Neuropsychological Function in Relation to Structural and Functional Brain Changes in Alzheimer’s Disease.

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Till moster Kurt
som har gått in i dimman
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..we don’t always remember that we forgot, so that to remember that we forgot is not exactly forgetting, is it?

What I loved by Siri Hustvedt
SUMMARY

Neuropsychological Functions in Relation to Structural and Functional Brain Changes in Alzheimer’s Disease.

The aim of this doctoral thesis was to study neuropsychological functions in relation to structural and functional brain changes in mild to moderate Alzheimer’s disease (AD).

In the first study relations between hippocampal volume, neuropsychological function and limbic-hypothalamic-pituitary-adrenal (LHPA) axis disturbances in AD were investigated. Hippocampal volume was measured with magnetic resonance imaging (MRI). Reduced hippocampal CA1 volume and suppressed cortisol levels in combination, best predicted the variation in neuropsychological performance. The conclusion was that reduced hippocampal volume and LHPA axis disturbances are associated with level of cognitive function in AD.

The second study focused on whether patients with mild to moderate AD showed an altered regional cerebral bloodflow (rCBF) pattern compared to healthy elderly. Correlation between performance on memory tests and rCBF in sub-lobar volumes of the brain were investigated. The rCBF was measured with single photon emission computed tomography (SPECT). AD patients showed a significantly lower rCBF in temporoparietal regions including left hippocampus compared to controls. The diagnostic sensitivity and specificity for AD was high in temporoparietal regions. AD patients had significantly lower performance on semantic and in particular episodic memory tests compared to the controls. Their performance on several episodic tests correlated with rCBF in parietal and temporal regions including the left hippocampus suggesting that abnormalities in the rCBF pattern underlie impaired episodic memory functioning in AD. The conclusion was that an observer-independent analyzing method for SPECT with sub-lobar volumes of interest (VOI) is promising in the diagnosis of AD.

In a third study possible differences in memory-related functional brain activation between persons with high versus low risk for AD were examined with functional magnetic resonance imaging (fMRI). The high-risk individuals performed worse than low-risk individuals on tests of episodic memory. Patterns of brain activity during episodic encoding and retrieval showed significant group differences. During both encoding and retrieval, the low-risk persons showed increased activity relative to a baseline condition in prefrontal and hippocampal brain regions that previously have been implicated in episodic memory. In contrast, the high-risk persons did not significantly activate any prefrontal region, but instead showed increased activity in visual occipito-temporal regions. The conclusion was that patterns of prefrontal brain activity
related to episodic memory differed between persons with high versus low risk for AD, and lowered prefrontal activity may therefore predict subsequent disease.

In a final study SPECT was used to map patterns of rCBF in an activated state (an episodic encoding task) and in a rest condition in persons with mild AD and in healthy elderly. A reduction of rCBF in temporoparietal regions was observed for the AD group. This reduction was more pronounced in the memory provocation state than in the rest condition. The conclusion of this is that there are rCBF differences between mild AD patients and healthy controls in temporoparietal regions, and that the temporoparietal reduction is more pronounced during activation than during rest which might be of importance in the early diagnosis of AD.

Taken together, these findings show that the level of neuropsychological function, notably episodic memory, can be systematically related to functional disturbances of the LHPA axis and to structural and functional characteristics of temporoparietal and prefrontal brain regions in AD patients. These changes are detectable in patients at risk for AD and in an early phase of AD which suggests that the obtained results might be important for early diagnosis of AD.

*Key words:* Alzheimer’s disease, neuropsychological function, episodic memory, hippocampus, prefrontal cortex, brain imaging, MRI, tMRI, SPECT, LHPA-axis, cortisol
SUMMARY IN SWEDISH
(SVENSK SAMMANFATTNING)


Att finna och utveckla känsliga och specifika markörer för tidig diagnos av Alzheimers sjukdom med möjligheter att bromsa eller helst avbryta den neurodegenerativa processen som föregår en demensutveckling. Den symptomatiska behandling som finns idag, t ex acetylcholinesterashämmare, antas vara mer effektiva i en tidig fas av sjukdomen. Tidig diagnos är svår i dagens läge då biologiska markörer saknas. Diagnostiken försvåras av att det finns en överlappning mellan den kliniska bilden av mild demens, normalt åldrande samt mellan Alzheimers sjukdom och andra demenssjukdomar.

Det övergripande syftet med denna avhandling är att studera neuropsykologisk funktion i relation till strukturella och funktionella förändringar i hjärnan hos personer med Alzheimers sjukdom.

I den första studien studerades relationer mellan hippocampus volym, episodiskt minnesfunktion och störningar i kortisol-axeln hos Alzheimer patienter. Hippocampusvolym mättes med magnetresonanskamera (MRI). Reducerad hippocampusvolym och kortisolnivå i kombination predicerade variationen i prestation i neuropsykologiska test. Slutsatsen var att reducerad hippocampusvolym och kortisol-axel störningar är associerade till grad av kognitiv funktion vid Alzheimers sjukdom.

Den andra studien fokuserade på om patienter med mild Alzheimers sjukdom uppvisade ett förändrat blodflöde jämfört med friska kontrollpersoner. Korrelationen mellan prestation i minnestest och blodflöde i sub-lobära volymer i hjärnan undersöktes. Blodflödet mättes med single photon emission computed tomography (SPECT) och tolkades i en datorbaserad


I en sista studie användes SPECT för att mäta hjärnaktivitet i en aktiverad betingelse (en episodisk inkodningssuppgift) och i en vilobetingelse hos personer med mild Alzheimers sjukdom och hos friska äldre. En reduktion i blodflöde i temporoparietala regioner observerades i Alzheimer gruppen. Denna reduktion var mer uttalad i den provocerade betingelsen än i vilobetingelsen. Slutsatsen var att det fanns blodflödesskillnader mellan milda ADpatienter och friska äldre kontroller i temporoparietala områden, och att den temporoparietala reduktionen var mer uttalad i aktiveringstillståndet än i vila. Detta kan vara av stor betydelse vid tidig AD.

Sammanfattningsvis visar dessa fynd att grad av neuropsykologisk funktion, främst episodiskt minne, systematiskt kan relateras till störningar i LHPA-axeln och till strukturella och funktionella karaktäristika i temporoparietala och prefrontala hjärnregioner vid Alzheimers sjukdom. Dessa förändringar finns hos personer med risk för Alzheimers sjukdom och hos personer i en tidig fas av Alzheimers sjukdom. De uppnådda resultaten kan öka möjligheterna att på ett tidigt stadium diagnostisera Alzheimers sjukdom.
The thesis is based on the following papers, referred to in the text by their roman numerals:


IV. Sundström, T., Elgh, E., Nyberg, L., Näsman, B. & Riklund, K. Å. Memory provoked rCBF-SPECT as a diagnostic tool! Manuscript.

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<td>AACD</td>
<td>Age-associative cognitive decline</td>
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<td>AAMI</td>
<td>Age-associated memory impairment</td>
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<td>AD</td>
<td>Alzheimer's disease</td>
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<td>ADRDA</td>
<td>Alzheimer's disease and related disorders association</td>
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<td>BA</td>
<td>Brodmann area</td>
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<td>BOLD</td>
<td>Blood oxygen level-dependent</td>
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<td>BPSD</td>
<td>Behavioural and psychological symptoms of dementia</td>
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<td>CBA</td>
<td>Computerised brain atlas</td>
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<td>CBF</td>
<td>Cerebral bloodflow</td>
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<td>CDR</td>
<td>Clinical dementia rating scale</td>
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<td>CT</td>
<td>Computed tomography</td>
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<td>DSM</td>
<td>Diagnostic and statistical manual</td>
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<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
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<td>HAROLD</td>
<td>Hemispheric asymmetry reduction in older adults</td>
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<td>HERA</td>
<td>Hemispheric encoding/retrieval asymmetry</td>
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<td>HMPAO</td>
<td>Hexamethylpropylene amine oxime</td>
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<td>LHPA</td>
<td>Limbic-hypothalamic-pituitary-adrenal</td>
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<td>LTM</td>
<td>Long term memory</td>
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<td>MADRS</td>
<td>Montgomery-Åsberg's depression scale</td>
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<td>MCI</td>
<td>Mild cognitive impairment</td>
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<td>MMSE</td>
<td>Mini-mental state examination</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>MTL</td>
<td>Medial temporal lobe</td>
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<td>NINCDS</td>
<td>National institute of neurological and communicative disorders and stroke</td>
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<td>PET</td>
<td>Positron emission tomography</td>
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<td>PFC</td>
<td>Prefrontal cortex</td>
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<td>rCBF</td>
<td>Regional cerebral bloodflow</td>
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<td>SPECT</td>
<td>Single photon emission computed tomography</td>
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<td>SPM</td>
<td>Statistical parametric mapping</td>
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<td>VOI</td>
<td>Volumes of interest</td>
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INTRODUCTION

When I introduce myself to older people as a clinical neuropsychologist working in the area of dementia diseases, they often complain about their bad memory irrespective of whether it is bad or not. During the years, I have met many persons in the clinic with subtle memory problems and persons with really bad memory. I consider myself as having a “good clinical eye” but I still haven’t figured out who will develop that bad memory and who will not. This has made me eager to understand more and contribute to the understanding of how the brain works when a person has contracted Alzheimer’s disease (AD). I also have a wish to contribute to a more accurate diagnosis of early AD. I have chosen to look at three different but equally important and interacting parts in the understanding of the AD process; neuropsychology, brain imaging, and the influences of stress hormones.

In my thesis I will start by describing dementia in general and AD in particular. After that I present the three parts that constitute the foundation of my research; first the neuropsychological basics and methods used in the diagnosis of AD, second the brain imaging methods used in my studies and third the theories behind stress hormones and their negative impact on AD. The use of neuroimaging in dementia may be divided into a diagnostic contribution (early and differential diagnosis) and a pathogenetic contribution, i.e. imaging findings that may contribute to the understanding of the pathophysiology of the dementia syndrome. This thesis has both those positions with a wish to contribute to the understanding of how the brain works when a person has contracted AD, and a wish to contribute to a more accurate diagnosis of early AD. After a review of prior findings in the research area I give a brief overview of my own studies, and in the last part I discuss the findings in the light of previous research.

Dementia

The most common cause of accelerated cognitive decline in old age is dementia. It is a devastating and costly disorder in elderly adults (Leung et al., 2003; Wimo et al., 1997). The worldwide number of persons with dementia in the year 2000 was estimated to about 25 million persons. The occurrence is associated with increasing age (Lobo et al., 2000; Skoog, 2004) and about 6% of the population 65 years of age or older suffer from dementia (Ott et al., 1995), a majority are female (Lobo et al., 2000). The number of new cases in the world in the year 2000 was estimated to be 4.6 million with an increase from 25 million to 63 million in 2030 and to 114 million in 2050 (Wimo et al., 2003). In the European union approximately 3.3 million persons
had dementia in the year 2000 and 824,000 new cases were expected to develop the disease in a year (Launer & Hofman, 2000). In Sweden between 150,000 and 200,000 people suffer from dementia at present. The cost for the individuals and their families is tremendous. A person with dementia is more often in institutional care and has over twice the risk of death compared to a healthy individual of the same age (Jagger et al., 2000).

Dementia means “without sense” (Latin de = without and mens = sense). In daily life the term refers to a condition where a person for some reason has changed from his or her premorbid functional and cognitive level to the present functional level with consequences for daily life. Dementia is not a disease in itself but a syndrome that, according to the criteria from the American psychiatric association (APA) Diagnostic and statistical manual of mental disorders (DSM IV) is characterized by multiple cognitive deficits including memory impairment that are due to the direct physiological effects of a general medical condition, to the persisting effects of a substance, or to multiple aetiologies. The disturbances must be sufficient to interfere with usual activities and relationships (DSM-IV, 1994).

There are several forms of dementia i.e. vascular dementia, frontotemporal dementia, dementia with Lewy bodies, and AD. AD is the most common form and accounts for between 50-70% of all dementia cases (Cummings & Benson, 1992; Fratiglioni et al., 1999 & 2000).

Alzheimer’s disease

AD was first described in 1907 by the German neuroscientist Alois Alzheimer. He reported his observations of a 51-year-old woman with rapidly increasing memory impairment, disorientation, and various psychiatric symptoms. She was severely ill for almost 5 years before death and terminally the patient was totally bedridden, incontinent and dependent on care (Alzheimer, 1907).

AD affects more than 12 million persons worldwide (Citron, 2002). The average incidence rates for AD across studies for ages 70 to 74 years is 0.5 %, increasing to 3.9 % at ages 85 to 89 (Petersen et al., 2001). The prevalence figures of AD differ between studies, but they increase continuously with age and were in one epidemiological survey in Europe 0.6% in the group age 65 to 69 years versus 22.2% at age 90 and older (Lobo et al., 2000). AD is associated with shorter survival, especially for subjects age 70 years or older, males, patients with greater impairment in daily activities of living, and those with more severe dementia (Heyman, 1996).

AD is characterized by a slow progressive loss of cognitive functioning and a long preclinical period during which deficits are observed in several cognitive domains and especially
in episodic memory (Bäckman et al., 2001; Chen et al., 2001; Grober et al., 2000; Howieson et al., 1997; Small et al., 1997 & 2000; Tierney et al., 1996). There are also observed changes in executive functioning (Albert et al., 2001; Chen et al., 2000), perceptual speed (Fabrigoule et al., 1998), attention (Linn et al., 1995), verbal ability (Jacobs et al., 1995), reasoning (Fabrigoule et al., 1996), and visuospatial skill (Small et al., 1997).

Figure 1. Gradual onset and functional decline over time in AD, MCI = mild cognitive impairment

The onset of AD is not sudden. It starts years before it is clinically manifest (Elias et al., 2000). Retroactively there might have been symptoms such as mild memory problems, language difficulties or personality changes long before the diagnosis (Almkvist et al., 1998) but not to such an extent that it made a diagnosis possible. Brain lesions gradually accumulate prior to the appearance of dementia. These preclinical neurodegenerative processes are probably present for decades at a non-symptomatic stage before precipitous decline occurs that eventually results in a clinical diagnosis (Nordberg, 2003). The gradual onset is illustrated in Figure 1.

Diagnosis of AD
Mild dementia is hard to demarcate from normal aging and mild cognitive impairment (MCI). There is an overlap between the clinical picture of mild dementia and normal aging. This overlap is also present in AD in regard to vascular dementia and depression (Laukka et al., 2004).

There are several diagnostic systems that deal with the diagnosis of AD and they are all based on the symptoms and course of the disease (DSM-III-R, 1987; DSM-IV, 1994; WHO, 1992). According to the Diagnostic and Statistical Manual (DSM-III-R) criteria for AD, the onset is gradual, there is a continuing cognitive decline, and there are no change of consciousness (DSM-III-R, 1987). The definition of probable AD is based on a number of criteria defined by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and
the Alzheimer's Disease and Related Disorders Association (ADRDA) (McKann, 1984). In the latter diagnostic manual there is a continuum that ranks the diagnosis of AD from possible to probable, and to definite AD. The criteria for the clinical diagnosis of probable AD are deficits in two or more areas of cognition, progressive worsening of memory and other cognitive functions, and no disturbance of consciousness. The onset of the symptoms should be between 40 and 90 year, and there should not be any systemic disorders or other brain diseases that in themselves could account for the progressive deficits in memory and cognition. Definite AD is obtained from histopathological data (Byrne et al., 1991; Khachaturian, 1985; Mirra et al., 1993).

There are no biological markers for most of the dementias. Clinical diagnostic criteria are therefore used for diagnosis (Fratiglioni, 1996). A clinical diagnosis is made by excluding other conditions (e.g.; depression, vascular dementia) on the basis of a thorough physical examination and a neuropsychological assessment. In a clinical investigation it is important to obtain a reliable and detailed history from the patient and a close relative, laboratory analysis of blood, urine and liquor, investigation of heart and lung, neuroimaging data, and a functional status with neuropsychological assessment and other methods describing behaviour.

Staging of AD
For staging of dementia in a clinical setting there are several methods; the clinical dementia rating scale (CDR) (Hughes et al., 1982) and the mini mental state examination (MMSE) (Folstein et al., 1975) are commonly used. MMSE is based on a set of 30 items dealing with orientation, calculation, memory functioning, language abilities, attention, and visuospatial function. MMSE itself does not indicate anything about the cause of the cognitive decline. A score of 23 points or less is typically seen as a cut-off for cognitive impairment, indicating that further diagnostic evaluation is recommended (Folstein et al., 1975). Score of 24-30 on the MMSE is considered as no dementia, 19-23 indicate mild dementia, and 13-18 indicate moderate dementia (Almkvist & Bäckman, 1993; Welsh et al., 1992). However, these limits are under discussion. MMSE has good psychometric properties (Pangman et al., 2000). It is easy and rapidly administrated and it is often used in both clinical settings and in research. It is suitable for quantitative comparisons across studies with regard to degree of dementia. The differentiation between mild dementia and normal aging is a problem with MMSE. It can not discriminate between these groups with certainty (Herlitz et al., 1989). CDR is a global rating scale. It rates impairment in six functional categories on a five point scale (i.e. memory, orientation, judgement and problem solving, community affairs, home and hobbies, and personal care). The outcome is CDR 0 – no dementia, CDR 0.5 – questionable dementia, CDR 1 – mild dementia, CDR 2 – moderate dementia, and CDR 3 – severe dementia (Berg, 1988).
Clinically, the course of AD has been divided into three stages (Cummings & Benson, 1992). In the first stage, new learning and remote recall are impaired besides topographic disorientation, anomia, and some alteration of personality, whereas CT and EEG may be normal. In the second stage, amnesia, apraxia, and aphasia are recognized as well as abnormal CT and EEG. In the third stage, most aspects of cognitive functioning are severely affected and motor symptoms are common.

There are also a range of noncognitive symptoms, behavioural and psychological symptoms of dementia (BPSD)-symptoms. That means that patients frequently show decreased emotional expression, increased stubbornness, diminished initiative, suspiciousness, delusions, hallucinations, depression and anxiety (Geldmacher & Whitehouse, 1996).

Risk factors
The aetiology of AD is yet unknown. A summary of risk factors for AD in epidemiological research revealed increasing age, family aggregation and apolipoprotein E (ApoE) ε4 allele as definite risk factors (Corder, 1993). Several studies have shown that persons with genetic risk for AD i.e. APOE ε4 carriers have a greater risk for developing AD (Rapoport, 2000; Small et al., 2000). It seems as if the ApoE ε4 allele is a stronger risk factor for men then for women (Qiu et al., 2004). (For an overview, see Morris, 2003).

Suggested risk factors are female gender, aluminium, hypertension, hypotension, head trauma, alcohol intake and low education. High caloric intake has also been suggested as a possible risk factor (Gustafson et al., 2003; Luchsinger et al., 2002).

There are suggested protective factors such as nonsteroidal anti-inflammatory drug use, postmenopausal estrogen therapy, smoking (Fratiglioni, 1998), an active and socially integrated lifestyle (Fratiglioni et al., 2004), and nutritional factors (Nourhashemi et al., 2000).

Neuropathology
The destructive process underlying AD is characterized by a typical distributed pattern of brain changes that is specific with respect to area, lamina, and even cell type (Braak & Braak, 1997). There is a progressive deposition of abnormal proteins both between the nerve cell (senile plaque) and within the nerve cell (neurofibrillary tangles). According to Braak & Braak there is a progression of neurofibrillary pathology in AD from medial temporal limbic areas early in the disease to neocortical association areas later. The brain destruction is subdivided in six stages and there is a linear correlation between the six stages of brain destruction and cognitive decline. Stage I and II include transenthorhinal regions and at this stage there is clinically no obvious cognitive impairment. Stage III and IV include limbic regions and minor changes in the hippocampus. Clinically there is an impairment of cognitive functions and presence of personality...
changes. Stages V and VI include isocortical association areas and correlate with fully developed AD. It takes many years from the first appearance of neuropathological changes in transenthorhinal areas to the end stage of AD (Braak & Braak, 1996).

Neurofibrillary tangles are also present in the brain of non-demented persons. The concentration, especially in hippocampus and parahippocampal areas, increase exponentially with age. Plaques distributed in neocortex and in limbic structures differed between non-demented individuals where one subgroup had few plaques and one subgroup changes that resembled those in very mild dementia cases, indicating preclinical AD (Price & Morris, 1998). Schmitt et al (2000) suggest that the aging brain may be able to withstand AD like structural changes without meaningful impact on mental functioning. The prevailing view seems to be that the neuropathological distinction between aging and AD is quantitative rather than qualitative while others suggest that aging and AD may differ qualitatively (Morris & Price, 2001).

Changes in the corpus callosum are also present in AD and these changes might cause a cortico-cortical disconnection that contributes to the severity of dementia (Pantel et al., 1999; Vermersch et al., 1994).

Neurochemical and neurophysiological changes
Disturbances in brain function and behaviour are reflected in altered neurochemical activity. A profound loss of cortical and hippocampal cholinergic innervation occurs early in the course of the disease (Coyle et al., 1983; Davies & Maloney, 1976). Nicotinic receptors seem to be of great interest in AD. They are important for cognition (Paterson & Nordberg, 2000) and a consistent loss has been observed in cerebral cortical areas of autopsy brain tissue from AD patients (Nordberg, 2003). Impairment of central cholinergic transmitter function led to the development of acetylcholinesterase inhibitors that are used as symptomatic therapy. Abnormalities in the noradrenergic and serotoninergic systems are also present. The cholinergic and glutamatergic neurotransmitter systems are associated with memory and higher brain functions (Nordberg, 2003).

Slowing of EEG background activity is a consistent finding in AD (Almkvist & Winblad, 1999).

Mild cognitive impairment
During the recent years a lot of attention has been drawn to the transitional stage of cognitive impairment between normal aging and mild AD. There is a confusion concerning the nomenclature regarding this transitional stage. A variety of terms exist to describe these cognitive changes. Mild cognitive impairment (MCI) seems to be the most common term and refers to the
clinical stage of individuals who are memory impaired but are otherwise functioning well and do not meet clinical criteria for dementia (Petersen et al., 2001). Other terms are age associated memory impairment (AAMI) (Crook et al., 1986), age-related cognitive decline and mild neurocognitive disorder. All these are criticised for being semantically inappropriate and imprecisely defined (Ritchie & Touchon, 2000). Age-associative cognitive decline (AADC), a similar concept to MCI is suggested to be related to normal cognitive aging processes rather than incipient dementia (Ritchie et al., 2001).

An issue under debate is whether MCI represent the preclinical stages of AD or a distinct and static cognitive aetiology (Arnaiz & Almkvist, 2003). There is a consensus emerging that MCI is common, that it is associated with significant mortality, with significant morbidity, to the development of AD, and to the same pathological processes responsible for AD (Bennett, 2003).

MCI is believed to be a high-risk condition for the development of probable AD. The likelihood of an MCI patient to develop AD in the long term is around 50% and the risk for persons with MCI to develop AD compared to cognitively intact persons is bigger only for the first 4 years (Frisoni et al., 2004). MCI is a poor predictor of dementia within a 3-year period with a conversion rate of 11.1%, in the AADC group the conversion rate was 28.6% (Ritchie et al., 2001).

MCI seems to be a heterogenous condition and there is a suggestion of a subclassification regarding the presence of isolated memory impairment or memory impairment together with other slight cognitive deficits (Elfgren et al., 2003; Petersen et al., 2001). Tests assessing new learning, delayed recall and attention/executive function seems to provide valuable information for screening and diagnosis not only for early AD but also for MCI (Arnaiz & Almkvist, 2003).

In conclusion MCI seems to be a heterogeneous condition with a neuropsychological performance between that of healthy elderly and demented patients. MCI includes many persons with high risk to develop AD. Sufficient evidence exist to recommend the evaluation and clinical monitoring of persons with MCI due to their increased risk for developing dementia (Petersen et al., 2001).

The impact of stress hormones on AD
The hippocampal region, with neurons showing both structural and functional plasticity, is an important structure for understanding the plasticity and resilience of brain cells to stress hormone action and aging (McEwen, 1999). CA1 area of the hippocampus is important since AD related neuron loss has been found early in that region (Bobinski et al., 1998) correlating significantly
with both the duration and the severity of AD (West et al., 1994). Hippocampus plays an important role for the activity and processing of memory, learning and mood (McEwen, 2000).

The LHPA-axis is the endocrine loop system controlling the secretion of stress hormones (glucocorticoids) (Lupien & Lepage, 2001). Main components of the axis include the hippocampus, the hypothalamus, the pituitary and the adrenal glands (Figure 2). Cumulative exposure to high levels of glucocorticoids can be particularly detrimental for the aged hippocampus (Lupien et al., 1999). Aged humans with significant prolonged cortisol elevations i.e. patients with Cushing’s disease showed reduced hippocampal volume and deficits in hippocampus dependent memory tasks compared to healthy elderly. The degree of hippocampal atrophy correlated strongly with both the degree of cortisol elevation over time and current basal cortisol levels. Basal cortisol elevation may cause hippocampal damage and impair hippocampus-dependent learning and memory in humans (Lupien et al., 1998). The LHPA axis plays an important role in maintaining homeostasis under both basal and challenging conditions. Glucocorticoids are essential for survival and for the stress response including energy mobilization, increased cardiovascular tone, suppressed growth and reproduction. In contrast, prolonged glucocorticoid elevation can present serious health risks including diabetes mellitus, hypertension, osteoporosis and changes in memory and mood (Lupien et al., 1998). Loss of corticosteroid receptors and neurons in hippocampus may underlie neurogenic LHPA dysfunction. This may cause progression of the disease since glucocorticoids exert toxic effects on pyramidal cells in the hippocampus (Jacobson & Sapolsky, 1991; Lupien et al., 1999; Näsman, 1994).

The “glucocorticoid cascade hypothesis”, introduced by Sapolsky 1986, suggests that age is associated with increasing LHPA axis dysregulation and that the dysregulation is the result of hippocampal neural loss, accelerated by LHPA axis hyperactivity (Sapolsky et al., 1986). This hypothesis is still of great interest and in both animal and human studies their results has been replicated (Gurevitch et al., 1989; Lupien et al., 1998; Rasmuson et al., 2001).

A lot of studies concerning stress have concentrated on hippocampus. More recent data suggests that stress has an impact on many cortical and subcortical brain structures other than hippocampus. It seems as if in addition to pituitary, hypothalamic sites and limbic structures (particularly hippocampus) cortical areas and particularly prefrontal cortex are involved in the regulation of LHPA activity, possible through an inhibitory role (Lupien & Lepage, 2001).
In response to stress the LHPA axis is strongly activated causing an increased release of glucocorticoids (cortisol in man) from the adrenal cortex. This occurs via release of corticotrophin-releasing hormone (CRH) and vasopressin from the hypothalamus, which in turn stimulates the anterior pituitary to secrete adrenocorticotrophic hormone (ACTH) that finally acts upon the adrenal gland to produce glucocorticoid hormones. The activity of the LHPA axis is regulated by negative feedback at the level of hippocampus, the hypothalamus, and the pituitary. Plasma levels of glucocorticoids vary during the day with peak levels around the onset of the active period of the day (morning for man) and trough levels just before the start of the inactive period.

The actions of corticosteroids are mediated by intracellular receptors of two different subtypes, i.e. the high affinity mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). When circulating levels of corticosterone are low, mainly MR is activated while GR is not (Seckl & Olsson, 1995). GR is only weakly detected in the hippocampus but strongly detected in the pituitary, cerebellum, hypothalamic paraventricular nucleus and in the prefrontal cortex. On the other hand MR was abundantly observed in the hippocampus. GR may change following stress, but might not be as important as previously thought in the human hippocampus (Lupien & Lepage, 2001). Tuvnes et al (2003) showed that lesions of the hippocampus in rats did not increase the concentration of corticosterone relative to control rats and suggest that
hippocampus is not necessary for tonic inhibition of adrenocortical activity. This implies that the LHPA axis receives efficient negative feedback inhibition from other brain systems (Tuvnes et al., 2003).

TOOLS FOR DIAGNOSIS

Neuropsychological assessment
As had been said earlier in this thesis there is still no unequivocal biological markers for AD. A variety of information has to be collected for diagnosis and a thorough neuropsychological assessment is important for a functional description of the patient’s cognitive function and behaviour. The neuropsychological evaluation is one of the most important tools when assessing early cognitive changes in AD. This has been shown in both cross-sectional (Almkvist et al., 1993; Almkvist et al., 1996; Arnaiz & Almkvist, 2003; Storandt & Hill, 1989; Welsh et al., 1991) and longitudinal studies (Herlitz et al., 1997). Performance on neuropsychological tests is affected by many factors i.e. education, cultural background, age, and diseases other than dementia. That sort of information has to be gathered and analysed in relation to test performance.

The neuropsychological evaluation raises a lot of questions to consider: What are the best neuropsychological markers for detection of dementia? Is it possible to differentiate between different dementia diseases, e. g. AD and vascular dementia? What are the neuropsychological markers for staging of dementia? I will not address all these issues in my own research which is mainly focused on the following question: What is the relationship between different expressions of brain functioning, i. e. neuropsychological markers, brain imaging and stress hormones?

In the following section I will go through the basic neuropsychological domains that I focus on in this thesis.

Memory
Human memory consists of multiple systems. A basic distinction is between short-term or working memory and long-term memory (LTM). In addition, LTM can be subdivided into multiple systems. A basic distinction is declarative versus non-declarative memory (Squire et al., 1993; Tulving, 1993). Declarative LTM is most affected in AD. This system can be separated into episodic and semantic memory. Episodic memory refers to the encoding and retrieval of information about personally experienced past events (Tulving, 1993). It deals with the acquisition and retrieval of information that is acquired in a particular place at a particular time.
In the laboratory, episodic memory is typically assessed by asking persons to recall or recognize some information learned in the experimental setting (Bäckman, 2001) i.e. sentences, stories, words, pictures.

Semantic memory concerns a network of associations and concepts of basic knowledge about the world, an organised body of knowledge regarding words and concepts, their meaning and associations. It can be measured by verbal fluency tests, picture naming, category listing and identification of semantically related pairs.

Attention
Attention is a multidimensional structure that involves processes that focus, select, divide, sustain and inhibit (Parasuraman & Davies, 1984; Rogers & Fisk, 2001). According to Parasuraman & Greenwood (1998) there are three primary components; selection, vigilance and control.

Attention is itself a process of interest but also provides a tool to investigate age-related changes in other aspects of human behaviour such as memory (Rogers & Fisk, 2001). It has been suggested that attentional decline is a primary reason for age-related differences in memory tasks (Craik & McDowd, 1987). Most studies have investigated selective attention and the typical findings are activation of prefrontal, midfrontal, and posterior parietal cortices in addition to the anterior cingulate and thalamus (Frackowiak et al., 1997). Attentional deficits are part of the AD disease and test of attention is almost always included in test batteries. Typical tests can for instance be to determine the number of A:s present in a small array of letters, reading a list of words aloud, a multiple choice visual matching test using nonsense shapes. It can also involve to measure simple response time. Tasks can differ and many tests are designed to detect decrements in concentration and ability to resist distraction rather than to determine the actual nature of the attentional dysfunction.

Visuospatial function
Visuospatial ability comprises analyzing spatial information associated with visual perception, a complex process that depends on information from multiple brain areas in the parietal, temporal, and occipital lobes, mainly in the right hemisphere (Benton, 1985). There is evidence to support the view that there are two main streams of visual information processing in the brain. An occipito-temporal stream (ventral system) subserving object identification, and an occipito-parietal stream (dorsal system) that is responsible for perceiving spatial relations between objects (Gazzaniga et al., 1998; Ungerleider & Haxby, 1994).
Assessment tools for visuospatial function are for instance Block design (Wechsler, 1987) and clock drawing (Hill et al., 1995). In Block design subjects are asked to reproduce a spatial pattern shown on a card by means of three-dimensional blocks with different patterns in two colours on different sides of each block. This task requires visuocstructional skill as well as visuoperceptual ability and there is also a dimension of novelty involved in the task.

Executive function
Executive function includes the abilities responsible for concurrent manipulation of information, cognitive flexibility, concept formation, and cue-directed behaviour. The test that is sensitive to executive dysfunction in the present studies are Trail making test B (Reitan, 1992) and Controlled oral word association (FAS and categories) (Spreen & Strauss, 1991).

Imaging
Brief history
The urge to understand the functional organization of the human brain using techniques to assess changes in brain circulation has occupied people for more than a century. In the end of the nineteenth century scientists reported work on brain bloodflow during mental activities (Raichle, 2001). The well known Paul Broca (1860) was interested in circulatory changes associated with mental activities manifested in brain temperature. At the end of World war II a quantitative method for measuring whole brain flow and metabolism in humans was developed (Raichle, 2001). The first rCBF method that permitted rCBF measurement to be made by using scintillation detectors arrayed like a helmet over the head was developed by David Ingvar and Neils Lassen in the 1960s. It was based on intracarotid injection of $^{133}$xenon and recorded by multiple stationary detectors. This method was replaced with rCBF-methods based on inhalation or i.v. injection of $^{133}$xenon and those methods were used in research and in clinical routine until replaced by tomographic rCBF methods, notably SPECT (Risberg, 1996). The first study of functionally induced regional changes in bloodflow using these techniques in normal humans was reported by Jarl Risberg and David Ingvar (Ingvar & Risberg, 1965).

Structural and functional imaging
Neuroimaging is a concept that includes structural techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) and functional techniques such as single photon emission computed tomography (SPECT), positron emission tomography (PET) and functional
MRI (fMRI). The structural scans produce highly detailed images of the anatomical features of the brain while functional techniques provide an indication of the activity of the brain, but do not produce high anatomical detail. CT, MRI and SPECT are available and used in clinical routine while PET and fMRI are mainly used for research purposes. This dichotomization between structural and functional might be an oversimplification, though (Small, 2003), and especially the term functional is opaque as even volumetric measurement might reflect neuronal function more than simply cell loss.

Imaging is widely used in the evaluation of AD (Burggren & Bookheimer, 2002). MRI, PET and SPECT are currently the most commonly used neuroimaging modalities in studies focusing on MCI (Wolf et al., 2003). One contribution is to exclude diseases that may be confused with AD, another contribution is the potential use in the early detection of AD and studies suggests that neuroimaging measures have the potential to become valuable in this effort (Scheltens & Korf, 2000; Wolf et al., 2003). Recently these techniques also have been found to be useful in monitoring cognitive and pathological progression of the disease, as well as monitoring response to clinical intervention treatment (Burggren & Bookheimer, 2002). The pattern of activation in AD depends on interactions between the clinical stage of patients, and the pattern of brain degeneration, as well as the task difficulty and specific networks necessary for solving the task (Almkvist, 2000).

In the following section I will concentrate on the techniques used in the studies presented in this thesis.

MRI

MRI provides an excellent tool for observing structural difference non-invasively; it makes it possible to quantify brain atrophy in vivo. After placing a subject into a strong and homogenous magnetic field, application of brief radio frequency electromagnetic pulses and gradient magnetic fields disturbs the equilibrium of the proton nuclei within a subject. This introduces a magnetization that can be detected as it induces current in a coil and formed into an image. Because the rate at which the MR signal decays in these protons depends on factors intrinsic to them, signals decay at different rates among the different tissue types. The resulting image, therefore, contains different signal intensities in various regions of the brain depending on the decay rate of the protons making up that area. This gives what is called “contrast” to the various regions in the MRI image and enables identification of various regions of the brain depending on their signal intensity (Burggren & Bookheimer, 2002).
fMRI

fMRI has many advantages as an activation imaging technique. It is non-invasive, it has a relatively high spatial resolution and a high temporal resolution and it is possible to repeat since it does not require radiation exposure. This functional imaging method monitors bloodflow, a marker for neural activity (Logothetis et al., 2001), during an active state in order to assess which regions are involved in completion of a task. When particular neural regions become more active, there is a corresponding change in glucose and oxygen utilization within that brain area to supply the neurons comprising that region. Oxygenated and deoxygenated haemoglobin have slightly different magnetization properties which may be exploited with fMRI with the purpose of visualizing changes in blood supply to active brain regions during a particular task. The rate at which the MR signal decays is more rapid for deoxyhemoglobin than for oxyhemoglobin. This naturally occurring blood oxygenation level-dependent (BOLD) contrast can be visualized and formed into an image. The increase in regional oxygenated blood levels following neural activity appears to be greater than is actually used by an active region. Thus, there is an excess of oxygenated blood on the venous side compared to a resting state which, when contrasted to the resting state indicates which neural regions have been active during a particular task. Long picture sequence are taken and the pictures are analysed statistically (Burggren & Bookheimer, 2002).

SPECT

Single photon emission computed tomography (SPECT) measurement of the brain is the internationally most widely available functional neuro-imaging method. It is a nuclear medical method in which the cerebral distribution of radiolabelled tracers is registered. These tracers are given in such amounts that they are almost without effect on the organism or the organism’s disposal of them. A series of important biological processes can be studied and the method has been used to study the rCBF in the brain and for mapping of the brain’s functional structure. One type of radioactive tracer substance is $^{99m}$Tc-hexamethyl propylenamine oxime (HMPAO) which when delivered by the rCBF into the brain is intracellularly converted to a hydrophilic compound that cannot leave the cell, and is irreversibly bound in the brain in an amount proportional to the rCBF (Ryding, 1996).
REVIEW OF PRIOR FINDINGS

Neuropsychology

There is an enormous amount of studies on AD and cognition and I will not be able to and I do not have the intention to account for them all. I will instead try to point out the main trends in the field.

Not all areas of cognition are equally impaired in AD, particularly not in mild and moderate stages of the disease. Even with regard to memory which is the main domain of impairment in AD, different memory functions are differentially degraded (Almkvist et al., 1996). The first obvious cognitive symptom to appear in AD is in episodic memory. This change can be detected in preclinical stages and many years before a clinical diagnosis (Bäckman et al., 2001; Small et al., 2003) and the memory deficits remain relatively stable until dementia diagnosis (Rubin et al., 1998; Small et al., 2000). It seems that the episodic memory deficit is present both for verbal (Tierney et al., 1996) and nonverbal (Fuld et al., 1990; Small et al., 1997) materials, as well as across different retrieval conditions such as free recall (Grober et al., 2000; Howieson et al., 1997), cued recall (Bäckman & Small, 1998), and recognition (Fuld et al., 1990; Small et al., 1997). It is also apparent that AD patients show deficits in utilizing different forms of contextual and cognitive support (Almkvist et al., 1999; Herlitz et al., 1991).

Scores of delayed recall could best discriminate between subjects with preclinical AD and subjects with nonprogressive MCI (Kluger et al., 1999; Visser et al., 2001). Delayed recall is not always the best discriminator, learning and immediate recall and test of executive function is also of importance (Albert et al., 2001; Elias et al., 2000). A number of studies have demonstrated that tests of memory can differentiate individuals in the prodromal phase of AD from those who have memory problems that will not progress to AD (Arnaiz & Almkvist, 2003; Howieson et al., 1997). Visser et al (Visser et al., 2000) investigated the course of objective memory impairment in non-demented subjects. After five years 42% had no memory impairment, 19% had memory impairment without dementia and 39% had AD type dementia.

Impairment in semantic memory tasks are seen in AD even though these are less pronounced than those seen in episodic memory (Almkvist & Bäckman, 1993). Tasks where semantic knowledge is needed together with episodic memory tests were superior in the differentiation between AD and normal aging (Petersen et al., 1994; Spaan et al., 2003). Language deficits are common in AD, and in some cases, the most prominent early symptom of the disease. AD appears to cause major deficits on tests of semantic abilities. It has even been
suggested that the semantic impairment underlies many other cognitive deficits (Nebes, 1992). A common language problem is word-finding difficulties.

Attention deficits are prominent symptoms in AD and many attention operations are clearly impaired in early AD. The major functions affected are disengagement, shifting, and dividing of attention. Other functions are less prominently affected or are affected only in later stages of the disease (Nebes, 1992). These attentional dysfunctions may represent the first cognitive indicator of neocortical dysfunction in early stages of AD. Intact focusing and impaired disengagement of visuospatial attention may be linked to dysfunction in early AD of cortico-cortical networks linking the posterior parietal and frontal lobes (Parasuraman et al., 1992). There is a certain inconsistency in the results which can be due to sensory modality used, the nature of the task, the type of data collected, or different subgroups of AD patients (Nebes, 1992).

Visuospatial ability generally tends to be impaired in AD and in some patients it is the most prominent symptom. It is involved in several cognitive areas and particular visual perception and constructional praxis. Complex visuoperceptual discrimination becomes difficult and left-right orientation remains relatively intact but fails when the task requires mental rotation. Unilateral visuospatial inattention is common. Line orientation judgement is often impaired. Constructional tasks are difficult, even simple ones as a clock drawing test (Lezak, 1995). Correlations between clock drawing score and global ratings of severity of dementia were highly significant (Sunderland et al., 1989) and might be useful in early diagnosis (Yamamoto et al., 2004). On more difficult copying tasks most of the performances are defect (Lezak, 1995).

Executive functions are impaired in patients with mild AD (Albert et al., 2001; Chen et al., 2000; Grady et al., 1988), especially those requiring set shifting, sequencing and self-monitoring while those requiring abstraction and concept formation were only marginally affected in very mild AD patients (Lafleche & Albert, 1995).

Early decline in odour identification has been demonstrated in questionable AD, in patients with MCI and in AD patients (Devanand et al., 2000; Morgan et al., 1995; Nordin & Murphy, 1998; Suzuki et al., 2004).

To conclude, neuropsychological tests seem to be useful in the early identification of Alzheimer’s disease. Using test that covers episodic memory, semantic memory, visuospatial functioning, verbal ability, and attention makes it possible to differentiate between normal aging and very mild AD to a large extent. This is supported in a meta analysis of 52 studies (1985-2003) concerning AD and preclinical cognitive markers revealed marked preclinical deficits in episodic memory, perceptual speed, executive functioning, and in global cognitive ability (i.e. MMSE).
Small deficits were seen in attention, verbal ability, and visuospatial skill, and no preclinical impairment was observed in short-term memory (Bäckman et al., 2004). For differential diagnosis neuropsychological tests might be less useful since difference in cognitive functions are hard to detect in an early phase (Laukka et al., 2004).

**Imaging**

MRI

Cerebral atrophy is a normal aging process. However, longitudinal MRI studies of global brain volume has shown that the rate of brain atrophy is greater in AD than in normal aging (Jack et al., 2000). On the other hand there is an overlap between healthy individuals and AD patients (de Leon et al., 1997). Hippocampus atrophy may be a risk factor for accelerated memory dysfunction in normal aging (Golomb et al., 1996). Temporal lobe structures are more atrophic in AD patients than in aged nondemented individuals (Jack et al., 1992; Kaye et al., 1997; Xu et al., 2000). Within the medial temporal lobe (MTL) the hippocampal formation and parahippocampal gyrus are most affected even at an early stage of AD, numerous studies have reported significant volume loss of the hippocampus in AD patients compared to nondemented elderly (Bobinski et al., 1999; de Leon et al., 1997; Golomb et al., 1994; Jack et al., 1992; Jack et al., 1999; Kaye et al., 1997; Killiany et al., 1993; Laakso et al., 2000; Narkiewicz et al., 1993; Scheltens et al., 1992). The mean volume loss is between 20% and 52% compared to age-matched controls (Mega et al., 2002). MTL atrophy is highly predictive of AD. There are reports that there is no apparent advantage of MTL over hemispheric rate measures for sensitivity to clinical conversion in early AD (Jack et al., 1999).

Volume loss has also been detected in persons at risk of developing AD (Chetelat & Baron, 2003; de Leon et al., 1997; Fox et al., 2001; Killiany et al., 2000; Mega et al., 2002). Chetelat and Baron (2003) suggested that a reduced association temporal neocortex volume combined with hippocampal or anterior cingulate cortex atrophy may be the best predictor of progression to AD. Atrophy rates have been shown to be greater among normal subjects who converted to MCI or AD than among those who remained stable (Jack et al., 2000; Jack et al., 1999; Jack et al., 2004; Laakso et al., 2000). Volume loss in both entorhinal cortex and hippocampus may be early signs of AD (Du et al., 2001). A group of persons with age associated memory impairment had significantly lower volumes of the right hippocampus and hippocampal head compared to controls (Mega et al., 2002).

Brain volumetric measures from MRI together with clinical variables can predict the rate of cognitive decline among normal elderly individuals (Adak et al., 2004). The volume of the
hippocampal formation correlated to quantitative and qualitative aspects of memory performance in patients with probable AD (Deweer et al., 1995) and to change in MMSE scores in a longitudinal study (Fox et al., 1999).

White matter hyperintensities are also related to cortical atrophy and neuropsychological impairment in AD (Capizzano et al., 2004).

fMRI and SPECT
There is a consensus that the most prominent rCBF pathology in AD is localised in posterior cortical regions bilaterally, notably in the temporoparietal association areas (Brown et al., 1996; Gustafson & Risberg, 1974; Ingvar & Gustafson, 1970; Small et al., 1999). The specificity for AD in these areas, in a Swedish study, was very good while the sensitivity was 75-80%, the majority of the mislabelled cases had selective incomplete white matter infarcts in addition to AD pathology (Risberg & Gustafson, 1997). Londos and colleagues found reductions in temporoparietal areas in AD patients but imaging results were strikingly similar between AD and Lewy body pathology (Londos et al., 2003).

One approach to study AD with imaging is to focus on examining tasks, such as encoding information into memory, that are known to be compromised in AD (Lee et al., 2003) and expect changes, decreases in brain activity that may provide an early marker of AD. Mainly decreases in bloodflow have been shown in several studies.

Changes in rCBF during verbal episodic memory activation were compared between AD patients and healthy volunteers. In AD patients a distributed system involving several leftsided ROI, especially the posterior inferior frontal region was found for listening-rest comparison and no significant changes were found in memory-listening comparison. During listening-rest significant activation was found in leftsided hypoperfused regions. A significant correlation between memory performance and rCBF was found only in right lateral frontal region, a region not hypoperfused. The conclusion was that the involvement of this region might relate to either retrieval effort or actual performance of the memory task (Cardebat et al., 1998).

In a memory encoding fMRI study Rombouts et al (Rombouts et al., 2000) observed significantly decreased activation in left hippocampus and parahippocampal gyrus bilaterally for AD patients.

Kato et al. (2001) studied individuals with mild AD patients during the learning of complex geometric figures. They found that AD patients showed an increased bloodflow during stimulus presentation only in visual association area and reduced signal in medial temporal and
frontal regions. The authors suggest that the reduced bloodflow might be an indicator of early AD.

Significant frontal deficits have been demonstrated in more severely demented AD patients with both PET and SPECT (Brown et al., 1996; Neary et al., 1987; Wyper et al., 1993) correlating significantly with change in cognitive function (Brown et al., 1996).

Impaired metabolism measured by activation imaging appears to precede cognitive decline in both AD and MCI patients. Studies of brain function during behavioural tasks revealed differences between AD patients and age matched controls (Herholz et al., 1999). In a SPECT study AD patients with focal temporal lobe dysfunction (FTLD), AD patients with diffuse cognitive impairment (AD) and normal controls were studied with respect to rCBF and neuropsychological testing. The patients with FTLD had a bilateral mesial temporal hypoperfusion and the patients with AD had a posterior parietal hypoperfusion. Patients with FTLD had a slower rate of decline (Cappa et al., 2001). High risk individuals also have reduced activation in the mid- and posterior inferotemporal regions bilaterally during naming and letter fluency tasks (Smith et al., 1999).

Garrido et al studied the relation between medial temporal atrophy and functional brain activity during memory processing and found that hippocampal atrophic changes in AD were associated with reduced functional activity in limbic and associative temporal regions during episodic memory processing. There was also increased activity in frontal lobes which was interpreted as compensatory acts (Garrido et al., 2002).

Another approach to study AD with imaging methods is to examine activation patterns that result from the performance of tasks that are completed successfully but may be more difficult in persons at risk for AD, cognitive tasks can serve as “stress test” with individuals at the earliest stages of the disease requiring more neural activity to reach the same level of performance (Lee et al., 2003). Here I present studies with mainly increases in bloodflow.

Bookheimer et al (2000) used fMRI of adults with genetic risk for AD (APOE ε4 allele carriers) and control persons (APOE ε3 allele carriers) to determine whether neural activation elicited by declarative memory is related to APOE genotype and whether such activation predicts later memory decline. It was found that the participants with risk for AD showed greater memory-related activation in multiple neural regions relative to controls. These regions included left prefrontal cortex and left medial temporal structures (Bookheimer et al., 2000).

With positron emission tomography, bloodflow was assessed in normal elderly and AD patients during two retrieval conditions. Both groups showed the same brain activity patterns during cued recall with increased bilateral activity in orbital and dorsolateral prefrontal cortex, left
precuneus, and right cerebellum, as well as decreased activity in distinct left temporal regions. During episodic retrieval the normal elderly alone activated the left parietal cortex and the left hippocampal formation while AD related increases were observed in the left orbital prefrontal cortex and left cerebellum (Bäckman et al., 1999). It was suggested that a large distributed network involved in episodic memory retrieval was relatively normal in early AD and that the AD related increases that were observed were compensatory reactions. Compensatory attempts has also been suggested for verbal recall when AD patients showed a greater activation in regions of cerebral cortex normally involved in auditory-verbal memory as well as areas not activated by normal elderly (Becker et al., 1996). AD patients demonstrate a reduced performance in many priming tasks compared to healthy individuals. There is a change in brain activity though that might be explained as a reflection of a compensatory action to balance the reduced ability to perform the task (Almkvist, 2000).

Various tasks requiring semantic processing appear to involve frontal as well as posterior areas, such as inferior temporal lobe in addition to areas involving stimulus and response processing. The areas of activation seem to be larger in AD patients than in controls. The temporoparietal regions are activated in controls but not in AD patients, maybe due to the typical bilateral morphological temporoparietal reduction of the brain seen in AD (Almkvist, 2000).

In AD patients and nondemented elderly, increased functional activation is proposed to reflect recruitment of neural and cognitive operations that compensate for a decline in the neural circuitry normally used for task performance (Cabeza et al., 1997; Wagner, 2000). AD patients used a unique network involving bilateral, dorsolateral, prefrontal and posterior cortices and activity in these networks correlated with better performance on both semantic and episodic memory tasks. This was interpreted as evidence that AD patients can use additional neural resources in prefrontal cortex, presumably those mediating executive functions to compensate for losses attributable to the degenerative AD process (Grady et al., 2003).

In a study with working memory paradigm activated regions were precentral gyrus, superior and middle temporal gyrus, postcentral gyrus, temporal cortex, and Broca’s area. Anterior and posterior cingulated cortex and precuneus showed decreased activation. No diseaserelated effects were found for the pattern of brain activation (Almkvist, 2000). Imaging studies of attention group comparison demonstrates that pattern of brain activation for AD patients resembled that of healthy aged individuals during sustained attention involving increased activation of the anterior cingulated cortex and in parietal areas. The main differences between AD patients and controls in divided attention tasks was lack of activation in AD patients which
has been explained in different ways i.e. misunderstanding of tasks demands, impaired task performance and destroyed neural tissue (Almkvist, 2000).

To conclude, studies have shown patterns of decreased bloodflow in temporoparietal regions of the brain for both AD patients and for persons at risk for AD. On the other hand, there are reports of increase of bloodflow in other parts of the brain. It seems as if multiple brain structures are involved early in the course of the disease or even before AD diagnosis, findings that are supported with other methods than SPECT and fMRI (Fox et al., 2001; Killiany et al., 2000; Kogure et al., 2000; van der Flier et al., 2002). Neuroimaging is important when identifying people who are in the preclinical or early phase of AD and recent advances have made it possible to identify regions of the brain that are affected early. Still we don’t know which set of brain regions will provide the most accurate identification of persons with questionable AD who will develop a definite AD. There are reports that the relationship between structural and functional changes in AD is not straightforward (Eagger et al., 1992), deficits on SPECT were seen both with and without associated changes on CT. Furthermore, we don’t know which brain regions are most sensitive to disease progression over time.

**Stress hormones**

Several studies on AD patients indicate a dysregulation in the LHPA axis on different levels (Davis et al., 1986; Näsman et al., 1995; Seckl et al., 1993). This dysregulation may contribute to hypercortisolism seen in AD patients (Näsman, 1994). Changes in the negative feedback of glucocorticoids in the LHPA axis are believed to be the link between hippocampal atrophy and hypercortisolism in AD (Landfield et al., 1978; Sapolsky et al., 1986). The insensitivity to glucocorticoid feedback in AD may contribute to hypercortisolemia which acts as a co-factor in further neurodegeneration. Not many studies have investigated the cognitive function related to LHPA axis change and hippocampus. O’Brien et al (O’Brien et al., 1996) have shown an association between LHPA axis change, depressive symptoms and hippocampal atrophy in AD.
No man is an Island, entire of itself; every man is a piece of the continent, a part of the main.

Devotions, XVII by John Dunne
RATIONALE AND AIMS OF THE THESIS

Discovering sensitive and specific markers for preclinical and/or early AD would be a major breakthrough, as it would make it possible to slow or even arrest the degenerative process before dementia develops. Furthermore, current symptomatic treatments, such as acetylcholine esterase inhibitors, may be more efficient when administered at an early stage of the disease. Early diagnosis is difficult to achieve, currently the clinical diagnosis of AD comes relatively late into the disease, mostly because there is an overlap between the clinical picture of mild dementia and normal aging, and between AD and vascular dementia.

The overall aim was to study level of neuropsychological function in relation to structural and functional changes in the brain of persons with AD.

The specific aims were:

* to investigate relations between hippocampal volume, neuropsychological function and disturbed feedback of the cortisol axis in AD

* to evaluate if patients with early AD show an altered regional cerebral bloodflow (rCBF) compared to control persons and to investigate the correlation between rCBF in sub-lobar volumes of the brain and performance on memory-tests

* to examine possible differences in memory-related brain activation between persons with high versus low risk for AD

* to investigate patterns of brain activity and cerebral bloodflow with SPECT in an encoding task of episodic memory in persons with early Alzheimer's disease and healthy elderly control persons
OVERVIEW OF STUDIES

This section gives a summary of methods and main results from the papers included in this thesis. Details are found in each paper respectively.

Table 1: A schematic overview of the studies including in the thesis

<table>
<thead>
<tr>
<th></th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of study</td>
<td>Cross-sectional</td>
<td>Cross-sectional</td>
<td>Cross-sectional</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>Participants</td>
<td>16 AD patients</td>
<td>14 AD patients</td>
<td>102 persons with subjective memory complaints screened fMRI – 6 high risk 6 low risk</td>
<td>18 AD 18 healthy controls</td>
</tr>
<tr>
<td>Severity of AD</td>
<td>Mild to moderate AD</td>
<td>Early AD</td>
<td>Subjective memory complaints</td>
<td>Early AD</td>
</tr>
<tr>
<td>Assessment scales</td>
<td>MMSE CDR MADRS</td>
<td>MMSE CDR</td>
<td>MMSE CDR MADRS FAST</td>
<td>MMSE CDR MADRS</td>
</tr>
<tr>
<td>Neuropsychological assessment</td>
<td>AVLT Word recall (ADAS) Logical memory (WMS) Paired associate (WMS) Rey 15 item test (RMT) Non-verbal memory test Block design (WAIS-R) Digit symbol (WAIS-R) Spatial span (WAIS-Rni)</td>
<td>SPT (Betula) Word recall (Kungsholm) RMT Face recognition Word fluency (Betula) Word comprehension (SRB)</td>
<td>Paragraph recall test RCFT Buschke Face recognition Verbal fluency (FAS) Digit span (WAIS-R) Block design (WAIS-R) Digit symbol (WAIS-R) Odor identification</td>
<td>Paragraph recall test RCFT Buschke Face recognition (Betula) Verbal fluency (FAS) Digit span (WAIS-R) Block design (WAIS-R) Digit symbol (WAIS-R) Trail making test A+B</td>
</tr>
<tr>
<td>Brain imaging method</td>
<td>Structural MRI</td>
<td>SPECT (rest)</td>
<td>Functional MRI</td>
<td>SPECT (activation)</td>
</tr>
<tr>
<td>Statistical analysis</td>
<td>Spearman correlation Multiple linear regression</td>
<td>ANOVA Pearson correlation t statistics CBA</td>
<td>t statistics SPM</td>
<td>t statistics SPM2</td>
</tr>
</tbody>
</table>


Table 2. Subject characteristics across study samples

<table>
<thead>
<tr>
<th>Study</th>
<th>Female/male</th>
<th>Age Mean (SD), range</th>
<th>Years of education, Mean (SD), range</th>
<th>MMSE Mean (SD), range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD patients</td>
<td>11/5</td>
<td>75.3 (7.1), 61-83</td>
<td>20.5 (5.8), 11-29</td>
<td></td>
</tr>
<tr>
<td>Study II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD patients</td>
<td>4/10</td>
<td>75.2 (8.8), 64-91</td>
<td>24.6 (2.9)</td>
<td></td>
</tr>
<tr>
<td>Healthy controls</td>
<td>7/8</td>
<td>71.4 (3.2), 66-75</td>
<td>28.3 (1.2)</td>
<td></td>
</tr>
<tr>
<td>Study III</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High risk for AD</td>
<td>3/3</td>
<td>73.3 (4.0)</td>
<td>6.8 (1.9)</td>
<td>26 (1.7)</td>
</tr>
<tr>
<td>Low risk for AD</td>
<td>2/4</td>
<td>74.7 (5.2)</td>
<td>6.5 (0.8)</td>
<td>28 (1.1)</td>
</tr>
<tr>
<td>Study IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD patients</td>
<td>10/8</td>
<td>73.3 (4.9),</td>
<td>9.4 (3.1),</td>
<td>26.0 (1.8),</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>14/4</td>
<td>69.4 (4.0),</td>
<td>13.4 (4.8)</td>
<td>29.7 (0.6)</td>
</tr>
</tbody>
</table>

**Study I**

The main purpose of this study was to examine the association between hippocampal volume (the CA1 region), episodic memory tests and the capacity to suppress cortisol in patients with mild to moderate AD (Table 2). The sixteen patients were healthy except for the AD diagnosis and had no medication. Hippocampal volume was measured with MRI. To evaluate LHPA axis, a dexamethasone suppression test was performed. A data reduction analysis for the neuropsychological battery was performed and revealed three main factors; Rey 15 item memory test (RMT), ADAS word recall and Spatial Span from WAIS-R NI, all representing episodic memory. Our hypothesis was that hippocampal CA1 volume and cortisol levels predicted the cognitive level. Predictors were the two different measures of hippocampal CA1 volume (footdx and footsin) and cortisol levels after DEX (0.25 mg).

Linear regression was performed for each of the three dependent variables separately; RMT, Spatial span and ADAS word recall. Age was included in the first step of the analysis as an independent variable but was excluded since it was non-significant in all models.
RMT scores correlated significantly to the size of the right CA1 hippocampal subregion (Figure 3) \((r_s=0.76; p=0.001)\) while Spatial span scores correlated significantly to left CA1 size \((r_s=0.63; p<0.01)\). Cortisol levels after DEX (0.25 mg) correlated significantly to Spatial span scores \((r_s=0.58; p<0.05)\). No associations were found between post-DEX cortisol levels after 0.5 mg DEX on one hand and memory tests/neuroradiological measurements on the other.

![Figure 3](image_url)

**Figure 3.** Correlation between episodic memory retrieval (RMT) and the diameter of the "foot" of the right hippocampus (as a proxy for the CA1 subregion of the hippocampus) in patients with AD, n=16.

The variance in both RMT and Spatial span was best explained by the variance in CA1 hippocampal volume and suppressed cortisol level in combination. ADAS word recall had no significant predictive variable.

This study gives support to the thesis of a link between hippocampal volume, dysfunction of LHPA axis and episodic memory dysfunction. Specific memory tests – notably Rey memory test and Spatial span correlate significantly to hippocampal CA1 atrophy and post-DEX cortisol levels in combination. The dysfunction of the LHPA axis may be of importance for cognitive dysfunction and progression of neurodegeneration in Alzheimer’s disease. Longitudinal studies of the LHPA axis in relation to hippocampal size and cognitive dysfunction are important to determine the causal directions of these associations.
**Study II**

To further explore memory related changes in the brain we wanted to study if patients with early AD show an altered regional cerebral bloodflow (rCBF) compared to control persons. To make a contribution to development of early diagnosis of AD we looked at the correlation between rCBF in sub-lobar volumes of the brain and performance on memory-tests.

**Figure 4.** Analysed Brodmann areas (BA) of the cortex (Thalamus and hippocampus are not shown). Blue = BAs in AD patients (n=14) with significantly lower rCBF (ANOVA) compared to healthy controls (n=16), Right and left regions are marked with DX and SIN, respectively.

Memory-tests were chosen to evaluate episodic and semantic memory. Fourteen patients with early AD and 15 control persons (Table 2) were included. rCBF measurements with single photon emission computerized tomography (SPECT) using $^{99m}$Tc-hexamethyl propylenamine oxime (HMPAO) were performed. The rCBF-SPECT images were spatially transformed to fit a brain atlas, computerized brain atlas software (CBA) (Thurfjell et al., 1995) and normalized for differences in rCBF. Cortical and sub-cortical volumes of interests (VOI) were analyzed and compared.
AD patients showed a significantly lower rCBF in temporoparietal regions including left hippocampus compared to the controls. A color-coded scheme showing significant reduction in the rCBF ratio in selected BAs (7, 10, 17, 20-22 and 37-40) is presented in Figure 4. (Thalamus and hippocampus are not shown). The diagnostic sensitivity and specificity for AD were high in temporoparietal regions. AD patients had significantly lower performance on semantic and, in particular, episodic memory-tests compared to the controls, and their performance on several episodic tests correlated with rCBF in parietal and temporal regions including left hippocampus. The correlation between rCBF and level of episodic memory performance suggests that abnormalities in rCBF pattern underlie impaired episodic memory functioning in AD.

Our conclusion was that an observer-independent analyzing method for SPECT with sublobar VOI’s is promising in diagnosis of AD.

Study III
In the third study we wanted to examine possible differences in memory-related brain activation between persons with high versus low risk for AD. This was achieved by combining a validated neurocognitive screening battery (the 7-minutes test) with memory assessment and functional magnetic imaging (fMRI). One hundred and two healthy community-living persons with subjective memory complaints were recruited through advertisement and tested with the 7-minutes test (Solomon et al., 1998). They were classified as having either high (N=8) or low risk for AD (N=194). Six high-risk individuals and six age, sex and education matched low-risk individuals (Table 2) were investigated with a neuropsychological testbattery and fMRI while engaged in episodic memory tasks (face/name).

The high-risk individuals performed at a lower level than low-risk individuals in all examined cognitive domains and especially on tests of episodic memory. Patterns of brain activity during episodic encoding and retrieval showed significant group differences. During both encoding and retrieval, the low-risk persons showed increased activity relative to a baseline condition in prefrontal brain regions that previously have been implicated in episodic memory. By contrast, the high-risk persons did not significantly activate any prefrontal regions, but instead showed increased activity in visual occipito-temporal regions (Figure 5). A group x condition ANOVA revealed higher encoding-related activity in right dorsolateral frontal cortex (Figure 6), and higher retrieval-related activity in right frontopolar cortex (Figure 7) for the low-risk group.
Figure 5. Statistical parametric maps of brain activity during encoding. The low-risk group showed increased activity in right dorsolateral frontal cortex while the high-risk group showed increased activity in bilateral occipital cortex and left cerebellum.
Figure 6. Results from group x condition ANOVA showing higher encoding-related activity in right dorsolateral frontal cortex for the low-risk group.

Figure 7. Results from group x condition ANOVA showing higher retrieval-related activity in right frontopolar cortex for the low-risk group.

It was concluded that patterns of prefrontal brain activity related to episodic memory differ between persons with high versus low risk for Alzheimer's disease. Lowered prefrontal activity may predict subsequent disease.

Study IV
The purpose of this final study was to evaluate if memory provoked rCBF-SPECT is a better diagnostic tool for early dementia than a standard rest condition. This was done by comparing the rCBF patterns for patients with mild AD and healthy elderly controls during a standard rest
condition and during a condition in which an episodic encoding task was used as memory provocation.

Patients with mild AD and healthy elderly (Table 2) were included in the study. The patients and controls were presented an episodic memory test at the same time as they were injected with technetium-99m hexamethylpropylene amine oxime (HMPAO). rCBF measurement was performed and the rCBF 99mTc-HMPAO SPECT images were spatially transformed to fit a brain atlas and analyzed in SPM2.

In the memory provoked rCBF-SPECT the AD group in comparison with healthy elderly showed an rCBF reduction in the left parietal cortex. No significant group differences were seen in the rest condition, although similar group differences were seen in the rest and memory conditions at lower statistical thresholds. In the healthy elderly group, increased rCBF during memory compared to rest was observed in extensive regions in bilateral occipital cortex, right temporal cortex (including a strong activation in the fusiform gyrus on the right side, i.e. the “face area”), and bilateral inferior parietal cortex. In the frontal lobes there were bilateral activations in medial caudal parts including the inferior and middle frontal gyri. More laterally there were bilateral activations in frontopolar cortex, including BA 10. The AD group had a similar but smaller activation pattern in the occipital region. Only one small fronto-temporal region in the right forebrain was activated. No increased rCBF was seen in the parietal cortex. At rest there were no significant differences in rCBF between the groups. During the memory provocation, reduced rCBF was observed for AD patients compared to healthy elderly in the left frontoparietal region, mainly localized to the postcentral gyrus and the inferior parietal lobule, extending over central sulcus to the precentral gyrus.

Memory provocation magnified group differences in rCBF. The fact that a similar pattern of rCBF reduction was seen in the rest condition and in the memory condition at a lower statistical threshold suggests that memory provocation primarily serves to improve sensitivity of the rCBF-SPECT. Thus, memory provoked rCBF-SPECT might be important for early diagnosis of AD when rCBF alterations are subtle.
GENERAL DISCUSSION

The overall aim of this thesis was to study level of neuropsychological function in relation to structural and functional changes in the brain of persons with AD. By using different neuroimaging techniques i.e. MRI, SPECT and fMRI and extensive neuropsychological evaluation we were able to show that neuropsychological level, notably episodic memory, can be systematically related to functional disturbances of the LHPA axis and to structure and function of temporoparietal and prefrontal brain regions in AD patients. These changes are detectable in patients with risk for AD and those in an early phase of AD, which suggests that the obtained results might be of importance for early diagnosis of AD.

Stress, hippocampus and AD

One of the aims was to investigate relations between hippocampal volume, neuropsychological function and the capacity to suppress cortisol in AD. The key finding in study I was the association between decreased episodic memory, decreased hippocampal CA1 volume and abnormal negative feedback in the LHPA axis in mild to moderate stages of AD. These data strengthens earlier experimental and human data suggesting an association between cognitive function, hippocampal function and LHPA activity (Lupien et al., 1998; Seckl & Olsson, 1995).

To our knowledge, this is the first study to document such an association in AD. Glucocorticoid production is increased in the earlier stages of AD (Linder et al., 1993; Näsman et al., 1996). Importantly, the findings of those prior studies as well as data reported in the current study was based on medication-free individuals without putative confounding factors such as known other diseases, institutionalisation etc. In experimental studies, the medial prefrontal cortex seems to be of importance for basal activity of the LHPA axis. The lack of association between post-DEX cortisol levels and hippocampal volume might be explained by dysfunction in other parts of the system e.g. medial prefrontal cortex. The association between episodic memory and glucocorticoid levels is interesting. Since this observation is based on cross-sectional data it is impossible to determine the causal relationship of the correlations described.

For future research there are several issues to consider. A natural diurnal variation of cognitive function could be related to circadian variation in cortisol levels which is important in the methodological refinement of the neuroendocrine protocols. It is also important to take into consideration the type of population studied when assessing the effects of corticoseroids on human cognitive function. Cognitive impairment in hypocortisolemic populations such as post...
traumatic stress disease or burn out patients might have a different cause than those observed in hypercortisolemic populations (AD, depression) (Lupien & Lepage, 2001).

There is a problem to recruit study patients in this area of clinical research, especially with the importance of high demands regarding health and non-medication. However, the strong links found between low hippocampal volume, disturbed negative feedback of the LHPA axis and memory dysfunction that was found in this study encourage future investigations on larger patient samples and furthermore, prospective studies of patients with mild cognitive impairment are of major interest.

rCBF - memory correlation in AD
The second aim was to evaluate if patients with mild AD show an altered regional cerebral bloodflow (rCBF) compared to control persons and to investigate the correlation between rCBF in sub-lobar volumes of the brain and performance on memory-tests. We were able to show that AD patients compared to controls, had a significantly lower rCBF ratio in temporoparietal regions, including left hippocampus and that the diagnostic sensitivity and specificity for AD were high in temporoparietal region. The AD patient’s performance on several episodic memory tests correlated with rCBF ratio in parietal and temporal regions, including left hippocampus. This was done by analyzing rCBF ratios in small volumes in combination with use of several accurately selected episodic and semantic memory tests. The use of focal volumes of interest (VOI)s increases the chance of detecting disease-related dysfunction and also increases the chances of detecting correlations between rCBF and memory performance. In our study the AD patients had decreased activity in the left hippocampus which is in line with other studies (Rombouts et al., 2000). Volume loss in the hippocampus has in several studies been detected in persons at risk of developing AD (Chetelat & Baron, 2003; de Leon et al., 1997; Fox et al., 2001; Killiany et al., 2000; Mega et al., 2002).

The neuropsychological profile with the more profound decrement in episodic than in semantic tests for AD patients, is in line with results from earlier studies (Almkvist et al., 1996; Nilsson, 1997). The sensitivity of 86% is equal or higher than that in studies based on visual interpretation (Bonte et al., 1997; Jagust et al., 2001; Jobst et al., 1997; Masterman et al, 1997) and the specificity of 93% for AD diagnosis is high.
Brain changes in persons at risk for AD

In a third study we wanted to examine possible differences in memory-related brain activation between persons with high versus low risk for AD. We found pronounced differences in functional brain activity associated with episodic memory between persons of high versus low risk for AD. The low-risk group consistently activated regions of the prefrontal cortex during the memory tasks whereas the high-risk group mainly activated posterior visual regions in the occipital lobes.

A right middle frontal region was strongly associated with memory encoding. This region was also activated during episodic encoding of nonverbal information in a previous fMRI-study (Brewer et al., 1998). The latter study found that activity in this right prefrontal region predicted subsequent memory performance, such that high activity in this region during encoding predicted that an item later on would be remembered. This observation is in line with the present finding that lowrisk persons, who had better subsequent memory performance, activated this right prefrontal region to a greater extent than highrisk persons during encoding.

The activation pattern associated with retrieval for the low-risk group included regions in the anterior cingulate cortex and right fronto-polar cortex. These regions have been associated with episodic memory retrieval in several previous studies (Cabeza & Nyberg, 2000b), and they have been seen as reflecting a neurocognitive state that is a necessary condition for remembering past events (Lepage et al, 2000). By this view, the high-risk persons who did not show frontal activation failed to engage in proper cognitive processing during retrieval. Instead, their activation of posterior visual regions during encoding as well as retrieval indicate that the high-risk participants were primarily engaged in extensive visual/perceptual processing. It is important to note that even if none had a clinical diagnosis of AD at the time of fMRI testing, as indicated by their neuropsychological profile the participants in the present study were likely to be in a relatively advanced stage of the disease process. It seems that some of them were at least mildly demented at the time of MRI scanning. After eight months, the difference between the groups was still manifest. At this point in time, six out of six (100%) in the high-risk group had an AD diagnosis whereas only two out of six (33%) in the low-risk group were demented. In contrast to our study, the participants with genetic risk for AD in a study by Bookheimer et al (2000) were presymptomatic and had comparable memory performance as their controls. Moreover, the specific testing conditions, notably the fast presentation rate, is likely to have made it more demanding to engage in self-initiated processing.

The stage of the disease process and the specific test conditions may thus have contributed to lower frontal activity for the high-risk persons. Nonetheless, a recent fMRI study
found reduced memory-related prefrontal brain activity in mild AD (Kato et al., 2001), and all persons in our high-risk group developed AD within eight months after fMRI testing. Collectively, these observations highlight the role of prefrontal cortex and indicate that a failure to activate task-relevant prefrontal brain regions is a predictor of subsequent disease. This pattern indicates that reduced memory-related prefrontal activity may be a biological marker for AD, possibly by reflecting problems with engaging in self-initiated higher-order processes during encoding and retrieval (Dick et al., 1989; Granholm, 1988).

Evidence exists which strongly suggests a role for frontal lobe functioning in declarative memory. The frontal lobes seems to be required to organize and program voluntary behaviour effectively, to suppress interfering stimuli, and to permit appropriate responses to rapidly varying environmental demands (Squire, 1987). According to this Stuss & Benson (1986) suggests that the role of frontal lobes in memory implies that lesions and neural loss do not cause a primary disturbance of memory, but that they do interfere with mnemonic activity. Luria proposed that memorizing is not affected by frontal lobe damage but by essential, associated, frontal lobe abilities of motivation, programming, regulation, attention and verification (Luria, 1973). Simensky & Abeles (Simensky & Abeles, 2002) investigated the relation between measures of frontal lobe functioning and verbal memory performance among healthy older adults and conclude that their data indicate a central role of frontal dysfunction in understanding age-related memory loss. Encoding and retrieval seem to be mediated by the functioning of the frontal lobes, leading to possible deficits in recognition and recall. Additionally, the frontal lobes appear to play a crucial role in the suppression of interfering stimuli, including controlling for the effects of previously acquired information (Simensky & Abeles, 2002).

**Increased diagnostic sensitivity in memory provocation**

Our final aim was to evaluate if memory provoked rCBF-SPECT is a better diagnostic tool for early dementia than a standard rest condition. Earlier studies have failed to find support for significant sensitivity improvement by using different activation tasks (Beversdorf et al., 1995; Sayit et al., 2000). In these previous studies, a region of interest (ROI) approach was used for statistical evaluation whereas we used a whole-brain SPM-approach. SPM analysis is a more sensitive tool to detect early AD changes than ROI analysis (Tonini et al., 2003).

Differences between patients and controls were most pronounced in the left parietal cortex. In accordance with this finding, Garrido and colleagues used rCBF-SPECT and MRI and found regions with reduced rCBF in bilateral temporoparietal cortex in a memory task (Garrido...
et al., 2002). A similar activation pattern was seen in an episodic retrieval task with decreased activity in left temporal regions during cued recall and increases in the left orbital prefrontal cortex (Bäckman et al., 1999). Cardebat et al. (1998) found that AD patients activated several left-sided ROIs especially in the posterior inferior region while listening. During the memory task, activation was found in the right lateral frontal region.

There is a consensus that the most prominent rCBF pathology in AD is localised in posterior cortical regions bilaterally, notably in the temporoparietal association areas (Brown et al., 1996; L. Gustafson & Risberg, 1974; Ingvar & Gustafson, 1970; S. A. Small et al., 1999). Reduced parietal rCBF in AD patients was observed during memory provocation in the present study. Several previous SPECT studies have found temporo-parietal reductions during rest (Elgh et al., 2002; Jagust et al., 2001; Ryding, 1996; Varrone et al., 2002). The contrast between controls and patients at rest revealed the same rCBF pattern as during memory provocation. The patterns were virtually identical which indicates that the memory provocation served to magnify group differences in AD-affected regions. This was evident at a more liberal statistical significance level. Provocation increased the sensitivity to detect group differences.

At the lower statistical level the AD patients also showed a reduction in the medial frontal cortex, in regions not typically associated with AD. However, frontal involvement is described in AD (Albert et al., 2001; Chen et al., 2000; Grady et al., 1988; Lafleche & Albert, 1995). In addition, recent studies have found reductions in frontal functional brain activity for AD patients and for patients at risk for AD (Elgh et al., 2003; Kato et al., 2001).

In this study we have demonstrated that memory provocation during rCBF-SPECT is more sensitive than rCBF-SPECT at rest. This might be of great importance for early diagnosis. The reduced rCBF in early AD was seen in the same regions in both rest and encoding, suggesting that memory provocation primarily serves to improve sensitivity of the rCBF-SPECT. Further studies are needed to evaluate “memory provoked rCBF-SPECT” as a diagnostic tool.

The overall aim in this doctoral thesis was to study level of neuropsychological function in relation to structural and functional changes in the brain of persons with AD. It was shown that episodic memory was associated with cortisol levels (study I), with structural hippocampal volume (study I) and with decreased bloodflow in temporoparietal brain regions (study II and IV) and hippocampus (study II and III). It was also shown that episodic memory was associated with brain activation in PFC for persons with low risk for AD while this activations were absent in persons with high risk for AD (study III).
Problems and limitations

There can be several explanations for difference in test performance, and it is for instance important that the level of education is equal between groups. In study IV there were differences between the groups in years of education which can be a confounding factor (Table 2). Gilleard (1997) suggested that education can be considered a socializing process promoting certain lifelong learning strategies enabling educated people to adapt and to perform more competently on neuropsychological tests and dementia screening batteries. As a result, surveys of elderly poorly educated populations in both developed and developing countries may lead to significant over-diagnosis of clinical dementia – and by implication a possible under-diagnosis of dementia in relatively well-educated populations (Gilleard, 1997). Another confounding factor can be sex, there are sex differences in episodic memory performance since women perform at a higher level than men (Herlitz et al., 1997). Sex differences have also been reported for regional cerebral bloodflow (Rodriguez et al., 1988). Our groups are not equal in sex distribution (Table 2).

Neuropsychological tests are sensitive to specific early cognitive dysfunctions and might be more accurate than global measures in distinguishing individuals who will subsequently develop symptomatic AD from those who will not (Chen et al., 2000) or differentiate between AD and vascular dementia (Almkvist et al., 1993; Starkstein et al., 1996). Brief general mental status tests (like MMSE) may be subject to ceiling effects (may be too easy) in early stages of disease and scores can remain high when underlying ability has started to decline. This is obvious in study III and study IV where the MMSE score are high for both groups in both studies. In study IV where the patients were diagnosed with AD, the patient group had a mean MMSE score of 26 points and the healthy control group 29 points. The neuropsychological test results for the same group revealed that the patients had a clear memory disturbance.

The usefulness of any neuropsychological battery for early diagnosis of AD will depend on its composition, size, and supporting data. An important aspect is the choice of memory tests. They need to be adapted to the stage of severity of the disease. Memory tests may differ in sensitivity because of the way in which the test is constructed (e.g. there may be differences in instructions, support and material to be remembered). It is important to take this into consideration when choosing tests, in order to be able obtain a range in the scores without either ceiling or floor effects. In study I several of the hippocampus sensitive tests reached floor effects i.e. too many of the patients scored zero or just above because the test was too difficult for the majority of the patients in that particular group, they had a range in MMSE of 11 – 29 points. There were no variation in test results and none of these tests were possible to use in the
correlation analysis. Equally important is the effort to minimise the sense of failure in the tests situation for persons with AD, and this can be done by selecting suitable tests.

In many studies there are positive correlations between aspects of different brain volume measures and stages of disease, even in preclinical phases of the disease (Deweer et al., 1995; Fox et al., 1999; Petersen et al., 2000). Clinically there is a problem with the large degree of overlap between groups i.e. MCI and AD or vascular dementia and AD, which is an everyday problem for the clinical neuropsychologist. Hippocampal and enthorhinal cortex atrophy in subjects with MCI are considered risk factors for development of AD, but also healthy elderly have hippocampal atrophy (Petersen et al., 2000). These measures cannot be regarded as being of high predictive value in an individual case (Wolf et al., 2003) which illustrates the importance of sharpening objective methods for diagnosis.

There are advantages and disadvantages with all neuroimaging methods. fMRI offers a method of indirectly measuring functional changes associated with neural activity. It may be performed in any clinical MRI scanner and does not require the injection of radioactive isotopes. Scanning technology allows robust and reproducible evaluation of both physiological and anatomical functions (Lee et al., 2003). A disadvantage is the relatively long duration of the scanning session when the subject is trapped in the machine. In my experience this has been a rather painful experience for some subjects, especially those with cognitive dysfunction. The subject may be unable to cooperate and may also move which is a problem in the analysis of data. This was the case in study III where persons at risk for AD were assessed with fMRI. Several of the persons felt really uncomfortable lying in the scanner. SPECT is used to provide in vivo tomographic images of how a radioactive tracer is distributed in the human brain. The scanner is widely available and relatively cheap (Lee et al., 2003) but there is a small amount of radioactivity involved which should not be underestimated. The scanning session is not very uncomfortable for the subject and the test session when the injection is given can be really calm and relaxed.

There are potential problems in functional studies of degraded brains that need to be considered. AD is seen as a unitary disease in terms of neuropathological characteristics and development. It is assumed that the disease has a continuous progression of brain degeneration (Almkvist, 2000). The continuous change occurring from healthy aging to AD is at present unknown and there are reports describing AD in terms of two or three subtypes (predominant left- or right hemispheric degeneration) (Grady et al., 1990). Therefore, there is a risk that findings are not valid for AD as a whole but rather to the specific sample of AD patients in that particular study.
It is important to take into consideration in the analysis of data how representative the persons in the controls groups are. Healthy control persons may not always be healthy. It is stated that the preclinical period of AD appear many years prior to the clinical diagnosis of the disorder (Bäckman et al., 2001). There is a possibility that preclinical cases of AD may contaminate the healthy control group. Healthy elderly are not representative in an elderly population. Subjects selected without evidence of clinical disease will differ a lot from a group of older persons that is chosen at random from a population because individuals chosen with the purpose to be representative of the average will include many individuals with serious medical illnesses. Some of these illnesses will include those with considerable impact on cognitive function (Odenheimer et al., 1994). Even though these optimally healthy individuals are not representative, they are important as comparisons to persons with early changes in cognitive function.

MCI groups are heterogeneous (Elfgren et al., 2003). Not all persons with mild memory impairment have incipient AD (Frisoni et al., 2004; Ritchie et al., 2001). Whereas MCI is frequently the earliest clinical manifestation of AD, other common conditions that cause dementia are also likely to manifest a prodromal or preclinical phase. A problem in early diagnosis is the exclusion of AD patients with non-amnesic presentations that obviously exist (Hodges, 1998) and the MCI patients that are not characterized by deficits in episodic memory (Bennett, 2003). Other problem is the use of age-adjusted cut off scores on neuropsychological tests. Only persons falling below the age-adjusted cut off will have the condition. This is probably underestimating the occurrence and importance of MCI (Bennett, 2003).

It is important to thoroughly assess the relationship between the volumetric data and clinical status. The lack of agreement in the precise anatomic limits of the hippocampus might be a problem when comparing different studies. Furthermore hippocampal atrophy is not specific to AD. Other diseases associated with memory impairment have also atrophic hippocampus (Lee et al., 2003).

Implications for diagnosis and future research

It is obvious that interesting brain areas for early diagnosis are temporoparietal regions. The finding that episodic memory is associated with structural and functional changes in temporoparietal and hippocampal areas are not surprising. Changes in these areas are well acknowledged (Jack et al., 1992; Kaye et al., 1997; Xu et al., 2000). What we were able to accomplish was a better diagnostic tool for temporoparietal regions. Measures of rCBF in a
memory provocation condition were better than measures of rCBF in a standard rest condition. This has a direct implication for diagnosis but further studies are needed to replicate our findings. Functional changes in prefrontal regions were found in two of our studies (study III and study IV). These changes were discovered in activated states. In future research the role of prefrontal cortex in early AD must be further explored. The dysfunction of frontal areas is supported by finding of executive dysfunction in early AD (Chen et al., 2001; Grady et al., 1988; Lafleche & Albert, 1995) but this is not in any systematic way connected to episodic memory. In younger adults brain imaging studies have associated episodic memory encoding and retrieval with prefrontal activations (Cabeza & Nyberg, 2000a). These activations tend to be left lateralized during encoding and right lateralized during retrieval, a pattern known as hemispheric encoding/retrieval asymmetry (HERA). This asymmetry has been found for both verbal and non-verbal materials (Nyberg et al., 1996). This model does not hold for older adults since, under similar circumstances, prefrontal activity during cognitive performance tends to be less lateralized in older adults than in younger adults. This empirical generalization is conceptualized in terms of a model called hemispheric asymmetry reduction in older adults (HAROLD) (Cabeza, 2002). The prefrontal dysfunction is interesting in relation to episodic memory and in future research episodic memory should definitely be tested in control of and maybe with higher demands of executive functioning. Brain imaging studies indicate that distinct neocortical regions might interact with medial temporal lobe structures to reinstate a memory. Frontal regions mediate the strategic retrieval attempt and monitor its outcome, with dissociated frontal regions making functionally separate contributions to retrieval (Buckner & Wheeler, 2001).

The prefrontal regions are also interesting regarding stress hormones and AD. A lot of studies concerning stress have concentrated on hippocampus, more recent data suggests that stress has an impact on many cortical and subcortical brain structures other than hippocampus. It seems as if in addition to pituitary, hypothalamic sites and limbic structures (particularly hippocampus), cortical areas and particularly the prefrontal cortex are involved in the regulation of LHPA activity, possible through an inhibitory role (Lupien & Lepage, 2001). The lack of association between post-DEX cortisol levels and hippocampal volume in our study might be explained by dysfunction in other parts of the LHPA axis. Both prefrontal and medial temporal structures should be considered when investigating the interaction between cortisol levels, brain measures, and cognition in the future.
CONCLUDING REMARKS

I would like to make a few concluding remarks based on the results presented in this thesis.

Of great importance and a major breakthrough for AD patients would be the discovery of sensitive and specific markers of early AD. Pharmacological therapy may make it possible to slow or even arrest the degenerative process before dementia develops.

Neuropsychological assessment of cognitive function and imaging play unique roles in the initial diagnosis and as a marker of the effects of therapy (Lee et al., 2003) but the key to success is the cooperation between different disciplines. Neuropsychology, cognitive psychology, neuroscience, brain imaging and medicine are all essential for the understanding of etiology, pathophysiology and clinical diagnosis of AD.

The LHPA axis is important in AD, and it is closely related to neuropsychological function and hippocampal volume. To further explore the role of stress hormones in relation to cognitive function and AD longitudinal studies are needed.

In the examination of possible differences in memory-related brain activation between persons with high versus low risk for AD we found interesting difference in prefrontal areas. The role of prefrontal cortex in AD is a topic for future research.

When comparing the rCBF patterns for patients with mild AD and healthy elderly during a standard rest condition and during a condition in which an episodic encoding task was used as memory provocation we were able to sharpen the diagnostic sensitivity which is indeed very interesting for early diagnosis.
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