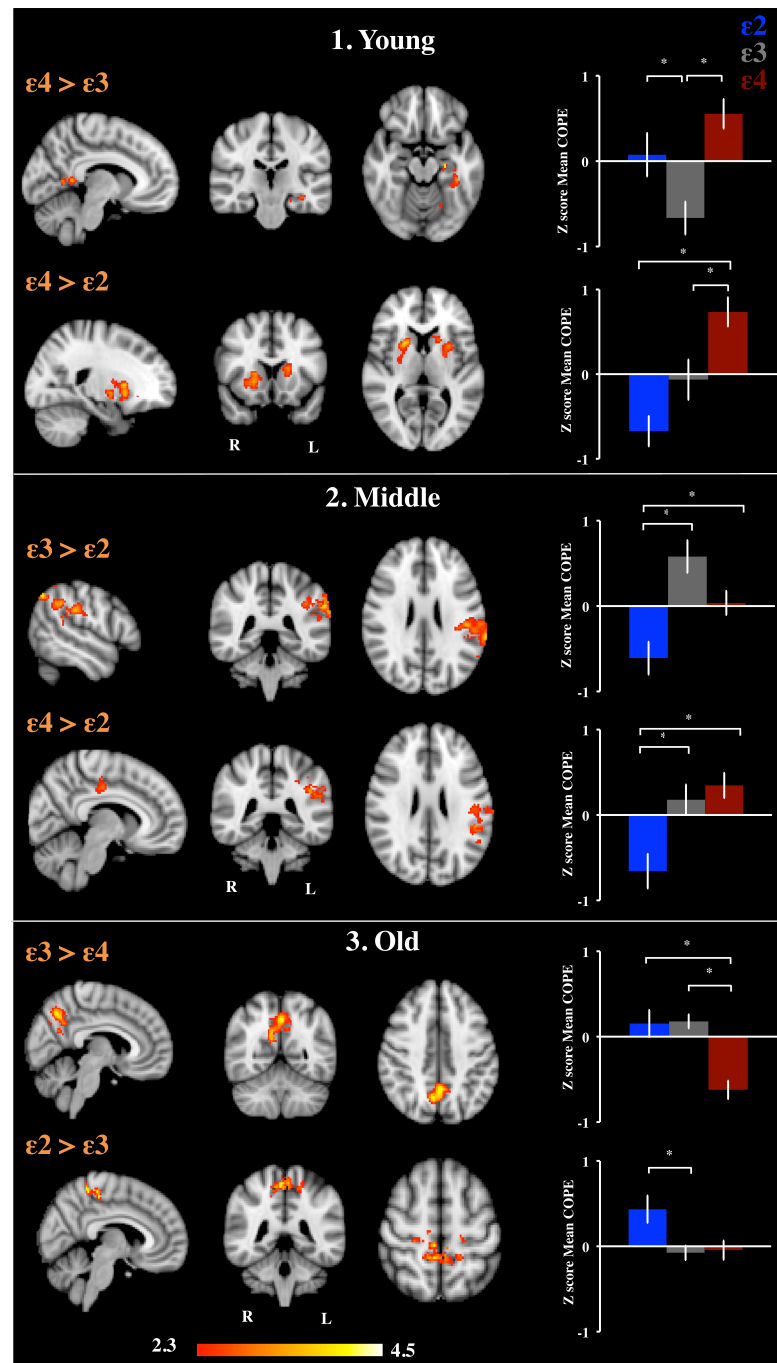


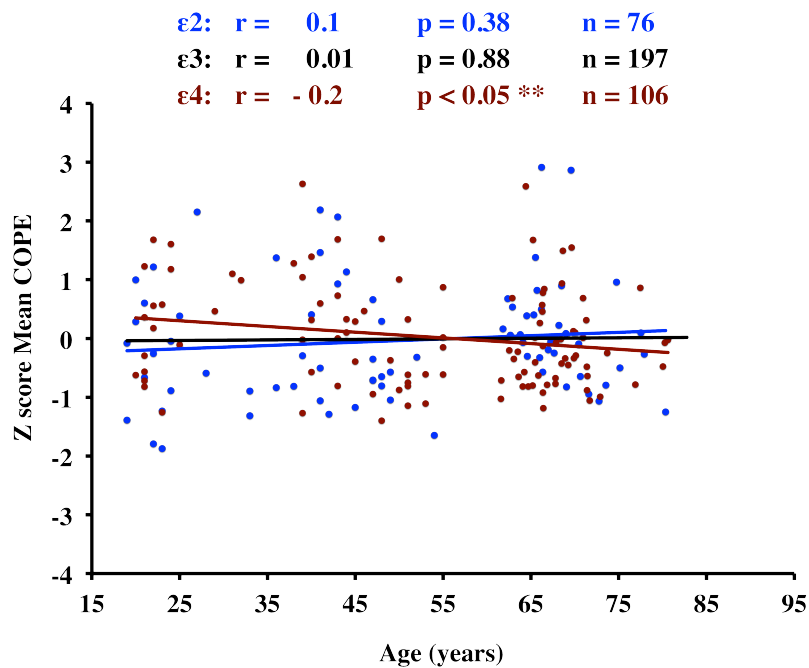
Figure 5.3: Seed-based analysis of the PCC.

Significant *APOE*-related differences in PCC connectivity were observed in the 1) young, 2) middle and 3) older age groups. Images are thresholded using $z > 2.3$ and corrected for multiple comparisons using a corrected-cluster significance threshold of $p < 0.05$. Z scores of mean COPEs extracted from the significant clusters are plotted on the right. Error bars represent standard errors of mean z-scored values. * denotes significant differences after post-hoc Bonferroni correction ($p < 0.05$). R and L: right and left hemisphere respectively.

(a) Details of significant clusters for PCC connectivity

Contrast	Cluster size	MNI coordinates x, y, z	Max Z	P	Location
Young					
$\epsilon 4 > \epsilon 3$	617	-24, -18, -16	4.18	<0.01	L hippocampus
$\epsilon 4 > \epsilon 2$	595	22, 10, 6	3.78	<0.01	R putamen
Middle					
$\epsilon 3 > \epsilon 2$	1443	-68, -36, 22	4.31	<0.001	Parietal operculum
$\epsilon 4 > \epsilon 2$	686	-52, -36, 28	3.25	<0.01	Parietal operculum
Old					
$\epsilon 3 > \epsilon 4$	802	6, -62, 42	4.26	<0.001	Precuneus
$\epsilon 2 > \epsilon 3$	615	6, -30, 52	5.27	<0.001	Precentral gyrus

(b) Correlation of mean PCC connectivity with age

 $\epsilon 2$ vs $\epsilon 4$: $p < 0.05^{**}$ **Figure 5.4: Statistical details of the effects of *APOE* on PCC connectivity.**

(a) Details of significant clusters for the PCC connectivity analysis. (b) Correlation of mean PCC connectivity with age. Only $\epsilon 4$ carriers show a significant negative correlation of the z-scored mean COPE with age and there is a significant difference in the correlation coefficients of $\epsilon 2$ and $\epsilon 4$ carriers. $^{**} p < 0.05$

5.3.4 Functional connectivity of the hippocampus

Like with the PCC, *APOE* modulated the functional connectivity of the hippocampus in an allele-specific and age-specific manner (Figure 5.5 and Figure 5.6).

Voxelwise analysis revealed that young $\epsilon 2$ carriers showed significantly lower hippocampal connectivity relative to $\epsilon 3$ homozygotes within the insular cortex. In the middle age group, similar $\epsilon 2$ -associated reductions in hippocampal connectivity were observed in the posterior DMN (posterior cingulate cortex, precuneus and lateral occipital cortex) when $\epsilon 2$ carriers were compared with $\epsilon 3$ and $\epsilon 4$ carriers. Conversely, in the older group, it was the $\epsilon 4$ carriers who had the lowest connectivity between the hippocampus and the posterior DMN. Post-hoc tests of the significant clusters revealed the following patterns of hippocampal connectivity across the age groups: *young and middle: ($\epsilon 3$ and $\epsilon 4$) > $\epsilon 2$; old: ($\epsilon 2$ and $\epsilon 3$) > $\epsilon 4$.*

None of the three *APOE* groups showed any significant correlations between age and whole-brain hippocampal connectivity ($\epsilon 2$: $r = 0.09$, $p = 0.42$, $n = 78$; $\epsilon 3$: $r = -0.09$, $p = 0.23$, $n = 198$, $\epsilon 4$: $r = -0.12$, $p = 0.23$, $n = 107$; Figure 5.5 and Figure 5.6) and there was no significant difference between the correlation coefficients of the $\epsilon 2$ and $\epsilon 4$ groups ($z = 1.39$; two-tailed $p = 0.17$).

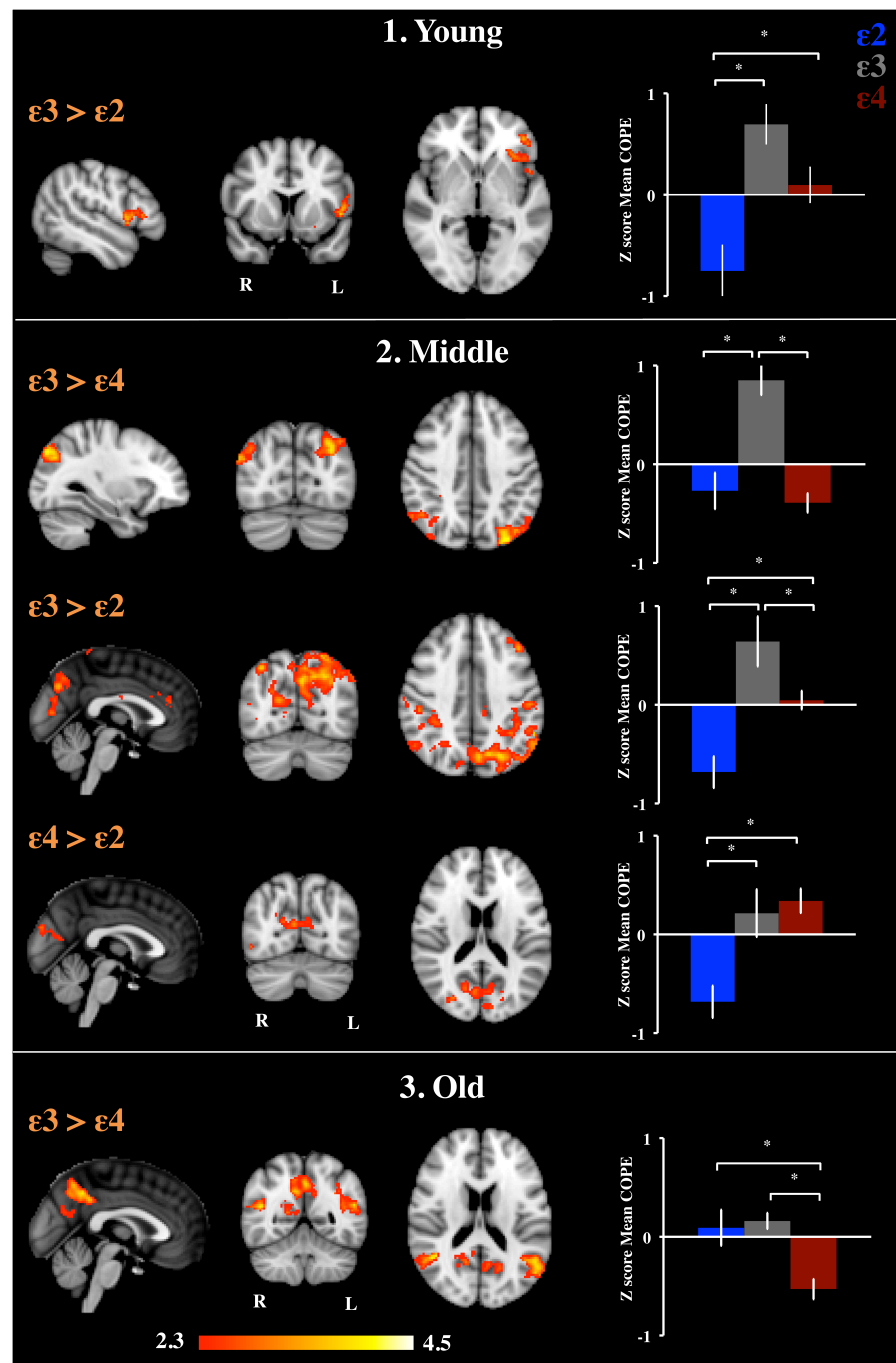
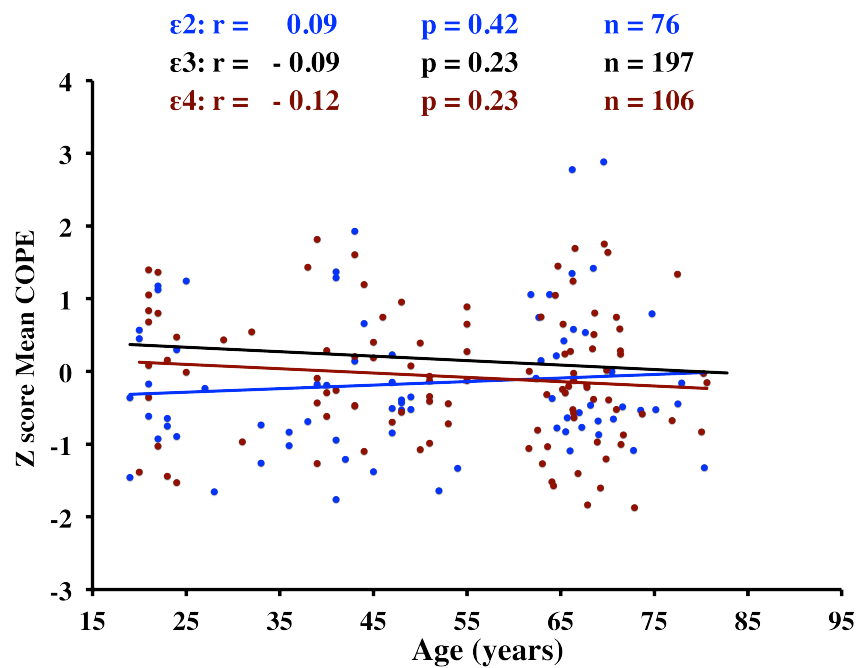


Figure 5.5: Seed-based analysis of the hippocampus.

Significant *APOE*-related differences in hippocampal connectivity were observed in the 1) young, 2) middle and 3) older age groups. Images are thresholded using $z > 2.3$ and corrected for multiple comparisons using a corrected-cluster significance threshold of $p < 0.05$. Z scores of mean COPEs extracted from the significant clusters are plotted on the right. Error bars represent standard errors of the mean z-scored values. * denotes significant differences after post-hoc Bonferroni correction ($p < 0.05$).

(a) Details of significant clusters for hippocampal connectivity

Contrast	Cluster size	MNI coordinates x, y, z	Max Z	P	Location
Young					
$\epsilon 3 > \epsilon 2$	725	-48, 10, 0	3.71	<0.01	Frontal operculum
Middle					
$\epsilon 3 > \epsilon 4$	742	-32, -80, -40	4.21	<0.01	Lateral occipital
$\epsilon 3 > \epsilon 2$	11674	38, -62, 48	4.71	<0.001	Lateral occipital
$\epsilon 4 > \epsilon 2$	599	8, -72, 16	4.08	<0.05	Precuneus
Old					
$\epsilon 3 > \epsilon 4$	1854	0, -50, 42	3.86	<0.001	Precuneus

(b) Correlation of mean hippocampal connectivity with age
 $\epsilon 2$ vs $\epsilon 4$: $p = 0.17$ **Figure 5.6: Statistical details of the effects of *APOE* on hippocampal connectivity**

(a) Details of significant clusters for the hippocampal connectivity analysis. (b) Correlation of mean hippocampal connectivity with age.

5.3.5 Functional connectivity of the DMN

Figure 5.1 showed that DMN was split into two components. Analysis of the first DMN component revealed significant *APOE* effects in the middle age group, with $\epsilon 2$ carriers showing lower functional connectivity than $\epsilon 4$ carriers in the right hippocampus and amygdala. In the second DMN component, older $\epsilon 2$ carriers had lower functional connectivity between the DMN and a small cluster in the visual cortex relative to $\epsilon 3$ homozygotes (Figure 5.7). There were no *APOE* differences in the young group for either component. Analysis of the sensory motor network (which served as a control group) revealed no *APOE* differences in this network in any age group.

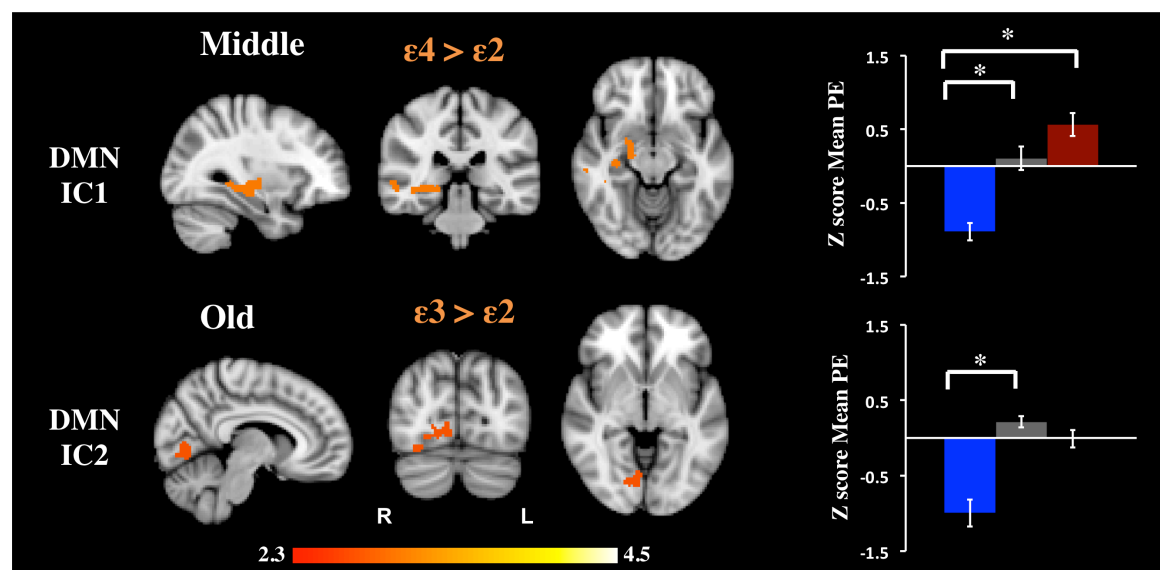


Figure 5.7: ICA analysis of the DMN.

Significant *APOE* differences in DMN connectivity were observed in the middle and older age groups. Images are thresholded using $z > 2.3$ and corrected for multiple comparisons. Z scores of mean COPEs extracted from the significant clusters are plotted on the right. Error bars represent standard errors of the mean z-scored values. * denotes significant differences after post-hoc Bonferroni correction ($p < 0.05$).

5.4 Discussion

This is the first detailed examination of the effects of the *APOE* $\epsilon 2$ allele on functional connectivity within the resting brain across a wide age-range. By studying three distinct and relatively large groups of young, middle-aged and older individuals we showed that the effects of the three *APOE* alleles on resting-state activity vary with age. Because the three age groups received different resting-state fMRI scan acquisitions, we were unable to combine their MRI data to study any interaction between *APOE* and age. All age-related trends discussed in this section are therefore observational. Our seed-based analysis revealed that $\epsilon 2$ and $\epsilon 4$ carriers displayed opposing age-related trends in functional connectivity of the hippocampus and PCC. Specifically, we found that of the three *APOE* groups, $\epsilon 2$ carriers had relatively lower functional connectivity of these seeds to the rest of brain in younger ages, but significantly higher connectivity in older ages, while $\epsilon 4$ carriers displayed the opposite pattern. These significant *APOE*-related differences were observed in key parts of the DMN. However, while we obtained allele- and age-specific effects of *APOE* using the seed-based analysis, we did not observe strong *APOE* effects using the ICA-based analysis of the DMN.

5.4.1 Seed-based vs. ICA-based analyses

Before interpreting our seed-based results, we must consider why we observed a lack of convincing *APOE* effects in the ICA analysis. This was surprising given that similar brain regions were examined using both seed and ICA-based analyses. However, other studies have also reported different outcomes with the two analysis methods (Westlye et al., 2011). Using ICA, we found effects in the hippocampus in the middle group that were

consistent with the seed results, but the small size and unusual location of the cluster in the old group suggests that we should interpret this result with caution (Damoiseaux et al., 2012). This is contrary to past ICA reports of robust changes in DMN connectivity between $\epsilon 4$ and $\epsilon 3$ carriers, including those from our own research group (Filippini et al., 2009; Damoiseaux et al., 2012). Several studies have highlighted the impact of analysis methods on the reproducibility of ICA analyses (Franco et al., 2013; Zuo et al., 2010) however these studies also seem to suggest that ICA analyses are generally more reproducible than seed-based analyses (Zuo and Xing, 2014). Thus, we expected to find convincing effects of *APOE* (at least for the $\epsilon 4$ allele) using an ICA-based analysis of the DMN, particularly since the DMN has been consistently implicated in AD.

The absence of strong ICA results in this study could be due to differences in the preprocessing protocol between this and previous studies. As discussed in Section 5.2.3, we used a rigorous “data-cleaning” approach (FIX) to remove non neural-related sources of inter-individual variability, which past studies have not used. FIX, in addition to removing noise, may also have weakened the signal, which could have resulted in an overall loss of statistical power. Our research group has recently shown that this can influence results, particularly in studies of small sample sizes (Griffanti et al., *in press*). This may be why we found no *APOE* effects the young group, which relatively smaller than the middle and older groups.

Our weak ICA results could also have been driven by the choice of our ICA template. Our research group has also shown that the method of creating ICA templates can impact the reproducibility of resting fMRI results (Griffanti et al., *in press*). Past ICA examinations of *APOE* have typically created a study-specific template for dual

regression. However, because all the three age groups in this study received different MRI scans, their data could not be combined to create a study-specific template. We therefore opted to use a template made from an external dataset. This template was created using subjects across a wide age-range (15 young, 15 middle and 15 old, Section 5.2.3). Thus, although it was representative of our dataset and proved to be the best alternative to a study-specific template, it is worth exploring whether it was the choice of template that impacted our results.

Therefore, while we were surprised to find no strong effects of *APOE* on DMN connectivity using ICA, we caution against interpreting this as a lack of an overall effect of *APOE* on the DMN, as this may have been driven by the limitations of our dataset.

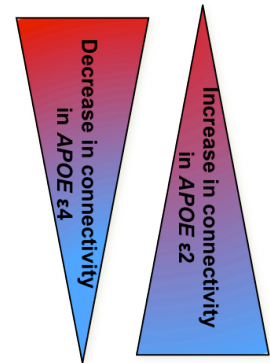
5.4.2 APOE $\epsilon 2$ and $\epsilon 4$ carriers show opposite ageing trajectories

Using more hypothesis-driven seed-based analysis, we observed that $\epsilon 4$ carriers show a reduction while $\epsilon 2$ carriers and $\epsilon 3$ homozygotes display a gradual increase in PCC functional connectivity from young to middle to older ages (Table 5.3). Age-related correlations revealed a negative ageing trajectory for $\epsilon 4$ carriers, *i.e.* the older the $\epsilon 4$ carriers, the weaker were their functional connectivity measures to the PCC. Similar trends were observed for the hippocampal seed, where $\epsilon 2$ carriers had an initial phase of low hippocampal connectivity in early and mid-adulthood that strengthened with age.

Table 5.3: Summary of seed-based analysis

Contrasts where $\epsilon 4$ -carriers have relatively higher and lower functional connectivity are depicted in red and blue respectively

Age group	Participants	Hippocampal connectivity	PCC connectivity
Young ~ 24 years	18 $\epsilon 2$, 17 $\epsilon 3\epsilon 3$, 18 $\epsilon 4$	$\epsilon 3 > \epsilon 2$ (frontal operculum)	$\epsilon 4 > \epsilon 3$ (hippocampus) $\epsilon 4 > \epsilon 2$ (putamen)
Middle ~ 45 years	25 $\epsilon 2$, 24 $\epsilon 3\epsilon 3$, 35 $\epsilon 4$	$\epsilon 4 > \epsilon 2$ (precuneus) $\epsilon 3 > \epsilon 2$ (lateral occipital) $\epsilon 3 > \epsilon 4$ (lateral occipital)	$\epsilon 4 > \epsilon 2$ (parietal operculum) $\epsilon 3 > \epsilon 2$ (parietal operculum)
Older ~ 68 years	33 $\epsilon 2$, 156 $\epsilon 3\epsilon 3$, 53 $\epsilon 4$	$\epsilon 3 > \epsilon 4$ (precuneus)	$\epsilon 3 > \epsilon 4$ (precuneus) $\epsilon 2 > \epsilon 4$ (precentral gyrus)

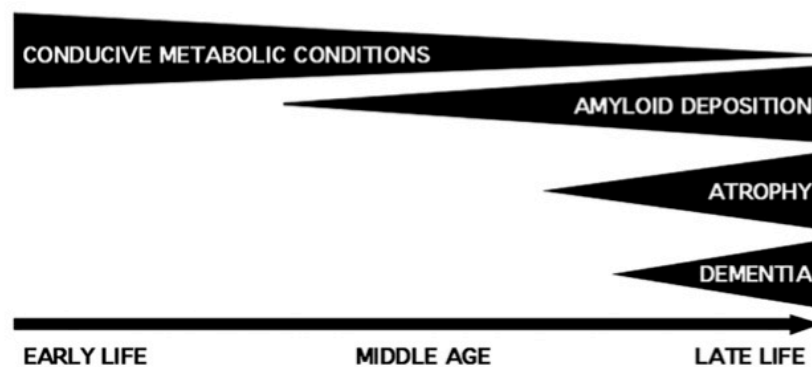


Based on this we propose that the point at which regional functional connectivity in $\epsilon 4$ carriers begins to decline and that in $\epsilon 2$ carriers starts to trend upwards occurs during mid-life, between 40-60 years. In this middle age-range we observed *APOE* patterns similar to both young and older groups, presumably because this group is in the midst of a transition phase from young to old. Indeed other groups have also estimated a “*turning point*” for $\epsilon 4$ at ~55 years (Shu et al., 2014), which we propose also applies to $\epsilon 2$ carriers. It is fitting then that studies on resting-state functional connectivity conducted specifically in this middle age-range find $\epsilon 2$ and $\epsilon 4$ similarities, perhaps reflecting a cross point in their trajectories (Trachtenberg et al., 2012b). We note that Shu et al., (2014) found these similarities even in a slightly older group of participants; however in their study they also stressed that $\epsilon 2$ and $\epsilon 4$ carriers showed opposite correlations of functional connectivity with age ($\epsilon 2$: positive correlation, $\epsilon 4$: negative correlation). Given that this is in line with our observation of opposite ageing trends, we suggest that rather than a single measure of mean connectivity, a life-long trajectory of how this connectivity varies with age may be a more accurate representation of how *APOE* influences risk and resilience for AD.

5.4.3 *Biological significance of the opposite ageing trajectories*

The main biological effect of *APOE* in relation to AD is that $\epsilon 4$ carriers appear to develop earlier and higher levels of $A\beta$ pathology whereas the amyloid burden is delayed and relatively lower in $\epsilon 2$ carriers (Chapter 2). Amyloid deposition begins in brain structures that are part of the DMN and gradually spreads across the brain as the AD progresses. In their seminal study on the role of the DMN in AD, Buckner *et al* (2005) demonstrated a correlation between patterns of activity in DMN hubs in young adults and the distribution of amyloid deposition in elderly patients with AD (Buckner *et al.*, 2005). Using FDG-PET, they found that parts of the DMN that were metabolically active in young adults were susceptible to increased amyloid deposition in older AD patients. Accordingly, they proposed that patterns of high functional activity in young adults might be conducive to late life cognitive decline (Figure 5.8).

A schematic illustration of one possible configuration of lifelong events that lead to AD. Conducive metabolic conditions, associated with default mode activity patterns, may lead to regionally specific amyloid deposition.



Randy L. Buckner *et al.* J. Neurosci. 2005;25:7709-7717

Figure 5.8: The possible lifelong events that lead to AD.

High metabolic activity within the DMN in young adulthood may lead to regionally specific late-life amyloid deposition. Figure from Buckner *et al.*, 2005.

Here, we suggest how *APOE* may fit into this hypothesis and put forth a model for the interaction between *APOE*, functional connectivity, amyloid burden and risk for AD (Figure 5.9).

In our study we found that increased resting-state activity in young adults was a characteristic of $\epsilon 4$ carriers, who are at a genetic risk of developing AD in the future. Notably, it was the same genetic group that also showed a decline in functional connectivity in late life, and this trend fits with the consequences of an age-related tapering of metabolic activity depicted in Figure 5.8. It could be, therefore, that a genetic predisposition to AD creates a functional environment in early adulthood that sets the stage for late-life amyloid deposition (Machulda et al., 2011). This amyloid burden could then in turn lead to a decline in functional connectivity, as seen in AD patients (Liu et al., 2008). If the interaction between a buildup of amyloid and reduction in functional connectivity surpasses a certain threshold, it could eventually lead to cognitive decline. Conversely, early-mid life hypoactivity in $\epsilon 2$ carriers may slow down and delay amyloid deposition, consequently enabling relatively stronger functional connectivity in later life and reducing the risk for developing AD (Shu et al., 2014). The fact that in this study we observed life-long *APOE*-specific alterations in connectivity in DMN centers like the hippocampus, PCC, precuneus and lateral temporal cortex, which are amongst the first regions to develop amyloid pathology only serves to bolster the idea that functional changes in these regions may influence susceptibility to AD. Our finding of a reduction in functional connectivity between the hippocampus and precuneus/PCC in old $\epsilon 4$ carriers lends additional support to the notion of AD as a “disconnection syndrome” that is preceded by a functional isolation of the hippocampus and other structures involved in memory (Delbeuck et al., 2003).

We note that although the participants in this study were cognitively healthy, they could in fact possess clinically silent amyloid plaques in the regions showing alterations in resting-state activity. Future studies using a combination of resting-state fMRI and amyloid PET imaging could help us understand how *APOE* mediates the relationship between connectivity within the resting brain and neuropathological burden.

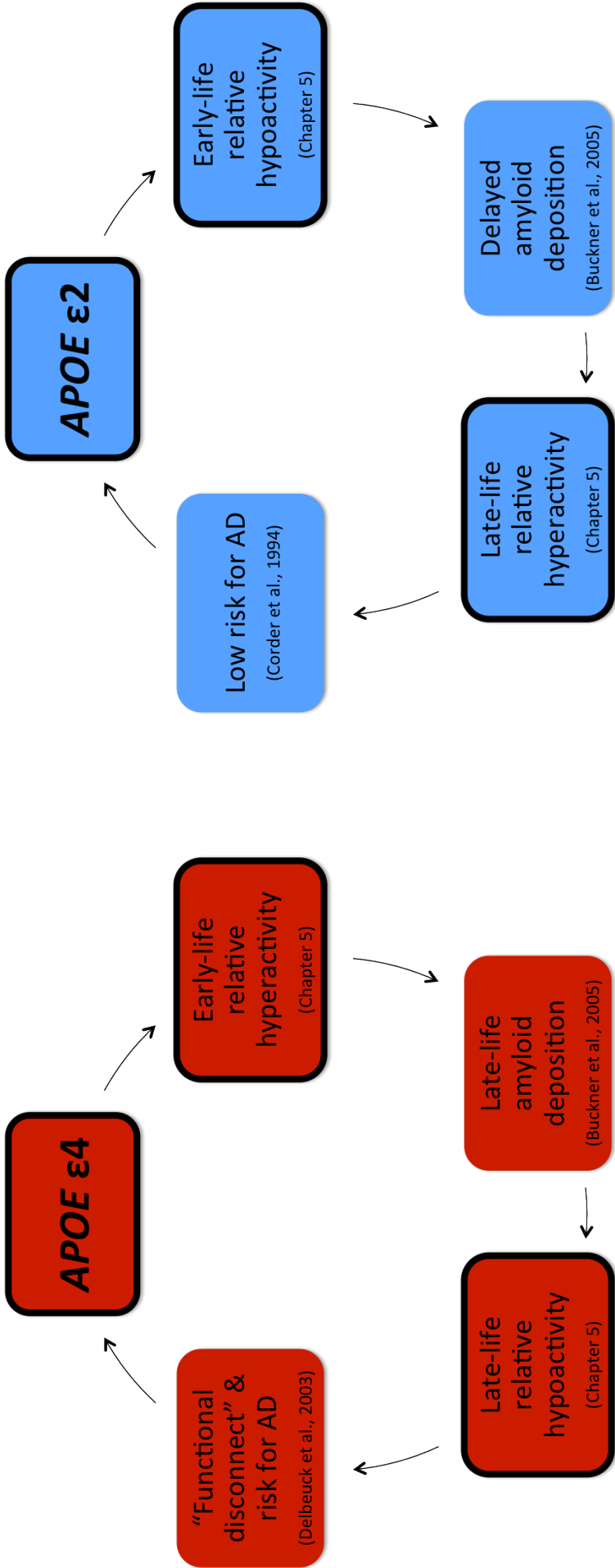


Figure 5.9: Proposed model for the interaction between APOE, functional activity, amyloid and risk for AD.

Based on the findings from this thesis (represented as boxes with dark outlines) and the relevant literature (boxes without outlines), we suggest how the two differing APOE alleles may influence risk and protection to AD via their effects on early-life events like functional activity.

5.4.4 Limitations and future considerations

Our main finding of hyperactivity/increased connectivity in young and hypoactivity in older $\epsilon 4$ carriers is in line with previous studies in our lab (Filippini et al., 2009) and elsewhere (Table 5.1). We have, for the first time, shown that the opposite trend is present in $\epsilon 2$ carriers. This study has its strengths in its relatively high sample size, its inclusion of the $\epsilon 2$ allele, and its wide age-range. However, it is limited by the fact that the three age groups underwent different fMRI scans. It is essential to corroborating our findings by performing age-related correlations in functional connectivity or quantifying interactions between *APOE* and age, which, because of our methodological constraints, we could only infer from observation alone.

Given that our PCC and hippocampal seeds correctly identified the DMN in all participants, we can be confident in our results from the seed analyses. However, our ICA results were less pronounced than seed-based findings. In Section 5.4.1, we discussed how this could have been driven by methodological differences between this and previous ICA studies. Another consideration is that the older group in this study was largely male (~85%). I discussed this concern in Chapter 4, and it may be also relevant here given that recent studies (Damoiseaux et al., 2012), including those from our lab (Heise et al., 2014) have suggested that the effects of *APOE* on resting-state connectivity may depend on gender, with females showing more pronounced changes in functional connectivity. A recent ICA analysis of the DMN found that female $\epsilon 4$ carriers showed significantly reduced DMN connectivity compared to $\epsilon 3$ homozygotes and male $\epsilon 4$ carriers (Damoiseaux et al., 2012). Notably, male $\epsilon 4$ carriers were no different from male $\epsilon 3$ homozygotes in their study. Similarly, in a hippocampal seed-based analysis similar to

ours, Heise et al. (2014) found that across participants aged 30-80 years, only the female $\epsilon 4$ carriers showed a significant negative correlation between age and hippocampal functional connectivity measures, while connectivity in male $\epsilon 4$ carriers and both male and female $\epsilon 3$ homozygotes did not correlate with age. The relatively low number of female $\epsilon 4$ carriers in our older group (8 out of 53 $\epsilon 4$ carriers) could therefore also explain why given our large overall sample size, we only observed a moderate negative correlation of age with PCC functional connectivity ($p < 0.05$, Figure 5.4) and no significant allele-specific ageing trajectory for the hippocampus seed (Figure 5.6). Although it is unlikely that gender is the only variable influencing the relationship between *APOE*, age and functional connectivity, it may certainly be one of the mediating factors given that female $\epsilon 4$ carriers are at a higher risk of developing AD (Farrer et al., 1997; Payami et al., 1996). While including more females in our older group may have strengthened our findings, we can be confident in our seed results given that we found consistent $\epsilon 4$ reductions in the voxelwise analyses for both seeds (Figure 5.3 and Figure 5.5) in a group that was largely male.

We also note that significant *APOE* effects were not limited to brain regions that are typically affected in early AD. As expected, we found *APOE*-related alterations in connectivity between the PCC, hippocampus and precuneus. But we also observed changes in connectivity between the PCC and the putamen and parietal operculum, and between the hippocampus and lateral occipital cortex (Table 5.3). This indicates a more widespread effect of the gene on global resting-state functional connectivity, beyond regions that make up the default mode network. It is becoming increasingly evident that maintenance of cognitive health relies on the coordinated activity of resting-state networks. It is not just the individual networks but also the *relationship* between networks

that may provide important insights into AD-related abnormalities within the resting-state brain. For instance, structural integrity of the salience network (a resting-state network comprising the anterior cingulate cortex and bilateral insulae) is essential for and predicts efficient functioning of the DMN (Bonnelle et al., 2012). Further, increasing AD severity is associated with widespread changes in both intranetwork and internetwork correlations between the DMN, sensory motor network and the dorsal attention network (Brier et al., 2012). Investigations of the effect of *APOE* on internetwork connectivity using advanced network modelling approaches like canonical correlation analyses (Smith et al., 2015, 2013) would provide additional information that would supplement the findings from seed-based correlations and ICA.

Further to our discussion on the timing of the crossover in ageing trajectories for $\epsilon 2$ and $\epsilon 4$ carriers, we suggest that future studies should perform a more detailed decade-by-decade analysis of resting state functional connectivity. Although our study included participants aged 20-85 years, we did not have a consistent number of participants in each decade to perform an in-depth analysis (for instance, we had relatively few participants in the 50-60 year age range, which our findings suggest may be a critical turning point in the trajectory).

Our study also raises a few questions. For instance, how does the effect of *APOE* on resting connectivity impact cognitive performance? Does early-life hyperconnectivity confer an early cognitive advantage to $\epsilon 4$ carriers? Likewise, does it influence cognitive performance in $\epsilon 2$ carriers? Relative to $\epsilon 3$, *APOE* $\epsilon 4$ has been associated with higher IQs and better cognitive performances in young adults (Rusted et al., 2013; Yu et al, 2000; Puttonen et al., 2003). Interactions between *APOE*, cognition and age have sparked the

idea that the varying effect of $\epsilon 4$ on cognitive performance can be reconciled through the antagonistic pleiotropy hypothesis, which suggests that the $\epsilon 4$ allele may actually be beneficial in early ages and exert deleterious effects on cognition in later life (Tuminello and Han, 2011). It would therefore be of interest to study how cognitive performance fits into the model proposed in Figure 5.9. Based on our findings of increased functional connectivity between regions involved in memory (*i.e.* PCC $\rightarrow$ hippocampus) in young $\epsilon 4$ carriers, we could speculate that these individuals may possess an early-life cognitive advantage that crosses over to a risk for dementia in mid-life due to their vulnerability to build-up of amyloid. In the following chapter, I will focus purely on the young group and ask what factors may mediate this link between *APOE* $\epsilon 4$, early life hyperactivity and amyloid burden (Figure 5.10).

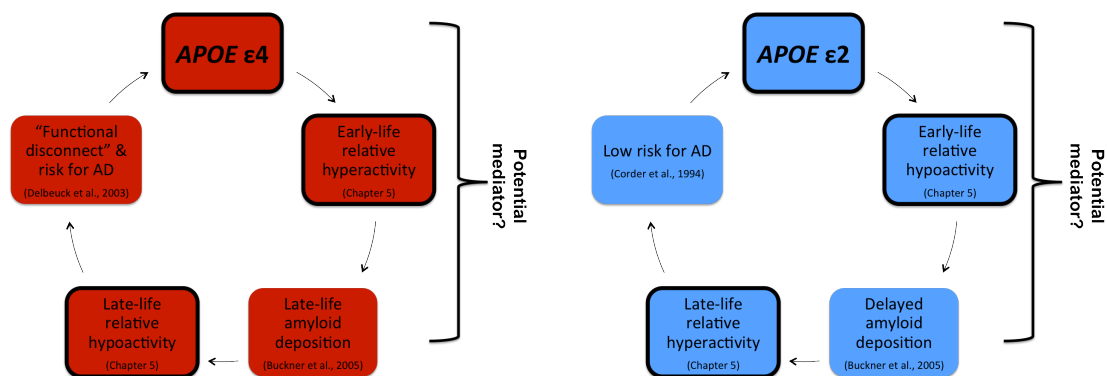


Figure 5.10: Questions to be addressed in Chapter 6.

Chapter 6

APOE and cerebrovascular health

In Chapter 5 I have shown that the three *APOE* alleles influence functional connectivity within the resting brain differently across the lifespan and that these effects are apparent even in early adulthood. Our lab and others have previously found that increases in BOLD activity are present not only at rest, but also during a memory-encoding task in young $\epsilon 4$ carriers. I have discussed the possibility of a link between early-life functional hyperactivity and susceptibility to late-life amyloid deposition in the previous chapter. Here, I further explore this link by studying the early effects of *APOE* on vascular health in the brain.

A modified version of this chapter has been published as an article titled “*Reduced cerebrovascular reactivity in young adults carrying the APOE $\epsilon 4$ allele*” in *Alzheimer’s & Dementia* (Suri et al., 2014a).

6.1 Introduction

APOE begins to modulate brain function in an allele-specific manner decades before potential cognitive decline. I have shown that its effects on resting-state functional connectivity are apparent even in individuals who are as young as 20 years old and that they vary during the lifespan (connectivity in young: $\epsilon 4 > (\epsilon 2 \text{ and } \epsilon 3)$; connectivity in old: $(\epsilon 2 \text{ and } \epsilon 3) > \epsilon 4$; Chapter 5). While the patterns in older $\epsilon 4$ carriers seem to fit with

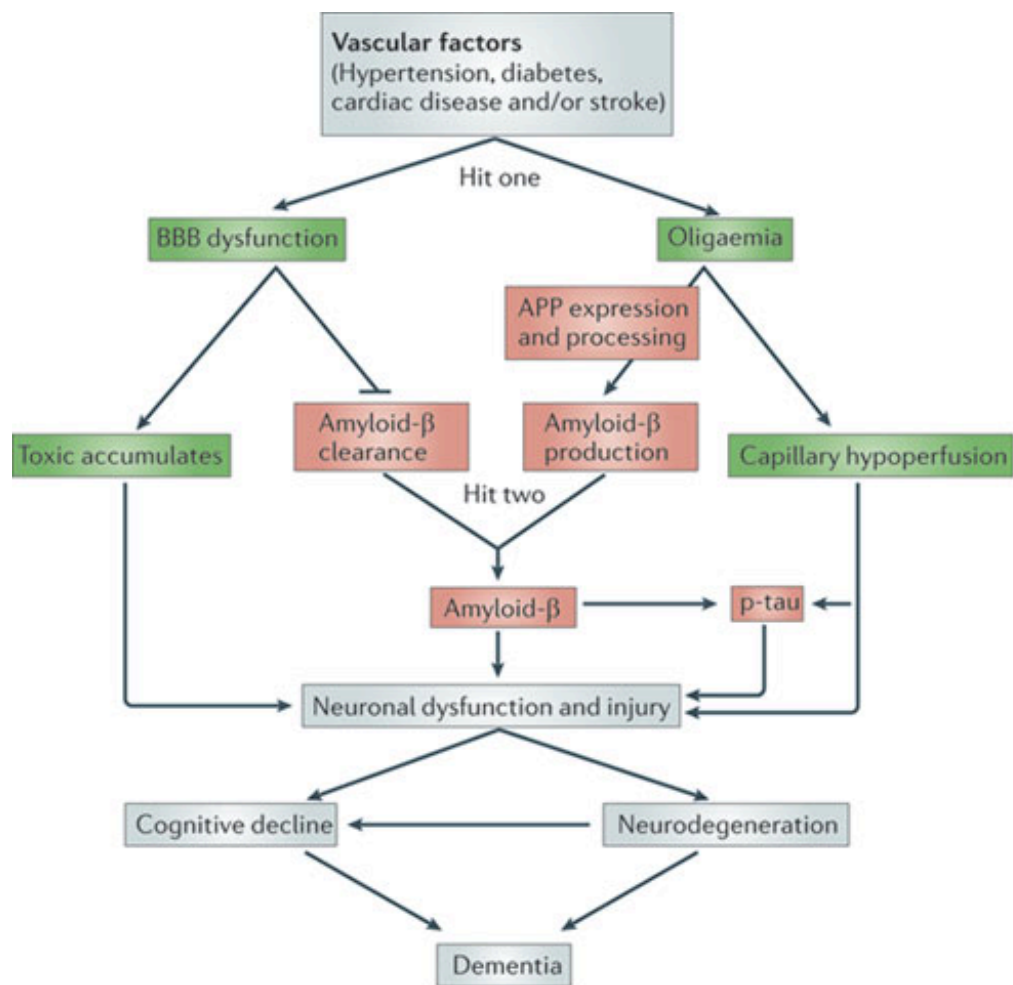
the trends observed in AD patients (connectivity in *healthy controls* > *AD patients*), those in younger adults contrast with the expected direction of activity. Interestingly, these findings are not limited to resting-state activity alone; our lab (Filippini et al., 2009) and others (Dennis et al., 2010; Filbey et al., 2006) have previously observed similar increases in BOLD activity during memory tasks in young $\epsilon 4$ carriers relative to $\epsilon 3$ homozygotes, and disproportionate reductions in BOLD activity in older $\epsilon 4$ carriers (Filippini et al., 2011; Trachtenberg et al., 2012c). Early-life hyperactivity has been attributed both to the antagonistic pleiotropic effect (*i.e.* $\epsilon 4$ carriers may actually have a cognitive advantage in early life) and neural compensation (*i.e.* $\epsilon 4$ carriers need to recruit more areas of the brain in order to maintain cognitive performance). At the end of this chapter, I will propose a new model to tie *APOE* in with BOLD activity and risk for AD. This model is based on Buckner *et al.*'s hypothesis that heightened BOLD activity in early life may lay the ground for the build-up of AD-related amyloid pathology (Figure 5.8). I will also draw from Zlokovic *et al.*'s discussion on the neurovascular pathways to AD outlined in Figure 6.1.

6.1.1 AD as a vascular disorder

Neurons are dependent on blood flow to the brain in order to maintain a constant supply of oxygen (O_2) and nutrients, and to remove and other toxins. When blood flow to the brain stops, neurons undergo irreversible damage within seconds. It is therefore important for us to consider neurons not as an isolated entity, but as a “neurovascular unit”, coupled with the vascular and circulatory system. This interaction between cerebral vasculature and neuronal integrity is particularly vital in disorders like AD, given that patients with AD often also present with cardio/cerebrovascular disorders and there is a recognized

overlap in the risk factors for the two conditions (Breteler, 2000; Claassen and Zhang, 2011; de la Torre, 2004). There is a general consensus that AD, in addition to being a neuropathological disorder is also a vascular one, strongly associated with impairments in circulation, blood-brain barrier function, cerebral microvasculature and cerebral blood flow (Austin et al., 2011; de la Torre, 2004; Liu et al., 2014; Zlokovic, 2011, 2005).

In Chapter 2, I discussed the amyloid hypothesis of AD, which describes a cascade of events initiated by amyloid deposition and culminating in neuronal loss and dementia. Recently, Zlokovic (2011) put forth the “*two-hit vascular hypothesis*” which incorporates a non-amyloid pathway that is instigated by vascular dysfunction (Zlokovic, 2011). This takes into account information from molecular, epidemiological, pharmacological, neuroimaging and clinical studies which suggest that disruptions to the brain’s microcirculation (“*hit one*”) lead to a series of downstream events that result in diminished amyloid- β clearance to the periphery and enhanced amyloid- β deposition (“*hit two*”) (Austin et al., 2010; Kalaria, 2009, 2000). Damage to the blood-brain barrier and impairments in small vessel blood flow can lead to the leakage and accumulation of neurotoxic molecules, and this eventually ties in with the neuronal damage caused by the amyloid cascade (Figure 6.1).



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Figure 6.1: The two-hit vascular hypothesis of AD.

Vascular risk factors contribute to amyloid pathology, which in turn leads to the pathological cascade culminating in dementia. The red boxes denote the amyloid pathway; the green boxes represent the non-amyloid vascular pathway; the grey boxes highlight the downstream events common to both pathways. Figure from Zlokovic, 2011.

6.1.2 APOE and the neurovascular unit

How does *APOE* tie in with the concept of AD as a vascular disorder? The apoE protein is primarily responsible for the transport of cholesterol, and is closely associated with risk for cardiovascular disorders. Like with AD, this risk manifests in an allele-specific manner, with $\epsilon 4$ carriers being most at risk for arteriosclerosis, coronary artery disease, and poor recovery from stroke, and $\epsilon 2$ heterozygotes being “protected” from these disorders (note that $\epsilon 2$ homozygotes may be at a risk for type III hyperlipoproteinemia) (Davignon et al., 1998; Mahley, 1988). The ApoE proteins mediate this risk for cardiovascular events regulating cholesterol levels: the $\epsilon 2$ allele is generally associated with lower plasma cholesterol than the other *APOE* alleles (Davignon et al., 1998; Ehnholm et al., 1986; Xhignesse et al., 1991). There is therefore a close association between *APOE* and lifetime risk for cardiovascular events (Bu, 2009). As vascular dysfunction can contribute to the AD cascade (Figure 6.1), it is worth asking how *APOE* affects vascular health specifically within the brain and how this ties in with the observed increases in neuronal (BOLD) activity in young adulthood.

6.1.3 Cerebrovascular reactivity: linking APOE, BOLD activity and AD

Our body maintains a constant supply of blood to the brain by dilating and constricting blood vessels in accordance with the demands. For instance, during a task, the heightened neuronal activity creates a demand for O_2 , which is met by an increase in the blood flow to the brain. As discussed in Chapter 3, this neurovascular coupling forms the basis of the BOLD signal. The ability of the brain’s blood vessels to dilate in response to a stimulus is called *cerebrovascular reactivity* (CVR), which serves as a surrogate marker of vascular

health in the brain (Ainslie and Duffin, 2009). This is most often measured using a vasodilatory stimulus like CO_2 . Within the brain, an increase in CO_2 is interpreted as a decrease in O_2 . This imbalance initiates a regulatory response mediated by an increase in perivascular pH, the activation of potassium channels and an influx of potassium ions, hyperpolarization of the endothelial cell, closure of calcium channels and a subsequent decrease in intracellular calcium, leading to the relaxation and dilation of vascular smooth muscles (Glodzik et al., 2013). The outcome of this cascade is an increase the regional cerebral blood flow in order to maintain constant tissue oxygen tension (Figure 6.2).

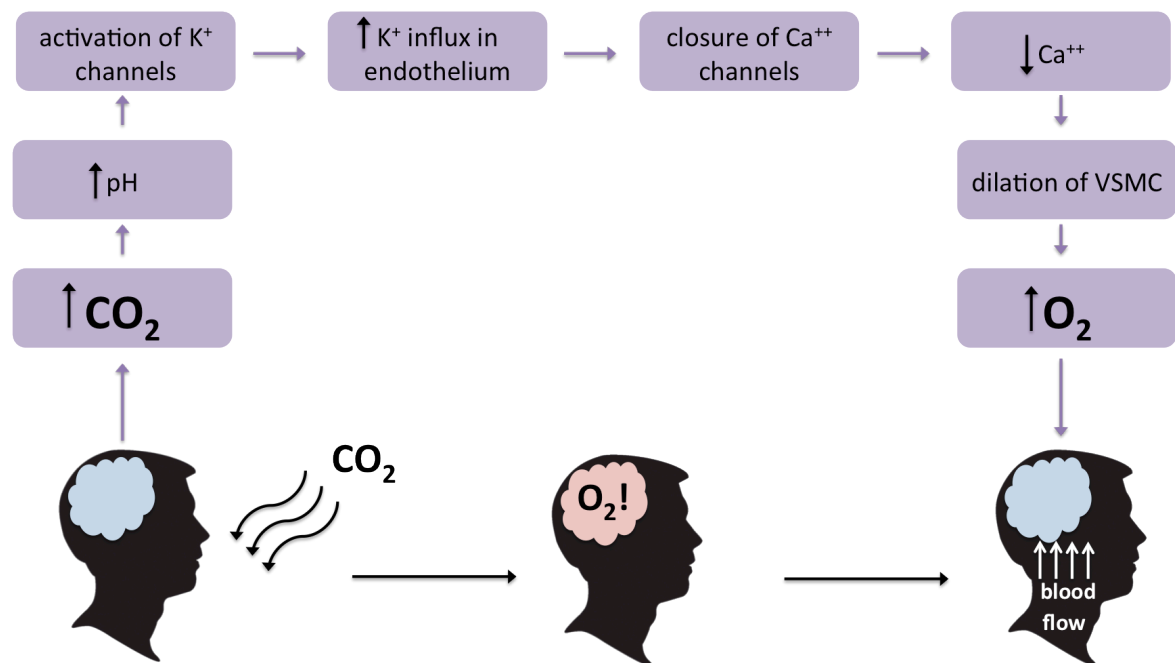


Figure 6.2: Normal regulatory response to CO_2 .

An increase in CO_2 creates a demand for O_2 in the brain. This sets in motion a series of cellular events that result in the dilation of blood vessels and a boost of blood flow to the brain. Impairments in this regulatory response have been observed in cardio and cerebrovascular disease as well as in neurodegenerative disorders like AD. VSMC: vascular smooth muscle cells

Cerebrovascular reactivity to CO₂ is a measure of the vascular system's potential to increase its performance in response to a challenge, and is representative of the body's vascular reserve (Boles Ponto et al., 2006; Glodzik et al., 2013). CO₂-CVR is reduced in a variety of vascular conditions like type 1 and type 2 diabetes, stroke, hypertension and atherosclerosis (Schwertfeger et al., 2006). Considerable evidence for AD-related impairment of cerebral autoregulation and CO₂-CVR has been derived from animal studies (Claassen and Zhang, 2011) and more recently from humans (Glodzik et al., 2013). Reduced blood flow in response to CO₂ has been reported in MCI and AD patients beyond that expected during healthy ageing, and CO₂-CVR has therefore been recommended as marker of early vascular dysfunction in people who are at a risk of developing AD (Liu et al., 2014). The mechanisms underlying this reduction in CO₂-CVR in AD patients are still unknown, but this measure is believed to provide useful prognostic information on the risk of subsequent cerebral events, primarily because impairments in CO₂-CVR are known to contribute to cerebrovascular disease if they persist over a long period of time (Schwertfeger et al., 2006).

Changes in CO₂-CVR have not yet been investigated in relation to *APOE*. Moreover, although CO₂-CVR (direct measure of CBF in response to CO₂) and the BOLD signal (indirect measure of neuronal activity) have different physiological origins, the two may be closely linked (Gonzales et al., 2014; Liu et al., 2013), and it would be a useful to examine fMRI signals in the context of potential *APOE*-related vascular alterations. We therefore studied the effects of the three *APOE* alleles on brain function and cerebrovascular health in cognitively healthy young adults. Specifically, we asked three questions:

1. Do the three *APOE* alleles influence cerebrovascular reactivity to CO₂ in a manner reflective of AD risk?
2. Are the increases in BOLD activity within the resting brain observed in Chapter 5 also present during a memory-encoding task in young $\epsilon 4$ carriers?
3. Does CO₂-CVR contribute to the effect of *APOE* on task-based BOLD activity?

6.2 Methods

6.2.1 *Participants*

Eighteen $\epsilon 2$ carriers, 17 $\epsilon 3$ homozygotes and 18 $\epsilon 4$ carriers aged 20-40 years were examined for this study. The neuroimaging protocol consisted of three scans acquired sequentially: (1) a structural MRI scan, (2) a “Novel vs. Familiar” task-based fMRI scan, and (3) a CO₂-challenge sequence to measure cerebrovascular reactivity to CO₂. (participant and scan sequence details provided in Chapter 3).

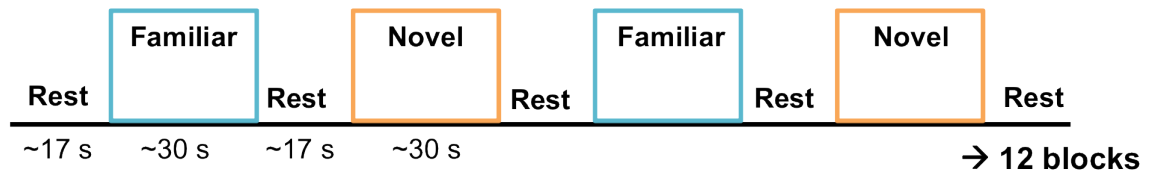
6.2.2 *Novel vs. Familiar task*

The experimental task was an explicit episodic memory-encoding paradigm (the “Novel vs. Familiar” task) performed using Presentation software (Figure 6.3). This task has been used by our group and others to study brain activity during memory encoding (Filippini et al., 2009). A set of emotionally neutral colour images of animals or landscapes (no humans), similar in complexity, brightness and contrast were shown to participants. Before the scan, subjects were presented with 8 images repeated 8 times (called “familiar” images), and tested to ensure images had been successfully encoded. During the scan, images were displayed in pseudorandom order in a block design alternating 6 blocks each of 8 familiar and 8 novel images. Familiar images were presented in a different order each time. There were a total of 48 “novel” images that were unseen before the scan (image presentation = 3.5 s, inter-stimulus interval = 0.67 s, block duration = 30 s). Between each block of images was 16.68 s of rest, during which subjects passively viewed a fixation cross (a total of 12 rest blocks). Subjects were instructed to

remember the images while in the scanner, and to ensure compliance, they were asked to indicate if each image contained an animal or not. After the scan, approximately 50 min after initial presentation, participants were given a memory test where novel and familiar images as well as “distractors” (images not seen either before or during the scan) were presented on a PC screen for 3 s each (inter-stimulus interval = 1 s). Participants had to indicate whether or not they remembered seeing the images inside the scanner.

6.2.3 CO₂ challenge

The paradigm consisted of delivering respiratory gases via a 2-tube nasal cannula at 8L/min (Figure 6.3). During resting blocks medical air was supplied, during hyperoxia 100% O₂, and during hypercapnia a mix of 10% CO₂, 21% O₂, and the balance nitrogen. Due to mixing with room air when using a nasal cannula, the inspired fractions were $FiO_2 \approx 0.5$ for hyperoxia and $FiCO_2 \approx 0.04$ for hypercapnia. The 2-tube nasal cannula and a Biopac MP150 with O₂ and CO₂ amps (Biopac Systems, USA) were used to produce a full respiratory trace for both O₂ and CO₂, from which end-tidal values were obtained. The respiratory paradigm consisted of 33 seconds of air followed by 2x2 minute blocks of hypercapnia interleaved with 2x3 minute blocks of hyperoxia. Each hypercapnia block was followed by 1 minute of air, whereas the hyperoxia blocks were followed by 2 minutes of air due to slower return to baseline following hyperoxia (Bulte et al., 2007).

(A) Block design during task fMRI**(B) Block design during CO₂ challenge****Figure 6.3: Task fMRI and CO₂ paradigm.**

Block design during the scan for (A) Novel vs. Familiar task and (B) CO₂-challenge. The CO₂-CVR scan was acquired after the task-fMRI scan.

6.2.4 MRI acquisition

All participants were scanned at the FMRIB Centre, Oxford using a 3T Siemens Verio scanner. The neuroimaging protocol included structural MRI, task-based fMRI, resting perfusion MRI and CO₂-CVR (details in Chapter 3). Whole-brain simultaneous perfusion and BOLD data were acquired using a dual gradient echo, pseudo-continuous arterial spin labelling (PCASL) echo planar imaging (EPI) sequence. A multi-TI MRI sequence was used for the resting scan to allow absolute quantitative measurement of resting cerebral blood flow (CBF₀) and arterial arrival time (AAT). A labelling duration of 1400 ms, and five post-labelling delay times (250/500/750/1000/1250 ms) were used. A single-TI

sequence (labelling duration = 1200 ms, post-labelling delay = 600 ms) was used for both the task-based and the CO₂-CVR sequences.

6.2.5 Image analysis

Data analysis was performed using FSL tools (Smith et al., 2004).

Structural MRI: A whole-brain investigation of *APOE*-related changes in GM volume was carried out using FSL-VBM, and brain GM, WM, CSF and hippocampal volumes were extracted as described in Chapter 4.

Task fMRI: Analysis of dual-echo fMRI data (BOLD and CBF) was carried out using FSL-FEAT. Pre-processing consisted of head motion correction, brain extraction, spatial smoothing using a Gaussian kernel of 6mm FWHM and high pass temporal filtering equivalent to 140 s. Any residual effect of CBF on the BOLD signal was accounted for by adding it as a covariate of no interest to the model. Time-series statistical analysis was carried out with local autocorrelation correction (Woolrich et al., 2001). A boxcar convolved with a gamma hemodynamic response function and its temporal derivative was used to model the activation time-course. The main contrast of interest for the task was “**Novel > Familiar**”. BOLD and CBF volumes were registered to the individual's structural and standard space images using FNIRT, then optimized using boundary-based-registration (Greve and Fischl, 2009). Group-level analysis was carried out using FLAME (Woolrich et al., 2004). An automatic outlier de-weighting tool was applied to reduce the effect of outliers. The cross-subject GLM included the three *APOE* groups. Z-statistic images were thresholded using clusters determined by $Z > 2.3$ and a FWE-corrected cluster significance threshold of $p < 0.05$ was applied to the suprathreshold clusters.

Resting Perfusion MRI: Resting perfusion maps in units of ml/(100g/min) were generated from the multi-TI PCASL data using in-house tools (Okell et al., 2013). Two calibration scans were used to calibrate the PCASL perfusion-weighted signal for equilibrium magnetization of blood. CBF was then quantified by carrying out a non-linear fit of the general kinetic model to the multi-TI PCASL data for all voxels in the brain using a variational Bayes approach (Buxton et al., 1998).

CO₂-CVR: Measurement of cerebral physiology was achieved using the method described in Bulte et al. (2012). This allows for the calculation of the resting CBF₀, AAT, cerebral blood volume (CBV), oxygen extraction fraction (OEF), cerebral metabolic rate of oxygen (CMRO₂), cerebrovascular reactivity to carbon dioxide (CO₂-CVR), and the fMRI BOLD calibration constant M (Davis et al., 1998).

Data analysis was performed using Matlab (MathWorks, Natick, MA, USA) and FSL tools. The short (13 ms) and long (30 ms) echoes were separated into 2 timecourses: ASL and BOLD data. Data from the multi-TI were used to calculate CBF₀ and AAT. For each participant, the GLM in the single-session FEAT was set up with five explanatory variables representing the following: 1) tag/control signal modulation, 2) CO₂ exposure, 3) O₂ exposure, 4) interaction of CO₂ exposure with tag/control signal, and 5) interaction of O₂ with tag/control signal. BOLD and ASL data were temporally filtered with a high pass temporal filtering of 270 s and 10 s respectively. CO₂-CVR in each voxel was determined by measuring the fractional change in CBF with pCASL, per mmHg of change in end-tidal CO₂ as a result of changing the inspired gas from air to a mixture of ~4% CO₂, 21% O₂, balanced with N₂ (Hare et al., 2013). The BOLD calibration constant

M was calculated on a voxelwise basis using the response to hypercapnia. This value was used along with the end-tidal oxygen and the BOLD response to hyperoxia to calculate the voxelwise OEF. The CMRO_2 was calculated from the OEF and the CBF_0 . The CBV was calculated from the BOLD response to hyperoxia. The cascade nature of these calculations means that the propagation of noise sequentially decreases the signal-to-noise ratio (SNR) for later parameters. Thus CBF_0 , AAT and CO_2 -CVR have the highest SNR, followed by CBV, then M, while OEF and CMRO_2 have the lowest.

Whole-brain analysis was performed on brain maps to investigate the effects of *APOE*. Images were smoothed using a Gaussian kernel of 6 mm FWHM and registered to standard space using FLIRT. The cross-subject GLM included the *APOE* groups and was applied using permutation-based non-parametric testing (5000 permutations) and a FWE-corrected significance threshold of $p < 0.05$ to the suprathreshold clusters. Due to high SNR variability in the BOLD signal response to the O_2 -inhalation task, OEF and CMRO_2 could not be reliably estimated for all the participants, and were therefore not further considered.

Regions of interest (ROIs): Masks of the left and right hippocampi were created for each subject using FIRST. ROIs were registered to native space and used to extract BOLD percentage signal changes from fMRI data using FSL Featquery. Hippocampal masks were registered to perfusion and CVR native space to extract: CBF_0 , AAT, CBV and CO_2 -CVR values for group comparisons.

Covariates: Structural images were used as additional covariates on a voxel-by-voxel basis to BOLD, CBF_0 , AAT, CBV and CO_2 -CVR maps to account for GM differences

(Oakes et al., 2007). Absolute and relative motion parameters were added to the GLM model as nuisance regressors to account for potential motion-related effects. Finally, to assess the contribution of CO₂-CVR to the BOLD signal, CO₂-CVR maps were added to the task analysis as nuisance covariates.

6.2.6 Statistical analysis

Statistical analyses of non-imaging variables were carried out using SPSS. ANOVA and Bonferroni correction for post-hoc tests were used for socio-demographic variables, memory performance, task-related reaction times and regional brain volumes. Exact Fisher's test was used for categorical variables (gender and family history of dementia).

6.3 Results

6.3.1 *Socio-demographics and brain structure*

VBM analysis revealed no effects of *APOE* on GM density. There were no *APOE*-associated differences in socio-demographic variables, memory task performance and regional brain volumes. There were also no effects of *APOE* on physiological parameters acquired pre- and post-scan (blood pressure and heart rate), and during the CO₂-challenge (end-tidal CO₂, arterial saturation and respiratory rates) (Table 6.1).

6.3.2 *Effect of APOE on CO₂-CVR*

We found a significant effect of *APOE* on CO₂-CVR using a whole-brain voxelwise analysis (Figure 6.4). *APOE* $\epsilon 2$ and $\epsilon 3\epsilon 3$ carriers had significantly higher CO₂-CVR than $\epsilon 4$ carriers and this pattern was widespread throughout the brain. The mean CO₂-CVR values were extracted from the hippocampi and a one-way ANOVA revealed a significant effect of *APOE* on the left ($F_{(2,50)} = 6.731$, partial $\eta^2 = 0.212$, $p < 0.005$) and right hippocampus ($F_{(2,50)} = 6.868$, partial $\eta^2 = 0.216$, $p < 0.005$), with the following pattern of CO₂-CVR values: $\epsilon 2 \geq \epsilon 3 > \epsilon 4$ (Figure 6.6 and Table 6.2). There were no *APOE*-related differences in resting CBF₀, CBV, and AAT.

6.3.3 *Effect of APOE on task-based fMRI*

The “Novel vs. Familiar” contrast robustly activated areas associated with encoding. Specifically, BOLD signal increases across all subjects were found bilaterally in the hippocampi, temporal fusiform cortices, parahippocampal gyri, as well as in association areas in frontal, parietal, and occipital lobes (Figure 6.5), as previously described (Filippini et al., 2009). Voxelwise analysis revealed greater left hippocampal, temporal and cerebellar activation in *APOE* $\epsilon 4$ carriers relative to $\epsilon 3$ homozygotes (Figure 6.5). The hippocampal signal change was extracted for each subject and a one-way ANOVA revealed a significant effect of *APOE* in the left ($F_{(2,50)} = 7.704$, $p < 0.005$, partial $\eta^2 = 0.236$) and right hippocampus ($F_{(2,50)} = 3.326$, $p < 0.05$, partial $\eta^2 = 0.117$). Post-hoc tests showed that the $\epsilon 4$ carriers had the highest hippocampal BOLD signal (Figure 6.6 and Table 6.2). No genotype differences were found either in whole-brain or ROI analyses of CBF during the task.

6.3.4 *Effect of CO₂-CVR on the BOLD signal*

To study the contribution of CO₂-CVR to the BOLD signal, CO₂-CVR maps were added as nuisance covariates in the task analysis. We found that the previously observed difference in hippocampal BOLD activation between $\epsilon 4$ carriers and $\epsilon 3$ homozygotes was attenuated after accounting for CO₂-CVR. Specifically, the number of active voxels within the hippocampus that survived the threshold was reduced from 215 to 66, resulting in a ~70% decrease in the significant difference between the two group (Figure 6.5 and Table 6.3).

Table 6.1: Socio-demographics, memory performance, brain volumes, and physiological parameters for the 3 *APOE* groups.

Values denote mean $\pm$ standard deviation. Tissue volumes are expressed as percentage of whole brain volume. End-tidal CO₂ is expressed as mmHg increase during CO₂-delivery relative to when participants were breathing medical air. ANOVA *P*-values are reported.

	ε2 N=18	ε3 N=17	ε4 N=18	P
Sociodemographics				
Age (years)	24.11 $\pm$ 5.31	24.11 $\pm$ 4.96	23.88 $\pm$ 4.75	0.99
Education (years)	17.38 $\pm$ 1.75	17.50 $\pm$ 2.89	17.05 $\pm$ 2.18	0.84
Body Mass Index	22.05 $\pm$ 2.95	22.42 $\pm$ 2.00	22.62 $\pm$ 2.72	0.80
Gender (% male)	38.89 %	47.06 %	44.44 %	0.74
Family history of dementia	2	1	0	0.53
Post-scan memory test (% of correct responses)				
Global performance	85%	85%	86%	0.91
Familiar images	97%	95%	98%	0.56
Novel images	78%	80%	79%	0.95
Distractor images	95%	93%	96%	0.51
Reaction time in scanner (seconds)				
Familiar images	0.90 $\pm$ 0.21	0.93 $\pm$ 0.17	0.87 $\pm$ 0.17	0.54
Novel images	1.24 $\pm$ 0.30	1.31 $\pm$ 0.31	1.27 $\pm$ 0.44	0.86
Brain volumes				
Whole brain (mm ³)	1439 $\pm$ 101	1465 $\pm$ 122	1449 $\pm$ 178	0.85
Grey matter (%)	43.6 $\pm$ 1.71	43.9 $\pm$ 1.60	44.3 $\pm$ 2.20	0.57
White matter (%)	36.8 $\pm$ 1.58	36.9 $\pm$ 1.35	37.1 $\pm$ 1.51	0.82
Cerebrospinal fluid (%)	19.5 $\pm$ 1.33	19.2 $\pm$ 2.09	18.6 $\pm$ 2.39	0.35
Left hippocampus (%)	0.26 $\pm$ 0.02	0.26 $\pm$ 0.03	0.27 $\pm$ 0.02	0.36
Right hippocampus (%)	0.26 $\pm$ 0.02	0.26 $\pm$ 0.03	0.26 $\pm$ 0.02	0.50
Physiological parameters				
Pre-scan Systolic / Diastolic BP	107 $\pm$ 15 / 68 $\pm$ 8	110 $\pm$ 10 / 69 $\pm$ 7	108 $\pm$ 10 / 70 $\pm$ 8	0.76-0.67
Post-scan Systolic/Diastolic BP	107 $\pm$ 13 / 69 $\pm$ 7	111 $\pm$ 10 / 71 $\pm$ 8	108 $\pm$ 9 / 69 $\pm$ 9	0.47-0.66
Pre-scan heart rate	76 $\pm$ 11	72 $\pm$ 16	72 $\pm$ 13	0.61
Post-scan heart rate	68 $\pm$ 8	64 $\pm$ 15	64 $\pm$ 7	0.50
End-tidal CO ₂ (mmHg)	2.32 $\pm$ 0.79	2.34 $\pm$ 0.83	2.91 $\pm$ 1.12	0.11
CO ₂ -challenge arterial saturation	98.1 $\pm$ 0.41	97.8 $\pm$ 0.63	98.1 $\pm$ 0.58	0.27
CO ₂ -challenge respiratory rate	13.9 $\pm$ 3.96	14.6 $\pm$ 3.18	13.2 $\pm$ 3.21	0.47

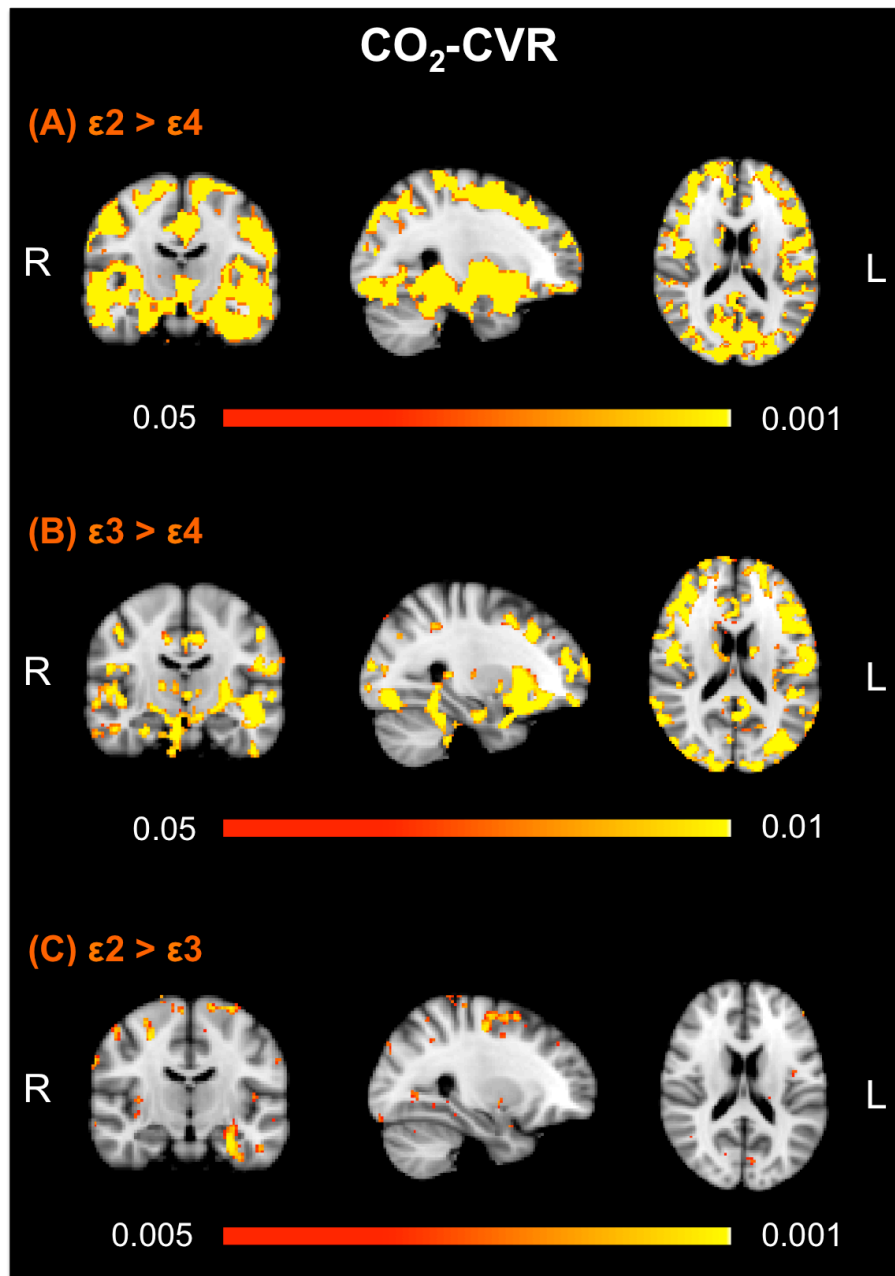


Figure 6.4: Effect of *APOE* on cerebrovascular reactivity to CO₂.

Increased CO₂-CVR in (A) $\epsilon 2$ relative to $\epsilon 4$ carriers, (B) $\epsilon 3$ homozygotes relative to $\epsilon 4$ carriers. Red-to-yellow colours define voxelwise significance at $p < 0.05$ corrected for multiple comparisons. For completeness, we also show results for (C) $\epsilon 2$ carriers relative to $\epsilon 3$ homozygotes at $P < 0.005$ uncorrected. R and L: right and left hemisphere.

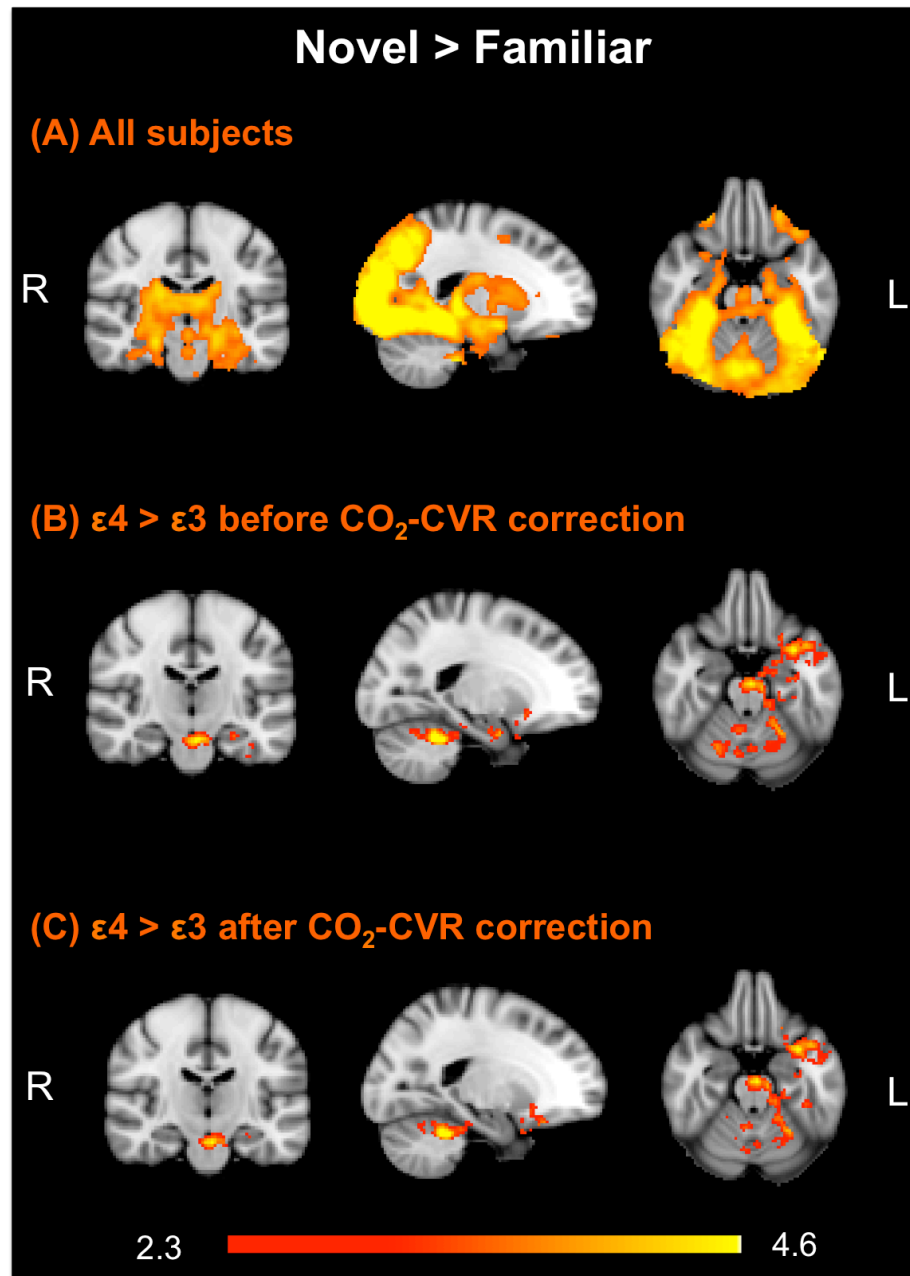


Figure 6.5: Effect of *APOE* on task-based BOLD activity.

(A) Mean activation for the Novel > Familiar contrast all 53 subjects, (B) regions of significantly increased BOLD activity for $\epsilon 4$ carriers relative to $\epsilon 3$ homozygotes before and (C) after accounting for differences in cerebrovascular reactivity to CO_2 (CO_2 -CVR). Red-to-yellow colours define the increases in brain activation ($p < 0.05$ corrected for multiple comparisons). R and L: right and left hemisphere.

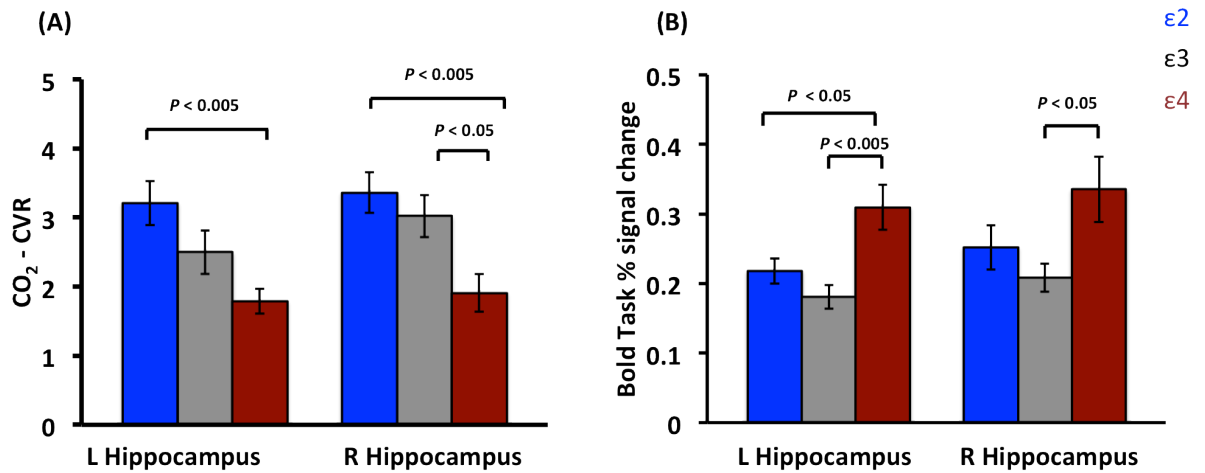


Figure 6.6: ROI analysis of the hippocampus.

Values extracted from the left and right hippocampus for (A) CO₂-CVR and (B) BOLD percentage signal change during the Novel > Familiar contrast. CO₂-CVR is measured as the change in CBF per unit mm Hg. Blue, grey and red bars represent mean $\pm$ standard errors for *APOE* ε₂, ε₃ and ε₄ carriers respectively. P values reflect Bonferroni-corrected post hoc comparisons across the three *APOE* groups.

Table 6.2: Extracted values for the hippocampal ROI analysis

Values denote mean $\pm$ standard error. ANOVA *P*-values are reported.

	ε ₂ N=18	ε ₃ N=17	ε ₄ N=18	P
CO₂-CVR (%ΔCBF/mm Hg)				
Left hippocampus	3.21 $\pm$ 0.32	2.49 $\pm$ 0.31	1.79 $\pm$ 0.18	<0.005
Right hippocampus	3.36 $\pm$ 0.30	3.02 $\pm$ 0.30	1.91 $\pm$ 0.27	<0.005
BOLD Task (% signal change)				
Left hippocampus	0.22 $\pm$ 0.02	0.18 $\pm$ 0.02	0.31 $\pm$ 0.03	<0.005
Right hippocampus	0.25 $\pm$ 0.03	0.21 $\pm$ 0.02	0.33 $\pm$ 0.05	<0.05

Table 6.3: Details of significant clusters for the Novel > Familiar contrast in the memory encoding task.

Values represent increased BOLD signal in $\epsilon 4$ carriers relative to $\epsilon 3$ homozygotes (A) before and (B) after accounting for differences in CO_2 -CVR. There was a $\sim 70\%$ reduction in the cluster size of the left hippocampus (from 215 to 66 voxels) after CO_2 -CVR maps were added as covariates to the task analysis.

Brain region	Max z score	MNI coordinates x, y, z	Cluster size
$\epsilon 4 > \epsilon 3$ before CO_2-CVR correction			
Left hippocampus	3.21	-18, -6, -26	215
Left temporal pole	4.01	-44, -2, -36	692
Left Cerebellum	4.53	-20, -48, -28	1026
$\epsilon 4 > \epsilon 3$ after CO_2-CVR correction			
Left hippocampus	2.99	-28, -30 -14	66
Left temporal pole	3.94	-32, 6, -22	493
Left Cerebellum	4.35	-20, -46, -28	801

6.4 Discussion

We have shown for the first time that *APOE* affects small-vessel cerebrovascular reactivity to carbon dioxide in young adults in a pattern that mirrors their lifetime risk for AD (*AD-risk*: $\epsilon 4 > \epsilon 3 > \epsilon 2$, *CO₂-CVR*: $\epsilon 2 \geq \epsilon 3 > \epsilon 4$). We have also successfully replicated past results that find increases in BOLD activity during a memory-encoding task in young $\epsilon 4$ carriers relative to $\epsilon 3$ homozygotes (Dennis et al., 2010; Filippini et al., 2009). Finally, we assessed the contribution of CO₂-CVR to the BOLD signal and observed a ~70% reduction in the extent of significant task-based hippocampal BOLD differences between $\epsilon 4$ carriers and $\epsilon 3$ homozygotes after accounting for *APOE*-associated differences in CO₂-CVR.

6.4.1 *APOE* modulates task-based hippocampal activity and CO₂-CVR in young adults

The novel versus familiar encoding task is a well-studied experimental paradigm that induces blood flow to the hippocampus, parahippocampal gyrus and fusiform and lingual gyri (Bondi et al., 2005; Filippini et al., 2009; Ringman et al., 2011). Activation in these regions, particularly within the hippocampus, is attributed to the novelty of the stimuli to be encoded. Our finding of an increased hippocampal activation in $\epsilon 4$ carriers is in line with past results (Filbey et al., 2006; Filippini et al., 2009; Fleisher et al., 2009; Ringman et al., 2011). The fact that we observed these increases in the absence of differences in task performance between the *APOE* groups seems to suggest that $\epsilon 4$ carriers may need to recruit more neural activity in order to perform at the same level as non-carriers. However, we cannot explain the relationship between high BOLD activity and cognition at this stage, since all groups nearly achieved a ceiling effect for task performance. More

sensitive cognitive tasks have found that $\epsilon 4$ carriers outperform non-carriers in this age-range in studies of memory, attention and speed of processing (for review, see (Rusted and Carare, 2015)) so our finding of increased hippocampal activation may fit with the antagonistic pleiotropic hypothesis suggesting a heightened cognitive reserve in young $\epsilon 4$ carriers.

Recently it has been suggested that increases in task-based BOLD signal in $\epsilon 4$ carriers may not be related to either cognitive compensation or reserve, but instead to “*an unidentified effect of the allele on cerebrovascular reactivity*” (Ringman et al., 2011). Here we found that young $\epsilon 4$ carriers also have relatively low cerebrovascular reactivity to CO_2 . In interpreting this result, it is important to note the distinction between the physiological origins of the BOLD signal (which measures variations in the level of oxygenation of the blood in response to task-related neuronal activation) and CO_2 -CVR (which measures changes in CBF in response to CO_2). It is entirely possible that fluctuations in vasodilation and/or CBF that occur as a result of task-related metabolic demand are regulated by different mechanisms than those induced by variations in the CO_2 (Lin et al., 2010). This would explain our findings of *APOE*-related differences in cerebral blood flow during CO_2 inhalation (*i.e.* CO_2 -CVR), but not in cerebral blood flow at rest, or during the memory task. In fact, it has been suggested that impairments in CO_2 -CVR could precede decreases in CBF, and reveal dysfunction earlier than CBF in still relatively healthy tissue because it serves as “stress test” for brain circulation (Glodzik et al., 2013).

However, this does not mean that CO_2 -CVR and the BOLD signal are independent of each other, because we observed a significant attenuation of the BOLD signal after

accounting for CO₂-CVR. Similar to this result, a recent study found that CO₂-CVR accounts for individual variance in the BOLD signal in healthy adults (Liu et al., 2013). Thus it is plausible that the two are linked even though they are initiated by different mechanisms. In this study, we do not have evidence directly linking CO₂-CVR and BOLD hyperactivity because we acquired the task fMRI and CO₂-challenge scans sequentially and not simultaneously. However our results suggest that the effect of *APOE* on cerebral vasculature may, at least partly, contribute to the pattern of *APOE*-related differences in the BOLD signal.

6.4.2 Linking CO₂-CVR, age, and amyloid: Can vascular dysfunction lead to neuronal damage?

In Chapter 5, we discussed Buckner and colleagues' finding that early-life functional hyperactivity might provide an environment conducive to the deposition of amyloid in $\epsilon 4$ carriers. Here, we suggest that this "environment" may be a product of a concomitant decline in cerebrovascular health, represented as a reduction in CO₂-CVR (Figure 6.7).

APOE $\epsilon 4$ carriers had relatively lower CO₂-CVR, which is reflective of an impaired cerebral regulatory response to hypercapnia. Over time, this insufficient blood (and therefore oxygen) supply to the brain during situations with similar physiologically demands could lead to an overall decrease in availability of oxygen in the cerebral tissue. This, together with the brain's inability to store excess oxygen, may eventually result in a state of chronic mild cerebral functional hypoxia (Morikawa et al., 2012). As CO₂-CVR is a marker of the brain's vascular reserve, the *APOE* $\epsilon 4$ -associated reduction in this measure could make these individuals more susceptible to "*hit one*" in the two-hit vascular hypothesis presented in **Figure 6.1**. Conversely, an elevated CO₂-CVR in $\epsilon 2$ -

carriers is suggestive of a highly efficient cerebral regulatory response to hypercapnia, a favourably high level of oxygen availability, potentially making them less vulnerable to hypoxia-induced pathways to amyloid deposition. Based on this idea, we build on the model put forward in Chapter 5 and incorporate a link between early-life cerebrovascular health and late-life amyloid deposition in Figure 6.7.

Collective evidence suggests that vascular dysfunction does in fact contribute to neuronal damage. Animal models have shown that hypoperfusion can amplify the deposition of amyloid- β (Koike et al., 2010; Wang et al., 2010), by increasing the activity of β -secretase and γ -secretase (Li et al., 2009; Sun et al., 2006). In humans, reductions in cerebral perfusion are present before cognitive decline, brain atrophy and notably, even before amyloid deposition in elderly individuals at a risk of AD, suggesting that impairments in circulation precede pathological changes in AD (Knopman and Roberts, 2010; Ruitenberg et al., 2005; Sheline et al., 2010; Smith et al., 1999; Zlokovic, 2011). This would explain why $\epsilon 4$ carriers, who have relatively poor cerebrovascular health, have an earlier age of onset and higher levels of amyloid burden than non-carriers, and conversely why $\epsilon 2$ carriers show delayed onset and lower plaque severity. The life-long build-up of damage to the microvasculature could also be why $\epsilon 4$ carriers have poorer outcomes following traumatic brain injury than non-carriers and an overall reduced ability to cope with neural insults (Mahley and Huang, 2012b).

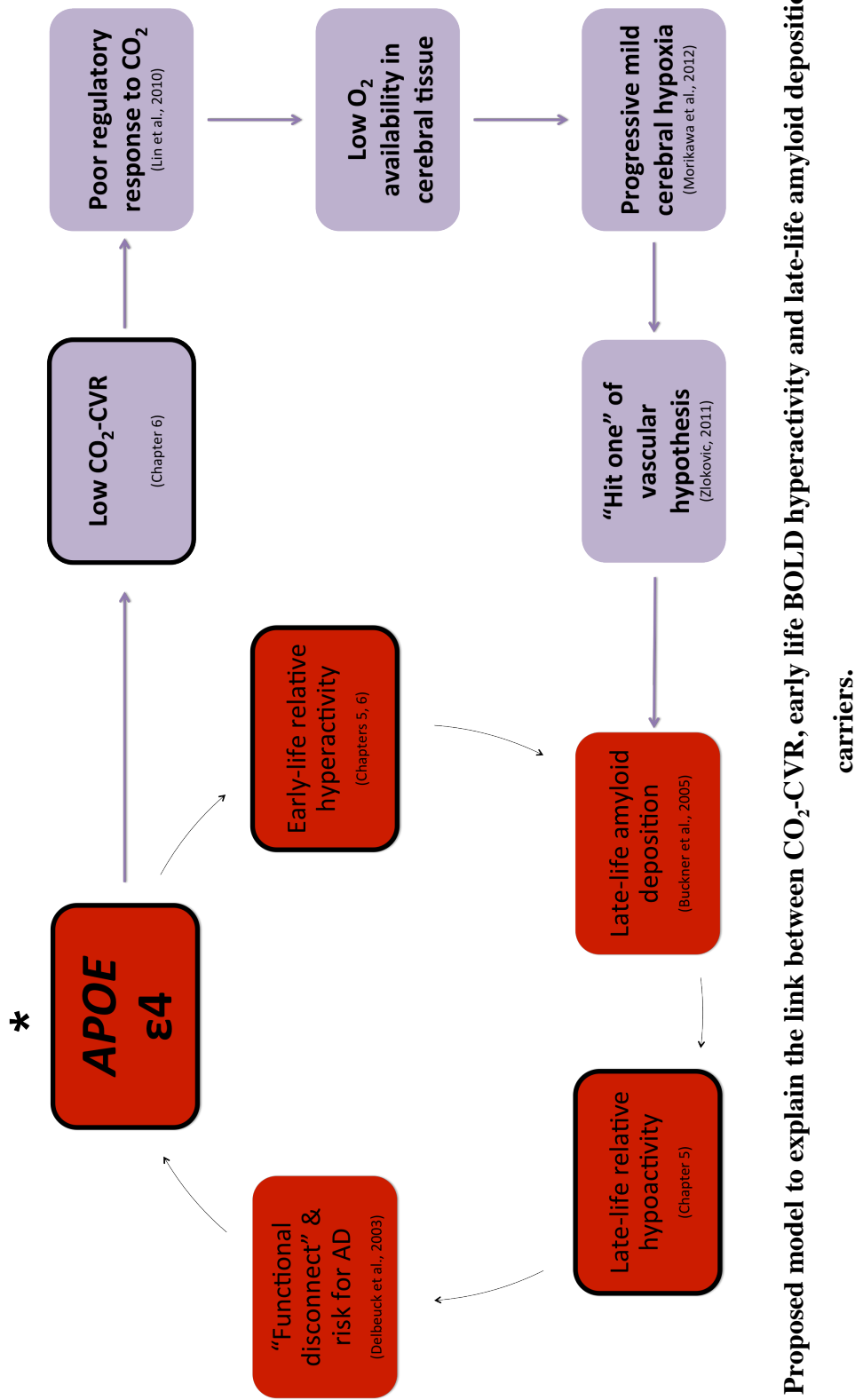


Figure 6.7: Proposed model to explain the link between CO₂-CVR, early life BOLD hyperactivity and late-life amyloid deposition in ε4 carriers.

The * denotes the starting point in this model. Based on the findings of this thesis (represented as boxes with dark outlines) and the relevant literature (boxes without outlines), we suggest that early-life reductions in CO₂-CVR may predispose ε4 carriers to amyloid deposition. We have shown that young ε4 carriers have low CO₂-CVR (purple cascade) as well as high BOLD activity at rest and during a task (red cascade) relative to non-carriers. This model describes how low CO₂-CVR may also serve as an entry point into the amyloid cascade by acting as “hit one” of the vascular pathway to neurodegeneration. We suggest that the opposite patterns are likely true for ε2 carriers.

The results of this chapter, together with those of Chapter 5 also give us an insight into the timeline of changes in the BOLD signal and CO₂-CVR across adulthood. The model in Figure 6.7 is based on an inverse association between CO₂-CVR and BOLD activity in early life (*young ε4 carriers: low CO₂-CVR and high BOLD activity*). However, two recent studies that have since measured CO₂-CVR in older populations lead us to suggest that this association may vary during the lifespan. Hajjar and colleagues replicated our finding of reduced CO₂-CVR in ε4 carriers in a large sample of non-demented older individuals (n=665, of which 147 were ε4+, mean age ~78 years) (Hajjar et al., 2015). More recently, a longitudinal study of 1629 non-demented participants (mean age ~ 70 years) found that lower CO₂-CVR at baseline was associated with a higher risk of AD at follow-up, and that this association was more pronounced in ε4 carriers (Wolters et al., 2016). Thus while the effect of *APOE* ε4 on resting-state (and less consistently on task-based) BOLD activity seems to vary with age, that on CO₂-CVR appears to remain relatively stable throughout life, with ε4 carriers consistently show reductions in this measure of cerebrovascular health (*old ε4 carriers: low CO₂-CVR and low BOLD activity*). Based on this, we hypothesise that early and sustained reductions in cerebrovascular reactivity may predispose ε4 carriers to amyloid deposition and that the late-life functional hypoactivity may be symptomatic of the neuronal injury caused by amyloid burden. Thus BOLD activity may begin to decline rapidly in ε4 carriers *after* a certain threshold of amyloid-based neuronal damage has been surpassed. Conversely, maintenance of cerebrovascular health in ε2 carriers may underlie their resilience to age-related neurodegeneration.

6.4.3 Limitations and future considerations

This is the first study to examine how *APOE* influences cerebrovascular health in young adults, and our findings have been strengthened by a recent replication of these results in older adults. However, we must consider some methodological limitations of our study.

First, although we find that CO₂-CVR may play some role in explaining the increased BOLD activity in young $\epsilon 4$ carriers, we cannot say with confidence that the two are linked unless we acquire a simultaneous measure of CO₂-CVR *during* a task-based fMRI acquisition. Before we can do that, we need a better understanding of direct effects of CO₂ inhalation on neural activity and oxygen metabolism. Quantifying the effect of CO₂ inhalation on oxygen metabolism is particularly important because it could influence our interpretation of a simultaneously measured BOLD signal, given that the intensity of the BOLD signal is dependent on the concentration of deoxyhaemoglobin in the blood. Some studies report that CMRO₂ remains constant while breathing CO₂, while others find changes in oxygen metabolism during CO₂ exposure (Xu et al., 2011). We could not reliably quantify CMRO₂ and OEF in this study due to poor signal-to-noise ratio of the measures derived from the hyperoxic blocks. Both these measures would have helped define the relationship between the BOLD signal and CO₂-CVR. CMRO₂ is typically quantified using ¹⁵O PET, which is an invasive, expensive and lengthy procedure. The MR-based approach used here therefore provides a useful alternative to PET but needs further work before we are able to quantify oxygen-derived measures (Bulte et al., 2012; Xu et al., 2009).

Second, while we found no *APOE*-related differences in baseline and post-scan heart rate and blood pressure, these physiological parameters were not continuously measured during CO₂-inhalation. However, a recent study using a similar approach to measure CO₂-CVR in AD, MCI, and healthy controls has reported no group differences in heart rate during CO₂-inhalation (Cantin et al., 2011). The low level of hypercapnia used in our study was specifically chosen to be below the threshold for healthy subjects to experience any physiological effects on breathing rate, heart rate, or blood pressure during the scan. Given that our subjects are all young and healthy, and that we found no genotype differences in end-tidal CO₂, arterial oxygen saturation and respiratory rates during the CO₂-challenge, we do not expect to find *APOE*-related differences in heart rate or blood pressure. Nevertheless, future studies may wish to consider these peripheral vascular responses to CO₂ as they could theoretically influence cerebrovascular reactivity.

Third, the BOLD signal depends on the concentration of haemoglobin in the blood, and it is possible that between-group variations in baseline haematocrit levels may influence it (Levin et al., 2001). There is no evidence for *APOE*-related differences in haemoglobin levels (Foley et al., 2014), and in order to limit the invasiveness of this study, we did not measure blood cell parameters. However, without these measurements, haematocrit cannot be completely excluded as a potential confound.

In summary, we have presented an imaging-marker of the early effects of *APOE*, *i.e.* CO₂-CVR, which may provide evidence for a vascular contribution to the vulnerability of ε4 carriers to late-life pathology and/or to the protective role of the ε2 allele. However the exact mechanism by which *APOE* modulates CO₂-CVR is still unknown. With reference to the cellular cascade in Figure 6.2 we need to identify the point during the regulatory

response at which $\epsilon 4$ exerts its deleterious effects. Future studies should examine whether the $\epsilon 4$ -mediated reduction in CO_2 -CVR stems from a weakened ability to respond to stressors like CO_2 (*i.e.*, impaired signalling between neurons and blood vessels) and/or early damage to microvasculature (*i.e.* inability of the vascular smooth muscle to dilate).

Chapter 7

APOE and brain metabolites

Proton magnetic resonance spectroscopy (^1H -MRS) is increasingly being used to characterize the neurochemical profile of AD, but its clinical utility has been limited by the lack of consistent information on how metabolite concentrations vary within the healthy ageing brain. There have also been very few examinations of whether AD-related metabolite changes are present before clinical onset, for instance in cognitively healthy *APOE* $\epsilon 4$ carriers. Moreover, these studies have produced conflicting results about the effects of $\epsilon 4$ and none have included the protective $\epsilon 2$ allele. In this chapter, I discuss how metabolite concentrations vary with age and *APOE* genotype in a relatively large dataset across a wide age-range.

A modified version of this chapter has been prepared as a manuscript titled “*The effect of age and APOE on metabolite concentrations in the posterior cingulate cortex*” (Suri et al., *submitted*).

7.1 Introduction

^1H magnetic resonance spectroscopy (^1H -MRS) is a non-invasive technique used to measure the concentration of brain metabolites *in vivo*. Over the last two decades, ^1H -MRS has routinely provided useful diagnostic information about brain tumors (Callot et al., 2008), multiple sclerosis (Narayana, 2005) and a wide range of metabolic disorders

(Cecil, 2006). It has also been used to study depression (Godlewska et al., 2015), mild cognitive impairment (Tumati et al., 2013), Alzheimer's disease (AD) (Graff-Radford and Kantarci, 2013), and other dementias (Kantarci et al., 2004).

7.1.1 AD and ¹H-MRS

The most widely studied metabolites in the brain are N-acetyl aspartate (NAA), creatine (Cr), choline, and myo-inositol, which serve as surrogate markers of neuronal health, energy metabolism, membrane turnover and glial proliferation respectively (Miller, 1991). MRS examinations of patients with sporadic late-onset AD and MCI have consistently revealed decreases in NAA/Cr and increases in choline/Cr and myo-inositol/Cr in the posterior cingulate cortex (PCC)/precuneus of patients relative to healthy controls (Graff-Radford and Kantarci, 2013; Kantarci et al., 2013, 2004, 2002a, 2000). Recent advances in ¹H-MRS techniques have also enabled the quantification of glutathione, an antioxidant, and neurotransmitters, such as GABA, glutamate and glutamine (Emir et al., 2011; Novotny et al., 2003), all of which have are reportedly reduced in AD patients compared to healthy controls (Antuono et al., 2001; Fayed et al., 2011; Mandal et al., 2012; Riese et al., 2015; Rupsingh et al., 2011; Saharan and Mandal, 2014).

Two decades of ¹H-MRS research suggest that these metabolic changes may be useful biochemical imaging markers of AD, possibly signifying the underlying oxidative, metabolic and neuronal damage (Graff-Radford and Kantarci, 2013). Several studies have investigated the ability of ¹H MRS to differentiate AD patients from controls and have reported up to 90% sensitivity in the temporoparietal regions, 95% specificity in the

medial occipital lobe and 73% sensitivity in the PCC (Kantarci et al., 2002b; Martínez-Bisbal et al., 2004; Zhu et al., 2006). These measures are further improved when MRS is used together with hippocampal volumes (Kantarci et al., 2002b). Given its non-invasiveness and the increasing availability of 3T MR scanners, ¹H-MRS has the potential to evolve into a useful diagnostic tool for AD, but it is still largely considered a research technique (Graff-Radford and Kantarci, 2013; Oz et al., 2014).

7.1.2 Inconsistencies in ¹H-MRS studies of *APOE*

Several drawbacks have limited the clinical utility of ¹H-MRS. The overall lack of standardized analysis approaches has led to varying results across studies. We also know very little about when the characteristic metabolite signatures of AD begin to manifest. For instance, do they precede clinical onset and are they present in cognitive healthy *APOE* ε4 carriers who are at a genetic risk of developing AD?

Thus far, only four studies have examined the effect of *APOE* on metabolite concentrations in healthy individuals, all investigating the PCC/precuneus regions and reporting conflicting results (Gomar et al., 2014; Kantarci et al., 2002a, 2000; Riese et al., 2015). Gomar et al (2014) found age-related increases in Cho/Cr and myo-inositol/Cr in the PCC of healthy older ε4 carriers but not in ε3 homozygotes (Gomar et al., 2014), and more recently, Riese et al. found effects of *APOE* ε4 on NAA/Cr but not on GABA, glutamate and glutamine (Riese et al., 2015). Conversely two earlier, smaller studies (with largely overlapping samples) found no *APOE*-related differences in an elderly population (Kantarci et al., 2002a, 2000). There have been no reports on the protective ε2 allele. Moreover all four studies were conducted in older individuals (>50 years). In

Chapters 5 and 6 I have shown that *APOE* exerts allele-specific effects on brain function and vascular health decades before potential cognitive decline, and it would be worthwhile to also study the gene's effect on metabolite concentrations in young adults.

7.1.3 Inconsistencies in ¹H-MRS studies of healthy ageing

Another factor restricting the ability of ¹H-MRS to accurately track disease progress is the inconsistent evidence on how metabolites change within the healthy ageing brain. Without this knowledge it is difficult to conduct longitudinal assessments of patients and disassociate alterations in metabolite levels that may be the result of disease progression from those that accompany the ageing process (Haga et al., 2009). Two recent literature reviews have highlighted the substantial variability in ¹H-MRS studies of metabolite changes during healthy ageing (Haga et al., 2009; Reyngoudt et al., 2012) (Figure 7.1). The most inconsistent reports have been for NAA, which has been found to decrease (Angelie et al., 2001; Brooks et al., 2001; Driscoll et al., 2003; Gruber et al., 2008; Harada et al., 2001; Lundbom et al., 1999), increase, (Charlton et al., 2007; Schuff et al., 1999) and remain unchanged with age (Chang et al., 1996; Leary et al., 2000; Pfefferbaum et al., 1999; Reyngoudt et al., 2012; Saunders et al., 1999), depending on the region of interest and choice of metabolite quantification technique. Similarly, there have been findings of age-related increases (Angelie et al., 2001; Chang et al., 1996; Chiu et al., 2014; Gruber et al., 2008; Leary et al., 2000; Pfefferbaum et al., 1999) or no changes (Brooks et al., 2001; Charlton et al., 2007; Harada et al., 2001; Reyngoudt et al., 2012; Saunders et al., 1999; Schuff et al., 1999) in the concentration of choline across different parts of the brain. Most studies on myo-inositol report higher concentrations in older people (Chang et al., 1996; Gruber et al., 2008; Raininko and Mattsson, 2010; Reyngoudt

et al., 2012; Ross et al., 2006), although there have also been reports of stable levels of myo-inositol within white matter with age (Leary et al., 2000; Saunders et al., 1999). By comparison, the evidence for glutathione and glutamate has been more consistent, both of which are known to decline with age across widespread brain regions (Chang et al., 2009; Emir et al., 2011; Grachev and Apkarian, 2001; Kaiser et al., 2005; Marsman et al., 2013; Sailasuta et al., 2008)

Such discrepancies have been attributed to differences in ¹H-MRS methods, such as voxel position, data acquisition, analysis and reporting techniques (Haga et al., 2009; Reyngoudt et al., 2012). For instance, in studies of ageing, some authors report absolute quantifications of cerebral metabolites, using internal water or external phantom concentration references while others report relative concentrations most often referenced to creatine. However, reporting metabolite concentrations as relative ratios of creatine has been considered misleading (Jansen et al., 2006), particularly in studies including wide age-ranges. Creatine may not be a suitable reference metabolite, because it has increasingly been found to vary with age and across different brain regions (Angelie et al., 2001; Chang et al., 1996; Charlton et al., 2007; Chiu et al., 2014; Gruber et al., 2008; Leary et al., 2000; Pfefferbaum et al., 1999; Reyngoudt et al., 2012; Saunders et al., 1999; Schuff et al., 2001) (Figure 7.1). Underpowered studies also contribute to the between-study variability; a systematic review of 18 ¹H-MRS publications, comparing healthy young (<60 years) and old (>60 years) people, found an average of *only ~16 older subjects per study*. There is, therefore, an evident need for large-scale studies in healthy older participants (Haga et al., 2009).

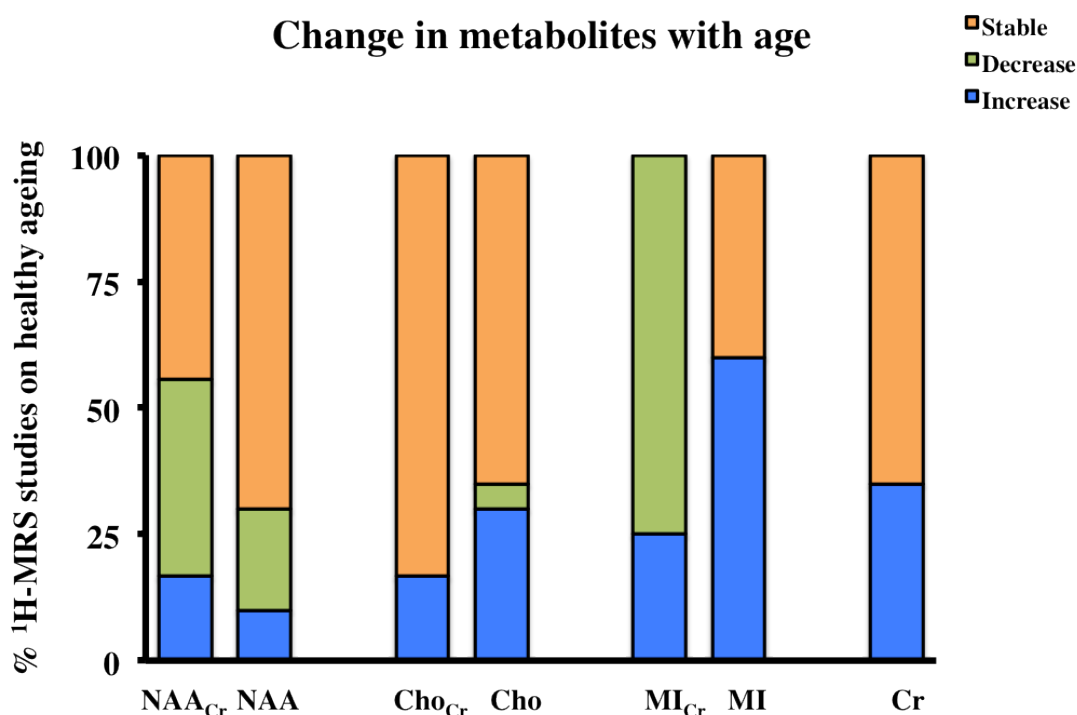


Figure 7.1: Variability in ¹H-MRS results within the healthy ageing brain.

This figure has been created using data from Haga et al., 2009 who performed a meta-analysis of 18 studies from 1980 to 2006 in which brain metabolite concentrations in young individuals (<60 years) were compared to healthy older subjects (>60 years). Across all brain regions studied, there have been reports of increases, decreases and no changes in NAA, choline (Cho), myo-inositol (MI) and creatine (Cr) with age. These inconsistencies may be driven by the choice of reporting technique, *i.e.*, if metabolites are reported as relative ratios of creatine (depicted as NAA_{Cr}, Cho_{Cr}, MI_{Cr}) or as absolute values referenced to water (depicted as NAA, Cho, MI, Cr). The increasing number of studies reporting changes in absolute creatine values with age suggest that creatine may no longer be a suitable reference metabolite.

7.1.4 Objectives

Given the need for a better understanding of (1) age and (2) genetic risk and resilience for late-onset AD on brain metabolite levels, we conducted a single voxel ^1H -MRS study in the PCC/precuneus of 30 young and 117 older individuals genotyped for *APOE*. We focused on the PCC because this region has been extensively examined in ^1H -MRS studies of AD (Kantarci et al., 2013, 2007, 2004, 2002a, 2000), but we know relatively little about how its metabolite profile is affected during normal ageing and in people who are at a genetic risk for developing AD (Chiu et al., 2014; Reyngoudt et al., 2012). We specifically chose the PCC over the hippocampus because ^1H -MRS studies report better reproducibility and precision of metabolite quantification in cortical regions compared to the hippocampus (Geurts et al., 2004). Moreover, regional variation in hippocampal metabolite ratios can confound interpretations of proton spectra obtained from this region, particularly at a field strength of 3T (Hsu et al., 2001).

7.2 Methods

7.2.1 Participants

Thirty young and 117 older participants were compared to study the effect of age on PCC metabolite concentrations. *APOE* information was only available for 101 out of the 117 older participants, of which one subject with the $\epsilon 2\epsilon 4$ genotype was subsequently excluded. Thus, 30 young (4 $\epsilon 2$ -carriers, 18 $\epsilon 3$ -homozygotes, 8 $\epsilon 4$ -carriers) and 100 old participants (10 $\epsilon 2$ carriers, 68 $\epsilon 3$ homozygotes and 22 $\epsilon 4$ carriers) were used to study the effect the *APOE*. The detailed participant selection protocol is presented in Chapter 3.

7.2.2 ^1H -MRS acquisition and analysis

The young and old groups underwent identical MRI acquisition and analysis protocols. All participants were scanned at the FMRIB Centre, Oxford using a 3T Siemens Verio scanner. The neuroimaging protocol included:

Structural MRI: FMRIB's automated segmentation tool (FAST) was used to segment the structural brain images into grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF) in order to compute the tissue composition of the PCC voxel (Zhang et al., 2001).

^1H -MRS: Data were acquired from a $2\times 2\times 2\text{cm}^3$ voxel located in the PCC and precuneus (Figure 3.4). The voxel was positioned manually by referring to anatomical landmarks on the structural scan, and a Short Echo Time Full Intensity Acquired Localized (SPECIAL)

sequence (Mekle et al., 2009; Near et al., 2013) with Variable Power radio-frequency pulses with Optimized Relaxation delays (VAPOR) water suppression was acquired (Tkáč et al., 2001). This sequence allows for the simultaneous quantification of several metabolites within a single acquisition without the need for spectral editing, and minimizes signal decay from T2 relaxation (Mekle et al., 2009; Near et al., 2013). We used an automated shim tool and outer volume suppression before each scan to saturate spins on all six sides of the PCC voxel. Eight averages of water un-suppressed data were acquired with the same outer volume suppression scheme (Near et al., 2013).

For all spectra, an in-house MATLAB (Natick MA, USA) based semi-automated processing protocol was applied as outlined previously (Near et al., 2013). Briefly, the processing chain involved correction for eddy currents, the removal of motion-corrupted averages and corrections for frequency drifts prior to signal averaging. All processed data were analyzed with LCModel (Version 6.3-1B) (Provencher, 2001), using a basis set that consisted of 21 simulated basis spectra. Macromolecule spectra acquired from the occipital cortex of five volunteers using an inversion recovery sequence (TR = 2 s, TI = 0.680 s) were included in the basis set (Emir et al., 2012). No baseline correction, zero filling or line broadening was applied to the *in vivo* data before input into LCModel. The spectra were fitted over the 0.5 to 4.2 ppm range.

Spectral quality was assessed using strict quality limits (Jansen et al., 2006; Kreis, 2004). The spectra of one young subject and 34 older subjects did not meet the guidelines and were removed from further analysis. All included spectra had line widths (measured as FWHM) <0.06 ppm (~8Hz) and SNR > 50, with no outliers for either of the measures. The spectra were inspected visually to ensure stable baselines and symmetry of the

metabolite peaks. We quantified reliability using a Cramer-Rao lower bound threshold of 20% (Jansen et al., 2006; Kreis, 2004). Accordingly, alanine, GABA and lactate could not be reliably measured in over 50% of the participants in the older group and were excluded from further analysis. We measured the concentrations of aspartate (Asp), glycerophosphocholine (GPC), phosphocholine (PCh), creatine, phosphocreatine (PCr), glucose (Glc), taurine (Tau), glutamine, glutamate, glutathione, myo-inositol, NAA, N-acetylaspartylglutamate (NAAG), and phosphoethanolamine (PE). GPC and PCh were combined to quantify total choline content (tCho), and similar measures were made for total creatine (Cr+PCr), total NAA (NAA+NAAG) and total glucose (Glc+Tau), as previously described (Emir et al., 2012). In agreement with past reports, we used creatine as a metabolite of interest, rather than as a reference for other metabolites in this study (Jansen et al., 2006).

The older subjects had generally higher linewidths than the younger subjects. Although the ^1H -MRS sequences were identical for the young and old groups, they were acquired at different times during the multimodal scan sequence. Where in the young group the sequence was acquired towards the start of the scan, the old group underwent the ^1H -MRS acquisition towards the end. Thus, in order to minimize the effect of longitudinal drift in scanner hardware on the spectra from the older group, line width was included as a confounding covariate in all the analyses. We performed an L2 normalisation of the signal intensity of metabolites measured by LCModel for young and old subjects. Spectra were normalized to account for the entire metabolic profile, *i.e.* the water-scaled signal intensity of each metabolite was divided by the sum of the water-scaled signal intensities of all metabolites reliably quantified by LCModel

Accordingly, for each subject:

$$\text{Metabolite}_{\text{corrected}} = \frac{\text{Metabolite}}{(\text{total choline} + \text{total creatine} + \text{glucose} + \text{total NAA} + \text{myo-inositol} + \text{glutamate} + \text{glutamine} + \text{glutathione} + \text{phosphoethanolamine} + \text{aspartate})}$$

7.2.3 Statistical analysis

Statistical analysis was performed in SPSS (SPSS, Chicago IL). Sociodemographic variables (age, education), spectral line width, and voxel tissue composition were compared using unpaired t-tests (between the two age groups) or ANOVA (across the three *APOE* groups). Exact Fisher's test was used for categorical variables (gender). χ^2 -test was used to confirm that the *APOE* distribution of our sample reflected that expected in a healthy Caucasian population. The effects of age, *APOE*, and the interaction of *APOE* x age on ^1H -MRS metabolite ratios were computed using a multivariate analysis of covariance (MANCOVA) with post-hoc Bonferroni correction for multiple comparisons across the three *APOE* groups. Correlations between age and metabolite concentrations are expressed as Pearson's product moment coefficients.

7.3 Results

7.3.1 Effect of age on metabolite concentrations in the PCC

The young and old groups did not differ in years of education or voxel WM content. There were significant age-group differences in gender, spectral line width, and GM and CSF concentrations within the voxel, and these variables were therefore included as covariates in the model for studying the effect of age on PCC metabolite content (Table 7.1). The MANCOVA revealed a significant effect of age on metabolite concentrations ($F_{(10,132)}=9.08$, $p<0.001$; Wilk's λ , partial $\eta^2=0.41$). Post-hoc comparisons showed that relative to the young group, the older group had significantly higher levels of myo-inositol ($F_{(1,141)}=5.73$, $p<0.05$; partial $\eta^2=0.04$) and total creatine ($F_{(1,141)}=36.31$, $p<0.001$; partial $\eta^2=0.18$), and significantly lower levels of glutamate ($F_{(1,141)}=31.28$, $p<0.001$; partial $\eta^2=0.184$) and glutathione ($F_{(1,141)}=5.07$, $p<0.05$; partial $\eta^2=0.04$) (Figure 7.2). To account for multiple comparisons across the metabolites, we performed an additional Bonferroni correction and accepted statistical significance at $p<0.005$. Accordingly, only age-related changes in total creatine and glutamate survive. There were no group differences in any other metabolite. Pearson's product moment correlation was used to determine the ageing trajectories of these four metabolites within each group (Figure 7.3). There was a significant negative correlation between glutamate and age in younger ($r_{28} = -0.37$, $p<0.05$) but not in the older group ($r_{115} = -0.16$, $p=0.09$). By contrast, there was a significant negative correlation between glutathione and age in the old ($r_{115} = -0.27$, $p<0.005$) but not in the young group ($r_{28}=0.273$, $p=0.144$). Myo-inositol and total creatine did not show any significant age-related correlations in either group.

Table 7.1: Effect of age on PCC metabolites

Values represent means $\pm$ standard deviations. P values indicate results of chi-square test (gender), t-tests (sociodemographics, PCC voxel) and MANCOVA (PCC metabolites). Age, gender, GM% , CSF% and FWHM were included as covariates in the MANCOVA. ** indicates metabolites which survived post-hoc Bonferroni correction for multiple comparisons across metabolites.

	Young N=30	Old N=117	P
Sociodemographics			
Age (yrs)	23.87 $\pm$ 4.95	68.86 $\pm$ 5.36	<0.001
Education (yrs)	17.08 $\pm$ 2.63	16.01 $\pm$ 3.29	0.10
Gender (% males)	56.7%	78.6%	<0.05
PCC voxel			
GM (%)	0.60 $\pm$ 0.03	0.52 $\pm$ 0.10	<0.001
WM (%)	0.23 $\pm$ 0.03	0.23 $\pm$ 0.04	0.72
CSF (%)	0.17 $\pm$ 0.03	0.23 $\pm$ 0.06	<0.001
FWHM (ppm)	0.026 $\pm$ 0.005	0.034 $\pm$ 0.006	<0.001
Neuropsychological tests			
CES-D	-	3.71 $\pm$ 3.98 (range 0-16)	
MOCA	-	28.35 $\pm$ 1.15 (range 26-30)	
PCC metabolites			
Aspartate	0.0607 $\pm$ 0.004	0.0614 $\pm$ 0.006	0.49
Glutamine	0.0430 $\pm$ 0.006	0.0519 $\pm$ 0.009	0.19
Glutamate	0.2072 $\pm$ 0.007	0.1893 $\pm$ 0.009	<0.001**
Glutathione	0.0232 $\pm$ 0.001	0.0215 $\pm$ 0.002	<0.05
Myo-inositol	0.1247 $\pm$ 0.007	0.1327 $\pm$ 0.010	<0.05
Total NAA	0.2396 $\pm$ 0.007	0.2321 $\pm$ 0.012	0.43
Total creatine	0.1667 $\pm$ 0.007	0.1747 $\pm$ 0.008	<0.001**
Total choline	0.0256 $\pm$ 0.002	0.0263 $\pm$ 0.003	0.11
Total glucose	0.0719 $\pm$ 0.006	0.0751 $\pm$ 0.014	0.66

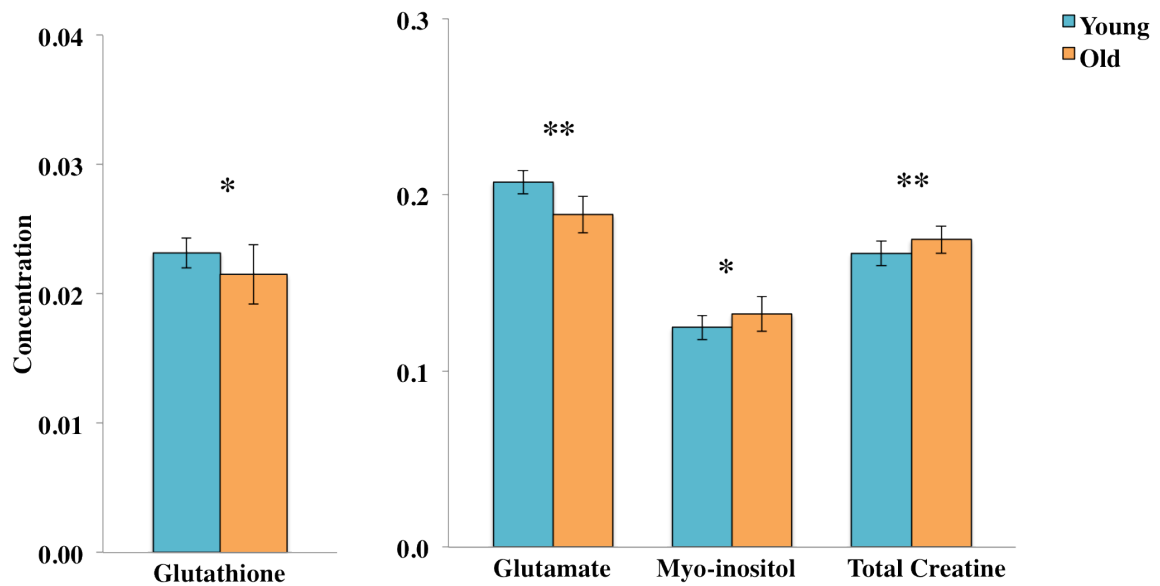


Figure 7.2: The effect of age on PCC metabolites.

Significantly lower glutathione and glutamate, and higher myo-inositol and creatine in the older (n=117) compared to the younger (n=30) group. Spectral linewidth, gender, voxel GM and CSF content were included as covariates in the MANCOVA. Bars represent means $\pm$ SD. * denotes $p < 0.05$, ** denotes $p < 0.005$.

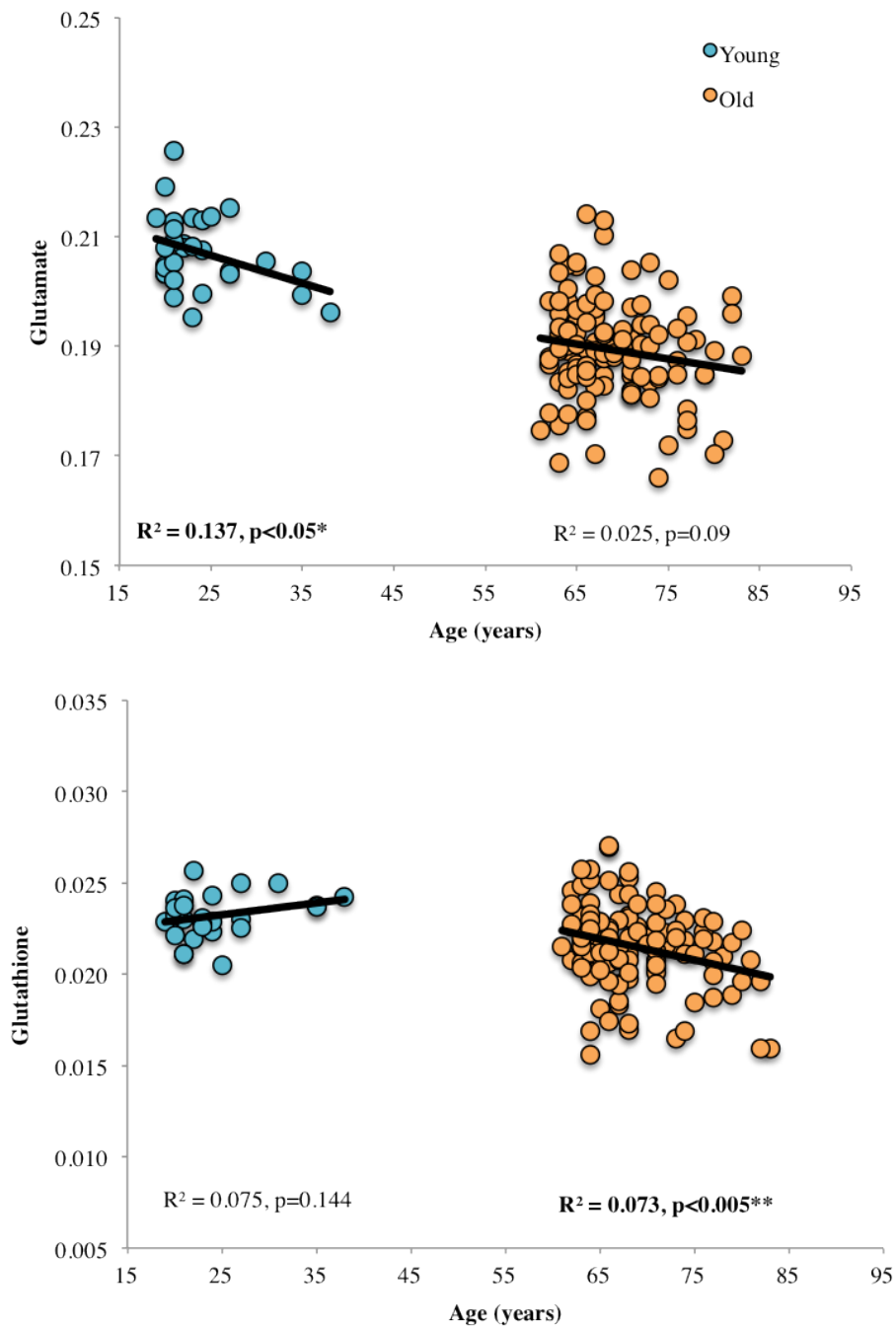


Figure 7.3: Correlations of metabolites with age.

Linear regression analysis of the effect of age on glutamate (top) revealed a significant negative correlation in the younger ($n=30$, $p<0.05^*$) but not the older group, and glutathione (bottom) revealed a significant negative correlation in the older ($n=117$, $p<0.005^{**}$) but not the younger group.

7.3.2 *Effect of APOE on metabolite concentrations in the PCC*

A total of 14 ϵ 2-carriers, 86 ϵ 3-homozygotes, and 30 ϵ 4-carriers aged 20 to 85 years were compared for effects of *APOE* on PCC metabolites. There was no significant difference in the ratio of young:old subjects in the three *APOE* groups ($\chi^2=0.68$, $df=2$, $p=0.71$). There were no significant *APOE*-related differences in age, gender ($\chi^2=4.42$, $df=2$, $p=0.11$), years of education, spectral line width, voxel CSF, GM or WM content. The multivariate analysis revealed no significant effect of *APOE* ($F_{(20,230)}=0.632$, $p=0.89$; Wilk's λ , partial $\eta^2=0.05$) or interaction between *APOE* and age ($F_{(20,230)}=0.736$, $p=0.79$, Wilk's λ , partial $\eta^2=0.06$) on metabolite concentrations (Table 7.2).

Table 7.2: Effect of *APOE* on PCC metabolites

Values represent means $\pm$ standard deviations. P values are results of chi-square tests (for gender) and one-way ANOVA (for sociodemographics, PCC voxel, PCC metabolites).

	ε2 N=14	ε3 N=86	ε4 N=30	P
Sociodemographics				
Age (yrs)	55.60 $\pm$ 19.57	59.93 $\pm$ 19.69	56.26 $\pm$ 20.68	0.57
% from old group	71.4%	79.1%	73.3%	0.71
Education (yrs)	17.36 $\pm$ 2.93	15.91 $\pm$ 3.19	16.40 $\pm$ 3.51	0.28
Gender (% male)	78.6%	67.4%	86.7%	0.11
PCC voxel				
GM (%)	0.53 $\pm$ 0.05	0.54 $\pm$ 0.10	0.55 $\pm$ 0.05	0.77
WM (%)	0.25 $\pm$ 0.06	0.23 $\pm$ 0.04	0.24 $\pm$ 0.04	0.28
CSF (%)	0.22 $\pm$ 0.07	0.22 $\pm$ 0.06	0.21 $\pm$ 0.06	0.88
FWHM (ppm)	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01	0.65
PCC metabolites				
Aspartate	0.062 $\pm$ 0.006	0.061 $\pm$ 0.004	0.062 $\pm$ 0.007	0.92
Glutamine	0.047 $\pm$ 0.008	0.050 $\pm$ 0.009	0.051 $\pm$ 0.007	0.52
Glutamate	0.194 $\pm$ 0.013	0.193 $\pm$ 0.011	0.193 $\pm$ 0.011	0.94
Glutathione	0.022 $\pm$ 0.003	0.022 $\pm$ 0.002	0.022 $\pm$ 0.002	0.75
Myo-inositol	0.133 $\pm$ 0.032	0.130 $\pm$ 0.010	0.132 $\pm$ 0.001	0.32
Total NAA	0.236 $\pm$ 0.013	0.235 $\pm$ 0.011	0.232 $\pm$ 0.010	0.42
Total creatine	0.169 $\pm$ 0.008	0.174 $\pm$ 0.008	0.174 $\pm$ 0.009	0.19
Total choline	0.026 $\pm$ 0.003	0.026 $\pm$ 0.003	0.026 $\pm$ 0.002	0.94
Total glucose	0.074 $\pm$ 0.010	0.074 $\pm$ 0.013	0.075 $\pm$ 0.012	0.98

7.4 Discussion

This is the first examination of metabolites in a relatively large sample of cognitively healthy individuals genotyped for *APOE*. We have shown age-related alterations in the concentrations of metabolites that are independent of *APOE* genotype. In particular we have found that creatine, which has widely been used as a reference metabolite, does not remain stable with age within the PCC and may therefore not be a suitable reference in studies involving a broad age-range.

7.4.1 Effects of age on metabolite concentrations in the PCC

The older group had significantly higher concentrations of total creatine and myo-inositol and lower levels of glutamate and glutathione within the PCC than younger individuals ($p < 0.05$). Group differences in total creatine and glutamate survived an additional conservative correction for multiple comparisons across metabolites ($p < 0.005$). These changes may point towards alterations in cellular efficiency that are characteristic of the normal ageing process.

Creatine and myo-inositol: The finding of a significant age-related increase in total creatine is supported by several previous reports both within the PCC (Chiu et al., 2014; Reyngoudt et al., 2012) and in other parts of the brain including frontal and parietal grey and white matter (Angelie et al., 2001; Chang et al., 1996; Charlton et al., 2007; Gruber et al., 2008; Leary et al., 2000; Pfefferbaum et al., 1999; Saunders et al., 1999; Schuff et al., 2001), although stable levels of creatine have also been noted in the frontal lobe of males (Brooks et al., 2001). Creatine is a marker of energy metabolism and it is predominantly

found in glia (Urenjak et al., 1993). Increases in creatine could be indicative of age-related glial proliferation (Charlton et al., 2007; Leary et al., 2000; Reyngoudt et al., 2012), particularly when observed together with a rise in the concentration of another prominent glial marker, myo-inositol (Brand et al., 1993), as is the case in this and other studies (Chang et al., 1996; Gomar et al., 2014; Gruber et al., 2008; Raininko and Mattsson, 2010; Reyngoudt et al., 2012; Saunders et al., 1999). Most importantly, however, our finding adds to the growing body of evidence expressing reservations about using creatine as a reference metabolite in ^1H -MRS evaluations of metabolite concentrations, particularly across a wide age-range (Jansen et al., 2006).

Glutathione: We also found a decline in glutathione in the older group. Glutathione is an antioxidant that regulates the elimination of toxic oxidative stressors (Forman et al., 2009) and has been reported to decrease with age (Emir et al., 2011). This decline may stem from an increase in intracellular glutathione consumption because of higher occurrences of reactive oxidative species with age. Alternatively, it could be suggestive of a shortfall in glutathione production, which would eventually lead to downstream deficits in protection from oxidative stress (Maher, 2005). Both ideas lend support to the free radical theory of ageing (Beckman and Ames, 1998).

Glutamate: In line with our findings, age-related reductions in glutamate have also been observed in ^1H -MRS studies of cortical grey matter (Chang et al., 2009; Grachev and Apkarian, 2001; Kaiser et al., 2005; Marsman et al., 2013; Raininko and Mattsson, 2010; Sailasuta et al., 2008). Glutamate is the main excitatory neurotransmitter in the brain, mediating key cognitive and motor functions that are impaired during the normal ageing process (Segovia et al., 2001). Unlike creatine and myo-inositol, glutamate is localized

primarily in neurons and its concentration in the brain is an indicator of neuronal integrity (Patel et al., 1982). Glutamate reductions have therefore often been attributed to neuronal loss, shrinkage, or a decline in neurotransmission (Kaiser et al., 2005). Here, we must exercise caution before citing neuronal loss as an underlying cause for the observed reduction in glutamate, essentially because we found no age-related changes in NAA, which is accepted as the most reliable marker of neuronal density (Urenjak et al., 1993).

NAA: NAA is perhaps the most widely studied and controversial metabolite in the brain. It is present exclusively in neurons (Urenjak et al., 1993). Levels of NAA are altered in neurodegenerative diseases (Graff-Radford and Kantarci, 2013), but reports within the healthy ageing brain have been far less consistent with studies finding increases, decreases and no change in NAA/Cr (Haga et al., 2009; Reyngoudt et al., 2012). However, while observed decreases in NAA/Cr have been interpreted as decreases in levels of NAA, they may in fact simply be methodological artefacts that are wholly explained by increases in creatine (Haga et al., 2009; Reyngoudt et al., 2012). Indeed, a recent study concluded that whole-brain NAA was actually conserved throughout normal ageing (Wu et al., 2012). In agreement with our findings, previous cross-sectional (Gomar et al., 2014; Reyngoudt et al., 2012) and longitudinal reports (Kantarci et al., 2007; Schott et al., 2010) within the PCC have found that NAA concentrations remain stable with age, although a relatively smaller study (n=30) found an age-related increase in PCC NAA levels across a wide age-range (Chiu et al., 2014). Our observation of stable PCC NAA levels points towards a lack of *measurable* age-related loss in number of neurons within the PCC, but it does not rule out the existence of neuronal loss or shrinkage in this region (Reyngoudt et al., 2012), the latter also being a salient feature of the ageing brain.

Lastly, we found that although both glutamate and glutathione were reduced in the older group, their individual correlations commenced at different stages. The decrease in glutamate is evident in early adulthood, whereas the decline in glutathione was apparent only in the older group. The older group was sufficiently powered (n=117) and the younger group was comparatively smaller (n=30) and any correlation in the young group should therefore be interpreted carefully. However, our findings are supported by previous evidence of early life declines in glutamate within the medial frontal cortex in 18-31 year old individuals (Marsman et al., 2013) and late life alterations in occipital glutathione (Emir et al., 2011) and are indicative of age-specific alterations in the point at which the concentrations of these two metabolites start to decline.

7.4.2 No effect of APOE on metabolite concentrations in the PCC

This is the first study to characterize the effects all three *APOE* alleles on PCC metabolites across a wide age-range. Although there is considerable evidence for metabolite signatures of late-onset AD, viz. decreases in NAA/Cr and increases in choline/Cr and myo-inositol/Cr in the PCC of patients relative to healthy controls (Kantarci et al., 2013, 2007, 2004, 2002a, 2000), we found no effect of *APOE* or *APOE* x age interaction on any metabolites. This is in line with two previous small studies (with largely overlapping samples) of healthy elderly $\epsilon 3$ homozygotes (n=53) and $\epsilon 4$ carriers (n=8), which also found no significant *APOE*-differences in NAA/Cr, myo-inositol/Cr and NAA/myo-inositol within the PCC (Kantarci et al., 2002a, 2000). In contrast, a larger study of 89 $\epsilon 3$ homozygotes and 23 $\epsilon 4$ carriers found no effect of *APOE* on NAA/Cr, but significant positive correlations of myo-inositol/Cr and choline/Cr and in $\epsilon 4$ carriers but

not $\epsilon 3$ homozygotes within the PCC (Gomar et al., 2014). More recently, Riese et al (2015) found no effects of *APOE* on GABA and glutamate+glutamine, but reported significantly lower NAA/Cr concentrations in $\epsilon 4$ carriers ($n=9$) relative to non-carriers ($n=27$) (Riese et al., 2015). However, 50% of the $\epsilon 4$ carriers in Riese and colleagues' study had amnesic mild cognitive impairment, which makes it difficult to disassociate the effects of *APOE* from any interaction between *APOE* and underlying amyloid pathology.

The lack of observable *APOE* effects in our study may be attributed to several factors. First, there may actually be effects of *APOE* on metabolite concentrations that our study did not pick up. We were limited by the usual constraints of a cross-sectional analysis. Our older group was screened for cognitive impairment and there is a possibility that we may have only included “survivors” who have sidestepped their genetic status for cognitive decline (Gomar et al., 2014). This may have diminished any potential modulatory effect of the $\epsilon 4$ allele. Alternatively, it could be that any potential underlying effects of *APOE* on brain metabolites are region-specific, and that the PCC may not be the ideal region of interest, particularly in cognitively healthy individuals. Recent studies point towards effects of $\epsilon 4$ on NAA/Cr and myo-inositol/Cr within the hippocampus in healthy individuals and MCI patients (Calderon-Garciduenas et al., 2015; Yin et al., 2015). Although the reproducibility of measurements obtained from ^1H -MRS of the hippocampus has been found to be lower than in other brain regions (Geurts et al., 2004), advances in acquisition techniques within the last year allow for more reliable quantification of hippocampal metabolites (Allaili et al., 2015; Bednarik et al., 2015). Given the relevance of the hippocampus in both AD and *APOE*-related changes, future studies investigating the pre-clinical effects of *APOE* should consider focusing on this

region. Our findings suggest that *APOE* may not strongly influence PCC metabolite concentrations in cognitively healthy individuals and that any effects on the PCC that are observed in AD patients (*e.g.* lower NAA/Cr, higher myo-inositol/Cr) may relate to later stages of the neuropathological cascade.

7.4.3 Limitations and conclusions

This study offers an improved understanding of age-related changes in metabolite concentrations. However, we must consider some limitations when interpreting our results.

First, metabolite concentrations vary between different parts of the brain and between grey and white matter (Angelie et al., 2001; Kreis, 2004; Schuff et al., 2001). As mentioned above, our findings of age-dependent changes are therefore likely to be specific to the examined brain region, and must be interpreted in the context of the PCC/precuneus.

Second, because of the increasing speculation about the use of creatine as a reference, particularly in studies of subjects with a wide age-range, we refrained from reporting metabolites referenced to creatine in this study. However, because the older group had higher linewidths than the younger group, we were not confident we could accurately quantify water-referenced values of metabolite concentrations in that group, and have therefore reported normalized values of metabolites for each subject. These values were referenced to the sum of all reliably estimated metabolites within the spectrum. Thus, although our findings are in line with previous reports, and it seems unlikely that this

approach would introduce systematic biases, they must still be interpreted with care. Third, we found significant differences in creatine and myo-inositol between young and old groups but there were no correlations between these metabolites and age in either group. Our study did not include participants in the 40-60 year age-range and so it may be that the turning point for elevations in total creatine and myo-inositol occurs in this mid-life period after which they remain relatively stable. Future studies should focus on this age-range.

This study has addressed the current lack of consistent information on age-related changes in the concentration of metabolites and the need for large-scale studies of older populations by examining ^1H -MRS measurements in a relatively large sample of cognitively healthy individuals. Moreover, we have focused on the PCC, which is a region of interest in studies of neurodegeneration but has thus far been understudied in the context of healthy ageing. We have shown that metabolites within the PCC are susceptible to the ageing process, and that these changes can begin in early adulthood and are independent of *APOE* genotype. Our findings can better inform studies involving longitudinal patient follow-up, where changes in metabolite levels resulting from disease progression may be confounded by those secondary to normal ageing.

Chapter 8

Discussion

This thesis presents the first comprehensive examination of the effects of the *APOE* $\epsilon 2$ allele on brain structure and function across adulthood. It also describes the early effects of the three *APOE* alleles on two novel markers of cognitive decline, *viz.* cerebrovascular health and neurochemical concentrations. In this summary, I answer the five questions outlined in Chapter 1 and suggest directions for future research.

Objective 1: To plug the gaps in the current *APOE* literature

8.1 What do we know about the *APOE* $\epsilon 2$ allele? (Chapter 2)

With a focus on the “forgotten *APOE* allele”, this chapter stressed the importance of including $\epsilon 2$ in studies of *APOE*-related effects on ageing (Suri et al., 2013). Collective evidence points towards neuroprotective effects of this allele, particularly on risk for late-onset AD, but also during the normal ageing process. This includes studies *in vitro*, in animal models, and in humans, with a general consensus of longer lifespans, and lower amyloid burden associated with possession of $\epsilon 2$. There are also isolated reports of relatively slower age-related decline in cognition and brain structural integrity in $\epsilon 2$ carriers. For all these measures, $\epsilon 2$ and $\epsilon 4$ carriers largely display opposite effects in the brain. However, recent MRI-measured similarities in white-matter microstructure and

resting-state brain function between these two groups suggest that we cannot rely exclusively on comparisons between $\epsilon 4$ carriers and $\epsilon 3$ homozygotes to explain risk for AD. The first two experimental chapters of my thesis addressed these nascent concerns by using advanced MRI techniques to perform a detailed comparison of brain structure and function between $\epsilon 2$ and $\epsilon 4$ carriers across a wide age-range.

8.2 Do *APOE* $\epsilon 2$ and $\epsilon 4$ differently influence structural integrity of the brain across adulthood? (Chapter 4)

Using structural MRI and DTI, we found no effects of *APOE* on whole-brain grey matter density, white matter microstructure or hippocampal volumes in young, middle or older individuals. A more localized analysis of the hippocampal branch of the cingulum revealed genotype-specific differences in young and middle-aged participants. Although subtle, these findings were consistent with more robust cingulum microstructure in $\epsilon 2$ and $\epsilon 3$ carriers than in $\epsilon 4$ carriers. Contrary to previous studies, we found no deleterious effects of $\epsilon 4$ on white matter in the older group. Given that the risk for AD in $\epsilon 4$ carriers is significantly more pronounced in females, the observed lack of $\epsilon 4$ effects may be due to the large proportion of males in this group. However, the overall absence of strong *APOE*-mediated changes in brain structure across life suggests that structural MRI and DTI may not be ideal imaging candidates to disassociate the effects of $\epsilon 2$ and $\epsilon 4$ in the brain. We therefore investigated measures of brain function in order to better understanding of how the two alleles may differently influence risk for AD.

8.3 Do *APOE* $\epsilon 2$ and $\epsilon 4$ differently influence functional connectivity of the resting brain across adulthood? (Chapter 5)

We observed that functional connectivity of the posterior cingulate cortex, a central hub of the default mode network, varied in an allele-specific and age specific manner (connectivity in young: $\epsilon 4 > (\epsilon 3 \text{ and } \epsilon 2)$; connectivity in old: $(\epsilon 2 \text{ and } \epsilon 3) > \epsilon 4$). The middle age-range seemed to serve as a transition phase from high to low connectivity for $\epsilon 4$ carriers and low to high connectivity for $\epsilon 2$ carriers. Similar age-related variations were observed for hippocampal connectivity. Accordingly, we proposed a model to explain how these opposing trajectories may contribute to *APOE*'s role in influencing risk for AD. Early-life hyper-connectivity may create an environment that is more vulnerable to earlier amyloid deposition. Over time, this progressive buildup of amyloid could lead to a functional disconnect between spatially distant regions, ultimately leading to a late-life decline in connectivity in $\epsilon 4$ carriers. This proposed association between early life functional hyperactivity and predisposition to AD was further investigated in the following chapter, using a novel marker of AD, *i.e.* cerebrovascular reactivity.

Objective 2: To extend our understanding of *APOE* by studying novel MRI techniques

8.4 Does *APOE* modulate cerebrovascular health in early adulthood?

(Chapter 6)

In this chapter, we focused on the vascular risk factors that contribute to neurodegeneration in AD. We showed for the first time that young $\epsilon 4$ carriers had the lowest and $\epsilon 2$ carriers the highest measures of cerebrovascular health during a CO_2 inhalation challenge, in a pattern that mirrored lifetime risk for AD (*AD-risk: $\epsilon 4 > \epsilon 3 > \epsilon 2$; $\text{CO}_2\text{-CVR: } \epsilon 2 \geq \epsilon 3 > \epsilon 4$*). Given that $\text{CO}_2\text{-CVR}$ is reduced in patients with AD, we suggested that the relatively low $\text{CO}_2\text{-CVR}$ in $\epsilon 4$ carriers and by contrast, the more robust $\text{CO}_2\text{-CVR}$ in $\epsilon 2$ carriers may contribute to their opposing effects on risk for AD (Suri et al., 2014a).

We also replicated past results showing increases in hippocampal activity during a memory task in young $\epsilon 4$ carriers. This functional hyperactivity was significantly attenuated when $\text{CO}_2\text{-CVR}$ was included as a nuisance covariate in the task analysis. Thus although $\text{CO}_2\text{-CVR}$ and the task-based BOLD signal represent different physiological mechanisms, it appears that the two measures may be linked in early adulthood. Accordingly, we anticipated that the way by which early-life functional hyperactivity may be conducive to late-life amyloid deposition in $\epsilon 4$ carriers is via the deleterious effects of this allele on cerebral vasculature.

8.5 Does *APOE* alter the neurochemical profile across life? (Chapter 7)

Studies investigating the change in metabolite concentrations during healthy ageing have thus far been conducted in small samples of elderly adults (~n=16 on average), and we do not have a strong understanding of how metabolites change within the healthy ageing brain. In the first examination of a relatively large group (n=117 older adults), we found significant alterations in the concentration of creatine, myo-inositol, glutamate and glutathione with age (Suri et al., *submitted*). However, we found no effect of *APOE*, or *APOE* x age interaction on any metabolites within the posterior cingulate cortex. AD has a characteristic neurochemical profile marked by lower levels of NAA and higher myo-inositol than healthy controls. The fact that we found no changes in these metabolites in healthy elderly $\epsilon 4$ carriers seems to suggest that neurochemical changes may be unique to the disease state.

8.6 Overall considerations

Our findings provide valuable insights into the effects of *APOE* in the brain, particularly with regards to the timing of allele-specific effects on brain structure, function, vascular health and metabolic profile. Chapter 6 shows that *APOE* $\epsilon 4$ is closely associated with the vascular dysfunction that is hypothesized to precede amyloid deposition. By contrast $\epsilon 2$ is linked to relatively superior measures of cerebrovascular health in early adulthood, which lends support to its status as a neuroprotective allele. However, although we have shown that cognitively healthy young $\epsilon 4$ carriers have significantly lower CO₂-CVR than non-carriers (Suri et al., 2014a) and others have subsequently reported similar declines in CO₂-CVR in older adults (Hajjar et al., 2015; Wolters et al., 2016), we also found that $\epsilon 4$

carriers have similar underlying brain structural and neurochemical profile as non-carriers through the lifespan (Chapters 4 and 7). Taken together, it is reasonable to suggest that *APOE* allele-specific changes in cerebrovasculature, resting-state and task-based function (Chapters 5 and 6) may reflect early stages of the pathogenic cascade and may *precede* and perhaps even contribute to late-life events like AD-related cognitive decline. By contrast, AD-related structural and neurochemical alterations may only become “measurable” at a later time point, *after* a certain threshold of neurodegeneration has occurred, which may not have been the case for the cognitively healthy participants in this study.

I have discussed the study-specific limitations in each chapter. A common limitation in all the chapters was that the three age groups belonged to different studies (young: this DPhil study, middle-aged: Oxford Biobank, older: Whitehall II cohort), and received different MRI scan protocols. For the structural and resting-state data, we were only able to show *APOE*-related trends in imaging variables across the lifespan as the MRI data for the three age groups could not be combined to perform a more detailed *APOE* x age analysis. Thus while we could speculate on the timing of the changes in resting-state activity across adulthood (*i.e.* high to low connectivity in $\epsilon 4$ carriers going from young to old), we could not evaluate the specific age-range during which these changes occur. A second limitation of this study is that it is cross-sectional. Even had we been able to combine the imaging data from all our participants, we would not have been able to pinpoint exactly when *APOE* begins to exert its allele-specific effects in the brain because the ageing trajectory is subject to inter-subject variability. There is a need for longitudinal examinations of *APOE* that would shed light on how the early effects of $\epsilon 4$ are associated

with late-life cognitive decline and by contrast how $\epsilon 2$ carriers lead relatively long and healthy lives.

8.7 Clinical relevance of MRI-based imaging markers of AD

Despite numerous efforts, a shocking 99.6% of clinical trials for AD have failed in the last 15 years and no new treatments for AD have been approved since 2003 (Cummings et al., 2014). This alarmingly high attrition rate is driven by several factors: the lack of disease modifying targets, sub-optimal methods of selecting participants into the trials, and inaccurate diagnoses. In fact, the disorder can only be affirmatively diagnosed at autopsy after an examination of the characteristic neuropathology, and a clinical diagnosis of “probable AD” is usually based upon patient history and performance on cognitive tests (Arnold et al. 1991). Because few patients present with clear-cut clinical symptoms, only 20-50% of dementia cases receive a formal diagnosis, potentially leaving a large proportion of affected individuals without access to appropriate treatment and care (Alzheimer’s Disease International, 2011). There is little scope for treatment even for the patients who do receive an accurate diagnosis, because by the time cognitive symptoms begin to manifest, much of the damage to the brain is already done. However, AD has a lengthy prodromal phase, with pathological changes beginning decades before clinical symptoms begin to manifest. This gives us a potential “*window of opportunity*” during which preventative disease-modifying treatments can be optimally effective (Lovestone, 2014). To develop these treatments, we first need to be able to identify individuals in this prodromal phase who are (1) likely to develop AD and (2) likely to benefit from AD treatment. In other words, there is a need for early-life biomarkers that will help us predict AD onset.

In this thesis, we used an individual's *APOE* status as a marker of their likelihood for developing AD (*APOE* ϵ 4: higher risk, ϵ 2: lower risk). We examined how *APOE* influenced known MRI-based imaging markers of AD to see if healthy ϵ 4 carriers displayed the same brain changes that have been well documented in AD patients (and vice versa for ϵ 2 carriers). Our main goal was to identify which MRI markers could serve as a stepping-stone to predicting genetic risk and resilience for AD. We found that the effects of *APOE* on brain function and vascular health may be more suitable as early predictors of risk for AD than brain structure and neurochemical levels. Notably, changes in the former are apparent even in individuals as young as 20 years old, making them potentially useful early-life biomarkers.

CO₂-CVR stood out as a particularly useful marker of predisposition to vascular and neurodegenerative disorders. What sets this measure apart from other MRI-based markers like structural atrophy or functional activity is that in addition to being a marker of risk, CVR could potentially serve as a clinical target. Animal studies have reported complete rescue of CVR dysfunction with pharmacological interventions (Nicolakakis et al., 2008). Impairments in CVR have also been reversed with treatment in healthy adults, patients with hypertension, and small-vessel cerebrovascular disease (Reinhard et al., 2011; Sander, 2005; Sterzer et al., 2001; Troisi et al., 1998). Mahley and Huang recently proposed that the development of treatments that can move *APOE* ϵ 4 to a more ϵ 2-like state may be a stepping-stone to exploiting the mechanisms by which the ϵ 2 allele confers a protection to AD (Mahley and Huang, 2012b), and because CO₂-CVR has been shown to be a target for drug discovery, it holds the potential to be translated into the clinical domain.

8.8 Directions for future research

8.8.1 Further work on CO₂-CVR

Taking Mahley and Huang's recommendation forward, future studies could ask exactly how we could increase CVR in $\epsilon 4$ carriers, and whether this lowers their risk for cognitive decline. The studies reporting drug-induced increases in CVR have largely used statins and anti-hypertensive medication, with one study reporting significant increases in CVR measurements after only 7 days of pravastatin treatment (Sander, 2005). It remains to be seen if improvements in CVR have downstream effects on cognition (*i.e.*, if there is an association between cerebrovascular and cognitive reserve) mainly because there are inconsistent reports on the cognitive benefits of statins themselves. There is compelling evidence for a lower risk for AD, as well as lower rates of cognitive decline in healthy controls and AD patients who are statin users relative to those who are not on statin medication. On the other hand, there are also reports demonstrating no cognitive benefit of statin use over time (for reviews see (Rojas-Fernandez and Cameron, 2012; Sabbagh and Sparks, 2012). Even amongst the studies that find improvements in cognitive performance in statin users, there is no suggestion of differences in the rates of improvement between *APOE* $\epsilon 4$ carriers and non-carriers (Steenland et al., 2013). However, the clinical efficacy of statins is typically evaluated in relatively older people, *i.e.* AD patients or healthy elderly controls. Our results show that $\epsilon 4$ carriers have relatively poorer measures of cerebrovascular health in people as young as 20 years. If this damage were to progressively build up over time, it is possible that the “*window of opportunity*” during which statins can exert a modulatory effect may already have been

missed by mid-late life, and studies focusing on a young age-range may be more informative.

Another therapy that has been reported to elevate CVR is exercise. There is a general consensus that exercise not only delays the onset of AD, but is also associated with enhanced cognition over time (Radak et al., 2010; Ratey and Loehr, 2011). Notably, the beneficial effects of exercise are most marked in *APOE* $\epsilon 4$ carriers (for review see (Raichlen and Alexander, 2014)). Athletic $\epsilon 4$ carriers have a significantly lower risk of developing AD than sedentary $\epsilon 4$ carriers (Kivipelto et al., 2008; Rovio et al., 2005; Schuit et al., 2001). Aerobic fitness is also positively associated with memory performance in $\epsilon 4$ carriers (Etnier et al., 2007). Recent work suggests that amyloid deposition may be central to the interaction between *APOE*, exercise and cognition; sedentary $\epsilon 4$ carriers have significantly higher levels of amyloid deposition than active non-carriers (Head et al., 2012), and higher exercise is associated with lower amyloid load in $\epsilon 4$ carriers (Brown et al., 2012). Our findings lead us to hypothesise that exercise may influence amyloid deposition via its intermediary effects on CO_2 -CVR. Indeed, significant elevations in CO_2 -CVR have been observed after only 12 weeks of exercise-training in previously sedentary individuals in both young (~23 years) and older (~63 years) ages (Murrell et al., 2013). Although the suggestion that early-life interventions of exercise-induced increases in CO_2 -CVR may result in lower levels of amyloid deposition and overall improved cognition in $\epsilon 4$ carriers may be simplistic, it is certainly attractive enough to warrant further examination.

We must stress that not all $\epsilon 4$ carriers go on to develop AD, and not all $\epsilon 2$ carriers are protected from AD, making one's *APOE* status alone an inadequate biomarker for disease

onset. Simply forming an association between the $\epsilon 4$ allele and reduced CO_2 -CVR is insufficient to predict which $\epsilon 4$ carriers will ultimately develop AD because inter-individual variation in CO_2 -CVR *within* the $\epsilon 4$ group confounds our ability to assess risk. There is a need for longitudinal studies that question exactly which $\epsilon 4$ carriers along the spectrum of low CO_2 -CVR are most likely to undergo cognitive decline. Moreover, given that $\epsilon 4$'s effects on risk for AD are gene-dose dependent, it would be worthwhile to see if it modulates CO_2 -CVR in a similar fashion.

As discussed in Chapter 6, we need an understanding of the mechanisms behind a reduction in CO_2 -CVR in $\epsilon 4$ carriers. Is a reduced ability to boost blood flow to the brain in response to CO_2 a consequence of impaired endothelial structure, or deficiencies in the cellular cascade responsible for signaling a change in pH? If $\epsilon 4$ carriers had early-life deficits in blood vessel structure, we likely would have observed a reduction in baseline cerebral perfusion during the task and at rest, which we did not find. We therefore hypothesize that inefficiency in the neuronal signaling cascade responsible for responding to vasodilatory stressors like CO_2 may drive the reduction in CO_2 -CVR in young $\epsilon 4$ carriers. Further, while recent studies find that older $\epsilon 4$ carriers also have reduced CO_2 -CVR relative to $\epsilon 3$ homozygotes, no such data is available for older $\epsilon 2$ carriers. A replication of our findings in older adults would help strengthen our assumption that life-long maintenance of cerebrovascular health underlies a protection to neurodegenerative disorders.

8.8.2 *APOE and the time course of cognitive decline*

Although $\epsilon 2$ and $\epsilon 4$ confer varying degrees of risk for AD, their effects on cognitive ageing in non-clinical populations has been less clear. In Chapter 2 I briefly touched upon some studies citing a protective effect of *APOE* $\epsilon 2$ on cognitive function and others reporting no effect of *APOE*. Similar contrasting evidence exists for the $\epsilon 4$ allele, which has been studied in much more detail (Rusted and Carare, 2015; Salvato, 2015). There is evidence for a positive effect on cognition in young $\epsilon 4$ carriers (Alexander et al., 2007; Marchant et al., 2010; Puttonen et al., 2003; Rusted et al., 2013) and accelerated cognitive decline in older individuals (Schiepers et al., 2012; Small et al., 2004; Wisdom et al., 2011), with a recent review finding no clear cognitive signature of $\epsilon 4$ in middle-aged individuals (Salvato, 2015). Although the cognitive effects reported in most of the studies are small, this pattern seems to support the antagonistic pleiotropy hypothesis (Rusted and Carare, 2015) and fits with the life-long trends in functional activity presented in Chapter 5. Future studies should question the direct association between BOLD activity and cognition, and ask if an initial phase of task-based BOLD hyperactivity is symptomatic of an early-life cognitive advantage in $\epsilon 4$ carriers and/or if it is an outcome of *APOE*'s effects on low CO_2 -CVR (as indicated in the model presented in Figure 6.7).

It is important for us to gauge the exact time point at which a decline in functional activity and cognition begins to occur in $\epsilon 4$ carriers, and what drives this turning point. Based on the results of Chapter 5 and the available literature on *APOE* and cognition, I propose that this decline begins to occur during mid-life (40-60 years), making $\epsilon 4$ carriers in this age-range a particularly relevant target for disease-modifying interventions, like cognitive enhancers (Husain and Mehta, 2011) or CO_2 -CVR elevators (Sabbagh and

Sparks, 2012). From the findings presented in Chapter 6, I suggest that this decline in function and cognition is driven by pre-existing early-life deficits in cerebrovascular health. The main downstream effect of cerebrovascular dysfunction is a chronic mild hypoxic state in the first few decades of life, which may predispose $\epsilon 4$ carriers to earlier and more severe amyloid deposition. The neuronal damage caused by the progressive build-up of amyloid causes may compromise BOLD activity at rest and during tasks, explaining the observed late-life functional hypoactivity. Finally, it could be that once this complex interaction between amyloid and brain function surpasses a certain threshold, it results in AD-related cognitive decline. It is likely that all $\epsilon 4$ carriers do not cross this threshold into cognitive decline, which may explain why some remain cognitively healthy in old age despite underlying changes in BOLD activity (Chapter 5) and CVR (Hajjar et al., 2015; Wolters et al., 2016).

8.9 Conclusion

In summary, this thesis shows that the *APOE* modulates brain function and vascular health in an allele-specific manner from a young age. In line with previous reports on cognition, the gene's effects on resting-state brain function were observed to vary with age with $\epsilon 4$ showing a decline and $\epsilon 2$ carriers an upward trend in functional connectivity across life. The turning point for this age-related trajectory is projected to occur during midlife. Early deleterious effects of $\epsilon 4$ on cerebrovascular health may contribute to the relatively faster decline in functional connectivity in this group, perhaps by increasing their susceptibility to amyloid deposition. Much more research is needed before we can breakdown the complex biological interactions that eventually culminate in AD, particularly those between *APOE*, age, brain function, cerebrovascular health, gender,

amyloid, cognition and other genetic and environmental risk factors for AD. Some of these variables have been examined for the first time here, however they were assessed cross-sectionally and there is an need for longitudinal studies across a wide age-range to test the models put forth in this thesis.

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