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Functional and Structural Magnetic Resonance Imaging Studies in Mild Cognitive Impairment and Alzheimer's Disease

Doctoral dissertation

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ABSTRACT

The most common form of dementia in the elderly population is Alzheimer's disease (AD), affecting millions of people worldwide. Aging is the most important risk factor for AD, and the apolipoprotein E (APOE) ε4 allele represents a major genetic risk factor. The first AD-related neuropathological changes appear in the medial temporal lobe (MTL) substructures already decades prior to the manifestion of dementia. Mild cognitive impairment (MCI) has lately been proposed as a possible prodromal state of AD. Recent research has demonstrated that subjects with MCI exhibit structural atrophy which is intermediate between aging and AD, and neuropathologically MCI has also been suggested as early-stage AD. However, little is known about the functional changes in brain activation in the MCI stage, or about the brain structures experiencing the most notable volume changes during conversion from MCI to AD.

This study aimed to investigate the changes in brain structure in relation to conversion from MCI to AD with magnetic resonance imaging (MRI) using voxel-based morphometry (VBM) as the analysis method. The effect of the APOE ε4 allele on the extent of atrophy in MCI subjects progressing to dementia was also evaluated. Changes in brain activation were investigated with functional MRI (fMRI) in MCI and AD subjects compared to controls during encoding and retrieval of word-picture pairs. This thesis also examined the correlations of MTL volumes obtained with manual delineation and hippocampal fMRI activations. The discriminating potential of hippocampal fMRI measures were compared to the discriminating power of MTL volumes and standard neuropsychological testing.

MCI subjects progressing to dementia were characterized by atrophy in the temporoparietal cortex, posterior cingulum and precuneus when compared to controls and stable MCI subjects. The APOE ε4 allele was associated with increased atrophy in the frontal and parietal cortices in MCI subjects progressing to dementia, while the effects were restricted to MTL structures in stable MCI subjects. The fMRI study demonstrated that MCI subjects expressed higher fMRI responses than the controls in the fusiform gyrus and posterior parts of the MTL during the encoding of word-picture pairs, while in AD, the fMRI activations were decreased. Correlations between MTL volumes and fMRI activation were detected only in MCI subjects such that MCI subjects with more entorhinal atrophy activated the hippocampus more during encoding, and those with hippocampal atrophy activated the posterior parahippocampal gyrus to a greater extent. Entorhinal volume was the best, and hippocampal fMRI activation the least successful measure for correctly classifying the subjects into their clinical groups.

The present study showed in MCI subjects originating from population-based cohorts that the posterior cingulum and precuneus undergo atrophy associated with the conversion to AD, and the presence of the APOE ε4 allele leads to excessive areas of atrophy. Functionally, the MCI subjects show increased fMRI responses which appear to be compensatory for the evolving atrophy of MTL structures whereas AD patients have already lost the capability for compensation. Entorhinal volume, and its combination with the delayed wordlist recall score seem promising ways for the identification of MCI and AD, whereas the applicability of hippocampal fMRI with the current method is limited by the high inter-individual variability in the fMRI responses, particularly in MCI subjects.

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Anne Jauhiainen

ABBREVIATIONS

Aβ	β-amyloid
AD	Alzheimer's disease
APOE	apolipoprotein E
APP	amyloid precursor protein
ASL	arterial spin labeling
ATLAS	Automated Medial Temporal Lobe Atrophy Scale
BBSI	brain boundary shift integral
BL	baseline
BOLD	blood-oxygen-level-dependent
CDR	Clinical Dementia Rating
CERAD	Consortium to Establish a Registry for Alzheimer's Disease
CSF	cerebrospinal fluid
CT	computed tomography
DBM	deformation-based morphometry
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders, fourth edition, text revised
DTI	diffusion tensor imaging
EC	elderly controls
EEG	electroencephalography
ENC	encoding
EPI	echo planar imaging
FCSRT	Free and Cued Selective Reminding Test
fMRI	functional magnetic resonance imaging
GM	gray matter
ICR	immediate cued recall
MCI	mild cognitive impairment
MMSE	Mini-Mental State Examination
MNI	Montreal Neurological Institute
MPRAGE	magnetization prepared rapid acquisition gradient echo
MRI	magnetic resonance imaging
MTL	medial temporal lobe
NFT	neurofibrillary tangle
NIA-R	National Institute on Aging and Reagan Institute
NINCDS-ADRDA	National Institute of Neurological Disorders and Stroke – Alzheimer's Disease and Related Disorders Association
NINCDS-AIREN	National Institute of Neurological Disorders and Stroke - Association Internationale pour la Recherche et l'Enseignement en Neurosciences
PET	positron emission tomography
PIB	Pittsburgh compound B
PMCI	progressive mild cognitive impairment
PSEN1	presenilin 1
PSEN2	presenilin 2
RF	radio frequency
ROI	region of interest
SD	standard deviation
SEM	standard error of mean
SMCI	stable mild cognitive impairment
SPECT	single photon emission computed tomography
SVC	small volume correction
TBM	tensor-based morphometry
TMT	Trail Making Test
VBM	voxel-based morphometry
WM	white matter
[^{18}F]FDDNP	2-(1-[6-[(2-[^{18}F]fluoroethyl)(methyl)amino]-2-naphthyl] ethylidene)-malononitrile

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to by their Roman numerals in the text.

I **Hämäläinen A**, Tervo S, Grau-Olivares M, Niskanen E, Pennanen C, Huuskonen J, Kivipelto M, Hänninen T, Tapiola M, Vanhanen M, Hallikainen M, Helkala EL, Nissinen A, Vanninen R, Soininen H. Voxel-based morphometry to detect brain atrophy in progressive mild cognitive impairment. NeuroImage 2007;37:1122-31.

II **Hämäläinen A**, Grau-Olivares M, Tervo S, Niskanen E, Pennanen C, Huuskonen J, Kivipelto M, Hänninen T, Tapiola M, Vanhanen M, Hallikainen M, Helkala EL, Nissinen A, Vanninen RL Soininen H. Apolipoprotein E ε4 allele is associated with increased atrophy in progressive mild cognitive impairment: a voxel-based morphometric study. Neurodegenerative Diseases 2008;5(3-4):186-9.

III **Hämäläinen A**, Pihlajamäki M, Tanila H, Hänninen T, Niskanen E, Tervo S, Karjalainen PA, Vanninen RL, Soininen H. Increased fMRI responses during encoding in mild cognitive impairment. Neurobiology of Aging 2007;28:1889-1903.

IV **Jauhiainen AM**, Pihlajamäki M, Tervo S, Niskanen E, Tanila H, Hänninen T, Vanninen RL, Soininen H. Discriminating accuracy of medial temporal lobe volumetry and fMRI in mild cognitive impairment. Hippocampus; in press.

CONTENTS

1. INTRODUCTION

Neurodegenerative diseases are a major public health concern in the developed countries, and the prevalence of dementia is constantly growing as the longevity of the population increases. Dementia has grievous effects on the individual patients' and their caregivers' lives, and in addition to the human suffering, it also imposes a major financial expense to society as well as a notable burden on the health care system. It has been shown that patients with dementia require longer hospitalisation times than comparable, non-demented patients (Erkinjuntti et al., 1986), require more care time (Nordberg et al., 2007) and the costs of their comorbid illnesses are higher than in control subjects with the same condition (Hill et al., 2002). The leading causes of dementia are Alzheimer's disease (AD), vascular dementia, frontotemporal dementia and dementia with Lewy bodies. With respect to these disorders, AD is the most common type of dementia constituting more than half of dementia cases. Symptomatic treatment for AD is currently available, and in addition to alleviating the symptoms, early treatment also seems to reduce the socioeconomic costs (Fillit et al., 2002). Future research advances may also yield disease-modifying treatments in AD, and thus the early diagnosis of these patients may become even more crucial.

Currently, the diagnosis of AD is based on clinical criteria without any definite biomarkers, and the patients are required to exhibit a progressive memory deficit at the time of diagnosis. However, recent concepts on the pathological cascade of AD suggest that the earliest neuropathological AD-related changes may appear already years, even decades prior to the manifestation of full-blown AD (Selkoe, 2001). Correspondingly, as the patients fulfil the diagnostic criteria, the amount of AD-related neuropathological changes is already abundant, leaving little scope for therapeutic interventions. Thus, substantial research interest has been focused on evaluation and follow-up of subjects with mild memory impairment not meeting the AD criteria in order to find markers that would predict later progression to AD and identify the appropriate subject group for treatment. This field of research has developed the concept of mild cognitive impairment (MCI) (Petersen et al., 1995) to describe subjects who possibly represent prodromal AD. The annual conversion rate from MCI to AD has been shown to range between 6 and 25 % (Petersen et al., 2001), which is clearly higher than that of cognitively healthy, age-matched subjects. The criteria for MCI have varied somewhat over the years, but generally the term refers to subjects having a memory - or another cognitive -

deficit but not meeting the criteria for dementia, and the subjects' activities of daily living are preserved. Research on MCI has, however, indicated that the subjects fulfilling the criteria constitute a rather heterogenous group with different etiologies, and especially in population-based studies, some of the subjects even seem to revert back to normal (Larrieu et al., 2002).

As knowledge on the biological basis of AD has increased since the diagnostic criteria were developed, a recent proposal for new research criteria for diagnostics of AD suggested certain biomarkers to be used as supportive features in the diagnostic workup (Dubois et al., 2007). These supportive features include evaluation of possible AD autosomal dominant genes, and imaging and cerebrospinal fluid (CSF) markers. Recent research suggests that imaging and CSF markers may also improve the detection of MCI subjects (de Leon et al., 2006). Structural magnetic resonance imaging (MRI) studies have revealed significant medial temporal lobe (MTL) atrophy (Jack et al., 1999; Killiany et al., 2002) and positron emission tomography (PET) studies have observed hypometabolism in the MTL areas (De Santi et al., 2001) and retrosplenial cortex (Nestor et al., 2003) in MCI. The diagnostic potential of changes in CSF markers has also been extended to MCI (Hansson et al., 2006). As imaging of the head, preferably with MRI, is already a part of the clinical routine in the diagnostics of AD to exclude other aetiological causes of dementia such as tumors or normal pressure hydrocephalus, an MRI biomarker would be advantageous in the early identification of AD and MCI. It is presumed that functional changes in neural tissue precede structural changes, and thus a functional MRI (fMRI) biomarker might be able to detect AD-related changes even earlier than structural MRI.

fMRI is a novel method which enables the assessment of brain function *in vivo* with excellent spatial resolution while subjects perform cognitive tasks. This study has utilized fMRI to investigate changes in brain function in MCI and AD patients compared to healthy controls during encoding and retrieval of word-picture pairs. However, initially the structural correlates of progression from MCI to AD were defined, as well as the modifying effect of a well-known genetic risk factor on the atrophy pattern related to conversion. Structural atrophy was then separately evaluated in MCI and AD subjects participating in the fMRI study in order to relate structural and functional changes in MCI and AD. Lastly, the discriminating potential of hippocampal fMRI was compared with MTL volumetry and standard neuropsychological test scores.

2. REVIEW OF THE LITERATURE

2.1. Cognitive effects of aging

Even healthy aging is associated with adverse effects on memory and cognition. Cognitive processes that remain relatively stable include short-term memory, autobiographical memory, semantic knowledge and emotional processing (Hedden and Gabrieli, 2004) whereas a longitudinal study reported that a regular decline in the speed of processing, working memory and long-term memory begins already from the age of twenty and continues to decline linearly across the life span (Park et al., 2002). Age-related cognitive decline is heterogeneous as demonstrated by a study showing that 120 healthy subjects with ages ranging from 55 to 85 could be divided into five distinct clusters according to their neuropsychological performance (Ylikoski et al., 1999). These clusters ranged from successful aging through normal or average performance to subjects at risk for dementia. In some studies, the age-related cognitive decline has shown a curvilinear slope such that the decline is more rapid at older ages, and when plotted as a function of time to mortality, the acceleration of cognitive decline begins 3-6 years before death (Hedden and Gabrieli, 2004). This phenomenon may reflect the evolvement of pathological processes, while normal aging processes might manifest in the linear slopes. Accordingly, the differentiation of effects caused by normal aging from pathological processes may be difficult especially at older ages since the risk of neural pathology increases, and studies of apparently normal subjects may in fact include subjects with prodromal neuropathology. When considering AD, aging is the most important risk factor - beyond the age of 65, the risk of AD doubles every 5 years and at the age of 85, the annual incidence of AD is 20-fold greater than at 65 (Drachman, 2006a). It has been estimated that 20 % of persons over 85 are demented, and nearly half are cognitively impaired (Drachman, 2006a).

Age-related cognitive decline is believed to be caused by changes in the frontostriatal system or in the MTL, in which the changes are primarily caused by AD (Hedden and Gabrieli, 2004). The frontal changes are considered to relate to normal aging, and include decreases in dopamine, noradrenaline and serotonin as well as decline in frontal volume and loss of integrity in the frontal white matter (WM) tracts (Hedden and Gabrieli, 2004). White matter hyperintensities in the brain are common in aged subjects, appearing increasingly from age 50 onwards (Salonen et al., 1997; Ylikoski et al., 1995). The white matter changes have been

associated to vascular risk factors (Yoshita et al., 2006) and deterioration of cognition (Breteler et al., 1994; Inzitari et al., 2007; Verdelho et al., 2007; Ylikoski et al., 1993; Ylikoski et al., 2000b; van der Flier et al., 2005). Although the volume of the MTL structures such as the hippocampus or entorhinal cortex may also decline to some extent during healthy aging, the difference between aging and AD is that the neuronal loss observed in aging MTL is not as severe as in AD (Drachman, 2006b). The small volume change seems to be unrelated to cognitive function according to one Finnish study (Ylikoski et al., 2000a), though some researchers have reported that hippocampal volume is related to memory performance (De Leon et al., 1997b; Hedden and Gabrieli, 2004).

2.2. Definition of dementia

The word dementia is derived from the Latin term “de mens”, i.e., “without mind”. Instead of being a single disease entity, dementia is a concept that refers to mental impairment severe enough to disrupt the individual’s activities of daily living. Thus, the cognitive deficit must be greater than could be expected from normal aging. The aetiology of dementia may be diverse, originating from either damage or disease in the brain. It is noteworthy that dementia must develop during adulthood, and should be differentiated from psychiatric illnesses and mental disability.

There are different sets of criteria for dementia but perhaps the most widely used definition is that presented in the Diagnostic and Statistical Manual of Mental Disorders, fourth edition, (DSM-IV-TR) (American Psychiatric Association, 2000). These criteria require the development of multiple cognitive deficits including memory impairment and at least one of the following cognitive disturbances: aphasia, apraxia, agnosia or disturbances in executive functioning. The cognitive impairment must disrupt occupational or social functioning, representing a decline from a previous level of functioning. If cognitive impairment is only present when the subject experiences delirium, then dementia diagnosis should not be set. However, both dementia and delirium can be diagnosed if dementia criteria are met when the subject does not suffer from delirium.

2.3. Clinical features of AD

The first AD patient was described at a meeting for psychiatrists by the German psychiatrist, Alois Alzheimer in 1906 as presenile dementia (Alzheimer, 1907). The patient was a 51-year-old woman, Auguste D, who had been admitted to an asylum for "delerium and frenzied jealousy of her husband". During her life, Auguste D experienced a progressive decline in cognitive function, disorientation, aphasia, delusions and psychosocial incompetence (Maurer et al., 1997). Alzheimer also had expertise in neuropathology, and when Auguste D died in 1906, Alzheimer arranged a neuropathological examination of her brain, which revealed plaques and neurofibrillary tangles (NFT) as well as arteriosclerotic changes (Maurer et al., 1997). This illness was later named as "Alzheimer's disease" by Alzheimer's mentor and colleague, psychiatrist Emil Kraepelin.

Initially, AD was considered as a rarity, affecting individuals in midlife. Only many decades later was it realised that AD is also the most common cause of senile dementia (Blessed et al., 1968). Currently, AD is recognized as the most common cause of dementia, accounting for 60-70 % of the cases, and as the longevity of the population increases, the prevalence of this disease is growing worldwide. In the year 2006, it was estimated that the worldwide prevalence of AD was 26.6 million, and researchers have predicted that the prevalence of AD will quadruple by 2050 (Brookmeyer et al., 1998). The annual costs of dementia in Europe were 55 billion euros in 2004 (Andlin-Sobocki et al., 2005). It has been suggested that if the onset of AD could be delayed by 5 years, the prevalence of AD would decrease by 50 % (DeKosky and Marek, 2003). Thus, the risk factors of AD as well as biomarkers permitting early detection and the introduction of possible disease-modifying treatments are the focus of enormous global research interest.

The onset of AD varies from 40 to 90 years of age, and typically the disease is characterised by a gradual, progressive loss of memory and other cognitive functions. The aetiology of AD is multifactorial, and the disease is a result of a complex interplay of genetic and environmental risk factors. Clinical variations are common, consisting of differences in onset age, rate of progression, composition of neuropsychological deficits as well as occurrence of neuropsychiatric symptoms. The disease is staged as mild, moderate or severe according to the degree of difficulty of the symptoms, and ultimately AD is fatal. The survival time after

the diagnosis is estimated to range between 5 and 12 years, and the cause of death is often an infectious disease such as pneumonia (Beard et al., 1996).

Neuropathologically the disease is characterised by the accumulation of extracellular deposits of β-amyloid (Aβ) plaques, intracellular neurofibrillary tangles (NFT) consisting of hyperphosphorylated tau protein, and synaptic and neuronal loss. The first neurofibrillary changes appear in the MTL already decades before the onset of dementia and can be detected from the third decade of life onwards (Ohm et al., 1995). Other pathological abnormalities observed in AD include chronic inflammation, mitochondrial dysfunction, oxidative stress and excitotoxicity (Pereira et al., 2005). Often there may also be some overlap with cerebrovascular disease, and the comorbidity may enhance the progression of cognitive decline (van der Flier et al., 2005). Understanding the pathological mechanisms of AD may yield novel possibilities for therapeutic strategies.

Neuronal loss is also a typical feature of AD, and it affects the neurotransmitter systems accordingly. The loss of cholinergic neurons in the cortex was reported in the 1970s (Bowen et al., 1976; Davies and Maloney, 1976) and later it was demonstrated that the severity of AD correlated with the loss of cholinergic function (Perry et al., 1981). The cortical cholinergic innervation originates mostly from the basal forebrain such that the hippocampus receives cholinergic input from the medial septal nucleus and the vertical limb of the diagonal band of Broca, and amygdala and the cortex receive input from other parts of the nucleus basalis of Meynert (Mesulam et al., 1983). In AD, the nucleus basalis of Meynert has been reported to suffer from up to 70 % neuronal loss (Arendt et al., 1983), and particularly the entorhinal cortex suffers from cholinergic dysfunction since as much as 80 % of the cholinergic axons can be depleted whereas the primary visual, somatosensory, and motor cortices display a relative preservation of cholinergic fibers (Geula and Mesulam, 1996). Thus, the cholinergic denervation occurs in a very selective manner.

The finding that loss of cholinergic function is the dominant neurotransmitter deficit in AD led to the development of cholinergic treatment. In 1986, it was reported that treatment with acetylcholinesterase inhibitors could achieve a symptomatic improvement in AD (Summers et al., 1986). Currently, three acetylcholinesterase inhibitors (donepezil, rivastigmine and galantamine) are available for the symptomatic treatment of AD, each of these showing a rather similar treatment effect at 6 months (Scarpini et al., 2003). Although the initial

cognitive improvement due to treatment cannot be expected to last indefinitely, it is probable that persistent treatment yields less cognitive decline over longer time periods (Geldmacher et al., 2006).

Other neurotransmitter mechanisms also become disrupted in AD, although the cholinergic system seems to suffer the most. It is thought that neurodegeneration of AD leads to excessive activation of the NMDA glutamate receptors, thus resulting in further neuronal death. Accordingly, the fourth currently available medication accepted for AD is memantine, an NMDA-receptor antagonist that possibly is neuroprotective (Scarpini et al., 2003). Additionally, the noradrenergic neurons in the locus coeruleus deteriorate significantly in AD compared to healthy aging (Tomlinson et al., 1981; Lyness et al., 2003), and deficits in the noradrenergic system can also be detected. The loss of dopaminergic neurons occurring in AD is variable as was discussed in a recent review (Lyness et al., 2003), and NFTs as well as neuronal loss can be detected in the serotonergic nucleus raphe dorsalis (Lyness et al., 2003; Yamamoto and Hirano, 1985), and serotonergic neural function is also impaired to some extent in AD.

2.3.1. Clinical diagnosis of AD

The diagnosis of AD in living human patients relies on clinical judgement, and often it is stated that AD is a diagnosis of exclusion. Nonetheless, sets of criteria have been developed to define the disease accurately. Perhaps the most widely used criteria for AD were developed by the National Institute of Neurological Disorders and Stroke – Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA), and these criteria divide AD into three categories: possible, probable and definite (McKhann et al., 1984). This staging is based on the available information and evaluation of how closely the patient's symptoms resemble classic AD. The NINCDS-ADRDA criteria include an insidious onset of symptoms and a progressive impairment of memory and other cognitive functions in the absence of other possible causes for dementia. Evidence for interference with social or occupational functioning is not required in these criteria, while more recent criteria based on the DSM-IV-TR (American Psychiatric Association, 2000) require the presence of both a memory disorder and impairment in at least one additional cognitive domain, both of which interfere with social function or activities of daily living. Thus, no definite diagnostic biomarkers are used in

the diagnosis of AD, which instead is based on probabilistic clinical criteria. However, the specificity and sensitivity of these criteria for possible or probable AD have been shown to be rather good exceeding 80 % (Blacker et al., 1994). When comparing the clinical diagnosis based on the NINCDS-ADRDA criteria and neuropathological diagnosis according to the National Institutes of Health (NIH) criteria, the diagnostic accuracy was 88%, the sensitivity was 98%, and the specificity was 69% (Kazee et al., 1993). A drawback for the NINCDS-ADRDA criteria is the rather low specificity against other dementias, e.g. many patients with frontotemporal dementia also fulfil the NINCDS-ADRDA criteria for AD (Varma et al., 1999).

As knowledge on the biological basis of AD has increased since the publication of the NINCDS-ADRDA criteria, a recent proposal for new research criteria for AD suggested that biomarkers could be used as supportive features in the diagnostics to improve the specific identification of AD (Dubois et al., 2007). According to this suggestion, the diagnosis of AD requires meeting the core criterion of significant episodic memory impairment, and at least one or more of the supportive biomarker criteria. The core criterion includes the following features: gradual and progressive change in memory function reported by the subject or an informant lasting over 6 months; objective evidence of significantly impaired episodic memory on testing; and the episodic memory impairment can be isolated or associated with other cognitive changes at the onset of AD or as AD advances. The supportive biomarkers include atrophy of the MTL structures such as entorhinal cortex or hippocampus in MRI, abnormal cerebrospinal fluid biomarkers (low Aβ or increased tau or phosphotau concentrations, or their combinations), a specific pattern of hypometabolism as indicated by PET or proven AD autosomal dominant mutation within the immediate family. In addition to these requirements, the proposal includes exclusion criteria consisting of sudden onset of symptoms, early occurrence of gait disturbances, behavioural changes or extrapyramidal signs, focal neurological features and evaluation of the presence of other medical disorders severe enough to account for memory and related symptoms. However, there are still limitations that complicate the use of these criteria in practice, such as lack of specific cut-off values and methodological uncertainties with respect to the biomarkers. Future research will likely provide answers to these matters.

2.3.2. Neuropathological diagnosis of AD

A diagnosis of definite AD can only be done by *post mortem* neuropathological evaluation. The currently used criteria are based on the initial work by Khachaturian according to which definite AD was diagnosed by an adequate number of amyloid plaques (Khachaturian, 1985). The neuropathological criteria were redefined in 1991 by the Consortium to Establish a Registry for Alzheimer's Disease (CERAD), providing definitions as normal and possible, probable or definite AD (Mirra et al., 1991). These criteria were also based on the presence of amyloid plaques, which was classified as frequent, moderate or infrequent. Another neuropathological hallmark feature of AD is NFTs, and also during 1991, Braak and Braak suggested that the progression of AD could be defined by the propagation of NFTs in the brain (Braak and Braak, 1991). According to their studies, AD pathology can be divided into 6 stages based on the location of NFTs: the transentorhinal stages I-II, the limbic stages III-IV and the isocortical stages V-VI. In contrast, the distribution pattern and packing density of amyloid deposits showed only limited significance for differentiation of neuropathological stages (Braak and Braak, 1991), and it has been reported that the intraneuronal cytoskeletal changes precede aggregation of amyloid protein (Braak and Del Tredici, 2004). The clinical status of AD has been linked to the Braak stages such that the transentorhinal stage represents clinically silent cases, the limbic stage corresponds to incipient AD and by the isocortical stage, fully developed AD is present (Braak and Braak, 1995).

More recently, the National Institute on Aging and Reagan Institute (NIA-R) working group developed consensus criteria for the neuropathological assessment of AD (The National Institute on Aging, and Reagan Institute working group on diagnostic criteria for the neuropathological assessment of Alzheimer's disease, 1997). These criteria recommend that both the CERAD assessment for amyloid, as well as the neuropathological staging according to the Braak and Braak criteria should be performed during the diagnostic process. The recommendation also defines the tissue processing methods to be used and localisation of the areas to be analysed, and also suggests that specific immunostains would be used in AD research centers. The diagnostic categories of the NIA-criteria are as follows: high likelihood of AD (including frequent amount of plaques according to CERAD and Braak stage V-VI), intermediate likelihood (CERAD moderate, Braak stage III-IV) and low likelihood (CERAD infrequent, Braak stage I-II).

AD-related neuropathological changes can sometimes be detected even in cognitively healthy elderly subjects. These changes are, however, always considered as abnormal and thus not related to healthy aging (The National Institute on Aging, and Reagan Institute working group on diagnostic criteria for the neuropathological assessment of Alzheimer's disease, 1997; Thal et al., 2004) and thus these subjects represent clinically silent cases. This finding reflects the problem of studying healthy aging since the study subjects may present early neuropathological alterations while still being clinically asymptomatic.

2.3.3. Risk factors for AD

Although aging is the most important risk factor for AD, there are both genetic as well as lifestyle risk factors that may influence the development of AD. Clinically, AD can be divided into familial and sporadic forms. Familial cases often have an early-onset, generally before the age of 65, and late-onset (after 65) is seen in both sporadic and familial cases.

2.3.3.1. Genetic risk factors

Currently, highly penetrant mutations in three risk genes have been detected for early-onset AD, including the amyloid precursor protein (APP), and presenilins 1 (PSEN1) and 2 (PSEN2) (Tanzi and Bertram, 2001). Genetic transmission of AD was first observed in 1981, and in that same study it was found that these families also had a great incidence of Down's syndrome, i.e. trisomy of chromosome 21 (Heston et al., 1981). Moreover, middle-aged subjects with Down's syndrome typically exhibit AD-related pathological changes. Thus, the APP gene was hypothesised to be found in chromosome 21, and in 1986 it was isolated by four different groups (Goldgaber et al., 1987; Kang et al., 1987; Robakis et al., 1987; Tanzi et al., 1987). The PSEN1 and PSEN2 are risk genes for early-onset AD localising to chromosomes 14 and 1 (Levy-Lahad et al., 1995; Rogaev et al., 1995; Sherrington et al., 1995).

Significant genetic linkage for the more common late-onset form of AD was reported in 1991 (Pericak-Vance et al., 1991). Two years later, the same group found that the ε4 allele of the gene coding for apolipoprotein E (APOE) was associated with an increased risk of late-onset

AD and was localised to the same chromosome region (Schmechel et al., 1993; Strittmatter et al., 1993). APOE is a plasma glycoprotein that is involved in lipid transport, and it may also have roles in the regeneration of nervous tissue; in AD it may be related to amyloid depositions or to the stabilisation of microtubules (Saunders et al., 2000). The association of APOE and AD has been investigated extensively, and it has been demonstrated that the ε4 allele of the APOE gene is a major susceptibility factor for AD in both genders over all ages between 40 and 90, although the risk tends to decrease after the age of 70 (Farrer et al., 1997). The ε4 allele is present in approximately 15 % of the population, with the frequency being twice as high in AD patients (Tanzi and Bertram, 2005). However, the presence of one or two ε4 alleles is neither sufficient nor necessary for the development of AD and thus there may be some other genetic or lifestyle factors that protect ε4 carriers against AD.

2.3.3.2. Lifestyle risk factors

Lifestyle factors that increase the risk of AD in later life seem to be similar as those involved in vascular diseases. The association of raised blood pressure and AD was first observed in a Swedish population and later in Japanese-American men (Skoog et al., 1996; Launer et al., 2000). The association of increased systolic blood pressure in midlife and AD has also been observed in a Finnish cohort (Kivipelto et al., 2001b). Moreover, it was first indicated that an increased serum cholesterol concentration during midlife is a risk factor for AD later in life in men (Notkola et al., 1998) and later the finding was extended to women as well (Kivipelto et al., 2001b). One noteworthy aspect of these risk factors is that their effect seems to be independent of the APOE ε4 allele, and elevated total cholesterol level and blood pressure appear to impose a higher risk of AD than the ε4 allele (Kivipelto et al., 2002).

Recently it has also been reported that metabolic syndrome including elevated blood pressure and cholesterol levels and impaired glucose tolerance is associated to AD in elderly subjects (Vanhanen et al., 2006). In a multicenter, multinational study examining 638 non-disabled subjects who were aged 65-84 and who had age-related white matter changes it was observed that diabetes could interfere with tests of executive function, attention, speed and motor control, memory and naming whereas hypertension affected executive functions and attention (Verdelho et al., 2007). Moreover, the effects of diabetes or hypertension were independent of age, education or white matter changes. The relation of diabetes and MTL atrophy has also

been demonstrated in the same group of subjects (Korf et al., 2007). When systematically reviewed, it has been shown that subjects with diabetes have a higher risk for dementia, including both AD and vascular dementia (Biessels et al., 2006). The association of diabetes and cognitive decline may be explained by vascular changes caused by increased serum glucose levels. Moreover, frequent alcohol consumption in midlife (Anttila et al., 2004) and moderate intake of saturated fats in midlife (Laitinen et al., 2006) have also been reported to increase the risk of AD, especially in ε4 carriers.

The degree of education may also relate to cognitive functions in older ages (Ylikoski et al., 1999) and low education, at least in women, is a risk factor for AD (Letenneur et al., 2000). It has been suggested that this association is explained by the unhealthy lifestyles of less educated subjects, however, subjects with higher education may also have a higher cognitive reserve that postpones the manifestation of dementia (Ngandu et al., 2007). Leisure time physical activity at least twice a week in midlife also reduces the risk of AD, and particularly in those subjects who are genetically susceptible by the APOE genotype (Rovio et al., 2005). There are controversial findings on the association of smoking and AD, however, it has been concluded that the possible protective effects of smoking detected in some studies may actually be a result of differential mortality in smokers and non-smokers, and that smoking would not be protective against AD (Wang et al., 1999). High levels of homocysteine and low levels of folic acid have also been associated with AD in cross-sectional studies (Clarke et al., 1998), and a longitudinal study showed that these factors are independent predictors for the development of AD and dementia (Ravaglia et al., 2005). Moreover, those individuals living alone and those without any close social ties had an increased risk of dementia, whereas having a rich social network seems to protect against dementia (Fratiglioni et al., 2000).

2.3.4. Possible biomarkers for AD

Originally, the term biomarker was used to represent cellular, biochemical or molecular alterations measurable in the human tissues, cells or fluids but more recently, the definition has been broadened to include biological characteristics that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to therapeutic interventions (Naylor, 2003). In practical terms, biomarkers are tools that aid in understanding the prediction, cause, diagnosis, progression,

regression or outcome of the treatment of a disease. Biomarkers can further be divided into biomarkers of exposure, including markers that are used in risk prediction, and biomarkers of disease that can be used in screening, diagnosis, and in monitoring disease progression (Mayeux, 2004). When considering AD, an ideal biomarker should be able to detect a fundamental feature of AD neuropathology, be diagnostically sensitive and specific and confirmed through neuropathological validation, as well as be precise with good reproducibility for monitoring treatment effects on the pathology (Kantarci and Jack, 2004). The biomarkers for AD presented here are limited to those having potential to be implemented in clinical use.

2.3.4.1. Imaging biomarkers

Currently, possible imaging biomarkers for AD include structural MRI of the MTL and hypometabolism patterns demonstrated with molecular imaging. PET imaging with specific ligands such as Pittsburgh compound B (PIB) (Klunk et al., 2004) or 2-(1-[6-[(2-[^{18}F]fluoroethyl)(methyl)amino]-2-naphthyl]ethylidene)malononitrile ([^{18}F]FDDNP) (Agdeppa et al., 2003) that visualize AD-related neuropathological changes may also be feasible as a biomarker for AD (Dubois et al., 2007). Control and AD subjects can be distinguished well with rating of hippocampal or MTL atrophy (de Leon et al., 1988; Seab et al., 1988; de Leon et al., 1989; Scheltens et al., 1992; Wahlund et al., 1999b; Wahlund et al., 2000) as well as by measuring the volumes in both young and old subjects (van de Pol et al., 2006). The hippocampal volume discriminates controls and AD patients with an accuracy greater than 85 % (Laakso et al., 2000; Pennanen et al., 2004) but the entorhinal volume may be an even more accurate marker, as it has been shown that subjects converting to AD were better identified by entorhinal than by hippocampal volume (Dickerson et al., 2001; Killiany et al., 2002). The controls and MCI subjects can also be distinguished better by entorhinal than by hippocampal volume (Pennanen et al., 2004). However, in clinical use, the delineation of MTL structures is very time-consuming, and thus methods that are based on visual rating may be more feasible, unless automated, accurate MTL measurements can be provided. Moreover, to enable the utilization of the MTL measurements as biomarkers, a specific cut-off value for a diagnostic amount of atrophy should be available.

Diminished cortical glucose metabolism is a characteristic PET finding in AD (de Leon et al., 1983a; de Leon et al., 1983b), and hypometabolism is especially seen in the temporoparietal cortex (Friedland et al., 1983). More recently, reduced glucose metabolism in the posterior cingulate cortex has also been described (Herholz et al., 2002; Minoshima et al., 1997). Left temporoparietal hypometabolism has been reported to predict conversion to AD in MCI subjects with an accuracy of 75 % (Arnaiz et al., 2001) and recently, hippocampal hypometabolism has also been shown to predict conversion to AD with an accuracy of 81 % (Mosconi et al., 2007). Thus, the measurement of hippocampal or temporoparietal metabolism appears useful in the early diagnostics of AD, and a combination of PET measures and neuropsychological testing may improve the classification accuracy (Arnaiz et al., 2001). However, these results need to be confirmed with larger studies, validated neuropathologically and a comparative norm for hypometabolism should be produced to enable its use as a biomarker for AD.

For the first time, PIB has been used to detect amyloid plaques in living human subjects in a study published in 2004 (Klunk et al., 2004). This study demonstrated PIB retention in AD patients in cortical areas known to be vulnerable to amyloid plaques, and correspondingly, regions that are not affected by amyloid did not show PIB binding. There also was an inverse correlation between PIB binding and cerebral glucose metabolism. One important question regarding PIB binding in human brain *in* vivo is how well it correlates with the neuropathological evaluation of amyloid at autopsy. Although the number of subjects undergoing PIB-PET imaging and subsequent *post mortem* neuropathological assessment is still rather small, preliminary reports on single cases provide promising information on the *in vivo* PIB binding and brain Aβ findings at autopsy (Ikonomovic et al., 2008). In addition to retention in fibrillar Aβ, PIB also binds to cerebrovascular amyloid deposits, thus reflecting cerebral amyloid angiopathy (Bacskai et al., 2007; Johnson et al., 2007). PIB imaging may also have potential in discriminating different dementias such as AD, Lewy body disease, frontotemporal dementia or semantic dementia based on the differential PIB binding in these disorders (Rowe et al., 2007; Drzezga et al., 2008). However, one major problem for using amyloid imaging as a biomarker for AD is the general understanding that neither the number nor the total area of amyloid plaques correlate well with cognition prior to death (Terry et al., 1991). Accordingly, a number of studies have shown that some cognitively normal controls can display relatively high amounts of cortical PIB binding (Klunk et al., 2004; Engler et al., 2006; Johnson et al., 2007; Rowe et al., 2007; Jack et al., 2008b). It has also been suggested

that the amount of amyloid may even decrease instead of increasing as the disease progresses (Hyman et al., 1993) and correspondingly, a recent study with serial PIB imaging reported that the PIB retention had remained rather stable in two years despite of a clear decline in brain glucose metabolism (Engler et al., 2006). Although there was some interindividual variance in the progression of both clinical status as well as PIB retention in these patients, the authors suggested that amyloid deposition in the brain may reach a plateau in the early clinical stages of AD. If so, amyloid imaging may not be an ideal biomarker for the disease progression, but instead might be more useful in the early detection of subjects at risk. Future longitudinal studies are warranted to further explore the progression of amyloid deposits in the human brain in relation to the development of AD, and to determine whether the PIB retention in control subjects is indicative of future cognitive decline or possibly related to aging.

Unlike PIB, [^{18}F]FDDNP binds also to tangle pathology in addition to amyloid plaques (Agdeppa et al., 2003), and neuropathological evaluations at autopsy in AD patients previously scanned with [^{18}F]FDDNP-PET show a close match of the distribution of plaques and tangles and [^{18}F]FDDNP-PET signal (Small et al., 2008). Initial studies have shown that this imaging method may discriminate subjects with MCI or AD from healthy controls and that the [^{18}F]FDDNP binding increases as cognitive symptoms progress (Small et al., 2006), thus making this imaging method a potential biomarker of AD.

Single photon emission computed tomography (SPECT), which allows the measurement of cerebral blood flow, is also a potential method in the early identification of AD. However, currently the diagnostic accuracy of this imaging method in AD generally falls below 80 % and thus this method is not considered as a supportive feature in the proposal for research criteria (Dubois et al., 2007). At present, it has been shown with SPECT that the cerebral blood flow in the posterior cingulum is decreased in MCI subjects progressing to dementia already two years prior to the clinical diagnosis of AD (Huang et al., 2002) and that temporoparietal hypoperfusion in AD may improve the diagnostic accuracy over the clinical diagnosis alone (Jagust et al., 2001). If future research succeeds in improving the diagnostic sensitivity and specificity of SPECT, it may well replace PET since it is both cheaper and more widely available.

2.3.4.2. CSF markers

CSF examination has been proposed as part of the diagnostic procedure for AD already in the NINCDS-criteria, however, not to detect AD but to exclude other causes for dementia (McKhann et al., 1984). Currently there are methods available for the analysis of the concentrations of $A\beta_{1-42}$, total tau or phospho-tau in the CSF, and in AD the amount CSF Aβ is low and the amount of tau high compared to controls (Vandermeeren et al., 1993; Motter et al., 1995). In a pooled analysis of 2500 patients and 1400 controls, total tau succeeded rather well in achieving a correct classification of subjects, having a sensitivity of 81 % and specificity of 90 % (Blennow and Hampel, 2003). Correspondingly, the pooled sensitivity of $A\beta_{42}$ was 86 % and specificity 90 % in 600 AD patients compared to 450 controls (Blennow and Hampel, 2003). Obtaining a CSF sample entails, naturally, an invasive procedure with a risk of adverse events including post puncture headache, however, the procedure is rather well accepted by the patients in clinical routines.

2.3.4.3. Plasma biomarkers

Since obtaining CSF samples may sometimes be difficult, it would be more convenient and patient-friendly to be able to analyse the concentrations of Aβ from venous blood samples. Accordingly, several studies have aimed to determine long-term changes in plasma or serum Aβ concentrations and their relation to the development or progression of AD. Recent longitudinal studies have shown that a high plasma $A\beta_{42}$ level at baseline is associated with cognitive decline in healthy elderly subjects during follow-up (Pomara et al., 2005) and an increased risk for AD (Mayeux et al., 2003). However, results are conflicting as another study has shown that instead of high $A\beta_{42}$, high baseline $A\beta_{40}$ was associated with an increased risk of dementia (van Oijen et al., 2006). One explanation for this discrepancy may lie in the timing of the measurements with regards to dementia diagnosis, since in newly acquired AD the plasma $A\beta_{42}$ concentration was claimed to decline (Mayeux et al., 2003), possibly due to changes in Aβ production or clearance from the brain. Measurement of the $A\beta_{42}$/ $A\beta_{40}$ ratio may also act as a premorbid biomarker of cognitive decline as it has been shown that subjects with a low ratio are at risk for AD (Graff-Radford et al., 2007). More work will be needed, however, until these markers can be validated into clinical use as there are no specific cut off-values for the amount of Aβ in plasma. More importantly, the exact source of plasma Aβ is

not known since amyloid precursor protein is also produced in platelets, and thus plasma Aβ level may represent the amount of amyloid produced in various parts of the body. There also seems to be high intra-person variability in the Aβ levels, particularly in control subjects, as shown in a study analysing serial blood samples taken within four weeks (Abdullah et al., 2007). Thus, more information on plasma Aβ is needed before it can be utilized as a biomarker for AD.

2.4. MCI as possible prodromal AD

Benign senescent forgetfulness was described by V.A. Kral in the 1960s (Kral, 1962), and he distinguished this entity from pathologic cognitive decline, which in his terms was called the "amnestic syndrome". Kral noticed that subjects with the amnestic syndrome performed worse than subjects with benign senescent forgetfulness in tests of memory, and also had higher mortality rates and increased risk for institutionalization. These findings laid the basis for the current MCI term, the history of which has been extensively described in a recent review (Reisberg et al., 2008).

In 1982, two distinct rating scales were published in order to be able to characterise the entire continuum from healthy cognitive aging to dementia, thus making it possible to grade mild memory impairment as well. These rating scales were the Clinical Dementia Rating (CDR) scale (Hughes et al., 1982), having a five stage rating, and the Global Deterioration Scale (GDS) (Reisberg et al., 1982), with seven different stages. In these rating scales, CDR 0.5 refers to questionable dementia, thus comprising the subjects subsequently defined as MCI, and the corresponding rating is GDS 3 stage, which was originally named as mild memory decline. However, in 1988, the term mild cognitive impairment was first used to refer to subjects in the GDS 3 stage, replacing the previous mild memory decline –term (Reisberg et al., 1988).

Several other terms have been used to describe memory impairment, including definitions such as age-associated memory impairment (Crook et al., 1986) and aging-associated cognitive decline (Levy and on behalf of the Aging-Associated Cognitive Decline Working Party, 1994), mild cognitive disorder (World Health Organization, 1993), mild neurocognitive decline (American Psychiatric Association, 1994) and cognitive impairment no dementia (Graham et al., 1997). Out of these definitions, MCI seems to have gained the most support

by the researchers working in the field of age-related cognitive decline and the early identification of AD.

MCI was initially considered as a possible transitional state between normal aging and AD, however, currently MCI refers more broadly to subjects with memory impairment beyond that expected for age and education but who are not demented. A longitudinal study performed in a community-based clinical setting showed that the MCI subjects were at an increased risk of developing dementia with an annual conversion rate of 12 % per year in comparison to the healthy elderly who had a conversion rate of 1 to 2 % annually (Petersen et al., 1999). At a memory clinic-based setting, the annual conversion rate of MCI to AD may even be as high as 25 % (Petersen et al., 2001). One explanation for the difference in the conversion rates may be that the subjects are more selected in the memory clinics, while community-based studies may include more heterogenous MCI subjects. In addition to neurodegenerative disease, there are factors such as education, vascular risk factors, psychiatric status, genetic background, hormonal changes or use of anticholinergic drugs that may explain the cognitive decline in the elderly population in the community samples. Correspondingly, it has been shown in community-based studies that a high number of MCI subjects remain stable or even revert back to normal (Larrieu et al., 2002; Ganguli et al., 2004; Gauthier et al., 2006). Since MCI is a heterogenous disorder, research interests are thus placed on finding biomarkers that would aid in correctly identifying those MCI subjects likely to convert to AD, as they are a target group for early therapeutic interventions in AD.

When it became evident that the MCI subjects constitute a broad, clinically heterogenous population, the diagnostic criteria were expanded such that MCI was divided into different subtypes depending on the specific cognitive deficits (Petersen, 2004; Winblad et al., 2004). Firstly, MCI subjects can be separated to amnestic or non-amnestic MCI depending on whether memory impairment is present, and these subtypes can be classified as single- or multiple domain MCI according to the number of cognitive domains affected (Petersen, 2004). The nonmemory cognitive domains include e.g. language, executive function and visuospatial skills (Petersen, 2004). The clinical phenotype of MCI may also be linked to the ultimate type of dementia to which the subject will progress. The aetiology of amnestic single- or multiple domain MCI is often AD, or possibly vascular dementia or depression, whereas non-amnestic single- and multiple domain MCI more often indicates dementia with Lewy bodies or frontotemporal dementia, although vascular dementia is also possible

(Petersen, 2004). Aetiological factors of MCI as well as probable outcomes are presented in Figure 1.

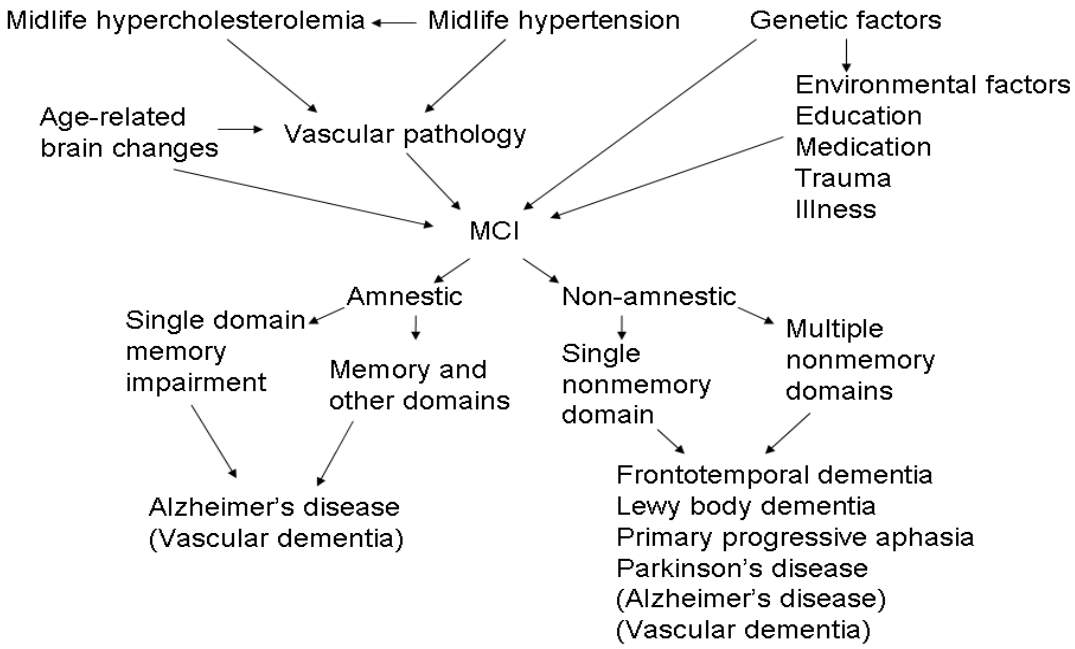

Figure 1. Aetiological factors and classification of MCI, and probable outcomes according to the classification.

2.4.1. Diagnosis of MCI

The initial MCI criteria, corresponding for the GDS 3 MCI, defined the subjects' cognitive status such that objective memory impairment was observed in a thorough clinical examination, concentration deficit could be observed on clinical testing and the subject may have displayed difficulties in remembering names; intimates had become aware of the subjects' problems in remembering words or names; decreased performance manifested itself in demanding employment or social occasions and co-workers had become aware of the subjects' poor performance; subjects may have got lost when travelling to unfamiliar locations; and the clinical symptoms may have been underestimated due to the subjects' denial of memory problems (Reisberg et al., 1982; Reisberg et al., 1988).

Later, a new set of criteria for MCI was suggested by the Mayo Clinic Alzheimer's Disease Research Center (Petersen et al., 1995). The following conditions were required for the diagnosis: 1) memory complaint by patient, family or physician; 2) normal activities of daily living; 3) normal global cognitive function; 4) objective impairment of memory or in one other area of cognitive function as evidenced by scores >1.5 standard deviation (SD) below the age-appropriate mean; 5) CDR score of 0.5; and 6) absence of dementia (Petersen et al., 1995; Smith et al., 1996).

The criteria were further revised to decrease the importance of psychometric cut-off values, and simultaneously the requirement of a CDR score 0.5 was omitted (Petersen et al., 1999). As CDR is fundamentally a severity scale instead of a diagnostic instrument, a CDR score of 0.5 included both subjects with MCI as well as subjects with very mild dementia and thus was not ideal for diagnostic purposes. The diagnostic criteria then became: 1) memory complaint; 2) normal activities of daily living; 3) normal general cognitive function; 4) abnormal memory for age; and 5) not demented (Petersen et al., 1999). Later, these criteria were modified to: 1) memory complaint, preferably corroborated by an informant; 2) objective memory impairment; 3) normal general cognitive function; 4) intact activities of daily living; and 5) not demented (Petersen et al., 2001). An international, multidisciplinary group of experts further revised the criteria some years later and their recommendation for the general criteria for MCI was as follows: 1) not normal, not demented; 2) cognitive decline by self or informant report and by objective cognitive testing; and 3) preserved basic activities of daily living or minimal impairment in complex instrumental functions (Winblad et al., 2004).

The numerous revisions of these criteria reflect the difficulty to define explicit criteria. The diagnosis of MCI is still controversial even after these criteria modifications, as there is insufficient evidence on specific tests or cut-off values to be used to define MCI. Although the degree of impairment can be assessed neuropsychologically, the ultimate diagnosis is determined through clinical judgement. It has also been suggested that it may be more useful to evaluate the subjects' cognitive decline by individual slopes instead of against age-specific norms (Winblad et al., 2004). This consideration has been taken into account in the most recent revision of MCI criteria developed by the European Consortium on Alzheimer's Disease Working Group in 2006, which define the criteria as follows: 1) cognitive complaints coming from the patients or their families; 2) the reporting of a relative decline in cognitive functioning during the past year by a patient or informant; 3) cognitive disorders as evidenced

by clinical evaluation; 4) absence of major repercussions on daily life; and 5) absence of dementia (Portet et al., 2006).

2.4.2. Neuropathological features of MCI

MCI is clinically considered as possible prodromal AD, and neuropathological studies support this hypothesis. In a community-based study, 15 subjects who had been diagnosed with amnestic MCI prior to their death underwent autopsy with a neuropathological examination being performed according to the criteria by Khachaturian, CERAD and NIA-R (Petersen et al., 2006). Most of the subjects with amnestic MCI in that study did not meet neuropathological criteria for AD, but 10 out of 15 subjects were classified as Braak stage II or III, and according to the NIA-R criteria, 1 subject had high likelihood, 3 subjects intermediate and 11 subjects had a low likelihood of AD. Concomitant pathology, such as argyrophilic grain disease, hippocampal sclerosis or vascular lesions was also present (Petersen et al., 2006). Thus, most of the amnestic MCI subjects exhibited AD-related neuropathological changes, suggesting that amnestic MCI might represent early AD. This finding has been confirmed later in another study including 10 subjects with amnestic MCI, and the neuropathological evaluation revealed that the MCI subjects had significantly elevated amounts of NFTs in ventromedial temporal lobe as well as in the parietal neocortex compared to controls (Markesbery et al., 2006). That study also included patients with early AD, and in the comparison of the amnestic MCI and AD patients, the AD patients had more NFTs and neuritic plaques in frontal and temporal lobes as well as in the amygdala and subiculum, suggesting that MCI indeed was a transitional state between normal aging and dementia (Markesbery et al., 2006).

In the aforementioned study of 15 amnestic MCI subjects, the mean age of the study subjects was 88.9 (Petersen et al., 2006). Another study including 116 very old subjects whose ages ranged from 90 to 104 found that 56 % of subjects having a CDR score of 0.5 were in Braak stage III, and 31 % in stage II and only 13 % in stage I (Gold et al., 2000). A CDR score of 0.5 was included in the earliest criteria for MCI, and generally this severity rating is considered to consist of subjects with MCI or very mild dementia.

The heterogeneity of MCI is also reflected in the neuropathological assessment. A community-based study of 34 amnestic MCI subjects who progressed to clinical dementia and underwent *post mortem* brain analysis showed that 29 % of the subjects exhibited non-AD primary pathological abnormalities in the brain (Jicha et al., 2006). A majority of the subjects progressed to AD both clinically and neuropathologically, but most of the subjects had secondary contributing pathologic abnormalities in addition to their primary pathologic diagnoses (Jicha et al., 2006). This study concluded that in the community-based cohorts, complex neuropathological findings consisting of two or more distinct pathologic entities may be common.

2.4.3. Risk factors for MCI

In general, the risk factors for MCI are similar as those recognized for AD with the most important factor being age. In familial AD with gene mutations, the subjects undergo a phase of mild deterioration until full-blown dementia is observed. The APOE ε4 allele is also a risk factor for cognitive decline and MCI, and for progression to AD from MCI (Hsiung et al., 2004; Tervo et al., 2004).

As in AD, midlife elevated cholesterol levels were noted as a risk factor for MCI, and the effect of elevated systolic blood pressure was close to being statistically significant in a Finnish population-based cohort (Kivipelto et al., 2001a). More recently, an association between hypertension and MCI was reported in a population-based study on American subjects (Reitz et al., 2007). Type 2 diabetes is also related to cognitive impairment in the elderly, and a population-based study showed that the risk of MCI attributable to diabetes was 8.8 % (Luchsinger et al., 2007). Lower levels of education may also be related to increased risk of MCI (Kivipelto et al., 2001a; Solfrizzi et al., 2004; Tervo et al., 2004). Alcohol consumption was reported to show a U-shaped curve with the risk of MCI in the elderly, such that those subjects drinking no alcohol or drinking frequently in midlife were twice as likely to have MCI in old age as subjects who drank infrequently (Anttila et al., 2004). Dietary intake of saturated fat also is a risk factor for MCI (Eskelinen et al., 2008). Frequent physical activity in midlife was a protecting factor for AD, and thus most likely for MCI as well. Moreover, it has been suggested that mild physical activity may improve executive functioning in elderly MCI subjects, although these results need to be confirmed with larger

studies (Scherder et al., 2005). Smoking MCI subjects have shown unfavourable serum cholesterol levels and decreased folic acid concentrations, both of which are risk factors for vascular damage and cognitive impairment (Stuerenburg et al., 2005).

2.4.4. Biomarkers for MCI

Essentially, the same biomarkers apply for MCI as in AD. With respect to MTL volumetry, entorhinal volume seems to be the best discriminator between controls and MCI subjects. The accuracy of entorhinal volume in being able to distinguish subjects with cognitive complaints (not specifically diagnosed as MCI) from controls was 69 % (Dickerson et al., 2001) and in another study with subjects diagnosed as MCI, the diagnostic accuracy of entorhinal volume was 65.9 % between MCI and controls (Pennanen et al., 2004). When using hippocampal volume, the discriminating accuracy was only 59.7 % (Pennanen et al., 2004). Another study followed the subjects after the MRI assessment, and it was shown that the baseline entorhinal volume had an accuracy of 84 % of differentiating those memory impaired subjects progressing to dementia or remaining stable (Killiany et al., 2002).

PET studies in MCI subjects have shown that reduced metabolism in the right temporoparietal cortex is related to subsequent conversion to AD (Chetelat et al., 2003), and that the hypometabolism is more accurate than neuropsychological testing in predicting conversion from MCI to AD (Chetelat et al., 2005a). Moreover, follow-up studies on control subjects have demonstrated that a baseline decline in entorhinal cortex metabolism predicted later cognitive decline as well as more widespread metabolic reductions in the temporal cortex and hippocampus (de Leon et al., 2001). PET was reported to have considerable diagnostic accuracy in recognising subjects likely to convert as shown in a longitudinal study following 77 cognitively normal elderly subjects for 6-14 years (Mosconi et al., 2007). The diagnostic accuracy of baseline hippocampal glucose metabolic rate was 81 % from normal controls to AD, 77 % from controls to other dementias, and 71 % from control to MCI (Mosconi et al., 2007).

PET imaging using PIB as a radioligand has also revealed that MCI subjects have an increased retention of PIB in a similar pattern as seen in AD patients (Kemppainen et al., 2007) while other studies have reported that MCI subjects fall into bi-modal groups

resembling either controls or AD patients (Price et al., 2005; Rowe et al., 2007; Jack et al., 2008b). In contrast, a recent study reported that the PIB retention in MCI was intermediate in comparison to normal elderly and AD, and that those MCI subjects converting to AD during a follow-up of 8 ± 6 months had significantly more PIB retention at baseline than stable MCI or control subjects (Forsberg et al., 2007). As the amount of amyloid may even decrease as AD progresses, it is possible that the maximum load of amyloid is observed in the prodromal phase as discussed in a recent review (Nordberg, 2008). Consistent with this hypothesis is the finding that in MCI and healthy elderly subjects, PIB binding and impairment in tests of episodic memory correlate strongly, whereas in AD the correlation is weaker (Pike et al., 2007). Thus PIB imaging may be more suitable to detection of MCI instead of being a marker of the progression in later disease stages. However, future longitudinal studies are needed to characterise the progression of amyloid deposits as subjects convert to MCI and further to AD, and to clarify the currently reported bi-modality in PIB binding in MCI subjects.

The potential of CSF Aβ and tau concentrations as biomarkers has also been investigated in MCI subjects. A recent study has reported that the combination of CSF $A\beta_{42}$ and total tau predicted conversion to AD in 4 to 6 years with hazard ratios of 17.7-19.8, and incipient AD was detected with 95 % sensitivity and 83 % specificity (Hansson et al., 2006), which highlights the potential benefits of these biomarkers in the early diagnostics of AD. Similarly as in AD, it was reported that a low plasma $A\beta_{42}$/ $A\beta_{40}$ ratio is also evidence of a risk for MCI (Graff-Radford et al., 2007), however, as previously discussed, there are still issues needing to be resolved before the plasma Aβ measurements can be utilized in clinical practise.

2.5. MRI as a tool to study the brain

The scientific principle behind MRI is nuclear magnetic resonance, which was initially investigated by Isidor Rabi (Rabi et al., 1938), and the phenomenon was investigated further in liquids and solids by Felix Bloch and Edward Purcell in the 1940s (Bloch et al., 1946; Purcell et al., 1946). The basis for MRI was developed later in the 1970s by two scientists, Paul Lauterbur and Sir Peter Mansfield (Lauterbur, 1973; Mansfield and Grannell, 1973). All five of these scientists are Nobel laureates – Rabi was awarded the Nobel Prize in Physics in 1944, Bloch and Purcell received the Nobel Prize in Physics in 1952 for their work on nuclear magnetic resonance, and Lauterbur and Mansfield shared the Nobel Prize in Physiology or Medicine in 2003 for developments in MRI.

The first human MRI images were obtained in 1977, and the MRI scanners have become increasingly popular in medical use since the 1980s. Until the development of MRI, tomography and computed tomography (CT) were two of the most important radiological methods. Tomography was invented in the 1930s by radiologist Alessandro Vallebona, and is based on moving the X-ray tube and the film synchronously in opposite directions. The development of computers in the 1970s led to the emergence of CT. Currently, CT is still widely used since it is rather inexpensive, has a short imaging time, and technical developments have enabled its usage e.g. in angiography. The disadvantages of CT compared to MRI are the moderate to high doses of ionising radiation, and inferior spatial resolution.

Medical MRI is based on the imaging of protons of hydrogen nuclei, as hydrogen is present in water which is abundant in human tissues. The nuclear spin of the proton has two possible energy states, and in an external magnetic field, two spin populations of protons with opposite nuclear magnetic moments develop. The magnetic field evokes a torque to the magnetic moments, producing precession of the nuclei around the field axis. The frequency of this precession, i.e. Larmor frequency, is directly proportional to the magnetic field strength and the gyromagnetic ratio, which is a nucleus-specific constant value. Since there is an excess of spins in the lower energy state, a weak magnetization aligned with the external magnetic field, or z axis, is created.

The spins in the lower energy state can be excited using radio frequency (RF) pulses at the Larmor frequency. The RF-pulse induces a magnetization component in the transversal xy-plane. Maximal transverse magnetization can be obtained with a so-called 90 degree pulse, and by adjusting the duration and power of the RF-pulse, the amount of transverse magnetization can be regulated. Simultaneously as the transverse component is formed, the longitudinal magnetization decreases. In the longitudinal magnetic field, the spins precess in different phases, but the RF-pulse makes the spins precess coherently. Relaxation starts after the energy pulse, i.e. the spin system begins to return to the initial state. Relaxation occurs in two components, T1 and T2. T1 relaxation refers to the recovery of the longitudinal magnetization, and T2 represents the decay of the transverse magnetization through dephasing of the spins. T2* relaxation corresponds to the transversal magnetization decay including the influence of local magnetic field inhomogeneities to the dephasing of spins. The T2* relaxation time is always shorter than T2 relaxation time. After the RF-pulse, magnetic gradients in x, y, or z directions are applied to select the plane of imaging. A receiver coil is

used to collect information on the magnetic field signal. The magnetic signal is composed of sine waves, and the MR image is created through Fourier transformations of the signal.

The image contrast is based on image acquisition parameters weighting the signal by T1, T2 or T2* relaxation, or no relaxation time at all when the resulting image is called a proton-density image. When scanning different regions of the body, differential imaging acquisition parameters, gradients and combinations of RF pulses, i.e. sequences, are used. The contrast can be further enhanced with specific imaging techniques such as fat suppression, or with contrast agents. Paramagnetic gadolinium compounds are most often used contrast agents in MRI, but superparamagnetic contrast agents, e.g. compounds including iron oxide nanoparticles have also become available.

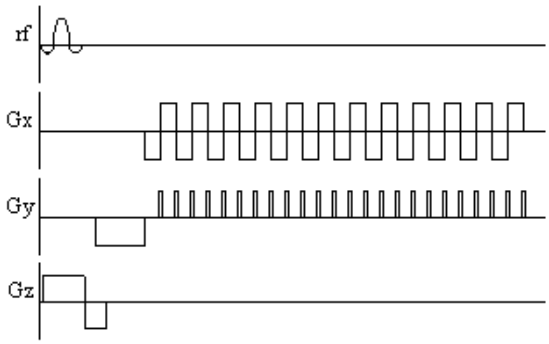

Figure 2. Example of a gradient echo echo-planar imaging sequence. After the radio frequency (rf) pulse is applied, reversal of the field gradients is used to generate the echo (G_z). The signal is collected during the rapid switching of read (G_x) and phase-encoding (G_y) gradients.

MRI is a feasible tool to study the brain as it provides excellent spatial resolution and is safe for the patient as no ionising radiation is produced, allowing multiple imaging sessions if needed. Contraindications for MRI include having a pacemaker or ferromagnetic metal prostheses in the body. Other biostimulation implants, such as insulin pumps or cochlear implants may also be contraindicated. Subjects with claustrophobia cannot usually be imaged with MRI, and the image quality may be low in anxious subjects due to motion artefacts. Anaesthesia is sometimes used to enable the imaging of anxious or claustrophobic subjects in extreme cases. Adverse effects of MRI include heating of the body or peripheral nerve stimulation, however, the occurrence of these events can be prevented with technical

modifications in the scanners. Loud acoustic noise is produced during imaging, and sufficient ear protection is needed.

2.5.1. Structural MRI

Various analysis methods are currently available to study the structure of the brain *in vivo*. Most often the structural analyses are performed using high-resolution three-dimensional T1-weighted images. The sequences used to obtain the images are vendor-specific, and examples of such sequences are magnetization prepared rapid acquisition gradient echo (MPRAGE) – sequence and gradient recalled acquisition in steady state –sequence. Volumetric analyses can be performed by manually delineating regions of interest (ROI), however, this method is highly dependent on the tracer's expertise, and is also time-consuming. Often the focus of interest is in the structural changes occurring in the whole brain instead of one specific structure and thus several methods that enable the assessment of the whole brain have been developed. These include techniques such as the brain or ventricular boundary shift integrals (BBSI and VBSI) (Freeborough and Fox, 1997), voxel-based morphometry (VBM) (Ashburner and Friston, 2000), deformation-based morphometry (DBM) (Ashburner et al., 1998), tensor-based morphometry (TBM) (Ashburner and Friston, 2000) and methods that enable the measurement of cortical thickness (MacDonald et al., 2000). Moreover, interindividual differences in brain structure or the effects of aging can be evaluated with the help of population-based probabilistic atlases (Thompson et al., 1996) or assessment of probabilistic subvolumes of the brain with the help of these brain atlases (Mega et al., 2005).

In the BBSI method, serial images are registered and a change of brain volume can be detected where the brain boundary has changed. The change is thus derived from voxel intensities of the images (Freeborough and Fox, 1997). VBM allows a voxel-wise comparison of local concentrations of gray matter (GM) between subject groups (Ashburner and Friston, 2000), and it can also be used for longitudinal evaluation of brain changes. It is also possible to assess changes in WM with VBM. DBM identifies differences in the brain structure by the relative positions within the subjects' brain, and TBM refers to methods that localise differences in the local shapes of brain structures.

2.5.2. Functional MRI and the BOLD contrast

Structural brain imaging allows the investigation of brain morphology *in vivo*, but functional MRI methods enable the non-invasive assessment of brain function during cognitive tasks *in vivo*. Accordingly, functional imaging has attracted huge interest worldwide, and has so far provided a wealth of information on human cognition and changes of cognitive processing in a variety of conditions and diseases.

Currently, the most widely used fMRI method is based on the blood-oxygen-level-dependent (BOLD) contrast which relies on neurovascular coupling. As early as 1890, Roy and Sherrington observed that the vascular supply of the brain varied locally corresponding to local variations of functional activity (Roy and Sherrington, 1890). Thus, blood flow and volume increase in those brain areas that are neurally active. Earlier it was considered that the increase in blood flow was driven by the increased oxygen demands of active brain tissue, but the oxygen delivery to the active brain areas actually exceeds the oxygen consumption even in the activated state (Fox and Raichle, 1986; Buxton and Frank, 1997). Accordingly, it has been suggested that the blood flow is regulated by a mechanism – either neuronal or biochemical – that is dependent on neuronal firing, but independent of the cerebral metabolic rate (Fox and Raichle, 1986) and more recently, it has been speculated that the haemodynamic responses might be regulated by neurotransmitter-related signalling (Attwell and Iadecola, 2002; Drake and Iadecola, 2007).

Nonetheless, more oxygenated haemoglobin, and relatively less deoxygenated haemoglobin is present in the venous blood in the active brain area. The magnetic properties of haemoglobin depend on its oxygenation state i.e. oxyhaemoglobin is diamagnetic, thus having little effect on its surroundings whereas deoxyhaemoglobin is paramagnetic, introducing magnetic inhomogeneity and distortions to its environment (Pauling and Coryell, 1936). Therefore, the relative decrease in the amount of deoxygenated haemoglobin enhances the MRI signal locally in the active brain areas and the BOLD contrast thus represents haemodynamic changes. The finding that paramagnetic deoxyhaemoglobin could be used as a naturally occurring contrast agent in fMRI was published in 1990 (Ogawa et al., 1990), and the first human fMRI experiment was performed shortly thereafter, observing activation in the primary visual cortex during a visual task (Belliveau et al., 1991). However, in that study, gadolinium was used as the contrast agent, and the first studies depending solely on the

BOLD contrast were published one year later. These studies utilized visual and motor tasks resulting in brain activation in the primary visual and motor cortices, correspondingly (Kwong et al., 1992; Ogawa et al., 1992).

The BOLD responses observed in fMRI studies correlate with neuronal local field potentials as demonstrated by simultaneous fMRI and intracortical recordings in monkey visual cortex, and thus the BOLD contrast reflects neuronal input and intracortical processing in the activated area (Logothetis et al., 2001). The BOLD haemodynamic response is a tri-phasic curve consisting of an initial dip followed by the maximum peak response and finally a post-stimulus undershoot (Buxton et al., 1998). The initial dip is considered to reflect a fast increase in the blood deoxyhaemoglobin concentration caused by the sudden increase of oxygen consumption in brain tissue before the blood flow increases to compensate for the increased oxygen need, and in some studies it has been speculated that the initial dip would better localise to the activated areas than the BOLD peak (Malonek and Grinvald, 1996; Thompson et al., 2003). However, in general the localisation of activation in fMRI studies is done by evaluating the active areas at the time when the BOLD response is at its peak. The undershoot reflects the time period when the perfusion and oxygen consumption gravitate to a new equilibrium state to compensate for the initial nonoxidative glucose consumption (Frahm et al., 1996). Partly, the undershoot can also indicate neural responses as it has been shown that neural inhibition precedes the undershoot (Logothetis et al., 2001).

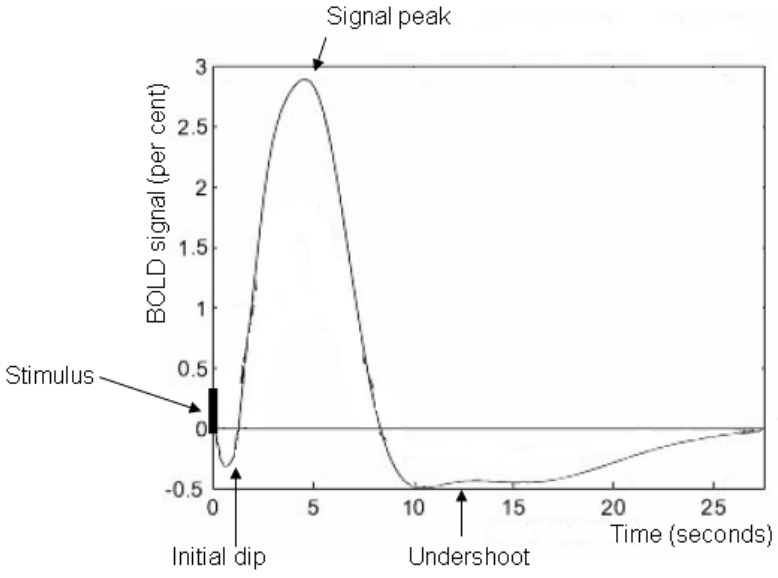

Figure 3. The shape of the BOLD signal response to a single stimulus applied at time point 0.

A linear relationship between haemodynamic responses and neural activity is required in order to be able to interpret perfusion-based imaging findings as indicators of actual brain activity. There is some ambiguity on the relations of haemodynamic and neural responses as one study reported that the coupling was linear (Logothetis et al., 2001), however, other studies have demonstrated that there are also nonlinear effects that may complicate the interpretation of fMRI results (Devor et al., 2003; Sheth et al., 2004). Another confounding factor for the construction of fMRI results is that cortical and subcortical structures produce differential BOLD responses in relation to changes in the metabolic rate of oxygen consumption (Ances et al., 2008). When fMRI is used in elderly individuals, the interpretation of the BOLD signal is further exacerbated by alterations in the cerebrovascular system that may affect neurovascular coupling (D'Esposito et al., 2003). These changes that may occur even in clinically asymptomatic elderly subjects include altered cerebrovascular ultrastructure, reduced elasticity of vessels, increased atherosclerosis, reduced cerebral blood flow in the resting state, decreased resting state cerebral oxygen consumption metabolic rate and reduced vascular reactivity to chemical modulators (D'Esposito et al., 2003; Bangen et al., 2007). However, despite these limitations, BOLD fMRI has produced important information on brain function, and continuing technical development will likely improve our understanding of the neurovascular coupling.

2.5.3. Other promising imaging methods to study cognitive impairment

Arterial spin labeling (ASL) is another functional MR imaging technique measuring cerebral blood flow using arterial water as an endogenous contrast agent (Detre et al., 1992; Williams et al., 1992). In this method, a saturation imaging sequence in the neck region is used to saturate the spins in the water of arterial blood flowing towards the brain, and the signal is derived by observing the effects of the spin inversions locally in the brain. The advantage of ASL compared to BOLD is that it is a direct measure of blood flow, and does not depend on many physiological effects in the same way as BOLD. Moreover, ASL may localise the activation areas more precisely than BOLD since it reflects changes in the arterial side of the vasculature whereas the BOLD signal is related to the venous side (Lee et al., 2001). The combination of these two techniques may prove beneficial by producing complementary information on the neurovascular coupling, and this has already been tested successfully to study encoding processes in healthy aging (Bangen et al., 2007; Restom et al., 2007).

ASL has been reported to underestimate the blood flow at low flow rates (Warmuth et al., 2003), and an alternative for the measurement of cerebral blood flow with MRI is provided by dynamic susceptibility-weighted perfusion MRI. However, this method requires administration of an intravenous contrast agent such as gadopentetate dimeglumine and therefore invasive procedures are needed. Perfusion MRI has been applied in MCI and AD subjects, and has detected decreases in the cerebral blood flow in MCI subjects in the MTL and anterior cingulum, whereas perfusion deficits in the posterior cingulum were only detected in AD (Luckhaus et al., 2007).

The spatial resolution of fMRI is excellent but its temporal resolution is limited by the physiological properties of the haemodynamic response and therefore the combination of fMRI with a method that has a superb temporal resolution appears tempting. Traditional electroencephalography (EEG) is non-invasive, directly coupled to neural activity and has a millisecond temporal precision, thus complementing BOLD fMRI extremely well. Recent advances in both hardware and software have enabled the simultaneous collection of EEG and fMRI data to provide a more accurate characterization of the location and timing of neurophysiological activity in the human brain (Bonmassar et al., 2001; Dale and Halgren, 2001; Debener et al., 2006). However, caution is necessary to ensure that the two methods refer to the same neural substrate (Debener et al., 2006; Fell, 2007). The quality of the EEG recording during fMRI has also been a major concern, but recent developments have improved the quality of EEG (Debener et al., 2006) and there are great hopes for the integration of these two methods.

Diffusion tensor imaging (DTI) can indicate the integrity of white matter tracts and local fibre orientation by providing quantitative data on the anisotropy (i.e. directionality) of water diffusion. Thus, this imaging method can be used to assess neuronal connectivity. When using DTI alone, it has been shown that the white matter in the temporal stem is affected in AD, possibly due to the secondary degeneration associated with the GM pathology in the MTL (Hanyu et al., 1998). With respect to MCI subjects, a reduction of the fractional anisotropy in the cingulum fibers has been observed, this effect being even stronger in AD patients (Zhang et al., 2007). Moreover, the correct differentiation between MCI and AD patients was improved by combining the assessment of DTI of the cingulum fibers with the measurement of hippocampal volumes (Zhang et al., 2007), thus suggesting that the combination of these

two measures might prove useful as a diagnostic tool for assessing the pathology of cognitive impairment.

2.6. Structural MRI findings in aging, MCI and AD

2.6.1. Structural MRI of healthy aging brain

The phylogenetically old brain structures in the medial temporal lobe also mature first during brain development in childhood (Gogtay et al., 2004). Cortical development appears to begin from the primary sensorimotor cortices and frontal and occipital poles and the remainder of the cortex matures in a parietal-to-frontal direction, i.e. higher-order association areas mature later than the lower-order somatosensory and visual cortices (Gogtay et al., 2004). The superior temporal cortex, containing association areas that integrate information from several sensory modalities, is the last brain structure to mature (Gogtay et al., 2004). During adulthood, there is a loss of cortical GM (Good et al., 2001; Sowell et al., 2003) and correspondingly, the amount of WM increases during the first four decades (Bartzokis et al., 2001) with no significant decline during aging (Good et al., 2001). However, although there is no significant reduction in the WM volume during aging, the WM integrity, particularly in the frontal areas, is affected (Head et al., 2005).

An age-related volume decline has consistently been detected in frontal and temporal lobes in cross-sectional studies (Coffey et al., 1992; Cowell et al., 1994; Tisserand et al., 2004). In a large cross-sectional study comprising 465 healthy adults with ages ranging from 18 to 79, it was shown that aging-related cortical GM volume decline was localised to the superior parietal and pre- and postcentral gyri, insula and the anterior cingulate, and decline in GM concentration was detected in the middle frontal gyrus, Heschl's gyri and left planum temporale (Good et al., 2001). Thus, the volume decline appears to begin in those areas maturing late in brain development. Longitudinal studies have also shown atrophy of the temporal lobe, and a decline in the whole-brain volume as well as an increase in the CSF volume (Mueller et al., 1998; Scahill et al., 2003). A longitudinal study investigating successful aging between the eighth and ninth decades of life found that the amount of white matter hyperintensities and the volume of CSF spaces increased with aging, but these changes did not correlate with alterations in neuropsychological performance (Wahlund et al., 1996).

Accordingly, a recent study of 79 subjects aged 65-100 with a 15-year follow-up observed that the ventricular volume increased with aging, but the annual increase of CSF volume declined with age (Carlson et al., 2008). When measuring cortical thickness, global thinning has been shown to occur by middle age with prominent atrophy in the prefrontal cortex and relative sparing of the parahippocampal cortex (Salat et al., 2004).

The findings of hippocampal atrophy in relation to aging are controversial as the study by Good et al. demonstrated that there is relative preservation of the amygdala, hippocampus and entorhinal cortex during aging (Good et al., 2001), and other studies have rather consistently shown that the MTL structures display age-related shrinkage (Convit et al., 1995; Du et al., 2006; Jack et al., 1997; Pruessner et al., 2001; Scahill et al., 2003; Schuff et al., 1999; Tisserand et al., 2004). These inconsistencies may partly be explained by the tremendous interindividual variability in the hippocampal volume which was demonstrated recently in a study showing that 25 % of young healthy adults had similar hippocampal volumes as those of average study subjects aged 60 to 75 years (Lupien et al., 2007), while some of the elderly subjects with hippocampal atrophy may in fact have early stage pathological processes. However, apparently the hippocampus does undergo some volume decline, as shown in longitudinal MRI studies demonstrating that the atrophy of the hippocampus increases along with aging (Mueller et al., 1998; Scahill et al., 2003; Wang et al., 2003), and shrinkage is specifically detected in the head of the hippocampus and subiculum (Wang et al., 2003). A correlation of MTL atrophy in post mortem MRI and neuropathological findings has been detected in a study of elderly subjects over 85 years of age, however, in addition to AD, notable MTL atrophy was also related to other primary pathologies such as hippocampal sclerosis and argyrophilic disease (Barkhof et al., 2007).

There are also gender differences in brain size and possibly also in age-related atrophy patterns. In general, males tend to have larger brains (Sowell et al., 2007) and more GM in both absolute terms and relative to the total intracranial volume (Good et al., 2001), however, another study in young adults revealed that men have larger global volumes of GM, WM and CSF but when correcting for the individual brain size, women had locally increased concentrations of GM (Luders et al., 2005). Consistent with that finding, a study on 176 healthy adults with ages ranging from 7 to 87 years showed that females had thicker cortical GM in the inferior parietal and posterior temporal regions independent of body or brain size (Sowell et al., 2007). Furthermore, the results on age-related atrophy according to gender are

inconsistent, as some studies have shown that atrophy is more prominent in men than in women (Cowell et al., 1994; Good et al., 2001), and other studies have not detected any gender-related effects (Scahill et al., 2003; Carlson et al., 2008).

2.6.2. Structural MRI in MCI

A characteristic structural finding in MCI is atrophy of the entorhinal cortex and/or hippocampus, which has been observed in several studies, mostly using ROI-based techniques (Becker et al., 2006; De Santi et al., 2001; Dickerson et al., 2001; Du et al., 2001; Jack et al., 1999; Killiany et al., 2002; Pennanen et al., 2004; Wolf et al., 2004). These findings are in line with neuropathological studies demonstrating the presence of neuropathological changes in the MTL in MCI (Petersen et al., 2006). In addition to the MTL atrophy, studies that have assessed the whole-brain have detected a more widespread cortical GM decline in areas including the anterior and posterior cingulate, lateral temporal and parietal cortices, insula and the thalamus (Chetelat et al., 2002; Karas et al., 2004; Pennanen et al., 2005). The heterogeneity of MCI is also reflected in the atrophy patterns as indicated by a study showing that different brain structures were affected according to MCI subtype (Whitwell et al., 2007). According to that study, amnestic single- and multiple domain MCI was characterised by GM loss in the medial and inferior temporal lobes, and the multiple domain –group also had atrophy in the posterior temporal lobe, parietal association cortex and posterior cingulate (Whitwell et al., 2007). In contrast, the non-amnestic single domain group with language impairment exhibited atrophy in the left anterior inferior temporal lobe, and those non-amnestic single domain MCI subjects with attention or executive deficits suffered from atrophy in the basal forebrain and hypothalamus (Whitwell et al., 2007).

Since some of the MCI subjects will remain stable and some will progress to dementia, great interest has been focused on attempts to identify the features predicting future conversion. A plethora of studies has shown the potential of baseline MRI hippocampal volume, assessed either by manual tracing or visually, to predict conversion during follow-up (Jack et al., 1999; Grundman et al., 2002; Visser et al., 2002; Csernansky et al., 2005; Apostolova et al., 2006; Geroldi et al., 2006; DeCarli et al., 2007; Devanand et al., 2007; Fleisher et al., 2008; Tapiola et al., 2008;). When investigating the hippocampal subfields more specifically, there is some evidence that the shrinkage of the CA1 field in particular corresponds to conversion (Csernansky et al., 2005; Apostolova et al., 2006). Some of these studies have also shown that

entorhinal volume predicts conversion as well (Devanand et al., 2007; Tapiola et al., 2008). In addition to baseline MTL atrophy, it has been demonstrated that the hippocampal atrophy rate increases in relation to the cognitive decline and further progression to AD (Rusinek et al., 2003; Rusinek et al., 2004; van de Pol et al., 2007). Investigation of the whole-brain alterations corresponding to conversion has indicated that the increased rate of whole brain shrinkage or ventricular enlargement is indicative of conversion to a more impaired cognitive state (Jack et al., 2005; Jack et al., 2008a; Fleisher et al., 2008). A recent longitudinal 15-year follow-up study of ventricular enlargement concentrating on healthy aging and MCI revealed that the rate of annual ventricular volume change was greater in subjects with MCI compared to controls, and that the rate of ventricular volume expansion accelerated further 2.3 years prior to MCI diagnosis (Carlson et al., 2008). The cortical areas observed to lose GM in relation to the conversion from MCI to AD are the inferior and middle temporal gyrus, posterior cingulum and precuneus (Chetelat et al., 2005b) and inferior frontal and supramarginal gyrus (Bozzali et al., 2006) according to two longitudinal studies using VBM. The studies assessing volumetric changes of the brain in relation to conversion from MCI to AD have, however, been conducted in subjects deriving from memory clinics, thus representing a selected sample of MCI subjects, and the results have not been replicated in MCI subjects originating from the general population. This issue is addressed in the current thesis which has investigated the atrophy with whole brain analytic methods in population-based MCI subjects converting to dementia (Study I).

2.6.3. Structural MRI in AD

In agreement with the stagewise distribution of neurofibrillary pathology in AD, the first structural changes are detected in the MTL structures. Volumetric MRI studies have shown a decrease of entorhinal volume of 39 to 61 % in AD compared to controls (Juottonen et al., 1998a; Bobinski et al., 1999; Du et al., 2001) and the entorhinal volume has appeared to be a good discriminator between controls and AD patients (Erkinjuntti et al., 1993; Dickerson et al., 2001; Killiany et al., 2002). Similarly, hippocampal atrophy as extensive as 40 % of the hippocampal volume is consistently detected in AD compared to controls (De Leon et al., 1988; Seab et al., 1988; De Leon et al., 1989; Jack et al., 1992; de Leon et al., 1993; Convit et al., 1993; Bobinski et al., 1998; De Leon et al., 1997a; Frisoni et al., 1999; Dickerson et al., 2001; Du et al., 2001; van de Pol et al., 2006). Additionally, the hippocampal atrophy rates

have been reported to increase as compared to the situation in controls (Jack et al., 1998; Jack et al., 2000; Barnes et al., 2007). In addition to quantitative measurements of the MTL volumes, hippocampal atrophy can feasibly be evaluated by visual rating in AD patients relative to controls (Scheltens et al., 1992). A recently developed, quantitative measure called Automated Medial Temporal Lobe Atrophy Scale (ATLAS), which compares the intensity of standardized perihippocampal volumes to pontine volumes, has also indicated that AD patients have lower ATLAS scores than controls both at baseline and at repeat scanning, and the rate of atrophy was greater in AD patients (Ridha et al., 2007).

Widespread cortical atrophy is also observed in AD, affecting the temporal and cingulate gyri, precuneus, and insular and frontal cortices (Baron et al., 2001; Fox et al., 2001; Frisoni et al., 2002; Karas et al., 2003; Karas et al., 2007). Progressive atrophy of these cortical and MTL areas has also been detected in subjects with autosomal dominant mutations for AD while following their clinical conversion to AD (Fox et al., 2001). Cortical thickness measurements yield similar results, showing cortical thinning in temporal, orbitofrontal and parietal regions, with the most pronounced changes being observed in the MTL (Lerch et al., 2005).

2.6.4. Effect of APOE on brain structure

Initially it was reported that the asymmetry of hippocampal volumes was decreased in nondemented elderly subjects due to the presence of the APOE ε4 allele (Soininen et al., 1995) and in subsequent studies, healthy subjects carrying at least one ε4 allele of the APOE consistently displayed increased atrophy of the MTL in comparison to subjects without the ε4 allele both in cross-sectional and longitudinal studies (Cohen et al., 2001; Geroldi et al., 1999a; Moffat et al., 2000; den Heijer et al., 2002). The effect of the ε4 allele could be detected already in children, with ε4 carriers having a thinner entorhinal cortex than non-carriers (Shaw et al., 2007). Despite the MTL atrophy, carriers of ε4 allele do not seem to suffer any excessive global atrophy (den Heijer et al., 2002). More recently, it has been shown that healthy ε4 carriers have a thicker cortex in frontal and temporal areas than non-carriers, but correspondingly show steeper age-related atrophy rates in other brain regions (Espeseth et al., 2008). It has been speculated that the effect of APOE genotype on brain structure might be particularly crucial at older ages, be more lateralized to affect the right hemisphere and be moderated by cardiovascular disease (Cherbuin et al., 2007).

In MCI subjects, the ε4 allele is also associated with increased MTL atrophy (Farlow et al., 2004; Fleisher et al., 2005; Pennanen et al., 2006), and women in particular seem to be vulnerable to this effect (Fleisher et al., 2005). According to some reports, the APOE ε4 allele also increases the amount of hippocampal atrophy in AD patients in a dose-dependent manner (Lehtovirta et al., 1995; Geroldi et al., 1999a). A decline in entorhinal volume in ε4 carriers has also been detected, and again, females exhibit more atrophy than males due to the effect of APOE ε4 allele (Juottonen et al., 1998b). In addition to ROI-based measurements, also voxel-based studies have observed MTL atrophy in AD subjects with the ε4 allele (Boccardi et al., 2004). Consistent with the study showing a thicker frontal cortex in healthy ε4 carriers (Espeseth et al., 2008), it has been reported that the ε4 allele is associated with similar or larger frontal lobe volumes in AD patients (Lehtovirta et al., 1995; Geroldi et al., 1999a). The global brain atrophy rate also appears to accelerate according to the ε4 allele dose in both healthy controls at late mid-life and in subjects with cognitive impairment (Wahlund et al., 1999a; Chen et al., 2007). However, the effect of the ε4 allele on brain atrophy in MCI subjects converting to dementia has not previously been assessed. This thesis now presents preliminary results of the brain atrophy patterns according to the ε4 status in MCI subjects progressing to dementia (Study II).

2.7. Functional MRI findings in aging, MCI and AD

2.7.1. Functional MRI of healthy aging brain

The initial imaging findings on functional changes related to aging originate from PET studies, indicating that old subjects recruit the prefrontal cortex to a larger extent or bilaterally in comparison to young subjects during retrieval or recognition tasks (Grady et al., 1994; Cabeza et al., 1997; Grady et al., 2002). Moreover, it has been shown that young subjects activate the frontal cortex more than the elderly subjects during encoding (Cabeza et al., 1997; Grady et al., 2002) and that the increase in frontal activation in elderly subjects reflects a general response to increased cognitive effort or the need for resources, whereas frontal activations increase in a more task-specific manner in young subjects (Grady, 2002). These findings have mostly been corroborated with later fMRI studies.

Under-recruitment of the frontal cortex in elderly subjects during intentional encoding was demonstrated with fMRI, and the same study also indicated that the elderly subjects recruit multiple areas of frontal cortex non-selectively for both verbal and non-verbal material (Logan et al., 2002). However, it was speculated that the non-selective activation might associate to cognitive decline in advanced aging. Consistent with the previous studies, another fMRI study confirmed the finding that elderly subjects elicited stronger responses than young subjects during working memory, visual attention and episodic retrieval tasks and recruited bilateral frontal areas while performing tasks of working memory and visual attention (Cabeza et al., 2004). Additionally, this study found that the elderly subjects activated the occipital cortex less than the young subjects, thus reflecting a decline in sensory processing which may functionally be compensated by the frontal areas (Cabeza et al., 2004). The posterior-to-anterior shift of activation in aging occurs while performing different tasks, and these age-related increases in frontal activity correlate positively with performance and negatively with the occipital decreases of activity (Davis et al., 2007). In addition to the previously mentioned cognitive tasks, increased frontal activity has also been observed during semantic classification (Dennis et al., 2007), and thus the increased frontal activation appears as a rather constant finding related to aging.

With regards to the MTL function, it has been shown that the elderly subjects had less hippocampal activity during working memory, visual attention and episodic retrieval tasks, but instead displayed increased parahippocampal activity during episodic retrieval, this being consistent with the age-related increase in familiarity-based recognition (Cabeza et al., 2004). This finding was later confirmed with a study investigating aging effects on recollection confidence levels and revealing decreased recollection-related activity in hippocampus and increased familiarity-related activation in the rhinal cortex (Daselaar et al., 2006). Age-related reductions in hippocampal activity have also been demonstrated when the subjects were performing semantic classification tasks, and frontal increases have also been detected (Dennis et al., 2007). A reduction in the hippocampal-retrosplenial/temporoparietal connectivity was observed when assessing functional connectivity during a recollection task whereas the activity of the rhinal-frontal network was increased (Daselaar et al., 2006). This finding is in line with the posterior-to-anterior shift, and it was speculated that elderly subjects compensate for the hippocampal deficits by relying more on the rhinal cortex, possibly through a frontal top-down mechanism (Daselaar et al., 2006).

Even at rest, some parts of the brain are still active, constituting a default mode network which consists of the posterior cingulate cortex, bilateral inferior parietal cortex, left inferolateral temporal cortex, ventral anterior cingulate cortex and the hippocampus (Gusnard and Raichle, 2001; Greicius et al., 2003; Greicius et al., 2004). The function of this network is thought to relate to monitoring the surrounding environment and possibly to self appraisal (Gusnard and Raichle, 2001). The resting state networks have also been demonstrated to be consistent across subjects (Damoiseaux et al., 2006). One typical finding in functional imaging studies is deactivation of these areas when the subjects perform cognitive tasks (Gusnard and Raichle, 2001) and apparently the deactivation of certain areas is critical to successful learning of new information (Daselaar et al., 2004). Aging also affects the function of the default mode network. During successful encoding and retrieval of visuospatial associates, older subjects have been reported to show a stronger deactivation of the anterior cingulate cortex than young adults (Gould et al., 2006). This finding was suggested to reflect the greater suppression of task-unrelated thoughts in older compared to young subjects. Correspondingly, another study found age-related reductions of the posterior midline deactivation, but the medial frontal deactivations were increased (Davis et al., 2007). The failure of parietal deactivation has been related to poor memory performance (Miller et al., 2008). In conjunction with increasing age, the structures of the default mode network seem to correlate less with each other, and the disruption of the anterior-to-posterior components of the default mode network appears to be caused by perturbation of white matter integrity (Andrews-Hanna et al., 2007).

2.7.2. Functional MRI in MCI

Since the first neuropathological lesions in AD appear in the MTL, this region has been the focus of imaging studies in MCI. However, the findings on MTL function in MCI have been controversial. Decreased hippocampal activation in MCI subjects in comparison to controls was detected while they were asked to encode pictures of people engaged in activities of daily living (Machulda et al., 2003). In contrast, increased hippocampal activity in MCI compared to controls was present during encoding of face-name pairs (Dickerson et al., 2005). The discrepancy between these findings may be related to differences in the definition of MCI or in the severity of cognitive decline. In support of the latter possibility, it has been shown that MCI subjects with greater impairment, as evaluated by the CDR Sum of Boxes scale, recruit

the parahippocampal gyrus to a larger extent during visual encoding (Dickerson et al., 2004) and thus the increased activation may reflect a compensatory mechanism for the progressive neuropathological burden in the MTL. Accordingly, it has been shown recently in a follow-up study that greater baseline hippocampal activity in MCI subjects predicted a greater degree and rate of cognitive decline, even when accounting for the baseline cognitive status, age, education, gender, hippocampal volume and APOE status (Miller et al., 2007). It has also been demonstrated that the hippocampal activity in the MCI subjects does not show dynamic attenuation associated with learning as in healthy controls, possibly reflecting the learning difficulties in MCI (Johnson et al., 2007). The direct relationships of MTL volume and fMRI activation have not, though, been previously investigated but are presented in the current thesis (studies III and IV).

Another brain structure that is reported to exhibit functional changes related to MCI in fMRI studies is the posterior cingulate cortex. When MCI subjects undertook a task of episodic recognition of previously learned information they showed less activation in this area compared to healthy older subjects (Johnson et al., 2006a; Ries et al., 2006). Increased prefrontal activity in MCI compared to controls has also been observed during correct recognition processing (Heun et al., 2007), and increased activation of the precuneus during an angle discrimination task with varying task demands has been detected in MCI subjects who later progressed to dementia (Vannini et al., 2007).

The integrity of the default mode network, or resting state activity, has also been investigated in MCI. Less deactivation in the anterior frontal, precuneus and posterior cingulum was observed in MCI compared to controls, with differences between these groups being particularly pronounced in the medial frontal cortex (Rombouts et al., 2005a). The more impaired MCI subjects also displayed a loss of deactivation of medial and lateral parietal regions during memory tasks, however, the less impaired MCI subjects still had the capability to deactivate these structures (Celone et al., 2006). The parietal deactivations were also correlated with hippocampal function, suggesting that the MTL pathology may have an impact on cortical function (Celone et al., 2006).

Several differences in brain activation patterns between control and MCI subjects have thus been detected as discussed above. Since the functional changes in the brain probably precede the structural alterations, it has been suggested that fMRI might detect subjects with cognitive

impairment in an earlier phase than structural imaging. However, the discriminating potential of fMRI in MCI (or AD) has neither been reported nor compared to the discriminating power of structural imaging. The current thesis thus provides new information on the classification capability of fMRI during a paired-associates encoding task between control, MCI and AD subjects.

2.7.3. Functional MRI in AD

A constant finding in fMRI studies in AD is diminished or absent activation of the MTL when processing novel compared to repeated visually presented stimuli (Small et al., 1999; Rombouts et al., 2000; Kato et al., 2001; Machulda et al., 2003; Sperling et al., 2003b; Dickerson et al., 2005; Golby et al., 2005; Pariente et al., 2005; Remy et al., 2005). In contrast, there is evidence for frontal hyperactivation during cognitive tasks in AD. This phenomenon was first observed with PET (Grady et al., 2003) and recently substantiated in an fMRI study during successful versus non-successful encoding (Pariente et al., 2005). Moreover, it has been reported that neuropsychological measures in AD correlate with fMRI activation in the left superior temporal and prefrontal cortices (Diamond et al., 2007). The default mode network also appears to be disrupted in AD. When AD patients undertook a face encoding and a working memory task they deactivated only anterior frontal regions of the default mode network unlike healthy controls who also displayed deactivations in the posterior cingulum and precuneus (Rombouts et al., 2005a).

When interpreting fMRI studies in AD, one relevant finding relates to the AD-related differences in the haemodynamical responses. Initially it was shown that elderly subjects with or without dementia present smaller haemodynamical response amplitudes in the occipital cortex in comparison to younger subjects (Buckner et al., 2000). Some years later it was detected that the haemodynamical response is delayed rather than decreased in the occipital cortex in AD patients compared to controls, and diminished early phase activation was detected in widespread cortical areas in AD compared to controls (Rombouts et al., 2005b). Thus, model-free analysis methods, which attempt to avoid the problems of time differences in the haemodynamical response, have recently been developed for the assessment of AD patients (Rombouts et al., 2007a). Another methodological problem encountered by fMRI in

AD patients is the fact that the BOLD signal may be affected by underlying structural atrophy and thus also the average baseline signal may be reduced in AD (Rombouts et al., 2007b).

2.7.4. Effect of APOE on brain function in fMRI studies

Young healthy subjects have been tested for the effect of APOE ε4 allele on brain function. Interestingly, the young ε4 carriers have decreased brain activity when they are learning whereas non-carriers display increased activity, and the same finding was replicated during retrieval with equal memory performance (Mondadori et al., 2007). In contrast to the economic use of neural resources found in young ε4 carriers, increased activation in brain areas vulnerable to AD-pathology has been observed in elderly ε4 carriers during encoding and recall of unrelated word pairs, and the degree of activation correlated with the later cognitive decline experienced by these individuals (Bookheimer et al., 2000). Increased parietal and frontal activation has also been observed during a working memory task in cognitively intact healthy adults (Wishart et al., 2006). These results are controversial, since another study using a semantic categorization task reported decreased parietal activity in healthy ε4 carriers (Lind et al., 2006). A family history of AD may also modulate the effects of APOE ε4 allele since ε4 carriers without a family history of AD showed increased hippocampal activity during encoding of novel items whereas ε4 carriers with a first-degree family history of AD displayed the smallest signal changes (Johnson et al., 2006b). Thus, there may be other genetic factors contributing to the changes in activation since investigation of subjects at familial risk for late onset AD revealed increased activation in the temporal and frontal lobes when compared to subjects with no familial risk, and the effect was unrelated to APOE genotype (Bassett et al., 2006).

Most of the fMRI studies assessing the effect of APOE genotype to brain activation have focused on healthy individuals, however, one study has examined ε4-positive subjects with mild memory dysfunction, and observed that the hippocampus had enhanced connectivity with the anterior cingulate, inferior parietal/postcentral gyri and caudate nucleus during encoding of face-name pairs in comparison to ε4 non-carriers (Bartres-Faz et al., 2007).

3. AIMS OF THE STUDY

The aim of this thesis was to investigate the structural correlates of conversion from MCI to AD, and to assess the structural and functional changes related to MCI and AD both in the whole brain level and more specifically, in the MTL structures. In addition, this work aimed to assess the potential of structural and functional MRI measures in discriminating subjects with MCI and AD from healthy controls.

The specific aims of the studies I-IV were:

1. To determine the structural changes associated with future conversion to AD in MCI subjects (study I).

2. To assess the effect of the APOE ε4 allele on the pattern of structural atrophy related to progression from MCI to AD (study II).

3. To investigate brain functional changes during an encoding task, to compare the functional and structural alterations in MCI and AD subjects, and to investigate the correlation between MTL volumetry and fMRI whole-brain activation (study III).

4. To investigate the MTL activation more specifically during encoding and retrieval tasks, and to correlate MTL volumetry with MTL function. Moreover, this study aimed to assess the potential of MTL volumetry and hippocampal activation during memory encoding and recall to discriminate subjects into their clinical groups in comparison to standard neuropsychological testing (study IV).

4. SUBJECTS AND METHODS

4.1. Subjects: studies I-II

Study I included both controls and MCI subjects, and study II only MCI subjects. Details on the clinical characteristics of the study subjects are presented in Tables 1 and 2. Both the controls and MCI subjects were recruited from two distinct population-based longitudinal studies being undertaken in the Brain Research Unit, University of Kuopio during the period 1997-2004. The first cohort (cohort I) was a random sample of 1,150 persons (age range 60-76) drawn from a population register including all the subjects living in the city of Kuopio as well as in nursing facilities (Hanninen et al., 2002; Tervo et al., 2004). Eighteen subjects had either died or moved out of the area before the evaluation, and out of the 1,132 eligible subjects 71.6 % (806 subjects) participated in the cohort. The second cohort (cohort II) was derived from a large population-based random sample within the framework of the North Carelia Project and FINMONICA (Finnish Multinational Monitoring of Trends and Determinants in Cardiovascular disease) originally studied in the 1970s and 1980s (Kivipelto et al., 2001a). A randomly selected subgroup of 2,000 subjects from this sample was invited for re-examination and altogether 72.5 % (1,449 subjects) were re-evaluated in 1998.

In both cohorts, the follow-up visits consisted of a structured interview including the CDR scale (Hughes et al., 1982), demographic information, medical history, current medication, history of smoking and alcohol consumption, and a subjective assessment of memory disturbances and depression as well as a clinical examination. Informant interviews were not included in the first two follow-up visits of cohort I, but during the third follow-up it was also possible to include an informant interview due to the smaller number of subjects to be assessed. The informants were interviewed to corroborate the subject's memory complaints, and they also completed their version of the CDR interview. The neuropsychological testing in cohort I included the following tests: *Memory:* Visual Reproduction Test (immediate and delayed recall) from the Wechsler Memory Scale (Russel, 1975), Logical Memory Test (immediate and delayed recall) from the Wechsler Memory Scale-Revised (Wechsler, 1987), Word List Recall (immediate and delayed recall) from the CERAD Neuropsychological Assessment Battery (Morris et al., 1989), Delayed Recall of the Constructional Praxis from CERAD (Morris et al., 1989), New York University Paragraph Recall (immediate and delayed recall) (Kluger et al., 1999); *Language:* Abbreviated (15 items) Boston Naming Test

Table 1. Demographic and neuropsychological data of the study subjects in study I.

	Controls		Stable MCI		Progressive MCI	
	n	mean ±SD	n	mean ± SD	n	mean ±SD
Age	22	72.9 ± 4.5	43	72.7 ± 4.1	13	72.1 ± 4.2
Female/male		11 / 11		33 / 10		8 / 5
Education, y	22	6.8 ± 1.7	41	6.6 ± 1.5	13	6.6 ± 1.9
Follow-up, months	22	48.3 ± 13.6	43	32.3 ± 12.3 †	13	30.7 ± 9.9 †
MMSE	22	26.9 ± 1.8	43	23.8 ± 2.6 †	13	23.3 ± 2.3 †
CDR total score	22	0.0 ± 0.0	43	0.5 ± 0.0	13	0.5 ± 0.0
CDR sum of boxes	22	0.2 ± 0.3	37	1.2 ± 0.4 †	10	2.1 ± 1.1 †¤
Trail Making Test A	20	60.1 ± 20.5	35	79.6 ± 32.9 *	12	93.3 ± 31.9 **
Trail Making Test C	20	168.0 ± 78.0	35	214.9 ± 67.5 *	12	257.2 ± 70.1 **
Boston Naming Test	19	12.2 ± 2.2	34	10.4 ± 1.8 *	12	10.8 ± 1.9
Verbal Fluency (animal)	19	16.5 ± 6.0	35	14.7 ± 4.0	12	15.1 ± 3.7
Verbal Fluency (PAS)	19	38.2 ± 9.2	35	27.1 ± 9.8 †	12	26.3 ± 9.2 †
Clock drawing test	19	4.5 ± 1.5	34	4.2 ± 1.5	11	3.9 ± 1.2
Logical memory, immediate	20	9.7 ± 3.2	35	4.7 ± 2.5 †	12	6.2 ± 3.2 **
Logical memory, savings (%)	20	90.0 ± 9.3	33	74.2 ± 22.6 *	12	60.5 ± 35.8 *
Word list, delayed recall	18	5.5 ± 1.8	35	3.9 ± 1.8 *	12	2.2 ± 1.9 †¤
Word list, savings (%)	18	76.1 ± 19.6	35	61.0 ± 22.9 *	12	38.5 ± 30.5 †

MMSE = Mini-Mental State Examination; CDR = Clinical Dementia Rating; Logical memory = logical memory test from the Wechsler Memory Scale-Revised; Word list = word list learning from CERAD. † $P < 0.001$ vs. controls; ** $P < 0.005$ vs. controls; * $P < 0.05$ vs. controls; ¤ $P < 0.05$ vs. stable MCI

Table 2. Demographic and neuropsychological data on MCI subjects in studies I and II.

	Stable MCI APOE ε4 non-carriers		Stable MCI APOE ε4 carriers		Progressive MCI APOE ε4 non-carriers		Progressive MCI APOE ε4 carriers	
	n	mean ± SEM	n	mean ± SEM	n	mean ± SEM	n	mean ±SEM
Age	21	73.1 ± 1.0	22	72.2 ± 0.7	5	73.4 ± 4.5	8	71.3 ± 4.1
Female/male		16/5		17/5		3/2		5/3
Education, y	21	6.8 ± 0.3	20	6.5 ± 0.4	5	6.6 ± 1.9	8	6.6 ± 2.0
Follow-up, months	21	34.4 ± 2.8	22	30.3 ± 2.5	5	26.0 ± 5.8	8	33.3 ± 11.3
MMSE	21	24.0 ± 0.6	22	23.5 ± 0.5	5	24.0 ± 2.0	8	22.9 ± 2.5
CDR total score	21	0.5 ± 0.0	22	0.5 ± 0.0	5	0.5 ± 0.0	8	0.5 ± 0.0
CDR sum of boxes	18	1.1 ± 0.1	19	1.3 ± 0.1	4	2.5 ± 1.6	6	1.8 ± 0.7*
Trail Making Test A	18	81.6 ± 8.2	17	77.5 ± 7.6	5	109.0 ± 22.6†	7	82.1 ± 34.2
Trail Making Test C	18	196.8 ± 16.1	17	234.1 ± 15.3	5	276.0 ± 52.3*	7	243.3 ± 81.6
Boston Naming Test	18	10.8 ± 0.4	16	10.0 ± 0.5	5	10.2 ± 1.9	7	11.3 ± 1.9
Verbal Fluency (animal)	18	13.9 ± 1.1	17	15.5 ± 0.8	5	15.4 ± 2.4	7	14.9 ± 4.6
Verbal Fluency (PAS)	18	26.3 ± 1.8	17	28.0 ± 2.9	5	20.2 ± 5.2	7	30.7 ± 9.1‡
Clock drawing test	17	4.5 ± 0.3	17	3.9 ± 0.4	4	3.5 ± 1.3	7	4.1 ± 1.2
Logical memory, immediate	18	5.3 ± 0.6	17	4.0 ± 0.6	5	6.6 ± 4.8	7	5.9 ± 1.7
Logical memory, savings (%)	17	73.3 ± 6.5	16	80.7 ± 7.1	5	74.9 ± 26.2	7	52.0 ± 42.8
Word list, delayed recall	18	4.1 ± 0.4	17	3.7 ± 0.5	5	3.2 ± 1.5	7	1.4 ± 1.9*†
Word list, savings (%)	18	61.6 ± 5.4	17	60.3 ± 5.8	5	60.7 ± 18.0	7	22.6 ± 28.0*†

SEM = standard error of mean; MMSE = Mini-Mental State Examination; CDR = Clinical Dementia Rating; Logical memory = logical memory test from the Wechsler Memory Scale-Revised; Word list = word list learning from CERAD. * $P < 0.05$ vs. stable ε4 non-carriers; † $P < 0.05$ vs. stable ε4-carriers; ‡ $P < 0.05$ vs. progressive ε4 non-carriers

(Kaplan, 1991), vocabulary subtest of the Wechsler Adult Intelligence Scale-Revised (Wechsler, 1981); *Attention and executive function:* Verbal Fluency Test (Borkowski et al., 1965; Butters et al., 1987), Trail Making Test (TMT) parts A and C (Reitan, 1958); *Visuospatial skills:* Constructional Praxis from CERAD (Morris et al., 1989), Block Design from the Wechsler Adult Intelligence Scale-Revised (Wechsler, 1981); *Global Functioning:*

Clock Drawing Test (Morris et al., 1989), Mini-Mental State Examination (MMSE) (Folstein et al., 1975). According to the national guidelines in Finland, the subjects have not been opted to perform the backwards spelling task in the MMSE assessment and the scoring for the corresponding section was based solely on the seven subtraction task.

The detailed neuropsychological evaluation in cohort II (Kivipelto et al., 2001a) included the Buschke Selective Reminding Test (Buschke and Fuld, 1974), the Logical Memory Test from the Wechsler Memory Scale-Revised (Wechsler, 1987), the Boston Naming Test (Kaplan, 1991), the Vocabulary subtest of the Wechsler Adult Intelligence Scale (Wechsler, 1981), the Verbal Fluency Test (Borkowski et al., 1965), the Copy a Cube Test (Goodglass, 1972), the Clock Setting Test (Goodglass, 1972), the Block Design subtest of the Wechsler Adult Intelligence Scale (Wechsler, 1981), the Wisconsin Card Sorting Test using the Nelson's version (Nelson, 1976), and the TMT (Reitan, 1958).

4.1.1. Controls

Study I included 22 controls with ages ranging from 63 to 80. They were identified as cognitively normal in cohorts I or II, and they did not have any neurological or psychiatric disorders or psychoactive medication. The controls were followed on average for 48 months until the end of the cohort or drop out. Hospital records for those subjects not participating in all study visits were checked in order to learn about possible changes in cognitive status.

4.1.2. MCI subjects

Studies I and II included 56 MCI subjects (age range 64-81) who originated from cohorts I and II and were followed on average for 32 months (range 10-54). The subjects were classified as either single- or multidomain amnestic MCI. All consecutive MRI eligible subjects fulfilling the diagnostic criteria for MCI used in the cohorts, and not having any other neurological or psychiatric diseases or medication affecting cognition were included in the MRI study. Conversion to dementia or end of the cohort was considered as the end-point of the follow-up. Medical records of subjects not attending all study visits were inspected to detect possible conversion.

The diagnosis of MCI in both cohorts was made by consensus by the attending doctors and neuropsychologists. The criteria for MCI (Petersen et al., 1995) were as follows: 1) memory complaint by patient, family, or physician; 2) normal activities of daily living; 3) normal global cognitive function; 4) objective impairment in memory as evidenced by a score 1.5 SD below the average of a normative age-matched sample group in at least one of the memory tests included in the neuropsychological test battery; 5) CDR score of 0.5; and (6) absence of dementia according to the NINCDS-ADRDA criteria for possible AD (McKhann et al., 1984). Both psychometric and clinical aspects were thus considered in the diagnostics of MCI, but the eventual diagnosis was based on clinical judgement.

During the follow-up of the cohorts, conversions to dementia from MCI were observed. The diagnosis of dementia was based on the DSM-IV criteria (American Psychiatric Association, 2000), and diagnosis of AD was based on the NINCDS-ADRDA criteria (McKhann et al., 1984). The diagnosis of vascular dementia was based on the National Institute of Neurological Disorders and Stroke - Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINCDS-AIREN) criteria (Roman et al., 1993).

4.2. Subjects: studies III-IV

The fMRI studies III and IV included healthy elderly controls, MCI subjects and AD patients. Demographic details are presented in Table 3. None of the subjects had a history of neurological or psychiatric disease other than MCI or AD. At the time of image acquisition, 9 out of the 15 AD patients were not receiving cholinesterase inhibitor treatment; 2 patients were on donepezil, 1 patient on rivastigmine and 3 patients on galantamine. One AD patient was being treated with 10 mg of citalopram daily. The other patients were not taking any medications known to affect cognition at the time of imaging. All the subjects were right-handed and did not need correction of visual acuity during functional imaging.

4.2.1. Controls

Studies III and IV included 21 right-handed controls (age range 64–79), who were cognitively normal in the neuropsychological tests. The controls were derived from the third follow-up visit of cohort I described in more detail in studies I and II (Chapter 4.1).

Table 3. Demographic and neuropsychological details of study subjects in studies III and IV.

	Controls		MCI		AD	
	n	mean ± SD	n	mean ±SD	n	mean ±SD
Age	21	71.2 ± 4.9	14	72.4 ± 7.3	15	73.1 ± 6.7
Female/male	21	17/4	14	10/4	15	10/5
Education, y	21	7.9 ± 2.9	14	8.1 ± 2.6	15	8.2 ± 2.7
MMSE	21	27.7 ± 2.0	14	25.6 ± 3.1[*]	15	21.7 ± 3.7[# †]
CDR total score	21	0.0 ± 0.0	14	0.5 ± 0.0 [#]	8	0.8 ± 0.3 [#]
CDR sum of boxes	21	0.1 ± 0.2	14	1.6 ± 0.6 [#]	4	2.9 ± 1.4 [#]
Trail Making Test A	20	47.6 ± 13.6	14	59.5 ± 19.3	11	75 ± 23.6 [#]
Trail Making Test C	20	116.3 ± 60.2	13	186.0 ± 78.6 [#]	9	287.1 ± 21.8 [# †]
Boston Naming Test	20	13.1 ± 1.7	13	10.7 ± 2.4 [#]	9	10.9 ± 2.4[*]
Verbal Fluency (animal)	20	23.20 ± 4.9	14	16.6 ± 5.5 [#]	11	14.6 ± 5.6 [#]
Verbal Fluency (PAS)	20	44.3 ± 11.9	12	36.3 ± 14.7	4	34.0 ± 14.9
Clock drawing test	20	5.6 ± 0.8	11	4.7 ± 1.1	3	3.7 ± 1.2
Heaton, immediate	20	11.5 ± 2.4	9	9.2 ± 2.5	4	6.0 ± 3.4 [*]
Heaton, delayed	20	9.7 ± 3.1	9	5.2 ± 2.4 [#]	4	2.5 ± 2.9 [#]
Heaton, savings (%)	20	81.8 ± 15.4	9	57.4 ± 25.5 [*]	4	36.5 ± 47.6
Logical memory, immediate	20	12.6 ± 3.3	13	6.7 ± 3.9 [#]	9	5.6 ± 2.6 [#]
Logical memory, savings (%)	20	90.5 ± 11.1	12	82.3 ± 16.4	8	25.6 ± 30.0 [# ‡]
Word list, delayed recall	20	6.7 ± 1.6	13	4.5 ± 1.5 [#]	11	1.6 ± 1.5 [# ‡]
Word list, savings (%)	20	85.1 ± 14.2	12	67.1 ± 19.0 [#]	8	38.8 ± 32.2 [# †]

MMSE = Mini-Mental state examination; CDR = Clinical Dementia Rating; [*] $P < 0.05$ vs controls; [#] $P < 0.005$ vs controls; [†] $P < 0.05$ vs MCI; [‡] $P < 0.005$ vs MCI

4.2.2. MCI subjects

The MCI group in studies III and IV consisted of 14 subjects (age range 57–81) who were recruited from the third follow-up visit of cohort I described in more detail in Chapter 4.1. The criteria for MCI in the longitudinal cohort were as described earlier, and for the fMRI studies, MCI subjects with a total CDR rating of 0.5, and at least 0.5 in the memory subcategory were selected. The subjects belonged to the category of amnestic multidomain MCI when diagnosing them more specifically.

4.2.3. AD patients

In studies III and IV, the AD group contained 15 subjects (age range 62-83) who were recruited from the neurological outpatient clinic in the Kuopio University Hospital. The patients underwent extensive diagnostic evaluation including neuropsychological testing, laboratory sampling, CT or MR imaging of the head as well as a clinical and neurological examination. Diagnoses were made by experienced neurologists according to the NINCDS-ADRDA criteria for probable AD.

4.3. fMRI activation paradigm

The fMRI activation paradigm used in studies III and IV (Figure 4) was a modification of the Free and Cued Selective Reminding Test (FCSRT) (Grober and Buschke, 1987). The subjects in studies III and IV underwent thorough training prior to scanning to be able to perform the cognitive task satisfactorily. The paradigm consisted of three different conditions that alternated as blocks: 1) encoding (ENC) 2) immediate cued retrieval (ICR) and 3) baseline (BL). In the ENC condition, pictures of black-and-white line drawings were presented with a categorical cue word, which was contextually associated to the picture. Two separate instruction slides were shown before each encoding block, instructing the subjects to name and encode the pictures, and to press a button with their index finger as a response when they had accomplished the task. In the ICR condition, the categorical cue words were shown to the subjects, and the instruction slides shown prior to each retrieval block encouraged the subjects to recall the pictures with the help of the cue. The subjects gave responses each time they succeeded in retrieval by pressing the response button. During the BL, subjects were

instructed to focus their gaze at a simple fixation cross-hair and not to rehearse previous word-picture pairs. Subjects were instructed to perform the tasks silently to avoid motion artefacts related to overt speech. Behavioural data with the button press were collected in order to verify that the subjects were attending properly to the task, and to obtain the subjects' own estimate of their performance. The responses also ensured that the AD patients were able to execute the task adequately. The duration of the stimulus slides during activation blocks was 4.5 sec and the stimuli were separated by a 0.5 sec interstimulus fixation. The entire activation block including 5 picture slides, as well as the baseline block, lasted 25 sec. Each instruction slide was shown for 9.5 sec and was followed by a 0.5 sec interstimulus fixation. The combination of the ENC / ICR / BL – block, including instruction slides preceding each block, was repeated six times during one functional run, thus leading to a total functional imaging time of 13 min 30 s. The visual stimuli were presented using a laptop computer outside the scanning room and the task was projected to the subjects via a video projector (Lite Pro 620, In Focus Systems Inc, Wisconville, OR, USA) onto a translucent screen. The subjects viewed the stimuli through a mirror attached to the head coil.

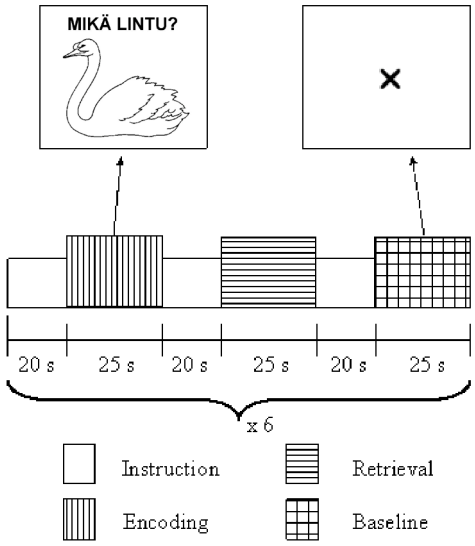

Figure 4. Schematic figure of the activation task. The task consisted of three different conditions: encoding, retrieval and baseline with instruction slides shown in between. The cycle of these three conditions was repeated six times during a single run. This article presents the results of the encoding-baseline contrast. During the encoding task, the subjects were instructed to encode and name the picture with the help of the contextually related categorical cue. The text above the picture translated from Finnish means "which bird?". During the baseline condition, the subjects were instructed to focus their gaze at the crosshair and not to rehearse the picture-word pairs.

4.4. MRI acquisition

4.4.1. Structural MRI acquisition

In studies I-IV, structural imaging was performed in Kuopio University Hospital with a Siemens Vision 1.5 T scanner (Erlangen, Germany) using a T1-weighted three-dimensional MPRAGE sequence with the following parameters: TR = 9.7 ms; TE = 4 ms; flip angle = 10°; field of view = 250 mm; matrix 256 × 256. The amount of vascular pathology was evaluated from T2-weighted, fluid attenuated inversion recovery (FLAIR) images in studies I-IV.

4.4.2. Functional MRI acquisition

Functional imaging in studies III and IV was conducted in Kuopio University Hospital with a 1.5 T scanner (Siemens Vision, Erlangen, Germany). A gradient echo echo planar imaging (EPI) sequence sensitive to BOLD contrast was used with the following parameters: TR = 2 500 ms; TE = 70 ms; flip angle = 90°; slice thickness = 5 mm plus 1 mm interslice gap; FOV = 256 mm; matrix size = 64 × 64; pixel size = 4.0 mm × 4.0 mm. The images were acquired in an oblique axial orientation aligned according to the anterior-posterior commissural line. The quality of the EPI images was visually inspected, and the overall image quality was reasonable and the in-plane distorsions insignificant.

4.5. Structural MRI data analysis

4.5.1. Measurement of entorhinal volume

The entorhinal volume was manually traced by a single tracer (AMJ) blinded to clinical data. A custom-made software for a standard Siemens work console was used for the tracing, and the volume of the outlined region was calculated with an in-house developed software. A trackball-driven cursor was used for the outlining, which proceeded in anterior-to-posterior direction. The boundaries of the entorhinal cortex were traced according to histology-based criteria designed for MRI volumetric measurements (Insausti et al., 1998). Outlining of the

entorhinal cortex started from the next slice after the appearance of the temporal stem, and the last slice measured was the one where the gyrus intralimbicus and uncus were not separable.

4.5.2. Measurement of hippocampal volume

Hippocampal volumes were also calculated by a single tracer (AMJ) blinded to clinical data with the same software as used for the measurement of the entorhinal cortex. The hippocampus included the dentate gyrus, the hippocampus proper and the subiculum. Tracing of the hippocampus started anteriorly where it first appears below the amygdala, and the last slice was the one where the crura of the fornices depart from the lateral wall of the ventricles.

4.5.3. Measurement of the intracranial area

The intracranial area was measured from a single coronal slice at the level of the anterior commissure to be used as an estimate of head size. It was used for the normalization of volumetric data as well as a nuisance variable in the VBM analyses. The entorhinal and hippocampal volumes were normalized to the intracranial area according to the following formula: (volume/intracranial area) × 100.

4.5.4. Voxel-based morphometry

An optimized VBM protocol was used (Ashburner and Friston, 2000; Good et al., 2001) under SPM2 (Wellcome Department of Imaging Neuroscience, London, UK; www.fil.ion.ucl.ac.uk/spm) in studies I-III. The first step of the analysis was to create a customized template. The origin of the spatial coordinates in the individual images was manually set to the anterior commissure and images were reoriented along the intercommissural line. Normalization to the Montreal Neurological Institute (MNI) T1-weighted template of SPM2 was conducted by using 12 parameter affine transformation, and resampling to a voxel size of 2 × 2 × 2 mm with a bilinear interpolation algorithm. The customized template was obtained by smoothing the normalized images with an 8 mm isotropic Gaussian kernel and averaging the smoothed images.

Customized prior probability maps were then created by partitioning the normalized unsmoothed images into GM, WM and CSF segments, smoothing with an 8 mm Gaussian filter and averaging the segmented images, which results in specific customized prior probability maps for each tissue segment.

Next, the original images were segmented, and parameters for normalization were determined from the obtained GM segments, customized prior probability maps and customized template. Using these parameters, original images were normalized to the customized template through affine and non-linear transformations, medium regularization, resampling to 2 × 2 × 2 mm and using no masking. The normalized images were further segmented into GM, WM and CSF utilizing the customized prior probability maps. The segmented GM images were modulated by multiplying the GM voxels with the Jacobian determinants derived from the non-linear step of spatial normalization. The modulated GM images were smoothed with a 12 mm Gaussian kernel.

In study I, the statistical analysis was performed with a "single subject: conditions and covariates" model including age, gender and intracranial area as nuisance covariates to compare the GM volumes between the study groups. Intracranial area was used to remove the effect of interindividual differences in head size, and it was measured with manual outlining from one coronal slice at the level of the anterior commissure in unnormalized T1-images. Between-group differences in study I were assessed using a t-test with a height threshold of $P < 0.05$, corrected for multiple comparisons by the family-wise error method. In study II, differences between the APOE ε4 carriers and non-carriers were assessed using a t-test with a height threshold of $P < 0.001$, uncorrected due to the small number of subjects. In study III, a t-test with a height threshold of $P < 0.01$ (uncorrected) and an extent threshold of 200 voxels was used for statistical analysis. The eventual threshold for significant differences in brain atrophy between study groups was a cluster-corrected $P < 0.05$.

The correlation of clinical and neuropsychological scores predicting dementia and GM in study I was performed using a "single subject: conditions and covariates" model including the corresponding individual neuropsychological test results as a covariate of interest, and age, gender and intracranial area as nuisance covariates. Due to a priori-hypotheses based on the between-group results, a lower threshold of $P < 0.005$, uncorrected was used. The detected correlations were further assessed with small volume correction (SVC), and voxel-level

family-wise error-corrected $P < 0.05$ was used as the criterion for significance. To detect correlations in other possible brain areas, a more stringent threshold of $P < 0.05$, corrected for multiple comparisons by the family-wise error, was applied. To examine the brain areas showing atrophy after the effects of differences in neuropsychological performance have been removed in study I, CDR and neuropsychological scores were included as nuisance covariates to the comparison of SMCI and PMCI. Due to having a priori –expectations on the brain areas revealing atrophy, the analysis was performed using a height threshold of $P < 0.005$ uncorrected. Clusters surviving SVC with a voxel-level FWE-corrected $P < 0.05$ were considered as significant. Effects on other possible brain areas were assessed using a threshold of $P < 0.05$ corrected for the whole brain.

4.6. Functional MRI data analysis

Functional image preprocessing and data analysis in study III and IV were carried out with SPM2 (Wellcome Department of Imaging Neuroscience, London, UK; www.fil.ion.ucl.ac.uk/spm). Spatial realigning of all functional volumes was performed first. Head motion was investigated using the data output of the motion correction algorithm and average values of the head motion in the three cardinal translational and rotational planes were calculated to assure that no group presented excessive movement in comparison to the other study groups. Slice-timing correction was performed to account for differences in acquisition order. Functional volumes were coregistered to T1-weighted structural volumes oriented along the intercommissural line. Coregistration success was visually controlled for each subject individually. Normalization parameters determined from the corresponding structural volumes were used to spatially normalize the functional volumes to the MNI reference brain. Spatial smoothing was performed with an 8 mm Gaussian filter.

In study III, functional image analysis was conducted by using general linear model on a voxel-by-voxel basis employing a random effects model implemented with a two level procedure. Functional volumes were sorted by condition and thus divided into ENC, ICR and BL blocks. The haemodynamical BOLD responses were modeled with a canonical haemodynamic response function. Only the ENC-BL contrast was assessed in study I, and it was first defined for each individual. In the within-group analysis, one-sample t-tests were carried out upon the resulting contrast images for each group, whereas in the between-group comparisons, we applied two-sample t-tests implementing SPM random effects analysis. A

height threshold of $P < 0.01$, uncorrected, and an extent threshold of 200 voxels was used for both the within-group and between-group data analysis. Statistical threshold for reporting final significance of fMRI results in the within-group and between-group analyses was a cluster-corrected $P < 0.05$. The anatomical location of activation areas was performed visually from the statistical activation maps superimposed on the spatially normalized T1 images, using the atlas of Duvernoy (Duvernoy, 1999) as a reference.

To inspect the relation of hippocampal volumes and fMRI activation in study III, a correlation analysis with a "single subject: conditions and covariates" model was performed for each study group. Individual normalized left and right hippocampal volumes were averaged together and used as the covariate of interest, and encoding performance as a nuisance variable. The search space for possible correlations was restricted by masking the correlation contrast with the between-group encoding-baseline comparison of MCI and control subjects. Both positive and negative correlations were tested. Local peak coordinates were inspected using an uncorrected threshold of $P < 0.01$ and further by performing SVC by placing a 10 mm sphere at the detected peak coordinates. SV-corrected voxel-level P values are reported for this analysis.

In study IV, the preprocessing steps of fMRI data were performed as described above. The hippocampal ROIs were defined by manual outlining from T1-weighted high-resolution 3D MPRAGE structural images for each individual using MRICro software (Rorden and Brett, 2000). The ROIs were outlined from coronal slices according to an identical procedure as described for hippocampal volumetry. The hippocampal ROIs were then applied to the functional EPI images using the MarsBaR-toolbox implemented in SPM2. The normalisation parameters acquired in the functional analysis were applied to the structural ROI to ensure the coregistration success. The extent of activation was defined as the number of voxels within each ROI that exceeded the statistical threshold of $P < 0.01$, uncorrected, in the ENC-BL and ICR-BL contrasts. A low statistical threshold was chosen as the analysis was restricted to an anatomically small area, and we had an a priori –hypothesis of finding activation. The extent of activation was also adjusted for the underlying hippocampal volume by dividing the number of activated voxels with the hippocampal volume.

4.7. Statistical analysis of demographic and fMRI ROI data

Statistical analysis of demographic and neuropsychological data in studies I-IV was performed using non-parametric Kruskal-Wallis and Mann-Whitney U. Results are expressed as means and standard deviation (SD) or standard error of mean (SEM). Criterion for statistical significance was set at $P < 0.05$.

In study I, the predictive accuracy of clinical and neuropsychological scores to conversion to dementia was assessed with a Cox regression model that included follow-up time as the time variable and conversion to dementia as the status variable. Results of the Cox regression were presented as the hazard ratio (HR) with 95 % confidence intervals.

In study IV, correlation analyses of volumes, hippocampal activations and neuropsychological test results were performed with Spearman's correlation. Discriminant analyses applying the enter-method were conducted to estimate the ability of the entorhinal and hippocampal volumes, neuropsychological tests and hippocampal activations to distinguish controls and MCI or AD patients, and MCI and AD patients from each other.

4.8. APOE genotyping

APOE genotype in study II was determined from blood leukocytes by extracting DNA with a standard phenol-chloroform extraction. APOE genotypes were analyzed by polymerase chain reaction and *Hha*I digestion as described previously (Tsukamoto et al., 1993).

4.9. Consent and ethical issues

Informed written consent was acquired from all the study subjects, and in the case of AD patients in the fMRI study, consent was obtained in the presence of a caregiver. The studies were approved by the Ethics Committee of Kuopio University Hospital.

5. RESULTS

5.1. Clinical characteristics of study subjects (studies I-IV)

In study I, the mean follow-up time of the 56 MCI subjects was 32 months (SD 11.8, range 10-54), and 48 months in controls (SD 14, range 26-60). Progression to dementia during this period was observed in 13 subjects (23.2 %; progressive MCI, PMCI), of whom 9 subjects had a clinical diagnosis of probable AD, 3 had vascular dementia and one had dementia of a mixed type. Two of the subjects with vascular dementia had a cortical stroke after the baseline imaging, and one subject suffered from subcortical disease which was detected after the baseline imaging. None of the controls converted to dementia during the follow-up. The mean follow-up time of the 43 stable MCI (SMCI) subjects from baseline MRI was 32 months (SD 12.3, range 10-52), and 31 months (SD 9.9, range 14-54) in PMCI with one subject converting within 18 months from the baseline, and from the remaining subjects, 7 subjects converted within 30 months, 4 within 36 months, with the final conversion being detected at 54 months. Two subjects in the SMCI group died during the follow-up. There were no significant differences between the controls and the SMCI or PMCI subjects in age or education at the baseline evaluation. The controls and MCI subjects differed significantly in their performance of several neuropsychological tests (for details, see Table 1). Compared to PMCI, the CDR Sum of Boxes score was significantly lower in SMCI subjects ($P < 0.017$), and correspondingly, the delayed recall of the wordlist included in the CERAD screening battery was significantly worse in the PMCI ($P < 0.023$).

Study II contained the same 56 MCI subjects as evaluated in study I. Among the 43 SMCI subjects, 21 were ε4 carriers and 22 non-carriers. Eight PMCI were either homozygous or heterozygous for the ε4 allele and five were non-carriers. Six PMCI ε4 carriers converted to AD and two to vascular dementia. Two of the converting non-carriers converted to vascular dementia and 3 to AD. Details are presented in Table 2.

In studies III and IV, the study groups did not differ significantly from each other in terms of age ($P < 0.40$) or years of education ($P < 0.90$), but there was a significant difference in the MMSE scores (controls vs. MCI, $P < 0.040$; controls vs. AD, $P < 0.001$; MCI vs. AD, $P < 0.007$). Details on other neuropsychological test scores are presented in Table 3.

5.2. Brain atrophy in stable and progressive MCI (study I)

5.2.1. Brain atrophy in stable MCI

The SMCI subjects displayed widespread atrophy in medial and lateral temporal areas, in the parietal and frontal cortices as well as in the thalamus and cingulate cortices compared to controls (for details, see Appendix: Study I, Table 4 and Figure 1). The controls did not reveal GM atrophy when compared to SMCI.

5.2.2. Brain atrophy in progressive MCI

Compared to controls, the PMCI subjects had a similar atrophy pattern as the SMCI, i.e. atrophy in the MTL, cingulum and in the temporoparietal and frontal cortical areas. In addition, the PMCI subjects exhibited atrophy in the precuneus compared to controls, unlike the SMCI. Details are presented in Appendix: Study I, Table 5 and Figure 1. Accordingly, atrophy in PMCI compared to SMCI was detected in the posterior cingulate cortex and precuneus bilaterally, and a large area of reduced GM was detected in the left hemisphere comprising the angular and middle temporal gyri extending to the occipital cortex. Details on the atrophy clusters are presented in Appendix: Study I, Table 6 and Figure 2. A trend for GM atrophy in the right posterior hippocampus was also observed, however, that cluster did not meet our criteria for statistical significance. The SMCI did not have atrophy relative to PMCI.

The comparison of those PMCI subjects converting to AD and SMCI yielded essentially similar results as the comparison of all the PMCI and SMCI subjects, but an additional statistically significant cluster of atrophy was detected in the right anterior hippocampus. No atrophy was detected in the SMCI when compared to PMCI who converted to AD. For image see Appendix: Study I, Figure 3.

5.2.3. Neuropsychological and clinical predictors of conversion to dementia

The CDR Sum of Boxes, TMT A, delayed recall of a wordlist and the savings of the wordlist were significant predictors of conversion to dementia in the univariate Cox regression analysis (for details, see Appendix: Study 1, Table 2). However, they only predicted conversion to dementia in general and when considering conversion to AD, none of these measures remained significant. Since the aforementioned test scores showed either significant correlations or trends for correlation with each other, none of them remained significant in a multivariate Cox regression analysis with respect to the prediction of dementia.

5.2.4. Correlation of clinical and neuropsychological measures to GM

A negative correlation between the TMT A performance and GM in the right precuneus in the PMCI was observed (peak coordinate: 6 -61 42; SV-corrected P = 0.002). In SMCI, the delayed wordlist recall score correlated positively with the GM bilaterally in the hippocampus (32 -26 -10; P = 0.005 and -31 -11 -20; P = 0.029) and in the right parahippocampal gyrus (23 -28 -24; P = 0.011). The delayed wordlist recall score correlated with the GM in the left hippocampus in controls as well, however, the correlation did not survive SV-correction (-20 -17 -20; P = 0.108). No correlations were detected outside the a priori-hypothesized brain regions.

5.2.5. Atrophy in progressive compared to stable MCI adjusted for cognitive status

Significant atrophy clusters in PMCI compared to SMCI were detected in the left middle temporal gyrus (peak coordinate -52 -60 3, SV-corrected P = 0.002) and in the left angular gyrus (-53 -76 7; P = 0.002) when introducing the CDR Sum of Boxes score as a nuisance covariate. After removing the effect of differences in the TMT A score, atrophy in PMCI compared to SMCI was found in the left middle temporal gyrus (-52 -66 6; P = 0.002), in the angular gyri bilaterally (-46 -67 21 and 53 -74 28; P = 0.013 and P = 0.005, correspondingly), in the right posterior cingulate cortex (6 -53 34; P = 0.013) and in the right precuneus (14 -58 48; P = 0.025). Significant atrophy clusters were detected in the left middle temporal (-52 -60 1; P = 0.042) and angular gyri (-52 -73 11; P = 0.008), and bilaterally in the precuneus (12 -56

47; P = 0.011 and -3 -59 46; P = 0.050) and in the posterior cingulate cortices (8 -45 34; P = 0.009 and -4 -43 35; P = 0.009) in the PMCI relative to SMCI when entering the delayed word list performance as a nuisance covariate in the analysis. Atrophy was not detected outside the a priori –expected areas.

5.3. The effect of APOE genotype on brain atrophy in stable and progressive MCI (study II)

The SMCI ε4 carriers revealed atrophy in the right amygdala and anterior hippocampus (peak coordinates 21 -6 -25, T score 3.57 and 23 -11 -26, T score 3.53) compared to stable non-carriers, who correspondingly did not show any atrophy relative to the ε4 carriers. In contrast, the PMCI ε4 carriers exhibited atrophy in the left inferior frontal gyrus (peak coordinate -56 17 9, T score 7.76) and around the banks of the left intraparietal sulcus (peak coordinate -43 -52 53, T score 6.44) compared to non-carriers (for image see Appendix, Figure 1). The PMCI non-carriers did not have grey matter atrophy compared to the ε4 carriers.

5.4. fMRI responses and underlying structural changes in MCI and AD (Study III)

5.4.1. Structural results

The MCI subjects revealed a significant GM volume reduction in the left superior frontal gyrus, in the left anterior hippocampus, and bilaterally in the thalamus relative to controls. GM volume reductions in AD patients relative to controls were detected in the right gyrus rectus, left amygdala, in the thalamus bilaterally and in the hippocampal formation bilaterally along its entire long axis. There was a trend towards more atrophy in frontal as well as parietotemporal cortices in AD but these differences did not meet our criteria for statistical significance. When compared to the MCI subjects, the AD patients showed a trend for GM volume loss in the left superior parietal cortex and in the right inferior temporal cortex. For details on atrophy clusters, see Appendix: Study III, Table 3 and Figure 2.

The volume of the hippocampus was obtained by manual outlining for each individual. The mean normalised volumes of the left and right hippocampi were 13.8 ± 1.9 (mean ± SD) and 14.5 ± 2.0 in control subjects, 13.4 ± 2.6 and 14.5 ± 2.2 in MCI subjects, and 11.5 ± 1.4 and

12.2 ± 2.6 in AD patients. There was a statistically significant difference in the hippocampal volume between the controls and AD subjects ($P < 0.005$), and between MCI and AD subjects ($P < 0.05$), but not between the controls and MCI subjects.

5.4.2. Behavioural data during fMRI

The behavioural data during functional imaging was collected completely in all 14 MCI, in 19 control and 11 AD subjects. The missing data in 2 control and 4 AD subjects are due to technical problems in the recording of the reaction times. Despite the missing reaction times, documentation of adequate button presses was completely recorded in all the subjects. The reaction times during encoding did not differ significantly between the three study groups ($P = 0.494$). Statistical analyses of the encoding responses, indicating the attention to the task and subjective evaluation of performance, showed no significant differences between the controls and MCI subjects ($P = 0.133$), but the controls performed significantly better than the AD patients ($P = 0.006$). The number of responses among the MCI and AD groups did not differ significantly ($P = 0.186$). The subsequent memory test, where the study subjects made a subjective evaluation of their memory performance, indicated that the controls felt they remembered 92.2 % of the picture-word pairs, whereas the MCI subjects remembered 82.6 % and AD patients recalled only 58.2 %. There were statistically significant differences in the memory performance between the controls and MCI ($P < 0.005$), AD and controls ($P < 0.002$) and MCI and AD ($P< 0.005$). For details, see Appendix: Study III, Table 2.

5.4.3. Within-group results during encoding

Detailed information on the within-group results is provided in Appendix: Study III, Table 4. In the control subjects, brain activations in the ENC-BL contrast were detected bilaterally in the superior frontal and precentral gyri, as well as in the left middle and inferior frontal and anterior cingulate cortices. There was a trend for bilateral activation of the middle and inferior frontal gyri, but the activations on the right hemisphere did not reach cluster-level significance. The left postcentral, supramarginal and superior temporal gyri were activated, as well as areas around the intraparietal sulci, and the fusiform, lingual and middle occipital gyri

bilaterally. Bilateral activations were detected in the hippocampus and parahippocampal gyrus, whereas the thalamic activation was only left-sided.

The within-group activation results of the MCI group in the ENC-BL contrast comprised bilateral activation in the middle and inferior frontal and precentral gyri. Postcentral gyrus and insula were activated on the left. Parietal activations were detected around the left intraparietal sulcus and in the angular gyrus. A trend for activation not meeting cluster-level significance was detected around the right intraparietal sulcus and bilaterally around the superior temporal sulci, and in the left superior temporal gyrus. MTL activations included regions in the parahippocampal gyrus bilaterally and in the left hippocampus. The thalamic activation was bilateral. Large occipital and inferotemporal activation areas were detected bilaterally in the superior, middle and inferior occipital, as well as fusiform and lingual gyri.

The within-group analysis of the AD group in the ENC-BL contrast revealed activation bilaterally in the inferior frontal gyri and in the right precentral gyrus, left insula, and in the left superior temporal gyrus. There was a trend towards activation around the right superior temporal sulcus. The right hippocampus and parahippocampal gyrus were activated, as well as the thalamus on the left. We also detected bilateral activations in the fusiform and middle and inferior occipital gyri, but no parietal activation areas were found in AD.

5.4.4. Increased fMRI responses in MCI

The MCI subjects compared to controls revealed increased activation along the left ventral visual stream in the fusiform gyrus extending further to the posterior parts of the parahippocampal gyrus and the hippocampus in the ENC-BL contrast. They also recruited the thalamus bilaterally to a larger extent than the controls. A trend towards increased activation in the right inferior frontal and left middle frontal gyri was also detected, which is consistent with the within-group results where the controls showed significant unilateral and the MCI subjects bilateral frontal activations. There were no activation areas greater in controls than in MCI but there was a trend for activation in the anterior cingulate gyrus. Details on the activation clusters are presented in Appendix: Study III, Table 5 and Figure 3.

5.4.5. Decreased fMRI responses in AD

Controls showed a trend for recruiting the left hippocampus, the left precentral and middle occipital gyri and the cuneus bilaterally more than the AD patients. The AD patients did not show greater activations than the controls. When compared to MCI, the AD patients did not reveal greater activations in any brain areas but there was a trend towards stronger activation in MCI relative to AD in the parietal areas, situated mostly around the intraparietal and intraoccipital sulcus. Details can be found in Appendix: Study III, Table 5.

5.4.6. Correlation of MTL volumes and fMRI responses in MCI

Interestingly, there was a negative correlation between the hippocampal volume and activity in the left parahippocampal gyrus (peak coordinate -24 -36 -12, uncorrected $P = 0.001$; SV-corrected $P = 0.044$) in the MCI subjects. A scatter plot of the correlation can be found in Appendix: Study III, Figure 4. The right fusiform gyrus also showed a trend for negative correlation (peak coordinate 32 -48 -18, uncorrected $P = 0.002$; SV-corrected $P = 0.101$). There were no positive correlations between the hippocampal volume and brain activations in the MCI subjects. No correlations between the hippocampal volume and parahippocampal activation were detected in either controls or AD patients.

5.5. Discriminative accuracy of MTL volumetry, hippocampal fMRI and wordlist recall in MCI and AD (study IV)

5.5.1. MTL volumetry

Details of the MTL volumes are presented in Appendix: Study IV, Table 1. The left and right entorhinal cortices were significantly smaller in MCI subjects than in controls ($P < 0.001$) whereas there were no differences in hippocampal volumes ($P = 0.80$). In contrast, bilateral atrophy was observed in the entorhinal cortex and hippocampus in the AD patients relative to controls ($P < 0.001$ and $P < 0.002$) and to MCI subjects ($P < 0.002$ and $P < 0.02$).

5.5.2. Behavioural and fMRI data during encoding and retrieval

Behavioural data during functional imaging were collected from 19 EC, 14 MCI subjects and 11 AD patients. The missing data from 2 control and 4 AD subjects are due to technical problems in the recording of reaction times. Despite the missing reaction times, documentation of adequate button presses was completely recorded in all of the subjects. A significant difference in the performance during encoding was only present between EC and AD ($P < 0.002$). In contrast, there were significant differences in the performance during retrieval between the EC and MCI ($P < 0.005$), AD and EC ($P < 0.002$) and MCI and AD ($P < 0.005$). The reaction times did not differ significantly between the study groups during encoding ($P = 0.494$) or retrieval ($P = 0.164$). For details on the behavioural measures see Appendix: Study IV, Table 2.

Details on the mean extents of hippocampal activation are presented in Appendix: Study IV, Table 2. During both encoding and retrieval of the word-picture pairs, all the groups showed bilateral hippocampal activation. Although the absolute extent of activation tended to be greatest in the EC and smallest in the AD patients, no significant differences were found between the groups during encoding or retrieval (all P values above 0.70) probably due to the extensive inter-individual variability in BOLD fMRI responses found within each group. When adjusting the extent of hippocampal encoding and retrieval activations with the corresponding hippocampal volumes, the MCI subjects' left hippocampal encoding activation was similar to EC whereas the retrieval activation was similar to that measured in AD patients. This finding suggests that the MCI subjects recruit the left hippocampus to a greater extent relative to the remaining volume than the EC during encoding of word-picture pairs.

5.5.3. Correlations of MTL volumes, hippocampal activation and wordlist retrieval

The left and right entorhinal and hippocampal volumes correlated with each other in the controls (entorhinal cortices: $r = 0.87$, $P < 0.001$, hippocampi: $r = 0.80$, $P = 0.001$). The hippocampal activations did not correlate with the MTL volumes. The left and right hippocampal encoding and retrieval activations correlated with each other (range of $r = 0.69$ - 0.88, $P < 0.02$).

In the MCI subjects as well, the entorhinal and hippocampal volumes correlated with each other (entorhinal cortices: $r = 0.84$, $P < 0.001$, hippocampi: $r = 0.82$, $P < 0.001$). One interesting finding was that the left entorhinal and the right hippocampal volumes correlated negatively with the left hippocampal activation during encoding of the word-picture pairs ($r = -0.59$, $P < 0.05$). In contrast, the right entorhinal volume correlated positively with the right hippocampal activation during retrieval of the pictures ($r = 0.74$, $P < 0.04$). The hippocampal encoding and retrieval activations did not correlate with each other in MCI subjects. A scatterplot of the correlation is presented in Appendix: Study IV, Figure 2.

The left and right entorhinal and hippocampal volumes correlated with each other also in the AD patients (entorhinal cortices: $r = 0.74$, $P < 0.002$, hippocampi: $r = 0.69$, $P < 0.005$). However, the hippocampal activations did not correlate with the MTL volumes, and similarly to the MCI subjects, there were no correlations between the hippocampal encoding and retrieval activations.

The delayed recall score of the CERAD wordlist correlated with the left entorhinal volume in MCI subjects ($r = 0.57$, $P < 0.05$) while correlations with the wordlist score were not detected in other subject groups.

5.5.4. Results of the discriminant analysis

When assessing the EC and MCI, the total entorhinal volume was the best discriminator with an overall correct classification of 85.7 % of the subjects. Similarly, the entorhinal volume was the best single discriminator between the MCI and AD subjects, with a correct classification of 86.2 % of the subjects, and also between EC and AD, distinguishing 97.2 % of the subjects correctly.

The hippocampal volume discriminated only 42.9 % of the control and MCI subjects, but succeeded better in MCI and AD subjects by differentiating 69.0 % correctly, and the discrimination between controls and AD was similar, i.e. 69.4 %.

The absolute extent of left and right hippocampal encoding and retrieval activation performed worst by discriminating correctly only 41.4-54.2 % of controls and MCI subjects. Between

MCI and AD subjects, the hippocampal encoding and retrieval activations classified 50.0-56.5 % of the subjects correctly, and between controls and AD subjects the discriminating accuracy ranged between 44.8 and 57.7 %. Using the atrophy-corrected extent measures of hippocampal encoding and retrieval activations did not improve the discriminating power of fMRI in any of the comparisons and thus these results are not presented in detail.

The traditional and validated neuropsychological test, delayed recall of a wordlist from the CERAD test battery, succeeded rather well by discriminating 81.8 % of the controls and MCI subjects correctly. Similarly, it was the second best discriminator between the controls and AD patients (correct classification 93.5 %). However, the correct classification between MCI and AD was only 75.0 %.

When testing the discriminating potential of combinations of these measures, the combination of entorhinal volume and wordlist recall score appeared to be best. This combination classified all the controls and AD patients correctly and also improved the correct classification of controls and MCI up to 87.9 %. However, the classification of MCI and AD subjects did not improve with this combination and the entorhinal volume alone was the best discriminator between these groups. Details of the discrimination results are presented in Appendix: Study IV, Table 3 and Figure 3.

6. DISCUSSION

6.1. Study subjects (studies I-IV)

The strength and novelty of studies I and II is that the study population is derived from population-based cohorts unlike in two other previously published MRI whole brain volumetric studies investigating brain structure alterations in relation to conversion to AD in where the MCI subjects originated from specialist memory clinics (Bozzali et al., 2006; Chetelat et al., 2005b). Subjects that have been randomly selected from the population may better reflect the course of memory disorders and conversion to dementia in the general population than a selected sample of subjects that have sought medical advice for cognitive complaints. Additionally, study subjects originating from memory clinics may be more impaired at the initial examination since they already are experiencing prominent memory problems. One drawback in the random selection of MCI subjects, though, is that there may be more variability both in the symptoms as well as aetiologies of clinical syndromes whereas subjects deriving from memory clinics may be more homogenous.

The subjects in studies I and II underwent repeated follow-up visits with extensive neuropsychological testing and the subjects were diagnosed with widely used criteria for MCI (Petersen et al., 1995). The controls and MCI subjects were well-matched according to age and education. The conversion rate to dementia in the present study was 23 % in three years, leading to an annual conversion rate of 7.7 % which is in keeping with previous research. In previous longitudinal population-based studies, the risk for developing dementia has ranged between 11 and 33 % over two years (Ritchie, 2004), and in a meta-analysis including both community-based and memory clinic studies, the conversion rate varied between 6 and 25 % (Petersen et al., 2001).

A limitation is the small number of PMCI subjects in studies I and II. Particularly in study II, the small number of subjects limited the statistical analysis and thus the results of study II need to be considered as exploratory. The prevalence of APOE ε4 allele in the MCI subjects included in studies I and II was actually high (29/56; 52 %) when compared to that seen in the general population (15 %) (Tanzi and Bertram, 2005) but the low number of converted subjects represented the limiting factor.

The control and MCI subjects in studies III and IV were also derived from the population-based cohort but the AD patients came from the neurological outpatient clinic in the Kuopio University Hospital. As the subjects originated from different sources, there also was variability in the administered neuropsychological test battery and thus some neuropsychological data acquired from MCI subjects is lacking from AD patients, complicating the comparison of the subjects. The controls, MCI subjects and AD patients did not differ according to age or education, indicating that the detected differences are not likely to be age-related or due to differences in education level.

6.2. Structural changes associated with conversion to dementia (study I)

Study I demonstrated that atrophy in the lateral temporoparietal, medial temporal and frontal cortices was detectable in both SMCI and PMCI subjects relative to controls at baseline, and that additional atrophy of the precuneus, posterior cingulate and temporoparietal cortices was characteristic to PMCI at baseline imaging performed on average two and a half years prior to conversion. According to the previous literature, aging-related structural changes are most prominent in the frontal cortex (DeCarli et al., 1994) whereas aging-related cognitive decline has been associated with reduced GM in the posterior parietal, prefrontal and (medial) temporal cortices (Tisserand et al., 2004). The posterior cingulum is also affected rather early by AD-related neuropathological changes, being one of the first regions to be affected after the initial Braak stages I and II (Braak and Braak, 1991). An early involvement of the posterior cingulum in MCI and AD has also been observed in functional imaging studies such that fMRI has shown abnormal, sustained activation during memory tasks in this area in AD patients (Lustig et al., 2003) and PET has shown hypometabolism of the posterior cingulate in MCI subjects (Nestor et al., 2003). Scahill and colleagues observed that the atrophy of the posterior cingulate cortex and the adjacent precuneus was significantly increased from the onset of symptoms in subjects at genetic risk for AD (Scahill et al., 2002) and a previous structural study on converting MCI subjects also reported atrophy of the posterior cingulum (Chetelat et al., 2005b). In addition, a recent study indicated that the expression of genes related to energy metabolism was notably reduced in the posterior cingulum, and the gene expression was even more affected than in the MTL (Liang et al., 2008). Thus, the finding of atrophy in the temporoparietal and posterior parietal cortices in PMCI subjects is in accordance with the previous literature, and these results indicate that this region may experience significant atrophy, particularly during the conversion process of MCI to AD.

In the comparison of SMCI and all PMCI subjects, hippocampal atrophy was not severe although atrophy of the MTL is a prominent finding in MCI and AD (de Leon et al., 1988; Seab et al., 1988; de Leon et al., 1989; Jack et al., 1992; de Leon et al., 1993; Convit et al., 1993; de Leon et al., 1997a; Bobinski et al., 1998; Frisoni et al., 1999; Juottonen et al., 1999; Dickerson et al., 2001; Du et al., 2001; Killiany et al., 2002; Pennanen et al., 2004; van de Pol et al., 2006). However, in vascular dementia hippocampal atrophy may not be so remarkable (Du et al., 2002), and thus the fact that some of the PMCI subjects converted to vascular or mixed dementia may confound the findings. Accordingly, when those PMCI subjects with later AD were compared to SMCI, a significant cluster of atrophy in the right hippocampus was found, which is congruent with the previous literature (Chetelat et al., 2005b; Bozzali et al., 2006). The small number of subjects developing VaD unfortunately prevented a valid direct comparison between those subjects converting to AD and those progressing to VaD.

The PMCI subjects were more impaired than the SMCI in tests assessing both frontal functions (TMT) and MTL functions (delayed word list recall), and had a significantly higher CDR Sum of Boxes score. However, when removing the effects of clinical and neuropsychological scores in the comparison of SMCI and PMCI, essentially the same brain regions emerged as in the comparison of the same subjects without these variables. Thus, structural differences were independent of the cognitive performance level, suggesting that analysis of the brain structure may provide important supplemental information to the prediction of conversion to dementia. However, the analyses performed in this study did not allow evaluation of the predictive value of the structural changes. Exploration of the brain areas related to conversion in this study may, nonetheless, be useful in the development of imaging markers for early diagnostics of dementia, perhaps more efficiently with ROI-based techniques.

6.2.1. Correlation of clinical and neuropsychological measures to GM

The predictors of conversion to dementia in study I were the CDR Sum of Boxes, TMT A and the delayed wordlist recall, and the correlation of these measures to GM was also investigated in all the subject groups. The only correlation found in PMCI was an inverse correlation between the TMT A score and the GM in the precuneus. Although it is normally considered as a test related to frontal functions, the TMT also includes a strong visual component through

which it can be linked to the precuneus. The negative correlation is interpreted to signify that those subjects performing better in the test have more GM in the precuneus, since in the TMT the result is better the smaller the resulting value, making this finding reasonable.

In contrast, differential correlations were detected in SMCI subjects and controls. In the SMCI, the performance in the delayed wordlist recall was related to GM in the MTL structures, in particular to the hippocampus. It is widely known that the MTL is active in the formation and consolidation of declarative episodic memory (for review, see (Squire et al., 2004)) which provides a logical explanation to account for this finding. Moreover, a trend towards a similar correlation was also present in control subjects. The lack of this correlation in PMCI subjects may thus reflect the disruption of the MTL memory system in subjects approaching dementia. Correlations of GM and clinical or neuropsychological measures were not detected for other tests in SMCI or controls.

6.3. Increased atrophy in APOE positive PMCI (study II)

In study II, it was found that the PMCI ε4 carriers had more atrophy in the left inferior frontal gyrus and in the parietal cortex compared to non-carriers whereas in SMCI, atrophy was limited to the MTL in ε4 carriers compared to non-carriers. Atrophy of the MTL is a common finding in relation to the APOE ε4 allele in healthy controls, and MCI and AD subjects (Lehtovirta et al., 1995; Juottonen et al., 1998b; Moffat et al., 2000; Cohen et al., 2001; den Heijer et al., 2002; Pennanen et al., 2006) whereas the ε4 carriers have not shown more global atrophy (den Heijer et al., 2002). One study has even reported larger frontal lobe volumes in ε4 positive AD patients (Geroldi et al., 1999b). The results of study II point to increasing atrophy in PMCI subjects carrying the ε4 allele which is in keeping with the previous literature indicating that the ε4 allele can make the brain more vulnerable to AD-related pathology by disrupting aging-related cellular mechanisms, impairing neuroreparative capability and promoting an earlier onset of symptoms in AD by enhanced deposition of abnormal protein aggregates (Berg et al., 1998). The linkage of amyloid processing and APOE has been shown in a study showing that the β-secretase levels are higher in MCI and AD subjects carrying the ε4 allele (Ewers et al., 2008). Accordingly, recent studies have demonstrated that the atrophy rate in cognitively impaired ε4 carriers is higher when compared to non-carriers (Chen et al., 2007; Jack et al., 2008a; Wahlund et al., 1999a). One interesting question remains - why do the ε4 positive SMCI not develop dementia?

Apparently there are other genetic or lifestyle factors that have protected them from developing AD. In addition, although the follow-up period was rather long, some of the SMCI may have converted after the study or will convert to AD in the future, i.e. they are not non-susceptable, but simply very slow converters.

6.4. Compensatory activation in MCI (studies III and IV)

Previous results from fMRI studies in MCI are conflicting, as both decreased (Machulda et al., 2003) and increased (Dickerson et al., 2004; Dickerson et al., 2005) fMRI responses have been reported. The seeming discrepancy in the previous studies may be explained by differences in the degree of cognitive decline in the subjects of these studies, the differential diagnostic criteria used for MCI, and additionally, the use of differential fMRI data analysis methods all of which lead to difficulties in comparing results of various studies with each other. In study III, using a whole brain fMRI data analysis, it was found that the MCI subjects exhibited significantly higher fMRI responses in the fusiform and posterior parahippocampal gyri and in the hippocampus when compared to controls. The increased activation in the aforementioned regions in MCI compared to controls may reflect compensatory mechanisms, i.e. activation of differential neuronal networks in order to compensate for the evolving dysfunction of the MTL while trying to achieve the level of control subjects in the behavioural performance. The analysis of behavioural data revealed no difference in the encoding performance between MCI and controls, but the MCI subjects performed poorly in retrieval, which suggests that they experience difficulties in encoding, possibly resulting from the increased cognitive efforts needed during the encoding process.

Compensatory parahippocampal activation during a memory task has earlier been reported in healthy elderly subjects (Cabeza et al., 2004). Study III did not include young control subjects and thus it is not possible to comment on the level of the activation of the elderly controls compared to young subjects. The increased MTL and fusiform activations in MCI subjects are, however, unlikely to be age-related as the mean ages of the control and MCI groups were similar. Support for the compensatory activation hypothesis comes from the previous literature reporting a loss of outer molecular layer neurons in the dentate gyrus in 75 per cent of MCI subjects when compared to the control group (Scheff et al., 2006), and thus the function of neural networks in the MTL in MCI may have altered to compensate for the synaptic and neuronal loss. In summary, the compensatory activations noted in MCI subjects

may result from a combination of aging-related shifts in the function of the MTL substructures (Daselaar et al., 2006) and the dysfunction caused by neurofibrillary pathology (Braak and Braak, 1991).

The hypothesis of compensatory activation was further supported by the finding of a negative correlation between hippocampal volume and fMRI activation in the posterior parahippocampal gyrus in study III. This correlation indicates that the MCI subjects with smaller hippocampal volumes elicit stronger parahippocampal activation. Importantly, this phenomenon was unique to the MCI subjects, since this correlation was not detected in the elderly controls or AD patients. As the structural VBM-results in study III demonstrated that hippocampal atrophy in MCI was located in the anterior part, the increased posterior MTL activation may be an attempt to compensate for the atrophy in the anterior MTL structures.

The correlation of MTL volumes and hippocampal activation was examined in more detail in study IV. In the MCI group, the hippocampal encoding activation correlated negatively with the entorhinal and hippocampal volumes such that the smaller the MTL volumes, the greater the fMRI activation. Thus, the hippocampal activation appeared to compensate for the atrophy in the entorhinal cortex. Once again, AD patients or controls did not reveal any correlations between MTL volumes and hippocampal activation. In contrast, the hippocampal encoding and retrieval activations correlated with each other in controls, but not in MCI or AD subjects, perhaps pointing to dysfunction of the MTL memory functions in the cognitively impaired groups. The findings of studies III and IV indicate that an increased, compensatory hippocampal or posterior parahippocampal activation may be related to emerging atrophy especially in the anterior parts of the MTL during the course of MCI, which is in accordance with previous studies suggesting that there is a phase of compensatory MTL hyperactivation preceding the failure of hippocampal function in clinical AD (Dickerson et al., 2004; Kircher et al., 2007). Apparently the MCI subjects are capable of eliciting compensatory activations that may be triggered by the evolving neuropathological deficits as indicated by the significant entorhinal atrophy in MCI in study IV. Alternatively, the compensatory activation may relate to a differential cognitive strategy or the increased efforts required in the MCI subjects to maintain a relatively good behavioral performance. However, the ability to compensate seems to disappear later in the AD stage. This can be concluded from fMRI studies showing diminished hippocampal activation in AD compared to controls (Dickerson et al., 2005; Rombouts et al., 2000; Small et al., 1999; Sperling et al., 2003b).

In study IV, it was observed that in contrast to fMRI responses during encoding, the MCI subjects showed levels of fMRI activation similar to AD patients during cued retrieval using both absolute and atrophy-corrected extent measures. Furthermore, the MCI subjects with greater entorhinal volumes showed more retrieval-related hippocampal fMRI activation in the correlation analyses. Thus, compensatory MTL activity was not observed during retrieval. Impaired encoding and storage of novel information are considered as the earliest cognitive deficits in AD (Petersen et al., 1992; Petersen et al., 1994), and thus compensatory activation may be present particularly during encoding tasks. However, it is worth noting that during the recall condition, the subjects only provided a subjective evaluation of the retrieval success or failure. Thus, the relatively easy recall task may not have required increased cognitive efforts from the MCI subjects and no compensatory activation was correspondingly observed. The interactions of objectively evaluated successful vs. failed encoding and retrieval performance and MTL activity in MCI cannot fully be explored by studies III and IV, and further event-related fMRI investigations will be needed to segregate the encoding and retrieval dysfunction in the earliest stages of AD.

6.5. Decreased activation in AD (studies III and IV)

In study III, the activation pattern observed at the within-group level in the AD patients was typical for picture naming and encoding tasks with activation in the inferior frontal and lateral temporal cortices, in the MTL, and in the fusiform and occipital cortices. In the between-group comparisons, the AD patients showed evidence for decreased activation compared to controls in the occipital and frontoparietal cortex and in the MTL, and towards both structural atrophy and diminished fMRI activation in the parietal cortices in comparison to MCI subjects. These findings are consistent with the known pattern of progressive deterioration in AD (Braak and Braak, 1991), however, these between-group comparisons did not meet the criteria set for significance and therefore must be interpreted with some caution. The AD patients also exhibited widespread atrophy in frontal, parietal and medial and lateral temporal regions, and according to recent knowledge, the baseline fMRI T2* signal may be attenuated because of underlying structural atrophy (Rombouts et al., 2007b). Thus, the decreased activations detected in AD may partly be associated with the atrophy. Moreover, it has been reported that the haemodynamic response may be delayed in AD (Rombouts et al., 2005b). The temporal dynamics of the haemodynamic response were not examined in studies III and

IV, and thus the detected activations may also be confounded by the possible differences in the haemodynamic response.

There were no significant differences between the AD and MCI subjects in MTL activations in the SPM random effects analysis in study III. The ROI-based analysis in study IV revealed that the AD patients had activated their hippocampus to a lesser extent than the MCI subjects during encoding, but the difference was not significant. During retrieval, the MCI and AD patients seemed to activate the hippocampus in a rather similar manner. The neuropsychological profile of the MCI subjects supports this finding as in some tests the MCI subjects performed almost as poorly as the AD patients. Some of these tests are also known to activate MTL structures as evidenced by a previous fMRI study investigating verbal fluency (Pihlajamaki et al., 2000). The MCI and AD subjects did, however, differ from each other in terms of compensatory MTL activations that were only detected in MCI subjects.

6.6. Discrimination of MCI and AD (study IV)

Study IV showed that the volume of the entorhinal cortex was the best discriminator between the controls and MCI subjects, which is consistent with the literature (Killiany et al., 2002; Pennanen et al., 2004). The entorhinal volume was the most successful discriminator in all the other group comparisons as well. Moreover, combining the entorhinal volume with the delayed wordlist recall score, which is one of the most sensitive neuropsychological indicators of early AD (Petersen et al., 1992; Petersen et al., 1994), further improved the classification between the controls and MCI. In the case of the delayed wordlist recall, one must be cautious not to make circular deductions since memory impairment is a part of the diagnostic criteria in MCI and AD. However, the good discriminative power of the combination of the entorhinal volume and wordlist recall suggests that these two parameters might prove useful in the early detection of MCI and AD.

In contrast, the hippocampal volume was not sensitive in discriminating amnestic MCI subjects from healthy elderly individuals in study IV, but succeeded somewhat better in the discrimination between MCI and AD subjects. Between-group differences in hippocampal volume were less prominent than in the entorhinal cortex, and thus the entorhinal volume appears to be a better discriminator since it possibly decreases more linearly across the groups. This finding is in accordance with the knowledge that the AD-related neurofibrillary

tangles spread from the entorhinal cortex to the hippocampus as the disease progresses (Braak and Braak, 1991), and is also consistent with the known involvement of these brain structures in episodic memory function, which is progressively impaired during the course of AD (Petersen et al., 1992; Petersen et al., 1994).

No significant differences in the ROI-based hippocampal activations were detected between the groups in study IV, and correspondingly, the hippocampal encoding and retrieval activations were the least successful discriminators between the groups. The extensive inter-individual variability of the fMRI responses in healthy controls as well as in cognitively impaired subjects (Dickerson et al., 2005; Machielsen et al., 2000; Machulda et al., 2003) complicates both the detection of significant differences between the study groups and weakens the discriminating potential of this MRI measure. The neurobiologically interesting finding of MTL hyperactivation in the MCI stage also makes the diagnostic use of fMRI in MCI and AD challenging, whereas measures such as the entorhinal volume or delayed recall scores apparently have better discriminating potential.

6.7. General considerations

The definite diagnosis of AD can only be made by neuropathological evaluation, and the diagnoses of the AD as well as the MCI subjects in studies I-IV are based on clinical examination. Thus, the eventual cause or causes of memory impairment in these subjects remain unknown. A community-based study of MCI subjects progressing to clinical dementia showed that 29 % of the subjects had developed non-AD primary pathological abnormalities when a *post mortem* brain analysis was conducted (Jicha et al., 2006). Moreover, most of the subjects progressing to clinical AD in the aforementioned study had secondary contributing pathologic abnormalities in addition to their primary pathologic diagnoses (Jicha et al., 2006), and thus it is possible that the MCI and AD subjects included in the current study also had a combination of pathologies. Furthermore, it is possible that some of the control subjects may have developed cognitive impairment after these studies were performed, and thus were neuropathologically in the initial stages of AD. It is equally possible that some of the MCI subjects in studies I-IV have reverted back to normal. Thus, neuropathologically confirmed MRI and fMRI studies with longer follow-up times are needed to clarify the structural and functional changes occurring in MCI and AD.

Only one structural MRI scan was used in the present study, and thus the results can be confounded by differences in pre-morbid brain shape. Analysis of serial MRI measurements would have allowed the investigation of structural changes associated with progression to dementia and made it possible to assess the rate of atrophy in stable and progressive MCI subjects in studies I and II. Literature reveals that the rate of atrophy in various brain regions is increased both in familial (Ridha et al., 2006) and sporadic AD when compared to controls (Jack et al., 2004). A previous longitudinal study on MCI subjects demonstrated significant GM loss in differential brain areas in converters compared to non-converters, but there was a notable overlap in the annual rate of GM loss between converters and non-converters (0 - 4.5 % in converters and 0 - 4 % in non-converters, respectively) (Chetelat et al., 2005b). Other studies have shown that the atrophy rates in MCI subjects progressing to AD increase, and that the atrophy rate is higher in younger MCI subjects (Jack et al., 2004; Jack et al., 2008a). A recent study with a particularly long follow-up time, 15 years, reported that the brain atrophy rate accelerated already 2.3 years prior to MCI diagnosis (Carlson et al., 2008), however, this article only reported the ventricular volumes and no volume changes in any distinct brain structures were evaluated. Future research with serial MRI measurements and long follow-up times is warranted to clarify the rate of brain atrophy in the progressive MCI subjects, and to investigate whether those regions experiencing most shrinkage vary as the disease progresses. However, with regards to the clinical diagnostic workup of memory disorders, cross-sectional measures seem more ideal than longitudinal imaging which is both tedious and expensive.

In studies III and IV, MTL volumetry was performed by manually tracing the MTL structures. This method is, however, very laborious. A less tedious alternative to manual outlining of the hippocampus would have been visual rating, which has been shown to correlate well with linear measurements of the MTL, as well as with memory tests (Scheltens et al., 1992) and may be more useful in the clinical diagnostic workup. A recently developed method called the ATLAS may also speed up the evaluation of MTL atrophy, although this method also appears to require manual outlining of MTL structures for a subset of the study sample (Ridha et al., 2007).

White matter lesions are also associated with cognitive decline (Breteler et al., 1994; Inzitari et al., 2007; Verdelho et al., 2007; Ylikoski et al., 1993; Ylikoski et al., 2000b; van der Flier et al., 2005), and also to progression from normal to MCI (Smith et al., 2008). In AD patients,

the presence and progression of white matter lesions are associated with progression of MTL atrophy (de Leeuw et al., 2006). The predictive value of white matter lesions for progression to dementia has previously been assessed for the subjects in studies I and II, showing that the lesions did not predict conversion to dementia (Tapiola et al., 2008). The amount of white matter lesions in the study subjects in studies III and IV was evaluated by an experienced neuroradiologist, and none of the subjects had excessive amounts of white matter lesions. However, the interactions between white matter hyperintensities and MTL atrophy have not been investigated in these study samples, and thus we cannot evaluate whether the presence of white matter changes would have affected hippocampal or entorhinal atrophy in these study subjects.

The results in study III derive from a relative comparison of active encoding and a more passive baseline condition. Therefore, sustained baseline activity might be misinterpreted as increased task-related activity. Task-induced deactivations have been detected in the so-called "default mode network" that comprises structures such as the posterior midline parietal and ventromedial frontal cortices, as well as the hippocampus (Greicius et al., 2004; Gusnard and Raichle, 2001). Alterations in the default mode network have been detected in MCI and AD subjects such that they show diminished deactivation responses when compared to controls (Lustig et al., 2003; Rombouts et al., 2005a). A recent study has also detected a linear increase in the default mode activity with age during memory tasks, as well decreased activity in the fusiform gyrus in relation to aging (Grady et al., 2006). When considering the main result of study III, *i.e.* increased activation in the fusiform, parahippocampal, and hippocampal regions in MCI, it appears possible that this finding might be explained by decreased deactivation in MCI in these areas as well. The technique used in study III does not provide direct evidence of deactivation findings, but inspection of the results in the opposite baseline-encoding contrast did not reveal significantly different MTL or fusiform deactivation responses in these three study groups. Thus, it is unlikely that the main findings of study III would primarily be due to differences in the default mode activity in the MTL or fusiform areas.

There are some possible limitations in relation to the fMRI paradigm used in studies III and IV. The comparison of an active task to a low-level control task such as visual fixation yields imperfect precision in evaluating specific cognitive processes. As a consequence of such a broad cognitive comparison, the observed activations may reflect many different underlying

processes, including simple motor responses given during the task but not during the fixation. However, the capability of AD patients to perform complex cognitive tasks is restricted, perhaps even more so under fMRI experimental conditions, and therefore the fMRI tasks need to be simple enough. Another possible limitation in studies III and IV is the block design paradigm, which does not allow the separation of successful and unsuccessful encoding trials. The published literature indicates that MTL activation relates to subsequent memory performance (Brewer et al., 1998; Sperling et al., 2003a; Wagner et al., 1998), and in MCI, both hippocampal and parahippocampal activation correlate positively to postscan memory performance (Dickerson et al., 2004). Correspondingly, decreased MTL activity is associated with relatively poor subsequent memory test results in AD (Rombouts et al., 2000; Sperling et al., 2003b) whereas an event-related study reported similar hippocampal fMRI responses for successful and unsuccessful encoding trials in AD patients (Pariente et al., 2005). Due to the block analysis used in study III it is not possible to investigate successful vs unsuccessful trials, and further fMRI studies with event-related designs are warranted to understand the neural underpinnings of successful and unsuccessful memory encoding in MCI.

There are advantages in using the fMRI stimulus as shown in studies III and IV. It is relatively brief and feasible to perform in elderly and cognitively impaired subjects. Moreover, the stimulus was modified from a widely used neuropsychological test, the FCSRT, which was recently claimed to be the most sensitive and specific neuropsychological test to detect prodromal AD in an MCI population (Sarazin et al., 2007). Additionally, a normal clinical scanner with a magnetic field strength of 1.5 T was used in order to achieve a clinically realistic experimental setting that could be performed in numerous clinical or research centers worldwide with respect to both the stimulus and the MRI scanner. The discriminating power of hippocampal fMRI using these methods was, however, relatively poor in study IV. The advantages of a 3.0 T scanner over 1.5 T imaging have been documented in a study which indicated that the statistical t values of cortical activations were 1.3 fold higher in 3.0 T compared to those obtained with 1.5 T imaging, and additional activation areas not detected at 1.5 T could be observed in 3.0 T scans (Hoenig et al., 2005). Thus, future fMRI studies using scanners with a higher field strength (3.0 T or more) with a better signal-to-noise ratio along with advances in imaging and data analysis methods may improve the applicability of fMRI in the clinically aimed research of memory disorders. In addition, an event-related design that allows more specific isolation of MTL activity related to

distinct memory processes may also reduce the inter-individual variability of BOLD fMRI responses.

7. CONCLUSIONS

This study aimed to investigate the structural alterations of the brain in relation to conversion from MCI to AD including the impact of the well-established susceptibility gene for AD, the APOE ε4 allele. The second aim of the study was to assess functional changes in fMRI activations in MCI and AD compared to controls and to relate the functional changes to the underlying structural changes, and to investigate the discriminating potential of functional and structural MRI measures in these subject groups. The following conclusions are drawn:

1. MCI subjects display atrophy of the entorhinal cortex, anterior hippocampus, and widespread cortical areas including tempoparietal and frontal areas compared to controls, and MCI subjects converting to AD are characterised by excessive atrophy of the posterior cingulum, precuneus and temporoparietal cortices.

2. The presence of the APOE ε4 allele leads to increased atrophy of the frontal and parietal cortices in those MCI subjects converting to AD.

3. MCI subjects show increased fMRI activation relative to controls while performing a word-picture pair encoding task whereas AD subjects exhibit decreased activation compared to controls.

4. The correlations of MTL volumes and hippocampal and parahippocampal fMRI encoding activations in MCI subjects indicate that they utilize compensatory activations both in the hippocampus and the posterior parahippocampal gyrus, probably due to the evolving neuropathological deficits in the MTL. The compensatory activation appears to be selectively related to encoding.

5. The volume of the entorhinal cortex is superior to hippocampal fMRI encoding or retrieval activation in the discrimination of controls, MCI and AD subjects. The combination of entorhinal volume and standard neuropsychological testing appears to be useful in the identification of MCI and AD.

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ORIGINAL PUBLICATIONS I-IV

I

Voxel-based morphometry to detect brain atrophy in progressive mild cognitive impairment

Hämäläinen A, Tervo S, Grau-Olivares M, Niskanen E, Pennanen C, Huuskonen J, Kivipelto M, Hänninen T, Tapiola M, Vanhanen M, Hallikainen M, Helkala EL, Nissinen A, Vanninen R, Soininen H

NeuroImage 2007;37:1122-31

II

Apolipoprotein E ε4 allele is associated with increased atrophy in progressive mild cognitive impairment: a voxel-based morphometric study

Hämäläinen A, Grau-Olivares M, Tervo S, Niskanen E, Pennanen C, Huuskonen J, Kivipelto M, Hänninen T, Tapiola M, Vanhanen M, Hallikainen M, Helkala EL, Nissinen A, Vanninen RL, Soininen H

Neurodegenerative Diseases 2008;5(3-4):186-9

III

Increased fMRI responses during encoding in mild cognitive impairment

Hämäläinen A, Pihlajamäki M, Tanila H, Hänninen T, Niskanen E, Tervo S, Karjalainen PA, Vanninen RL, Soininen H

Neurobiology of Aging 2007;28:1889-1903

IV

Discriminating accuracy of medial temporal lobe volumetry and fMRI in mild cognitive impairment

Jauhiainen AM, Pihlajamäki M, Tervo S, Niskanen E, Tanila H, Hänninen T, Vanninen RL, Soininen H

Hippocampus; in press

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