How can Magnetoencephalography and Magnetic Resonance Imaging improve our understanding of genetic susceptibility to Alzheimer's disease?



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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Oxford

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Trinity Term 2013

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Abstract

The Apolipoprotein E (*APOE*) ϵ4 allele is the best-established genetic risk factor for sporadic Alzheimer's disease (AD) while the ϵ2 allele confers a reduced risk compared with the most common ϵ3 allele. Neuroimaging studies using magnetic resonance imaging (MRI) have shown that *APOE* genotype affects brain structure and function. The aims of the research presented in this DPhil thesis were twofold: 1) to investigate the effect of *APOE* genotype on brain function in healthy adults using magnetoencephalography (MEG), which is a direct measure of neuronal activity and 2) to explore interactions between the AD risk factors *APOE* ϵ4 allele, age and female gender and their effects on brain structure and function using resting-state functional MRI and diffusion tensor imaging. MEG revealed similar neuronal activity at rest for *APOE* ϵ2 and ϵ4 carriers, i.e. those with opposite AD risk, indicating a more general effect on the functional architecture of the brain that is not directly linked to AD risk. However, differences between *APOE* ϵ2 and ϵ4 carriers became apparent when reactivity to stimuli was explored using the excellent temporal resolution of MEG. *APOE* ϵ4 carriers showed faster sensory processing and *APOE* genotype effects were found for functional networks associated with attention. In the second part of this project, female *APOE* ϵ4 carriers showed overall significantly reduced functional connectivity between the hippocampus and precuneus and a significant age-related decrease in connectivity of these regions. Increased vulnerability of this connection might be one reason for increased AD risk and interventions targeting hippocampal connectivity might be especially effective in at-risk populations. The research presented in this DPhil thesis showed a complex pattern of *APOE* genotype effects on brain structure and function. While global *APOE* genotype effects on functional and structural connectivity do not follow patterns of AD risk, more specific measures of connectivity and task-related brain function could be of use in the development of preclinical markers for AD development.

Acknowledgements

This DPhil project would not have happened without the help and support of many people.

First of all, I would like to thank my supervisors Clare Mackay and Klaus Ebmeier. Thanks to Clare for teaching me to see the big picture and not get lost in the mystery of wriggly lines and for making this DPhil project possible with the successful application for an Alzheimer's Research UK PhD scholarship. Without their financial support I would not have been able to return to Oxford. And thanks to Klaus for always being there when I needed support, especially in the last couple of months. I would also like to thank Kia Nobre, my unofficial supervisor, for all her support and advice during the development and analysis of the MEG project.

The MRI analyses would not have happened without the help of Nico Filippini, thanks for teaching me all I know about MRI analyses and always being there when I had a problem. Also a massive thank you to Aaron Trachtenberg who did a fantastic job with the patient project that I took over from him and for letting me analyse his MRI data.

I would never have managed to analyse the MEG data without the help of Henry Luckhoo, a simple thank you is not enough for the countless hours we spent in front of my computer screen trying to get our scripts to work. Also thanks to Mark Woolrich for his helpful advice and to George Wallis, the best deskmate ever, who kept me sane when I was getting exasperated with bugs in the matlab code.

I am very grateful to Giovanna Zamboni, Ellen McCulloch and others at OPTIMA for all their support with the patient study and helping out with patient recruitment.

Many thanks to all the participants and colleagues who took part in my studies, especially to the patients and carers who spent so much time with me at OCMR. Thanks also to Steve Knight for his patience as scan operator and getting even the most difficult participants into the scanner. Thanks to all those people who helped me out with the MEG data acquisition: Sven Braeutigam for his technical support, Anling Rao for helping out with the scans and Jenni Swettenham for getting everything organised.

Thanks to the best colleagues in the world at FMRIB, OHBA and Psychiatry for stimulating discussions and fun pub crawls that we will continue at SFN and OHBM conferences.

A special thank you to my friends, the relatives I made for myself, a family of Neuros, musicians, sportspeople and others with who I had the best days of my life in Heidelberg and Oxford.

Finally, I want to thank my family, my parents and my sister for their incredible and constant support, for always believing in me, for being the best family anyone could ever have.

Contents

List of Abbreviations **ix**

List of Figures **xi**

List of Tables **xiii**

List of Publications and Presentations **xv**

Preface **1**

Introduction **5**

1 *APOE* and Alzheimer's disease - From Molecular to Psychiatry **11**
1.1 Alzheimer's disease . . . 13
1.1.1 Ageing . . . 13
1.1.2 Metabolism and Cardiovascular Health . . . 15
1.1.3 Systemic Inflammation . . . 17
1.1.4 Oxidative stress and Mitochondria - the common denominator? 18
1.1.5 Amyloid . . . 20
1.1.6 Tau . . . 22
1.1.7 Brain resilience . . . 24
1.1.8 The extended mitochondrial cascade hypothesis . . . 26
1.2 Apolipoprotein E . . . 28
1.2.1 What is apolipoprotein E? . . . 28
1.2.2 ApoE isoforms - Availability . . . 29
1.2.3 ApoE isoforms - Structure . . . 30
1.2.4 ApoE isoforms - Function . . . 31
1.2.5 Amyloid . . . 32
1.2.6 Tau . . . 34
1.2.7 Ageing and Mitochondria . . . 35
1.2.8 Metabolism and Inflammation . . . 36
1.2.9 Brain resilience . . . 38
1.2.10 Other genetic factors . . . 43
1.2.11 Summary . . . 44

2 Neuroimaging techniques **47**
2.1 Magnetic Resonance Imaging 48
2.1.1 Image contrast 50
2.1.2 BOLD fMRI 52
2.1.3 White matter structure 57
2.1.4 MRI acquisition protocol for the studies presented in this thesis 58
2.2 Magnetoencephalography 60
2.2.1 MEG acquisition protocol for the studies presented in this thesis 64
2.3 Summary 65

3 *APOE* genotype and the resting brain **67**
3.1 Introduction 67
3.1.1 AD, *APOE* and resting fMRI 67
3.1.2 AD and resting MEG 69
3.1.3 Aims 70
3.2 Methods 71
3.2.1 Participants 71
3.2.2 Analysis protocols 71
3.2.3 Statistics 76
3.3 Results 77
3.3.1 Demographics 77
3.3.2 ICA 77
3.3.3 Resting-state fMRI 80
3.3.4 Resting-state MEG 80
3.4 Discussion 89
3.4.1 Comparison of fMRI and MEG ICA maps 89
3.4.2 Default-mode network 90
3.4.3 Other resting-state networks 90
3.4.4 MEG functional connectivity 91
3.4.5 Biological mechanisms 92
3.4.6 Methodological considerations 93
3.5 Conclusion 93

4 *APOE* genotype and basic visual processing **95**
4.1 Introduction 95
4.1.1 AD and brain function 95
4.1.2 *APOE* and brain function 96
4.1.3 AD, *APOE* and the oddball task 97
4.1.4 Aims 99
4.2 Methods 100
4.2.1 Participants 100
4.2.2 Oddball task 100
4.2.3 Analysis protocols 102
4.3 Results 104

4.3.1 Sensor-space analysis . . . 104
4.3.2 Task ICA . . . 105
4.4 Discussion . . . 106
4.4.1 Sensor-space analysis . . . 106
4.4.2 Task ICA . . . 108
4.4.3 Limitations . . . 109
4.5 Conclusion . . . 110

5 *APOE* genotype and brain structure 111
5.1 Introduction . . . 111
5.1.1 AD and grey matter . . . 112
5.1.2 *APOE* and grey matter . . . 113
5.1.3 AD and white matter . . . 114
5.1.4 *APOE* and white matter . . . 114
5.1.5 Aims . . . 115
5.2 Methods . . . 116
5.2.1 Participants . . . 116
5.2.2 Analysis protocols . . . 116
5.2.3 Statistics . . . 117
5.3 Results . . . 118
5.3.1 Structural MRI . . . 118
5.3.2 Diffusion tensor imaging . . . 118
5.4 Discussion . . . 121
5.4.1 Grey matter . . . 121
5.4.2 White matter . . . 121
5.4.3 White matter and AD risk . . . 122
5.4.4 Biological mechanisms . . . 122
5.5 Conclusion . . . 124

6 *APOE* genotype, gender and age modulate connectivity of the hippocampus in healthy adults 125
6.1 Introduction . . . 125
6.2 Materials and Methods . . . 128
6.2.1 Participants . . . 128
6.2.2 Image Acquisition . . . 129
6.2.3 Image Analysis . . . 129
6.2.4 Statistics . . . 133
6.3 Results . . . 134
6.3.1 Demographics . . . 134
6.3.2 Functional connectivity . . . 134
6.3.3 White matter integrity . . . 137
6.3.4 Combining functional connectivity and WM integrity . . . 137
6.3.5 Structural MRI . . . 137
6.4 Discussion . . . 140
6.4.1 Reduced functional connectivity in female *APOE* ϵ4 carriers . 140

6.4.2 *APOE* genotype and gender affect WM integrity 142
6.4.3 Correlation between functional connectivity and WM integrity 142
6.4.4 Implications for AD risk 143
6.5 Conclusion 144

7 Final Discussion and Conclusions **145**
7.1 Summary of Objectives and Results 145
7.1.1 *APOE* genotype and the resting brain (chapter 3) 145
7.1.2 *APOE* genotype and basic visual processing (chapter 4) . . . 146
7.1.3 *APOE* genotype and brain structure (chapter 5) 146
7.1.4 *APOE* genotype, gender and age modulate connectivity of the hippocampus in healthy adults (chapter 6) 146
7.1.5 Summary 147
7.2 *APOE* and brain resilience 147
7.3 Neuroimaging markers of preclinical AD and possible intervention strategies 148
7.4 Future Directions 149
7.4.1 What is the difference between *APOE* ϵ2 and ϵ4 carriers? . . . 149
7.4.2 What are the mechanisms underlying antagonistic pleiotropy? 150
7.4.3 How does *APOE* interact with other AD risk factors? 151
7.5 Conclusion 151

References **153**

List of Abbreviations

Aβ, amyloid-β; AD, Alzheimer's disease; *APOE*, apoE, apolipoprotein E; ApoER, apoE receptor; APP, amyloid precursor protein; AUN, auditory network;

BBB, blood-brain barrier; BBR, boundary-based registration;

COPE, contrast of parameter estimate; CSF, cerebrospinal fluid; CVD, cardiovascular disease;

DA, axial diffusivity; DMN, default-mode network; DR, radial diffusivity; DTI, diffusion tensor imaging;

ECG, electrocardiogram; ECN, executive control network; EEG, electroencephalography; EOG, electrooculogram; ERD, event-related desynchronisation; ERF, event-related field; ERP, event-related potential; ERS, event-related synchronisation;

FA, fractional anisotropy; FAST, FMRIB's automated segmentation tool; FEAT, FMRI expert analysis tool; FILM, FMRIB's improved linear model; FIRST, FMRIB's integrated registration and segmentation tool; FLAME, FMRIB's local analysis of mixed effects; FLIRT, FMRIB's linear image registration tool; FNIRT, FMRIB's non-linear image registration tool; FSL, FMRIB software library; FWHM, full-width at half-maximum;

GABA, γ-aminobutyric acid; GLM, general linear model; GM, grey matter; GSK, glycogen synthase kinase; GWAS, genome-wide association study;

HDL, high-density lipoprotein; HPI, head position indicator;

LDL, low-density lipoprotein; LDLR, LDL receptor; LFPN, left fronto-parietal network; LTP, long-term potentiation; LRP, low-density lipoprotein receptor related protein; LVN, lateral visual network;

MD, mean diffusivity; MEG, magnetoencephalography; MNI, Montreal Neurological Institute; MRI, magnetic resonance imaging; MTL, medial temporal lobe; MVN, medial visual network;

NFT, neurofibrillary tangles;

PCC, posterior cingulate cortex; PET, positron emission tomography; PFC, prefrontal cortex;

RF, radiofrequency; RFPN, right fronto-parietal network; RNS, reactive nitrogen species; ROS, reactive oxygen species; RSN, resting-state network;

SMN, sensory-motor network; SNP, single nucleotide polymorphism;

TBSS, tract-based spatial statistics; TFCE, threshold-free cluster enhancement; TE, echo time; TI, inversion time; TOR, target of rapamycin; TR, repetition time;

VBM, voxel-based morphometry;

WM, white matter;

List of Figures

1.1 Amyloid hypothesis . . . 13
1.2 Extended mitochondrial cascade hypothesis . . . 27
1.3 Structural differences between the apoE isoforms . . . 31
1.4 *APOE* and the mitochondrial cascade . . . 46

2.1 Resolutions of MRI and MEG . . . 48
2.2 T_1-weighted imaging . . . 51
2.3 T_2-weighted imaging . . . 53
2.4 Image weighting . . . 53
2.5 BOLD imaging . . . 55
2.6 Resting-state networks . . . 56
2.7 Diffusion ellipsoids . . . 58
2.8 Basis of MEG . . . 60
2.9 Elekta sensors . . . 61
2.10 MEG data . . . 63

3.1 ICA components DMN . . . 78
3.2 ICA components other RSNs . . . 79
3.3 *APOE* genotype differences in DMN connectivity . . . 82
3.4 Frequency contribution to DMN connectivity differences . . . 83
3.5 *APOE* genotype differences in RSN connectivity using fMRI maps . 84
3.6 *APOE* genotype differences in RSN connectivity using MEG maps . 85
3.7 *APOE* genotype differences in oscillatory power 4-30Hz . . . 87
3.8 *APOE* genotype differences in oscillatory power 4 frequency bands . 88

4.1 ERP example . . . 99
4.2 Oddball task design . . . 101
4.3 ERFs in the oddball condition . . . 103
4.4 *APOE* genotype effects on ERFs . . . 104
4.5 *APOE* genotype effects on task ICA . . . 105

5.1 WM structure . . . 120

6.1 Resting-state MRI hippocampal connectivity . . . 136
6.2 DTI analysis of the cingulum . . . 138
6.3 Correlation between structural and functional connectivity . . . 139

List of Tables

2.1 Relaxation times 50
2.2 Imaging Parameters 59

3.1 Demographics MEG subjects 77

5.1 General brain morphology 118

6.1 Demographics 135

List of Publications and Presentations

Publications arising from this Thesis

1. **V Heise**, N Filippini, A J Trachtenberg, S Suri, K P Ebmeier, C E Mackay "APOE genotype, gender and age modulate connectivity of the hippocampus in healthy adults", *In submission*

2. H Luckhoo, S Palazzo Corner, M J Brookes, **V Heise**, C E Mackay, E Duff, S M Smith, M W Woolrich "A framework for group comparisons of functional networks in MEG using ICA and Dual Regression", *In preparation*

Other Publications during DPhil

1. **V Heise**, N Filippini, K P Ebmeier, C E Mackay "The APOE ϵ4 allele modulates brain white matter integrity in healthy adults", *Molecular Psychiatry*, 16: 908–916, 2011

2. K P Ebmeier, **V Heise**, C E Sexton "Neuroimaging chapter", *Oxford Textbook of Old Age Psychiatry*, 5th edition, 2013

3. K P Ebmeier, N Filippini, **V Heise**, C E Sexton "Other magnetic resonance imaging techniques", *International Psychogeriatrics*, 23 Suppl2: S50–S57, 2011

4. S Suri, **V Heise**, A J Trachtenberg, C E Mackay "The forgotten APOE allele: a review of the evidence and suggested mechanisms for the protective effect of APOE ϵ2", *In submission*

Conference Presentations

1. **V Heise** "The effect of APOE genotype on human brain structure and function", *Talk at Alzheimer's Research UK conference*, Belfast, UK, 2013

2. **V Heise** "Resting-State and APOE - why add MEG to fMRI? ", *Talk at MEGUK conference*, Cambridge, UK, 2013

3. **V Heise** "Resting-State and APOE - why add MEG to fMRI? ", *Talk at Donders Discussions*, Nijmegen, Netherlands, 2012

4. **V Heise**, N Filippini, A J Trachtenberg, K P Ebmeier, C E Mackay "APOE genotype and gender modulate functional and structural connectivity of the hippocampus in healthy adults", *Poster at Annual Meeting of the Society for Neuroscience*, New Orleans, LA, USA, 2012

5. **V Heise**, N Filippini, K P Ebmeier, C E Mackay "The APOE ϵ4 allele modulates brain white matter integrity in healthy adults", *Talk and Poster at Alzheimer's Research UK conference*, Leeds, UK, 2011

6. H Luckhoo, M J Brookes, **V Heise**, K P Ebmeier, C E Mackay, P G Morris, M Woolrich "Extracting resting state networks from Elekta Neuromag MEG data using independent component analysis", *Poster at Annual Meeting of the Organization for Human Brain Mapping*, Beijing, China and *International Conference on Biomagnetism*, Paris, France, 2012

7. H Luckhoo, M J Brookes, **V Heise**, K P Ebmeier, C E Mackay, P G Morris, M Woolrich "Investigating the frequency composition of resting state networks in MEG using ICA", *Poster at Annual Meeting of the Organization for Human Brain Mapping*, Beijing, China and *International Conference on Biomagnetism*, Paris, France, 2012

8. H Luckhoo, M J Brookes, J R Hale, **V Heise**, K P Ebmeier, C E Mackay, M Woolrich "Development of methods for imaging resting state connectivity in MEG using independent component analysis", *Poster at MEGUK conference*, London, UK, 2011

Preface

This DPhil thesis contains the results of my original work carried out since the commencement of my research higher degree candidature at the University of Oxford Department of Psychiatry and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. It contains no material previously published or written by another person except where due reference has been made in the text.

My DPhil project was supported by a PhD scholarship from Alzheimer's Research UK and consisted of three parts. In the first part I investigated the effects of *APOE* genotype on brain function using MEG as a complimentary technique to fMRI. Although MEG has been used as a neuroimaging technique for decades, the recent developments in computer technology now offer new opportunities for the analysis of complex MEG data. The renewed interest in MEG as a neuroimaging tool has led to the relatively recent start of OHBA, the MEG centre in Oxford, and its analysis group that is now developing advanced MEG analysis tools. For the analysis of resting-state MEG data I teamed up with Henry Luckhoo, a DPhil student in the methods group, and over the course of the last year we developed a pipeline for resting-state MEG analysis that is now also used by other members of OHBA. In addition, I assembled a pipeline for MEG data preprocessing that can be used for resting-state as well as task-based data. This pipeline is now also part of

the OHBA Software Library (OSL), a software package for MEG analysis that is still in development. In particular, the OSL pipelines for analysis of task-based MEG data are not fully functional yet. Therefore, the analysis of task-based MEG data presented in this thesis is focused on methods that are available at the moment.

In the second part of my DPhil project I used resting-state fMRI and DTI data to investigate effects of *APOE* genotype on functional and structural connectivity and the relationship between these two measures. Thanks to the extensive MRI data that has been collected by previous DPhil students in our group, I was able to conduct the analysis presented in the last experimental chapter of this thesis. There is some indication of interactions between gender and *APOE* genotype because female *APOE* ϵ4 carriers appear to be at the highest AD risk. Large numbers of subjects were needed to investigate whether there is an interactive effect of these factors on functional and structural connectivity. Combining two MRI datasets collected by Nico Filippini and Aaron Trachtenberg I was able to use around 90 datasets for this analysis.

In the third part of my project I wanted to determine whether resting-state fMRI could be used as a diagnostic tool to differentiate between different types of dementia - Alzheimer's disease (AD), fronto-temporal dementia, Lewy-Body dementia and vascular dementia. Unfortunately, it turned out to be incredibly difficult to recruit patients for this study, such that the group sizes for dementia types other than AD were < 10 patients per group. Rather than presenting very preliminary data for this study I have omitted the analysis of clinical data from this thesis to focus on the MEG and MRI datasets for the first two parts of my DPhil project.

Due to my background in molecular biology, the focus of the first chapter of this thesis is on the biological mechanisms that lead to AD development. Since it is a very complex disease, AD research spans many different medical disciplines,

from immunology via endocrinology to neurology. There are many reviews that collate the information for specific areas, but to my mind the ”big picture” of how these mechanisms interact is not well understood. Therefore, in the first chapter I will outline a model that aims to describe this ”big picture” to explain what causes the neurodegeneration seen in AD. This also allowed me to explore how *APOE* genotype can interact with processes that lead to neurodegeneration. Although such an extensive ”theoretical” chapter is rather unusual in a scientific DPhil thesis, it allowed me to put the results of the experimental chapters into perspective to tackle the question of whether neuroimaging can be used to investigate the biological mechanisms underlying AD risk.

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder and most common type of dementia (de Lau and Breteler, 2006; Reitz et al., 2011). The vast majority of AD patients present with sporadic AD, for which the most important risk factor is ageing. From the age of 65 years AD prevalence and incidence rates increase exponentially (Mayeux and Stern, 2012). For a definitive AD diagnosis a post-mortem analysis has to be carried out showing the hallmark AD pathologies - extracellular plaques of amyloid β (Aβ) protein and intracellular neurofibrillary tangles (NFTs) made up of the protein tau. However, the diagnostic criteria for AD according to the Diagnostic and Statistical Manual of Mental Disorders IV are memory impairment and loss of other cognitive abilities with gradual onset and continuing cognitive decline that cannot be accounted for by conditions such as other neurodegenerative disorders or cardiovascular disease.

The Apolipoprotein E (*APOE*) ϵ4 allele is the best-established risk factor for sporadic AD conferring a 3-fold increased risk of developing AD in around 20% of the population that carry this allele whereas the *APOE* ϵ2 seems to be protective compared with the most common *APOE* ϵ3 allele (Corder et al., 1993; Corder et al., 1994; Farrer et al., 1997; Corbo and Scacchi, 1999). Interestingly, *APOE* genotype not only affects AD risk but the *APOE* ϵ4 allele is also associated with an earlier age of AD onset and accelerated cognitive decline (Corder et al., 1993; Caselli et al., 2009).

Although there is this strong link between *APOE* genotype and AD risk, the exact mechanisms by which the *APOE* alleles affect AD development are only barely established. In order to understand how *APOE* genotype and AD interact it is vital to define what AD actually is and what causes the disease. In the first chapter of this thesis entitled "***APOE* and Alzheimers disease - From Molecular to Psychiatry**" I will develop a model that aims to explain what causes the neurodegeneration seen in AD and how environmental and genetic factors interact to influence AD risk. Once this model is established I will outline how APOE genotype can interact with the different routes to neurodegeneration.

The aim of research into AD and *APOE* is not only to investigate the mechanisms behind this interaction. At the point when AD patients present with clinical symptoms of AD, neurodegeneration is so advanced that it seems impossible to expect that a "cure" can be developed. Strategies aimed at preventing neurodegeneration could be a lot more promising. However, for them to work it is paramount on the one hand to gain an understanding of how AD risk factors exert their deleterious effects and on the other hand to reliably identify individuals who will develop AD at very early, preclinical stages. Neuroimaging could not only provide insights into the mechanisms linking *APOE* and AD but could also be of use in the search for biomarkers of preclinical AD stages. Many neuroimaging studies have investigated brain structural and functional differences between healthy older individuals and AD patients (Weiner et al., 2012). Interestingly, there are striking similarities between the results of these studies and the comparison of brain structure and function between healthy carriers of the *APOE* ϵ4 allele and non-carriers (Reiman et al., 2005) supporting the idea that neuroimaging markers could be used to define preclinical AD stages.

The neuroimaging techniques used in this DPhil project to investigate brain structure and function will be introduced in chapter 2. In the following experi-

mental chapters 3 to 6 I show how these methods were applied to identify *APOE* genotype effects on brain structure and function in healthy adults.

In chapter 3 of this thesis I will focus on the effects of *APOE* genotype on resting-state functional connectivity using two complementary neuroimaging techniques - functional magnetic resonance imaging (fMRI) and magnetoencephalography (MEG). Many resting-state fMRI studies have reported that *APOE* genotype affects functional connectivity of the brain at rest, i.e. when people are not asleep but not engaged in a particular task. Brain areas that show correlated resting-state activity are termed resting-state networks (RSNs). *APOE* genotype effects have been shown for several of these networks (Filippini et al., 2009a; Trachtenberg et al., 2012). However, RSN connectivity does not directly reflect AD risk because *APOE* ϵ2 carriers and ϵ4 carriers, i.e. those with the lowest and highest AD risk, show similar RSN connectivity but differences compared with individuals homozygous for the ϵ3 allele (Trachtenberg et al., 2012).

APOE genotype is not only associated with AD risk but also with risk for cardiovascular disease (CVD) (Mahley et al., 2009). Since fMRI is an indirect measure of brain function taking into account changes in blood flow, blood volume and blood oxygenation, it is possible that vascular factors and not necessarily altered brain function underlie *APOE* genotype effects on RSN connectivity. Therefore, I will investigate differences in neuronal function and RSN activity directly using MEG as a complementary neuroimaging technique. MEG measures changes in the magnetic field around the head that are caused by neuronal activity. Therefore, it is a more direct measure of neuronal function that is independent of vascular changes. This allows me to test the hypothesis that differences in neuronal function underlie *APOE* genotype effects on functional connectivity reported in fMRI studies.

In chapter 4 I will investigate whether *APOE* genotype affects neuronal function

during a visual oddball task. Investigations of *APOE* genotype effects on task-related brain function have mainly involved cognitive domains that are impaired early during AD development such as memory functions and attention/ executive control (Trachtenberg et al., 2011). However, *APOE* genotype has very widespread effects on brain function at rest. Since there is evidence of impaired sensory processing in AD patients (Pollock et al., 1989), I will test the hypothesis that not only "higher" cognitive functions but also basic sensory processing is affected by *APOE* genotype. Making use of the excellent temporal resolution of MEG, I will use a visual oddball paradigm to investigate sensory processing as well as attentional processes in this chapter. Using MEG will also allow me to investigate whether patterns in task-related neuronal activity follow AD risk and can distinguish between *APOE* ϵ2 and ϵ4 carriers.

In chapter 5 I will determine whether putative difference in neuronal function investigated in the previous chapters are associated with alterations in brain structure using structural MRI and diffusion tensor imaging (DTI). AD is accompanied by many changes in grey matter (GM) and white matter (WM) structure and there is a close correlation between brain atrophy and cognitive deficits (Frisoni et al., 2010). However, there are many inconsistencies in reports of *APOE* genotype on GM structure in healthy adults, possibly because of an interaction between age and genotype (Cherbuin et al., 2007). Although there are not many reports of WM structural changes associated with *APOE* genotype, the emerging picture is that carriers of the *APOE* ϵ2 and ϵ4 allele show reduced WM integrity compared with ϵ3 homozygotes independent of age (Heise et al., 2011; Westlye et al., 2012). The aim of the study presented in this chapter was to replicate previous results showing that there are no *APOE* genotype effects on GM structure but significant effects on WM structure in the age group studied here.

In the final experimental chapter I combined resting-state fMRI and DTI data

to investigate a possible interaction of gender and *APOE* genotype on functional and structural connectivity. Female $\epsilon 4$ carriers have the greatest AD risk and show increased Aβ and NFT pathology (Payami et al., 1996; Farrer et al., 1997; Bretsky et al., 1999; Corder et al., 2004). However, few neuroimaging studies have investigated an interaction between gender and APOE genotype. In AD patients, female $\epsilon 4$ carriers show decreased volumes of the entorhinal cortex compared with males (Juottonen et al., 1998), and a recent study in healthy older adults found decreased functional RSN connectivity in the default-mode network (DMN) of female $\epsilon 4$ carriers (Damoiseaux et al., 2012). To investigate a possible interaction between gender and *APOE* genotype, data of a large number of participants is required. Whereas there was not enough statistical power to study gender effects in the studies presented in chapters 3 to 5, the MRI data that has been acquired by previous DPhil students in my group allowed me to investigate differential effects of gender and age in addition to *APOE* genotype. The focus of this study was on functional and structural connectivity of the hippocampus because of its connection with AD as one of the structures that shows NFTs and atrophy early during disease development (Braak and Braak, 1991). I tested the hypothesis that in particular female *APOE* $\epsilon 4$ carriers show reduced connectivity of the hippocampus, which could make them more susceptible to AD development.

In summary, over the following chapters I will provide a theoretical framework for AD research and mechanisms linking *APOE* genotype and AD development (**Chapter 1**) followed by an introduction into the neuroimaging techniques (**Chapter 2**) used in the experimental chapters. The following two chapters focus on the effects of *APOE* genotype on brain function using MEG to determine whether *APOE* genotype directly affects neuronal function at rest (**Chapter 3**) and during basic visual processing (**Chapter 4**). In the next chapter (**Chapter 5**) I present a study on the influence of *APOE* genotype on brain GM and WM structure. In the

last chapter (**Chapter 6**) I will focus on the interaction of AD risk factors female gender and *APOE* genotype and how they affect hippocampal connectivity. I finish with a general discussion of the findings and implications for *APOE* research.

Overall, this thesis provides an overview of how genetic risk for AD affects brain structure and function even in healthy adults. Insights into these changes are one important step towards understanding the mechanisms leading to AD with the ultimate goal to develop neuroimaging biomarkers for preclinical AD and intervention strategies to prevent or slow down AD progression.

Chapter 1

APOE and Alzheimer's disease - From Molecular to Psychiatry

More than 100 years ago Alois Alzheimer described the pathological hallmarks of AD - extracellular amyloid plaques consisting of Aβ peptide and intracellular NFTs mainly formed by hyperphosphorylated microtubule-associated protein tau (Small and Duff, 2008).

Most of the research trying to elucidate the mechanisms that lead to neurodegeneration in AD has focused on how these protein aggregates can promote neurodegeneration. Based on the familial form of AD where genetic mutations lead to an increased and faster build-up of pathology, the "amyloid hypothesis" was formulated around 20 years ago and states that Aβ plaque accumulation is the primary driver of AD pathogenesis (Fig. 1.1). Even though this hypothesis is useful to understand AD pathogenesis in familial AD, it is unclear how it applies to sporadic forms. The "amyloid hypothesis" states that an imbalance in Aβ production and clearance leads to the build-up of protein aggregates. This is certainly true for familial AD where mutations affect the processing of Aβ, which will be discussed in more detail later. However, it does not explain what the cause for the

imbalance is in sporadic AD. What is the reason for increased Aβ production and/or decreased clearance?

Since sporadic AD accounts for approximately 99% of AD cases (Reitz et al., 2011), I would like to approach the question of what this upstream mechanism might be from a slightly different angle. There are many risk factors for AD, the most important being ageing itself, and I would like to focus on what these risk factors have in common to develop a hypothesis of the underlying cause for AD. I will come back to the question how this links in with the familial forms of AD to investigate whether this hypothesis is applicable to those cases as well. In the second part of this chapter I will outline how *APOE* genotype fits in with this hypothesis and investigate the different routes by which *APOE* genotype could influence AD risk.

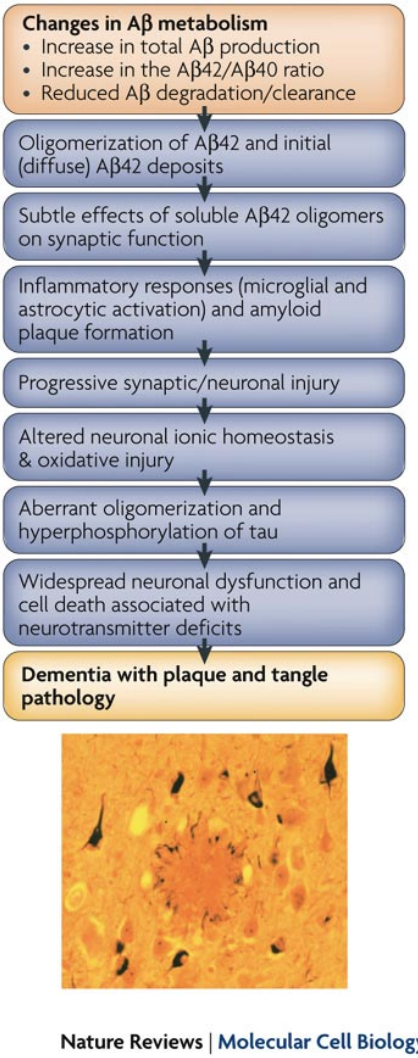

Figure 1.1: Summary of the amyloid hypothesis that states that the accumulation of Aβ leads to neurodegeneration seen in AD. (Figure from Haass and Selkoe (2007))

1.1 Alzheimer's disease

1.1.1 Ageing

The most important risk factor for sporadic AD is ageing. Whereas the prevalence rate of AD is around 1% at age 65 it increases to 25% for the age of 80 and above (Ferri et al., 2005). There is not only an exponential increase in cognitive decline but also in prevalence of AD pathology with age in cognitively healthy individuals

(Savva et al., 2009; Corrada et al., 2010; Rowe et al., 2010). Therefore, I would like to suggest that the hallmark AD pathologies are side-effects of a common upstream mechanism that is cellular ageing itself. Cellular ageing can basically be seen as the accumulation of damage in DNA, proteins and lipids that occurs within the lifetime of cells (Rattan, 2008). This is especially a problem in post-mitotic cells like neurons because they cannot just be recycled and there is very limited capacity for neurogenesis. It is the balance of two processes that defines how quickly this damage occurs: on the one side external and internal processes that cause cellular damage and on the other side systems for maintenance, repair and turnover. One of the internal processes associated with age-related damage is the production of reactive oxygen species (ROS). ROS are a normal byproduct of cell energy metabolism and mainly produced in mitochondria but ROS are very unstable and cause oxidative damage to DNA, proteins and lipids (Rajawat et al., 2009). There are many systems in the cell to combat oxidative damage, from antioxidants that act as scavengers of ROS to DNA repair mechanisms and degradation pathways for faulty proteins. According to the "free radical theory" accumulation of oxidative damage is what determines ageing (Harman, 1956). It seems plausible that accumulative damage leads to alterations in gene transcription, protein synthesis and turnover and thus cellular dysfunction with age. Downstream events include inflammation in response to cellular damage and ultimately cell death either via toxic molecules released by surrounding cells or apoptosis (programmed cell death) as a last resort to combat malfunctioning cells (Andersen, 2004). Increased oxidative damage has been shown at early stages of AD and might be a trigger for downstream pathological events (Nunomura et al., 2001).

I would argue that cellular damage is cause, not effect of neurodegeneration. Analogous to the "amyloid hypothesis" I would suggest that an imbalance between

production of toxic molecules and systems for cellular maintenance and repair is the cause of cellular damage, neurodegeneration and ultimately AD. In this scenario the determinant of AD development is how "fast" people age as indicated by the amount of cellular damage and how well their brains can cope with this damage. What are the internal and external factors that influence accumulation of damage and thus the rate of ageing?

While genetic (internal) factors will be discussed in the context of *APOE*, environmental (external) AD risk factors appear to have one thing in common: they influence the rate of cellular damage. Most of the research into the influence of environment on ageing to date has focused on a phenomenon that has been replicated in many species: dietary or calorie restriction, i.e. restriction of food intake, extends the life span of an organism (Fontana et al., 2010). Therefore, the links between metabolism, ageing and AD development will be explored in the next section.

1.1.2 Metabolism and Cardiovascular Health

CVD has consistently been associated with cognitive decline and AD (Breteler et al., 1994; Newman et al., 2005). It has been suggested that CVD might affect AD risk via atherosclerosis that leads to a reduced blood supply to the brain or it might simply be an effect of shared risk factors for CVD and AD (Stampfer, 2006).

Hypertension, especially in mid-life, is among those shared risk factors as well as elevated plasma homocysteine levels and cholesterol levels. Total plasma cholesterol, low plasma high-density lipoprotein (HDL) and high low-density lipoprotein (LDL), the "bad" cholesterol, levels have been associated with both lower cognition and AD (Launer et al., 2000; Seshadri et al., 2002; Dufouil et al., 2005; Whitmer et al., 2005). Again, mid-life plasma lipid levels in particular seem to play a key role in AD risk. Another well-established risk factor for cognitive

impairment and AD is type 2 diabetes (Cukierman et al., 2005). Even without clinical diabetes increased insulin levels have been associated with higher AD risk (Luchsinger et al., 2004).

All of these processes (hypertension, dyslipidaemia, glucose intolerance, insulin resistance and obesity) are part of the metabolic syndrome that leads to increased CVD risk (Eckel et al., 2010). However, ageing research suggests that differences in metabolism through dietary restrictions not only affect CVD risk. Studies in non-human primates have shown that dietary restriction leads to healthy ageing in much more general terms, for example with delayed ageing of the immune system and delayed onset of cancer (Messaoudi et al., 2006; Colman et al., 2009). Importantly, restricted calorie intake also affects brain ageing with preserved subcortical GM volume and reduced age-related GM atrophy (Colman et al., 2009). What could be the link between metabolism, ageing and neurodegeneration?

Nutrient-signalling such as insulin signalling pathways and mammalian target of Rapamycin (mTOR) pathways appear to be an important link between metabolism and ageing. They do not only act in the periphery but also mediate signalling in the brain. For example, insulin receptors can be found in the hippocampus and their function depends on insulin transport from the periphery to the brain (Craft, 2005). Direct effects of calorie restriction on the brain include increased production of neurotrophic factors and neurogenesis in the hippocampus (Lee et al., 2000; Maalouf et al., 2009). The nutrient-signalling pathways on the one hand affect cellular metabolism, which intuitively makes sense: when food is scarce the organism needs to be able to enter a stand-by mode of energy saving to support only those processes that are vital for survival (Fontana et al., 2010). On the other hand, there is also a link between these pathways and cellular damage because they affect production of antioxidants and proteins involved in cellular maintenance and repair mechanisms

(Kenyon, 2010). This might explain the strong links between hypertension, dyslipidaemia and oxidative stress with studies showing increased ROS production and decreased antioxidant activity in CVD patients (Briones and Touyz, 2010; Hopps et al., 2010). Indeed, some antihypertensive drugs and cholesterol-lowering statins probably act via decreasing ROS production (Touyz, 2004). There is also a link between metabolism and the immune system because increased peripheral insulin levels are associated with increased pro-inflammatory markers in the CSF (Fishel et al., 2005). Inflammation itself is an important risk factor for AD and will be discussed in the next section.

1.1.3 Systemic Inflammation

One side-effect of AD pathology is inflammation in response to accumulation of Aβ plaques. However, plasma levels of inflammatory proteins are also increased before the clinical onset of AD (Engelhart et al., 2004). Ageing in general is associated with upregulated inflammation, possibly because the immune system has to deal with recurrent or chronic systemic infections and imbalances between pro- and anti-inflammatory networks (Franceschi et al., 2007). Following injury or infection the immune response to systemic insults is not restricted to the periphery but also affects the brain. This becomes most evident in the behavioural symptoms of sickness such as lethargy and poor concentration, which require communication between the periphery and the brain (Holmes, 2013). Several studies have shown that systemic inflammation could promote AD development. Increased markers of systemic inflammation during midlife lead to higher risk of cognitive decline, long-term use of nonsteroidal antiinflammatory drugs seems to decrease AD risk and incidence of systemic infections can promote AD onset (in t' Veld et al., 2001; Dunn et al., 2005; Laurin et al., 2009). AD is characterised by chronic inflammation in the brain and this might not only be an effect but also cause

of AD. Chronic systemic inflammation accompanies many chronic diseases such as rheumatological conditions and CVD. This also leads to an increased state of activation of the brain immune response. If the immune cells in the brain then also have to respond to insults caused by AD pathology, their overactivity could contribute to neurotoxicity in AD (Perry et al., 2003). This neurotoxicity is possibly mediated at least in part via ROS and reactive nitrogen species (RNS): ROS/RNS are used by the immune system to destroy microbial pathogens but in the brain their role as signalling molecules might even be more important. Increased ROS/RNS production by damaged neurons and Aβ plaques attract microglia that in turn also produce ROS/RNS. ROS/RNS signalling pathways could switch on apopotosis-related genes with the result of apoptosis not only of damaged neurons but also of healthy cells in the surrounding area (Akiyama et al., 2000; Dedon and Tannenbaum, 2004).

1.1.4 Oxidative stress and Mitochondria - the common denominator?

The brain is particularly susceptible to cellular damage because of its limited capacity for regeneration and its high metabolic rate. Many neurodegenerative diseases have in common that post-mortem studies show increased oxidative stress and reduced levels of antioxidants in brain regions affected by pathology (Montine et al., 1999; Andersen, 2004). I would argue that this is cause, not effect of neurodegeneration. The AD risk factors ageing, metabolic syndrome and inflammation all have in common that they lead to increased oxidative stress and cellular damage. This makes the brain more susceptible to neurodegeneration that may or may not be caused by the AD hallmark pathologies Aβ plaques and NFTs. If oxidative damage plays a main role in neurodegeneration, a closer look at the main production site of ROS, the mitochondria, might be in order.

Mitochondria are the powerhouse of the cell. They use oxygen and carbohydrates, lipids or proteins to produce adenosine triphosphate (ATP), the energy currency of the organism. One by-product of the use of oxygen is the production of ROS. Therefore, mitochondrial dysfunction that leads to increased leakage of ROS into the cell can be detrimental because it damages proteins, lipids and DNA, causes cellular dysfunction and ultimately cell death. Mitochondria also play an important role in calcium signalling thus influencing numerous functions from protein function to gene transcription (Rimessi et al., 2008). Evidence for an association between mitochondria and ageing comes from genetic studies in centenarians. Two-thirds of Japanese centenarians carry a mitochondrial gene variant that is possibly associated with decreased mitochondrial leakage of ROS (Tanaka et al., 2000). Strong links between metabolic syndrome and mitochondrial dysfunction exist as impaired mitochondrial function is associated with type 2 diabetes, obesity and high-fat diets (Ballinger, 2005; Schapira, 2012). Similarly, inflammation leads to mitochondrial dysfunction via signal transduction cascades and direct damage caused by release of ROS (Volobouevа and Giffard, 2011).

Several researchers have suggested that mitochondrial dysfunction and oxidative stress are at least partly responsible for AD development (Swerdlow and Khan, 2004; Zhu et al., 2004). Expanding on the "mitochondrial cascade hypothesis" proposed by Swerdlow et al. (2004) I would suggest that it is not only genetic factors determining mitochondrial function and durability that predict susceptibility to AD. Rather, mitochondrial dysfunction and increased cellular damage is also a consequence of environmental AD risk factors. Therefore, I propose a model termed the "extended mitochondrial cascade hypothesis" where environmental and genetic factors interact to influence mitochondrial function and cellular repair mechanisms thereby defining the rate of brain ageing and age-of-onset of sporadic AD.

This hypothesis offers a framework for development of sporadic AD but could it be useful for familial AD as well? This will be discussed in the next sections.

1.1.5 Amyloid

The different genetic mutations that cause familial AD all lead to an increased accumulation of Aβ in the brain (Tanzi, 2012). Mutations occur in three different proteins: amyloid precursor protein (APP), presenilin 1 and 2. APP is a protein found in the plasma membrane and membranes of intracellular compartments. Proteolysis of APP can occur via two different pathways (LaFerla et al., 2007). The non-amyloidogenic pathway is mediated by proteins of the α-secretase family and precludes Aβ formation. In contrast, sequential cleavage by β-secretase and the γ-secretase complex (made up of presenilin and other proteins) result in release of Aβ40 or the more pathogenic Aβ42. Although APP and Aβ are important proteins in AD development, surprisingly little is known about their physiological function. Cleavage of APP protein and release of soluble Aβ protein appears to be a normal metabolic process and positively correlates with neuronal and synaptic activity (Haass et al., 1992; Shoji et al., 1992; Kamenetz et al., 2003; Cirrito et al., 2005). Therefore, it has been hypothesised that the physiological role of secreted Aβ protein is to regulate neuronal activity through acting on glutamatergic and GABAergic synaptic transmission (Hsieh et al., 2006; Shankar et al., 2007; Yang et al., 2009; Palop and Mucke, 2010). In familial AD the mutations in APP or the γ-secretase complex all favour the amyloidogenic pathway of APP cleavage and/ or increase the production of Aβ42, which is more likely to form Aβ plaques (Jarrett et al., 1993). According to the "amyloid hypothesis" the increased accumulation of Aβ then causes downstream events leading to neurodegeneration and AD.

What are the downstream events of Aβ accumulation? Interestingly, there is no

study to date describing direct neurotoxicity exerted by Aβ plaques, they rather appear to be biologically inert (Martins et al., 2008). This fits in with the notion that severity of AD symptoms and Aβ plaque burden do not always correlate and might explain why 20 - 40% of cognitively healthy older subjects show Aβ pathology (Cummings and Cotman, 1995; Sperling et al., 2011). It rather seems to be the soluble protofibrils and oligomers formed by Aβ that are neurotoxic and their levels correlate highly with AD severity (McLean et al., 1999; Klein et al., 2001; Haass and Selkoe, 2007). Therefore, it has been suggested that sequestration of Aβ into plaques might be beneficial to protect from the toxic soluble forms. Mechanisms for toxicity of soluble Aβ species might include inhibition of long-term potentiation (LTP), an important process for memory formation, and interaction with postsynaptic receptors (Eisenberg and Jucker, 2012). Activation of signal transduction cascades that lead to apoptosis might be one link between soluble Aβ and neuronal cell death (Chong et al., 2006). Insertion of Aβ oligomers into membranes has also been reported leading to the formation of ion channels that disrupt normal cell homeostasis (Eisenberg and Jucker, 2012). Nevertheless, according to the "amyloid hypothesis", it is not necessarily direct neurotoxicity that leads to synaptic loss in AD. Aβ plaque formation induces several downstream events that might be detrimental.

One of these downstream events is neuroinflammation. A strong activation of microglia and astrocytes has been shown within and around Aβ deposits, possibly in an attempt to internalise and remove these plaques. This leads to a chronic state of neuroinflammation in AD. Since both astrocytes and microglia can produce neurotoxic molecules, this might promote neurodegegernation in AD (Wyss-Coray and Rogers, 2012). Another proposed downstream event is the hyperphosphorylation of tau, which will be discussed in the next section.

1.1.6 Tau

Tau is a protein that is mainly expressed in neurons and regulates microtubule assembly and stability (Schneider and Mandelkow, 2008). Microtubules are an integral part of the cytoskeleton especially in axons where they promote structural stability and function as tracks for cellular transport between the cell body and synapse. In AD, hyperphosphorylation of tau occurs at different sites of the protein but mostly in the microtubule binding domain (Anderton et al., 2001). This leads to impaired microtubule binding, which results in destabilisation of microtubules and thus impairment of axonal transport and synaptic function (Schneider and Mandelkow, 2008). Additionally, hyperphosphorylated tau forms intracellular aggregates that might also be neurotoxic (Schneider and Mandelkow, 2008). These effects of tau hyperphosphorylation could be one reason why, in contrast to Aβ plaques, the number of NFTs is strongly associated with severity of AD in most studies (Thal et al., 2000). It also fits in with the patterns of pathology: NFT pathology is found in and around the hippocampus at early AD stages, which could lead to the main hallmark symptoms memory loss and disorientation. On the other hand Aβ plaques are much more prevalent in the cortex and only occur in the hippocampus at later stages (Braak and Braak, 1991).

Although the "amyloid hypothesis" states that tau phosphorylation occurs downstream of Aβ accumulation, there are surprisingly few studies linking those two events. Most evidence is supplied by studies in transgenic mice where Aβ injection induces NFT formation (Götz et al., 2004). However, it is important to note that NFTs and Aβ plaques are found in different areas of the human brain (Braak and Braak, 1991). One hint that it is probably not simply Aβ plaques that are driving neurodegeneration and the cognitive decline in AD comes from clinical trials that used immunisation strategies to target Aβ pathology. Although all of these trials were unsuccessful in preventing degeneration, a post-mortem analysis

of patients who took part in one trial showed a reduction of Aβ plaques in their brains. These patients only had mild AD at the beginning of the trial but showed severe cognitive impairment prior to their death within 6 years after the trial start. Additionally, they also showed heavy tau pathology. Therefore, a reduction of Aβ pathology does not seem to be sufficient to halt progression of tau pathology or cognitive decline (Holmes et al., 2008). The dissociation between Aβ plaque load and cognition also fits in with the findings of Aβ pathology in cognitively healthy older adults (Rowe et al., 2010).

Therefore, it has been proposed that both AD hallmark pathologies are an effect of common upstream mechanisms (Small and Duff, 2008). Small and Duff (2008) suggest that this common driver could be overactivity of glycogen synthase kinase 3 (GSK3), which causes both tau hyperphosphorylation and affects APP processing leading to increased Aβ production (Small and Duff, 2008). However, again the question remains what causes increased GSK3 activity.

This is where mitochondrial dysfunction comes into play. First of all, there is a clear connection between Aβ and mitochondria. Aβ is transported to mitochondria where it causes dysfunction leading to reduced energy production and increased ROS formation (Coskun et al., 2012). Additionally, the APP and presenilin proteins themselves have been found in the mitochondrial membrane where APP appears to "clog" pores that are important for mitochondrial function (Lin and Beal, 2006). This begs the question whether mutated APP and presenilins in familial AD might cause neurodegeneration via mitochondrial dysfunction, which would make Aβ plaque formation rather a side effect of this process. On the other hand, there is also a link between mitochondrial dysfunction and tau phosphorylation via GSK3 activity. GSK3 plays an important role in apoptosis (Beurel and Jope, 2006). Mitochondrial dysfunction might well lead to activation of intrinsic apoptosis pathways, which in turn activate GSK3. Among many other functions of GSK3

this leads to tau hyperphosphorylation, microtubule destabilisation and ultimately cell death. Therefore, tau hyperphosphorylation might be a part of the normal apoptosis pathway for dysfunctional neurons. In this way tau does not "cause" neurodegeneration, it is only a symptom of a dysfunctional neuron undergoing apoptosis.

The "extended mitochondrial cascade hypothesis" therefore offers a unifying hypothesis of AD development that could underlie both familial and sporadic forms of AD and defines Aβ plaques and NFTs as effects rather than cause of neurodegeneration (Fig. 1.2).

I would like to add that a sole focus on the mechanisms that lead to neurodegeneration in AD might be too narrow. Many studies have reported discrepancies between the degree of brain pathology, for example due to stroke or AD, and the clinical manifestation of that damage. This led to the hypothesis of brain and cognitive reserve that defines the ability of the brain to compensate for pathology (Stern, 2002), which will be discussed in the following section.

1.1.7 Brain resilience

According to Stern there are two main mechanisms that can lead to increased reserve: passive and active (Stern, 2002). To my mind, a more accurate division would be: pre- and post-pathological. The idea behind passive or pre-pathological mechanisms for "brain reserve" is that brain size or, probably more accurately, brain complexity determine how well a patient can cope with pathology. One example of that would be the loss of synapses in AD: it is not the total amount of synapse loss that determines clinical manifestations but rather the synapse loss with respect to the amount of synapses that are still fully functional. The higher the synapse count before injury the less cognitively affected a patient will be. The post-pathological mechanism that Stern describes as active mechanism is the concept of "cognitive

reserve". Here, the focus is not so much on underlying structural differences but functional differences. People who use brain networks in a more efficient way and can use alternative networks for accomplishing similar tasks have some protection from disruptions of a specific network due to pathology. Additionally, the ability to compensate for insults by developing new brain networks also contributes to "cognitive reserve". I would describe this with the term "functional and structural plasticity": the better patients can develop and use alternative pathways for accomplishing a task, the more they can compensate for pathology.

The theoretical concept of brain reserve is very interesting when it comes to risk factors for AD (Esiri and Chance, 2012). The assumption here would be that the more brain reserve people develop during their lifetime the more they will be protected from AD. Therefore, the question is: which factors determine brain reserve, brain complexity and plasticity?

There are many hypotheses to explain how genetic and lifetime environmental factors interact to influence brain resilience in old age (Whalley et al., 2006). However, evidence is scarce, which is not that surprising since it is very difficult to quantify and measure environmental factors that influence brain development from fetus to adulthood. One factor that has been proposed in this respect is education. However, education is difficult to distinguish from socieeconomic status and general health inequalities associated with it (Huisman et al., 2005). This probably explains the conflicting reports on associations between education and AD risk (Whalley et al., 2006). In general, cognitive and social stimulation have been proposed as factors that could influence cognitive reserve but evidence of direct effects on brain function and structure and AD risk is very limited (Stern, 2012). Nevertheless, genetic influence on brain structure and function are probably important and will be discussed in the context of *APOE*.

1.1.8 The extended mitochondrial cascade hypothesis

In summary, the "extended mitochondrial cascade hypothesis" offers a framework for AD development that can be applied both to familial and sporadic forms of the disease (Fig. 1.2). Central to this hypothesis is the idea that the amount of mitochondrial dysfunction and cellular damage determines the rate of brain ageing and therefore the rate of neurodegeneration. Mitochondrial dysfunction leads to cellular damage via production of ROS, imbalance in energy production and calcium homeostasis and ultimately to cell death via induction of apoptotic pathways. The hallmark AD pathologies - Aβ plaques and NFTs - are only a symptom of brain ageing, although soluble forms of Aβ might contribute to mitochondrial dysfunction. When neurodegeneration reaches a certain threshold it leads to cognitive decline and AD. This threshold is determined by brain resilience, i.e. the degree to which the brain is capable of plastic remodelling of functional and structural pathways. Genetics, lifestyle and environmental factors influence the rate of brain ageing via two main routes: they affect the development of mitochondrial dysfunction and brain resilience. In the case of familial AD, genetics also directly influence the amount of Aβ production leading to increased mitochondrial dysfunction. For the sake of completeness there is also a direct link between genetics and lifestyle and environmental factors. It is clear that environmental factors influence gene expression via epigenetics. On the other hand genetics also affect e.g. immune system function and the risk for CVD development, both of which can be seen as lifestyle risk factors for AD.

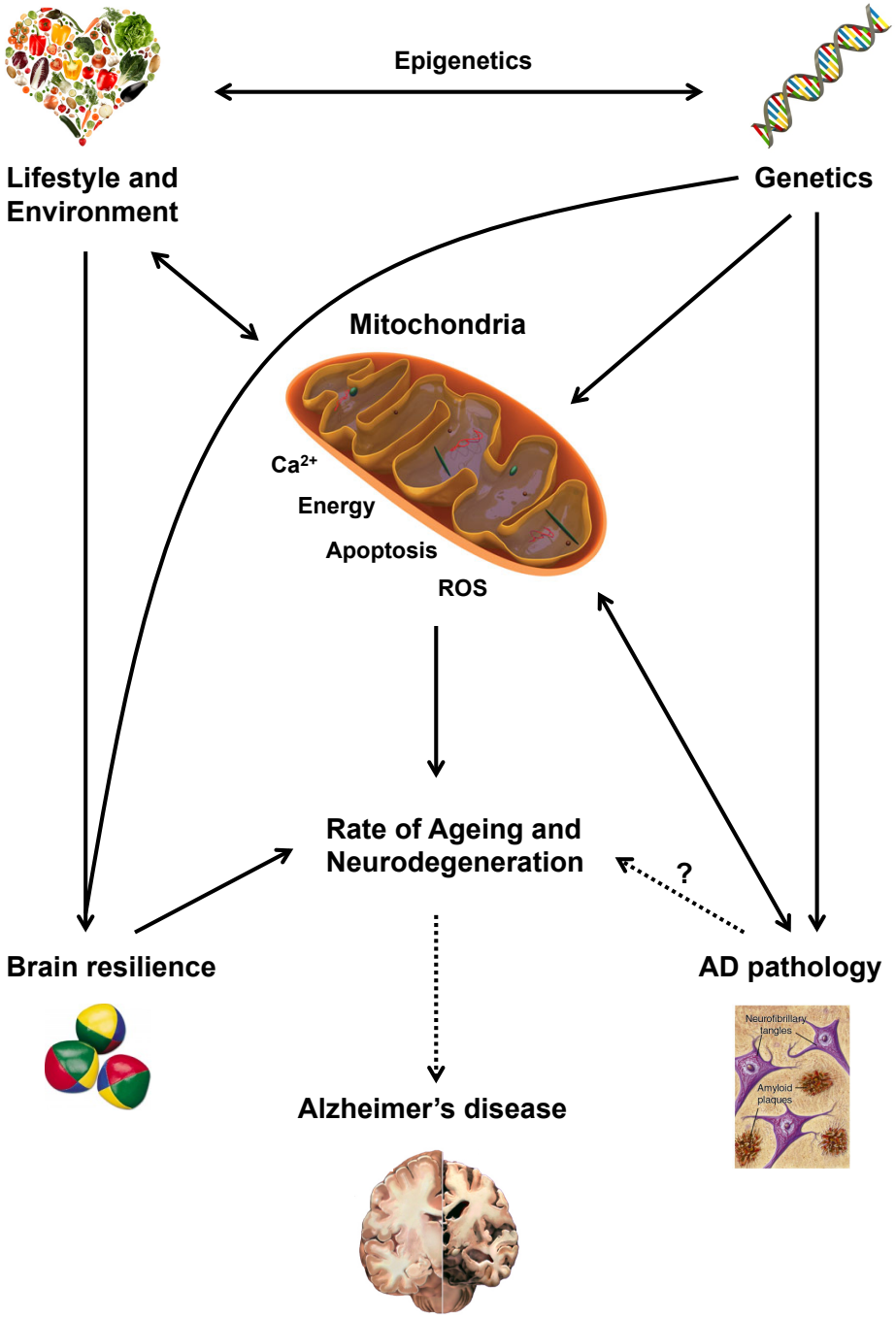

Figure 1.2: *The extended mitochondrial cascade hypothesis.* The extent of mitochondrial dysfunction defines the rate of brain ageing and neurodegeneration that ultimately leads to AD. Mitochondrial dysfunction is detrimental disrupting different pathways including calcium homeostasis, energy production, signalling cascades (apoptosis) and ROS production. Genetic and lifestyle factors such as CVD and inflammation influence the extent of mitochondrial dysfunction as do the pathological hallmarks of AD, Aβ plaques and NFTs. Brain resilience is influenced by genetic, lifestyle and environmental factors and determines the capacity to compensate for degenerative processes.

1.2 Apolipoprotein E

In this section I would like to explore in more detail how genetics and particularly *APOE* genotype affect the rate of brain ageing and AD development. Gene mutations, in this case the different *APOE* alleles, can affect cellular functions in two main ways: either they influence how much protein is available in the cell or they lead to changes in protein structure and thus protein function. Regarding *APOE* genotype I will start off with the question what the impact of the different alleles is on protein availability and protein structure. Then I will explore the different routes how *APOE* genotype could affect the rates of brain ageing and thus AD risk. Based on the "extended mitochondrial cascade hypothesis *APOE* genotype could have a direct impact on mitochondrial dysfunction or an indirect impact via interactions with environmental factors. Another important influence could be the effect on brain structure and function and thus brain resilience. In particular, effects on brain structure and function will be discussed in more detail because they are the basis of the work presented in the following experimental chapters. In the end I will also briefly discuss how other genetic polymorphisms that have been reported to contribute to AD risk in genome-wide association studies (GWAS) might affect the different processes underlying brain ageing.

1.2.1 What is apolipoprotein E?

ApoE was discovered in the 1970s as part of particles transporting triglycerides and cholesterol in the plasma and interstitial fluid (Mahley, 1988). It is synthesised and secreted by many tissues, especially liver, brain, skin and tissue macrophages, where it plays a role in functions ranging from cardiovascular to infectious and neurological disease (Mahley et al., 2009). ApoE is a glycoprotein with a molecular mass of 34 kDA containing 299 amino acids. Its gene is located on the long

arm of chromosome 19 and exhibits three different alleles $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$. The resulting protein isoforms apoE2, apoE3 and apoE4 differ in primary structure at two sites, residues 112 and 158. In humans, all six phenotypes ($\epsilon 2/\epsilon 2$, $\epsilon 3/\epsilon 3$, $\epsilon 4/\epsilon 4$, $\epsilon 2/\epsilon 3$, $\epsilon 2/\epsilon 4$, $\epsilon 3/\epsilon 4$) can be found (Zannis and Breslow, 1981). In Western and Japanese populations the most common allele is $\epsilon 3$ with a frequency of around 80%, followed by $\epsilon 4$ (10-20%) and $\epsilon 2$ (5-10%) (Gerdes et al., 1996). In AD patients this ratio is altered with around 60% of patients carrying the $\epsilon 4$ allele and only around 2% carrying the $\epsilon 2$ allele (Corder et al., 1994; Farrer et al., 1997).

The *APOE* $\epsilon 4$ allele is the best-established risk factor for sporadic AD conferring a 3-fold increased risk of developing AD whereas the *APOE* $\epsilon 2$ seems to be protective compared with the *APOE* $\epsilon 3$ allele (Corder et al., 1993; Corder et al., 1994; Farrer et al., 1997). Interestingly, *APOE* genotype not only affects AD risk but the *APOE* $\epsilon 4$ allele is associated with an earlier age of AD onset and accelerated cognitive decline (Corder et al., 1993; Caselli et al., 2009). *APOE* genotype is also the most important predictor of longevity with a decreased life span in *APOE* $\epsilon 4$ carriers and longer life span in *APOE* $\epsilon 2$ carriers (Schächter et al., 1994). However, the effect on longevity could well be a "side-effect" of *APOE* genotype effects on risk for AD and CVD. Although the *APOE* $\epsilon 4$ allele is a risk factor for AD it is neither necessary nor sufficient for AD development. On the one hand this might be a consequence of the complex interactions of *APOE* genotype with many processes that determine AD risk. On the other hand interactions with other genetic polymorphisms might play a role, which could be particularly important with regards to protein availability.

1.2.2 ApoE isoforms - Availability

The *APOE* alleles show single nucleotide polymorphisms (SNPs) in the coding region of the gene and lead to differences in protein structure. Neverthe-

less, these SNPs might be in linkage disequilibrium with other SNPs in the promoter region, which could influence gene transcription directly or stability of mRNA. Both of these processes determine how much protein is produced, which again has an impact on the functions for which this protein is needed. Several studies have shown that polymorphisms in the *APOE* promoter affect AD risk and *APOE* transcription (Bullido et al., 1998; Lambert et al., 2002; Laws et al., 2003). Particularly, increased *APOE* expression appears to be associated with AD risk (Artiga et al., 1998). This fits in with some post-mortem studies reporting increased *APOE* mRNA and protein levels in AD brain but the results appear to depend on brain region and analysis method (Bekris et al., 2010; Akram et al., 2012). Additionally, it is unclear whether expression of a particular isoform is altered in AD, whether upregulated expression is cause or effect of AD, or whether it might be a response e.g. to combat AD pathology and neurodegeneration. In healthy subjects some studies show increased transcription of the *APOE* $\epsilon 4$ vs the $\epsilon 3$ allele while others report no significant differences or the opposite findings (Lambert et al., 1997; Bray et al., 2004). In transgenic mice apoE levels are increased in $\epsilon 2/\epsilon 2$ mice and decreased in $\epsilon 4/\epsilon 4$ mice compared with the $\epsilon 3/\epsilon 3$ genotype but this appears to be caused by an increased degradation of apoE4 (Riddell et al., 2008). In summary, it is currently unclear whether apoE levels are affected by *APOE* genotype in humans but reports on the *APOE* promoter suggest that transcription and protein levels might play a role in AD development. However, most research on the effects of *APOE* genotype have focused on the structural differences between apoE isoforms and their functional consequences.

1.2.3 ApoE isoforms - Structure

The less common isoforms apoE2 and 4 differ from apoE3 in only one residue (Mahley and Huang, 2012). Whereas apoE4 contains an arginine at residue 112

compared to cysteine in the two other isoforms, a cysteine can be found at residue 158 in apoE2, which is an arginine in the other two. This causes structural differences: in apoE4 there is a domain interaction between the N- and C-terminus of the protein (Fig. 1.3). This leads to a more compact structure but decreased protein stability, possibly because the compact structure targets the protein for proteolysis. The substitution in apoE2 is associated with an increased protein stability but it also leads to defects in the lipoprotein-receptor binding domain.

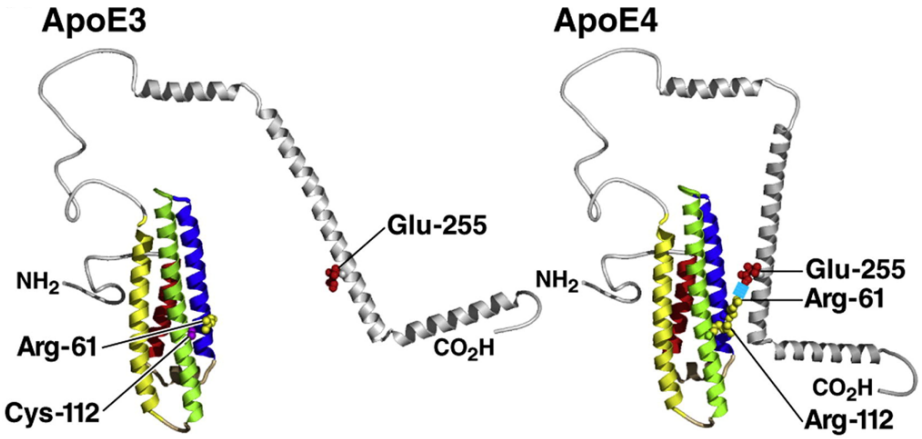

Figure 1.3: *Structural differences between the apoE isoforms.* A new salt bridge in apoE4 alters orientation of Arg61 leading to domain interaction with Glu255 and a more compact structure compared with apoE3. (Figure from Mahley and Huang (2012))

1.2.4 ApoE isoforms - Function

The major role of apoE in the brain is the transport of cholesterol (Mahley, 1988). Containing around 20% of body cholesterol, the brain is the human organ with the highest amount of cholesterol (Dietschy and Turley, 2004). However, this is mostly due to the accumulation of cholesterol in the myelin sheath of axons accounting for around 70% of brain cholesterol in mice (Snipes and Suter, 1997). The remaining 30% are mainly used as building blocks for membranes. ApoE is predominantly expressed by astrocytes but also by microglia and in neurons in response to injury (Holtzman et al., 2012). ApoE-mediated transport of cholesterol from astrocytes

to neurons plays an important role in maintaining neuronal membranes because lipoprotein particles cannot cross the blood-brain barrier and neurons show a reduced *de novo* synthesis of cholesterol compared with astrocytes (Saito et al., 1987; Jurevics and Morell, 1995; Nieweg et al., 2009).

ApoE transports lipids to cells that express receptors of the low-density lipoprotein receptor (LDLR) family (Herz and Bock, 2002). Even though all receptors undergo endocytosis upon ligand binding to transport cholesterol inside the cell, the endocytosis rates differ significantly between the receptor types. Therefore, some receptors are thought to be of higher importance in extracellular signal transduction than in actual cholesterol transport also through binding ligands other than apoE (Li et al., 2001; Bu, 2009). The structural differences between apoE isoforms lead to different affinities for specific lipid particles and receptors. This causes alterations in the cellular uptake of cholesterol and downstream signalling cascades and might affect AD risk in many different ways.

Since the "amyloid hypothesis" has been the predominant explanation for AD development, much of the research published on functional difference between the apoE isoforms to date has focused on links between *APOE* genotype and the hallmark AD pathologies Aβ plaques and NFTs rather than mitochondrial dysfunction. In the following sections I will discuss how the apoE isoforms differentially affect those two hallmark pathologies.

1.2.5 Amyloid

Immunostained apoE colocalised with Aβ plaques and intra- as well as extracellular NFTs in brains of AD patients (Namba et al., 1991). The *APOE* ϵ4 allele is associated with increased amyloid plaque formation in AD patients and in cognitively healthy adults (Polvikoski et al., 1995; Kok et al., 2009). On the one hand, apoE has been shown to catalyse Aβ monofibril formation with apoE4 being the more efficient

isoform (Sanan et al., 1994). This might explain the increased formation of amyloid plaques in *APOE* ϵ4 carriers. There also appears to be a synergistic effect of apoE4 and Aβ oligomers that leads to increased neuronal death compared to the other isoforms (Manelli et al., 2007).

Additionally, clearance of the Aβ peptide might be affected: increased binding of apoE2 and 3 to Aβ peptide, especially the oligomeric form, enhances clearance of this peptide from the brain (LaDu et al., 1997; Manelli et al., 2004). There are four main ways of Aβ clearance: uptake by neurons and glia and degradation in lysosomes, clearance along the interstitial fluid drainage pathway or through the blood-brain barrier and proteolytic degradation of extracellular plaques (Bu, 2009). The first three pathways require receptor-mediated uptake of Aβ peptide. ApoE acts as a chaperone for this uptake and the isoforms might differentially affect Aβ clearance. Slower clearance of Aβ peptide by the apoE4 isoform has been shown for the blood-brain barrier (Deane et al., 2008). Proteolytic degradation of soluble Aβ also seems to be affected by apoE genotype. Within microglia and the extracellular milieu, apoE2 and 3 increase the rate of proteolysis compared with apoE4 (Jiang et al., 2008).

However, indirect effects on APP processing could also play a role. Isoform differences in apoE-mediated cholesterol transport influences membrane protein function (discussed below), which could have an impact on APP processing and formation of Aβ (Osenkowski et al., 2008). Additionally, several apoE receptors in the brain have been shown to influence APP trafficking and processing and differential affects of apoE isoforms might play a role in receptor function or availability (Holtzman et al., 2012).

1.2.6 Tau

In studies of post-mortem AD brains apoE associates with NFTs but it is unclear whether this affects neurotoxicity. First of all, it is possible that apoE directly binds to tau especially at phosphorylation sites thus preventing hyperphosphorylation. It has been shown that all apoE isoforms bind to tau at different sites and that apoE4 fails to protect sites that are required for microtubule binding (Gibb et al., 2000). Additionally, apoE3 binds more strongly to tau than apoE4, which may increase its protective effects as well (Strittmatter et al., 1994). In concordance with this idea it has been shown that apoE4 overexpression in neurons but not in astrocytes increases tau phosphorylation in mice (Tesseur et al., 2000). Nevertheless, it is unclear whether this effect is caused by the apoE4 isoform and/or the unnatural high expression levels of apoE in these mice.

However, there is another mechanism by which apoE might act on NFT formation. It has been shown *in vitro* that truncated fragments of degraded apoE directly bind to cytosolic hyperphosphorylated tau and can induce NFT formation with increased NFT aggregation induced by apoE4 truncated fragments (Huang et al., 2001). As truncated fragments of apoE were also found in AD patients in the same study, this mechanism might play a role *in vivo* in AD development. It has also been reported that cells treated with Aβ peptide show an increase in numbers of truncated fragments of both apoE3 and 4. This might provide a link between the two hallmarks of AD pathology and apoE isoforms. Nevertheless, it is unknown how Aβ peptide might act on the proteolysis of apoE.

It is also possible that apoE has an indirect effect on NFT formation by acting on tau phosphorylation pathways. This could either be through a direct interaction with kinases or through an upstream mechanism via apoE receptor-mediated activation of kinases. One of the major kinases responsible for phosphorylation of tau is GSK3 (Hong and Lee, 1997). A link between apoE and GSK3 activity

has been established in *in vitro* studies. ApoE activates GSK3 with apoE4 to a greater extent than apoE3 leading to an upregulation of GSK3 activity and thus tau phosphorylation (Cedazo-Mínguez et al., 2003; Small and Duff, 2008).

Although all of these effects of *APOE* genotype on Aβ plaques and NFTs offer explanations for increased pathology in *APOE* ϵ4 carriers I would argue that this pathology is only a side-effect of faster brain ageing in *APOE* ϵ4 carriers. *APOE* genotype effects on the different routes to faster brain ageing will be explored in the next sections.

1.2.7 Ageing and Mitochondria

Some of the most interesting concepts regarding functional differences between apoE isoforms come from gene expression studies. Post-mortem analyses of gene expression in AD patients and cognitively healthy middle-aged adults without AD pathology show differences in gene expression patterns between *APOE* ϵ4 carriers and non-carriers (Xu et al., 2006; Xu et al., 2007; Conejero-Goldberg et al., 2011; Cooper-Knock et al., 2012). In healthy subjects, *APOE* genotype affects pathways involved in mitochondrial function, calcium regulation, apoptosis, proteasome functionality, cell-cycle regulation, axon guidance and insulin signalling pathways (Conejero-Goldberg et al., 2011). This suggests that mitochondrial dysfunction is not only at the centre of the "extended mitochondrial cascade hypothesis" but might also differentiate *APOE* ϵ4 carriers from non-carriers. Calcium regulation and apoptosis signalling are two functions of mitochondria. Functionality of the proteasome is important e.g. to protect from oxidative stress via degradation of faulty proteins. Insulin signalling is a very important link between metabolism, ageing and mitochondrial dysfunction whereas differences in axon guidance might affect brain structure and resilience. All of these processes could contribute to *APOE* genotype related differences in the rates of brain ageing and AD risk.

Several direct links between apoE and mitochondria have been established. As mentioned above, apoE4 is the least stable isoform and is targeted by the proteasome because the domain interaction makes it appear misfolded (Mahley and Huang, 2012). However, the cleavage of apoE4 produces truncated fragments that can be neurotoxic. In neurons these fragments translocate to mitochondria where they cause dysfunction (Chang et al., 2005). Mitochondrial dysfunction in the brain has been shown in a post-mortem analysis of young, cognitively healthy adults carrying the *APOE* ϵ4 allele (Valla et al., 2010). ApoE4 also appears to disrupt axonal transport of mitochondria, which is important to keep up energy production in different parts of the cell (Huang and Mucke, 2012). Additionally, apoE possesses antioxidant activity with E2>E3>E4 (Miyata and Smith, 1996). Interestingly, apoE production is increased by oxidative stress (Mahley et al., 2009), which could lead to a vicious cycle where increased production of apoE4 and apoE4 fragments increases mitochondrial dysfunction and oxidative damage. ApoE is also involved in apoptotic signalling cascades with apoE3 being more efficient than apoE4 at preventing apoptosis (Hayashi et al., 2007).

1.2.8 Metabolism and Inflammation

ApoE is an important player in triglyceride and cholesterol transport and clearance in the plasma. The apoE isoforms show differences in lipid transport and receptor binding, therefore it is not surprising that they are associated with different risks for CVD and atherosclerosis (Mahley et al., 2009). The apoE2 isoform differs from apoE3 and 4 in the receptor binding domain, which leads to reduced cellular uptake of cholesterol. This explains the association of apoE2 with type III hyperlipoproteinemia, a lipid disorder characterised by increased plasma levels of cholesterol and triglycerides that leads to premature atherosclerosis, particularly in people homozygous for the *APOE* ϵ2 allele (Mahley et al., 1999). ApoE4 on the

other hand shows structural differences in the lipid binding domain, which leads to preference for different lipids and thus increased plasma LDL levels. Therefore, *APOE* ϵ4 carriers have an increased risk for CVD and atherosclerosis. Increased or earlier development of atherosclerosis could lead to decreased blood supply and thus altered energy metabolism in the brain (Kim et al., 2010). However, post-mortem studies in young adults appear to suggest differences in mitochondrial function and energy metabolism before one would expect onset of atherosclerosis (Valla et al., 2010). Importantly, apoE isoforms differentially affect blood supply to the brain through direct actions on the blood-brain barrier. In transgenic mice expressing apoE4, chronic degeneration of the blood-brain barrier has been shown, which leads to reduced cerebral blood flow and neuronal dysfunction through uptake of blood-derived toxic molecules (Bell et al., 2012).

Interactions between diabetes and *APOE* genotype have also been shown because carrying the *APOE* ϵ4 allele increases AD risk in patients with type 2 diabetes (Peila et al., 2002). Underlying mechanisms might be crosstalk between insulin and apoE. Insulin alters activity of apoE receptors leading to increased cellular uptake of apoE (Descamps et al., 1993). Insulin also reduces *APOE* transcription and plays a role in apoE degradation at least in the periphery (Ogbonna et al., 1993). *APOE* genotype dependent differences in reponse to insulin have been shown in some studies and might be relevant for AD risk but the underlying mechanisms are largely unclear (Reger et al., 2006).

APOE genotype also plays a role in susceptibility to infection with *APOE* ϵ4 carriers being more susceptible to viral infections (Mahley and Rall, 2000; Mahley et al., 2009). ApoE is an important player of the immune system, e.g. it binds antigens and delivers them to antigen-presenting cells so that they can activate an immune response (van den Elzen et al., 2005). In the brain there seems to be an intricate interaction between inflammation and apoE in that inflammation

triggers apoE production which in turn affects neuroinflammation. Interestingly, apoE3 suppresses inflammation to a greater extent than apoE4 (Kim et al., 2009). ApoE4 is also associated with increased production of RNS of immune cells in the periphery and CNS and thus increased oxidative damage in transgenic mice and humans (Colton et al., 2002). Plasma levels of inflammatory markers are increased in cognitively healthy adults who carry the *APOE* ϵ4 allele and decreased in *APOE* ϵ2 carriers (Ringman et al., 2012). Since systemic inflammation is important for AD risk, this might contribute to increased risk in *APOE* ϵ4 carriers.

On the other hand inflammation caused by AD pathology, especially Aβ, could be exacerbated in *APOE* ϵ4 carriers and contribute to neurodegeneration (LaDu et al., 2001; Guo et al., 2004). Interestingly, ibuprofen appears to slow down AD progression particularly in *APOE* ϵ4 carriers (Pasqualetti et al., 2009), which might indicate detrimental overactivity of the immune system associated with the ϵ4 allele.

1.2.9 Brain resilience

Many neuroimaging studies have shown effects of *APOE* genotype on brain structure and function. These studies will be discussed in the next chapter but here I would like to outline the biological basis for *APOE* genotype dependent differences in brain structure and function. These could lead to reduced brain resilience in *APOE* ϵ4 carriers e.g. via affecting neurodevelopment and/ or plasticity and thus the ability to compensate for insults such as AD pathology.

Neurodevelopment

Two main mechanisms explaining how apoE could influence brain structure are cholesterol transport and activation of signalling cascades. Since neurons do not produce much cholesterol they are dependent on cholesterol supply by astrocytes

(Nieweg et al., 2009). But why is cholesterol transport so important? Cholesterol is a component of cellular membranes where it plays an essential part in determining their permeability and fluidity. It thus influences properties and functions of integral membrane proteins (Burger et al., 2000). Additionally, the concentration of cholesterol in microdomains, termed lipid rafts, is important for the clustering of proteins e.g. different components of signalling cascades (Korade and Kenworthy, 2008). Cholesterol is also essential for the formation of synaptic vesicles (Thiele et al., 2000). During neurodevelopment synapse formation depends on apoE-mediated cholesterol transport from astrocytes to neurons and this process might also play a role in synaptic plasticity of adult brains (Barres and Smith, 2001; Koudinov and Koudinova, 2001; Mauch et al., 2001). Since cholesterol plays an important role in synapse formation, plasticity and stability (Pfrieger, 2003), differences in cholesterol transport between the apoE isoforms probably affect those mechanisms but there are no published studies directly investigating how apoE isoforms differentially affect synaptogenesis.

However, there are some studies reporting differential effects of apoE isoforms on neuron structure. They showed that apoE3 increases while apoE4 decreases or only has weak effects on neurite outgrowth (Nathan et al., 1994; Holtzman et al., 1995; Teter et al., 2002). ApoE interacts with neurotrophic factors and regulates interactions between neurons and the extracellular matrix (Huang et al., 1995; Gutman et al., 1997), which might account for differences between the apoE isoforms in neurite outgrowth. This outgrowth requires the binding of apoE to its receptors, which not only promotes cholesterol uptake into the cell but also influences signalling cascades (Nathan et al., 1994; Holtzman et al., 1995).

ApoE receptor functions are very diverse and include mechanisms ranging from cytoskeleton dynamics to neuronal survival, synaptic plasticity, dendrite and spine formation, which are important for neurodevelopment (Holtzman et al., 2012).

Most of the research on apoE receptor functions has been done using a different ligand (reelin) and not apoE. However, apoE expression in astrocytes is strongly upregulated from postnatal day 1 in mice, so there are indications that apoE also plays a role in neurodevelopment around the time when most synapses are formed (Cahoy et al., 2008). Effects of apoE isoforms on dendrites have been reported in transgenic mice where the *APOE* ϵ4 allele leads to reduced spine density and dendritic arborisation whereas the ϵ2 allele increases arborisation compared with the ϵ3 allele even in young mice (Dumanis et al., 2009). A very small study in humans found reduced spine density in the hippocampus of older cognitively healthy *APOE* ϵ4 carriers vs non-carriers (Ji et al., 2003). Interestingly, there is evidence for crosstalk between spine plasticity, apoE and mitochondria. Energy supply by mitochondria in dendrites is crucial for spine development (Li et al., 2004). Upon stimulation of mitochondrial function by rosiglitazone dendritic spine loss caused by apoE4 and its toxic fragments can be rescued (Brodbeck et al., 2008). Therefore, the negative or weak effects of apoE4 on neurite outgrowth could at least in part be mediated via mitochondrial dysfunction (Huang, 2010).

Plasticity

Several apoE-mediated mechanisms might not only play a role in neurodevelopment but also continuously throughout the lifespan. ApoE isoforms differentially affect synaptic plasticity with decreased glutamate receptor mediated signalling and reduced induction of long-term potentiation (LTP) in mice expressing the *APOE* ϵ4 allele (Trommer et al., 2004; Korwek et al., 2009; Chen et al., 2010; Klein et al., 2010). ApoE also affects GABAergic signalling with impaired survival and thus reduced signalling of GABAergic interneurons in apoE4 expressing mice, particularly in the hippocampus (Li et al., 2009b). Learning processes are associated with increased GABA release and inhibitory synaptic plasticity in the

hippocampus (Nitz and McNaughton, 2004). Therefore, in apoE4 expressing mice an age-dependent decrease in GABAergic interneurons leads to deficits in learning and memory (Andrews-Zwilling et al., 2010). Interestingly, this apoE isoform specific effect is limited to the hilar region of the dentate gyrus and not found in other areas of the hippocampus (Leung et al., 2012).

These mechanisms might be relevant in humans because age-related decreases in GABA levels have also been shown in the CSF of healthy adults (Bareggi et al., 1982). GABAergic interneuron dysfunction is directly linked to differential effects of apoE isoforms on adult neurogenesis that requires GABAergic input (Li et al., 2009b). Active neurogenesis in adults occurs in two regions: the subgranular zone of the dentate gyrus in the hippocampus and the subventricular zone from where neurons migrate to the olfactory bulb (Ming and Song, 2011).

Hippocampal neurogenesis is enhanced by training of hippocampus-dependent tasks (Gould et al., 1999). In transgenic mice apoE4 expression inhibits neuronal maturation and neurogenesis in the hippocampus and might even be associated with increased hippocampal cell death (Levi and Michaelson, 2007; Li et al., 2009b). Increased apoptosis in response to apoE4 has also been shown *in vitro* and might be a toxic gain-of-function, especially in conditions when apoE levels are normally increased (Hashimoto et al., 2000; DeKroon et al., 2003; Teter, 2004; Levi and Michaelson, 2007). Interestingly, it has been shown in mice that apoE appears to play a particular role in adult rather than embryonic neurogenesis contributing to hippocampal development and maintenance later during life (Gilley et al., 2011; Yang et al., 2011).

ApoE isoforms not only differentially affect brain structure but functional consequences on cognitive performance have been reported in transgenic mice with poorer performance of apoE4 expressing mice that further decreases with age (Grootendorst et al., 2005; Bour et al., 2008). This leads me to speculate that

in humans effects of *APOE* genotype on brain structure and function might be larger in adults than in children and might become even more pronounced during ageing. Studies investigating differential effects of *APOE* genotype in children, adolescents and adults will be discussed in the next chapters.

Neuroregeneration

Adult neurogenesis is also induced in response to injury to compensation for brain insults. In AD patients neurogenesis is increased in the hippocampus (Jin et al., 2004) but differential effects of apoE isoforms have not yet been investigated. Traumatic brain injury is another risk factor for AD and this risk is further increased in carriers of the *APOE* ϵ4 allele (Fleminger et al., 2003; Koponen et al., 2004). The *APOE* ϵ4 allele is also associated with poorer recovery from brain injury (Zhou et al., 2008a), which might indicate reduced capacity for compensation in *APOE* ϵ4 carriers. How could apoE be involved in neuroregeneration? ApoE expression is upregulated after peripheral nerve and CNS injury and then also occurs within neurons (Laskowitz et al., 1998; Aoki et al., 2003). ApoE isoforms might differentially affect recovery from brain injury via mechanisms that modulate inflammation and oxidative stress as discussed above. More direct impacts on neuroregeneration involve cholesterol scavenging at the site of injury and redistribution to the injured cell where cholesterol can be used for membrane remodelling (Ignatius et al., 1987). In the peripheral nervous system apoE is also used to deliver cholesterol to Schwann cells to facilitate remyelination (Boyles et al., 1989). Although the general assumption is that apoE also facilitates remyelination in the CNS (Bartzokis, 2011), there does not appear to be direct evidence for that. However, apoE3 is much more successful in providing neuroregeneration and neurite outgrowth after injury than apoE4 (Teter et al., 1999; White et al., 2001). This might also play a role in AD development because growth

and reorganisation of dendrites have been shown in response to neurodegeneration, possibly in an effort to compensate for degeneration (Geddes et al., 1985). This response is decreased in AD patients carrying the *APOE* ϵ4 allele who show reduced dendritic remodelling (Arendt et al., 1997).

1.2.10 Other genetic factors

Some studies have reported that SNPs in the *TOMM40* gene might be associated with AD risk independent of *APOE* genotype, although these are adjacent genes on chromosome 19 and therefore in high linkage disequilibrium (Yu et al., 2007; Roses et al., 2009; Hong et al., 2010; Deelen et al., 2011; Cervantes et al., 2011; Cruchaga et al., 2011). In part this association might be explained by effects of *TOMM40* SNPs on *APOE* expression (Bekris et al., 2010). However, *TOMM40* itself is a very interesting gene because it provides a direct link between AD risk and mitochondria function. It codes for a channel in the Translocase of the Outer Mitochondrial Membrane (TOM) complex that is essential for protein transport into mitochondria (Humphries et al., 2005). Since most mitochondrial proteins such as those required for mitochondrial DNA damage repair are produced in the cytosol, protein transport into mitochondria is essential to maintain mitochondrial function (Szczesny et al., 2003; MacKenzie and Payne, 2007). Therefore, SNPs in the *TOMM40* gene could directly affect rates of brain ageing and thus AD risk via altered mitochondrial function.

In addition to the rather controversial links with *TOMM40* several genome-wide association studies (GWAS) comparing AD patients with healthy controls have reported SNPs in other genes that might also contribute to AD risk (Harold et al., 2009; Lambert et al., 2009; Seshadri et al., 2010; Carrasquillo et al., 2011; Hollingworth et al., 2011; Naj et al., 2011). Although the contribution of these SNPs to AD risk is several orders of magnitude lower than that of *APOE* (Tanzi,

2012), they can give us an idea of pathways that are important in AD development. Two main pathways that have emerged are functions of the immune system (*CR1, CLU, ABCA7, CD33, EPHA1*) and transmembrane transport of molecules such as nutrients, growth factors and neurotransmitters (*BIN1, PICALM, CD33, CD2AP*) (Harold et al., 2009; Hollingworth et al., 2011). Interestingly, some genes have overlaps with *APOE* function. *CLU/ APOJ* is one of the apolipoproteins of the CNS other than *APOE* that is involved in very similar functions to *APOE* such as cholesterol transport, response to inflammation and APP processing (Harold et al., 2009; Lambert et al., 2009; Pfrieger and Ungerer, 2011). *ABCA7* mediates the efflux of lipids from cells to lipoproteins such as *APOE* and regulates APP processing (Hollingworth et al., 2011). The roles of *EPHA1* are not entirely clear but might also involve axon guidance and synaptic plasticity (Hollingworth et al., 2011).

1.2.11 Summary

APOE genotype and other genetic risk factors can affect AD risk in many different ways ranging from direct effects on cellular damage to effects on lifestyle factors and brain resilience (Fig. 1.4). *APOE* genotype has direct effects on mitochondrial function and cellular repair and maintenance mechanisms. Links with environmental and lifestyle factors include effects on the immune system, metabolism and CVD risk. *APOE* genotype also affects brain structure and function and thus brain resilience in many ways including effects on neurodevelopment, plasticity and neuroregeneration. All of these processes could contribute to faster brain ageing in *APOE* ϵ4 carriers, which is indicated by increased Aβ plaque and NFT pathology.

However, it is important to note that most of the studies investigating effects of *APOE* genotype have been done *in vitro* or *in vivo* in transgenic mice. There might be many differences compared with the human brain, e.g. regarding expression levels, cell types expressing *APOE* and its receptors and apoE functions in the

periphery. Nevertheless, a recent study reported that *APOE* ϵ4 carriers who maintain vascular health, have a higher education and lead an active life have an AD risk that is similar to non-carriers (Ferrari et al., 2013). Therefore, it is important to study the mechanisms that could lead to differences in AD risk in humans. The focus of my work is on the effects of *APOE* genotype on brain structure and function and thus brain resilience in humans. Understanding the mechanisms behind brain resilience offers the opportunity to develop interventions that could change the rates of brain ageing and AD development. To investigate *APOE* genotype related differences in brain structure and function, several neuroimaging methods can be applied. MRI and MEG were used in this DPhil project and these methods will be introduced in the next chapter followed by the experimental chapters where I investigated *APOE* genotype effects on several aspects of brain structure and function.

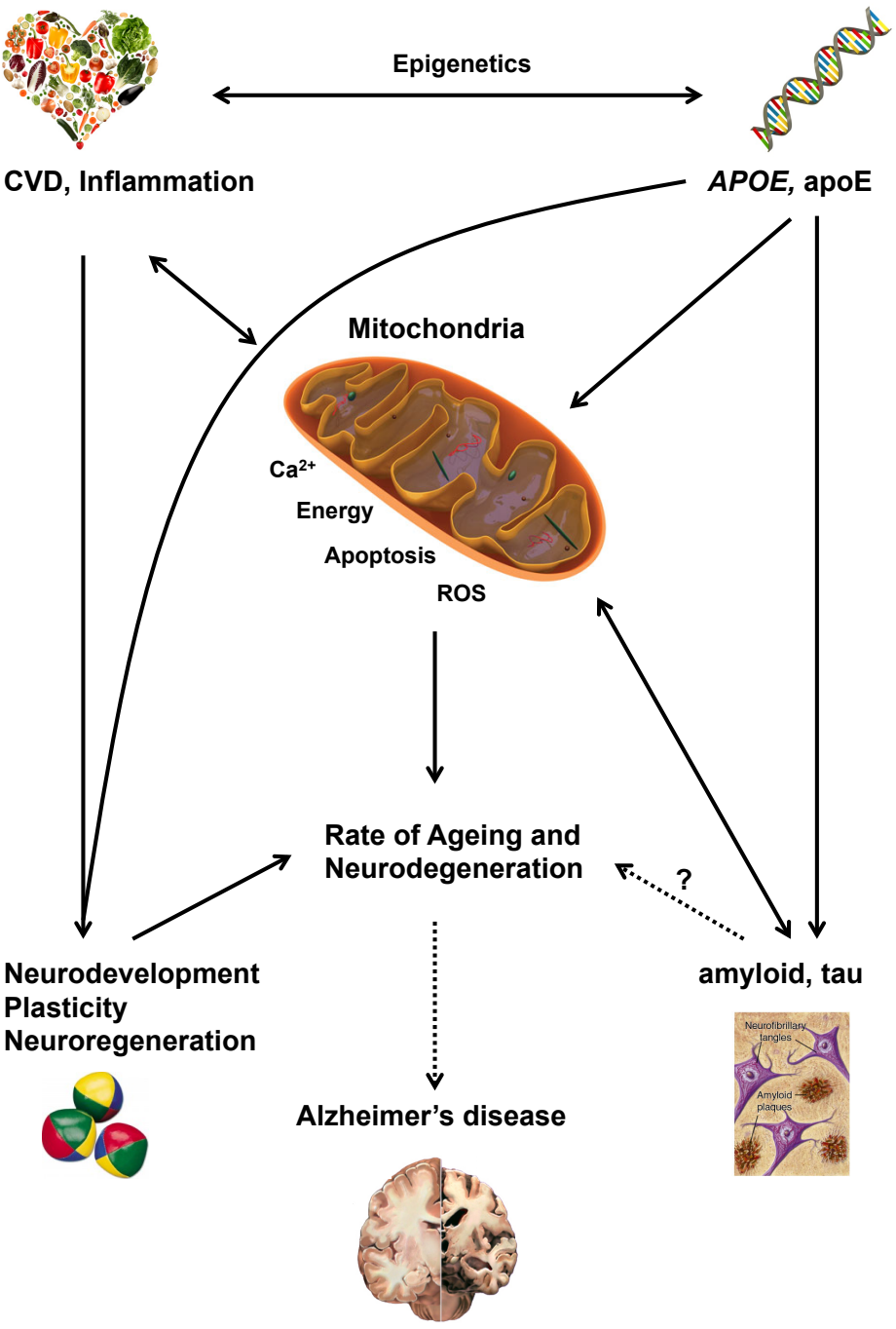

Figure 1.4: APOE *and the extended mitochondrial cascade hypothesis*. ApoE isoforms and protein availability influence the rate of mitochondrial dysfunction and brain ageing via effects on cholesterol transport and signalling cascades. *APOE* genotype affects CVD risk, inflammation and the amount of amyloid deposition. ApoE fragments directly affect mitochondrial function and tau phosphorylation. *APOE* genotype influences brain resilience via effects on neurogenesis, neurite outgrowth, glutamate and GABA signalling and neuroregeneration. All of these mechanisms contribute to higher risk of brain ageing and neurodegeneration in *APOE* ϵ4 carriers.

Chapter 2

Neuroimaging techniques

There are a variety of neuroimaging methods that allow the non-invasive investigation of brain structure and function. MRI has evolved as the most important tool to study brain structure in detail. To study brain function there are two main branches of methods with different underlying principles: measurement of the brain's electrical activity, for example MEG, and indirect measurements of metabolic consequences of brain activity, for example functional MRI. While MEG allows for great temporal resolution on the order of milliseconds it comes at the price of low spatial resolution. FMRI on the other hand has low temporal resolution of a few seconds but high spatial resolution on the millimeter scale (Fig. 2.1) (Matthews and Jezzard, 2004; McRobbie et al., 2007). In this chapter I will outline the principles underlying data acquisition from the different types of structural and functional MRI to MEG.

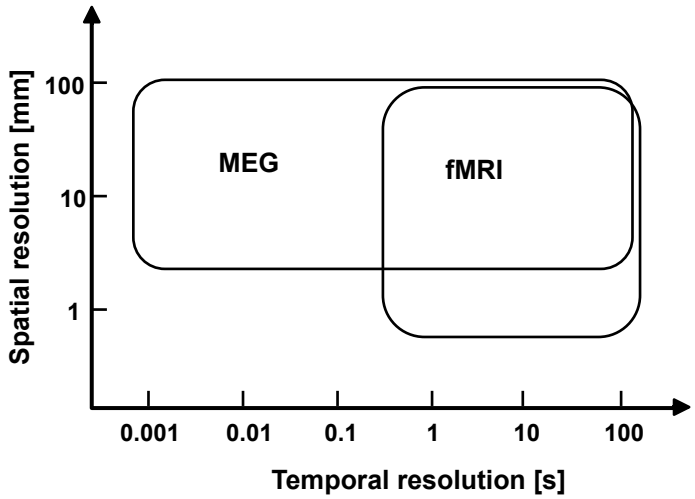

Figure 2.1: Whereas fMRI has great spatial resolution with 1mm or less, MEG has a great temporal resolution on the order of ms.

2.1 Magnetic Resonance Imaging

The basic principle underlying MRI is that it uses natural properties of hydrogen primarily in water molecules, which makes up around 80% of the human body. How hydrogen behaves in different tissues depends on the composition, e.g. the amount of water within a tissue and how freely water molecules can move. Within the brain, hydrogen in fluids such as CSF behaves differently from hydrogen in WM, where a hydration layer is bound to lipids, and GM. The nucleus of a hydrogen atom consists of a proton precessing on its own axis. This moving positive charge, i.e. this electric current, has an associated magnetic field, called the magnetic moment. In biological samples such as the brain the individual hydrogen atoms are randomly oriented so that the magnetic moments cancel each other out leading to a net magnetic moment of zero for the sample. When the sample is placed in an external magnetic field (B_0) such as the one created by an MRI scanner the spin of the proton will try to align itself with the direction of the field so that it precesses around the direction of B_0. The magnetic moment of the proton

can be oriented in two ways: parallel or anti-parallel to B_0 where the anti-parallel orientation requires slightly more energy. The energy difference between those two orientations or the frequency of electromagnetic radiation required to switch from one state to the other is called the Larmor frequency or resonance frequency and is directly proportional to the strength of B_0. This relationship between Larmor frequency and magnetic field strength is important to localise the MR signal and achieve 3D imaging. By inducing small short-term variations or gradients in magnetic field strength in the x-, y- and z- direction, protons at different locations within a sample will resonate at slightly different frequencies. This information can be used to obtain MR signal from a defined position in space.

When all spins in the biological sample are aligned with B_0 (conventionally labelled the z direction) their net magnetisation (M_0), i.e. the sum of all spins, is also aligned. This net magnetisation is the signal that is measured with MRI. However, when M_0 is parallel to B_0 it is impossible to measure because it is so small compared with B_0 (on the order of μT vs 3T in my study). Therefore, M_0 is tipped into the transverse (x,y) plane using a radiofrequency (RF) pulse at Larmor frequency with a flip angle of $90°$. The RF pulse is created in the transmit coil and produces a magnetic field perpendicular to B_0. As long as the RF pulse is on, M_0 will align with the new magnetic field that is now perpendicular to B_0. This leads to the induction of voltage in the receiver coil that is sensitive only to magnetisation in the transverse plane. The amplitude of this signal is greatest right after excitation because the RF pulse causes a phase coherence of spins. However, the signal decays exponentially, which is driven by two mechanisms: the realignment of spins with B_0 and their dephasing because of small differences in precession frequencies.

So how does all this help to acquire images of the brain? As mentioned above different tissue properties determine how protons behave. In this case they define

how fast the signal decays, i.e. how fast spins realign with B_0 and how quickly they dephase. In the next section I will explain how different proton behaviour can be utilised to produce images with visible contrasts between tissue types, e.g. CSF, WM and GM.

2.1.1 Image contrast

The two different processes that lead to signal decay - realignment with B_0 and dephasing - depend on tissue properties in different ways. The time it takes for protons to realign with B_0 is called spin-lattice relaxation time T_1. More specifically, T_1 is the time it takes for 63% of net magnetisation to be restored along the z-axis, i.e. the direction of B_0, following a $90°$ RF pulse. When they relax, protons transfer the energy they absorbed during the RF pulse to the surrounding environment ("lattice"). This energy transfer depends on how "freely" protons can move within a given tissue. If they are moving relatively unrestricted, e.g. in CSF, the energy transfer is very inefficient leading to a very long T_1. If they are bound to larger macromolecules, e.g. the myelin sheath in WM, the energy transfer is very efficient leading to a short T_1. The T_1 of GM lies between CSF and WM (Table 2.1).

Table 2.1: Relaxation times, proton densities and diffusion values of different brain tissues at 3T. (Data from FMRIB graduate course and http://www.lems.brown.edu/∼msj/cs292/assign2/mr.html)

	T_1 [ms]	T_2 [ms]	Proton density	FA / MD
Grey Matter	1200	80	0.85	Low / Low
White Matter	800	70	0.70	High / Low
CSF	4000	600	1.0	Low / High
Arterial blood	1700	120	-	-
Venous blood	1500	40	-	-

The tissue differences in relaxation times mean that shortly after an RF pulse

the restored net magnetisation along the z-axis (M_z) will be highest in WM with WM > GM >> CSF. If a second RF pulse is applied while M_z is not fully restored, this gives rise to differences in signal intensities between the tissue. A higher M_z will lead to a higher magnetisation in the x,y- plane and therefore a higher signal intensity. The time between two RF pulses is called repetition time or TR. By shortening the TR a good contrast between WM, which has the highest signal intensity and appears bright on the image, CSF (low signal intensity = dark) and GM (intermediate intensity = grey) can be achieved (Fig. 2.2). This so-called T_1-weighted imaging is used for structural imaging in my study.

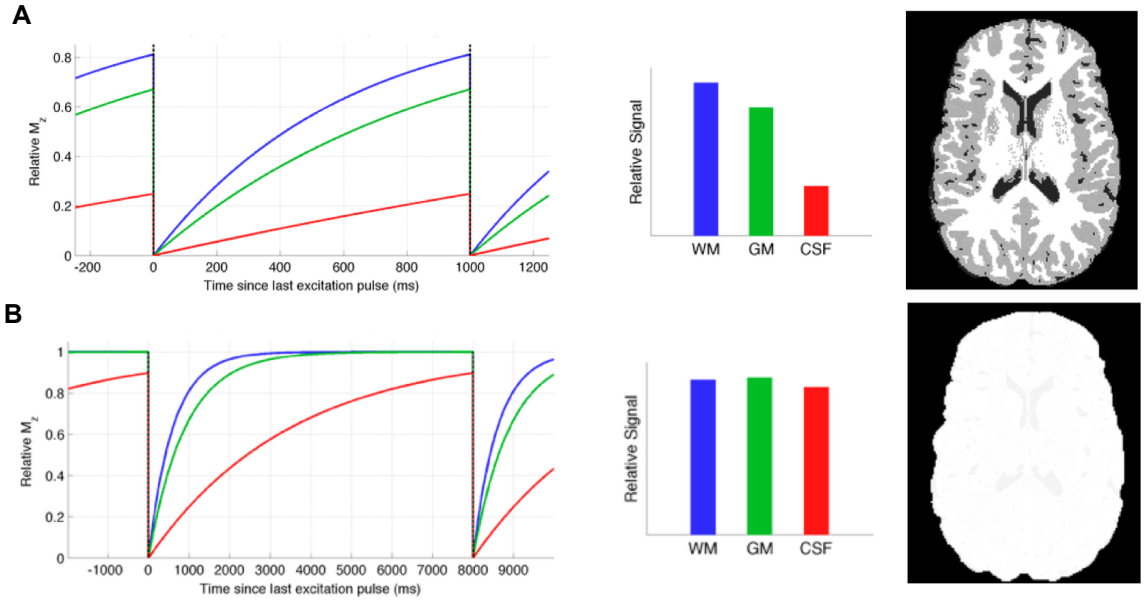

Figure 2.2: *T_1-weighted imaging.* (**A**) WM (blue) has a shorter T_1 and recovers more than GM (green) if a short TR is chosen. (**B**) A long TR leads to full recovery of M_z in WM and GM and does not produce any contrast. (Figure from FMRIB graduate course)

The second contribution to signal decay comes from dephasing. Dephasing occurs because spins have small differences in precession frequencies. The two effects that have an impact on precession frequencies are inhomogeneity in the magnetic field and interaction between the spins. This second effect gives rise to tissue differences in spin-spin relaxation time or T_2. T_2 is the time it takes for the

transverse magnetisation to drop to 37% of the initial size. Again, this is due to an energy transfer but this time energy is transferred between two protons or spins. Similarly to spin-lattice relaxation, energy transfer between spins is most efficient in WM (short T_2) and least efficient in CSF (long T_2) with GM lying inbetween (Table 2.1). T_2-weighted imaging is normally achieved using a so-called spin-echo sequence. This means that at a certain time after the $90°$ RF pulse, i.e. at a certain time during dephasing, a second RF pulse is transmitted but this time at an angle of $180°$. This refocusing does not stop the dephasing but reverses the phase angles. It means that the spins are now dephasing in the same direction instead of some going clockwise and some anti-clockwise. After a time equal to the delay between the $90°$ and $180°$ RF pulse the spins come into phase again, which leads to a second signal in the receiver coil called "echo". The time between the $90°$ RF pulse and the peak of this signal is called echo time or TE. As tissue differences in T_2 only become relevant when spins are left to dephase for some time, a long TE provides T_2-weighted images (Fig. 2.3).

Additionally, contrast is not only achieved using tissue differences in T_1 or T_2 but the amount of protons within a tissue or proton density also plays a role. Proton density imaging is especially important if two tissues have very similar T_1 and T_2 characteristics and is achieved using long TRs (no T_1 contrast) and short TEs (no T_2 contrast) (Fig. 2.4). CSF has the highest proton density, WM the lowest and GM intermediate (Table 2.1). Therefore CSF appears bright, WM dark and GM grey on proton density weighted images (Fig. 2.4).

2.1.2 BOLD fMRI

Using a spin-echo sequence for T_2-weighted imaging one does not need to take into account inhomogeneities of the magnetic field or differences in magnetic susceptibilities of tissue that affects the spins. Magnetic susceptibility is a measure

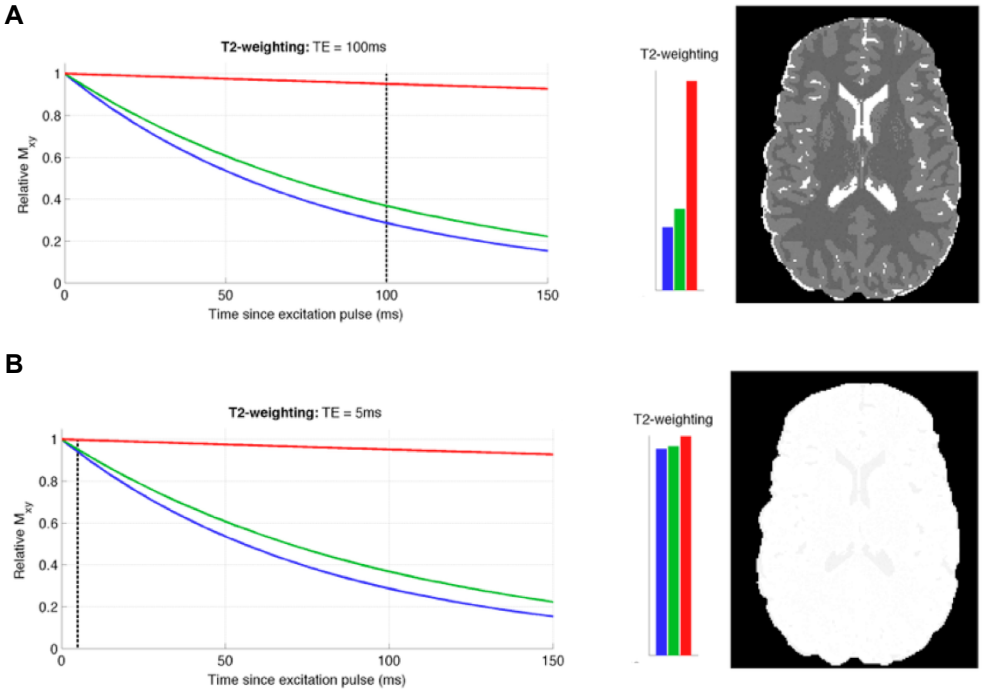

Figure 2.3: *T_2-weighted imaging.* (**A**) WM (blue) has a shorter T_2 and loses $M_{x,y}$ faster than GM (green) but this only shows after a long TE. (**B**) A short TE does not produce any contrast between WM and GM. (Figure from FMRIB graduate course)

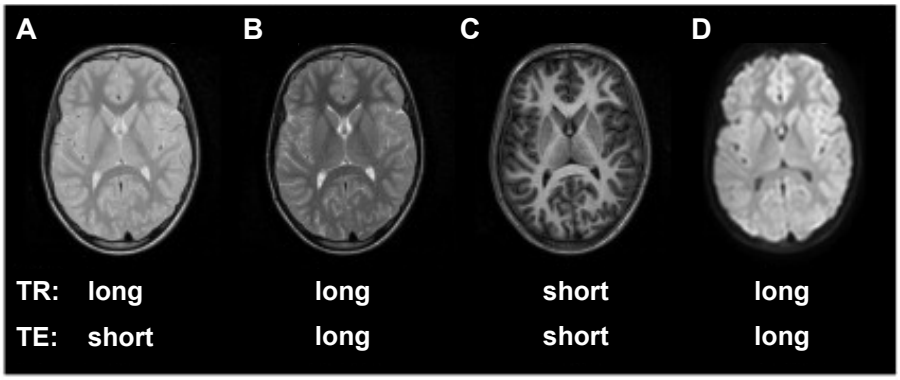

Figure 2.4: *Different image weighting.* (**A**) Proton density image with WM in dark, GM in grey and CSF in white. (**B**) T_2-weighted image with WM in dark, GM in grey and CSF in white. (**C**) T_1-weighted image with WM in white, GM in grey and CSF in dark. (**D**) Diffusion-weighted image with WM in grey, GM in white and CSF in dark. (Figure adapted from Mori and Zhang (2006))

of how magnetised a tissue becomes when it is placed in a strong magnetic field. In the body air pockets such as sinuses have very low susceptibility whereas blood has high susceptibility because of the iron-containing haemoglobin and its breakdown products. This leads to small gradients in the magnetic field at tissue boundaries, which in turn speeds up the dephasing of protons on either side of those boundaries. The relaxation time taking into account faster dephasing because of inhomogeneities of the magnetic field and susceptibility effects is denoted T_2*. T_2*-weighted imaging is achieved in a similar way to T_2-weighted imaging. The only difference is that instead of a spin-echo sequence a gradient-echo is used. Around the excitation a gradient is applied, which leads to faster dephasing of protons. Then another gradient is applied which means that spins now dephase in the same direction so that the signal (”echo”) can be read out when all spins are realigned.

T_2*-weighted imaging is especially important for functional MRI. The basic idea behind fMRI is that neuronal activity requires energy (mainly in the form of glucose and oxygen) that is supplied to neurons via the blood stream. Increased neuronal activity, e.g. while doing a particular task, requires an increase in blood supply and leads to changes in blood flow, blood volume and oxygenation. These three factors influence the blood oxygen level dependent (BOLD) signal because they alter the rate of diamagnetic (essentially non-magnetic) oxyhaemoglobin (oxygenated blood) to paramagnetic deoxyhaemoglobin (deoxygenated blood) (Fig. 2.5). A higher ratio of deoxyhaemoglobin to oxyhaemoglobin leads to increased magnetic susceptibility and therefore shorter T_2* and lower MR signal. Although one might expect that increased neuronal activity is associated with an increase in oxygen consumption and therefore increased amount of deoxyhaemoglobin this does not show on MR images where areas of increased neuronal activity show a higher MR signal. The explanation for this is that neuronal activity leads to a disproportionate increase in

supply of oxygenated blood which reduces the concentration of deoxyhaemoglobin and therefore increases MR signal.

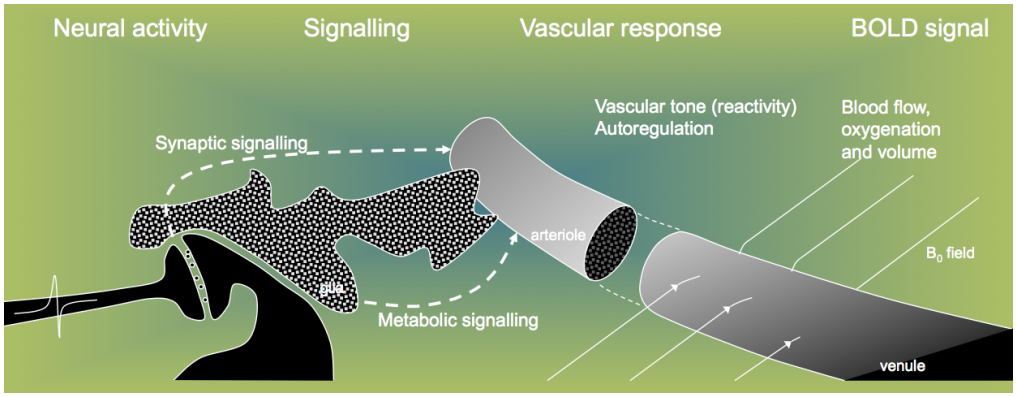

Figure 2.5: *BOLD imaging*. Neuronal activity causes increased metabolic demand leading to changes in blood flow, blood volume and blood oxygenation that affect T_2* and thus the BOLD signal. (Figure from FMRIB graduate course)

For my studies resting-state fMRI was acquired, which means that neuronal activation was recorded while subjects were lying in the scanner with their eyes open and not doing a particular task. Although participants are not doing a task there are spontaneous fluctuations in the BOLD signal that can be used to investigate functional connectivity in the brain (Fox and Raichle, 2007). Brain areas are functionally connected if they show correlated fluctuations in BOLD signal. One way of exploring the spatial and temporal characteristics of these fluctuations is independent component analyis (ICA) (Beckmann et al., 2005). This allows the identification of resting-state networks (RSNs), i.e. brain areas that are functionally connected at rest (Damoiseaux et al., 2006). Most of these networks are also relevant for performing particular tasks (e.g. visual networks) while some of them, e.g. the default-mode network (DMN), are particularly active during rest and deactivated when the brain is engaged in tasks (Fig. 2.6) (Raichle et al., 2001; Smith et al., 2009).

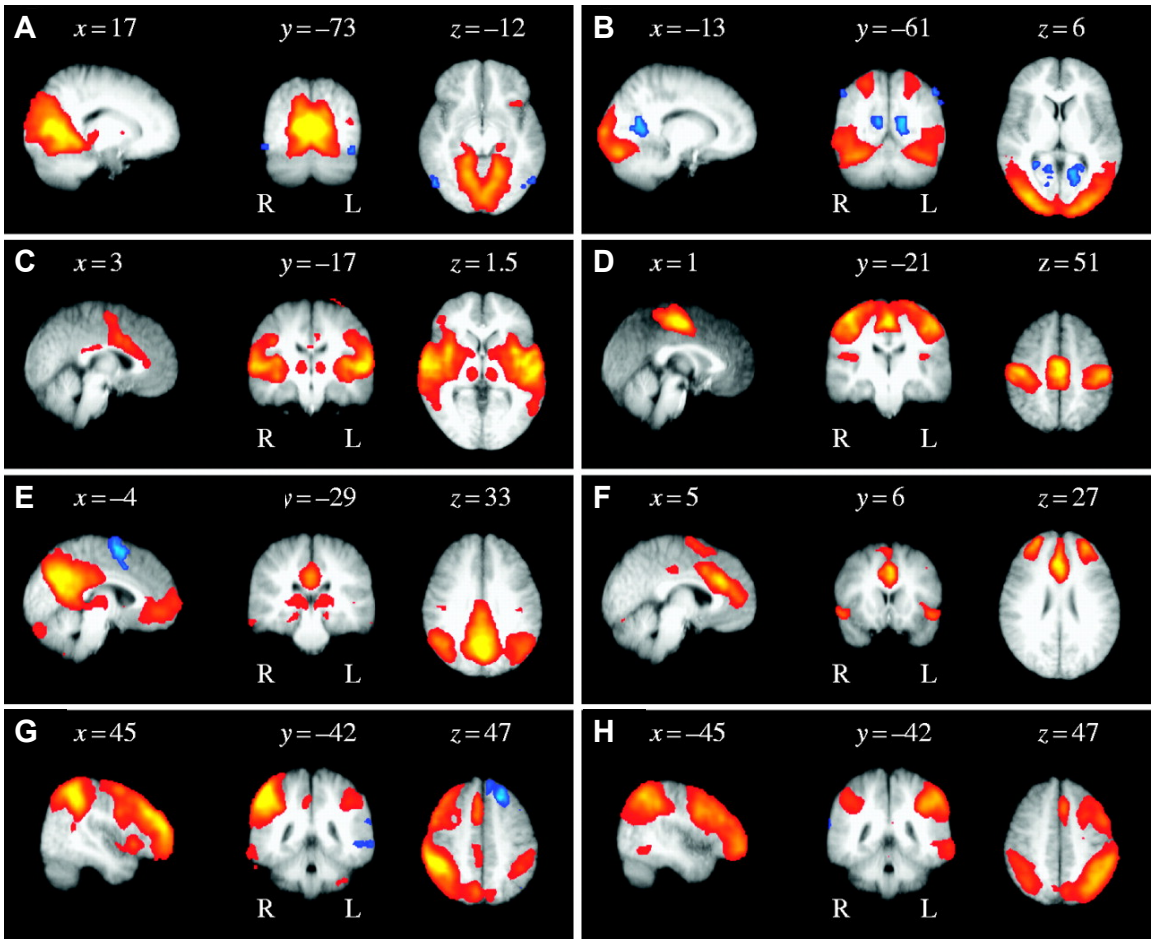

Figure 2.6: *Resting-state networks.* Using ICA several RSNs can be observed in resting-state fMRI. (**A**) medial visual network, (**B**) lateral visual network, (**C**) auditory network, (**D**) sensory-motor network, (**E**) default-mode network, (**F**) executive control network, (**G**) right fronto-parietal network (dorsal visual stream) and (**H**) left fronto-parietal network. (Figure from Beckmann at al. (2005))

2.1.3 White matter structure

In addition to proton density, T_1- and T_2-weighted imaging, image contrast can also be achieved using diffusion weighting (Mori and Zhang, 2006). Diffusion-weighted MRI or diffusion tensor imaging (DTI) is based on the idea that there are tissue differences in water diffusion. Within and between cells random water diffusion is restricted by membranes and within tissues such as WM the main direction of water diffusion is along the axon because the myelin sheath leads to restricted motion perpendicular to the axon. Therefore, DTI is especially used to investigate white matter structure. The magnitude and direction of water diffusion in each voxel is measured in the x-, y- and z-dimension and the diffusion tensor is modelled as an ellipsoid with symmetry across any one of its three axes (Fig. 2.7).

The largest axis or eigenvalue is denoted $\lambda 1$ or axial diffusivity (DA). The two minor axes ($\lambda 2$, $\lambda 3$) are usually averaged to compute radial diffusivity (DR). The average of all three eigenvalues is called mean diffusivity (MD). Fractional anisotropy (FA) is a ratio of axial to radial diffusivity, and thus provides a measure of the directionality of diffusion. Higher directionality of water diffusion in axons gives rise to a high FA in WM, whereas less restricted diffusion, e.g. in GM or CSF leads to lower FA. GM and WM have similar MD values, whereas MD is higher in CSF because diffusion is less restricted in fluid than within or around cells (Fig. 2.7).

To achieve diffusion weighting, MRI sequences need a long TR and TE (Fig. 2.4). DTI sequences are similar to T_2*-weighted imaging sequences in that two gradient pulses are applied. The second gradient pulse leads to a refocusing to get the spins into the same phase again. However, this refocusing is not perfect because protons move around so that they experience a slightly different gradient during the second gradient pulse. The imperfect refocusing leads to a signal loss, therefore the higher the diffusion the more signal is lost. To compute the amount of signal loss,

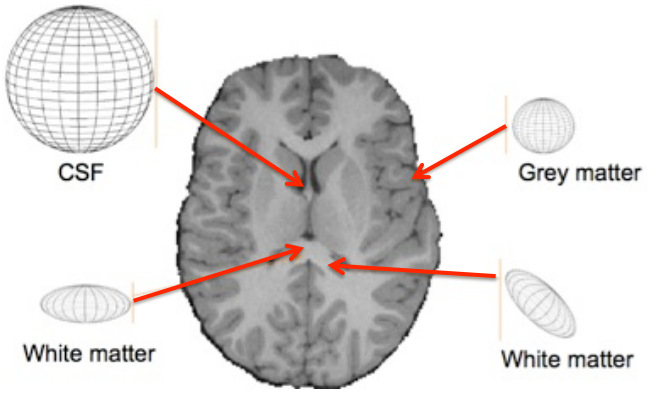

Figure 2.7: *Diffusion ellipsoids.* CSF and and GM have low FA, i.e. a spherical diffusion ellipsoid, whereas FA is high in WM so that the diffusion ellipsoid has the shape of a rugby ball. MD is higher in CSF than in GM indicated by a larger diameter of the sphere. In every voxel the main direction of diffusion is modelled, so that axon bundles can be traced in WM. (Figure from FMRIB graduate course)

diffusion-weighted images have to be compared with a non diffusion-weighted image. The image that is not diffusion-weighted is termed the b_0 image. The b value (measured in s/mm^2) is determined by a combination of gradient pulse strength and duration and describes how far water molecules can diffuse between two gradient pulses.

Acquiring two images - one with diffusion-weighting, one without - only allows the measurement of the amount of diffusion. However, it is not only the total amount of diffusion but the amount of diffusion in a particular direction that is of interest to compute the diffusion tensor for each voxel. Therefore, gradients are applied in multiple directions and these diffusion-weighted images are compared with the b_0 image to model the diffusion tensor as accurately as possible.

2.1.4 MRI acquisition protocol for the studies presented in this thesis

MRI scans were performed at the University of Oxford Centre for Clinical Magnetic Resonance Research (OCMR) using a 3-T Siemens Trio scanner (Siemens AG, Erlangen, Germany) with a 12-channel head coil. The different imaging protocols

used for T_1-weighted structural MRI, resting-state fMRI and DTI are shown in Table 2.2.

Table 2.2: Imaging parameters used in this study for structural MRI, resting-state fMRI and DTI. EPI = echo planar imaging; MPRAGE = magnetisation-prepared rapid gradient echo; TI = inversion time.

	Structural MRI	**Resting-state fMRI**	**Diffusion Tensor Imaging**
Sequence	MPRAGE	gradient echo EPI	diffusion-weighted spin echo EPI
TR [ms]	2040	2000	9300
TE [ms]	4.7	28	94
Flip Angle	8°	89°	90°
Voxel Dimension	1mm isotropic	3 x 3 x 3.5mm	2mm isotropic
Field of View [mm]	192	224	192
TI [ms] (T_1)	900	-	-
B-value (DTI)	-	-	1000
Gradients applied (DTI)	-	-	60 isotropically distributed
Number of Acquisitions	2	1	1
Acquisition Time	12 min 02 sec	6 min 04 sec	10 min 31 sec

2.2 Magnetoencephalography

MEG is a neuroimaging technique similar to electroencephalography (EEG) that is used to study brain function. Neural activity induces electric currents within neurons, which in turn leads to changes in the electric and magnetic fields around the head that are measured by EEG or MEG, respectively (Hansen et al., 2010). The recorded changes in the magnetic field are generated by populations of thousands of neurons that have a similar orientation and fire synchronously (Fig. 2.8). The pyramidal neurons of the cortex have these properties and therefore their activity is thought to underlie (most of the) MEG signal. For an example of raw MEG data, see Fig. 2.10.

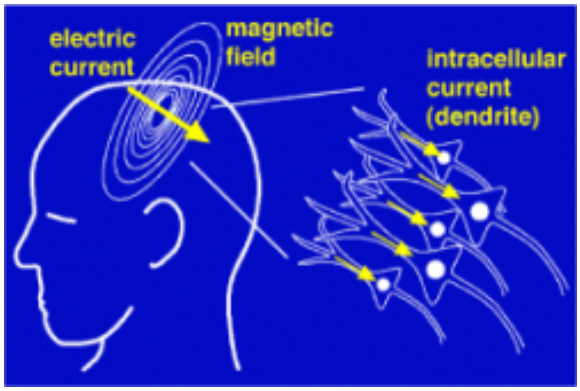

Figure 2.8: *Basis of MEG.* Intracellular electric currents in neurons induce changes in the magnetic field around the head that are picked up by sensors in the helmet of the MEG scanner. Figure from `http://psychology.wikia.com/wiki/Magnetoencephalography`.

While the temporal resolution of MEG is much higher compared with fMRI - signals can be recorded only milliseconds after the presentation of a stimulus - the spatial resolution is greatly reduced (Fig. 2.1). One advantage of MEG over EEG is that the magnetic field is less influenced by changes of conductivity between brain, skull and scalp, which improves localisation of neuronal activity. A disadvantage of MEG is that it is most sensitive to signals from neuronal pathways that run in parallel to the skull. Therefore, the main contribution to MEG signals comes from neurons located within cortical sulci. In contrast, EEG measures activity

particularly in the gyri and is, therefore, more sensitive to sources located deeper within the brain. Changes in the magnetic fields around the head are measured using superconducting quantum interference devices as sensors that are arranged in a helmet placed over the head. Signal recording is very similar to MRI in that the changes in the magnetic field around the head lead to induction of an electric current in the sensors. The amount of electric current is proportional to the amount of change in magnetic field, which in turn is dependent on how many neurons close to the sensor are active and how synchronously they fire. The Elekta Neuromag sensor system used in the studies presented in chapters 3 and 4 uses 306 MEG channels in 102 sensor arrays (Fig. 2.9).



Figure 2.9: This figure shows the outline of the helmet of the Elekta Neuromag system with 102 sensor arrays. Every sensor array consists of one magnetometer and two orthogonal gradiometers. (Figure from the Elekta Neuromag System Description)

Every channel consists of two orthogonal planar gradiometers and one magnetometer. It is useful to combine these two sensor types for several reasons. A magnetometer consists of just one pick-up coil that is sensitive to quite distant sources with the disadvantage that it is also very sensitive to noise. That is why in the Elekta system every magnetometer is coupled with two orthogonal planar gradiometers. Planar gradiometers compensate for distant noise contributions to the signal with the disadvantage that they are most sensitive to sources right be-

neath them, i.e. less sensitive to activity of deeper brain structures. As the sensors are in a fixed position in the helmet, they do not necessarily record signal from the same brain location in every individual because of different brain shapes and sizes. However, using individual structural MRI images it is possible to reconstruct the source of neuronal activity with methods such as beamformer approaches. Source reconstruction techniques use specific constraints to locate neuronal activity because theoretically there are an infinite number of solutions to explain the MEG signal, which is termed the ”inverse problem”.

There are several ways of analysing MEG data. One might be interested in the electrophysiological response with respect to the onset of a specific stimulus. This response is called event-related field (ERF) and occurs phase-locked to stimulus onset. The average of many synchronised MEG traces removes noise and emphasises any regular responses to the stimulus. Alternatively, it is possible to study changes in the power of oscillations in specific frequency bands. More synchronised neuronal firing is thought to underlie increased power of oscillations. Typical frequency bands of interest are delta (<4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-30 Hz) and gamma (>30 Hz). The power of oscillations can be measured with respect to a stimulus onset (induced activity), which is called event-related synchronisation (ERS) or desynchronisation (ERD). It can also be measured independently of a stimulus (spontaneous activity) when subjects are resting during the measurement and not engaged in a specific task. Further details of the MEG data analysis will be discussed in the relevant chapters of this thesis.

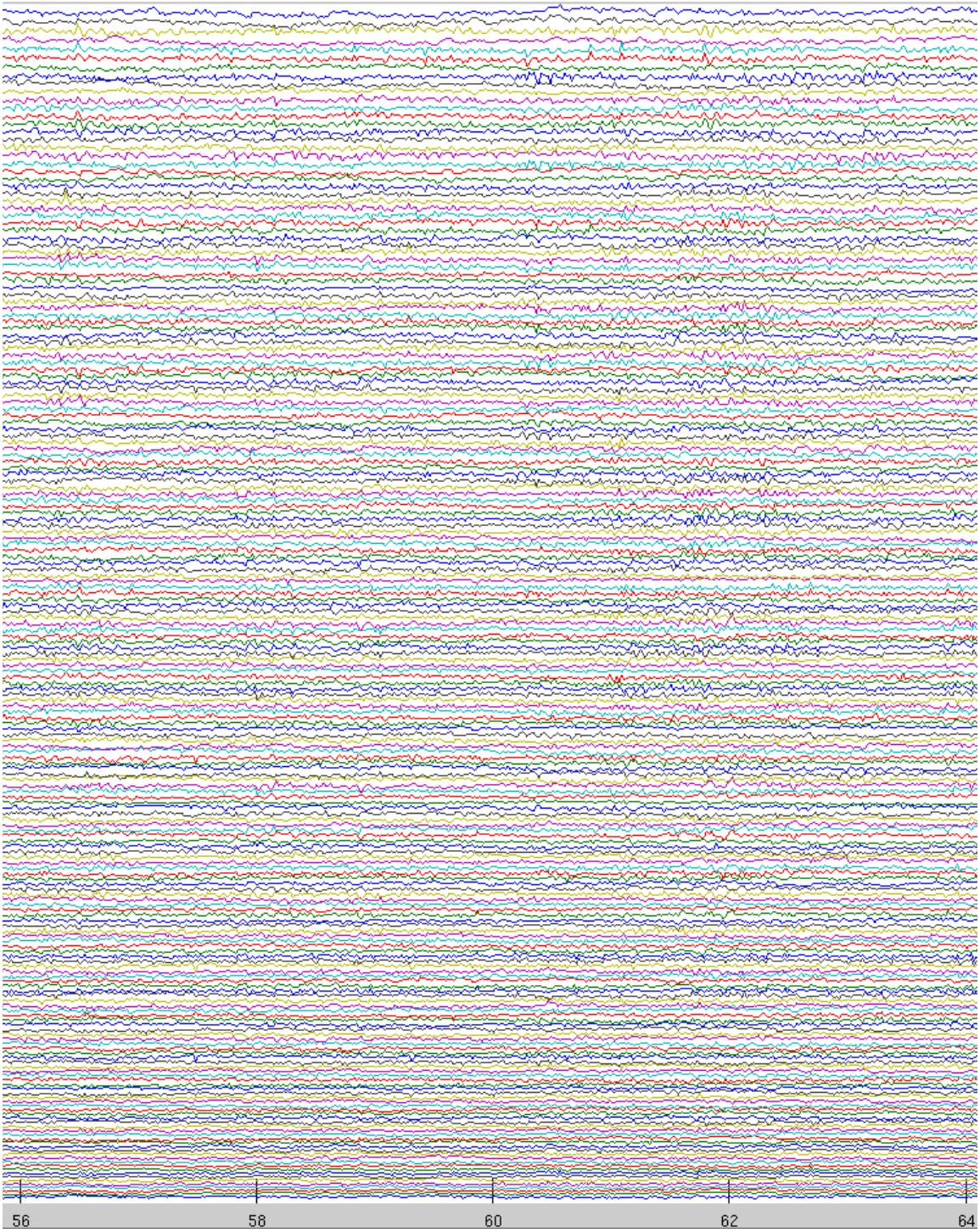

Figure 2.10: Example of a MEG recording. Only the data recorded by the gradiometers is shown in this figure.

2.2.1 MEG acquisition protocol for the studies presented in this thesis

MEG measurements were performed at the Oxford Centre for Human Brain Activity (OHBA) using an Elekta Neuromag scanner (Elekta AB, Stockholm, Sweden) with a 306-channel VectorView system. The data was sampled at 1000 Hz in a frequency range of 0.03-300 Hz. Electrooculogram (EOG) and electrocardiogram (ECG) were used to identify artefacts due to eye blinks and heart beat. In addition, eye movements were recorded using an eye tracker (EyeLink 1000, SR Research Ltd., Mississauga, Canada). Head movement was monitored by measuring the head position continuously during data acquisition. Head localisation within the MEG helmet was achieved using four electromagnetic head position indicator (HPI) coils. Two of these coils were placed on the forehead and two behind the ears. The subject's head position within the MEG sensor array was identified by energising these coils. Before data acquisition, the HPI coil locations and the subject's head shape were digitised using a Polhemus Isotrack system. By matching the digitised head surface to the head surface extracted from the structural MRI image it was possible to co-register the MEG sensor location to the subject's brain anatomy.

During the scan, participants were seated in the scanner and instructions were projected onto a screen placed 1.5 meters away from the participant. The MEG protocol comprised three different tasks and two resting periods as follows: 6 minutes resting period, 12 minutes memory task practise and encoding phase, 6 minutes resting period, 12 minutes Stroop task practise and task, 12 minutes visual oddball task practise and task, 10 minutes memory task recognition phase. All stimuli were presented and recorded using the software Presentation 14.5 (`http://www.neurobs.com`).

2.3 Summary

The different neuroimaging techniques introduced here were used to identify *APOE* genotype effects on brain function and structure. Resting-state fMRI and MEG were combined to study changes in functional connectivity and neuronal function at rest (chapter 3) and a MEG study on basic visual processing (chapter 4) made use of the excellent time resolution. GM and WM structure were investigated using structural MRI and DTI (chapter 5). In chapter 6 I present a study where combined resting-state fMRI and DTI allowed the investigation of hippocampal connectivity. Using different but complementary neuroimaging techniques is vital to understand the overall picture of the relationship between brain structure and function and how *APOE* genotype contributes to susceptibility to AD.

Chapter 3

APOE genotype and the resting brain

3.1 Introduction

In this chapter I will present an analysis of functional connectivity of the resting brain and the effects of *APOE* genotype on resting-state connectivity. Two complementary neuroimaging techniques - fMRI and MEG - were used with analogous analysis techniques to identify resting-state networks of interest. This makes it possible to directly compare the two techniques. Additionally, I will describe the added value of using MEG as a complementary neuroimaging method to understand the mechanisms that drive *APOE* genotype effects on brain function.

3.1.1 AD, *APOE* and resting fMRI

Many neuroimaging studies of brain function at rest have been carried out in AD patients. These include studies of brain metabolism at rest measured with positron emission tomography (PET) and studies using resting-state fMRI to investigate RSNs, i.e. brain areas that are functionally connected at rest because they show correlated fluctuations in BOLD signal.

Alterations of brain glucose metabolism have been shown in many brain regions

in AD patients. Brain areas that are affected at early AD stages include the precuneus, posterior cingulate cortex (PCC), parietal and temporal cortices and at later stages the frontal cortex and the whole brain (Reiman and Jagust, 2012). Interestingly, glucose metabolism is decreased in these regions not only in AD patients compared with healthy adults but also in healthy *APOE* ϵ4 carriers compared with non-carriers (Reiman et al., 1996). The particular regions that show this reduction in glucose metabolism, i.e. the precuneus/ PCC, parietal and temporal regions, are also the ones showing amyloid deposition in healthy older people and AD patients and overlap with those that are part of the DMN (Buckner, 2005; Sperling et al., 2009). The DMN is a RSN that is particularly active when people are awake but not engaged in a specific task. Buckner et al. (2005) hypothesised that the high metabolism of the DMN at rest across the lifespan predisposes these brain regions to AD-related changes such as amyloid depositions and brain atrophy. Importantly, the DMN is also functionally connected with the medial temporal lobe (MTL) and particularly the hippocampus. Reduced connectivity with MTL regions, that are affected early by NFT pathology in AD (Braak and Braak, 1991), might be one further explanation for reduced metabolism in the DMN in AD patients (Greicius et al., 2004). Altered hippocampus - DMN connectivity has been shown in AD patients (Greicius et al., 2004) as well as healthy younger and older *APOE* ϵ4 carriers using fMRI (Filippini et al., 2009a; Westlye et al., 2011). In cognitively healthy *APOE* ϵ4 carriers these connectivity differences are not caused by but precede Aβ plaque formation (Sheline et al., 2010).

Although a lot of research has focused on the DMN, functional connectivity of other brain regions is also altered in AD patients. In particular, long-distance connections, e.g. between prefrontal and parietal areas, appear to be affected by AD and show reduced connectivity compared with healthy controls (Wang et al.,

2007; Sanz-Arigita et al., 2010). There is only one study that investigated RSN connectivity differences associated with *APOE* genotype in networks other than the DMN. Trachtenberg et al. (2012) reported that *APOE* ϵ3 homozygotes showed significantly increased connectivity of the auditory network compared with both *APOE* ϵ2 and ϵ4 carriers. Additionally, differences between *APOE* ϵ2 carriers and ϵ3 homozygotes were found in the left fronto-parietal network (Trachtenberg et al., 2012). The most important findings of this study were that *APOE* genotype affects RSN connectivity beyond the DMN and that this effect does not follow the pattern of AD risk because *APOE* ϵ2 and ϵ4 carriers, i.e. those with the lowest and highest AD risk, showed similar connectivity but differences compared with ϵ3 homozygotes.

3.1.2 AD and resting MEG

FMRI is an indirect measure of brain function because it takes into account changes in blood flow, volume and oxygenation. Cerebral blood flow has been shown to be affected by *APOE* genotype both in cross-sectional studies (Scarmeas and Stern, 2006) and a longitudinal study that showed greater age-related cerebral blood flow decline in *APOE* ϵ4 carriers (Thambisetty et al., 2010). Therefore, *APOE* genotype effects in resting-state fMRI studies might not necessarily reflect altered neuronal activity but could be caused by differences in neurovascular coupling.

A more direct measure of neuronal function is MEG. Studies using MEG at rest in AD patients have shown a consistent slowing down of oscillatory activity with increased power in lower frequency bands, especially delta (0-4Hz) and theta (4-8Hz) (Stam, 2010). In the alpha band (8-13Hz), there appears to be an anterior shift of sources of alpha activity with increases in temporal regions of AD patients and decreases in parietal-occipital regions compared with healthy controls (Osipova et al., 2005). These findings are similar to EEG studies reporting increased delta and

theta activities and decreased alpha and beta (13-30Hz) activities in AD patients (Jeong, 2004).

In MEG, functional connectivity between different brain regions can be defined as how synchronous their patterns of oscillations are. A loss of synchronisation of brain regions in the alpha, beta and gamma ($>$ 30 Hz) band has been shown for AD patients (Stam et al., 2002). This affects mainly long distance connections within the left hemisphere in the alpha and beta band while local connectivity in parietal areas was increased in the theta, beta and gamma band in AD patients (Stam et al., 2006). Again, these results are similar to EEG studies showing reduced coherence in AD patients especially in the alpha and beta band (Jeong, 2004).

In a comparison between AD patients who carried the *APOE* ϵ4 allele and non-carriers, ϵ4 carriers showed reduced alpha activity in the left inferior parietal areas and decreased interhemispheric alpha connectivity between frontal and parieto-temporal areas (Canuet et al., 2012). Similar findings have been reported in resting-state EEG studies of AD patients where patients carrying the *APOE* ϵ4 allele showed overall reduced alpha power compared with non-carriers and healthy controls (Ponomareva et al., 2008). However, there are no published studies of *APOE* genotype effects on MEG or EEG resting-state activity in healthy participants.

3.1.3 Aims

The aims of the study presented in this chapter were twofold: 1) to reproduce the findings of *APOE* genotype on RSN connectivity using fMRI and 2) using MEG to determine the *APOE* genotype effect on neuronal function at rest with a similar analysis method to compare the findings with fMRI.

3.2 Methods

3.2.1 Participants

Imaging data for 40 right-handed subjects (20 males) aged 33 to 57 years were acquired. A priori exclusion criteria were: current or past history of neurological or psychiatric disorders, memory complaints, head injury, substance abuse (including alcohol), corticosteroid therapy, and diabetes. Subjects were recruited from the Oxford Biobank, an age-stratified random sample of healthy 30 to 55 year old men and women from Oxfordshire with a current pool of 5000 participants. All subjects were pre-screened for APOE genotype (using a blood or cheek swab sample) and selected for the study on the basis of either having an APOE ϵ4 allele (APOE ϵ4 carrier), having an APOE ϵ2 allele (APOE ϵ2 carrier) or being an ϵ3 homozygote. There was no subject homozygous for the ϵ2 or ϵ4 allele and those with the ϵ2/ϵ4 genotype were excluded from this study. Genotyping was conducted at the Oxford Biobank. DNA was extracted from blood or cheek swab samples of subjects according to standard procedures to allow PCR for the characterization of APOE genotype. The study was approved by the local Ethics Committee, and written informed consent was obtained from all participants.

3.2.2 Analysis protocols

Resting-state fMRI

For the resting-state fMRI scan, subjects were instructed to lie in dimmed light with their eyes open, to think of nothing in particular, and not to fall asleep. For data analysis FMRIB Software Library (FSL) tools v5.0 were used (`http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/`).

Preprocessing Preprocessing of imaging data consisted of motion correction, brain extraction, spatial smoothing using a Gaussian kernel of 6mm full-width at half-maximum (FWHM), and high-pass temporal filtering equivalent to 150s (0.007 Hz). Functional data were aligned to the anatomical image initially using FLIRT (Jenkinson and Smith, 2001; Jenkinson et al., 2002), then optimized using the Boundary-Based Registration (BBR) approach (Greve and Fischl, 2009). Registration from anatomical to the Montreal Neurological Institute (MNI) standard brain was carried out using FNIRT.

ICA After preprocessing, fMRI analysis was carried out using Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC) (Beckmann et al., 2005). The fMRI data containing 180 time points for each subject were temporally concatenated across subjects to create a single 4D data set. Group independent component analysis (ICA) was used to identify spatially independent large-scale patterns of functional connectivity in the population of all subjects independent of *APOE* genotype. The concatenated multiple fMRI datasets were decomposed into 25 components. RSNs of interest were selected after comparison against a set of previously defined maps (Beckmann et al., 2005).

Dual Regression For the RSNs of interest the between-subject analysis of the resting data was carried out using a regression technique (dual regression) that allows for voxelwise comparisons of resting functional connectivity (Filippini et al., 2009a). Within each subject's fMRI dataset, subject-specific temporal dynamics and associated spatial maps were identified. This involved the following steps. First, the full set of group-ICA RSN spatial maps were used in a linear model fit (spatial regression) against the separate fMRI datasets for each subject, resulting in matrices describing temporal dynamics for each RSN of interest. Then, these time-course matrices were used in a linear model fit (temporal regression) against

the associated fMRI dataset to estimate subject-specific spatial maps. Next, the different RSN maps were collected across subjects into single 4D files (1 per original ICA map, with the fourth dimension being subject identification). To determine *APOE* genotype effects on functional connectivity, voxelwise general linear modelling (GLM) was applied using permutation nonparametric testing with 5000 permutations and $p < 0.05$ correcting for multiple comparisons across space using threshold-free cluster enhancement (TFCE), which avoids using an arbitrary threshold for the initial cluster-formation (Nichols and Holmes, 2002; Smith and Nichols, 2009). Age was used as a covariate of no interest in the resting-state fMRI analysis. To exclude the possibility that connectivity differences between the *APOE* genotype groups were influenced by structural differences, structural images were used as covariates of no interest. GM images for each subject were extracted using FMRIBs Automated Segmentation Tool (FAST) (Zhang et al., 2001), registered in standard space, smoothed to match the resting-state fMRI data, demeaned within each group and added to the GLM as covariates.

Resting-state MEG

During the measurement of resting-state activity, participants were seated in the MEG scanner, asked to keep their eyes open and fixed on a white cross in the centre of a black screen. Subjects were asked to think of nothing in particular and not to fall asleep.

Preprocessing MEG data analysis was carried out using the MATLAB (The MathWorks, Inc., Natick, USA) based toolbox Statistical Parametric Mapping 8 (`http://www.fil.ion.ucl.ac.uk/spm/`) and OHBAs Software Library (OSL). The preprocessing pipeline consisted of the following steps. First, channels containing excessive noise were identified manually before applying MaxFilter 2.2, a software

provided by Elekta, with signal space separation and movement compensation (Taulu et al., 2004). MaxFilter is a method for rejecting signal from sources outside the helmet, i.e. it reduces noise from external sources. Additionally, MaxFilter was used to compensate for the effects of head movement. After applying MaxFilter the data was downsampled to 250Hz and time periods that showed artefacts associated with body movement and external sources were removed manually. Then, a semi-automatic artefact rejection method was applied using ICA that identified noise components associated with line noise (50Hz), eyeblinks and heartbeat. Interference components were identified by inspection of their spatial topography, timecourse and frequency spectrum and were removed from the data. The last step of preprocessing consisted of a high-pass filter at 1Hz to remove low-frequency fluctuations and a further manual data check to remove time points with artefacts that had survived the preprocessing stage.

Source reconstruction The source reconstruction of MEG data consisted of several steps. First, the subject's headshape (measured with the Polhemus Isotrack) was co-registered to their structural MRI and to the MNI template brain. Then, several modelling steps were taken to approach the inverse problem, i.e. the problem that there is not only one solution to explain the MEG signal. An overlapping local spheres forward model implemented in FieldTrip (`http://fieldtrip.fcdonders.nl/`) was chosen as head model to define the head shape of each subject (Huang et al., 1999). The forward model was then used to estimate the leadfield, i.e. to model the MEG signal at a specific sensor that is induced by neuronal activity at a particular location of interest in the brain. The final step of source reconstruction was achieved using a linearly-constrained minimum variance beamformer (Van Veen et al., 1997; Woolrich et al., 2011). Beamformers act as spatial filters that estimate the contribution to the MEG signal for specific

locations of interest, in this case at each vertex of a 6mm grid spanning the entire brain. This is achieved by combining the leadfield estimations with the actual MEG signal picked up by the sensors. MEG data in different frequency bands of interest (alpha, theta, beta and broadband (4-30Hz)) were projected into source space. For each 6x6x6 mm voxel a timecourse of oscillatory activity in the frequency band of interest was computed.

Hilbert Enveloping and ICA Following beamformer projection, a Hilbert transform was applied to each voxel timecourse to derive the amplitude of oscillatory activity, termed the Hilbert envelope. The envelope was downsampled temporally by dividing each envelope timecourse into 2s non-overlapping windows and averaging the data within those windows. This has consistently been shown to be a robust way of detecting stationary functional connectivity (Brookes et al., 2011b; Brookes et al., 2011a; Luckhoo et al., 2012). Envelope data were smoothed spatially with a 4mm Gaussian kernel and the voxel size was resampled to an 8mm grid. Then, data were transformed to standard (MNI) space using FLIRT before datasets from all subjects were temporally concatenated.

After Hilbert enveloping, the MEG data were analysed in two different ways. For a direct comparison between MEG and fMRI results, the RSN ICA maps derived from the fMRI analysis were used as input for the regression step. Additionally, an ICA was run on the MEG data across all subjects to decompose the concatenated envelopes into 25 temporally independent timecourses (implemented using fastICA) (Hyvärinen, 1999). The 25 spatial maps resulting from this step were compared with those identified with fMRI. The MEG derived ICA maps of interest were then used as input for the regression. For both approaches a frequency band of 4-30Hz was chosen. If components showed significant *APOE* genotype effects, the contribution of specific frequency bands (theta, alpha and

beta) was investigated as a post-hoc test.

Regression For the fMRI and MEG RSNs of interest the between-subject analysis of the resting data was carried out using a regression technique that allows for voxelwise comparisons of MEG data. Similar to the second step of dual regression for resting-state fMRI data, the subject-specific spatial maps were estimated by using the time-course of the ICA maps in a linear model fit against the subject's MEG dataset. As in the analysis of fMRI data, voxelwise GLM was applied using permutation nonparametric testing with 5000 permutations and $p < 0.05$ correcting for multiple comparisons across space using TFCE. Age was used as a covariate of no interest.

3.2.3 Statistics

SPSS software version 19 (SPSS Inc., Chicago, USA) was used for non-image-based statistical analyses. One-way ANOVAs were used for continuous sociodemographic variables and a Fisher-Freeman-Halton exact test for categorical variables. An alpha of $p < 0.05$ was considered significant.

3.3 Results

3.3.1 Demographics

Subject demographics are shown in Table 3.1. There were no differences in age, gender distribution, years of education or family history of dementia between the three *APOE* genotype groups.

Table 3.1: Sociodemographic features of the three *APOE* genotype groups. Values denote mean (±Standard Deviation) or number of subjects, p-values refer to one-way ANOVAs (parametric data) and Fisher-Freeman-Halton exact tests (categorical data).

	ϵ**2/**ϵ**3**	ϵ**3/**ϵ**3**	ϵ**3/**ϵ**4**	**P**
n	12	15	13	-
Age [years]	45.25 (6.85)	46.87 (4.27)	48.92 (5.24)	0.25
Gender [m/f]	5/7	8/7	7/6	0.85
Education [years]	16.00 (1.82)	15.87 (2.56)	15.42 (1.89)	0.78
Family history of dementia	2	7	5	0.29

3.3.2 ICA

DMN

The DMN component of the fMRI ICA consisted of the PCC, prefrontal cortex (PFC), two lateral parietal areas and MTL areas including the hippocampus. For the MEG data, the DMN was split up into five different components: PCC, PFC, a bilateral parietal component and left and right MTL components (Fig. 3.1).

Other RSNs

The ICA analysis of fMRI data yielded 7 additional RSNs and similar networks were obtained for the MEG ICA analysis (Fig. 3.2).

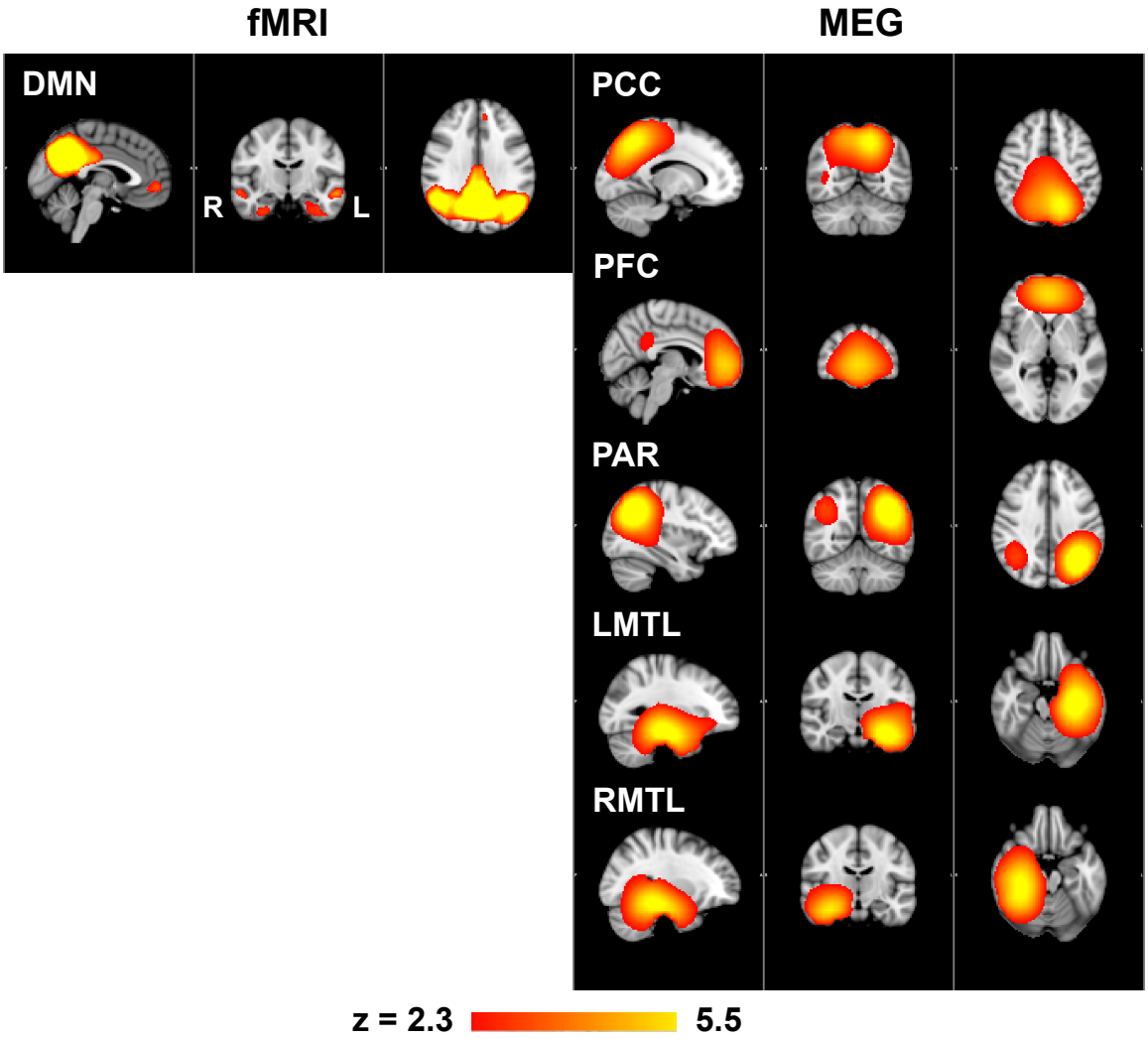

Figure 3.1: *DMN ICA components.* The fMRI analysis showed one DMN component (left) whereas the different areas of the DMN were split up into 5 components in the MEG analysis (right): PCC, PFC, the lateral parietal areas (PAR) and a left and right MTL components. (R=right, L=left)

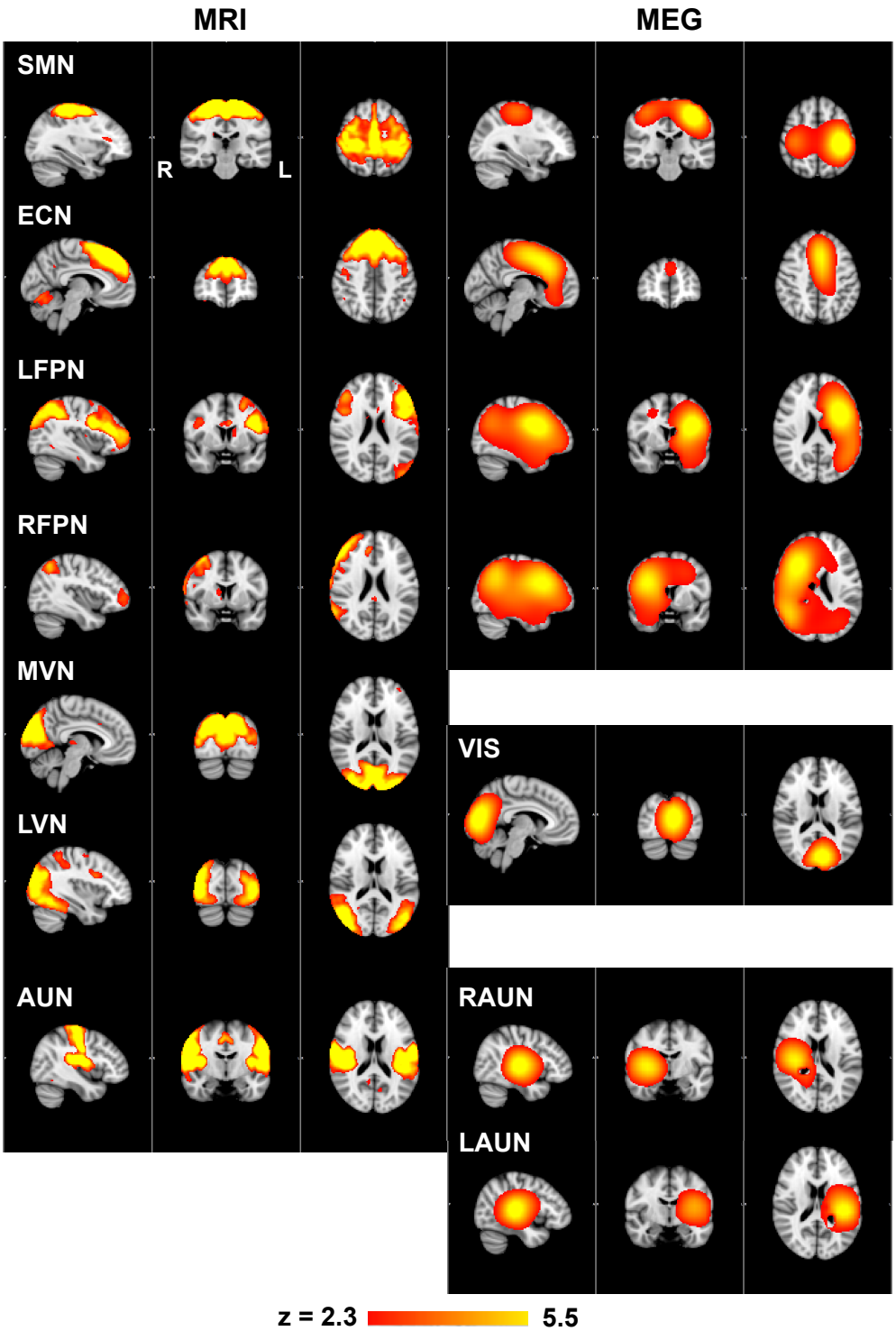

Figure 3.2: *Comparison of ICA components between fMRI and MEG.* The fMRI analysis showed typical RSN components (left): sensory-motor network (SMN), executive control network (ECN), left and right fronto-parietal networks (LFPN, RFPN), a medial and lateral visual network (MVN, LVN) and auditory network (AUN). Similar networks were found for the MEG ICA analysis but only one visual network (VIS) was found and the auditory network was split into the left and right component.

3.3.3 Resting-state fMRI

DMN

There were no significant *APOE* genotype differences in DMN connectivity using fMRI. However, with a more lenient statistical threshold of $p<0.1$, a trend to significantly increased DMN connectivity in *APOE* $\epsilon 4$ carriers compared with $\epsilon 3$ homozygotes was found in the right lateral parietal area (Fig. 3.3). No differences between *APOE* $\epsilon 2$ carriers and $\epsilon 3$ homozygotes or $\epsilon 2$ carriers and $\epsilon 4$ carriers were found.

Other RSNs

There were no significant *APOE* genotype differences in fMRI resting-state connectivity for the other RSNs.

3.3.4 Resting-state MEG

DMN

Using fMRI ICA map *APOE* $\epsilon 4$ carriers showed significantly reduced connectivity in the PCC and lateral parietal areas, bilaterally, compared with $\epsilon 3$ homozygotes (Fig. 3.3). The post-hoc comparison in specific frequency bands indicated that this was mainly due to differences in the theta band for *APOE* $\epsilon 4$ carriers. Although the broadband analysis did not show significant differences between *APOE* $\epsilon 2$ carriers and $\epsilon 3$ homozygotes, there were significant differences in the alpha band (Fig. 3.4). There were no differences between *APOE* $\epsilon 2$ and $\epsilon 4$ carriers in connectivity of the DMN.

Using MEG ICA map There was significantly reduced connectivity in the lateral parietal DMN component in *APOE* $\epsilon 2$ and $\epsilon 4$ carriers compared with $\epsilon 3$ homozy-

gotes (Fig. 3.3). Additionally, *APOE* ϵ4 carriers had reduced connectivity in the PCC component and between the PFC and PCC (Fig. 3.3). The post-hoc comparison in specific frequency bands indicated that this was mainly due to differences in the alpha band (Fig. 3.4). For the PFC component differences between *APOE* ϵ4 carriers and ϵ3 homozygotes did not reach significance in any frequency band but were just below significance threshold for the theta, alpha and beta band. No differences between *APOE* ϵ2 and ϵ4 carriers were found.

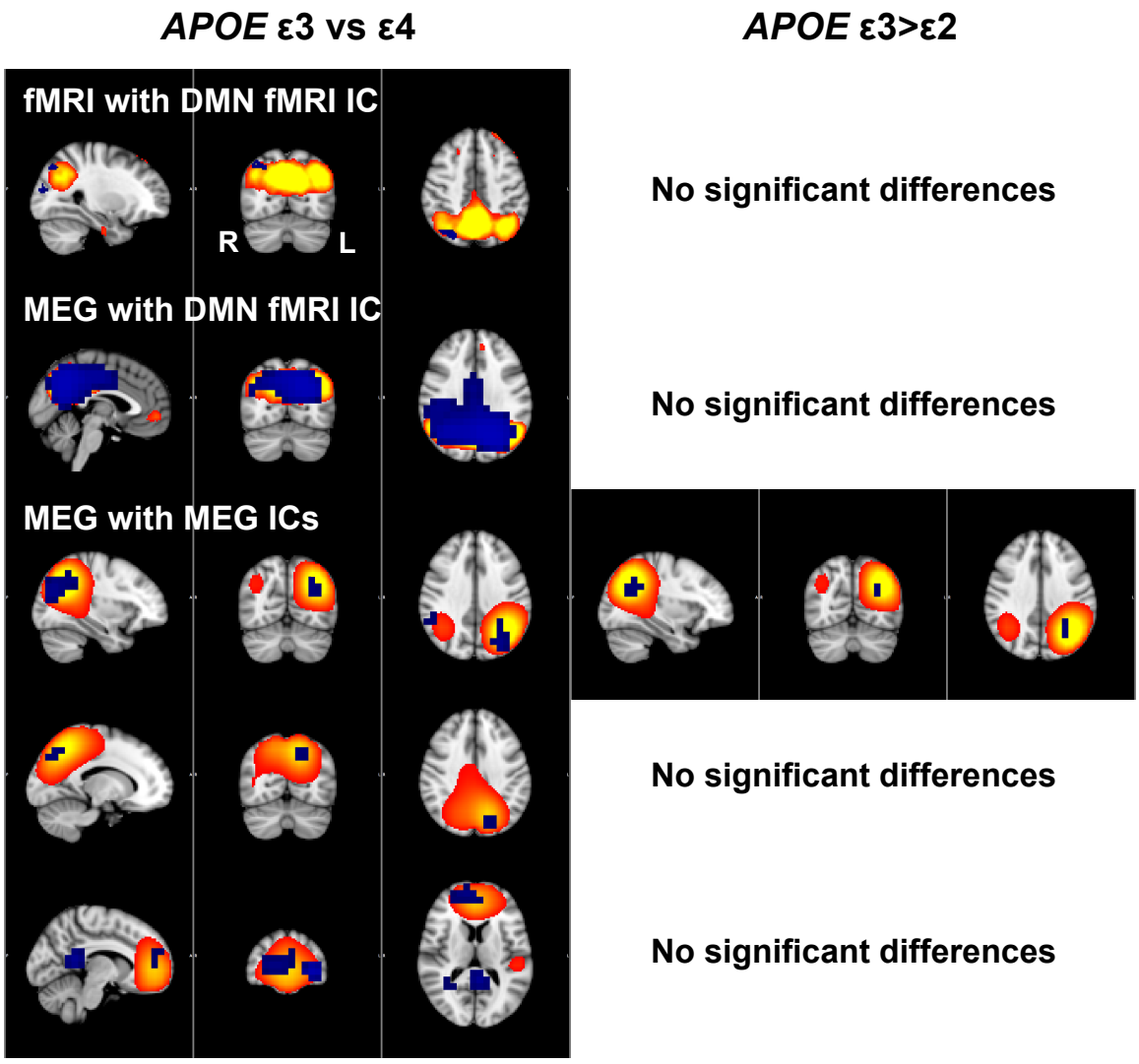

Figure 3.3: APOE *genotype differences between APOE ϵ3 homozygotes and ϵ4 carriers (left) and ϵ2 carriers (right)*. For fMRI there was a trend to increased connectivity in ϵ4 carriers (blue). Using the fMRI maps as input for dual regression on MEG data, there was decreased connectivity in *APOE* ϵ2 and ϵ4 carriers. For the MEG analysis with the five MEG ICA maps, there were significant differences in the lateral parietal component with increased connectivity in ϵ3 homozygotes compared with the other two groups. *APOE* ϵ4 carriers also showed decreased connectivity in the PCC and PFC component.

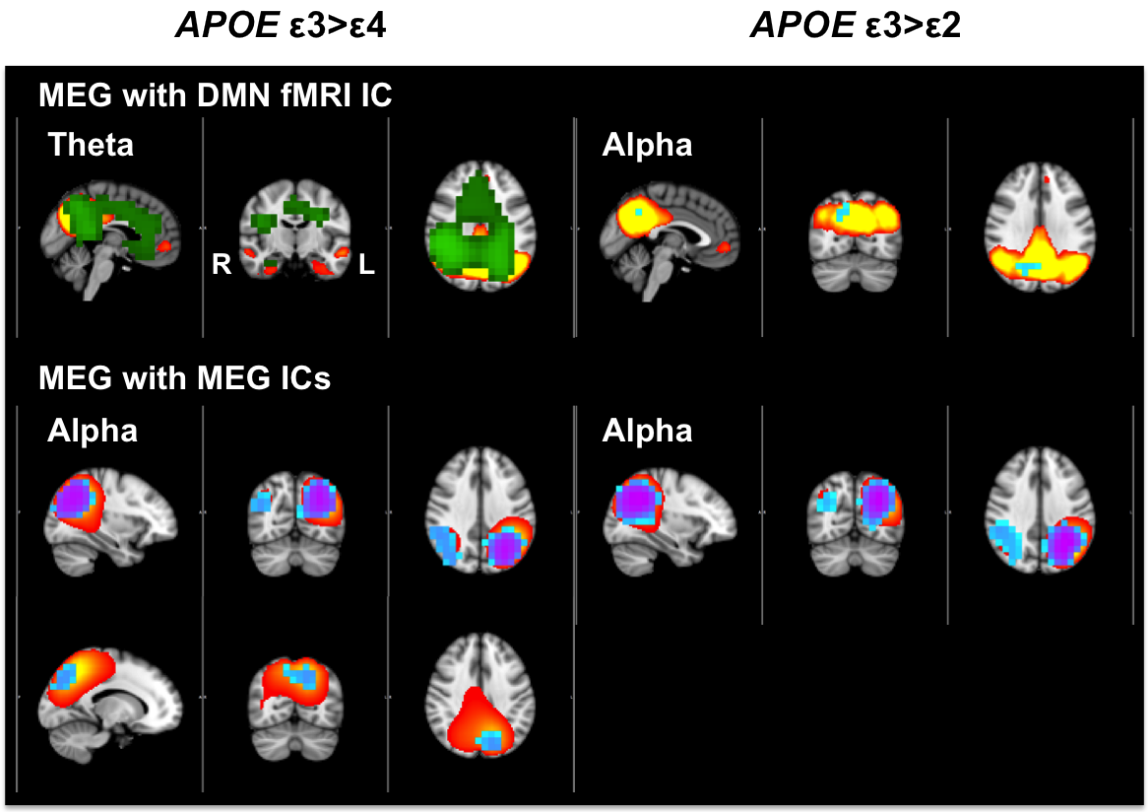

Figure 3.4: *Frequency contributions to DMN connectivity differences.* Using the fMRI maps, significant differences were seen in the theta band (*APOE* $\epsilon 3 > \epsilon 4$) and alpha band (*APOE* $\epsilon 3 > \epsilon 2$). For the MEG maps, the biggest contribution to *APOE* genotype differences was in the alpha band.

Other RSNs

Using fMRI ICA map *APOE* $\epsilon 2$ and $\epsilon 4$ carriers showed significantly reduced connectivity within the lateral visual network. Additionally, differences between *APOE* $\epsilon 4$ carriers and $\epsilon 3$ homozygotes were found in the left fronto-parietal network (Fig. 3.5). The post-hoc comparison showed that this was mainly due to differences in the alpha band (data not shown). Again, no differences between *APOE* $\epsilon 2$ and $\epsilon 4$ carriers were found.

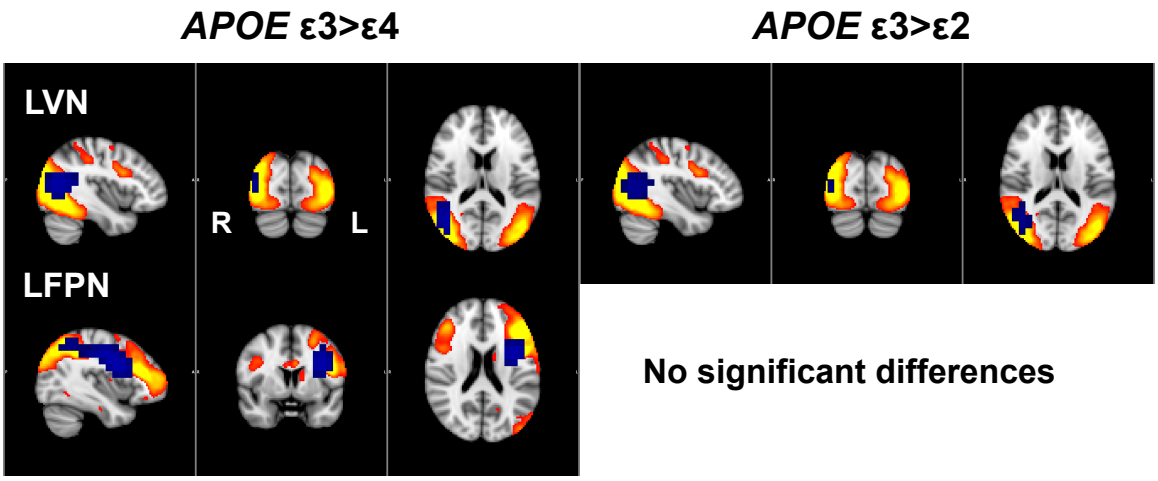

Figure 3.5: APOE *genotype differences in RSN connectivity between APOE $\epsilon 3$ homozygotes and $\epsilon 4$ carriers (left) and $\epsilon 2$ carriers (right) using the fMRI ICA maps.* There were significant differences in the LVN with increased connectivity in $\epsilon 3$ homozygotes compared with the other two groups. Additionally, *APOE* $\epsilon 3$ homozygotes had significantly increased connectivity compared with $\epsilon 4$ carriers in the LFPN.

Using MEG ICA map Similar to the analysis using fMRI ICA maps, there was significantly reduced connectivity in *APOE* $\epsilon 2$ and $\epsilon 4$ carriers in the left fronto-parietal network, executive control network and right auditory network. Additionally, significant differences between *APOE* $\epsilon 4$ carriers and $\epsilon 3$ homozygotes were found in the right fronto-parietal network and between $\epsilon 2$ carriers and $\epsilon 3$ homozygotes in the sensory-motor network (Fig. 3.6). Again, this was mainly due to differences in the alpha band and there were no differences between *APOE* $\epsilon 2$ and $\epsilon 4$ carriers.

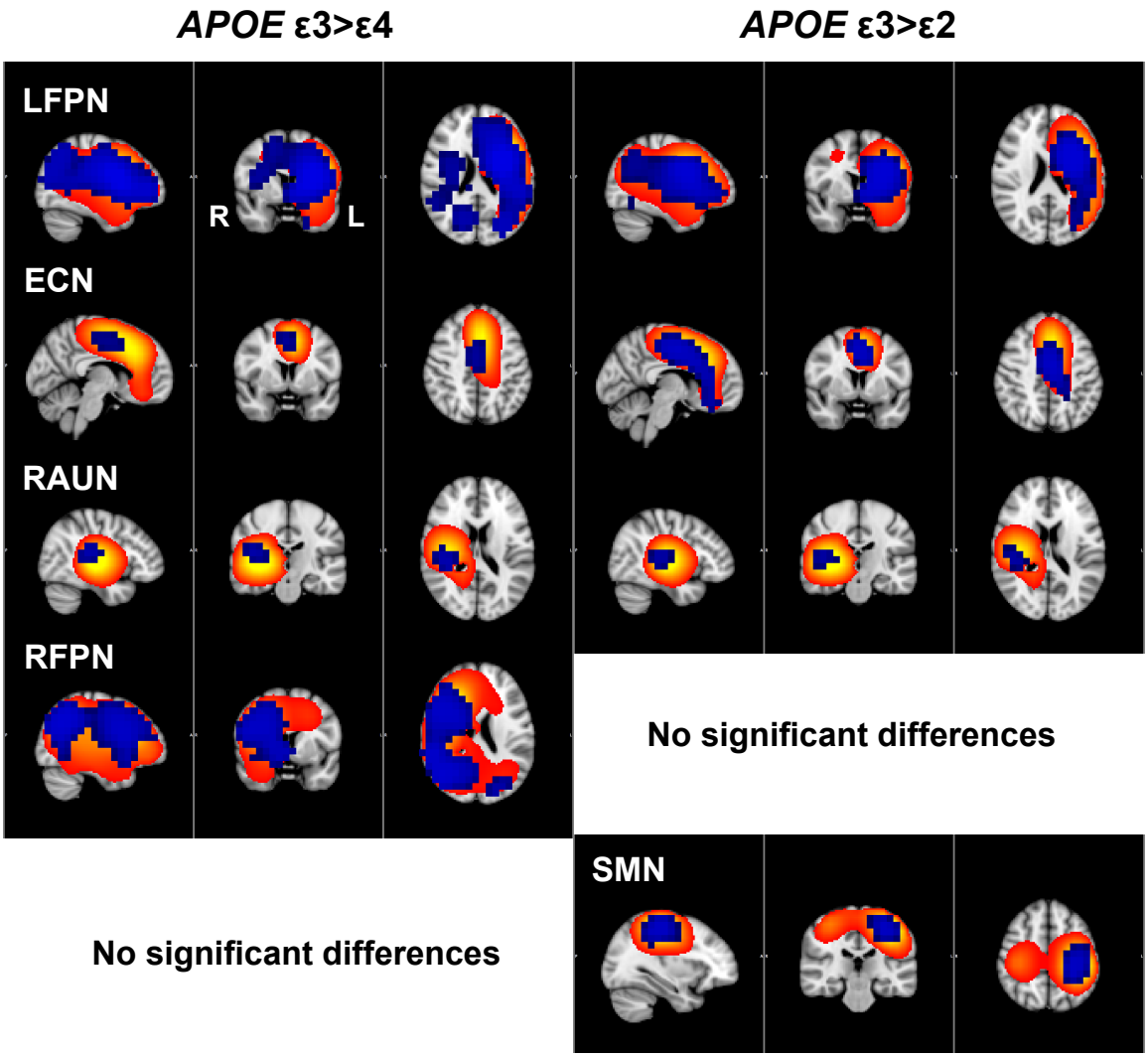

Figure 3.6: APOE *genotype differences in RSN connectivity between APOE ϵ3 homozygotes and ϵ4 carriers (left) and ϵ2 carriers (right) using the MEG ICA maps.* There were significant differences in the LFPN, ECN and RAUN with increased connectivity in ϵ3 homozygotes compared with the other two groups. Additionally, *APOE* ϵ3 homozygotes had significantly increased connectivity compared with ϵ4 carriers in the RFPN and in the SMN compared with ϵ2 carriers.

Power analysis To determine if mean oscillatory power contributed to the *APOE* genotype related differences, a general analysis of mean power averaged across time was carried out. First, oscillatory power was investigated across the whole 4-30Hz range in frequency bins of 0.5Hz. In a voxelwise analysis no significant differences between the three *APOE* genotype groups were found. When averaging across the whole GM and across the fMRI map of the DMN, greater power was found in *APOE* ϵ3 homozygotes compared with the two other groups particularly around 10Hz (Fig. 3.7). However, due to high inter-individual variability this difference did not reach statistical significance.

Then, oscillatory power was averaged across the 4 different frequency bands (4-30Hz, theta, alpha and beta) again across the whole GM and the fMRI map of the DMN. No significant differences were found for the mean oscillatory power across the whole GM. For the DMN, *APOE* genotype related mean power differences were close to significance for the alpha band ($F_{2,37}$=3.23, p=0.051) (Fig. 3.8). A post-hoc Bonferroni test showed that this was due to a trend to reduced mean alpha power in *APOE* ϵ2 carriers compared with ϵ3 homozygotes (p=0.063).

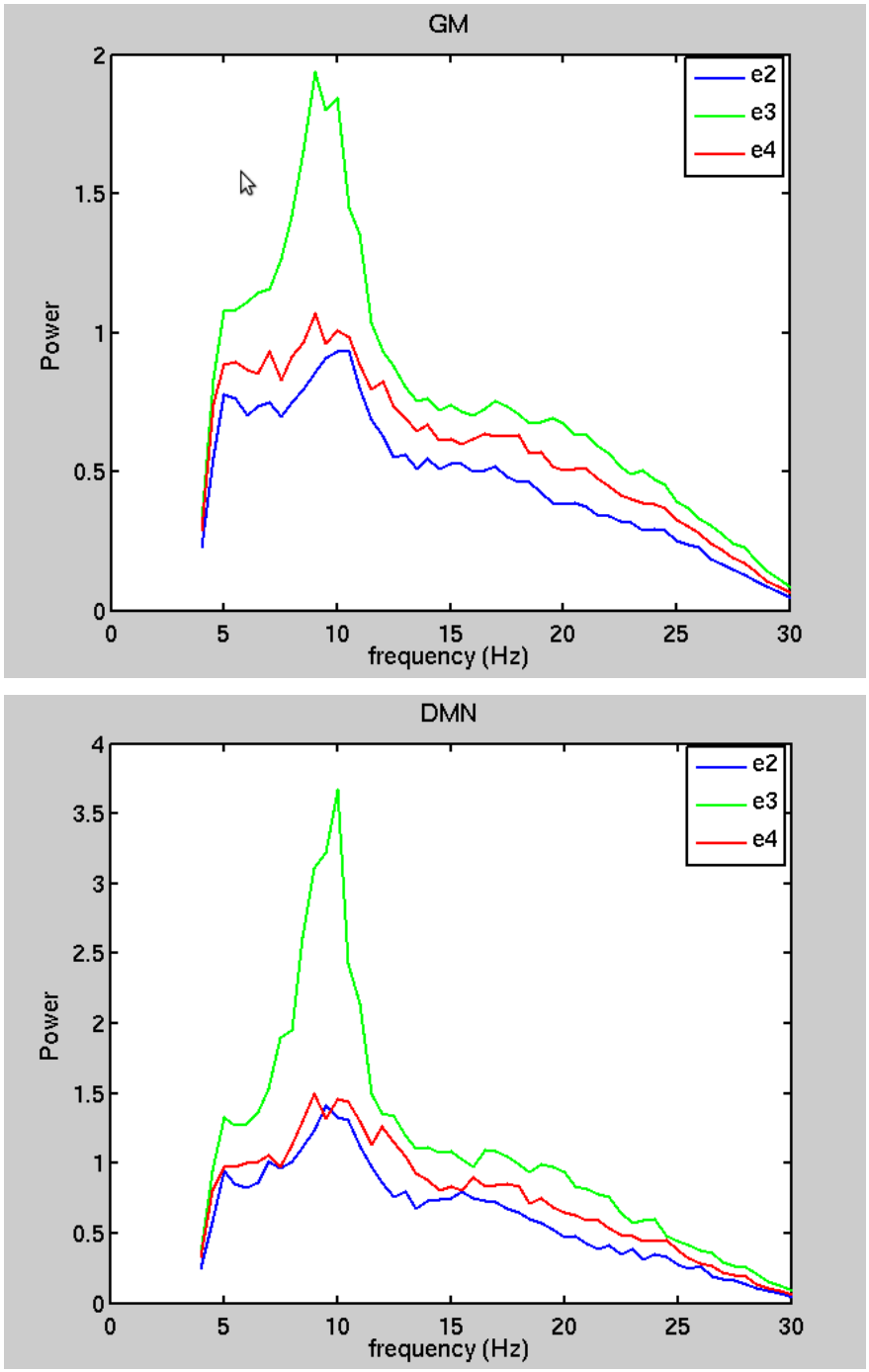

Figure 3.7: APOE *genotype differences for the 4-30Hz range averaged across the whole brain GM (top) or the fMRI map of the DMN (bottom).* Although mean power was higher around 10Hz in *APOE* ϵ3 homozygotes, there were no significant differences between the *APOE* genotype due to high inter-individual differences.

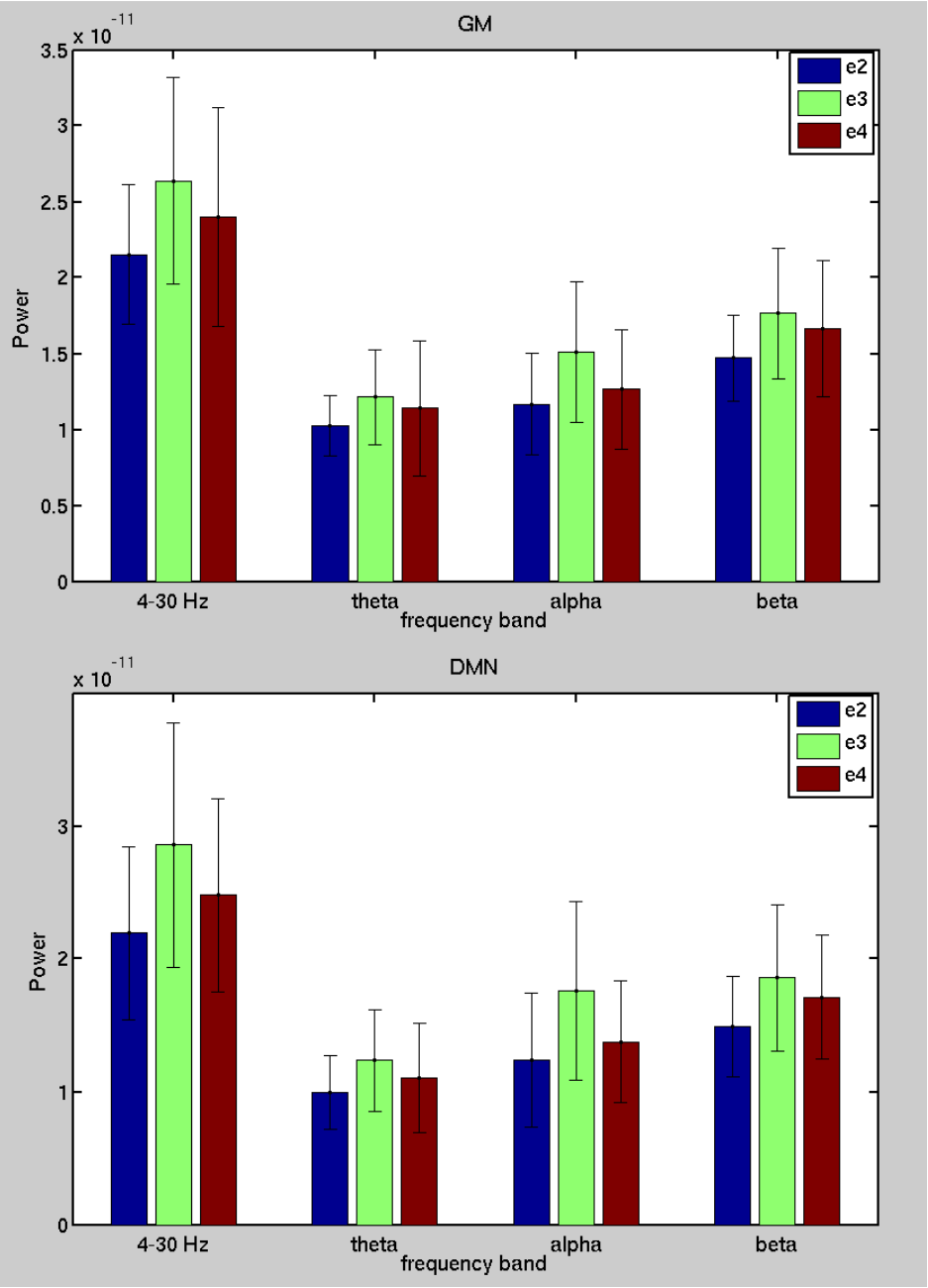

Figure 3.8: APOE *genotype differences in mean oscillatory power in 4 frequency bands averaged across the whole brain GM (top) or the fMRI map of the DMN (bottom).* There were no significant differences between the *APOE* genotype groups when averaging across the whole GM. Averaged across the DMN, there was a trend to reduced alpha power in *APOE* $\epsilon 2$ carriers compared with $\epsilon 3$ homozygotes (p=0.063). Error bars indicate standard deviation.

3.4 Discussion

In this study the effect of *APOE* genotype on resting-state connectivity was tested using fMRI and MEG. No significant effects on fMRI connectivity were observed although there was a trend to increased DMN connectivity in *APOE* ϵ4 carriers compared with ϵ3 homozygotes. Using MEG, there was a significant effect of *APOE* genotype on resting-state connectivity on several RSNs with increased connectivity in ϵ3 homozygotes compared with the two other groups. This was mainly driven by connectivity differences in the alpha band.

3.4.1 Comparison of fMRI and MEG ICA maps

The comparison of ICA maps obtained by spatial ICA in fMRI and temporal ICA in MEG revealed that some of the RSNs that were well defined using the fMRI data were split up into several components in the MEG data, e.g. two separate components for the left and right auditory network were found. For the DMN, this looked similar to the division into anterior, posterior and ventral DMN subnetworks, which has been reported in previous fMRI studies (Damoiseaux et al., 2012). However, other networks such as the sensory-motor network, executive control network and bilateral fronto-parietal networks were similar for fMRI and MEG data. These similarities between RSNs derived from fMRI and MEG data have been shown before. The MEG networks reported by Brookes et al. (2011b) were more clearly defined than those reported here. One possible explanation for that are the differences between MEG system. Brookes et al. (2011b) used a CTF scanner system with a different MEG channel configuration from the one of the Elekta Neuromag system used in this study. Nevertheless, the data presented here shows that RSNs can be reliably identified using MEG data and stresses the neuronal origin of fMRI RSNs.

3.4.2 Default-mode network

The finding that *APOE* genotype related differences did not reach statistical significance in the fMRI analysis is not necessarily surprising because of the small number of subjects included in this analysis. Nevertheless, there was a trend to increased functional connectivity within the DMN in *APOE* ϵ4 carriers compared with ϵ3 homozygotes. More widespread differences between *APOE* ϵ4 carriers and ϵ3 homozygotes were seen in the analysis of MEG data while differences between *APOE* ϵ2 carriers and ϵ3 homozygotes were only observed for the ventral DMN component, i.e. the lateral parietal areas. Most resting-state fMRI studies investigating differences associated with *APOE* genotype have reported altered connectivity of the hippocampus and DMN (Filippini et al., 2009a; Sheline et al., 2010; Westlye et al., 2011). Although these effects were not shown here, there are two other studies that also found *APOE* genotype effects in regions other than the hippocampus. Machulda et al. (2011) reported decreased connectivity between PCC and lateral parietal areas in *APOE* ϵ4 carriers. Damoiseaux et al. (2012) investigated DMN subnetworks and found *APOE* genotype effects for the anterior and posterior network very similar to those reported here for the PFC and PCC MEG components. This shows the validity of the MEG results and, more importantly, indicates that there is a neuronal basis for functional connectivity differences reported in fMRI studies investigating *APOE* genotype effects.

3.4.3 Other resting-state networks

APOE genotype effects on MEG functional connectivity were not only observed in the DMN but also in several other RSNs. The results here are very similar to those presented by Trachtenberg et al. (2012) who found *APOE* genotype effects on fMRI resting-state connectivity in the auditory network, left fronto-parietal

network and lateral visual network. Therefore, the effects of *APOE* genotype are not restricted to the DMN, i.e. brain areas that show early AD related pathology. Instead, *APOE* genotype affects brain function in a fundamental way leading to widespread differences in neuronal function. In addition, the results here indicate that *APOE* ϵ2 and ϵ4 carriers show similar functional connectivity but differences compared with ϵ3 homozygotes, which has also been shown by Trachtenberg et al. (2012). Therefore, the patterns of functional connectivity differences do not follow that of AD risk. Instead, those with the highest and lowest AD risk due to their *APOE* genotypes show similar functional connectivity.

3.4.4 MEG functional connectivity

In fMRI an increase in correlated BOLD activity is normally interpreted as increased functional connectivity. With the MEG study presented here it is possible to define at least one neuronal mechanism that is underlying functional connectivity and that is synchronicity of neural oscillations. Synchronised oscillatory activity in the brain is thought to be one mechanism underlying integration of information on a small scale, i.e. within local networks, as well as on larger scales, i.e. between distant brain sites (Varela et al., 2001). It is tempting to speculate that *APOE* genotype related differences in synchronised activity could lead to differences in the ability to integrate information. Interestingly, this does not affect one specific type of network because more localised networks such as the executive control network are affected as well as connections between distant brain areas (fronto-parietal network) and the two hemispheres (sensory-motor network).

A loss of synchronisation of brain activity has also been shown in AD patients but this seems to particularly affect long distance connections (Stam et al., 2006). Although one would like to hypothesise that decreased ability to integrate information over distant brain areas in *APOE* ϵ4 carriers could be one reason for increased

AD susceptibility, it is difficult to reconcile with the idea that $\epsilon 2$ carriers with the lowest AD risk show similar patterns of synchronised brain activity. However, the architecture of the brain is only one part of cognitive reserve that plays a role in AD susceptibility. Another part is the response to insults and remodelling abilities, which could be completely different between *APOE* $\epsilon 2$ and *APOE* $\epsilon 4$ carriers.

3.4.5 Biological mechanisms

The biggest *APOE* genotype related differences in synchronous oscillations can be found in the alpha band. It has been suggested that alpha synchronisation reflects rhythmic activity of GABAergic inhibitory neurons and, therefore, active inhibition such as inhibition of sensory information during internally directed tasks (Klimesch et al., 2007). The alpha rhythm is thought to originate from interactions between the thalamus and the cortex (Stam, 2010). However, not only the thalamus but also cortico-cortical projections have been reported to control alpha oscillations, and especially the precuneus appears to be a generator of alpha oscillations (Cantero et al., 2009). Altered activity and structural connectivity of the precuneus have often been found in *APOE* $\epsilon 4$ carriers (Reiman et al., 1996; Brown et al., 2011) and might be associated with *APOE* genotype related differences in synchronous oscillations.

Although the *APOE* genotype related differences are mostly caused by differences in synchronous activity, there is also a small effect on alpha power. Overall decreased alpha power has been associated with AD and the *APOE* $\epsilon 4$ allele in particular in previous MEG and EEG studies (Ponomareva et al., 2008; Canuet et al., 2012). However, there were no significant effects associated with the *APOE* $\epsilon 4$ allele in this study, which could be due to the small number of subjects. Nevertheless, there was a trend to decreased alpha power in *APOE* $\epsilon 2$ carriers. One of the biological mechanisms associated with reduced alpha power is reduced

cholinergic transmission. This is based not only on studies in AD patients where deficits in cholinergic transmission can be seen but also on studies in healthy subjects after administration of anticholinergic drugs (Jeong, 2004). Nevertheless, the cholinergic system is probably not the only driver of alpha power because of its interactions with other neurotransmitter systems.

3.4.6 Methodological considerations

For this study resting-state data was investigated using ICA. One main reason for choosing this method was that it made it possible to directly compare MEG and fMRI data, which made interpretation of results easier. Nevertheless, other methods such as seed-based correlation analyses would also have been applicable but were not carried out because of the limited time for methods development. Following on from the results presented here, it would be very interesting to make use of the finer timescale of MEG data. For example, resting-state EEG research has shown fast fluctuations on the order of 100ms of stable topographies, so-called microstates (Britz et al., 2010). It would be very interesting to investigate with MEG whether RSN connectivity fluctuates in a similar way and whether *APOE* genotype affects those temporal dynamics.

3.5 Conclusion

This study showed that differences in neuronal function underlie *APOE* genotype effects on resting-state connectivity. Increased synchronised neuronal oscillations can be found in *APOE* $\epsilon 3$ homozygotes compared with the two other genotype groups in many different RSNs such as the DMN and fronto-parietal networks. Since *APOE* $\epsilon 2$ and $\epsilon 4$ carriers show similar patterns of synchronised activity, *APOE* genotype has a more general effect on neuronal connectivity that is not

directly linked to AD risk.

Chapter 4

APOE genotype and basic visual processing

4.1 Introduction

In this chapter I will present an analysis of *APOE* genotype effects on basic visual processing using MEG during a visual oddball paradigm. On the one hand this makes it possible to explore which types of neuronal function are affected by *APOE* genotype. On the other hand the high temporal resolution allows me to investigate whether *APOE* genotype affects the speed of neuronal communication. However, the analysis presented here can only be considered preliminary because of the limited methods available for MEG analysis at the moment.

4.1.1 AD and brain function

One of the first cognitive areas affected by AD is memory function. Therefore, it is not surprising that regions involved in memory formation such as the MTL show reduced fMRI activation during memory tasks in AD patients (Schwindt and Black, 2009). Interestingly, other brain regions, in particular prefrontal regions, show

increased activity in AD patients during memory tasks. This has been interpreted as compensatory activity, using alternative networks to approach cognitive tasks (Schwindt and Black, 2009). However, AD does not only affect memory processes. Brain activations during other cognitive functions such as attention have also been shown to be affected by AD (Li et al., 2009a). Additionally, AD patients show alterations in basic visual processing, which could be due to the visual system degeneration in AD seen in the visual cortex as well as optic nerve and retinal ganglion cells (Pollock et al., 1989). Basic auditory processing also appears to be affected by AD in one of the few MEG studies investigating deficits in task-related cognitive function in AD patients (Pekkonen et al., 1999). Other MEG studies predominantly used memory tasks and reported reduced activity in temporal and parietal regions but increased activity in motor regions in AD patients (Maestú et al., 2001; Maestú et al., 2003). Again, it has been hypothesised that increased activity could reflect compensatory processes in AD patients (Maestú et al., 2001). However, a high proportion of AD patients (around 50%) are usually carriers of the *APOE* ϵ4 allele while one would assume that only around 17% of healthy controls are ϵ4 carriers (Strittmatter et al., 1993). Therefore, it might be possible that altered neuronal activity is not simply an effect of neurodegeneration but could be associated with altered brain function in *APOE* ϵ4 carriers. The higher percentage of *APOE* ϵ4 carriers in the group of AD patients compared with healthy controls could contribute to the reported differences in brain function in addition to the effects of neurodegeneration.

4.1.2 *APOE* and brain function

Similar to research into AD, most studies investigating effects of *APOE* genotype on brain function have focused on aspects of memory functions. Most of these studies reported *APOE* genotype related differences in brain activity although

there are some inconsistencies as to whether carriers of the *APOE* ϵ4 allele show increased or decreased BOLD responses compared with non-carriers (Trachtenberg et al., 2010). However, Trachtenberg et al. (2011) showed that *APOE* genotype not only affects memory processing but also brain activation during a task probing attention and executive function. Similar to studies investigating *APOE* genotype effects on functional connectivity, the patterns of task-related brain activations did not follow AD risk. While brain activations were very similar for *APOE* ϵ2 and ϵ4 carriers, both groups showed increased activity compared with ϵ3 homozygotes (Trachtenberg et al., 2011).

However, as mentioned in the previous chapter, differences in BOLD activation might be driven by *APOE* genotype effects on neurovascular coupling. Therefore, MEG could be a more sensitive measure of differences in neuronal function. There are only two MEG studies investigating *APOE* genotype effects on neuronal function. These studies again focused on memory functions and found longer ERF latencies (Deeny et al., 2008) as well as increased ERS in the theta and gamma band in *APOE* ϵ4 carriers (Filbey et al., 2006). However, they did not include *APOE* ϵ2 carriers as a separate group and thus did not investigate whether there are similar patterns of task-related neuronal function in *APOE* ϵ2 and ϵ4 carriers. Additionally, it is unclear whether *APOE* genotype affects cognitive processes other than memory function and more basic sensory processing. Therefore, a visual oddball paradigm was chosen in the MEG study presented here to investigate *APOE* genotype effects on attention and sensory processing.

4.1.3 AD, *APOE* and the oddball task

In the oddball paradigm, occasional target stimuli occur in a train of frequent non-target stimuli (Linden et al., 1999). In active oddball paradigms, a reaction to the target stimuli, e.g. a motor response, is required while in passive paradigms

participants only see or hear the stimuli without reacting to them. This paradigm is used to test the orienting response that is necessary to perceive and react to sudden changes in the environment (Debener et al., 2005). The conscious process of target detection takes place around 300-500ms after stimulus onset (Ritter and Vaughan, 1969). In the EEG literature a specific component of the event-related potential (ERP), termed P3 or P300 (and M300 for ERFs in MEG), is associated with this target detection (Fig. 4.1). FMRI studies have shown that activity in the inferior parietal and frontal lobes and the insular cortex are associated with this response (Linden et al., 1999; Bledowski et al., 2004).

Using visual and auditory oddball paradigms, several EEG and MEG studies have reported smaller amplitudes and longer peak latencies of the P300 in AD patients (Kimiskidis and Papaliagkas, 2012). There are several EEG studies investigating the effects of positive family history of AD or *APOE* genotype on ERPs using the oddball paradigm. Higher AD risk in those studies was associated with increased P300 latencies and reduced P300 amplitudes (Green and Levey, 1999; Ally et al., 2006; Liu et al., 2009; Irimajiri et al., 2010; Lai et al., 2010)

However, the P300 is not the only ERP component that can be investigated using this paradigm. Early components of the ERP such as the P100 (or M100) occur around 100ms after stimulus onset (Fig. 4.1) and are associated with sensory processing of the visual stimulus (Perry and Hodges, 1999). EEG research has shown that AD patients show longer latencies of the visual evoked P100 (Pollock et al., 1989), which is generated by neuronal activity in the extrastriate cortex (Di Russo et al., 2002). Lower amplitudes and longer latencies of early sensory components have also been reported in healthy older adults who carry the *APOE* ϵ4 allele (Espeseth et al., 2009). However, there are no published studies investigating whether *APOE* ϵ2 carriers show similar task-related neuronal activity to ϵ4 carriers during a visual oddball task.

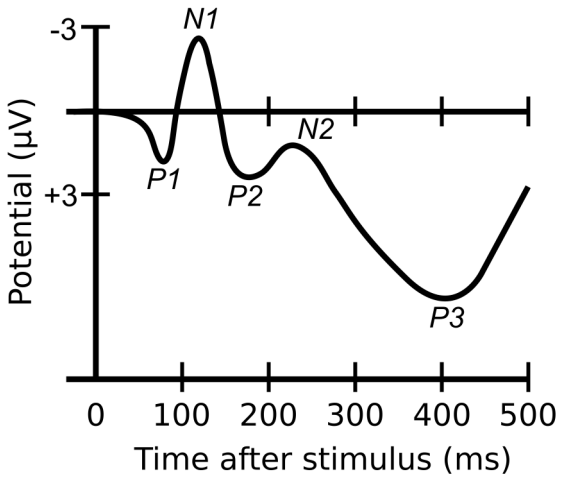

Figure 4.1: *Example of an ERP elicited by a stimulus.* While the early component (P1 or P100) is associated with sensory processing, later components reflect cognitive processes such as reorientation of attention and target detection (P3 or P300). (Figure from `http://en.wikipedia.org/wiki/Event-related_potential`)

4.1.4 Aims

The aims of the study presented here were twofold: 1) to investigate the effect of *APOE* genotype on latency and amplitude of neuronal responses to visual stimuli and 2) to determine whether *APOE* genotype effects on task-related neuronal activity reflect AD risk by including carriers of the *APOE* ϵ2 allele.

4.2 Methods

4.2.1 Participants

The participants were the same as those described in the previous chapter but some datasets had to be excluded because of excessive artefacts. Therefore, the group sizes were as follows: *APOE* ϵ2 carriers = 9, *APOE* ϵ3 homozygotes = 15 and *APOE* ϵ4 carriers = 11.

4.2.2 Oddball task

For the visual oddball task two different types of stimuli were used on a black background, either circles with a checkerboard pattern or a red circle (Fig. 4.2). Participants were asked to keep their eyes on a fixation cross in the center of the screen while stimuli were shown. The checkerboard stimuli were presented for 200ms in one of four corners of the screen (upper left, lower left, upper right or lower right) at 6° eccentricity with a random interstimulus interval of between 750 and 1250ms (average 1000ms) and subjects were asked not to react to these stimuli. In 20% of the trials a red circle was shown for 200ms in the center of the screen instead of a checkerboard and participants were asked to count the number of red circles quietly in their head. In total, 200 stimuli were presented in a pseudorandom order with 40 checkerboards in each of the four corners and 40 red circles in the center. The stimuli were presented in blocks of ten after which participants could take a break for eye blinks and other indispensable movements. Upon a button press, participants resumed the task.

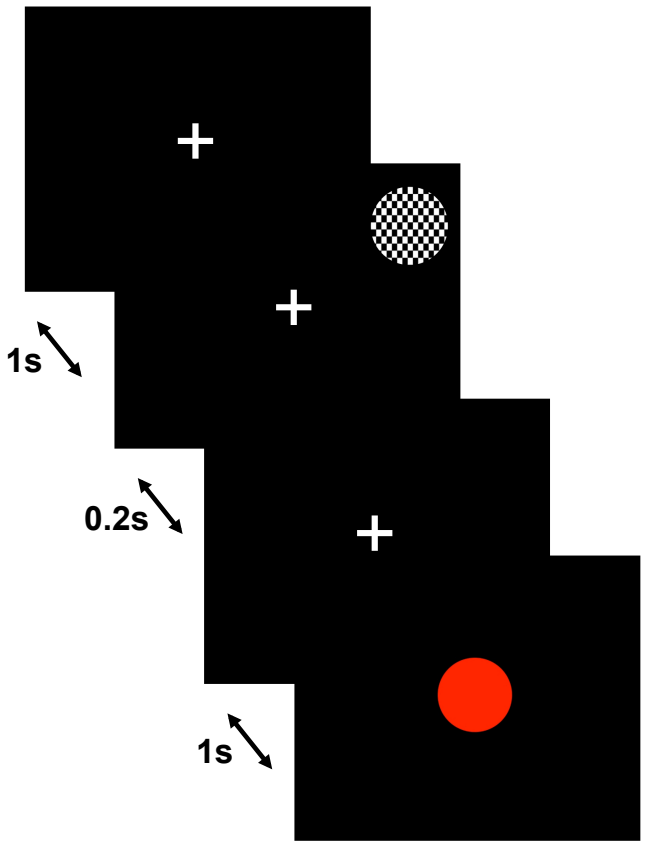

Figure 4.2: *Design of the oddball task*. In each trial either a checkerboard or a red circle was shown. Checkerboards appeared in the four corners of the screen and a red circle (the oddball) in the centre of the screen for 200s with a randomised interstimulus interval between 750ms and 1250ms (average 1s).

4.2.3 Analysis protocols

MEG data analysis was carried out using SPM8, FieldTrip and OSL. Data pre-processing was done on the whole dataset in the same way as described in the previous chapter. Since only timepoints around stimulus onset were of interest for the following analyses, data were epoched with respect to stimulus onset (-200ms to 800ms around the stimulus). Artefactual epochs were removed after visual inspection using FieldTrip visual artefact rejection routines. Then, task MEG data was used for two different analyses. To obtain simple measures of ERF latencies and amplitudes a sensor-space analysis was carried out using the OSL pipeline. In addition to the sensor-space analysis, a task-based ICA was applied to identify components of interest that were compared between the three *APOE* genotype groups.

Sensor-space analysis

Within the framework of the OSL pipeline, a GLM was performed as the first level, i.e. within-subject, analysis to investigate main effects of different conditions (target and non-target conditions) in a frequency band of 1-48Hz (Hunt et al., 2012). In the study presented here, the focus was on the oddball condition because it elicited the most prominent ERFs. After the first-level GLM, data were inspected visually and for each individual subject occipital gradiometers were identified that showed clear ERFs. *APOE* genotype effects on amplitude and latency of the M100 peak (maximum between 80-120ms) and M200 peak (maximum between 150-250ms) (Fig. 4.3) were investigated using one-way ANOVAs and age as a covariate of no interest.

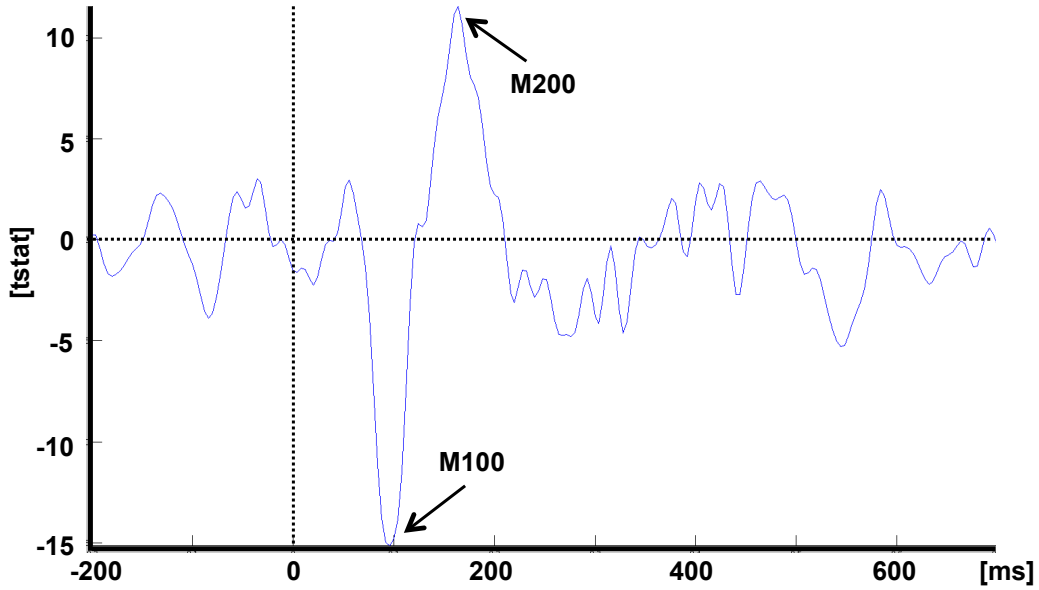

Figure 4.3: *ERFs in the oddball condition.* An example of the ERF recorded in an occipital gradiometer of a single subject. The M100 peak was defined as the maximum peak between 80-120ms and the M200 peak as the maximum between 150-250ms.

Task-based ICA

Task-based ICA was run as described in Luckhoo et al. (2012) in the same way as for resting-state data for a frequency band of 4-30Hz. Instead of running the ICA on the whole dataset that included break periods, only the concatenated epochs from stimulus onset to 600ms after onset were used as input. A between-subject GLM was carried out to identify ICs that showed *APOE* genotype related differences during the oddball trials. A multi-step-up test equivalent to false-discovery-rate correction was applied to correct for multiple comparisons (Nichols and Hayasaka, 2003).

4.3 Results

4.3.1 Sensor-space analysis

Investigating the ERFs, there was a significant *APOE* genotype effect on latency of the M100 peak ($F_{2,32}$=3.68, p=0.037). A post-hoc Bonferroni test showed that this was due to reduced M100 latency in *APOE* ϵ4 carriers compared with ϵ3 homozygotes (p=0.046) (Fig. 4.4 A). The *APOE* genotype effect on M100 amplitude was close to significance ($F_{2,32}$=3.23, p=0.053) with a trend to reduced amplitude in *APOE* ϵ4 carriers compared with ϵ2 carriers (p=0.096). No *APOE* genotype effect on amplitude or latency of the M200 peak was found (Fig. 4.4 B).

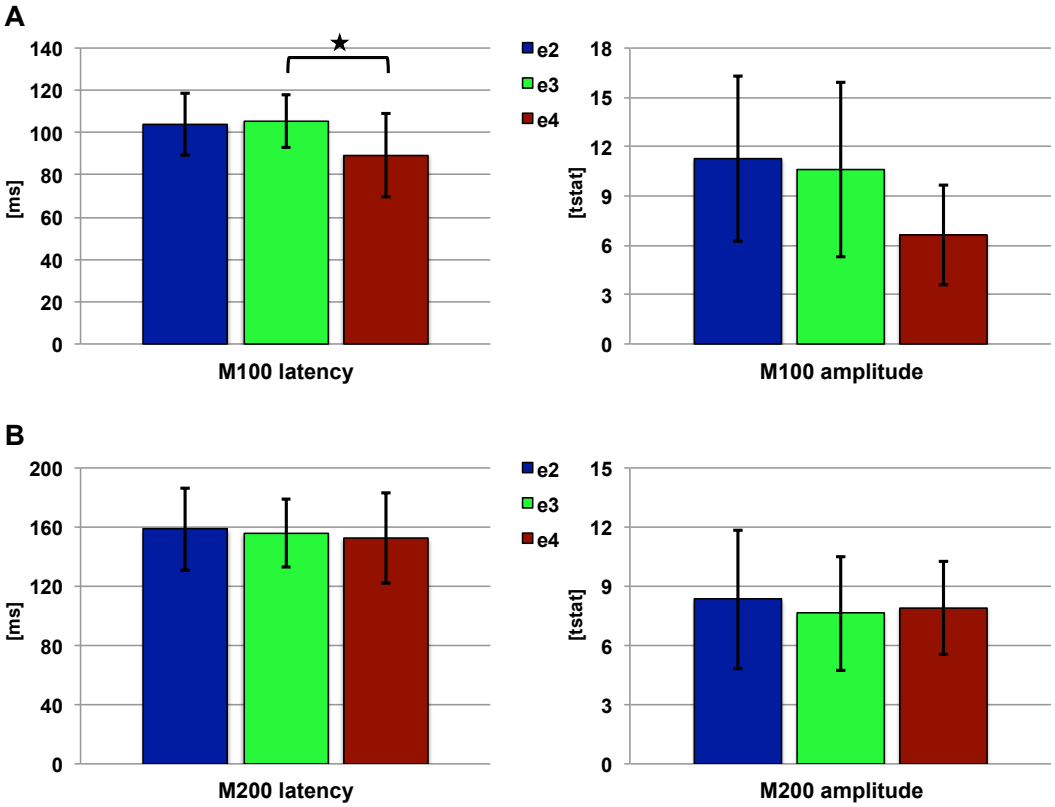

Figure 4.4: *APOE genotype effects on ERFs.* (**A**) There was a significant *APOE* genotype effect on M100 latency with reduced latency in *APOE* ϵ4 carriers compared with ϵ3 homozygotes (p=0.046). For M100 amplitude, there was a trend to reduced amplitude in *APOE* ϵ4 carriers compared with ϵ2 carriers (p=0.096). (**B**) No *APOE* genotype effects on M200 amplitude or latency were found.

4.3.2 Task ICA

The task ICA showed significant *APOE* genotype differences between *APOE* $\epsilon 4$ carriers and $\epsilon 3$ homozygotes for the right MTL component (Fig. 4.5). The same component showed differences between *APOE* $\epsilon 2$ carriers and $\epsilon 3$ homozygotes and additional differences between those two groups were found for a right inferior parietal lobe component and the right optic radiation. Significant differences between *APOE* $\epsilon 2$ and $\epsilon 4$ carriers were observed for a right insula component and the right inferior parietal lobe.

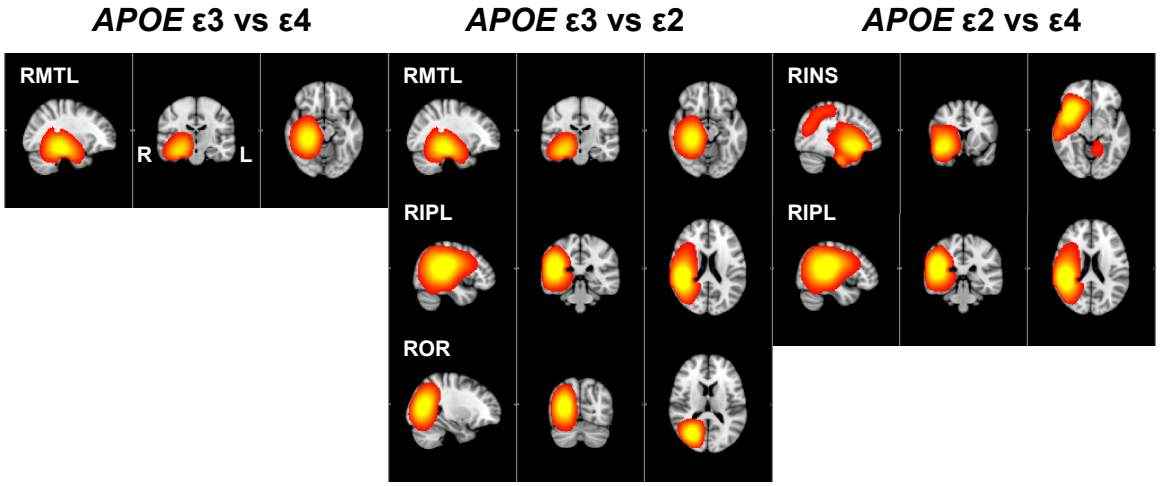

Figure 4.5: *APOE genotype effects on task ICA*. Differences between *APOE* $\epsilon 3$ homozygotes and the two other groups were found for the right MTL component while differences between $\epsilon 2$ carriers and the two other groups were observed for a right inferior parietal lobe (IPL) component. Additional differences between $\epsilon 2$ carriers and $\epsilon 3$ homozygotes were found for the right optic radiation (OR) and between $\epsilon 2$ and $\epsilon 4$ carriers for the right insula (INS).

4.4 Discussion

In the sensor-space analysis *APOE* ϵ4 carriers showed reduced M100 latency indicating that the ϵ4 allele is associated with faster sensory processing. The task ICA data showed several components that were differentially associated with the *APOE* genotype groups. In contrast to the analysis of resting-state functional connectivity, differences were also observed between *APOE* ϵ2 and ϵ4 carriers.

4.4.1 Sensor-space analysis

APOE genotype effects were found for the M100 peak of the ERF. *APOE* ϵ4 carriers showed significantly reduced M100 latency compared with ϵ3 homozygotes and a trend to reduced M100 amplitude compared with ϵ2 carriers. This early component of the ERF has been localised to the extrastriate cortex (Di Russo et al., 2002). Therefore, this indicates that the *APOE* ϵ4 allele is associated with faster sensory processing of the visual stimulus. This is slightly surprising because longer latencies of early ERP components have been shown in AD patients as well as older *APOE* ϵ4 carriers (Pollock et al., 1989; Espeseth et al., 2009). Additionally, studies using neuropsychological test batteries to assess *APOE* genotype effects on cognition have shown slower performance in *APOE* ϵ4 carriers (O'Hara et al., 2008).

However, age differences between participants in these studies and the one presented here might play an important role. Data from our lab and others suggests that the *APOE* ϵ4 allele could have opposite effects depending on the age of subjects studied. The *APOE* ϵ4 allele has been associated with advantages in infant brain development and better cognitive performance in children and young adults (Wright et al., 2003; Alexander et al., 2007b), including attentional mechanisms (Rusted et al., 2013). This led to the suggestion that the *APOE* ϵ4 allele

shows antagonistic pleiotropy with the idea that the allele is beneficial during early life and shows negative effects on survival in old age (Tuminello and Han, 2011). In an fMRI study it was shown that *APOE* genotype effects on BOLD activity during a memory task were dependent on age: while young *APOE* ϵ4 carriers showed increased BOLD signal compared with *APOE* ϵ3 homozygotes older subjects showed the opposite effect (Filippini et al., 2011). Therefore, the increased processing speed in middle-aged *APOE* ϵ4 carriers shown in this study here could be associated with antagonistic pleiotropy and might indicate advantageous effects of the *APOE* ϵ4 allele with regards to sensory processing in this age group.

In the study presented here there also was a trend to reduced M100 amplitude in *APOE* ϵ4 compared with ϵ2 carriers. A similar effect has been reported in an EEG study of slightly older subjects (mean age 63 years) than in this study where *APOE* ϵ4 carriers showed significantly reduced N100 amplitude compared with ϵ3 homozygotes (Espeseth et al., 2009). A decreased amplitude of ERP components is presumably caused by either reduced neuronal activity per se and/ or reduced synchronicity of neuronal firing (Shinba, 1999). It is possible that reduced amplitude in *APOE* ϵ4 carriers is an early sign of previously reported age-related decline in P100 amplitude (Lorenzo-López et al., 2002). In older adults this effect might become more pronounced and thus reach statistical significance.

Interestingly, the patterns of *APOE* genotypre related M100 amplitude and latency are more reflective of AD risk than functional connectivity differences presented in the previous chapter. In particular, this is true for amplitude differences with a trend to differences between *APOE* ϵ2 and ϵ4 carriers and ϵ3 homozygotes inbetween these two groups. This might indicate that although the functional architecture of the brain is similar for *APOE* ϵ2 and ϵ4 carriers there are some differences with regards to task-related neuronal activity that could have an impact on AD risk.

4.4.2 Task ICA

The task-based ICA showed significant differences for target trials in several components. *APOE* ϵ3 homozygotes showed differences compared with the two other genotype groups in the right MTL. Activity in the hippocampus has consistently been shown during the oddball task and hippocampal activity is thought to contribute to the P300 ERP component (Brázdil et al., 2005). The ICA here confirms involvement of MTL structures in the oddball task and adds to many other studies showing *APOE* genotype related differences in hippocampal activity for different types of memory and attention tasks (Trachtenberg et al., 2010; Trachtenberg et al., 2011). There are no differences between *APOE* ϵ2 and ϵ4 carriers with regards to involvement of the MTL component. Using fMRI, similar activations of the hippocampus for these two *APOE* genotype groups and differences compared with ϵ3 homozygotes have been shown before (Trachtenberg et al., 2011).

APOE ϵ2 carriers showed differences in involvement of a right inferior parietal lobe component compared with the two other genotype groups. Again, this is a region where activity during oddball tasks has often been found and is thought to contribute to the P300 (Bledowski et al., 2004). The same is true for the other component around the right insula that showed significant differences between *APOE* ϵ2 and ϵ4 carriers. Differences between *APOE* ϵ2 carriers and ϵ3 homozygotes were also found for a component around the optic radiation. This suggests differences between those two genotype groups during very early sensory processing that would occur even earlier than the ERP P100 component. This could be visible in a sensor-space analysis but data presented in this study were not clean enough to investigate these very early ERP components.

The results of the ICA indicate that most of the components that show *APOE* genotype related differences are those suggested as generators of the P300 even

though no clear M300 component could be identified in the sensor-space analysis. This implies *APOE* genotype related differences in attention and the way in which target stimuli are detected. This result is backed up by a fMRI study that reported *APOE* genotype effects on attention (Trachtenberg et al., 2011). The most interesting finding with regards to *APOE* genotype related differences was the component around the insula showing differences between *APOE* ϵ2 and ϵ4 carriers. Based on the results of other studies and the previous chapter of this thesis, one would expect very similar neuronal activity for those two genotype groups. Differences between those groups affecting the insula might indicate that these two genotype groups respond differently to salient stimuli in the environment and/ or show differences in task coordination (Eckert et al., 2009). Interestingly, the right insula also plays a role in task-related switching between DMN activity at rest and ECN activity during task (Sridharan et al., 2008). This might imply that AD risk is associated with alterations in the ability to respond to task demands.

4.4.3 Limitations

The study presented in this chapter can only be considered preliminary. One of the most important limitations is the small number of subjects, in particular of *APOE* ϵ2 carriers. In addition, it was surprising to see that no clear M300 component was discernible in the sensor-space analysis . It is certainly possible that this is due to some problems with the study design. A clear M300 component was seen in the pilot study for this investigation but the subjects included in the pilot study were mostly researchers who had taken part in many experiments before. Particularly in MEG research, it has a big impact on data quality if subjects are used to minimising eye blinks and body movements. The subjects included in the main study were recruited from the normal population and very inexperienced with regards to the experimental setup. Therefore, data were not as clean and it would have been

useful to record at least twice as much data for this particular task. Additionally, it would have been very interesting to conduct an analysis of task data in source space to determine with high temporal resolution which brain areas show activity differences between the *APOE* genotype groups. Unfortunately, these methods are still in development and not ready to be applied to this dataset yet.

4.5 Conclusion

In summary, this study showed that *APOE* genotype affects early sensory processing with faster processing in *APOE* ϵ4 carriers, which might be one example of antagonistic pleiotropy. Additionally, an ICA analysis of task-related brain networks indicated that *APOE* genotype affects cognitive processes associated with attention. In contrast to the analysis of the functional architecture of the brain, task data revealed some differences between those with the highest and lowest AD risk. *APOE* genotype might particularly affect reactivity to stimuli, which only becomes apparent using the excellent temporal resolution of MEG data.

Chapter 5

APOE genotype and brain structure

The focus of this thesis so far has been on brain function. However, neurodegeneration in AD leads to many structural changes in the brain. Some of these changes have also been reported in healthy *APOE* ϵ4 carriers. In this chapter I will investigate whether functional changes reported in the previous chapters were accompanied by structural changes in the same group of subjects as a replication of results that have been published previously by our group and others.

5.1 Introduction

The pathological hallmarks of AD are accompanied by progressive brain atrophy. Many MRI studies have investigated this progression of tissue loss from mild to later AD stages and found a close correlation between brain atrophy and cognitive deficits (Frisoni et al., 2010). At early AD stages, brain atrophy is seen in the entorhinal cortex, hippocampus and PCC together with memory and orientation deficits. At later stages, atrophy can also be found in the temporal, parietal and frontal cortices underlying loss of language and visuospatial skills and behavioural impairments. Therefore, it is not surprising that a decline in structural measures such as volumes of the whole brain, entorhinal cortex, hippocampus and temporal

lobe correlate closely with decline in cognitive functions in AD patients (Frisoni et al., 2010). Although volume loss is a useful marker of brain atrophy, it is possible to measure in more detail how GM (i.e. neuronal cell bodies, dendrites, and glial cells) and connections between neurons (i.e. myelinated axons in WM) are affected by AD. Methods to investigate GM structure include measures of cortical thickness and voxel-based morphometry (VBM) to compute GM density. WM density can also be measured using VBM but WM integrity is mostly studied using DTI-derived measures such as FA and MD, which are sensitive to disease-related WM changes and provide more detailed information than volumetric measurements (Johansen-Berg and Behrens, 2009).

5.1.1 AD and grey matter

Methods for comparing GM density and cortical thickness across the whole-brain are very accurate in discriminating between AD patients and healthy controls (Cuingnet et al., 2011). The most consistently reported GM differences between AD patients and healthy controls are thinning and reduced GM density of the MTL cortex and limbic structures (Dickerson et al., 2009; Schroeter et al., 2009). In longitudinal studies, AD participants show greater decline in GM density than healthy controls especially in the left MTL but also the frontal and parietal lobe (Risacher et al., 2010). Similar results have been reported for a longitudinal study of cortical thinning where AD patients show accelerated thinning compared with healthy controls (Li et al., 2012). However, the patterns of cortical thinning were similar for AD patients and healthy controls affecting particularly the parahippocampal cortex and middle/inferior temporal gyrus (Li et al., 2012).

5.1.2 *APOE* and grey matter

In studies of AD patients, there are many effects of *APOE* genotype. *APOE* ϵ4 carriers have smaller baseline hippocampal volumes and higher rates of hippocampal volume loss as well as entorhinal cortex thinning (Schuff et al., 2009; Risacher et al., 2010). Other findings include reduced GM density of MTL structures and the orbitofrontal cortex in *APOE* ϵ4 carriers (Filippini et al., 2009b).

Reports of *APOE* genotype effects on GM structure in healthy adults are inconsistent. There are some longitudinal studies that report increased hippocampal atrophy not only in AD patients carrying the *APOE* ϵ4 carriers but also in older healthy controls and decreased atrophy rates in *APOE* ϵ2 carriers (Chiang et al., 2010; Risacher et al., 2010). Healthy older *APOE* ϵ2 carriers also show greater cortical thickness in the superior temporal cortex compared with ϵ4 carriers and ϵ3 homozygotes (Fan et al., 2010). However, *APOE* genotype effects are not restricted to adults because a MRI study in children and adolescents reported that entorhinal cortex thickness depends on *APOE* genotype with a pattern that reflects AD risk, i.e. *APOE* ϵ2 carriers $>$ ϵ3 homozygotes $>$ ϵ4 carriers (Shaw et al., 2007). In a recent study of GM structure in neonates, *APOE* ϵ4 carriers showed reduced GM volume in temporal regions including the hippocampus but increased volume in superior parietal areas (Knickmeyer et al., 2013). Some cross-sectional studies in adults comparing *APOE* ϵ4 carriers and non-carriers report decreased hippocampal, frontotemporal and MTL GM volume in healthy ϵ4 carriers (Cherbuin et al., 2007; Wishart et al., 2006) while others do not (Cherbuin et al., 2008; Fan et al., 2010).

One reason for the inconsistency between studies could be that *APOE* genotype effects vary with age, which has been shown in studies of brain function (Filippini et al., 2011) and might explain the lack of effects on GM structure especially in several studies including younger subjects (Cherbuin et al., 2007; Filippini et al., 2009a). Other reasons might be the inclusion of *APOE* ϵ2 carriers as non-

carriers, who show similar brain function to $\epsilon 4$ carriers (Trachtenberg et al., 2011; Trachtenberg et al., 2012), and the number of homozygous $\epsilon 4$ carriers who could be driving effects on brain structure (Lemaître et al., 2005).

5.1.3 AD and white matter

AD pathology not only involves GM atrophy but also changes in WM structure. Studies of post-mortem tissue have shown demyelination in AD (Roher et al., 2002) that is greater in frontoparietal than in occipital areas (Sjöbeck et al., 2005). Compared with the number of studies investigating GM changes associated with AD, studies of WM changes have not been carried out in a lot of patients. Therefore, meta-analyses are quite useful to summarise the effects of AD on WM. Volumetric studies have shown that WM volume is decreased in AD patients, particularly in the parahippocampal gyrus, temporal lobe and posterior corpus callosum (Li et al., 2012). This pattern is not surprising given that these are also the regions of greatest GM atrophy, and neuronal loss should be associated with loss of axons and thus WM atrophy. When investigating WM structure in more detail using DTI, effects on WM integrity in AD are more widespread than GM changes. Decreased FA and increased MD have been found in many WM tracts especially in the frontal and temporal lobe but also the the posterior cingulum and corpus callosum (Sexton et al., 2011). That these WM changes are of relevance when it comes to cognitive function in AD has been shown in a study that found correlations of WM integrity with disease severity (Duan et al., 2006).

5.1.4 *APOE* and white matter

There are only a few studies investigating *APOE* genotype effects on WM structure. This is surprising considering the function of apoE in the brain discussed in the

first chapter. For example, its role in cholesterol transport for remyelination and neuroregeneration suggests a direct link between WM structure and apoE.

Although there is one study that did not find *APOE* genotype effects on WM structure (Bendlin et al., 2010), the majority of studies report reduced WM integrity in *APOE* ϵ4 carriers. Those effects have been reported in brain tracts such as parahippocampal WM (Nierenberg et al., 2005; Honea et al., 2009) and the corpus callosum (Persson et al., 2006; Smith et al., 2008). We showed widespread *APOE* genotype related changes in a whole-brain analysis of FA and MD even in a group of healthy young adults aged 18-35 years (Heise et al., 2011). We compared *APOE* ϵ4 carriers with people homozygous for the ϵ3 allele and found reduced WM integrity in both younger and older healthy *APOE* ϵ4 carriers (Heise et al., 2011). A recent study extended these findings and included *APOE* ϵ2 carriers (Westlye et al., 2012). Similar to reports on brain function (Trachtenberg et al., 2011), there were no WM differences between *APOE* ϵ2 and ϵ4 carriers but both showed reduced WM integrity compared with ϵ3 homozygotes (Westlye et al., 2012). In a cross-sectional graph theoretical analysis of DTI data in healthy adults, Brown et al. (2011) showed accelerated age-related decline of interconnectivity particularly in the precuneus, lateral parietal cortex and orbitofrontal cortex in *APOE* ϵ4 carriers compared with ϵ3 homozygotes.

5.1.5 Aims

The aims of the study presented in this chapter were twofold: 1) to investigate possible *APOE* genotype effects on GM structure that could influence the results of the functional studies presented in the previous chapters and 2) to replicate and extend our findings of *APOE* genotype effects on WM structure with the inclusion of *APOE* ϵ2 carriers.

5.2 Methods

5.2.1 Participants

The participants were the same as those described in the chapter 3 but one participant (female *APOE* $\epsilon 3$ homozygote) had to be excluded because no DTI data was available.

5.2.2 Analysis protocols

For data analysis FSL tools v5.0 were used.

Structural MRI

Total brain volume and GM, WM and CSF volumes were calculated using FAST. Individual hippocampi were identified and their volumes calculated using FMRIBs Integrated Registration and Segmentation Tool (FIRST), an automatic subcortical segmentation program (Patenaude et al., 2011). To ensure that hippocampal voxels only contained GM, voxels that contained WM or CSF as identified by FAST were excluded from the analyses. A VBM approach was used to study GM morphology. Whole-brain analysis was carried out with FSL-VBM (Douaud et al., 2007), using default settings. Brain extraction and tissue-type segmentation were performed, and resulting GM partial volume images were aligned to standard space using FLIRT and then FNIRT. The resulting images were averaged, modulated, and smoothed with an isotropic Gaussian kernel of 3mm ($\sim$7 mm FWHM) to create a study-specific template. Finally, voxelwise GLM was applied using nonparametric testing with 5000 permutations and $p < 0.05$ correcting for multiple comparisons across space using TFCE. Age was used as a covariate of no interest in the VBM analyses.

Diffusion tensor imaging

Raw images were pre-processed using Eddy Current correction in order to correct for distortions due to the applied gradient directions. FA and MD maps were generated using DTIFit, part of FMRIBs Diffusion Toolbox, that fits a diffusion tensor model at each voxel (Behrens et al., 2003).

The FA output images were used as input for tract-based spatial statistics (TBSS), a voxelwise approach for analysis of FA data (Smith et al., 2006). All subjects' FA data were aligned into a common space using FNIRT. 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 were then projected onto this skeleton and the resulting data fed into voxelwise GLM cross-subject statistics. A voxel by voxel nonparametric test with 5000 permutations was used to assess group-related differences. All results are shown at $p < 0.05$ corrected for multiple comparisons across space using TFCE. In addition to FA data, MD, DA and DR were also compared using TBSS in an analogous fashion. Age was added to the TBSS analyses as covariate of no interest.

5.2.3 Statistics

For non-image-based statistical analyses SPSS software version 19 (SPSS Inc., Chicago, USA) was used. One-way ANOVAs were used to compare brain volumes and an alpha of $p < 0.05$ was considered significant.

5.3 Results

5.3.1 Structural MRI

No differences between the three *APOE* genotype groups were found for GM, WM, CSF and hippocampal volumes (Table 5.1). No differences in GM density were observed using a whole-brain VBM approach.

Table 5.1: General brain morphology of the three *APOE* genotype groups. Values denote mean (±Standard Deviation) or number of subjects, *Values are expressed as percentage of whole-brain volume; p-values refer to one-way ANOVAs.

	ϵ**2/**ϵ**3**	ϵ**3/**ϵ**3**	ϵ**3/**ϵ**4**	**P**
n	12	14	13	-
Grey matter*	39.63 (1.44)	39.76 (1.60)	39.25 (1.51)	0.67
White matter*	38.30 (1.46)	38.31 (0.86)	38.37 (1.33)	0.99
CSF*	22.07 (1.53)	21.93 (1.36)	22.38 (2.44)	0.81
Left Hippocampus*	0.29 (0.02)	0.28 (0.03)	0.29 (0.02)	0.67
Right Hippocampus*	0.29 (0.02)	0.28 (0.03)	0.29 (0.02)	0.65

5.3.2 Diffusion tensor imaging

There were significant differences in FA between the three *APOE* genotype groups with decreased FA in *APOE* ϵ2 and ϵ4 carriers compared with ϵ3 homozygotes (Fig. 5.1). No differences between *APOE* ϵ2 and ϵ4 carriers were observed. WM tracts that showed significantly decreased FA in *APOE* ϵ2 and ϵ4 carriers comprised the left forceps minor, inferior fronto-occipital fasciculus, uncinate fasciculus, anterior and superior corona radiata, anterior thalamic radiation and the genu and body of the corpus callosum. Additionally, *APOE* ϵ2 carriers had decreased FA in the left external capsule and anterior limb of the internal capsule.

APOE ϵ2 carriers also showed a decreased DA compared with ϵ3 homozygotes

but this effect was only at trend level ($p < 0.1$) and did not reach statistical significance. There were no significant *APOE* genotype effects on MD and DR.

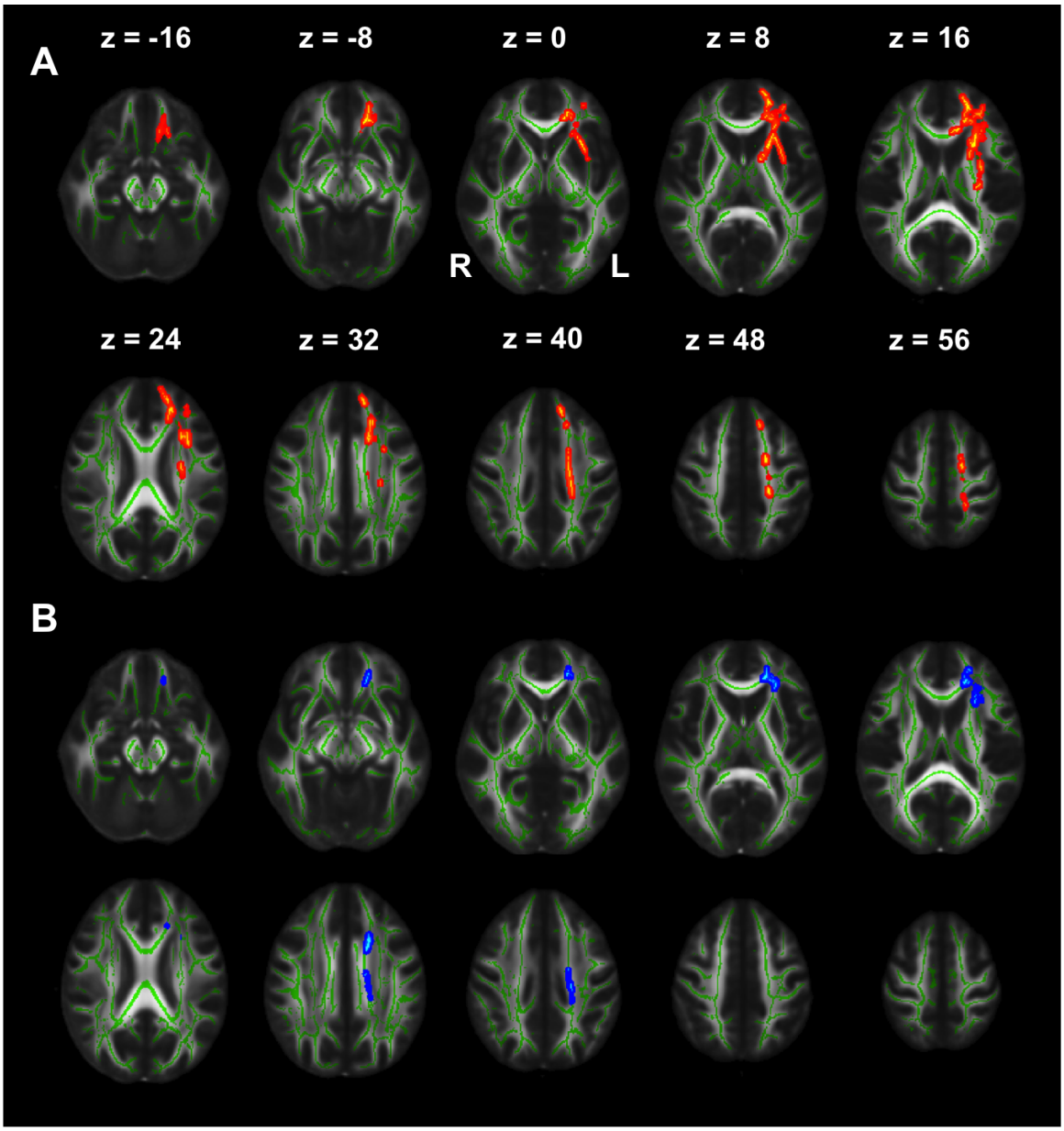

Figure 5.1: *WM structure*. Mean FA image with the TBSS skeleton shown in green. (**A**) Regions that showed significantly decreased FA in *APOE* $\epsilon 2$ carriers compared with $\epsilon 3$ homozygotes are shown in red and (**B**) significantly decreased FA in *APOE* $\epsilon 4$ carriers compared with $\epsilon 3$ homozygotes in blue.

5.4 Discussion

For this study the effect of *APOE* genotype on GM and WM structure in healthy individuals was investigated using structural MRI and DTI. There was no *APOE* genotype effect on GM structure but *APOE* ϵ4 carriers and ϵ2 carriers showed significantly reduced WM integrity compared with ϵ3 homozygotes.

5.4.1 Grey matter

There were no *APOE* genotype effects on GM structure in this study. Although some other studies have reported *APOE* genotype effects on GM volume or cortical thickness, these studies were conducted in older adults where decreased GM volume might be related to faster age-related decline in *APOE* ϵ4 carriers (Espeseth et al., 2008; Donix et al., 2010). On the other hand, several studies of middle-aged or younger participants have not found *APOE* genotype related alterations in GM structure (Cherbuin et al., 2007; Filippini et al., 2009a).

5.4.2 White matter

Compared with *APOE* ϵ3 homozygotes, ϵ2 and ϵ4 carriers showed significantly reduced WM integrity with reduced FA. This effect was most pronounced in frontal WM tracts of the left hemisphere and was found in commissural fibres (corpus callosum, forceps minor) as well as association fibres (externl capsule, inferior fronto-occipital fasciculus, uncinate fasciculus) and thalamocortical projection fibres (anterior thalamic radiation, corona radiata, internal capsule) (Wakana et al., 2004; Orrison, 2008). These widespread *APOE* genotype effects on integrity of different types of WM tracts have been shown before although these studies found significant effects across the whole brain and not only in left frontal regions (Heise et al., 2011; Westlye et al., 2012). Compared with these studies the numbers

of participants presented here were quite low, which leads to decreased statistical power. At a trend level statistical threshold of $p < 0.1$, differences between *APOE* genotype groups were widespread in both hemispheres including posterior regions.

5.4.3 White matter and AD risk

These findings are quite difficult to interpret with regards to AD risk. It is tempting to speculate that decreased WM integrity in *APOE* ϵ4 carriers could underlie greater AD risk. WM alterations are at the centre of a hypothesis put forward by Bartzokis. He suggests that AD and other disorders are primarily caused by myelin breakdown and deficient myelin repair mechanisms (Bartzokis, 2011). WM integrity in general decreases from age 40 (Westlye et al., 2010) and, interestingly, the trajectories for WM changes with age do not seem to differ between *APOE* ϵ4 carriers and ϵ3 homozygotes (Westlye et al., 2012). However, since WM integrity is consistently lower in *APOE* ϵ4 carriers this might make them more susceptible to environmental insults and thereby accelerate AD development.

Unfortunately, no longitudinal or cross-sectional data is available for *APOE* ϵ2 carriers to determine whether the trajectory of age-related WM change is the same as in the other two genotype groups. Although *APOE* ϵ2 carriers show decreased WM integrity at age 50, they might be protected from ageing effects and show slower age-related decline in WM integrity, which might be one mechanism to protect them from AD.

5.4.4 Biological mechanisms

Similarities between *APOE* ϵ2 and ϵ4 carriers in WM integrity do not necessarily imply that the same biological mechanisms are underlying those differences. The

degree of myelination certainly plays a role but measures like axon integrity, density and diameter, and density of oligodendrocytes also influence directionality of water diffusion in WM (Beaulieu, 2002; Alexander et al., 2007a). It is, therefore, certainly possible that different mechanisms are driving the FA alterations in *APOE* ϵ2 and ϵ4 carriers. One indication that this might be the case is the trend to a decreased DA in *APOE* ϵ2 carriers, which was not seen in ϵ4 carriers. One might speculate that reduced myelination in *APOE* ϵ4 carriers could underlie their increased AD risk while reduced DA in *APOE* ϵ2 carriers could indicate differences in axon morphology rather than alterations in myelination (Alexander et al., 2007a).

However, a recent GWAS analysis has shown that in particular genes involved in biological pathways such as neuronal transmission and cell adhesion are related to WM integrity (Lopez et al., 2012). As discussed in the first chapter, there are many links between the different apoE isoforms and processes related to neuronal transmission. Therefore, *APOE* genotype effects on WM integrity do not necessarily have to reflect direct influences on myelination but could have an impact on WM via differences in neuronal transmission.

This is an important point when discussing the relationship between WM structure and brain function. The *APOE* genotype effects on WM integrity seen here are very similar to those reported for resting-state and task-based fMRI data (Trachtenberg et al., 2011; Trachtenberg et al., 2012). The reciprocal relationship between structure and function, i.e. Hebb's rule of "fire together wire together" (Hebb, 1949), makes it impossible to determine whether functional or structural differences are driving the *APOE* genotype effects but it is reassuring that different modalities show a similar tendency, i.e. brain structure and function distinguish *APOE* ϵ3 homozygotes from the two other genotype groups.

5.5 Conclusion

This study demonstrates that individuals carrying the *APOE* ϵ2 and ϵ4 allele have reduced WM integrity compared with ϵ3 homozygotes but show no differences in GM structure. These findings are in line with previous findings for both WM integrity and brain function but difficult to interpret with respect to AD risk. Nevertheless, low FA levels in those with the lowest and highest AD risk do not necessarily have to reflect similar biological mechanisms because there are many measures and pathways that influence FA. It does not rule out that decreased myelination might be one reason for increased AD risk in *APOE* ϵ4 carriers but more studies are required to determine mechanisms that protect from AD in *APOE* ϵ2 carriers. This study emphasises the importance of including *APOE* ϵ2 carriers in analyses of *APOE* effects on brain function and structure because otherwise neuroimaging results might be mistakenly interpreted with regards to their value as predictors of AD development.

Chapter 6

APOE genotype, gender and age modulate connectivity of the hippocampus in healthy adults

The study presented in this chapter has been submitted as a paper.

6.1 Introduction

The *APOE* ϵ4 allele is the best established genetic risk factor for developing sporadic Alzheimer's disease (AD) (Corder et al., 1993). Interestingly, there is a gender effect, with female ϵ4 carriers having the greatest risk compared with the most common ϵ3 allele (Payami et al., 1996; Farrer et al., 1997; Bretsky et al., 1999). In addition, female ϵ4 carriers are at higher risk for AD related pathology, amyloid plaques and neurofibrillary tangles (Corder et al., 2004). Understanding how the AD risk factors *APOE* ϵ4 allele, female gender and age interact is important because a fuller understanding of the mechanisms that lead to increased AD risk is crucial for the development of interventions and to determine who would benefit most from them.

Moreover, neuroimaging biomarkers could be of clinical use to assess efficacy of interventions and to identify individuals at preclinical AD stages. Neuroimaging studies have shown that the *APOE* ϵ4 allele is associated with modification of brain function, grey matter (GM) and white matter (WM) structure, both in AD patients (Cosentino et al., 2008; Filippini et al., 2009b) and in healthy subjects (Bookheimer et al., 2000; Small et al., 2000; Reiman et al., 2004; Persson et al., 2006; Filippini et al., 2011; Trachtenberg et al., 2011; Trachtenberg et al., 2012). These differences are already evident in young carriers of the ϵ4 allele aged 20 to 35 who are not in prodromal stages of AD (Filippini et al., 2009a; Heise et al., 2011). However, few neuroimaging studies have investigated an interaction between gender and *APOE* genotype. In AD patients, female ϵ4 carriers show decreased volumes of the entorhinal cortex compared with males (Juottonen et al., 1998), and a recent study in healthy older adults found decreased functional connectivity in the default-mode network (DMN) of female ϵ4 carriers (Damoiseaux et al., 2012). The DMN is a resting-state network, i.e. a network of functionally connected brain areas that are active when people are awake but not engaged in a specific task. This network is of particular interest because alterations in functional connectivity have been shown both in AD patients (Greicius et al., 2004) and healthy *APOE* ϵ4 carriers (Sheline et al., 2010). The DMN is functionally connected with the hippocampus, which is one of the first regions to show AD related pathology (Braak and Braak, 1991). Altered functional connectivity between the hippocampus and the DMN has been reported in healthy younger and older *APOE* ϵ4 carriers (Filippini et al., 2009a; Westlye et al., 2011). However, it is unclear whether *APOE* genotype only affects connectivity of the hippocampus and DMN or whether *APOE* genotype has a more general effect on functional connectivity of the hippocampus with the whole brain. Additionally, it is unknown whether gender modulates hippocampal connectivity in a pattern that is similar to AD risk, i.e. female *APOE* ϵ4 carriers show greatest

changes in hippocampal connectivity. Diffusion tensor imaging (DTI) studies have shown that *APOE* genotype is also associated with changes in WM structure (Heise et al., 2011; Westlye et al., 2012). However, there are no published studies investigating a possible interaction of *APOE* genotype and gender on WM integrity and the relationship between functional and structural connectivity. Therefore, the aims of this study were threefold: 1) to determine the effect of *APOE* genotype on functional and structural connectivity of the hippocampus 2) to study the relationship between functional and structural connectivity and 3) to investigate differential effects of gender and age on hippocampal connectivity.

6.2 Materials and Methods

6.2.1 Participants

Imaging data for 86 right-handed subjects (37 males) aged 30 to 78 years were acquired. Data for these participants has been published previously in Filippini et al. (2011) (older group) and Trachtenberg et al. (2012). *A priori* exclusion criteria were: current or past history of neurological or psychiatric disorders, memory complaints, head injury, substance abuse (including alcohol), corticosteroid therapy, and diabetes. In order to exclude possible confounds due to cognitive complaints subjects aged over 55 years underwent a pre-screening cognitive test [Addenbrookes Cognitive Examination-revised version (ACE-R) [(Mioshi et al., 2006)]. Subjects with an ACE-R score < 95 were excluded. Younger subjects were recruited from the Oxford Biobank, an age-stratified random sample of healthy 30 to 55 year old men and women from Oxfordshire with a current pool of 5000 participants. Subjects above the age of 55 years were recruited by advertisement from the general public. All subjects performed a memory task, described in Trachtenberg et al. (2011) for the younger subjects and in Filippini at al. (2011) for the older participants. All subjects were pre-screened for *APOE* genotype (using a blood or cheek swab sample) and selected for the study on the basis of either having an *APOE* ϵ4 allele (*APOE* ϵ4 carrier) or being an ϵ3 homozygote (non-carrier). Carriers of the ϵ2 allele were not included in this study. Eight *APOE* ϵ4 carriers (5 female) were homozygous for the ϵ4 allele. Genotyping was conducted either at the Oxford Biobank or at the Wellcome Trust Centre for Human Genetics in Oxford. DNA was extracted from blood or cheek swab samples of subjects according to standard procedures to allow PCR for the characterization of *APOE* genotype. The study was approved by the local Ethics Committee, and written informed consent was obtained from all participants.

6.2.2 Image Acquisition

Scans were performed at the University of Oxford Centre for Clinical Magnetic Resonance Research (OCMR) using a 3-T Siemens Trio scanner (Siemens AG, Erlangen, Germany) with a 12-channel head coil. The neuroimaging protocol included a 3D T1-weighted structural scan (repetition time (TR) = 2040 ms, echo time (TE) = 4.7 ms, flip angle = 8°, field of view = 192 mm, voxel dimension = 1 mm isotropic, scan time = 12 min 02 sec), whole-brain resting functional imaging using a gradient echo EPI sequence (TR = 2000 ms, TE = 28 ms, flip angle = 89°, field of view = 224 mm, voxel dimension = 3 x 3 x 3.5 mm, volumes = 180, scan time = 6 min 04 sec) and diffusion weighted imaging (echo planar imaging, TR = 9300 ms, TE = 94 ms, field of view = 192 mm, voxel dimension = 2 mm isotropic, B-value = 1000, gradients applied = 60 isotropically distributed, scan time = 10 min 31 sec).

6.2.3 Image Analysis

For data analysis we used FMRIB Software Library (FSL) tools v5.0 (`http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/`).

Structural MRI

Total brain volume, GM, WM and cerebrospinal fluid (CSF) measurements were calculated using FMRIBs Automated Segmentation Tool (FAST) (Zhang et al., 2001). Individual hippocampi were identified using FMRIBS Integrated Registration and Segmentation Tool (FIRST), an automatic subcortical segmentation program (Patenaude et al., 2011). To ensure that hippocampal voxels only contained GM, voxels that contained WM or CSF as identified by FAST were excluded from the analyses. Functional and structural connectivity changes might be downstream events of

altered GM structure. To rule this out we used a voxel-based morphometry (VBM) approach to study GM morphology. Whole-brain analysis was carried out with FSL-VBM (Douaud et al., 2007) using default settings. Brain extraction and tissue-type segmentation were performed, and resulting GM partial volume images were aligned to standard space using FMRIB's Linear (FLIRT) and then Nonlinear Image Registration Tools (FNIRT). The resulting images were averaged, modulated, and smoothed with an isotropic Gaussian kernel of 3mm (7 mm full-width at half- maximum - FWHM) to create a study-specific template. Finally, voxelwise general linear modelling (GLM) was applied using permutation nonparametric testing (5000 permutations) and $p < 0.05$ correcting for multiple comparisons across space using threshold-free cluster enhancement (TFCE) (Smith and Nichols, 2009). The GLM included the four groups (male and female *APOE* ϵ4 carriers and non-carriers). We tested for genotype and gender effects as well as an interaction between those factors. Post-hoc tests were performed to compare means between the four groups. Age was used as a covariate of no interest in the VBM analysis.

Resting-state functional Magnetic Resonance Imaging (rs fMRI)

For the resting-state scan, subjects were instructed to lie in dimmed light with their eyes open, to think of nothing in particular, and not to fall asleep. Pre-processing of imaging data consisted of motion correction, brain extraction, spatial smoothing using a Gaussian kernel of 6mm FWHM, and high-pass temporal filtering equivalent to 150s (0.007 Hz). Functional data were aligned to the anatomical image initially using FMRIB's Linear Image Registration Tool, (FLIRT) (Jenkinson and Smith, 2001; Jenkinson et al., 2002), then optimized using the Boundary-Based Registration (BBR) approach (Greve and Fischl, 2009). Registration from anatomical to the Montreal Neurological Institute (MNI) standard brain was carried out using FNIRT. To determine functional connectivity of the left and right hippocampus, a seed-based

correlation approach was carried out using FMRI Expert Analysis Tool (FEAT) Version 6.00. Correlation maps were computed separately for the left and right hippocampus using WM and CSF signal as covariates of no interest. Individual hippocampi were used as seeds for every subject to account for differences in hippocampal shapes and sizes and ensure that only voxels within the individuals hippocampus were selected for analysis. Time-series statistical analysis was carried out using FMRIBs Improved Linear Model (FILM) with local autocorrelation correction (Woolrich et al., 2001). Voxel-wise group analysis was carried out in MNI standard space using FMRIBs Local Analysis of Mixed Effects (FLAME) stage 1 with automatic outlier detection (Beckmann et al., 2003; Woolrich et al., 2004; Woolrich, 2008). The z statistic images were thresholded using $z > 2.3$ and a family wise error corrected cluster significance threshold of $p < 0.05$. Age and hippocampal volumes were included as covariates of no interest. To exclude that connectivity differences between the four groups were influenced by structural differences, structural images were used as covariates of no interest. GM images of each subject were extracted using FAST, registered in standard space, smoothed to match the resting-state fMRI data, demeaned within each group and added to the GLM as covariates. In addition, head movement was taken into account but there were no group differences in subject motion. To extract the contrast of parameter estimate (COPE) value for each participant, significant clusters were used as binary masks. Mean COPE values were calculated for the four groups male and female *APOE* ϵ4 carriers and non-carriers.

Diffusion Tensor Imaging

Raw images were pre-processed using Eddy Current correction in order to correct for distortions due to the applied gradient directions. Fractional anisotropy (FA) and mean diffusivity (MD) maps were generated using DTIFit, part of FMRIBs

Diffusion Toolbox, that fits a diffusion tensor model at each voxel (Behrens et al., 2003). The magnitude and direction of tissue water mobility is measured in the three dimensions (Beaulieu, 2002) and the diffusion tensor is modelled as an ellipsoid with symmetry across any one of its three axes. The largest axis or eigenvalue is denoted $\lambda 1$ or axial diffusivity (DA). The two minor axes ($\lambda 2$, $\lambda 3$) are usually averaged to compute radial diffusivity (DR). The average of all three eigenvalues is called mean diffusivity (MD). Fractional anisotropy (FA) is a ratio of axial to radial diffusivity, and thus provides a measure of the directionality of diffusion. All subjects' FA data were aligned to the FMRIB58_FA standard space image using FNIRT to compare FA, MD, DA and DR values across subjects. Following up on the results of the resting-state analysis we focused on the white matter tract connecting the hippocampus and PCC. A mask of this tract labelled cingulum of the hippocampal area was derived from the Johns Hopkins University ICBM-DTI-81 white-matter labels atlas used in FSLView (Hua et al., 2008). To compare only the voxels of this tract that are common to all subjects, the FA output images were used as input for tract-based spatial statistics (TBSS), a voxelwise approach for analysis of FA data (Smith et al., 2006). All subjects' FA data were aligned into a common space using the nonlinear registration tool FNIRT. The mean FA image was generated and thinned to create a mean FA skeleton, which represents the centers of all tracts common to the group. The cingulum part of the skeleton was used for a voxel by voxel permutation nonparametric test (5000 permutations) to assess group-related differences in FA, MD, DR and DA of this tract correcting for multiple comparisons across space with TFCE (Smith and Nichols, 2009). An alpha of $p < 0.05$ was considered significant. Age was included as covariate of no interest. To investigate correlations between resting-state functional connectivity and WM integrity, extracted COPE values of the resting analysis were used as covariate of interest in this analysis.

6.2.4 Statistics

We used SPSS software version 19 (SPSS Inc., Chicago, USA) for non-image-based statistical analyses. One-way ANOVAs were used for continuous sociodemographic variables, cognitive performance and brain volumes. A Fisher-Freeman-Halton exact test was used for categorical variables (family history of dementia). Correlations between age, functional and structural connectivity were computed using two-tailed Pearson correlations. An alpha of $p < 0.05$ was considered significant.

6.3 Results

6.3.1 Demographics

Subject demographics are shown in Table 6.1. The four groups - male and female *APOE* ϵ4 carriers and non-carriers - did not differ in terms of age, years of education, family history of dementia, memory task performance or ACE-R (the latter for subjects aged 55 and older only).

6.3.2 Functional connectivity

In the first part of this analysis we investigated functional connectivity of the left and right hippocampus with the whole brain. There were significant effects of gender and *APOE* genotype. We also found a significant interaction between gender and *APOE* genotype on functional connectivity of the precuneus/ PCC with both the left and right hippocampus (Fig. 6.1 A). Post-Hoc analysis revealed that this was driven by significantly reduced connectivity of these regions in female *APOE* ϵ4 carriers compared with male *APOE* ϵ4 carriers and female non-carriers (Fig. 6.1 B). Only in female *APOE* ϵ4 carriers there was significant negative correlation between age and connectivity of the left hippocampus and precuneus/ PCC (male $\epsilon3\epsilon3$: r = 0.05, p = 0.84; female $\epsilon3\epsilon3$: r = 0.06, p = 0.80; male ϵ4 carriers: r = -0.21, p = 0.38; female ϵ4 carriers: r = -0.59, p = 0.001) (Fig. 6.1 C). No other region showed significant age-related lower functional connectivity with the hippocampus in female *APOE* ϵ4 carriers or in the other three groups.

Table 6.1: Sociodemographic features and general brain morphology of the four different groups. Values denote mean (±Standard Deviation) or number of subjects, *Values are expressed as percentage of whole-brain volume; p-values refer to one-way ANOVAs (parametric data) and Fisher-Freeman-Halton exact tests (categorical data).

	Male $\epsilon3\epsilon3$	**Female $\epsilon3\epsilon3$**	**Male $\epsilon4$ carriers**	**Female $\epsilon4$ carriers**	**P**
n	18	22	20	26	-
Age [years]	54.44 (10.35)	57.82 (10.09)	49.20 (8.69)	54.21 (12.75)	0.082
Education [years]	15.67 (3.38)	16.09 (3.19)	14.40 (3.42)	14.81 (3.45)	0.345
Family history of dementia	3	7	3	8	0.452
Grey matter*	39.20 (0.92)	39.64 (1.24)	39.98 (1.11)	39.34 (1.43)	0.184
White matter*	38.13 (2.02)	37.87 (1.50)	38.54 (1.46)	38.82 (1.65)	0.221
CSF*	22.68 (2.10)	22.48 (2.15)	21.48 (1.82)	21.83 (2.41)	0.267
Left Hippocampus*	0.27 (0.03)	0.30 (0.03)	0.28 (0.03)	0.29 (0.02)	0.168
Right Hippocampus*	0.28 (0.03)	0.30 (0.03)	0.29 (0.03)	0.29 (0.02)	0.393
ACE-R total (max 100)	99.25 (1.75)	99.31 (0.95)	98.50 (1.29)	98.90 (0.99)	0.635
Memory task performance (z scores)	0.20 (0.87)	-0.20 (0.82)	-0.10 (1.21)	0.13 (0.99)	0.504

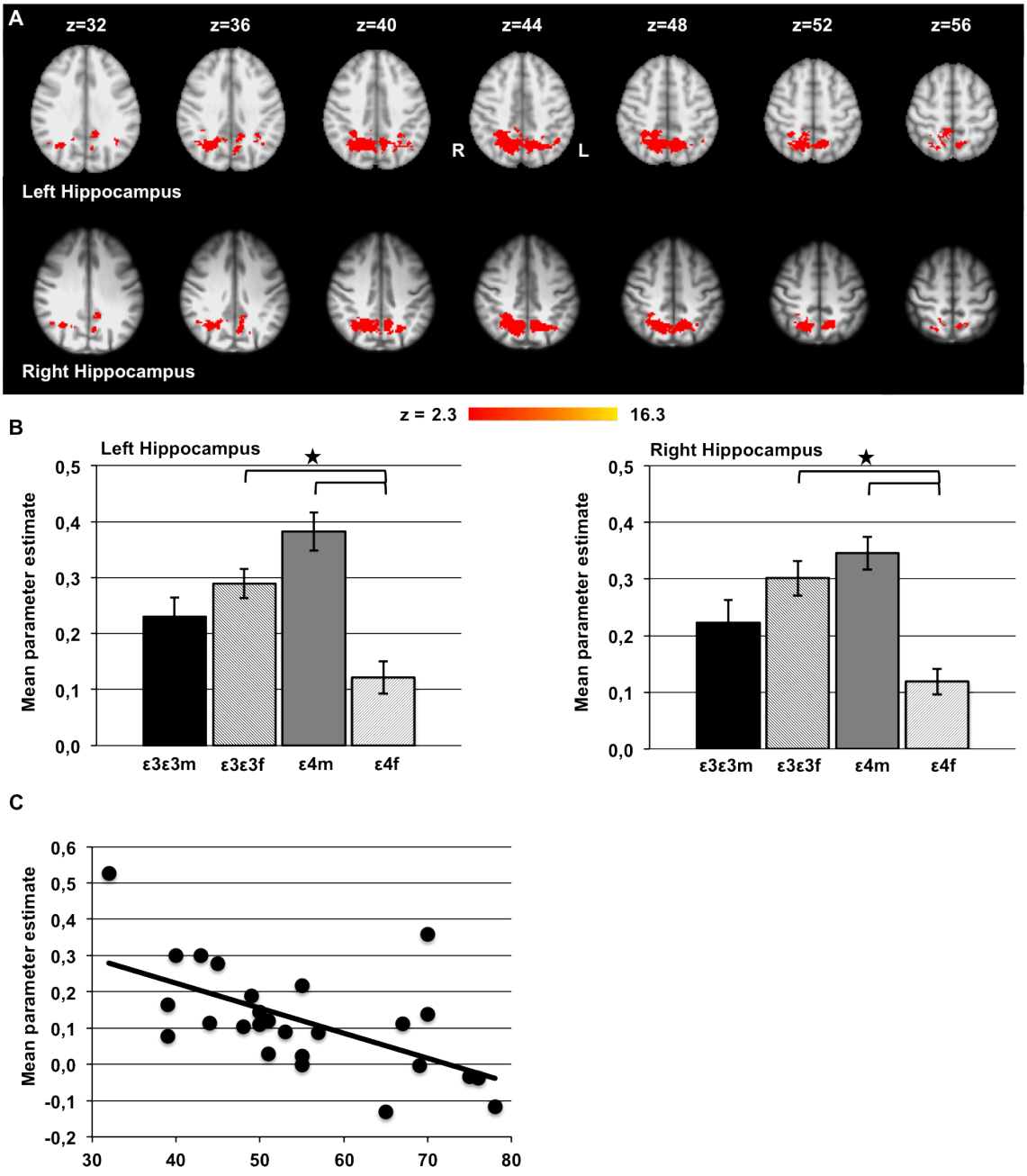

Figure 6.1: Resting-state MRI hippocampal connectivity. (**A**) Regions in the precuneus/ PCC that showed a gender by genotype interaction for connectivity with the left (top) and right (bottom) hippocampus. (**B**) Post-hoc analysis revealed significantly reduced connectivity of the precuneus/ PCC in female *APOE* $\epsilon 4$ carriers compared with male $\epsilon 4$ carriers and female non-carriers (error bars indicate standard error of the mean). (**C**) Only in female $\epsilon 4$ carriers resting-state connectivity of the left hippocampus and precuneus/ PCC decreased significantly with age.

6.3.3 White matter integrity

There was a significant effect of gender and *APOE* genotype on WM integrity of the left and right cingulum tract that connects the hippocampus and PCC (Hua et al., 2008). Female subjects and *APOE* ϵ4 carriers showed increased MD, DA and DR but no interaction between gender and genotype was found (Fig. 6.2 A-D). In all subjects there was a significant age related increase in MD, DA and DR.

6.3.4 Combining functional connectivity and WM integrity

There was a significant positive correlation of the extracted mean parameter estimates with FA of the cingulum tract in all subjects (left cingulum: r = 0.34, p = 0.002, right cingulum: r = 0.25, p = 0.02) but no differential effects of gender or *APOE* genotype on this correlation (Fig. 6.3 A and B).

6.3.5 Structural MRI

No differences between the four groups were found for GM, WM, CSF and hippocampal volumes (Table 6.1). Using a whole brain VBM approach significant gender differences in GM density were observed with increased density in males in a cluster within the visual cortex. There were no effects of *APOE* genotype and no interaction between gender and *APOE* genotype on GM density.

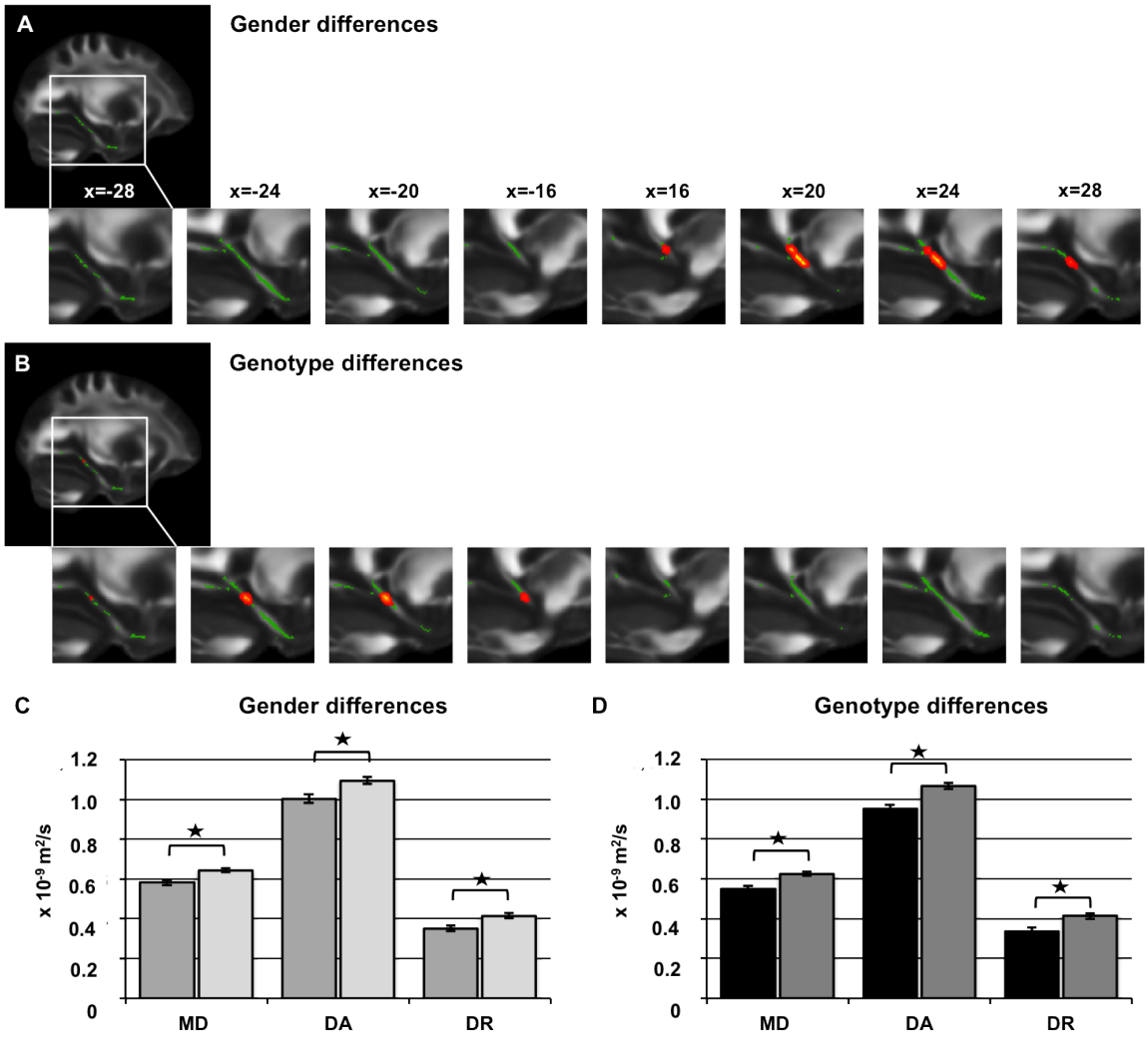

Figure 6.2: DTI analysis of the skeleton of the cingulum (green) connecting the hippocampus and PCC. Significant results are inflated and shown in red-yellow. The MNI coordinate of the respective brain slice is shown in mm. There was a significant effect of gender (**A**) and genotype (**B**) on MD. Effects on DA and DR were in similar regions of the skeleton. (**C**) Female subjects (light grey) showed increased MD, DA and DR compared with male subjects (dark grey). (**D**) MD, DA and DR was significantly increased in *APOE* ϵ4 carriers (grey) compared with non-carriers (black). Error bars indicate standard error of the mean.

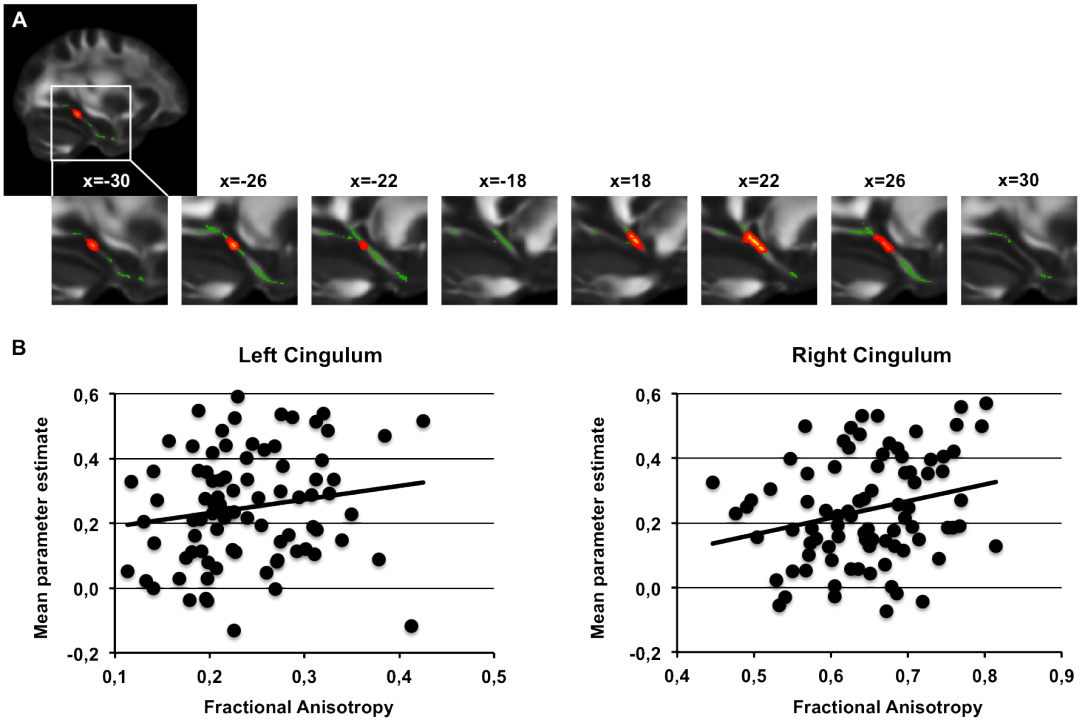

Figure 6.3: Correlation between structural and functional connectivity. Resting-state MRI mean parameter estimates correlate significantly with FA (**A**) for both the left ($r = 0.34$, $p = 0.002$) and right cingulum ($r = 0.25$, $p = 0.02$) (**B**).

6.4 Discussion

Possession of an *APOE* ϵ4 allele leads to a threefold increased AD risk in around 15% of the population who carry this allele, and female carriers of the *APOE* ϵ4 allele are at the highest AD risk (Payami et al., 1996; Farrer et al., 1997; Bretsky et al., 1999). Given the importance of understanding the mechanism of risk, it is surprising how few studies have taken gender into account when investigating the effect of *APOE* genotype on the healthy brain. We investigated connectivity of the hippocampus and found that female carriers of the *APOE* ϵ4 allele show reduced functional connectivity of the hippocampus with the precuneus/ PCC and an age-related functional connectivity decline. Functional connectivity correlated significantly with white matter integrity of the cingulum tract connecting those regions with reduced WM integrity in females and *APOE* ϵ4 carriers. Decreased WM integrity may underlie greater age-related decline of functional connectivity in female *APOE* ϵ4 carriers creating an increased vulnerability of this critical connection, and potentially contributing to the increased risk of AD.

6.4.1 Reduced functional connectivity in female *APOE* ϵ4 carriers

Our analysis of functional connectivity between the hippocampus and the precuneus/ PCC region showed a significant gender by *APOE* genotype interaction, even in a group of middle-aged healthy participants (mean age 54 years). This is consistent with a recent study that reported a gender by *APOE* genotype interaction on functional connectivity of the precuneus/ PCC in the DMN with decreased connectivity in older (mean age 79 years) female *APOE* ϵ4 carriers (Damoiseaux et al., 2012). The hippocampus is coactivated with the DMN and shows early AD related pathology (Braak and Braak, 1991). Therefore, it is possible that a loss of connectivity between the hippocampus and precuneus/ PCC leads

to decreased precuneus/ PCC activity within the DMN. Similar effects have been shown in epilepsy patients where functional connectivity of the precuneus with other brain regions is altered due to hippocampal sclerosis (Frings et al., 2009). We found decline in functional connectivity with age only in female *APOE* ϵ4 carriers, suggesting increased vulnerability to ageing effects in female *APOE* ϵ4 carriers. Expanding on the idea put forward by Damoiseaux at al. (2012) it might not only be an interaction between *APOE* genotype and gender but differential effects of age that underlie inconsistencies between studies of *APOE* genotype effects on functional connectivity. However, as this is a cross-sectional study the decrease in functional connectivity with age observed in female *APOE* ϵ4 carriers might be caused by a cohort effect. Longitudinal studies would be required to rule this out. We can only speculate what the underlying mechanisms for an interaction between gender and the apoE isoforms are. One possibility could be a link between estrogen and apoE function. Several studies have shown that estrogen promotes neurite outgrowth by upregulating apoE expression (Stone et al., 1998; Struble et al., 2003) and that this process depends on apoE isoforms. Although apoE4 expression increases following estrogen administration, apoE4 does not promote neurite outgrowth (Nathan et al., 1994; Nathan et al., 2004). Dendritic remodelling in AD has been interpreted as an attempt to counteract neurodegeneration and reduced remodelling has been reported in AD patients who carry the *APOE* ϵ4 allele (Arendt et al., 1997). Estrogen also influences neuroinflammation and serves as an anti-inflammatory agent (Pfeilschifter et al., 2002). However, this anti-inflammatory activity is reduced in mice carrying the *APOE* ϵ4 allele (Brown et al., 2008). Reduced responsiveness to estrogen might explain increased AD risk in female *APOE* ϵ4 carriers and why estrogen replacement therapy does not prevent or might even accelerate their cognitive decline (Yaffe et al., 2000; Kang and Grodstein, 2012).

6.4.2 *APOE* genotype and gender affect WM integrity

Previous studies have shown alterations in WM integrity associated with *APOE* genotype (Heise et al., 2011; Westlye et al., 2012). Here, we report alterations in the specific WM connections between the hippocampus and precuneus/ PCC, where the *APOE* ϵ4 allele is associated with increased MD, DA and DR and thus reduced white matter integrity. We found a significant effect of gender with increased MD, DA and DR in female subjects. Gender differences in WM integrity have been reported previously and develop during childhood and adolescence when gender differences in WM maturation can be observed (De Bellis et al., 2001; Hsu et al., 2008). We also found an age related decline in WM integrity in all subjects independent of gender or *APOE* genotype. Reduced WM integrity with age has been reported before and might be attributed to age related alterations in axon myelination (Westlye et al., 2010).

6.4.3 Correlation between functional connectivity and WM integrity

We found a significant positive relationship between functional connectivity and WM integrity across all subjects, i.e. higher WM integrity was associated with increased functional connectivity. Previous studies have shown this positive correlation, with increased WM integrity predicting higher functional connectivity (Andrews-Hanna et al., 2007; Van Den Heuvel et al., 2009) but to our knowledge, this is the first study to show a correlation for this particular tract connecting the hippocampus and PCC. Reduced DMN activity in the hippocampus and PCC and decreased functional and structural connectivity between those regions has also been shown in neuroimaging studies in AD patients (Greicius et al., 2004; Wang et al., 2006; Zhou et al., 2008b). Although the subjects studied here are all

healthy, it is possible that increased AD related pathology, i.e. amyloid plaques and neurofibrillary tangles, in female *APOE* ϵ4 carriers underlies those age-related changes in functional connectivity and WM integrity because AD related pathology is also found in non-demented subjects (Corder et al., 2004; Price et al., 2009). The hippocampus is one of the structures that shows early signs of neurofibrillary tangle pathology and the precuneus/PCC shows early amyloid pathology, even in healthy subjects (Braak and Braak, 1991; Buckner, 2005). Amyloid pathology in healthy subjects has been associated with reduced functional connectivity between the hippocampus and precuneus/ PCC (Hedden et al., 2009). Therefore, AD related pathology might be one explanation for our findings. Importantly, changes in functional connectivity and WM integrity were not accompanied by differences in GM morphology and are therefore more sensitive to processes associated with increased AD risk.

6.4.4 Implications for AD risk

Impaired connectivity between the hippocampus and precuneus/ PCC could be detrimental to cognitive processes. We have reported that hippocampal activation during resting-state and a memory task similarly depend on *APOE* genotype without affecting performance of a memory task (Filippini et al., 2009a). Therefore, we would predict that resting-state differences reported here should be associated with altered activation during memory formation. Although female *APOE* ϵ4 carriers showed no differences in performance of a memory task, the task was relatively simple and it might be possible to tease apart effects on cognition in future studies using more difficult tasks. Successful memory formation depends on functional connectivity between the hippocampus and PCC (Ranganath et al., 2005) and age-related decline of connectivity could underlie the faster rates of memory decline from the age of 60 reported in healthy *APOE* ϵ4 carriers compared

with non-carriers (Caselli et al., 2009). Although carrying an *APOE* ϵ4 allele is not necessary or sufficient for AD development, these observations may point to increased vulnerability of this critical pathway, particularly in female *APOE* ϵ4 carriers. Therefore, functional connectivity and WM integrity could be measures that are more sensitive to subtle changes in people who are at risk of developing AD. Improving hippocampal connectivity could be a target for interventions that might be especially effective in female *APOE* ϵ4 carriers. It has been shown that AD patients can benefit from cognitive interventions (Sitzer et al., 2006). In healthy participants memory training leads to increased BOLD activity in parietal regions (Olesen et al., 2004) that are very similar to those that show decreased functional connectivity with the hippocampus in female *APOE* ϵ4 carriers here. Memory training also leads to changes in white matter integrity (Takeuchi et al., 2010). Therefore, one might speculate whether reduced functional and structural hippocampal connectivity in people at increased AD risk could be restored using interventions such as memory training.

6.5 Conclusion

This study demonstrated an interaction between the AD risk factors *APOE* ϵ4 allele and female gender on connectivity of the hippocampus and the precuneus/ PCC. Functional connectivity decreased with age only in female *APOE* ϵ4 carriers indicating that increased vulnerability of this connection might be associated with higher AD risk. Interventions targeting hippocampal connectivity might be especially effective in this at risk population.

Chapter 7

Final Discussion and Conclusions

7.1 Summary of Objectives and Results

The overall aim of this thesis was to investigate the effects of *APOE* genotype on brain structure and function in healthy adults. In more detail, the objectives and results of the experimental chapters were as follows.

7.1.1 *APOE* genotype and the resting brain (chapter 3)

The focus was on the functional architecture of the brain at rest and *APOE* genotype effects were investigated using MEG. This made it possible to explore neuronal function directly to rule out that altered neurovascular coupling was driving the *APOE* genotype related differences reported in previous fMRI studies. Increased synchronised neuronal oscillations were found in *APOE* ϵ3 homozygotes compared with the two other genotype groups in many different RSNs such as the DMN and fronto-parietal networks. This confirms the neuronal origin of differences in brain function at rest that have been reported in fMRI studies. No differences in resting-state functional connectivity were observed between *APOE* ϵ2 and ϵ4 carriers.

7.1.2 *APOE* genotype and basic visual processing (chapter 4)

In this chapter *APOE* genotype effects on task-related brain function were explored using MEG during a visual oddball task. It was shown that *APOE* genotype affects early sensory processing with faster visual processing in *APOE* ϵ4 carriers. Additionally, an influence of *APOE* genotype on cognitive processes associated with attention was found. In this analysis of task-related brain function differences between *APOE* ϵ2 and ϵ4 carriers were found for both sensory processing as well as attentional mechanisms.

7.1.3 *APOE* genotype and brain structure (chapter 5)

Structural MRI and DTI were used to explore the question of whether *APOE* genotype related differences in brain structure were associated with functional changes. This was a replication of previous results and in concordance with these studies no *APOE* genotype effects on GM structure were found. WM integrity was influenced by *APOE* genotype and *APOE* ϵ2 and ϵ4 carriers showed reduced WM integrity compared with ϵ3 homozygotes.

7.1.4 *APOE* genotype, gender and age modulate connectivity of the hippocampus in healthy adults (chapter 6)

In this chapter resting-state fMRI and DTI data were used to investigate possible interactions between AD risk factors *APOE* ϵ4 allele, age and female gender on functional and structural connectivity of the hippocampus. There was overall reduced functional connectivity of the hippocampus and PCC in female *APOE* ϵ4 carriers and they were also the only group to show an age-related decline in functional connectivity of these areas.

7.1.5 Summary

Taken together, these results show a complex influence of *APOE* genotype on brain structure and function that is difficult to interpret with regards to AD risk. While the global functional and structural architecture of the brain is very similar for those with the highest and lowest AD risk, differences between those groups become apparent when task-related brain function is studied in more detail. In addition, *APOE* genotype is not the only variable that affects brain structure and function. Interactions with other risk factors such as age and female gender also play a role and might more specifically affect local connections that could be relevant for AD development. In the remainder of this chapter I will revisit the different aspects of brain resilience explored in the first chapter of this thesis to investigate whether the findings of this DPhil project might offer an explanation for the association of *APOE* genotype and AD risk.

7.2 *APOE* and brain resilience

The studies presented in chapters 3 and 5 showed that differences between the three *APOE* genotype groups were not restricted to particular functional networks or WM tracts indicating that *APOE* genotype has a widespread effect on the functional and structural architecture of the brain. This could be a result of *APOE* genotype related differences in neurodevelopment. In addition, these studies showed that functional and structural connectivity is very similar for *APOE* $\epsilon 2$ and $\epsilon 4$ carriers, i.e. those with the lowest and highest AD risk. The results of chapter 4 seem to imply that it might not be the global brain architecture but the way in which brain networks are used and respond to cognitive tasks that predispose to AD. *APOE* genotype not only affects "higher" cognitive functions such as memory and attention but also basic sensory processing. The concept of

antagonistic pleiotropy is very interesting in this respect. The mechanisms that could underlie advantages in cognitive processing in *APOE* ϵ4 carriers early in life might also predispose to faster brain ageing because of increased wear and tear.

The hypothesis that faster brain ageing might underlie increased AD risk in *APOE* ϵ4 carriers is backed up by the study presented in chapter 6. The results of chapter 6 also indicate that faster age-related deterioration might only affect particular brain circuits, in this case the connection between the hippocampus and PCC that is important for memory processing. Nevertheless, *APOE* ϵ2 carriers were not included in this study, which makes it difficult to determine whether faster ageing is only found in *APOE* ϵ4 carriers and might be one explanation for their increased risk. Alternatively, faster ageing could simply be a downstream event of altered global brain architecture and might also be found in *APOE* ϵ2 carriers.

7.3 Neuroimaging markers of preclinical AD and possible intervention strategies

The aim of this research is not only to investigate mechanisms leading to increased AD risk but also to develop possible markers of preclinical AD. The results presented here indicate that global measures of brain structure and function might not offer the most promising approach. Localised measures such as hippocampal connectivity might be a potential marker of neurodegeneration. Large-scale longitudinal datasets will be required to investigate this further and potentially develop useful thresholds at which age-related hippocampal connectivity decline has to be considered "abnormal". Intervention strategies targeting specific networks such as hippocampal connections should be explored and might potentially be of benefit to those at the highest AD risk. If connections can be strengthened e.g. by cognitive

interventions, which has been shown in some studies investigating plasticity, this might reduce AD risk.

The research presented here also indicates that MEG might be a useful neuroimaging marker in addition to MRI. This applies in particular to task-related neuronal function where differences between *APOE* ϵ2 and ϵ4 carriers were found. However, the preliminary findings presented here will need to be confirmed in larger studies before the clinical use of MEG can be fully explored. In addition, standardised MEG analysis protocols, which are in development at OHBA and other MEG centres at the moment, will be needed to ensure comparability between MEG studies.

7.4 Future Directions

Most importantly, the results presented here indicate that *APOE* ϵ2 carriers have to be included in future studies of *APOE* genotype effects on the brain. Otherwise, the results cannot be interpreted with regards to AD risk because there is now considerable evidence that low and high AD risk groups show very similar brain function and structure. In the following I will present some of the questions that need to be addressed in future studies.

7.4.1 What is the difference between *APOE* ϵ2 and ϵ4 carriers?

One of the main questions is in how far *APOE* ϵ2 and ϵ4 carriers differ in brain function and structure. If it is not global functional and structural connectivity that predisposes to AD development, what are the factors that lead to increased AD risk in *APOE* ϵ4 carriers? One answer might be that they show different trajectories in functional and structural decline with age, which would strengthen the hypothesis that faster brain ageing in *APOE* ϵ4 carriers leads to earlier AD

development. To investigate differences in age trajectories it will be necessary to conduct longitudinal studies. There are many between-subject differences that could affect the results of cross-sectional studies and within-subject designs would be much more sensitive to explore factors that affect brain ageing. One such factor could be the ability to compensate for injuries. Future studies should explore possible *APOE* genotype effects on functional and structural plasticity and neuroregeneration. For example, this could involve intervention studies to investigate plasticity mechanisms associated with learning or exploring the capability for adult neurogenesis in the different *APOE* genotype groups.

7.4.2 What are the mechanisms underlying antagonistic pleiotropy?

Another mechanism that needs to be investigated further is the hypothesis of antagonistic pleiotropy in *APOE* ϵ4 carriers. There are only a few studies exploring the effects of *APOE* genotype on brain development in children and adolescents. Those studies mostly focus on GM structure or brain function but there are no published studies investigating whether *APOE* genotype affects WM development in children. Therefore, it is unclear whether *APOE* genotype affects global brain function and structure or whether effects are limited to particular cognitive functions and brain structures such as the hippocampus. Even if one assumes that carrying the *APOE* ϵ4 allele is beneficial early in life, a further question is when this advantage turns into a disadvantage. This would presumably be the point at which AD prevention strategies would have the biggest impact.

7.4.3 How does *APOE* interact with other AD risk factors?

A further point to consider is that brain structure and function are not solely dependent on *APOE* genotype. Interactions with other risk factors play an important role because not all *APOE* ϵ4 carriers go on to develop AD later in their lives. Only two additional risk factors were considered in the study presented in chapter 6. Nevertheless, many other factors influence AD risk. In particular, interactions with other genetic factors such as the *APOE* promoter would be very interesting to study but large subject numbers will be needed to investigate interactions between different genetic factors. In addition, investigating how *APOE* genotype interacts with environmental and lifestyle factors could lead to interventions that are targeted at particular at-risk populations but again these questions can only be explored with large datasets.

7.5 Conclusion

In summary, the research presented in this DPhil thesis showed a complex pattern of *APOE* genotype effects on brain structure and function. Most importantly, differences in brain function were not driven by altered neurovascular coupling but clear differences in neuronal function were observed using MEG. While *APOE* genotype effects on global structural and resting-state functional connectivity do not follow patterns of AD risk, more specific measures of local connectivity and task-related brain function could be of use in the development of preclinical markers for AD development. The mechanisms leading to increased AD risk need to be explored further targeting particular aspects of brain resilience such as neurodevelopmental differences, functional and structural plasticity and taking into account interactions with other genetic and environmental risk factors.

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