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MEMORY, GENES, AND BRAIN IMAGING: RELATING THE *APOE* GENE TO BRAIN FUNCTION AND STRUCTURE

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To my parents

Abstract

Alzheimer's disease (AD) is the most common form of dementia. An important goal for current AD research is to find preclinical markers of impending disease. *Apolipoprotein E* ε4 (*APOE* ε4) is the chief known genetic risk factor for AD. A number of neuroimaging studies have reported structural and functional brain alterations in non-demented *APOE* ε4-carriers. Such results have tentatively been interpreted as early signs of impending dementia, but the findings have been inconsistent across studies. To further address this issue, the overall aim of this thesis was to examine asymptomatic cognitively well-functioning *APOE* ε4-carriers with magnetic resonance imaging (MRI) techniques, together with longitudinal neuropsychological testing. Study I revealed that carriers of *APOE* ε4 expressed reduced functional brain activity during incidental episodic encoding. In the parietal cortex, a genetic dose-effect was seen such that the activity reduction was more pronounced for homozygous than heterozygous *APOE* ε4-carriers. In addition, it was found that *APOE* ε4-carriers had structural changes in white-matter tracts in the hippocampus and the posterior corpus callosum (Study II), and grey matter reductions in the hippocampus (Study III). Study IV demonstrated that the degree of functional activity in the parietal cortex predicted subsequent episodic memory decline within the group of *APOE* ε4-carriers. Collectively, the results suggest that a combination of genetic, neuropsychological, and neuroimaging strategies is beneficial in predicting AD development.

LIST OF PUBLICATIONS

I. Lind J., Persson J., Ingvar M., Larsson A., Cruts M., Van Broeckhoven C., Adolfsson R., Bäckman L., Nilsson L-G., Petersson K. M., and Nyberg L. (2006) Reduced functional brain activity response in cognitively intact *apolipoprotein E* ε4 carriers. *Brain*, 129, 1240-48.

II. Persson J., Lind J., Larsson A., Ingvar M., Cruts M., Van Broeckhoven C., Adolfsson R., Nilsson L-G., and Nyberg L. (2006) Altered brain white matter integrity in healthy carriers of the *APOE* ε4 allele: A risk for Alzheimer's disease. *Neurology*, 66:1029-33.

III. Lind J., Larsson A., Persson J., Ingvar M., Nilsson L-G., Bäckman L., Adolfsson R., Cruts, M., Sleegers K., Van Broeckhoven C., and Nyberg L. (2006) Reduced hippocampal volume in non-demented carriers of the *apolipoprotein E* ε4: Relation to chronological age and recognition memory. *Neuroscience Letters*, 396:23–27.

IV. Lind J., Ingvar M., Persson J., Sleegers K., Van Broeckhoven C., Adolfsson R., Nilsson L-G., and Nyberg L. (2006) Parietal cortex activation predicts memory decline in *apolipoprotein E* ε4 carriers. *NeuroReport*, 17:1683-1686.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AD	Alzheimer's disease
APOE	Apolipoprotein E (protein)
APOE	*apolipoprotein E* (gene)
BOLD	Blood Oxygen Level Dependent
CBF	Cerebral Blood Flow
CNS	Central Nervous System
DTI	Diffusion Tensor Imaging
FA	Fractional Anisotropy
fMRI	Functional Magnetic Resonance Imaging
MRI	Magnetic Resonance Imaging
MTL	Medial Temporal Lobe

1 INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia. It is a chronic neurodegenerative disorder that is characterized by a gradual loss of cognitive functions such as episodic memory. More than 12 million persons are affected worldwide and with an aging population the number of AD cases is steadily increasing. Early diagnosis is crucial in relation to development of treatments to prevent or delay disease onset. However, at present, methods for identifying asymptomatic individuals who will go on to develop AD are still imperfect.

Genetic studies have identified the *apolipoprotein E* ε4 (*APOE* ε4) as a major susceptibility gene for AD. Results of brain imaging studies suggest that non-demented *APOE* ε4-carrires have alterations in memory-related brain activity, and that these changes occur in brain regions that are pertinent to the disease. Given that the AD process likely begins years prior to the onset of cognitive problems, it is tempting to interpret these findings as preclinical markers for impending dementia. However, the existing studies are equivocal with regard to a number of issues and the impact of *APOE* ε4 remains unclear. For instance, there is inconsistency regarding in what direction (decreased versus increased) activity alterations are seen, and due to the lack of longitudinal follow-ups it is still obscure how the observed changes correspond to subsequent cognitive impairment (and ultimately AD development).

To further address how genetic risk for AD may translate into preclinical brain alterations, the overall aim of this thesis was to study healthy *APOE* ε4-carriers with functional as well as structural neuroimaging techniques, together with longitudinal neuropsychological testing.

1.1 APOLIPOPROTEIN E

The apolipoproteins represent a diverse set of proteins that have in common their presence on plasma lipoproteins that transport cholesterol, triglycerides, and phospholipids from one tissue or cell type to another. Thus, the apolipoproteins play a large role in lipid homeostasis, particularly in determination of the levels of cholesterol. Several types of apolipoproteins, including type A, B, C, D, E, H, and J, and a number subclasses, have been reported. Of these, APOE has been the subject of particular scrutiny. Initial interest centred on its role as a regulator of plasma lipid levels.

However, in 1993, APOE was identified as a major risk factor for the neurodegenerative disorder AD (Corder et al., 1993; Strittmatter et al., 1993) which sparked extensive research on the role of APOE in neurobiology. Since then, APOE has emerged as an important factor for several processes not obviously related to lipid metabolism, including neurite outgrowth and differentiation, neuronal repair, and immunoregulation (for reviews, see Mahley, 1988; Mahley and Rall, 2000).

The APOE protein is a 299 amino acid peptide with a molecular weight of approximately 34 kD. It is synthesized in most organs, with the largest quantity found in the liver (about two-thirds of the total plasma APOE) and the second largest concentration (about one-third the level seen in the liver) is found in the brain (Elshourbagy et al., 1985). In the central nervous system (CNS), APOE is synthesized and secreted primarily by astrocytes (Boyles et al., 1985), although recent results suggest that APOE may also be produced by neurons (albeit at a lower level) under diverse physiological and pathological conditions (Xu et al., 2006). The encoding gene (*APOE*) is located at chromosome 19. It is a polymorphic gene with three common alleles (ε2, ε3, ε4), coding for three APOE protein isoforms (E2, E3, E4) (Zannis et al., 1981). The protein isoforms differ at only two amino acid residues: 112 and 158 (Figure 1). E3 contains cysteine at 112 and arginine at 158, whereas E4 has arginine at both positions, and E2 has a cysteine at both sites (Rall et al., 1982). The *APOE* ε3 is by far the most common allele: frequencies for Caucasian samples are approximately 5-10% (ε2), 70-80% (ε3), and 10-15% (ε4), with corresponding genotypic frequencies of 0.5% (ε22), 11% (ε23), 59% (ε33), 2% (ε42), 25% (ε34), and 3% (ε44).

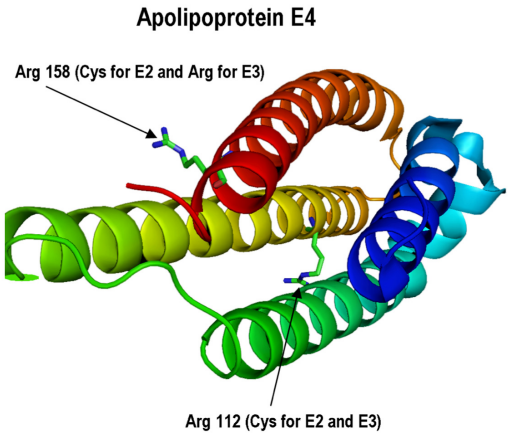

Figure 1. *APOE4 differs from E2 and E3 at only two amino acid residues (arrows).*

Although the structural difference between the APOE isoforms may seem small, it has significant impact on ultimate function. The single amino acid change appears to alter the overall conformation of the protein and thereby modifying its preference for specific type of lipoproteins, affecting its intracellular handling by cells, and modulating its biological activity with respect to *in-vitro* effects on neurons and *in-vivo* effects on CNS structure and function (Mahley and Huang, 1999). In general, E3 seems to be the normal isoform in every known function of the protein, whereas E4 and E2 can each be dysfunctional (or in some cases protective) (Hill et al., *in press*). For instance, E2 has been associated with inheritance of type III hyperlipoproteinemia (Utermann, 1987), but also with *decreased* incidence for both AD (Corder et al., 1994) and cardiovascular disease (Davignon et al., 1988). APOE4 has been associated with increased risk for and/or severity of a number of clinical conditions, with AD being the most acknowledged (Corder et al., 1993; Strittmatter et al., 1993). Other examples include cardiovascular disease (Frank et al., 2002), poor recovery after brain injury (Sundström et al., 2004) and stroke (Martinez-Gonzalez and Sudlow, 2006), Lewy body disease (Hardy et al., 1994), vascular dementia (Frisoni et al., 1994), and frontotemporal dementia (Verpillat et al., 2002), to mention some, and the list is still growing. Furthermore, studies of healthy subjects have found that E4 is associated with diminished CNS glucose utilization (e.g. Reiman et al., 2004) and increased decline in cognitive function (e.g. Deary et al., 2002). Taken together, APOE4 appears to have a global detrimental effect on the CNS.

The precise underlying mechanism(s) by which the APOE isoforms affect these and other processes remains unknown. However, it has been suggested that a key to further insight probably resides in determining how APOE modulates neuronal repair, remodelling, or protection; one theory is that E4 is less effective than E3 and E2 in mediating these processes (Mahley and Rall, 2000).

1.2 *APOE* AND RISK FOR ALZHEIMER'S DISEASE

AD is the most frequent neurodegenerative disease worldwide and accounts for 60-80% of all dementia cases. It is characterized phenotypically by progressive cognitive decline, in particular loss of episodic memory, learning, and attention, inevitably accompanied by alterations of personality (e.g. Lanctot et al., 2003). The tentative diagnosis of AD is based mainly on these behavioural features, and can only be confirmed post-mortem by the presence of three defining neuronal hallmarks: (1)

abnormal aggregation of the tau protein inside nerve cell bodies known as neurofibrillary tangles; (2) extracellular deposits of an abnormal protein (beta-amyloid) forming so called amyloid plaques; (3) significant brain atrophy (The National Institute on Aging and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's, 1997; Braak et al., 1993); reported morphological changes include decreases in dendritic arborisation (Einstein et al., 1994) and loss of neurons and synapses (Hamos et al., 1989; Masliah et al., 1994). Typically, the pathology progresses from the medial temporal lobe (MTL) (critically, the entorhinal cortex and hippocampus) to neocortical association areas, with relative preservation of the occipital area (Braak and Braak, 1991; Braak et al., 2006). As the neural degeneration accumulates, subsequent behavioural symptoms are initiated until a generalized cognitive decline prompts a diagnosis of dementia. AD is invariably fatal.

The ultimate cause of AD is unknown in most cases (DeKosky, 2003). Probably, several environmental and biological factors act in concert and affect each person differently. A number of risk factors have been recognized, with advanced age traditionally being held as the most powerful, followed by familial history of the disease (e.g. Turner, 2006). AD is commonly divided into early-onset AD (before the age of 60-65 years), and late-onset AD. Late-onset AD represent the vast majority of all cases (>95%). Demarcation between these two types has never been clear, although point mutations in any of three specific autosomal dominant genes have been identified in patients who suffer from familial early-onset AD, namely in the amyloid precursor protein (*APP*), presenilin1 (*PSEN1*), and presenilin2 (*PSEN2*) (Selkoe, 2000; Tanzi et al., 1996). These three genes are deterministic genes, meaning that anyone who inherits a mutated copy of them will most likely develop the disease (for this reason, early-onset AD is often called “true familial” AD). In contrast to early-onset AD, late-onset AD (which may be sporadic or familial) is not inherited in any simple, mendelian pattern. Instead, certain risk genes (i.e. genes that increase the likelihood of developing the disease but not guaranteeing it will happen) may influence whether someone is more or less susceptible to the disease. Indeed, a large population-based twin study recently showed that the influence of inheritance in late-onset AD may be as much as 79% (Gatz et al., 2006; see also Ashford and Mortimer, 2002).

A large number of AD associated risk genes has been reported (Tanzi, 1999), although the only one that has been consistently repeated is the *APOE* gene (Saunders et al., 1993; Strittmatter et al., 1993; for a review, see Tanzi and Bertram, 2005). Strikingly, the *APOE* ε4 allele occurs in approximately 60% of AD patients,

compared to approximately 20% of the general population. In contrast, those individuals with the ε33 genotype constitute 60% of the population but only 35% of AD cases (Saunders et al., 1993; Farrer et al., 1997). It has been calculated that *APOE* ε4 accounts for at least 50% of all AD cases in the United States (Saunders et al., 1993). Considering the rarity of AD among carriers of the ε2 allele, some researchers even suggest that the *APOE* gene by itself accounts for over 95% of AD cases (Raber et al., 2004; but see Daw et al., 2000). This would make *APOE* the most powerful AD risk factor of all. Furthermore, *APOE* ε4 appears to have a genetic dose effect on the risk and age at onset of AD: as the number of *APOE* ε4 alleles increases from 0 to 1 to 2, the risk of developing late-onset AD increases from 20% to 90%, and the mean age at onset decreases from 84 to 68 years (Corder et al., 1993). However, it appears that the *APOE* polymorphism determines susceptibility for AD without strongly influencing the clinical course of the disease (Corder et al., 1995; Basun et al., 1995), and also that *APOE* ε4 confers it maximal effect on risk before the age of 70 (Blacker et al., 1997).

Biochemical, cell biological, and transgenic animal studies have suggested several potential mechanisms to explain the contribution of *APOE* ε4 to the increased risk of AD (for reviews, see Huang et al., 2004; 2006). However, the underlying mechanisms of the implied effects are yet largely unclear. Likewise, it is not known whether the observed effects result directly or indirectly from APOE activities or to what extent they contribute to the pathogenesis that characterize AD clinically. Nevertheless, four main hypotheses regarding the action of APOE4 in AD pathophysiology exist: (1) The E4 isoform bind more strongly to the beta-amyloid peptide which may facilitate plaque formation (Strittmatter et al., 1993). This hypothesis is supported by findings of increased beta-amyloid deposition in the cortex of both demented and non-demented *APOE* ε4-carriers (Strittmatter et al., 1993; Polvikoski et al., 1995; Nagy et al., 1995; Yamaguchi et al., 2001); (2) Compared to E2 and E3, E4 binds less efficiently to microtubule-associated protein tau which renders tau vulnerable to phosphorylation and therefore neurofibrillary tangles formation (Strittmatter et al., 1993; Polvikoski et al., 1995); (3) APOE4 appears to be deficient in stimulating neurite outgrowth in response to cellular injury and degeneration, and moreover, to interfere with the normal protective function of E3 (Buttini et al., 2000). This may lead to loss of synaptic connections and in turn, a progressive decline in the efficacy of neuronal network function with associated cognitive impairment (Turic et al., 2001); (4) In a study of transgenic mice, Harris et al (2003) demonstrated that

APOE4 is particularly vulnerable to cleavage by a serine protease, generating a biologically active truncated product that can produce neurofibrillary tangle-like intra-neuronal inclusion bodies and elicit neurodegenerative and behavioural deficits.

1.2.1 *APOE* and the search for antecedent biomarkers of Alzheimer's disease

Currently, no reliable methods exist for early detection and treatment of AD. New techniques for preventing, slowing the progression of, and treating AD are being urgently sought. Such attempts would be aided considerably if impending AD could be detected prior to when clinical signs appear and before irreversible brain damage occurs. However, to date, little is known about the earliest "asymptomatic" stages of the disease, although post-mortem studies have revealed that neuropathological changes probably develop decades before the onset of behavioural symptoms (Braak and Braak, 1991). It has also been shown that the AD diagnosis is preceded by a long period during which deficits to cognitive performance are present (Bäckman et al., 2001; Elias et al., 2000).

In search for antecedent markers of AD, one strategy would be to take an epidemiological, population-based approach and obtain biological and/or neuroimaging measures from large numbers of cognitively intact, middle-aged individuals, and follow them clinically over time to determine who ultimately develops AD, then retrospectively analyze and compare the samples as a function of their final dementia status in order to find best predictors of disease. However, such prospective population-based studies of younger individuals would be too costly and require too many research subjects and years to be pursued as a primary strategy. Instead, a more fruitful approach would be to compare cohorts that differ *a priori* in their risk for AD. One would then hypothesize that the group at higher risk for AD would express a pattern more similar to AD cases than the group at lower risk, and that the observed alterations might be indicative markers of impending AD.

High-risk groups are typically defined by genetic variables (any of the three deterministic genes *APP*, *PSEN1*, *PSEN2*, or the risk-gene *APOE* ε4) or cognitive status [mild cognitive impairment; MCI (Petersen et al., 1999)]. Of these, *APOE* ε4 is arguably the best choice, since the rare autosomal dominant form of AD may differ in natural history from the most common (>95%) late-onset form, and because of the ill-defined transition between MCI and early-stage AD. (Recruiting at-risk subjects on the basis of advanced age is likely not meaningful given the many co-morbidities that come

with aging.) Indeed, both functional and structural alterations, similar to those seen in AD patients, have been observed among non-demented carries of *APOE* ε4, sometimes even decades before the normal onset-age of AD (e.g. Reiman et al., 2004; Tohgi et al., 1997; c.f. section "*APOE* and brain imaging findings" below). However, due to a great deal of inconsistency, and because most studies to date have been cross-sectional, it is difficult to argue that the observed changes actually forecast clinical AD. Cross-sectional studies may reveal deficits associated with the *APOE* ε4 allele *per se*, but (since many of the *APOE* ε4-carriers remain healthy) to be able to identify alterations that can ultimately be associated with subsequent development of AD, longitudinal follow-ups are required. Further, in order to increase accuracy, careful sample selection, consistent methods, and a combination of different tools are desirable together with longitudinal follow-ups.

1.3 *APOE* AND COGNITION

A number of studies have demonstrated that non-demented individuals with a copy of the *APOE* ε4 allele perform worse on neuropsychological tests than non-carriers, in particular on tasks that assess memory (e.g. Bondi et al., 1995; Jonker et al., 1998; O'Hara et al., 1998; Caselli et al., 1999; Staehelin et al., 1999; Dik et al., 2000; Flory et al., 2000; Rosen et al., 2002; Wilson et al., 2002b; Driscoll et al., 2005; Greenwood et al., 2005; Nilsson et al., 2006). However, other studies have failed to find such effects (Plassman et al., 1997; Smith et al., 1998; Small et al., 2000a; Bennett et al., 2005).

Several factors may account for the contradictory findings. First, the influence of the *APOE* genotype may vary for different cognitive domains or subdomains, meaning that the nature of the tests may affect the likelihood of observing *APOE* ε4-related deficits. A meta-analysis (including 38 studies) by Small et al (2004) showed that *APOE* ε4-carriers, as compared to non-carriers, had lower scores on tests of global cognitive functioning, executive functioning, and episodic memory, while no differences were observed for the domains of primary memory, attention, visuospatial skill, verbal ability, or perceptual speed. In a recent longitudinal population-based study by Nilsson et al (2006), *APOE* ε4-related deficits were primarily observed in tests of episodic memory, whereas little or no effects were seen in tests of semantic memory, primary memory, or priming. Further, episodic memory impairments were more pronounced in tests of recall than in tests of recognition memory. Nilsson et al (2006) interpreted the finding of more salient deficits in recall than in recognition as reflecting

problems with executive processes, such as attention and working memory. Indeed, these abilities have been found to be impaired among ε4-carriers (Parasuraman et al., 2002; Greenwood et al., 2005; Reinvang et al., 2005).

A possible explanation for the selective cognitive impairments concerns the neural seat of *APOE* ε4-related alterations. *APOE* ε4 has been associated with increased regional atrophy (e.g. Plassman et al., 1997; Tohgi et al., 1997) and hypometabolism (e.g. Small et al., 1995; Reiman et al., 2005), in frontal, parietal and temporal regions (c.f. section "*APOE* and brain imaging findings" below), including the hippocampal structure which is known to be crucial for episodic memory functioning (Squire and Zola-Morgan, 1991; Nyberg et al., 1996b; Cabeza and Nyberg, 2000b). Parasuraman et al (2002), who found *APOE* ε4-attentional deficits, emphasized temporoparietal brain areas as possible locus of *APOE* ε4-related cognitive impairments by implicating the cholinergic neurotransmitter system (see also Espeseth et al., 2006). However, overall, the biological mechanisms through which the *APOE* genotype might affect cognitive function remain largely unknown. Although several neurobiological deficits have been associated with the *APOE* ε4 allele as compared to the ε2 and ε3 alleles, including less effective neuronal repair mechanisms (c.f. section "*APOE* and risk for AD" above), a link between these effects and memory decline has not been conclusively demonstrated.

Other factors that might add to the inconsistent findings include small or unequal sample sizes, recruitment methods, and sample demographics. For instance, both Small et al (2004) and Nilsson et al (2006) found that *APOE* ε44 homozygotes performed significantly worse than ε4 heterozygotes. Moreover, the pattern of cognitive deficits observed among *APOE* ε4-carriers may not be the same in younger as compared to older subjects: it has been found that the *APOE* ε4 allele loses some importance with age (Corder et al., 1993; Farrer et al., 1997; Breitner et al., 1999; Small et al., 2004), and tests on children and young subjects have revealed no significant effects of the *APOE* genotype on intelligence quotient (Yu et al., 2000), or on general cognitive ability (Turic et al., 2001; Deary et al., 2002). It may also be that longitudinal studies that assess rate of cognitive decline are more sensitive than cross-sectional studies in finding *APOE*-related effects. In fact, many longitudinal studies that report significant between-group differences in memory change over time see no or only small absolute differences at baseline in relation to *APOE* genotype (e.g. Jonker et

al., 1998; O'Hara et al., 1998; Wilson et al., 2002a; Blair et al., 2005; Nilsson et al., 2006).

Further, considering the strong association between *APOE* ε4 and increased risk for AD, and that AD-related memory deficits commonly appear many years prior to clinical diagnosis (Elias et al., 2000; Bäckman et al., 2001), a given question is whether the observed memory decline among *APOE* ε4-carries reflects yet undiagnosed dementia symptoms, or an influence of the *APOE* genotype by itself. For example, Bondi et al (1999) found that the presence of *APOE* ε4-related deficits in memory performance was greatly reduced once subjects who would go on to develop AD were excluded from the analyses. In another study, Smith et al (1998) found a significant influence of the *APOE* genotype on cognitive phenotype in AD and MCI patients, but not in healthy subjects. Indeed, if no ubiquitous relationship between *APOE* genotype and cognitive performance exists, it may have clinical implications for the early detection of AD. This was noted by Small and colleagues (2000b), who suggested that since *APOE* ε4 is a risk factor for AD and impaired cognitive function is an early sign of AD, the interaction between these two characteristics may enhance early detection of impending dementia. However, strong longitudinal results by Nilsson et al (2006) revealed that *APOE* ε4-related memory deficits remained significant even after eliminating individuals who were diagnosed with dementia within 10 years after testing. Clearly, in order to ensure unambiguous interpretations of the source of *APOE* ε4-related cognitive deficits, careful sample selection and follow-ups are warranted.

1.4 *APOE* AND BRAIN IMAGING FINDINGS

1.4.1 Structural studies

Grey matter findings

As already noted, clinical AD is associated with severe brain atrophy, particularly in the MTL (Braak et al., 1993). Structural imaging studies of AD patients have revealed that morphological loss is most pronounced in carriers of the *APOE* ε4 allele (e.g. Lehtovirta et al., 1995; Juottonen et al., 1998; Geroldi et al., 1999; 2000; Mori et al., 2002). However, in studies of non-demented subjects, results have been mixed. For instance, Jack et al (1998) studied hippocampal volumes in 62 demented and 125 cognitively normal subjects (mean age 79 years) and found no differences on the basis of *APOE* genotype. Neither did a study by Killiany et al (2002), which investigated

entorhinal and hippocampal volumes among subjects with normal cognition, memory difficulties, and mild dementia (see also Small et al., 1995; Schmidt et al., 1996; Jernigan et al., 2001; Han et al., 2007). In contrast to these negative findings, Plassman et al (1997) reported that non-demented *APOE* ε4-carriers (mean age 63 years) had smaller left and right hippocampal volumes as compared to non-carriers, despite the fact that the two groups did not differ in performance on neuropsychological tests. Tohgi et al (1997) extended this finding by observing significantly reduced right hippocampal size and a trend towards smaller left hippocampal volume in cognitively intact *APOE* ε4-carrires as young as in their forties (age range 39-80 years). Further, in a large-scale (N=750) VBM study, Lemaitre et al (2005) found a significant decrease of grey matter bilaterally in the MTL, including the hippocampus, extending over the superior temporal gyrus in *APOE* ε4-carriers (age range 63-75 years), as compared to non-carriers. The effect was however limited to *APOE* ε44 homozygotes. This study also found that the relative risk of cognitive impairment over a four year follow-up period was substantially greater in homozygous carriers relative to both heterozygotes and non-carriers.

In addition, there are studies of non-demented subjects that have failed to detect differences in absolute sizes between *APOE* genotype groups, but instead observed other alterations associated with the *APOE* ε4 allele (Soininen et al., 1995; Moffat et al., 2000; Cohen et al., 2001). For instance, Soininen et al (1995) found no differences in normalized measurements of right or left hippocampal volume in elderly (mean age 69 years) *APOE* ε4-carriers, but reported that normal hippocampal right > left asymmetry was diminished. In a longitudinal study, Moffat et al (2000) found no *APOE* -related difference in absolute size at the start of the study, whereas *APOE* ε4-carrires (mean age 69 years) in comparison to non-carriers displayed a significantly steeper rate of hippocampal atrophy over a 3 year follow-up period. Similar results were reported by Cohen et al (2001).

Overall, findings of *APOE* ε4-related atrophy indicate that the MTL region is most affected, particularly on the right side (Tohgi et al., 1997; Soininen et al., 1995; Lehtovirta et al., 1995), whereas total cerebral volume seems not to differ by genotype (e.g. Cohen et al., 2001; Moffat et al., 2000; Lehtovirta et al., 1995; but see Yasuda et al., 1998, who reported larger global brain volume for *APOE* ε4-carriers). The finding of *APOE* ε4-related atrophy in relatively young subjects (Tohgi et al., 1997) raises the question whether smaller hippocampal volume in *APOE* ε4-carriers

represents early-onset atrophy or an inherent trait. Support for the former was presented by Rose et al (referred to by Scarmeas and Stern, 2006), who studied 50 healthy pediatric subjects at two occasions, at age 10 and 12, and found no evidence for a relationship between *APOE* status and hippocampal volume.

White matter findings

Although AD has traditionally been considered a disease of the grey matter, there are several reports of abnormal white matter integrity in AD patients (Englund et al., 1988; Meyer et al., 1992; Hanyu et al., 1998; Bronge et al., 2002; Bozzali et al., 2002; Yoshiura et al., 2002). In contrast to normal aging, which is commonly associated with white matter atrophy in frontal regions of the brain (Janowsky et al., 1996; Head et al., 2004), AD patients display greatest changes in posterior regions, preferentially in callosal fiber systems (Teipel et al., 1999; 2003).

In a study by Skoog et al (1998), it was found that the combination of white matter lesions and the presence of an *APOE* ε4-allele increased the risk for AD in an elderly population at the age of 85. This finding led Bronge and colleagues (1999) to further evaluate the relation between white matter lesions and the *APOE* genotype. Bronge et al (1999) studied T2-weighted MR images of AD patients and found that homozygous *APOE* ε44-carriers had more extensive white matter lesions than did heterozygous carriers and non-carriers. The authors also noticed the absence of an age correlation for white matter lesions in *APOE* ε4-carrires (in contrast to non-carriers), and concluded that white matter lesions in *APOE* ε4-carriers may represent a pathological process related to the aetiology of AD. De Leeuw et al (2004) investigated a possible interaction effect of *APOE* genotype and hypertension on white matter lesions, and found that ε4-carrires had significantly increased subcortical white matter lesion volume as compared to non-carriers, irrespective of hypertension. In a recent study, Nierenberg et al (2005) used diffusion tensor imaging (DTI) to compare healthy *APOE* ε4-carriers (mean age 68 years) and non-carriers (mean age 67 years). It was found that ε4-carriers had disrupted white matter microstructure in the parahippocampal gyrus, in the absence of regional or global atrophy.

Taken together, not many studies have examined white matter alterations in healthy *APOE* ε4-carriers. Hence, additional studies are needed before further conclusions can be drawn in this matter.

1.4.2 Functional studies

Resting-state findings

Resting-state studies of AD patients show a highly consistent pattern of reduced blood flow and glucose utilization in parietal, temporal, and posterior cingulate regions with later spreading to prefrontal cortices (e.g. Frackowiak et al., 1981; Smith et al., 1992; Lehtovirta et al., 1996; 1998; Mielke et al., 1998; Alexander et al., 2002). This typical distribution can differentiate AD from the metabolic abnormalities of other dementias, including Lewy body dementia, vascular dementia, and frontotemporal dementia (Silverman et al., 1999). Strikingly, similar reductions have been observed among non-demented *APOE* ε4-carriers (Small et al., 1995; 2000b; Reiman et al., 1996; 2001; 2004). For instance, Small et al (2000b) studied subjects with normal memory performance (mean age 66 years) and found that a single copy of the *APOE* ε4 allele was associated with lowered inferior parietal, temporal, and posterior cingulate cortical metabolism. Moreover, a longitudinal follow-up revealed that lower baseline metabolism predicted cognitive decline among the *APOE* ε4-carriers years later, and also that the greatest metabolic decline over time was observed in the parietal and temporal regions. Reiman et al (2004) extended these findings by observing similar metabolic reductions in cognitively intact *APOE* ε4-carriers even before the age of forty (age range 20-39 years).

Task-associated findings

In line with resting-state studies, it has been found that AD patients (Rombouts et al., 2000; Bäckman et al., 2001; Kato et al., 2001; Grossman et al., 2003; Machulda et al., 2003), as well as non-demented *APOE* ε4-carrires (Smith et al., 1999; 2005; Trivedi et al., 2006), display reduced neuronal activation in MTL and temporoparietal regions during performance of various cognitive tasks. Smith et al (1999) compared fMRI activation between *APOE* ε4-carriers with a family history of AD versus non-carriers with no family history of AD (mean age 52 years) during an object naming tasks (relative to a low-level control task). Carriers of *APOE* ε4 showed diminished task-related activity in the inferotemporal cortex, and a follow-up four years later revealed a greater longitudinal decline in fMRI response in the same region among the ε4-carriers (Smith et al., 2005). Trivedi et al (2006) examined the effects of *APOE* genotype on fMRI brain activation patterns during an episodic encoding task in cognitively normal individuals with a family history of AD (mean age 53 years). They found that *APOE*

ε4-carriers displayed an abnormal hippocampal response, i.e. reduced activation to novel relative to familiar items as compared to non-carriers. This was found in the absence of cognitive and hippocampal volume differences.

However, observations of reduced task-associated brain activity among at-risk subjects have not been consistent; several studies have reported increased, rather than decreased, response (Bookheimer et al., 2000; Smith et al., 2002; Bondi et al., 2005; Fleisher et al., 2005; Wishart et al., 2006; Han et al., 2007). Bookheimer and colleagues (2000) performed fMRI while 14 *APOE* ε4-carriers and 16 matched non-carriers (age range 47-82 years) with mild memory complaints but normal cognitive performance memorized and recalled unrelated pairs of words. When contrasted against resting periods, the magnitude and spatial extent of brain activation during memory performance was greater in several regions, including hippocampus, parietal and prefrontal regions among *APOE* ε4-carriers, as compared to non-carriers. In addition, *APOE* ε4-carrires performed worse on a delayed-recall test, and longitudinal assessment indicated that greater baseline brain activation correlated with verbal memory decline two years later. The authors suggested a compensatory hypothesis, whereby subjects at increased risk for AD need to perform additional cognitive work in order to accomplish the task. Bondi et al (2005) found that cognitively normal (mean age 76 years) *APOE* ε4-carriers showed greater fMRI response in the fusiform gyrus, parietal cortex and frontal gyrus compared to *APOE* ε33 carriers, when subjects had to discriminate novel pictures from a single repeating picture. This study also reported that *APOE* ε4-carrires displayed greater activation in the right MTL, but reduced activation in the left MTL, compared to ε33 carriers. In addition, there was a correlation between memory ability on a word list learning task and right and left hippocampal activation during picture encoding (positive in ε33-carires and negative or zero in ε4-carrires). Han et al (2007) assessed brain activity while subjects memorized novel and familiar word-pairs. They found that non-demented *APOE* ε4-carriers (mean age 77 years) displayed greater activation than non-carriers in multiple right hemisphere regions for previously encoded word pairs relative to fixation. Moreover, in contrast to non-carriers, *APOE* ε4-carriers displayed greater response to familiar words than to novel words in the right hippocampus, which is inversed to what is normally seen (i.e. novel > repeated).

In summary, there is conflicting data regarding the influence of *APOE* genotype on task-induced brain activations. Several demographic and methodological

differences might account for the incongruity, e.g. small or unequal sample sizes, recruitment methods, age and cognitive status of subjects, or proportion of heterozygous versus homozygous *APOE* ε4-carrires. Moreover, differential atrophy or definitions of regions of interest (ROIs) (c.f. Han et al., 2007; Vandenbroucke et al., 2004), choice of cognitive task (c.f. Burggren et al., 2002) and/or contrasting baseline task (high- or low-level) (c.f. Trivedi et al., 2006), task difficulty (c.f. Scarmeas et al., 2004; 2005), or failures to consider more than one gene (c.f. Espeseth et al., 2006) have been suggested to add to the inconsistency. Another critical factor that should be considered is individual variance among the *APOE* ε4-carriers. For instance, it may be that ε4-carriers that are predestined to develop AD differ from ε4-carriers that will remain healthy. Longitudinal large-scale studies, including well-characterized and individually matched subjects, are necessary to clarify this issue.

Nevertheless, taken together, there is some coherence in that the most prominent alterations appear in regions known to be early affected by AD pathology, including MTL and temporoparietal regions, and that these changes may occur in the absence of any morphological or behavioural differences. Thus, functional brain imaging seems to be a more sensitive tool than both structural imaging and neuropsychological testing in revealing early and subtle changes in subjects at increased risk for AD. However this conclusion needs to be verified in longitudinal studies.

2 HUMAN MEMORY

2.1 GENERAL OVERVIEW

This section will provide a general overview of human memory systems, with particular focus on episodic memory since it is of most relevance for the present empirical work.

Human memory consists of multiple systems that can be classified in several ways. Based on duration of retention, a basic distinction is made between sensory memory, short-term memory, and long-term memory (Purves et al., 1997). Sensory memory is characterized by the duration of memory retention from milliseconds to seconds and short-term memory from seconds to minutes, whereas information that can be retrieved in a period of time (from days to years) is called long-term memory. Additionally, the term working memory is used to refer to the short-term memory capacity needed for certain mental tasks – thus, it is not a synonym for short-term memory, since it is defined not in terms of duration, but rather in terms of purpose (Baddely and Hitch, 1974). The conversion of transient short-term forms of memories – that requires only covalent modification of pre-existing proteins – to more stable and self-maintained long-term memories is accompanied by morphological changes, including growth of new synaptic connections and increased protein synthesis (Frankland and Bontempi, 2005).

Long-term memory is commonly subdivided into different components and although the concepts and terminology used to characterize these memory systems has varied, there is a consensus concerning the broad division of human memory into non-declarative and declarative memory (Squire and Knowlton, 1995a) (Figure 2). Non-declarative memory subsumes gradual acquisition of various abilities that are not readily expressible in verbal form, as retention is not necessarily accompanied by conscious recollection of the original learning situation. Motor and cognitive skills and habits, perceptual priming, classical conditioning, and non-associative learning are commonly denoted non-declarative memories (Squire and Zola-Morgan, 1991). In contrast, declarative memory is under conscious control, in that some conscious process must retrieve the information (Cohen and Squire, 1980; Squire et al., 1993). Declarative memory can be further sub-divided into semantic memory, which concerns facts taken

independent of context, and episodic memory, which concerns information specific to a particular context, such as a time and place, and associated emotions (Tulving, 1972).

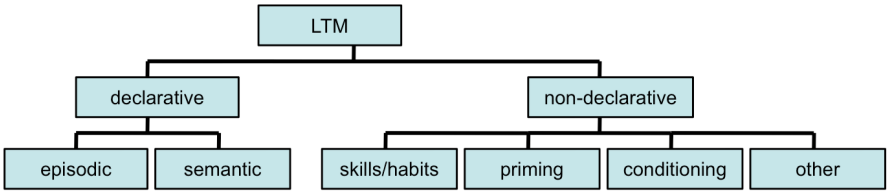

Figure 2. *Taxonomy of human memory systems.*

Episodic memory is the youngest memory system, phylogenetically and ontogenetically; it is assumed to exist in man and monkey only and is present at about two years of age in humans (Tulving, 2002). According to Tulving (2002) many operations of later memory systems depend on, and are supported by, the earlier systems, whereas earlier systems can operate essentially independently of the later ones. Following this assumption, the latest system to develop, namely the episodic memory, is most vulnerable to brain injury or disease. Indeed, this notion is supported by the current dementia research, indicating that episodic memory impairment are the earliest and most prominent clinical symptom of AD (Tierney et al., 1996; Bäckman et al., 2001).

From an information processing perspective there are three main stages in the formation and retrieval of declarative memory: (1) *Encoding*: the processing and combining of received information. Encoding can be intentional (i.e. done with intention or on purpose) or incidental (i.e. happen in a subordinate conjunction with something else). It is not clear what determines what will be encoded into memory, but attention-capturing or novel events tend to be more effectively encoded than repeated events (Nyberg, 2005; Ranganath and Rainer, 2003); (2) *Storage/consolidation*: creation of a permanent record of the encoded information. Storage requires changes to neural synapses, thought to be mediated through a cascade of molecular and microstructural events by which short-term synaptic modifications lead to permanent changes in connectivity between neurons (Eichenbaum, 2002); (3) *Retrieval/recall*: calling back the stored information in response for use in some process or activity. Retrieval can be unsupported (free recall) or supported with a cue or indication (cued recall). Another form of retrieval is recognition, i.e. to decide whether an encountered stimulus has been previously perceived or not.

Lesion-studies, together with functional neuroimaging of the healthy brain, have converged on the view that different memory systems are dependent on different brain structures (although some overlapping clearly exists) (Cabeza and Nyberg, 2000a). Declarative memory is critically dependent on MTL areas, in particular the hippocampal formation (Squire and Zola-Morgan, 1991; Nyberg et al., 1996b; Cabeza and Nyberg, 2000b), and has also been found to consistently engage prefrontal and parietal areas (Cabeza and Nyberg, 2000b). In contrast, non-declarative memory does not depend on temporal lobe function but rather involves the same sensory, motor, or associational pathways used during the learning process.

The following sections will give a basic overview of the main structures engaged by episodic memory processing, with main focus on the hippocampal region.

2.2 BRAIN REGIONS ASSOCIATED WITH EPISODIC MEMORY

2.2.1 The medial temporal lobe; the hippocampus

The MTL has multiple components; in addition to the hippocampal formation, the MTL also includes the parahippocampal region (including the perirhinal and entorhinal cortices) and the amygdala. Although the amygdala appears not to have an essential role in declarative memory, it is important for the emotional aspects of episodic memories (for reviews, see Sarter, 1985; Phelps and Anderson, 1997).

The hippocampal formation has a curved tubelike appearance and extends from the lateral neocortex of the MTL towards the midline of the brain. It consists of the dentate gyrus, Ammon's horn (divided into subfields CA1-CA3, and CA4, frequently called the hilus and commonly considered part of the dentate gyrus), and the subiculum. The hippocampal region is reciprocally connected to the posterior neocortical structures by the perforant path and to the frontal cortex and several subcortical areas by the fimbria-fornix. More anterior parts of the parahippocampal region project to anterior cortical areas and more posterior parahippocampal areas to more posterior cortical sites. (Eichenbaum, 2002; Kolb and Whishaw, 2003).

The hippocampal region was first identified as critical for declarative memory when Scoville and Milner (1957) reported severe loss of memory following bilateral removal of the MTL in their patient H.M. The case of H.M. – together with numerous subsequent reports – shows that the most prominent deficit following MTL damage is profound forgetfulness, so-called anterograde amnesia. Anterograde amnesia refers to the incapacity to encode and store new information in long-term memory, e.g.

information held in short-term memory is rapidly forgotten and not transferred to long-term memory trace. In addition to anterograde amnesia, MTL damage may also result in partial loss of memory for information aquired before the damage occurred, so-called retrograde amnesia. This type of amnesia is particularly prominent when the lesion is large. When the damage is restricted to the hippocampus and its related structures, the retrograde memory impairment appears to be temporally graded (Squire and Alvarez, 1995; Reed and Squire, 1998). In other words, the retrograde extent of the amnesia shrinks with the passage of time, often leaving a residual amnesia of only a few seconds to a minute for events immediately preceding the injury. This implies that more recently formed memories are relatively more impaired than remotely acquired information. In line with this, a widely held theory is that the hippocampus interacts with the neocortex in order to establish, maintain, and retrieve long-term memory, and that ultimately, declarative memories become (relatively) independent of the hippocampus (Squire and Alvarez, 1995).

The finding from human amnesia that the hippocampus is a key-structure for declarative memory functioning has been complemented by studies using brain imaging techniques; hippocampal activations have been consistently observed during various declarative memory performances (e.g. Tulving et al., 1996; Lepage et al., 1998; Cabeza and Nyberg, 2000b; Killgore et al., 2000; Davachi and Wagner, 2002). In addition, brain imaging studies have revealed that the degree of activation correlates with the nature of stimuli processing: during episodic retrieval, activations are greater for well-remembered than for poorly remembered words; during encoding, the degree of hippocampal activation appears to index stimulus novelty, as initially perceived stimuli typically evoke stronger neuronal activity than repeated stimuli (Stern et al., 1996; Tulving et al., 1996). Another common finding is that encoding activations tend to engage more anterior hippocampal regions, and retrieval activations more posterior hippocampal regions – a pattern called the hippocampal encoding/retrieval (HIPER) model (Gabrieli et al., 1997; Lepage et al., 1998; Zeineh et al., 2003).

2.2.2 The frontal lobe

In a meta-analysis by Wheeler et al (1995), it was shown that frontal lobe damage was associated with memory deficits for both recognition and recall, with a more pronounced impairment for recall. These results indicate that the prefrontal cortex (PFC) contributes to both encoding and retrieval processes. Indeed, several

neuroimaging studies have demonstrated consistent frontal activations during episodic memory encoding as well as retrieval (Cabeza and Nyberg, 2000a; Fletcher and Henson 2001; Rossi et al., 2001), and moreover, that frontal engagement is particularly prominent during retrieval (Cabeza and Nyberg, 2000a). Another common finding is that episodic memory encoding (and semantic memory retrieval) activations usually are left-lateralized, whereas episodic memory retrieval activations are mostly right-lateralized (Cabeza and Nyberg, 2000a; Rossi et al., 2001). This pattern is commonly referred to as the hemispheric encoding-retrieval asymmetry (HERA) model (Tulving et al., 1994; Nyberg et al., 1996a).

Although the precise functional role of the PFC in long-term memory is still under debate, a more general view states that the PFC subserves high-level "executive" processes needed for voluntary goal-directed behaviour – sometimes called *cognitive control* (Miller and Cohen, 2001). In line with this assumption, the engagement of the frontal lobe during declarative memory tasks have been referred to as *working-with-memory* (Moscovitch, 1992) or control processes that aid and optimize encoding and retrieval memory, rather than storage processes per se (Fletcher and Henson, 2001). Accordingly, damage to the frontal lobe does not result in global amnesia, but may cause significant deficits on declarative memory tasks that require more complex strategic reasoning or problem-solving (Poldrack and Gabrieli, 1997; Gabrieli, 1998). Indeed, this notion is supported by the findings of Wheeler et al (1995) that frontal patients exhibit greater deficits on (the more demanding) recall tests, than recognition tests.

2.2.3 The parietal lobe

Lesions to the parietal cortex are not generally associated with robust episodic memory deficits. Hence, traditionally, the parietal lobe has not been considered a critical contributor to declarative memory expression. However, recent brain imaging studies suggest that the parietal lobe indeed support declarative memory, in particular episodic memory retrieval processes (Cabeza and Nyberg, 2000a; Wagner et al., 2005). Considering the well-known involvement of the parietal lobe in (spatial) attention (e.g. Colby and Goldberg, 1999), a natural interpretation would be that memory-related effects in this region reflect attentional, rather than mnemonic, processes. However, several findings argue against this view; for instance, the engagement of the parietal lobe is frequently modulated by level of retrieval success (Cabeza and Nyberg, 2000a),

and parietal regions show increased activity when individuals correctly recognize items to be old, as compared with correctly rejecting new items [*old/new effects* (Wagner et al., 2005)]. Moreover, parietal activation seems to increase when retrieval is oriented towards event-specific details, as compared with just detecting item familiarity.

Based on anatomical connections and similarities in activation patterns, it has been suggested that the parietal lobe participates to mnemonic processes by integrating prefrontal executive functions and MTL declarative memory functions (Wagner et al., 2005; Naghavi and Nyberg, 2005).

In addition to the regions mentioned above, other brain structures have also been reported as important for long-term declarative memory, including the lateral temporal lobes (Murray and Bussey, 1999; Cabeza and Nyberg, 2000a), the medial portion of the diencephalons [thalamus and the mammillary bodies (Squire and Knowlton, 1995b; Vann and Aggleton, 2004)], the anterior cingulate cortex (ACC) (Cabeza and Nyberg, 2000a), as well as the cerebellum (Andreasen et al., 1999).

3 BRAIN IMAGING

3.1 GENERAL OVERVIEW

Brain imaging includes the use of various techniques to either directly or indirectly image the brain and falls into two broad categories: structural imaging and functional imaging. Structural imaging is used to measure brain volume or the volume of subregions, or to look at diffuse changes in grey or white matter or to assess localised lesions. Functional imaging detects changes in regional cerebral blood flow or metabolism as an indirect measure of neural activity. This can be used to map patterns of brain activity that corresponds to various mental operations.

Common techniques used for structural imaging are computed tomography (CT) or magnetic resonance imaging (MRI). CT is likely one of the least expensive imaging modalities, but involves radiation. Hence, there are limits to the total number of scans that can be performed safely within an individual. Also, CT scanning displays very prominently those things which most deflect the x-ray, such as bone, which makes CT excellent for examining skull fractures but also very difficult to read for the cerebellum and base of the skull. MRI does not involve any radiation, but uses magnetic fields and radio waves to produce high quality two- or three dimensional images. Hence, in contrast to CT, MRI can be used repeatedly on a single subject. This is highly valuable since it permits longitudinal studies and also improves the signal-to-noise ratio. Structural MRI makes it possible to image both surface and deep brain structures with a high degree of anatomical detail.

For functional brain imaging there are several techniques; usually, the choice is based on whether high temporal versus high spatial resolution is of main interest. Electrophysiological methods can provide near real-time temporal accuracy (10-100 ms) for the recorded neuronal activity by measuring either the electric field change [e.g. electroencephalogy (EEG)] or magnetic field change [magneto-encephalogy (MEG)] associated with the neuronal depolarization. However, because the measurement is performed outside the skull, the signal is distorted and “smoothed” by the bone with loss of spatial resolution in result. In contrast, the so called hemodynamic techniques [positron emission tomography (PET); single-photon emission computerized tomography (SPECT); functional magnetic resonance imaging (fMRI)] measure neuronal activity indirectly through the associated changes in

metabolism or blood flow. This provides a relatively high spatial resolution (1-10 mm) but rather low temporal resolution (hundreds of milliseconds for fMRI, several seconds for PET and SPECT), being limited by the rate of the much slower hemodynamic changes that accompany neuronal activation. Measure of the cortical blood flow is also performed by optical imaging methods (near infrared spectroscopy; NIRS). However, this technique is limited by light scattering that occurs in the skull and by the inability to measure brain structures below the surface. Another useful technique is to measure activity in single neurons by single-cell recordings in patients and in animals, such as rats or monkeys. This makes it possible to study how single cells fire in response to different kinds of stimuli. Since this technique is mainly used on animals, it is not optimal for studying higher cognitive functions.

3.2 MAGNETIC RESONANCE IMAGING

MRI is a completely non-invasive method that can be used to study both brain anatomy and function (fMRI). It is named for its use of a large magnet (M) and a radiofrequency pulse of a certain resonance (R) to generate a signal from the brain in order to produce an image (I). During the MRI session, the subject is placed horizontally into the bore of a high field magnet, typically with a field strength of 1.5 Tesla (which is more than 25000-times the Earth's magnetic field) or greater. The technique is based on the principle that a hydrogen atom's nucleus, which consists of a single proton, behaves like a spinning dipole bar magnet with a north- and a south pole. (All soft tissues contain water, which contains hydrogen.) Ordinarily, protons are oriented at random, meaning that any given piece of tissue has no net dipole. However, when placed in a magnet field, the spinning protons orient themselves with respect to the field's lines of force and thus all line up in parallel. When a radio frequency force field that resonates with the target protons is applied on the magnetic field the atoms are transferred to higher energy state – the tiny bar magnets are "flipped". Such flipped protons will now have two motions: their spin around their own axes and a spin around their longitudinal orientation. The latter spin is like a wobble in a spinning top and is called precession. Precession creates a rotating magnetic field that changes in time and according to Faraday's law generates an electrical current. When the radio frequency pulse is turned of, the excited protons relax back to their lower energy state, whereby a small amount of energy is transferred to the environment. Ultimately, it is this change in electric current that is measured in MRI.

Two kinds of relaxation processes take place: the protons that were rotating together are beginning to fall out of synchrony with one another, and their axes become aligned with the original magnetic field. The two relaxation processes are characterized by time constants T1 and T2. The T1 relaxation component is the "righting" of flipped protons as they realign with the original magnetic field. The rate of this relaxation is quite long (from tens of ms to seconds), and influenced by non-excited molecules in the surrounding tissue. The T2 relaxation component is the "dephasing" of the rotated protons. Dephasing occurs relatively quickly (from a few ms to tens of ms) and results largely from loss of energy to nearby spinning nuclei. Due to magnetic field inhomogeneity in the scanner, spins also loose energy to the surrounding lattice; this variant of the T2 relaxation is called T2*. The T2* time is less than the T2 time. In T2 imaging, spins are refocused to compensate for local magnetic field inhomogeneities, whereas T2* imaging is performed without refocusing. T2* imaging sacrifices some image integrity (resolution) in order to provide additional sensitivity to spin dephasing (as compared to T2) and is commonly used for fMRI.

Protons have different relaxation rates and corresponding T1 and T2 time constants depending on their surroundings, i.e. whether they are in fat, cerebro spinal fluid, neurons, bones, or other tissues. These differences in time constants can be translated into image contrast. Either T1 or T2 can be used, though one may be more suitable than the other in a given situation. Modifications to the pattern of radio frequency excitation (the "pulse sequence") modulate the contribution of T1 versus T2 to the resulting MR signal.

Three-dimensional readings are accomplished by using magnetic gradients, i.e. magnetic fields in which the strength of the field changes gradually along an axis. One magnetic gradient is used to excite a single "slice" of the subject's brain; two more gradients subdivide that slice into rows and columns. Applying gradients along three axes thus subdivides the tissue into individual cuboid elements (called voxels), each having a unique signal.

3.3 DIFFUSION TENSOR IMAGING

Diffusion-weghted MRI – or diffusion tensor imaging (DTI) – measures the amount of non-randomness (anisotropy) of water diffusion within tissues. Diffusion contrast is introduced into images by the application of two field gradient pulses between the excitation of the protons and the acquisition of the signal. The first gradient pulse

causes a dephasing of the spinning protons, where the phase is dependent on their positions along the field gradient. A shorter time later, a second gradient pulse of equal shape and size but opposite polarity is applied. If the protons have not moved in the period between the two pulses then the second gradient pulse will completely rephrase the effects of the first gradient pulse. Protons that have diffused a distance along the gradient will not be fully rephrased by the second gradient and the result is a loss of MR signal in regions of molecular diffusion. By applying diffusion gradient pairs in a number of directions a full map of the diffusion coefficient in every direction is possible. Such information is known as the diffusion tensor. Based on the diffusion tensor, the degree of fractional anisotropy (FA) can be determined and mapped.

The degree of anisotropy is particularly high in brain white matter, as the oriented axons allow less restricted diffusion of water along the axons, but more restricted diffusion perpendicular to them. Neurodegenerative processes that cause changes at the microstructural level (such as the rate of myelination or demyelination, degradateion of microtubules, or loss of axonal structure) cause a significant measurable decrease in anisotropy. Hence, DTI is a powerful technique for the assessment of white matter structural integrity and connectivity (Moseley, 2002; Moseley et al., 2002; Masutani et al., 2003).

3.4 FUNCTIONAL MRI

FMRI detects changes in regional cerebral blood flow (rCBF) as an indirect measure of neural activity. The basis for this inference is an assumtion of a roughly linear coupling between neural activity, metabolic activity, and rCBF (Scannell and Young, 1999; Logothetis et al., 2001). In other words, increases in neural activity in a given brain region will increase the energy consumption, which in turn implies increased blood flow in order to supply the neurons with glucose and oxygen. However, for unknown reasons, the increased supply of oxygenated blood exceeds oxygen utilization (Fox and Raichle, 1986). As a consequence, the ratio of oxygenated to deoxygenated blood will be higher than normal in areas of increased metabolism. Oxygenated hemoglobin is diamagnetic (i.e. essentially nonmagnetic) whereas deoxygenated hemoglobin is paramagnetic, meaning that deoxyhemoglobin disturbs the applied magnetic field more than oxyhaemoglobin does. Thus, as the relative amount of deoxyhemoglobin decreases in activated brain areas – due to unproportionally increases in blood flow – the MR signal increases. This effect is termed the BOLD (blood-oxygen-level-

dependent) effect (Ogawa et al., 1992) and is the major source of contrast in most fMRI experiments.

Noteworthy, although both excitatory and inhibitory neural activity is energy-consuming (Nudo and Masterson, 1986), several lines of evidence suggest that the BOLD effect is more closely related to excitatory, rather than inhibitory, neural processes (Shinohara et al., 1979; Waldvogel et al., 2000).

3.4.1 Experimental considerations in fMRI

This section will give a short overview of some of the methodological issues that must be considered when performing fMRI research.

Choice of task

The brain is always highly active, even at times of rest. Hence, functional brain maps are typically generated by calculating the BOLD-signal *differences* between two states of neural activity. One way to design an experiment is to create two experimental conditions that differ only with regard to the cognitive function of interest. This is sometimes called a "tight" task comparison and is particularly useful for testing specific hypothesis about the activation pattern in a single brain region. In contrast, a "low-level control task", such as simple visual fixation or rest, is particularly useful for seeing the simultaneous activation of many regions of the brain. A loose task comparison may also serve as an important point of reference for the observed differences within the tight task comparison. For instance, a difference between two conditions in a tight task comparison could reflect either an increase in activity in one condition, or a decrease in activity in the other. The addition of a loose task comparison provides a means of disambiguating such a situation by providing a baseline against which the two tight conditions can be compared.

Experimental design

There are two basic design paradigms used in fMRI research: block and event-related (e.g. Friston et al., 1999; Birn et al., 2002). In the block design, series of the same type or stimuli are presented during a specified length of time ("blocks"). Blocks of the experimental task are alternated with blocks of rest or a control task. The idea here is to set up a "steady state" of neural and hemodynamic change; the fact that there is a brief delay in the hemodynamic response – and hence in the BOLD signal – to increased

neural activity is often unimportant when analysing a long block of steady-state activity. This technique maximizes statistical power and is thus optimal for *detecting* small changes in brain activity. The major weakness of block design is that it precludes randomized presentation of individual stimuli, which makes the stimulation susceptible to habituation effects. The second and more recently developed design paradigm is called event-related (e.g. Buckner et al., 1996). Event-related studies observe the change in activity immediately after a single stimulus has been introduced. Because the BOLD responses to single events generally summates linearly, it is possible to then go back and isolate the BOLD responses related to individual trials. An event-related design has less statistical power than a block design, but has the great advantage that it allows researchers to link specific responses to specific behavioural stimuli and to randomize trials. Typically, block designs are used to measure sustained processes related to a state, while event-related designs are used to measure transient processes related to specific items.

Preprocessing of data

In a typical fMRI session a low-resolution functional volume is acquired every few seconds. Usually, 100 volumes or more are collected over the course of the experiment. Each volume is made up of cuboid voxels. Hence, an fMRI data set can either be seen as *t* volumes, one collected every few seconds, or as a large set of voxels, each with an associated time-series of *t* time points.

The fMRI measurements are always contaminated with artefacts, such as head movement and physiological vascular changes. To overcome these difficulties, the images need to be preprocessed before further analyses. The usual steps includes: (1) *Realigning* the slices to reduce head movement related artefacts. This makes the voxels of different time points match spatially and removes some of the empty area surrounding the head in the images. (2) *Normalizing* the volumes into standard standard brain shape and size. This allows data to be averaged across scans and subjects, wich is necessary for group studies. (3) *Smoothing* or low-pass filtering whereby each voxel is replaced with an avereage of that and the surrounding voxels. This increases the signal-to-noise ratio of the hemodynamic effects and makes detection of interesting phenomena easier. The size of the area that is smoothed is referred to as a “kernel”.

Statistical analyses

After the preprocessing steps, statistical analysis is carried out to detect those parts of the brain (voxel-by-voxel) that show increased MR signal at the specific points in time

when the subject responded to the experimental condition of interest (as compared to a resting state or control condition). The general linear model (GLM) (Worsley and Friston, 1995) is currently the most popular statistical approach; it reveals those time-series (voxels) that best correspond to a reference time-course. The reference time-course is based on the experimental design (i.e. the stimulation pattern over time) and the shape of the hemodynamic response. The predetermined pattern of activation that one expects to see in the data is referred to as the "model". A good fit between the collected data and the model means that the activation was probably caused by the stimulation. The main output from these statistical analyses is a functional image which indicates those points where the brain was activated in response to the stimuli. A following step is to segment the image into inactive and active areas, by using a statistical threshold for the probability of activation. (See Figure 3 for an overview of functional neuroimaging data analysis.)

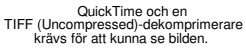

Figure 3. *General outline of functional neuroimaging data analysis. The primary functional neuroimaging data are commonly preprocessed, i.e. realigned, anatomically normalized, and spatially and temporally low-pass filtered; a statistical model for the data is created; model parameters are subsequently estimated and a test statistic is chosen in order to conduct for statistical interference. (From the SPM homepage http://www.fil.ion.ucl.ac.uk/spm/ with permission.)*

The results are represented both graphically, by superimposing thresholded statistical maps of functional activity upon high-resolution anatomical images, and numerically, in terms of neuroanatomical peak activations within an area of the brain. The location of peak activations is indicated by three-dimensional

coordinates (x, y, z) in reference to stereotaxic brain atlases, such as Talairach and Tournoux (1988) or the atlas from the Montreal Neurological Institute (MNI). The use of a standard metric for localization makes it possible to compare results across experiments and between research groups.

Analyses of fMRI data can be performed on the whole brain (whole-brain approach) or only on specified regions (regions-of-interest; ROIs) of the brain that are selected and considered relevant by the experimenters. Generally, the whole-brain approach is more exploratory, whereas the ROI approach is much more specific or hypothesis-driven. Each approach has pros and cons and the type of analyses chosen should be based on the type of questions the study is aimed at answering.

There are a variety of statistical methods for combining results across sessions or subjects to either create a single result for a group of subjects, or to compare different groups of subjects. These methods include “fixed-effects” and “random-effects” analysis. Fixed-effects assume that all subjects activate equally, and are only interested in within-subject variability. Random-effects analysis also takes into account between-subject variability, and therefore makes fewer assumptions about the data; random-effects results can thus be seen as valid for the whole population from which the group of subjects was drawn (whereas fixed-effects results can only be seen as valid for the particular group of subjects that was sampled). The random-effects results tend to give more conservative results.

4 AIMS

The general aim of this thesis was to study structural and functional brain alterations in relation to the *APOE* genotype in healthy adults (*APOE* ε4-carriers versus non-carriers, and also homozygous versus heterozygous carriers of *APOE* ε4). Since *APOE* ε4 is associated with increased risk for AD, these results may be of importance for attempts at identifying markers of impending dementia.

The specific objectives were:

- To investigate how the *APOE* genotype would influence functional brain activity during the performance of an incidental episodic encoding task. (Study I)
- To investigate the influence of the *APOE* genotype on brain white matter. (Study II)
- To compare hippocampal volume and recognition memory performance for *APOE* ε4-carriers and non-carriers. (Study III)
- To examine whether functional brain alterations among *APOE* ε4-carriers forecast longitudinal episodic memory decline. (Study IV)

5 METHODS

5.1 THE BETULA PROJECT

All research subjects were collected from the ongoing longitudinal *Betula Prospective Cohort Study: memory, health, and aging*. For a detailed description of the Betula study and its design, sampling procedures, and battery of measures, see Nilsson et al (1997; 2004). In short, the Betula data-base contains more than 4000 persons that were randomly selected from the population register in Umeå – a city with a population of about 100,000 inhabitants located in the northeast of Sweden. The main purpose of the Betula project is to explore the development of memory functions in adulthood, and to determine risk factors and preclinical signs of dementia. All included participants go through extensive cognitive testing, medical examination (including *APOE* genotyping), and interviews regarding critical life events and socio-economical issues. The memory examination covers a wide variety of processes and hypothetical memory systems; included tests assess, for example, short-term and long-term memory processing, semantic memory, episodic memory, priming, and attention.

Several independent samples are included in the project: S1, S2, S3, and S4. Subjects in each sample belong to ten different age groups (cohorts): from 35-40 years to 80-85 years. Participants in the first sample (S1) have been tested at four occasions (1988-90, 1993-95, 1998-2000, and 2003-2005). S2 and S3 were included in the 1993-1995 wave of testing and S4 1998-2000. Every cohort contained 100 participants at the first assessment, except for sample S4 which comprised 50 participants per cohort. Participants were contacted by mail and participation was voluntary. Those with severe visual and auditory handicaps, mental retardation, dementia, or whose first language was not Swedish were not included.

5.2 STUDY SAMPLE

All studies included in this thesis are based on data that were collected at one occasion from the same study sample, including in all 60 healthy individuals with normal memory performance (age range 49-79 at time of recruitment). Thirty subjects were carriers of at least one copy of the *APOE* ε4: 10 were homozygous (ε44) and 20 were heterozygous (ε34). The remaining 30 subjects carried two copies of *APOE* ε3 and

served as controls. To examine possible genetic dose-effects (Study I and II), three subgroups consisting of 10 subjects each were composed: *APOE* ε44, *APOE* ε34 and *APOE* ε33. The extensive Betula sample pool served as an excellent base for selecting homogenous subgroups; participants were closely matched according to sex, age and length of education. Critically, all accessible data imply that all participants were cognitively intact at time of recruitment (c.f. Study I for details). Sample (N=60) characteristics are given in Table1.

Table 1 Sample characteristics

	APOE ε4[1] (N=30)	*APOE* ε33 (N=30)	*APOE* ε44 (N=10)	*APOE* ε34 (N=10)	*APOE* ε33 (N=10)
Female/Male	19/11	18/12	9/1	7/3	8/2
Age	65.3 (7.9)	66.6 (8.3)	61.2 (9.4)	65.0 (8.5)	64 (11.1)
Range	49-74	49-79	49-74	49-74	50-79
Education (yrs)	10.6 (3.5)	10.2 (3.3)	11.7 (3.1)	10.7 (4.0)	11.8 (3.1)
Range	6-17	6-16	8-16	6-17	9-16
MMSE	28.2 (1.5)	27.9 (1.7)	28.5 (1.4)	28.4 (1.4)	28.1 (2.1)
Range	24-30	24-30	26-30	26-30	24-30
SRB	25.0 (2.4)	22.6 (4.8)	23.7 (2.8)	25.3 (2.4)	23.2 (3.8)
Range	16-29	11-29	16-26	22-29	17-28
AD in family (N)	2	0	0	1	0

Note. Means and standard deviations (in parenthesis). MMSE = Mini Mental State Examination (Maximum = 30). SRB = Word comprehension (Maximum = 30). AD in family = 1st degree family history of AD. The three right-most columns represent the matched subgroups. [1]Carriers of at least one copy of the *APOE* ε4 allele: 10 with *APOE* ε44; 20 with *APOE* ε34.

Study I-III included the whole study sample (N=60), while Study IV only included 18 *APOE* ε4-carriers that were collected and divided into two groups on basis of their longitudinal memory performance ("decline" versus "non-decline"; measured at two occasions approximately five years apart, as a part of the Betula project). Three episodic memory tests were used to determine longitudinal memory performance: (i) yes/no face recognition; (ii) recall of performed tasks; (iii) verbal recall (for detailed descriptions of the tests, see Nilsson et al 1997).

5.3 COLLECTION OF BRAIN IMAGING DATA

Three types of MRI data were collected and analysed: (1) fMRI, to study task-associated functional brain activity (Study I and IV); (2) DTI, to investigate brain white matter (Sudy II); and (3) structural MRI, to measure hippocampal volume (Study III).

This section will provide a brief description of the methodologies, please see each study for more details.

5.3.1 fMRI

Measurements were taken while subjects performed semantic categorization (abstract or concrete), in order to promote incidental encoding of a word list (Wagner et al., 1998; Kirchhoff et al., 2000). Semantic categorization was chosen as the experimental task because it is fairly simple and robust; it produces a consistent activation pattern, including temporal, parietal, and (in particular) frontal regions, mainly left-lateralized (Wagner et al., 1998). A blocked-task paradigm was used, alternating between the experimental task and a low-level control condition (visual fixation) (Demb et al., 1995). Of main interest was whether genetic risk would modulate activity in task-engaged regions. In addition, we were interested to see if *APOE* genotypes would affect hippocampal responding, as this region is one of the earliest to show pathological signs in AD (Braak and Braak, 1997; Fox et al., 2001). An effective way to assess hippocampal activation is by contrasting processing of novel items versus familial items (Knight, 1996; Tulving et al., 1996; Hariri et al., 2003). Therefore, two (of four) categorization blocks included novel words (i.e. words that had not been presented previously during the study phase) and the other two included words that had been presented twice prior to functional scanning.

During functional scanning, subjects indicated their categorization decisions (abstract or concrete) by pressing one of two buttons, using the right index and middle finger. Behavioural performance was recorded for categorization accuracy and reaction times. In addition, a word recognition test was administered after the scanning session had ended (consisting of previously studied and unstudied words).

All functional images were pre-processed and analyzed using SPM99 (Wellcome Department of Cognitive Neurology, UK, http://www.fil.ion.ucl.ac.uk/spm/) implemented in Matlab 6.1 (Mathworks Inc.). Prior to analysis, all images were realigned to the first image volume required. The images were then normalized to a standard brain shape as defined by the SPM99 MNI EPI template (Evans et al., 1993; Friston et al., 1995), and finally spatially smoothed with a 6.0 mm full-width at half-maximum Gaussian kernel. First-level single-subject statistical contrasts were created using the general linear model. Statistical parametric maps were generated using t-statistics to identify regions activated according to the

model. Second level (random-effects) exploratory whole-brain contrasts were thresholded at $P < 0.001$ (uncorrected) with an extent threshold of 50 contiguous voxels (Study I) or $P < 0.05$ corrected for multiple comparisons (Study IV). Group contrasts with prior anatomical hypotheses were investigated at high sensitivity by defining spherical search volumes encompassing the region of interest; reported activations survived a small volume false discovery rate correction at $P < 0.05$ (Study I and Study IV). A ROI approach was used to further characterize the regions in which the BOLD response was found to differ between groups as a function of genotype (Study I) or longitudinal memory-performance (Study IV).

5.3.2 DTI

The DTI sequence was repeated four times and included six sets of diffusion gradients. The images were averaged using a script implemented in Matlab 6.1 (Mathworks Inc.) and subsequently processed using a custom toolbox in SPM99 (Wellcome Department of Cognitive Neurology, UK, http://www.fil.ion.ucl.ac.uk/spm/) that calculated the diffusion tensor eigenvalues voxel-by-voxel. FA maps were then generated and smoothed with an 8.0 mm full-width at half maximum Gaussian kernel. Three ROIs – including the genu, splenium, and body of the corpus callosum – were manually outlined on multiple slices on non-diffusion images acquired along with the DTI images. The ROIs were then superimposed on the FA maps and mean values for each region and for each subject were calculated. In addition, a whole-brain analysis was performed to examine the effects of *APOE* status on a map-wise basis by contrasting the FA maps of *APOE* ε4 non-carriers versus carriers. Effects from these analyses were regarded as significant if they reached a threshold of 0.005, uncorrected for multiple comparisons.

5.3.3 Structural measurements; Hippocampal volume

Manual tracing of the hippocampal formation was performed on T1-weighted structural images (c.f. Study III for MRI details). The right and left hippocampus formation was manually traced on every other coronal slice (Figure 4) using a computer mouse and measured with NIH Image public domain software (version 1.20; http://rsb.info.nih.gov/nih-image/). Beginning rostrally, the first slice used was the one where the mammilary bodies were clearly visible, whereas the caudal boundary was

marked by the slice showing the fornices rising from the fimbria. To separate the rostral part of the hippocampus from the adjacent amygdala, the temporal horn of the lateral ventriclewas used as a border-indicator. Medially, the subiculum was demarcated from the cortex of the parahippocampal gyrus by tracing the subiculum to its most medial position and drawing a horizontal line at its medial curve. Any part of the subiculum above this line was included as a part of the hippocampus. The total number of slices used to outline the hippocampus varied between 17 and 23 per subject. All measurements were performed by the same operator (J.L.) who was blind to the characteristics of the participants. Body height was used to adjust the hippocampal volumes via the analysis of covariance formula (c.f. Rodrigue and Raz, 2004; Persson et al, 2006).

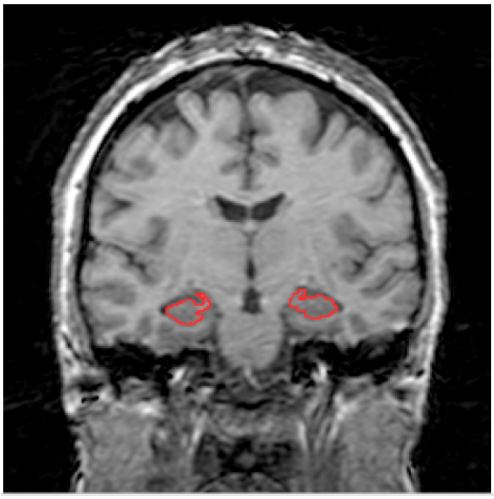

Figure 4. *The right and left hippocampus formation was manually traced on coronal T1-weighted images.*

6 SUMMARY OF STUDIES I-IV

6.1 THE INFLUENCE OF THE *APOE* GENOTYPE ON FUNCTIONAL BRAIN ACTIVATION

At present there is conflicting data on the relationship between *APOE* ε4 and brain activity in non-demented subjects; while some studies report that *APOE* ε4 is associated with reduced functional brain activation (e.g. Smith et al., 1999), others have found increased activation (e.g. Bookheimer et al., 2000). In order to address this issue further, we conducted this large-scale fMRI study of healthy carriers and non-carriers of *APOE* ε4.

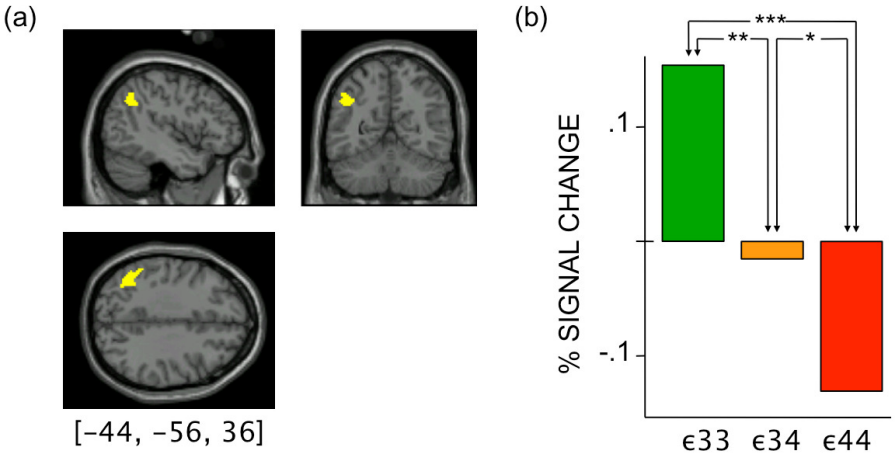

Figure 5. *Dose-dependency of the APOE ε4 in the activation pattern: subgroups comparison (ε33 vs. ε34 vs. ε44) of left parietal response:* ***(a)*** *The anatomical search was constrained by means of a functional ROI derived from the main group contrast (ε4 non-carriers > ε4 carriers; N=60).* ***(b)*** *The dose of APOE ε4 predicted failure to recruit the left parietal region (BA 39).*

Measurements were taken while participants performed a simple semantic categorization task (in order to promote incidental encoding). Behavioural results [word classification accuracy, response time, and post-scan recognition memory (hits-false alarms)] showed no differences between groups. In contrast, fMRI data analyses revealed that *APOE* ε4-carriers had significantly lower task-associated brain activity in the left inferior parietal cortex and bilaterally in the anterior cingulate region,

as compared to non-carriers. In addition, for the first time with fMRI, a genetic dose-related effect was observed in the parietal region such that homozygous *APOE* ε4-carriers exhibited greater reduction than heterozygous carriers (Figure 5). Moreover, contrasts of processing novel versus familiar items revealed an abnormal response in the right hippocampus in the *APOE* ε4 group, mainly expressed as diminished sensitivity to the relative novelty of stimuli.

Collectively, these observations demonstrate that genetic risk for AD, in symptom free individuals, translates into reduced functional brain activity in regions pertinent to the disease.

6.2 THE INFLUENCE OF THE *APOE* GENOTYPE ON BRAIN WHITE MATTER INTEGRITY

Not many studies have addressed the influence of the *APOE* genotype on brain white matter in healthy subjects, and no study before us has used DTI in this matter. Of main interest was whether *APOE* ε4-carriers would exhibit alterations in posterior parts of the brain, as this has been demonstrated for patients with AD (e.g. Teipel et al., 2003).

Three ROIs were examined (the genu, body, and splenium of the corpus callosum). As hypothesized, carriers of the *APOE* ε4 allele showed a significant decline in FA values in the posterior part of the corpus callosum (splenium). This difference was significant for both young (< 65 years of age) and old (> 65 years of age) individuals. We also tested for an allelic dose effect but found no differences between heterozygous and homozygous *APOE* ε4 carriers. Exploratory whole-brain analysis revealed reduced FA values in *APOE* ε4-carriers as compared to non-carriers in the occipitofrontal fasciculus and the body/posterior corpus callosum. We also found a difference between carriers and non-carriers in the left hippocampus.

These findings are in line with previous observations in AD patients, and suggest that the presence of *APOE* ε4 may influence microscopic white-matter integrity before onset of AD.

6.3 THE INFLUENCE OF THE *APOE* GENOTYPE ON HIPPOCAMPAL VOLUME AND RECOGNITION MEMORY

Hippocampal atrophy is a critical structure feature of AD but may also be induced by normal aging. Several studies indicate that *APOE* ε4 is associated with more prominent hippocampal atrophy as compared to other *APOE* alleles, both in AD patients (e.g. Lehtovirta et al., 1995) and in non-demented subjects (e.g. Soininen et al., 1995; Tohgi et al., 1997). In AD patients, the degree of hippocampal atrophy tends to correlate with clinical status (Jack et al., 1992; Laakso et al., 1995; De Leon et al., 1997), whereas studies of a similar structure-function relationship in non-demented subjects have yielded very varying results (for a review, see Van Petten, 2004).

We compared hippocampal volume and recognition memory performance between carriers and non-carriers of *APOE* ε4. The hippocampal volume was manually outlined on T1-weighted MRI images. We observed reduced right hippocampal volume in *APOE* ε4-carriers compared to non-carriers, and found that the difference was most pronounced before the age of 65. Furthermore, the *APOE* ε4-carriers made significantly more false alarms in the recognition-memory test, and the number of false alarms correlated significantly with right hippocampus volume.

These results indicate that relatively young non-demented individuals at genetic risk for AD have smaller hippocampal volume and lower performance on hippocampal-dependent cognitive tasks, relative to control individuals.

6.4 DOES FUNCTIONAL BRAIN ALTERATIONS PREDICT MEMORY DECLINE?

Functional abnormalities in the parietal cortex have been reported for AD patients as well as for those at genetic risk. In line with this, we observed diminished functional activation in a left-sided parietal region in non-demented *APOE* ε4-carriers (Study I). The purpose of this study was to examine whether diminished BOLD fMRI response in the same parietal area longitudinally predicted episodic memory decline within the group of *APOE* ε4-carriers.

Behavioural data was collected at two test occasions, approximately five years apart (before and after the fMRI data collection), and the participants were divided into two groups based on their longitudinal memory performance ("decline" versus "non-decline"). Both groups showed equal behavioural performance at the initial

examination as well as at the time for fMRI data collection. Analyses of fMRI data showed significantly lower parietal activation for those *APOE* ε4-carriers that later dropped in episodic memory performance, as compared to those that remained stable. The locus of this effect overlapped with the previously observed region (Study I). Further analyses revealed that the parietal activation correlated significantly with the relative change in episodic memory performance over time (Figure 6). In other words, lower BOLD fMRI response in the left inferior parietal area was related to poorer subsequent memory performance.

This is the first fMRI study to report an actual relationship between functional brain alterations and future cognitive decline in persons at genetic risk for AD. Our results emphasize the value of the parietal cortex for preclinical detection of AD, and further support the notion that a combination of genetic information with neuroimaging and behavioural data represent a promising route for early diagnosis and for monitoring disease progression.

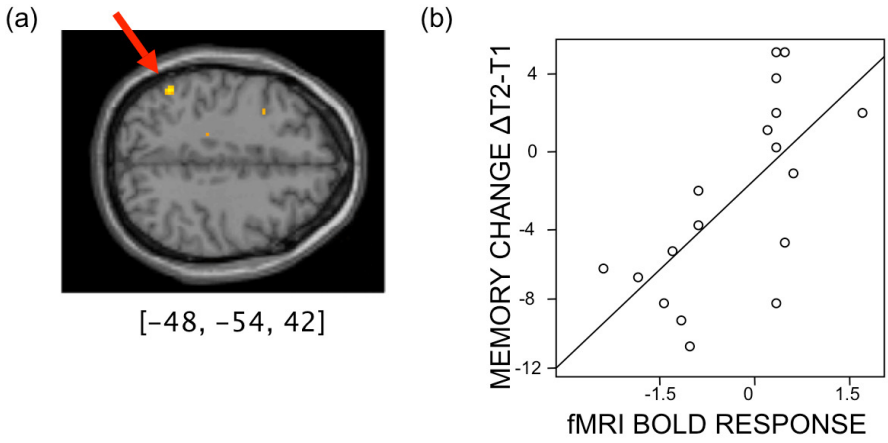

Figure 6. ***(a)*** *The results showed diminished parietal response (BA 39) for APOE ε4-carriers who later experienced memory decline. The location of this effect overlapped with that of a region in which activity previously was found to differentiate cognitively intact APOE ε4-carriers from non-carriers (Figure 5a).* ***(b)*** *The parietal response correlated significantly with subsequent episodic memory change, such that lower parietal activity was related to poorer subsequent memory.*

7 DISCUSSION

The general aim of this thesis was to study structural and functional brain alterations in relation to the AD risk gene *APOE* ε4 in healthy adults. By using MRI techniques, we found that carriers of *APOE* ε4 expressed reduced functional brain activity during the performance of an incidental episodic memory task (Study I), and also that *APOE* ε4-carriers had structural changes in both white (Study II) and grey matter (Study III) as compared to non-carriers. Moreover, we were able to demonstrate that functional brain alterations predicted longitudinal episodic memory decline within the group of *APOE* ε4-carriers (Study IV). Specifically, alterations were seen in the hippocampus and the parietal cortex, i.e. regions that are known to be affected early in the course of AD.

7.1 *APOE* AND CHANGES IN THE HIPPOCAMPAL REGION

In Study II, by using DTI, we observed that carriers of *APOE* ε4 had reduced FA values in the hippocampal region as compared to non-carriers. This finding likely relates to microstructural white matter changes, e.g. disrupted hippocampal-parahippocampal connectivity (Powell et al., 2004). White-matter changes in *APOE* ε4-carriers could possibly be explained by grey matter hippocampal atrophy affecting the surrounding white matter integrity through Wallerian degeneration (Pierpaoli et al., 2001).

Support for this assumption was presented in Study III, where we demonstrated that the *APOE* ε4-carriers had reduced right-sided hippocampal volume compared to non-carriers. This finding is consistent with several studies showing that *APOE* ε4 is associated with increased hippocampal atrophy, both in AD patient (e.g. Lehtovirta et al., 1995, Juottonen et al., 1998) and in non-demented subjects (e.g. Plassman et al., 1997; Cohen et al., 2001). We also noted that the hippocampal volume difference was most pronounced before the age of 65 (possibly as a function of normal age-related hippocampal atrophy among non-carriers in older ages). This is in keeping with findings reported by Tohgi et al (1997), who observed right-sided hippocampal atrophy in non-demented *APOE* ε4-carriers as early as age 40.

Another observation in Study III was that carriers of *APOE* ε4 made more false alarms in a recognition memory test, and also that the right hippocampal volume correlated with numbers of false alarms. The increased tendency to say that

non-studied items are familiar may relate to difficulties assessing relative novelty/familiarity. There is ample evidence that hippocampus, in particular the right hippocampus, is crucial for novelty detection (Tulving et al., 1996; Martin, 1999; Strange et al., 1999). Related to this, Weiss et al (2004) reported that right hippocampal atrophy in schizophrenic patients correlated with false alarm rate, and studies of AD patients have demonstrated that intact hippocampal function is important for correctly rejecting previously non-studied words in tests of recognition memory (Lekeu et al., 2003).

The observations of structural changes in the hippocampal region in Study II and III converge with the functional findings in Study I that carriers of *APOE* ε4 displayed a diminished novelty response in the right hippocampus. Similar findings were recently reported by Trivedi et al (2006). They used a comparable fMRI paradigm (i.e. contrasted processing of novel versus familiar items) and found a reduced novelty response in the right hippocampal region for *APOE* ε4-carriers (see also Han et al., 2007). In addition, previous imaging studies have demonstrated that hippocampal damage selectively reduces the hippocampal response to novel (but not familiar) items (Grunwald et al., 1998; Weiss et al., 2004).

7.2 *APOE* AND CHANGES IN THE PARIETAL CORTEX

A main finding in Study I was that carriers of *APOE* ε4 displayed reduced functional activity in the left inferior parietal cortex. In addition, we found a genetic dose-effect, such that the parietal reduction was most pronounced in homozygous compared to heterozygous ε4-carriers. This dose effect strengthens the association between reduced activity in this parietal region and genetic risk for AD.

Altered functional activity in parietal cortex for *APOE* ε4-carriers may be related to low rates of cerebral glucose metabolism. Several studies have found substantial hypometabolism in the parietal cortex in AD patients (e.g. Frackowiak et al., 1981; Smith et al., 1992) and similar observations have been reported for non-demented *APOE* ε4-carrires (e.g. Small et al., 1995; Reiman et al., 2001; 2004). Previous research has demonstrated that regional metabolic rate is coupled to regional cerebral blood flow, and both of these features are coupled to the neural response (Mosconi et al., 2004; Logothetis and Wandell, 2004).

An underlying mechanism to the observed reduction may be a localized neuronal pathology. This is supported by findings of Mega et al (1999), who reported a

correlation between density of amyloid plaques in AD and hypometabolism in the parieto-occipital lobe. Elevated levels of insoluble beta-amyloid (the main constitute of amyloid plaques) have been observed post-mortem in non-demented *APOE* ε4-carriers already at the age of 40 (Morishima-Kawashima et al., 2000). Further, Boxer et al (2003) reported that atrophy in AD patients was particularly prominent in the inferior parietal cortex, suggesting that this region is affected very early in the course of AD. The reduced parietal response could also reflect disruption of upstream regions of the functional network, resulting in decreased synaptic input. It has been shown that hypometabolism in the parietal cortex is induced by damage to the densely connected hippocampal region (c.f Meguro et al., 2001). In turn, this demonstration can be related to our findings of altered structural (Study II and III) and functional (Study I) hippocampal response in *APOE* ε4-carriers. Taken together, it is possible that early structural and metabolic brain abnormalities coexist or interact to produce the observed activation pattern in subjects at genetic risk for AD.

It should also be stressed that white matter tracts of the splenium originate from temporo-parietal regions of the brain (Conturo et al., 1999). Study II provided evidence for microstructural changes of white matter integrity in the posterior corpus callosum in carriers of *APOE* ε4. This is a novel finding but well in line with previous reports of posterior macroscopic white matter lesions in AD patients (Biegon et al., 1994; Teipel et al., 1999; 2003). The observation of altered parietal activation in *APOE* ε4-carriers may, at least in part, be related to atrophy in the posterior corpus callosum.

Further support that genetic risk for AD is related to changes in parietal cortex was provided in Study IV, in which we were able to demonstrate that fMRI measures of parietal integrity predicted subsequent memory decline (and hence possibly progression to AD) within the group of *APOE* ε4-carriers. In other words, the parietal diminution was most pronounced for those who later experienced memory decline. This finding supports the notion (c.f. section "*APOE* and brain imaging findings") that individual differences among the examined *APOE* ε4-carriers could add to the discrepancy between studies. Importantly, our results indicate that examination of parietal cortex function in *APOE* ε4-carriers can provide information that goes beyond that of genetic risk in predicting subsequent dementia progression. In line with our observation, Small et al (2000b) found that hypometabolism in inferior parietal, lateral temporal, and posterior cingulate regions decreased significantly over time in

non-demented *APOE* ε4-carriers, and that lower metabolism at baseline predicted memory decline two years later. Correspondingly, Smith et al (2005) studied non-demented *APOE* ε4-carriers over time, and found that reduced fMRI response in infero-temporal and occipital regions at baseline was even more pronounced at follow-up four years later.

7.3 DECREASED VERSUS INCREASED FUNCTIONAL RESPONSE IN *APOE* ε4-CARRIERS

Our findings of reduced regional BOLD fMRI response in non-demented subjects at increased risk for AD are consistent with several previous results (e.g. Smith et al., 1999; Elgh et al., 2003), but at odds with studies that report increased, rather than decreased, activations in *APOE* ε4 carriers (e.g. Bookheimer et al., 2000; Fleisher et al., 2005; Wishart et al., 2006). The exact reason for these contradictory findings is not known, although there are several demographic and methodological differences that may add to the inconsistency. Such differences include choice of task as well as age and cognitive status of the participants (c.f. section "*APOE* and brain imaging findings"). For example, Bookheimer et al (2000), who observed a relative increase in fMRI signal in *APOE* ε4-carrires, used a relatively demanding task (to memorize and recall unrelated pairs of words), whereas we used a fairly simple task (semantic categorization). In addition, the *APOE* ε4-carriers in the Bookheimer et al (2000) study performed worse than controls on a delayed recall test and also showed a significant decline in memory performance two years later. In contrast, the *APOE* ε4-carries in our study had cognitive test results that were equal to their non-carrier counterparts, both at time for scanning and two years later (c.f. Study I).

Strikingly, with few exceptions (Smith et al., 2002), studies that relate *APOE* ε4 to decreased brain activation (Smith et al., 1999; 2005; Trivedi et al., 2006) have used relatively simple tasks [picture naming (Smith et al., 1999; 2005) or picture recognition (Trivedi et al., 2006)], and also examined subjects at a relatively young age (mean age 52-57 years). In contrast, a majority of the studies that report increased brain activation for *APOE* ε4-carriers (Bookheimer et al., 2000; Bondi et al., 2005; Fleisher et al., 2005; Wishart et al., 2006; Han et al., 2007) have used relatively demanding tasks (e.g. intentional encoding), and examined either elderly *APOE* ε4-carrires [mean age > 75 years (Bondi et al., 2005; Han et al., 2007)] or *APOE* ε4-carriers that express

lower memory performance than controls (Bookheimer et al., 2000; Wishart et al., 2006).

This leads to the tentative conclusion that genetic risk for AD in asymptomatic individuals initially translates into decreased brain activation in regions that are pertinent to the disease (the MTL and parietal regions), but eventually, with emerging pathology and/or in response to more demanding cognitive tasks, compensatory processes and associated brain activity come into play. In particular, frontal and temporal areas that are important for executive functions and semantic memory might be engaged in order to achieve comparable task performance (c.f. Bookheimer et al., 2000). However, after a limited period of time, i.e. when a critical threshold of brain pathology is reached, this additional activation can no longer be sustained and the subject exhibits a period of rapid decline in episodic memory abilities and reduced functional brain activity. In support of this cascade model, functional brain imaging studies commonly observe significantly decreased brain activity in clinical AD patients (Bäckman et al., 1999; Kato et al., 2001; Grossman et al., 2003; see also Persson and Nyberg, 2006).

7.4 LIMITATIONS AND DIRECTIONS FOR THE FUTURE

This thesis considered isolated effects of the *APOE* genotype on brain structure and function. However, it is conceivable that the reported results are not strictly *APOE*-related, but may be influenced by other factors such as promoter polymorphisms, genes other than *APOE*, and environmental factors (Lahiri et al., 2004; Espeseth et al., 2006). For instance, it has been demonstrated that *APOE* interact with the nicotinic acetylcholine receptor gene *CHRNA4*, so that individuals possessing the *APOE* ε4 allele in combination with a *CHRNA4* TT genotype show reduced white matter volume and slower reaction time on tests of attention than *APOE* ε4-carriers without *CHRNA4* TT (Espeseth et al., 2006). Such gene-gene interactive effects on brain function and structure may ultimately modulate the predicted *APOE*-related risk for AD.

Further, a natural caveat in studies of *APOE* ε4-carriers is that the examined sample includes yet undetected cases of dementia, which would then violate the assumption that the observations relate to impending, rather than developed, AD. However, follow-up data from the fourth wave of Betula testing showed that all participants still remained free of AD symptoms two years after the MRI data collection.

Due to the low frequency of homozygous *APOE* ε44 carriers in the population and hence in the Betula cohort, analyses for determining the effects of *APOE* ε4 zygosity were based on relatively few subjects (N = 10 in each subgroup). Similarly, the sub-sample in Study IV included only 18 subjects. However, in comparison with other papers in this field our sample sizes compare very well, and the significant associations noted indicate that there were effects strong enough to be demonstrable even within a limited amount of subjects. Nevertheless, like in all continuous building of knowledge, the reported findings should be repeated in independent studies.

Longitudinal fMRI studies are warranted in order to determine at what time point brain activity is altered, in what direction (decreased versus increased), and how this relates to the emergence of cognitive decline. A follow-up fMRI data collection has already been planned for in connection with the fifth way of Betula testing, which will be carried out during 2008-2010. In order to further resolve the discrepancy of increased versus decreased activation in *APOE* ε4 carries, a future study should be designed to include both a simple and a more demanding fMRI task (c.f. discussion above). However, note that differential brain activity pattern should not be considered a general effect of *APOE* genotype because certain tasks (e.g. digit span forward) induces no differences of either direction in *APOE* ε4-carrires, regardless of difficulty level (Burggren et al., 2002).

Finally, it is important to stress that follow-up behavioural assessment is crucial in order to determine whether the reported results ultimately forecast clinical AD.

7.5 GENERAL CONCLUSIONS

In closing, this thesis provides evidence that a combination of genetic, neuropsychological, and neuroimaging strategies can be beneficial in assessing risk for AD development. The presented results demonstrate that asymptomatic *APOE* ε4-carriers have functional as well as structural brain alterations in regions associated with the clinical manifestations of AD. Specifically, in addition to the hippocampal region, the findings highlight the importance of examining the parietal cortex for early detection of impending dementia.

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