Human brain function evaluated with rCBF-SPECT
Memory and pain related changes and new diagnostic possibilities in Alzheimer’s disease

by
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Cover illustration: A transaxial slice of a normal regional cerebral blood flow (rCBF) acquired with single photon emission computed tomography (SPECT) using the tracer technetium-99m (99mTc) hexamethylpropyleneamine oxime (HMPAO). The slice is mapped to a brain atlas with drawn volumes of interest (VOIs). The three blue fields illustrate the three-headed gamma camera.

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To my lovely wife Lena and the treasures of my life Ebba Alva and Johan

"Fantasi är viktigare än kunskap"
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ABSTRACT

The aim of this doctoral thesis was to study the influence of memory, pain, age and education on the regional cerebral blood flow (rCBF), i.e. brain function, in early Alzheimer’s disease (AD) and in chronic neck pain patients in comparison to healthy controls and in healthy elderly per se. This was done by optimizing single photon emission computed tomography (SPECT) as a method to study rCBF with the tracer Technetium-99m (99mTc) hexamethylpropyleneamine oxime (HMPAO) and by matching all image data to a brain atlas before evaluation. The rCBF-SPECT was evaluated and developed to obtain higher diagnostic accuracy in AD and in chronic neck pain patients it was used to study basic pain related cerebral processes in chronic pain of different origin. A new semimanual registration method, based on fiducial marker, suitable for investigations with low spatial resolution was developed. The method was used to reconstruct images with an improved attenuation and scatter correction by using an attenuation-map calculated from the patients’ previously acquired CT images.

The influence of age and education on rCBF was evaluated with statistical parametric mapping, SPM in healthy elderly. The main findings were age related changes in rCBF in regions close to interlobar and interhemispheric space but not in regions typically affected in early AD, except for the medial temporal lobe. The theory of a ‘cognitive reserve’ in individuals with a longer education was supported with findings in the lateral temporal lobe, a region related to semantic memory, and in the frontal lobe.

A cross-sectional study of chronic neck pain patients showed extensive rCBF changes in coping related regions in a non-traumatic pain patients compared to both healthy and a pain group with a traumatic origin, i.e. whiplash syndrome. The whiplash group displayed no significant differences in rCBF in comparison with the healthy controls. This suggests different pain mechanisms in these groups.

The AD-patients showed a significantly lower rCBF in temporoparietal regions including left hippocampus. These changes were associated to episodic memory performance, and especially to face recognition. The diagnostic sensitivity for AD was high. The face recognition test (episodic memory) was used in AD patients to improve the sensitivity of method, i.e. memory-provoked rCBF-SPECT (MP-SPECT). The results were compared to healthy controls and the reductions of rCBF in temporoparietal regions were more pronounced in mild AD during provocation. Memory provocation increased the sensitivity of AD-related rCBF changes at group level. If a higher sensitivity for AD at the individual level is verified in future studies, a single MP-SPECT study might then be of help to set diagnosis earlier.

In conclusion rCBF in temporoparietal regions are associated to an impaired episodic memory in early AD. Changes in these regions do not have a strong connection to chronological age. The diagnostic sensitivity of rCBF-SPECT in AD is high and there is a potentially higher sensitivity if memory provoked investigations are used. The findings in this thesis have given an increased knowledge of underlying cerebral pain processing in non-traumatic and traumatic (whiplash) neck pain. Preliminary results supporting the theory of ‘cognitive reserve’ by showing a correlation between long education and preserved rCBF was found in healthy elderly.

Key words: brain imaging, rCBF, SPECT, early diagnosis, brain atlas, HMPAO, Alzheimer’s disease, Whiplash, neuropsychological function, episodic memory
**SUMMARY IN SWEDISH**

Hjärnans blodflöde avspeglar dess funktion och ökar av lång utbildning, minskar av långvarig kronisk nacksmärta och minskar vid försämrat minne p.g.a. Alzheimers sjukdom.

Grunden i avhandlingen utgörs av att fördelningen av hjärnans blodflöde (rCBF) avspeglar hjärns funktion. Med hjälp av en nuklearmedicinsk skiktbaserad undersökningsteknik (SPECT) kan rCBF mätas med spårämnet \( { }^{99m} \text{Tc-HMPAO} \) som injicerats i blodet och dess fördelning sedan studeras med en s.k. gammakamera. Undersökningen används rutinmässigt för diagnostik av olika demenssjukdomar. Mer än 100 000 svenskar över 65 år ålder lider idag av Alzheimers sjukdom vars mest kända symptom är den tidiga minnesförtslost som sedan följs av en långsam försämring av hjärnans samtliga funktioner. Den sjuke blir då småningom helt beroende av sin omgivning och sjukdomen belastar sjukvård och samhälle med stora kostnader. Idag finns mediciner som, om de sätts in tidigt, kan lindra symtomen och ge den sjuke en bättre livskvalitet under en tid. Därför är det viktig med en tidig och korrekt diagnos.

I avhandlingen med titeln "*Human brain function evaluated with rCBF-SPECT - Memory and pain related changes and new diagnostic possibilities in Alzheimer’s disease*”, översatt, "*Den mänskliga hjärnans funktion utvärderad med rCBF-SPECT- Minne och smärtrelaterade förändringar och nya diagnostiska möjligheter vid Alzheimers sjukdom*" visas att rCBF är försämrat i både hjässloben och tinningloben hos tidigt Alzheimersjuka. Blodflödet i dessa delar av hjärnan är kopplad till neuropsykologiska testresultat som visar ett försämrat minne för tidigare händelser i livet (episodiskt minne). Genom att provocera hjärnan med en minnesinlärningsuppgift kunde dessa rCBF försämringar visas i ett än tidigare stadium av sjukdomen. Detta är en viktig upptäckt som kan komma att användas för att förbättra rCBF-SPECT diagnostiken av Alzheimer.

Det finns idag ett stöd för att ett aktivt liv är bra för hjärnfunktionen och kan ha en skyddande effekt för utveckling av demens. I den frågan lägger avhandlingen ytterligare en pusselbit på plats som visar att lång utbildning kan öka hjärnbloodflödet i olika delar av hjärnan hos friska. Ett annat intressant fynd är att de områden som vanligen drabbas av rCBF-SPECT förändringar vid Alzheimers inte drabbas av normala åldersförändringar. Detta gör det lite enklare att bedöma undersökningar med avseende på förekomst av sjukdomen.

The thesis is based on the following papers which are referred to by their Roman numerals in the text.

I. Larsson A, Johansson L, Sundstrom T, Ahlstrom KR
   A method for attenuation and scatter correction of brain SPECT based on computed tomography images.

II. Sundström T, Larsson A, Nyberg L, Riklund ÅK
    Influences of chronological age and length of education on rCBF-SPECT patterns in a healthy sample: Diagnostic implications
    *Manuscript*

III. Sundström T, Guez M, Hildingsson C, Toolanen G, Nyberg L, Riklund ÅK
    Altered cerebral blood flow in chronic neck pain patients but not in whiplash patients: a $^{99m}$Tc-HMPAO rCBF study.
    *Accepted for publication in the European Spine Journal*

IV. Elgh E, Sundström T, Näsman B, Åhlström K, Nyberg L
    Memory functions and rCBF $^{99m}$Tc-HMPAO SPET: developing diagnostics in Alzheimer's disease.

V. Sundström T, Elgh E, Näsman B, Nyberg L, Riklund ÅK
    Memory-provoked rCBF-SPECT as a diagnostic tool in Alzheimer's disease?

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### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ACC</td>
<td>Anterior cingular cortex</td>
</tr>
<tr>
<td>AChEIs</td>
<td>Acetylcholinesterase inhibitors</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>APA</td>
<td>American psychiatric association</td>
</tr>
<tr>
<td>APP</td>
<td>Amyloid precursor protein</td>
</tr>
<tr>
<td>ApoE</td>
<td>Apolipoprotein E</td>
</tr>
<tr>
<td>ADRDA</td>
<td>Alzheimer's disease and related disorders association</td>
</tr>
<tr>
<td>BA</td>
<td>Brodmann area</td>
</tr>
<tr>
<td>BAs</td>
<td>Brodmann areas</td>
</tr>
<tr>
<td>CBA</td>
<td>Computerized brain atlas</td>
</tr>
<tr>
<td>CBF</td>
<td>Cerebral blood flow</td>
</tr>
<tr>
<td>CDR</td>
<td>Clinical dementia rating scale</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>DSM</td>
<td>Diagnostic and statistical manual of mental disorders</td>
</tr>
<tr>
<td>ECD</td>
<td>Ethylcysteinate dimer</td>
</tr>
<tr>
<td>FDR</td>
<td>False discovery rate</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full width at half maximum</td>
</tr>
<tr>
<td>GLM</td>
<td>General linear model</td>
</tr>
<tr>
<td>FBP</td>
<td>Filtered back projection</td>
</tr>
<tr>
<td>HE</td>
<td>High education</td>
</tr>
<tr>
<td>HMPAO</td>
<td>Hexamethylpropyleneamine oxime</td>
</tr>
<tr>
<td>IASP</td>
<td>International association for the study of pain</td>
</tr>
<tr>
<td>LE</td>
<td>Low education</td>
</tr>
<tr>
<td>LEHR</td>
<td>Low energy high resolution</td>
</tr>
<tr>
<td>LTM</td>
<td>Long-term memory</td>
</tr>
<tr>
<td>MADRS</td>
<td>Montgomery-Åsberg’s depression scale</td>
</tr>
<tr>
<td>MCI</td>
<td>Mild cognitive impairment</td>
</tr>
<tr>
<td>MIP</td>
<td>Maximum intensity projection</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>MMSE</td>
<td>Mini-mental state examination</td>
</tr>
<tr>
<td>MNI</td>
<td>Montreal neurological institute</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MSU</td>
<td>MNI space utility</td>
</tr>
<tr>
<td>MTL</td>
<td>Medial temporal lobe</td>
</tr>
<tr>
<td>NINCDS</td>
<td>National institute of neurological &amp; communicative disorders &amp; stroke</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>PFC</td>
<td>Prefrontal cortex</td>
</tr>
<tr>
<td>PMTs</td>
<td>Photomultiplier tubes</td>
</tr>
<tr>
<td>PS2</td>
<td>Presenilin 1</td>
</tr>
<tr>
<td>PS2</td>
<td>Presenilin 2</td>
</tr>
<tr>
<td>QTF</td>
<td>Quebec task force,</td>
</tr>
<tr>
<td>rCBF</td>
<td>Regional cerebral blood flow</td>
</tr>
<tr>
<td>RCFT</td>
<td>Rey complex figure test</td>
</tr>
<tr>
<td>rCMR&lt;sub&gt;Glc&lt;/sub&gt;</td>
<td>Regional cerebral metabolic rates for glucose</td>
</tr>
<tr>
<td>rCMR&lt;sub&gt;O2&lt;/sub&gt;</td>
<td>Regional cerebral oxidative metabolism</td>
</tr>
<tr>
<td>RMT</td>
<td>Rey memory test</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>SI</td>
<td>Primary somatosensory cortex</td>
</tr>
<tr>
<td>SII</td>
<td>Secondary somatosensory cortex</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single photon emission computed tomography</td>
</tr>
<tr>
<td>SPET</td>
<td>Single photon emission tomography</td>
</tr>
<tr>
<td>SPM</td>
<td>Statistical parametric mapping</td>
</tr>
<tr>
<td>SPT</td>
<td>Subject performed task</td>
</tr>
<tr>
<td>SW</td>
<td>Software</td>
</tr>
<tr>
<td>TMT</td>
<td>Trail making test</td>
</tr>
<tr>
<td>TSU</td>
<td>Talairach space utility</td>
</tr>
<tr>
<td>WAD</td>
<td>Whiplash-associated disorders</td>
</tr>
<tr>
<td>VOI</td>
<td>Volume of interest</td>
</tr>
<tr>
<td>VOIs</td>
<td>Volumes of interest</td>
</tr>
<tr>
<td>VT</td>
<td>Verbal task</td>
</tr>
</tbody>
</table>
INTRODUCTION

In this thesis the diagnostic potential and possibilities of measuring human regional brain blood flow (rCBF) with single photon emission computed tomography (SPECT) will be presented and discussed. The focus is on Alzheimer’s disease (AD) and chronic pain, which represent two large problems in health care, i.e. memory complains and pain [1]. The question of dementia and notably AD is the most common reason for referral to an rCBF-SPECT investigation [2-6]. In pain rCBF-SPECT is used as a research tool to increase the knowledge of brain response. All data handling from the raw image data file acquired with the scintillation camera to the evaluation of the images by statistical methods and obtained results will be discussed.

Radionuclide based techniques such as SPECT and positron emission tomography (PET) can provide information of the regional cerebral functional activity, and constitute an important tool in the study of the pathophysiological aspects of dementia and normal aging. They are most helpful for the differentiation between different types of dementia and an accurate diagnosis of Alzheimer's disease. SPECT is readily available, while the availability to PET studies in Sweden is restricted.

The brain

The brain uses glucose as substrate for its energy metabolism [7] and the neurones depend almost exclusively upon oxidative metabolism to support metabolic activity and normal function. Thus, adequate delivery of glucose via blood flow is essential for normal neuronal activity [8]. The average human rCBF in brain tissue is about 50 ml/100g/min [9], approximately fourfold higher in grey than in white substance. In PET studies of humans at rest it has been shown that cortical grey matter values of rCBF, regional cerebral oxidative metabolism (rCMR$_{O_2}$), and regional cerebral metabolic rates for glucose (rCMR$_{Glc}$) are regionally linked [10]. Therefore rCBF is a reliable estimation of rCMR$_{O_2}$, i.e. brain function [11, 12].

The blood brain barrier, (BBB) maintains a constant environment in the brain and protects it from blood borne chemical substances that may cause injury, for example circulating drugs, immunogenic antigens and changes of electrolyte concentration. It is a semi-permeable barrier between blood and the brain consisting of endothelial cells with tight intracellular junctions, localized mainly in the capillaries. The BBB is permeable to non-polar materials having a small molecular size, as well as to lipid soluble compounds. For other substances such as glucose there are specific carrier systems or pumps. Most of the diseases in the brain can affect the BBB, i.e. cerebral neoplasm, inflammation or oedema of any kind [13].
Brain blood flow and its relation to activity

Although the brain represents only 2% of the total body weight, it consumes about 20% of the total body oxygen and utilizes about 25% of the total body glucose. The brain adjusts its own blood supply due to local needs, i.e. auto-regulation. Furthermore, the rCBF can remain constant at a mean arterial pressure between 60-160 mmHg [14]. This intrinsic capacity of cerebral blood vessels to maintain the cerebral blood flow constant over a wide range of altered blood pressures, provides adequate nutritive blood flow despite large fluctuations in arterial blood pressure. There are data demonstrating that the auto-regulation is mediated by astrocytes sensing the neural activity and releasing dilator metabolites which shunt blood flow to active neurones [8]. This explain how the regional blood flow locally is correlated to neuronal activity and why increased activity in a small active brain region is accompanied by an increase in metabolism and therefore also in rCBF [15]. This correlation between rCBF and neuronal activity constitute the basic principle for both PET and SPECT [16, 17]. Brain damage such as ischemia or trauma impairs the auto-regulation and the CBF will then be more proportional to the perfusion pressure [18].

How does the brain work – network or regions

Since a day at the Société d’Anthropologie de Paris in 1861 where Broca and other researchers had a discussion about language localization, there has been a debate on whether the main functions in the brain can be related to specialized regions or to distributed networks in the brain. The theories have been referred to as localizationism or (dis)connectionism.

The latter theory has had a big influence on the neurological research during the mid 20th century when the specialized regions were nearly ignored. Since the eighties the localization theory has regained its importance mostly due to modern neuroimaging, although it is important not to diminish the role of the anatomical neuronal network. The current view appears to be that the brain function is based on both these fundamental principles of
functional organization with hierarchical organized neurons both in specialized regions as well as in larger networks in the brain [19, 20].

Brodmann [21] divided the human brain into 52 functional areas based on its cytoarchitecture, which can be seen in Fig. 1. The Brodmann areas (BAs) are still widely used in neurophysiology research even if the anatomical division into lobes or gyri and sulci is the best known classification of different brain regions. The relation between lobes and alternative descriptive names of some of the functional BAs is presented in Table 1.

**Table 1.** Slightly modified version of table 10. p. 373, Nolte (1993) The Human Brain

<table>
<thead>
<tr>
<th>Lobe</th>
<th>Brodmann Area</th>
<th>Location</th>
<th>Alternative functional descriptive names</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal</td>
<td>4</td>
<td>Precentral gyrus, paracentral lobule</td>
<td>Primary motor area</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Superior and middle frontal gyri, precentral gyrus</td>
<td>Premotor area, supplementary motor area</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Superior and middle frontal gyri</td>
<td>Inferior portion = frontal eye field</td>
</tr>
<tr>
<td></td>
<td>44, 45</td>
<td>Opercular and triangular parts of inferior frontal gyrus</td>
<td>Broca’s area</td>
</tr>
<tr>
<td>Parietal</td>
<td>3, 7</td>
<td>Postcentral gyrus, paracentral lobule</td>
<td>Primary somatosensory area; S1</td>
</tr>
<tr>
<td></td>
<td>5, 7</td>
<td>Superior parietal lobule</td>
<td>Somatosensory association area</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>Inferior parietal lobule</td>
<td>Angular gyrus</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>Inferior parietal lobule</td>
<td>Supramarginal gyrus</td>
</tr>
<tr>
<td>Occipital</td>
<td>17</td>
<td>Banks of calcarine sulcus</td>
<td>Primary visual area; V1</td>
</tr>
<tr>
<td></td>
<td>18, 19</td>
<td>Surrounding 17</td>
<td>Visual association area; V2, V3, V4, V5</td>
</tr>
<tr>
<td>Temporal</td>
<td>41</td>
<td>Superior temporal gyrus</td>
<td>Primary auditory area; A1</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>Superior temporal gyrus</td>
<td>Auditory association area; A2</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>Superior temporal gyrus</td>
<td>Wernicke’s area</td>
</tr>
</tbody>
</table>

**Normal aging versus pathology in Alzheimer’s disease**

Several recent studies have shown that the cerebral metabolism and cerebral blood flow declines with aging [22-24], although after correction for structural changes the decline in metabolism is not always significant [24, 25]. The rCBF in normal subjects has been evaluated in several studies that all conclude that age has a considerable effect on rCBF [26-28]. Furthermore it is shown that normal aging also progressively affect the structure of the brain [29]. Aging and AD are related to each other and there are evidence suggesting that the brain actively adapts to a progressive fuel deprivation characterized by hypometabolism and oxidative stress which finally result in neuronal loss in both conditions [30]. Especially the grey matter is affected during aging and the amount of grey matter seems to decrease linearly with age [26, 28]. In contrast, the amount of white matter is reported to be intact or even increase until the mid-50s, after which it declines with an accelerated rate [26, 28]. In studies of global rCBF with Xenon-133 ($^{133}$Xe) [31, 32] as well as in estimated whole brain volume in structural studies [33, 34] it has been shown that the loss is about 0.3–0.5% per year in healthy elderly. In very early AD the global loss of brain tissue has been reported to be increased to approximately 1% yearly [34] with an even more accelerated loss when the disease progresses [35]. Some brain regions are more severely affected and AD patients can as an example show up to 20-30% less tissue in temporoparietal regions compared to healthy elderly [36].

9
Dementia

The prevalence of dementia worldwide is approximated to about 24 million people, of which 150,000 are in Sweden, and if the current trend with 4.6 million new cases of dementia each year continuous, the population of patients with dementia will nearly double every 20 years until 2040 [37, 38]. Dementia is a psychosocial tragedy to the growing elderly population and a financial burden for the society. The mean annual cost per patient in Swedish healthcare including caregiver time has been calculated to around 200,000 SEK [39]. Dementia, which means “without sense” in Latin, is a progressive disease with final loss of all functions, and is most often defined by the criteria of the Diagnostic and statistical manual of mental disorders (DSM IV) [40], which is created by the American Psychiatric Association (APA). It is a syndrome with loss of function in multiple cognitive domains including memory impairment and at least one of the following: aphasia, apraxia, agnosia and disturbances in executive functioning causing impairment in social and occupational functioning.

There are many conditions leading to dementia although the majority is degenerative diseases such as AD followed by vascular causes [41]. Other conditions that may cause dementia is toxic reactions, nutritional deficiencies, infections, hydrocephalus, injuries and brain tumours.

Alzheimer’s disease

AD is a progressive neurodegenerative disorder first affecting memory functions and then gradually affecting all cognitive functions with behavioural impairments and eventually causing death [42]. It is today the most common cause of dementia, and accounts for 60–70% of all cases [43, 44]. The incidens of dementia increases with age, and is reported to be approximately 0.5% in ages of 65–74 rising to 4% in ages of 85–89 [45]. The prevalence of AD in Europe is reported to be 0.6% in ages of 65–69 years, rising to more than 20% at ages above 90 [46]. Furthermore, the prevalence has been reported to be higher in women and especially among the oldest [47]. The clinical onset of dementia before 65 years of age is rare and represents less than 10% of all AD cases [48, 49]. The majority of the patients in this small subgroup of AD aggregate within families [50].

It is today 100 years since Alois Alzheimer discovered and described the disease after he had followed the patient that got the first AD diagnosis, during her last five years of life. Besides the symptoms he described and the characteristic plaques and neurofibrillary tangles that he found at necropsy, the brain had a thinner cerebral cortex than normal [51, 52]. During a long period of time AD was thought to be a rare disease and it was not until 1976 it was suggested to be the single most likely neuropathological correlate of dementia.
At the same time two British research teams independently reported an association between AD and severe loss of cholinergic function/neurons in the cerebral cortex [54, 55]. The discovery of a link between neuronal loss with a deficiency of acetylcholine and its relation to memory performance in AD marked the beginning of modern AD research. The previously obscure disease with plaques and tangles was now transformed into a disease with a transmitter-based pathophysiology which challenged the modern research of neuroscience.

**Memory and Alzheimer’s disease**

An impaired memory performance is often the symptom that brings the AD patient to the health care. Since memory complains are central in AD an understanding of human memory function is crucial in AD research and especially if attention is put on the relation between rCBF disturbances and human brain function in early AD. The human memory can be divided into short-term or working memory and long-term memory (LTM). The LTM constitutes of the declarative memory which is the form of the memory that stores facts (semantic memory) and events (episodic memory) and non-declarative memory which includes the procedural memory (skills and habits) and priming [56-59], Fig. 2.

![Fig. 2. The figure illustrates different forms of memory and their relationship.](image-url)
The declarative memory which depends on hippocampus [60] is almost always affected in AD and especially the episodic memory, which includes encoding and retrieval of information about personally experienced past events at a particular time and place [57]. The episodic memory can be assessed by asking persons to recall or recognize sentences, stories, words and pictures given at an experimental setting [61]. The semantic memory is our acquired knowledge about the world in form of words and concepts tied by associations and meanings. The semantic memory can be assessed by verbal fluency tests, picture naming, category listing and identification of semantically related pairs.

The functions of each of these memory systems have important implications for the ability of learning and coping with the activities of daily life. In AD a neuropsychological evaluation of memory and other cognitive functions is one of the most important tools when assessing early cognitive changes. This has been shown in both cross-sectional [62-64] and longitudinal studies [65, 66].

**Mild cognitive impairment or MCI**

The first preclinical stage of AD which precedes the dementia is characterized of an affected episodic memory, Fig. 2 [67]. This stage is often referred to as mild cognitive impairment or MCI [68-70]. Even though MCI has received a lot of attention there is still no consensus on appropriate clinical criteria for this preclinical stage [71]. The original criteria for a MCI required presence of memory complaint (preferably confirmed by an informant), objective memory impairment, preserved general cognitive function, in combination with normal functional activities, and no dementia. The predictive strength of MCI is quite high and annual conversion rates to dementia of about 12% summing up to about 80% at 6 years follow-up is reported [70].

**Risk factors**

The pathologic process in AD is often precipitated by genetic disorders (amyloid precursor protein (APP) and presenilin mutations and Apolipoprotein E (ApoE)), environmental risk factors and the aging process, which lead to a manifestation of the disease [30, 72]. The most potent genetic risk factor is the presence of the epsilon4 (ε4) allele of the ApoE gene, which has been suggested to by itself account for 50% of the nonfamilial AD cases in US [72]. The presence of the gene is linked to a smaller hippocampal volume and a lower performance on hippocampal-dependent cognitive tasks [73]. The other forms of the allele, ε2 and ε3 have not been shown to increase the likelihood of developing AD. The lifetime risk of developing AD for an individual without the ε4 allele is approximately 9% compared to 29% for individuals carrying at least one ε4 allele. The ε4 genotype is however not sufficiently sensitive or specific to be used on its own for the diagnosis of AD [74].
Besides this, at least three other genes, amyloid precursor protein (APP, chromosome 21), presenilin 1 (PS1, chromosome 14) and presenilin 2 (PS2, chromosome 1) have been identified as responsible for AD in familial dementia. PS1 is the most frequently mutated gene with a mutation frequency of 18–50% in autosomal dominant AD with onset before 65 years of age [50].

Besides the genetic factors, MCI, Down syndrome, abnormal blood pressure, aluminium intake, alcohol abuse, head trauma and low education level has been proposed as risk factors for AD [75, 76]. A developed social, mental and physical lifestyle as well as education has on the other hand been suggested to have a beneficial effect on cognition with a protective effect against dementia [77-80].

**Diagnosis of Alzheimer’s disease**

The diagnosis of AD is based on a clinical examination since there is no biological marker except for brain biopsy [81]. In 1980, APA published clinical criteria for AD, i.e. Diagnostic and Statistical Manual of Mental Disorders third edition, DSM-III. These criteria were revised in 1987, DSM-III-R [82] and again in 1994, DSM-IV [40]. The DSM-III-R defines AD as a dementia disease with a gradual onset with progressive cognitive decline including memory impairment and personality deficits which become severe enough to interfere with social life or work, but without any disturbance of consciousness. In clinical research the “National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association” (NINCDS-ADRDA) [42] criteria, published 1984, for AD has been widely used. These criteria make it possible to diagnose AD earlier than with DSM-III. In the NINCDS-ADRDA diagnostic manual the diagnosis of AD is ranked from being possible or probable to definite. The criteria for clinical diagnose of probable AD demands deficits in two or more areas of cognition, progressive memory impairment and other cognitive functions with no disturbance of consciousness. The onset of the symptoms has to be between 40 and 90 years of age, and there should be no systemic disorders or other brain diseases that by themselves can account for the progressive deficits in memory and cognition. The diagnostic criteria for a definite AD according to NINCDS-ADRDA are a fulfilling of the clinical criteria for probable AD, plus histopathologic evidence. The new DSM-IV criteria are very similar to the NINCDS-ADRDA criteria.

In clinical practise the diagnosis is based on information from a careful clinical examination, a thorough interview of the patient and relatives, and a neuropsychological assessment. An rCBF study is frequently used as a diagnostic tool in addition to the clinical findings. To exclude other conditions causing the symptoms, laboratory analysis of blood, urine and liquor,
investigation of heart and lung and neuroimaging, i.e. CT of the brain, can be included in the investigation.

The clinical dementia rating scale (CDR) [83] and the mini mental state examination (MMSE) [84] are the most common used methods for staging of dementia in a clinical routine. The CDR is used as a global measure for staging the severity of dementia [83]. It was primarily developed for use in persons with dementia of the Alzheimer type (the equivalent of probable AD) and it can also be used to stage dementia in other illnesses as well. It is usually completed by a clinician with detailed knowledge of the individual patient. The information is gathered, either as part of normal clinical practice or as part of a research study. If a specific interview is carried out, about 40 minutes is needed to gather the relevant information. Six domains are assessed, i.e. memory; orientation; judgement and problem-solving; community affairs; home and hobbies; and personal care. The five-point scale of CDR has a rating of 0 for healthy people, 0.5 for questionable dementia and 1, 2 and 3 for mild, moderate and severe dementia. Notable is that a rating of 0.5 may indicate either MCI or probable AD [85].

MMSE is a quick global assessment of many domains including: orientation to time and place (10 points), registration of three words (3 points), attention and calculation (5 points), memory recall of three words (3 points), language ability (8 points) and visual construction (1 point). A full score is 30 points. A score of 23 points or less is typically used as a cut-off for cognitive impairment, indicating that further diagnostic evaluation is recommended [84]. A limitation of the MMSE is the floor effect with low sensitivity of scores in the low range, which can be seen in advanced dementia, low level of formal education and in patients with severe language problems as part of their dementia [86]. There is also a ceiling effect in very well educated with high scores even though a clinical examination meets the criteria for dementia [87]. The specificity of the MMSE is high (96%) but the sensitivity is poor (63%), indicating that by itself the test (using a standard cut-off score of 23) will leave a substantial proportion of cases of early dementia undetected [88]. The sensitivity increases with a maintained high specificity if the cut-off is adjusted for age and education [89]. MMSE and CDR are often used to compare the degree of dementia between research studies.

**Histopathology, neuropathology and neurochemical changes**

According to NINCDS-ADRDA, a histopathological verification of an elevated amount of both senile neuritic plaques and neurofibrillary tangles in the brain is needed for a definite AD diagnosis [42, 90]. The neuritic plaques consist of a central core of amyloid protein which is surrounded by astrocytes and microglia. The neurofibrillary tangles contain paired helical filaments of
abnormally phosphorylated tau protein which are localized in the cell body and dendrites. In addition AD is characterized by a reduced synaptic density, loss of neurons and degenerated hippocampal neurons. The neuronal loss in the nucleus basalis of Meynert, locus ceruleus, and raphe nuclei of the brainstem leads to deficits in cholinergic, noradrenergic and serotonergic neurotransmitters. The cholinergic deficit which is related to memory function is the most common neurochemical abnormality in AD [91].

**Treatment of Alzheimer’s disease**

It is only during the last years medical treatment of AD has become available. Short-term treatment with acetylcholinesterase inhibitors (AChEIs) has been shown to improve cognition and other symptoms in mild-to-moderate AD [92]. Early treatment with AChEIs may have a beneficial neuroprotective effect in AD by increasing the neurotransmission through an increase of acetylcholine availability. As AD progresses, cholinergic deficits become more severe and long-term pharmacologic treatment with AChEIs is suggested to be beneficial for maintaining and reducing the rate of cognitive decline in patients with AD [92, 93]. In mild-to-moderate AD, three different AChEIs, i.e. donepezil, rivastigmine and galantamine are approved in Sweden. During moderate to severe stages of AD a glutamate inhibitor, memantine, is approved for treatment. This is a receptor-targeted therapy (N-methyl-D-aspartate) which is thought to work by blocking the action of glutamate that may be overactive in people with AD.

Epidemiologic data suggest non-steroidal anti-inflammatory agents, hormonal treatments, antihypertensive agents, and statins to decrease the likelihood of developing AD [94].

Future hope for a cure or improved disease-modifying treatment is in the field of gene-therapy [95], intervention in the β-amyloid process or the hyperphosphorylation of tau proteins [96], which are under evaluation in ongoing clinical trials [97].

**Pain**

Pain is a subjective and psychological experience which is most useful to protect the body from potentially harmful events and by protecting already damaged tissue while it heals. However, the subjective pain experience may lead to aberrant pain processing which affects our ability to function optimally. The typical experience of pain is a consequence of a noxious stimulus that is transmitted along nociceptive pathways to the brain where there is an integration of sensory, cognitive, and emotional information with an appropriate response.
To understand the aetiology in organic and functional pain syndromes there is a need to increase the knowledge of the specific functions and integration of the many brain regions which are involved in pain processing. The knowledge in this area has been enhanced by functional brain imaging by functional magnetic resonance imaging (fMRI), PET and SPECT. Although these techniques have allowed researchers to identify the anatomy of the cortical pain matrix [98, 99] further research is required to be able to understand the dysfunctional aspects of the pain related cerebral processes.

**Definition of pain and brief pathophysiology**

The definition of pain by the International Association for the Study of Pain (IASP) is “An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such”. This definition has been changed after a paper published in Pain 1996 [100] that led to a revision in the IASP definition, which now also includes pain in infants and other people who are unable to express their pain verbally. Note that by definition pain is always subjective and allows the pain experience to be without stimuli, although pain is always an experience associated with actual or potential tissue damage.

**Nociceptive versus neuropathic pain**

Nociception is the sensation caused by painful stimuli of peripheral endings of primary sensory neurons, called nociceptors, whose cell bodies are in the dorsal root ganglia. The nociceptive pain usually is characterized by an aching, sharp or throbbing sensation. It is perceived as an alarm of a potentially damaging or lethal stimulus in the environment and it signals the body to move away from danger. Nociceptive pain is typically a somatic painful condition such as sprains, bone fractures, burns, bumps and bruises [101]. A subtype of the nociceptive pain is the visceral pain in internal organs that tends to be episodic and not easily localized. The visceral pain is most often a consequence of a disease [102]. The neuropathic pain on the other hand, is caused by an injury or malfunction in the peripheral or central nervous system. The pain can be produced by damage to the nerve itself or by pathological changes within the nerve. It is often felt as a burning or tingling sensation or as hypersensation to touch or cold. Diabetic neuropathy, phantom limb pain and postherpetic neuralgia are typical examples of neuropathic pain [101].

**Acute pain versus chronic pain**

Acute pain is often caused by an obvious injury or trauma with a good response to simple analgesic therapy. The pain gradually disappears as the region of injury heals, often within less than 1 to 3 months [103]. Pain that lasts longer than 3 months or exceeds the expected recovery time is usually considered
chronic [101, 104]. Chronic pain has often an unclear pathogenesis where the pathology may be absent or insufficient to explain the persistence of pain [101]. The recovery is often much less predictable and behavioural, psychological, social and environmental factors may affect the clinical course and treatment outcomes of the chronic pain [105]. The chronic pain is frequently accompanied by emotional stress, irritability, depression, social withdrawal, loss of libido, disturbed sleep patterns, diminished appetite and weight loss. The acute pain seems to have a positive protective biological function which is not the case in chronic pain, especially when no tissue injury is verified [101].

Perception of pain and modulation

Pain typically begins with a noxious stimulus of terminal branches of nerve fibres, typically in skin, blood vessels or viscera. The signal in these receptors (nociceptors) passes the cell body in the dorsal root ganglia and terminates at a secondary neuron in the dorsal horn of the spinal cord. In the spinal cord the nociceptor input also activates a reflex withdrawal from the acute painful stimulus followed by a cerebral response [101, 106].

![Schematic figure of the human cortical pain matrix (PAG stands for periaqueductal gray)](image_url)

The pain signal travels by sensory neurons via the thalamus to the primary sensory cortex, where the discriminative component of pain is perceived, and
to cortical areas in which the affective and emotional aspects of the pain is processed and perceived, Fig. 3 [98, 107, 108].

The perception of pain is coloured by emotional, cultural, psychological, behavioural and social state [109].

**Modulation and sensitization**

The nociceptive system is complex and consists of different, partially independent pathways ascending from the spinal cord to the cortex. A modulation of the pain input by descending projections can occur at all levels of these ascending pathways of the nociceptive system [99, 110].

Central sensitization is an increase in the excitability of neurons within the central nervous system. This produces an increased synaptic efficacy established in somatosensory neurons in the dorsal horn of the spinal cord and leads to an abnormal response to normal inputs. The increased excitability is typically triggered by a burst of activity in nociceptors which alter the strength of synaptic connections between the nociceptor and the neurons of the spinal cord. Central sensitization can lead to a decreased pain threshold and a spread of hypersensitivity to regions beyond injured tissue [111]. Modulation by supraspinal descending facilitatory [112] and inhibitory [113] influences appear to contribute to the development and maintenance of central sensitization [114].

Peripheral sensitization on the other hand is produced by injury to the primary afferent neuron in the periphery due to neurohumoral modulators arising in response to injury. Both peripheral as well as central sensitization can contribute to a post injury hypersensitivity that is responsible for a decrease in pain threshold.

Chronic pain syndromes including chronic Whiplash-Associated Disorders (WAD) and fibromyalgia show evidence of hyper-excitability in the central nervous system induced by central sensitization [115-117]. This sensitization might explain the diffuse pain or ‘pain all over’ that fibromyalgia patients feel [112, 118].

**Chronic neck pain**

Chronic neck pain is a common disorder in the western world [119, 120] and nearly one fifth of the population in northern Sweden reports chronic neck pain, defined as continuous neck pain with more than 6 months duration [121]. Several factors such as female-gender, increasing number of children, psychological status, educational level, mental stress and musculoskeletal pain in general have been associated with the development of chronic neck pain [122-124]. Cross-sectional and longitudinal population studies have shown that
a history of cervical spine injury including whiplash trauma constitute a risk factor for persistent neck pain [122, 123, 125].

The cause of neck pain is most often not identified to a specific origin and is often classified as soft-tissue, muscular, mechanical or postural related pain. In most of these cases, radiological methods like X-ray, CT or MRI have not been able to identify the origin of pain. Age-related degenerative findings are often seen although just as frequently as in asymptomatic subjects and they do not usually cause the pain [126]. In several studies it has been shown that degenerative changes do not correlate to pain and the clinical relevance of these changes is therefore low [127, 128]. Less common but more specific causes are diseases like rheumatoid arthritis, infections, tumours, verified traumatic cervical spine injuries or cervical disk hernia affecting nerve roots [126].

**Whiplash-associated disorders**

Whiplash is the result of a sudden stretching of the spine, often as a result of a rear-end traffic collision. The Quebec Task Force (QTF) on WAD, has defined whiplash as follows: “Whiplash is an acceleration-deceleration mechanism of energy transfer to the neck which may result from rear-end or side impact, predominantly in motor vehicle collisions, but also from diving accidents, and from other mishaps. The energy transfer may result in bony or soft tissue injuries (whiplash injury), which in turn may lead to a wide variety of clinical manifestations, i.e. WAD [129].

Whiplash is the most common type of traffic injury [129-132] and has become the most common disabling disorder following traffic accidents with a significant burden of disability in the population of the western society [129, 130]. The annual incidence of reported whiplash injury is high in most western societies and is shown to be about 0.4% in northern Sweden. In a resent review it has been shown that approximately 5–8% of the patients with acute whiplash injury at follow-up more than 6 month later, have disabilities, negatively affecting work capacity [133]. At follow-up more than 1 year after the whiplash trauma, as much as about one-third of the patients report some form of persisting symptoms [130, 134], often leading to long term sick leave and disability. The incidence and prevalence of WAD varies considerably worldwide, indicating that socioeconomic, psychological and cultural factors other than biological ones may play a role [130, 135].

The QTF classifies WAD in a scale from 0 to 4, with increasing grade of severity. The grade 3 level has neurological signs and grade 4 covers cervical fractures and dislocations. Common symptoms after a whiplash trauma is neck pain, headache, stiffness and impaired mobility of the neck, radiating pain in the arms, and cognitive problems [136].
The aetiology and pathophysiological mechanisms of the chronic pain in WAD is still unknown and the diagnosis remains clinical. Some anatomical localizations of injuries to the cervical spine have been suggested [137, 138] although no specific organic lesion has been verified and neither imaging, nor physiological, or psychological studies have been able to provide a diagnostic test for WAD [138, 139]. The psychological influence has been suggested to be a more reliable predictor of the duration and severity of symptoms than the collision severity [130, 140].

**Neuroimaging**

There is an incredible drive to understand the function of the brain and modern technologies such as PET, SPECT, and fMRI have brought a mass of new knowledge of the location of human brain function, even if the knowledge of brain functions before modern neuroimaging was impressive. This is illustrated by an image from 1957, Fig. 4 [141]. The mapped functions in Fig. 4 are only based on lesion studies and studies of direct cortical stimulation during neurosurgery [142].

![Fig. 4. Schematic summary of the state of knowledge of the location of human functional brain in 1957. (Reproduced with permission from the publisher from Fig. #275, p. 456 of “The Vertebrate Visual System”, by Stephen Polyak). Today the research in the field of Human Brain Mapping is basically done by indirect measurements of metabolism by evaluation of rCBF such as in fMRI, PET and SPECT. Impressive knowledge of the brain function has been maintained over time, and modern technologies have provided new insights into brain function.](image-url)
achieved with the non-invasive highly sensitive fMRI method, which also has a high temporal resolution. This method allows the human brain function to be studied simultaneously as it is stimulated. There are however no doubts that molecular or biologic imaging are important for future neuroimaging, since it has the possibilities to map function and communication of neurons, neurotransmitters and even genes. This requires imaging techniques with a very high sensitivity to detect small differences in concentrations, typically SPECT and PET, Fig. 5. The threshold for these techniques is in the nanomolar to the picomolar range [143, 144]. Both PET and SPECT allow in vivo quantification of biochemical processes by measuring the concentration of radiotracers that reflect the activity of proteins receptors or even genes [145, 146].

Fig. 5. A schematic drawing of the sensitivity and specificity of nuclear medicine techniques i.e. PET and SPECT within the spectrum of medical imaging.

To produce scintigraphic images two major components are needed; the radionuclide and the detector. The first natural radionuclide was detected by Marie Curie and Henri Becquerel [147, 148] in the beginning of the 20th century. Hal Anger developed the scintillation camera for medical imaging during the fifth decade (www.lbl.gov/Science-Articles/Archive/nuclear-med-history.html). The next major step towards modern neuronuclear imaging took place during the seventies when the computerized tomography (CT) technique was introduced and tomographic scintigraphic studies became possible. The SPECT development in combination with the development of radiotracers dedicated to functional brain imaging during the eighties gave a new possibility in nuclear medicine imaging of cognitive function. During the same time, PET
and several neuroimaging tracers were developed. Furthermore the imaging of
cognitive functions has developed in MRI and especially in fMRI [149].

**Basic principles of a rCBF-SPECT HMPAO investigation**

In nuclear medicine the most widely used radionuclide is $^{99m}$Tc. It has an
optimal half-life of six hours which is long enough to examine metabolic
processes and short enough to keep the radiation dose to the patient low.
Furthermore $^{99m}$Tc decays without high energy beta emission which also
contributes to a relatively low radiation dose to the patient. The low energy
gamma rays, which are not attenuated in the human body, can escape and be
detected by the scintillation camera. The chemistry of $^{99m}$Tc allows it to be
incorporated into a range of biologically active substances that can interact in
human tissue or organs of interest.

A $^{99m}$Tc generator is a simple lead pot enclosing a glass tube containing
Molybdenum-99, ($^{99}$Mo), which has a half-life of 66 hours. Molybdenum-99
decays to $^{99m}$Tc which can be washed out of the generator by a saline solution
when it is required. After decay the generator is returned for recharging at the
nuclear reactor where the radionuclides are made.

To obtain an rCBF-SPECT image there is a need of a radiopharmaceutical that
reflects the rCBF. The most used radiopharmaceuticals for rCBF studies are
$^{99m}$Tc-hexamethylpropyleneamine
oxime (HMPAO, Ceretec®, General
Electric Healthcare) and $^{99m}$Tc-
ethylcysteinate dimer (ECD,
Neurolite®, Bristol-Myers Squibb
Medical Imaging) which both are
lipophilic with the ability to pass the
BBB. These lipophilic compounds,
which following intravenous
administration, are rapidly protein
bound have a high degree of first
pass extraction in the brain. The
extraction factor is about 80% for
HMPAO and 60-70% for ECD, and
the distribution of the tracers is
proportional to the rCBF. For
HMPAO the ratio of grey to white
matter activity is about 2-3 to 1
compared to 4 to 1 for ECD [150].
Both tracers appears to overestimate

![Fig. 6. Principal sketch of the three-headed scintillation camera used in this thesis. The red photons are detected, the blue are attenuated, the green is screened off and the yellow is scattered.](image-url)
low blood flow slightly, and underestimating high blood flow [150-152].

The patient has to be prepared with an intravenous line and kept in a calm environment with low light, reduced noise and minimal disturbance to achieve a standardized condition. The eyes could either be kept open or closed depending on the clinical routine. It is important to keep a record of the eye status during the injection point since it will affect the uptake to the primary visual cortex with a variability of 30% [3, 150]. This effect of increased rCBF in the visual cortex is clearly seen in study V in this thesis, in the comparison of the rest and test condition where the eyes were open during injection. Before and after the injection the environmental conditions should be maintained for about 10 minutes. The HMPAO tracer reaches the brain after approximately 15 seconds and the major uptake into the brain takes place during the following 40 seconds [153]. An equilibration between brain and blood is fully achieved after 5 minutes [154]. The image acquired is therefore representing the cerebral perfusion during these first minutes after injection. This unique feature is often called the “frozen image”, since it allows the injection to be separated from the gamma camera acquisition which is specifically useful for detecting critical short lasting changes in rCBF for instance in activation studies [155].

Some of the gamma ray photons emitted from the radiopharmaceutical will be detected by collimated radiation detectors, i.e. the scintillation camera. It uses a scintillating material, usually NaI, to convert the emitted gamma photons to light photons which are converted to electrical signals with photomultiplier tubes (PMTs). An electronic circuit evaluates the relative signals from all the PMTs and determines the location of each registered gamma photon. A collimator made of lead in front of the detector usually allows only photons perpendicular to the surface to hit the detector. All other gamma photons are usually screened off. In the body, the emitted photons interact mainly by Compton scattering, but also by the photoelectric effect. In Compton scattering part of the energy is transferred to an electron and the remaining photon is scattered into a new direction with reduced energy. These photons can also be detected by the camera causing blurring of the image, if they are left uncorrected in the reconstruction at image, Fig. 6. The information collected from one detector at a fixed position is called a projection. SPECT images are often reconstructed from 64 or 128 projections. The 3-dimentional distribution can be calculated from these projections, acquired from different angles covering the whole circumference of the patient. Following sampling of enough imaging statistics with the scintillation camera, images are reconstructed. Two different reconstructions techniques can be used, the iterative reconstruction (ordered subset expectation maximization, (OSEM)) or the filtered back projection (FBP) technique [156-158].
Functional imaging in early Alzheimer’s disease and MCI

Functional imaging with PET or SPECT has a high sensitivity and can detect subtle pathophysiologic changes in the brain before structural changes are present [159, 160]. The methods are used both for verification of dementia as well as for discrimination of different types of dementia. The diagnostic accuracy of PET and SPECT to distinguish patients with AD from healthy elderly has in an evidence-based review, been found to be comparable to the accuracy of a histopathologically confirmed diagnosis [161]. Concerning the discrimination between different types of dementia i.e. AD, Lewy body dementia and frontotemporal dementia, both PET and SPECT have a higher sensitivity than clinical evaluation [2, 161]. A recent systemic review suggests that clinical criteria may have a higher sensitivity to detect AD than SPECT (81% versus 74%) but SPECT studies provide a much higher specificity against other types of dementia (91% versus 70%). SPECT may therefore be helpful in the differential diagnosis of AD [2]. It has also been shown that addition of rCBF-SPECT findings typical for AD to a clinical criteria diagnosis, increases the probability of accurate diagnosis (definite AD) from 84% to 92% in case of “probable AD” and from 67% to 84% in patients with "possible AD" [162]. Reduction of rCBF in the temporoparietal regions is the most consistent changes in mild to moderate AD [3, 5, 6]. In a recent three year follow up rCBF-SPECT study of MCI subjects, a reduced rCBF in the inferior parietal lobule, angular gyrus or precunei had a high predictive value and high discriminative ability to detect MCI subjects that converted to AD [163]. Furthermore it seems to be a difference of onset regions between early and late onset of AD where elderly AD patients tend to present with involvement of the medial temporal lobes (MTL) associated with a marked memory loss whereas early onset patients predominantly have the decrease of rCBF in posterior cortical association regions [164]. This difference has also been shown in voxel based morphometric studies with MRI [165]. This should be considered in clinical routine although as presented in this thesis the uptake from the MTL is largely affected by age.

Structural imaging in early Alzheimer’s disease and MCI

Structural neuroimaging modalities such as MRI and CT have a limited role in the clinical evaluation of AD patients and are most often used to exclude structural cause to dementia, i.e. brain tumours, subdural haematoma or normal pressure hydrocephalus [166]. The most common structural neuroimaging findings in AD are a widespread cortical atrophy with a thinning of MTL [167]. This is also seen in normal aging although with a much slower progression. Therefore MTL atrophy is not pathognomonic for AD and has not been able to discriminate between different types of dementia [168].
The development of functional based MR imaging, i.e. fMRI, spectroscopy, perfusion and diffusion has shown possibility to detect pathophysiologic changes in the brain in mild or moderate AD and might be implemented in clinical routine [169].

**Neuroimaging in pain**

Structural and functional imaging of the brain is typically not used clinically in pain patients, and for research purposes mainly functional imaging methods are used.

Since the beginning of the nineties, neuroimaging has been used to study cerebral response to noxious and non-noxious stimuli in normal subjects. An increased knowledge of ‘normal’ brain processing of pain has been achieved, and a large field of research in pain patients has developed. The current view is that pain sensation is influenced by a multi-dimensional integration of sensory-discriminative, cognitive and affective-motivational processes. Furthermore, the interpretation of image data in pain is complicated since all aspects of the sensory encoding (intensity, location, modality), affective (fear, unpleasantness) and cognitive processes (attention, memory) has to be considered in the understanding of pain experience.

Acute experimental noxious stimuli have shown to cause rCBF responses with changes in the secondary somatosensory cortex (SII), anterior cingular cortex (ACC), and with slightly less consistency, in both the contralateral thalamus and the primary somatosensory cortex (SI). Changes in these regions are suggested to reflect the sensory, cognitive and affective dimensions of pain [98, 170]. In studies of rCBF at rest in chronic pain patients, reductions or asymmetric changes in the thalamus and reduction in the frontal, temporal, parietal and occipital regions have been shown [171-174]. A parietooccipital hypoperfusion has been shown in chronic WAD patients at rest, and a possible activation by nociceptive afferent nerves from the cervical spine has been suggested [175]. Coping strategies can change the perception of pain during noxious stimulation [176]. Pain studies manipulating the context of the administered noxious stimulus with a change in pain anticipation indicated that there might be a cognitive coping mechanism with a suppression of the activity in limbic and paralimbic structures to attenuate pain-related stress response [177, 178]. A recent study shows such a connection of emotional pain modulation with a deactivation of amygdale and the anterior medial temporal lobe [179].
AIMS OF THIS THESIS

This thesis aimed to:

• develop the method of rCBF-SPECT
  o by developing and evaluating a method for attenuation and scatter correction of the rCBF-SPECT images using an attenuation map calculated from the patients’ previously acquired CT images (Study I)
  o by developing and evaluating a method for manual matching of SPECT and CT images i.e., registration to a common space by using fiducial markers that only need to be present during the SPECT acquisition (Study I)
  o by recruiting a normal material of rCBF-SPECT in healthy elderly (Study II)

• increase the knowledge of brain processing in pain and education
  o by evaluating rCBF-SPECT in patients with chronic pain with or without previous neck trauma in comparison to findings in healthy subjects (Study III)
  o by evaluating the influence of education length on rCBF-SPECT in healthy elderly (Study II)

• increase the knowledge of disturbances in brain function in AD
  o by evaluating altered rCBF-SPECT in predefined volumes of the brain in patients with early AD compared with age-matched healthy control persons (Study IV)
  o by relating rCBF and memory performance by correlating the estimated rCBF ratio in sublobar brain regions with memory performance (Study IV)

• evaluate and improve the diagnostic sensitivity of rCBF-SPECT in AD
  o by evaluating the sensitivity of rCBF-SPECT in early AD at rest condition (Study IV) on an individual level
  o by using an episodic memory task provocation, i.e. memory provoked rCBF-SPECT (MP-SPECT) (Study V) on group level
MATERIAL AND METHODS

Overview of the studies

The studies included in this thesis are shortly summarized in Table 2. Paper I is a methodological work, and II-V are cross-sectional prospective studies. Each set of patient data is used in only one study.

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<td>SPM t statistics, Mann-Whitney U test</td>
<td>ANOVA, Pearson`s corr t test</td>
<td>SPM t statistics</td>
</tr>
</tbody>
</table>

Ethical considerations

All studies, except for number I, are approved by both the Ethics as well as the Radiation Protection Committee of Umeå University. Written informed consent has been obtained according to the Declaration of Human Rights of Helsinki 1975 from all healthy controls and patients involved in the studies. Study number I is considered as a technical development project not affecting patient handling.

Subjects

Normal material

Healthy subjects in age between 50-75 years were consecutively recruited from a large prospective research project in the community of Umeå, the ‘Betula project’ [180]. The purpose of the ongoing Betula project is to explore the development of memory functions in adulthood and to determine risk factors and preclinical signs of dementia. All subjects in the Betula project take part in an extensive health and memory examination every fifth year including cognitive testing and interviews regarding critical life events and socio-
economical issues. The inclusion criteria in Betula as for all healthy controls in all studies in this thesis are a good subjective and objective health without history of psychiatric disorder, stroke or dementia.

**Patients**

All 82 patients in the studies were recruited from Norrlands University Hospital in Umeå, Sweden and are summarized in Table 3. In study I, patients referred to an rCBF-SPECT examination were recruited. The inclusion criterion was a CT examination prior to the rCBF.

The patients in study III were referred to the department of Orthopaedic surgery during the years 1997–2001, all with chronic neck pain. The inclusion criteria were disabling chronic neck pain leading to full or halftime sick leave, or changed profession.

In study IV, and V the patients were recruited from the Department of Geriatrics. They were referred for memory complaints, and investigated by a specialist in geriatric medicine prior to the rCBF-SPECT examination. All patients had an early AD diagnosis according to the NINCDS-ADRDA [42], and were in a good physical health.

**Table 3.** Subject characteristics across study samples.

<table>
<thead>
<tr>
<th>Study</th>
<th>Material</th>
<th>Female/Male</th>
<th>Age Mean ± SD range</th>
<th>Symptom duration Mean ± SD range</th>
<th>Years of education Mean ± SD range</th>
<th>MMSE Mean ± SD range</th>
<th>CDR 0.5/1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I</td>
<td>Brain phantom 5 pat. with SPECT &amp; CT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study II</td>
<td>45 Healthy controls</td>
<td>20/25</td>
<td>63.2 ± 7.9 50-75</td>
<td>10.3 ± 4.0</td>
<td>28.1 ± 1.3 25-30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study III</td>
<td>27 Chronic whiplash 18/9</td>
<td>40.9 ± 11.6 26-65</td>
<td>7.1 ± 5.0 3-20</td>
<td>11</td>
<td>28.1 ± 1.5 25-30</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18 Chronic neck pain 13/5</td>
<td>44.0 ± 9.6 29-61</td>
<td>8.5 ± 3.2 3-15</td>
<td>10.3</td>
<td>28.1 ± 1.5 25-30</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 Healthy controls</td>
<td>7/8</td>
<td>55.5 ± 4.4 50-61</td>
<td>12.8</td>
<td>28.1 ± 1.5 25-30</td>
<td></td>
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</tr>
<tr>
<td>Study IV</td>
<td>14 AD 4/10</td>
<td>75.2 ± 8.8 64-91</td>
<td>1.9 ± 0.8</td>
<td>24.6 ± 2.9</td>
<td>28.3 ± 1.2 4/10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 Healthy controls</td>
<td>7/8</td>
<td>71.4 ± 3.2 66-75</td>
<td>28.6</td>
<td>26.0 ± 1.8 21-29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study V</td>
<td>18 AD 10/8</td>
<td>73.3 ± 4.8 62-81</td>
<td>9.4 ± 3.0 6-15</td>
<td>26.0 ± 1.8 21-29</td>
<td>29.7 ± 0.6 28-30</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18 Healthy controls</td>
<td>14/4</td>
<td>69.4 ± 3.9 65-78</td>
<td>13.4 ± 4.6 6-25</td>
<td>29.7 ± 0.6 28-30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Image acquisition and processing**

**Instrumentations, gamma camera**

All rCBF-SPECT studies were performed on a three-headed gamma camera, Neurocam (General Electric, Milwaukee, WI, US) equipped with low energy high resolution (LEHR) collimators, at the department of Nuclear Medicine, Diagnostic Radiology. A special carbon fibre head holder has been developed to prevent motion of the patient's head to avoid serious artefacts in the reconstructed images. An individually formed thermoplastic face mask with
punched holes for the eyes is fastened with Velcro tapes underneath the carbon fibre head holder. It also has a possibility to place a plastic fiducial marker with 0.1 MBq $^{57}$Co at the root of the nose, which can combined with plastic markers to be placed 1 cm into the external auditory canal in order to facilitate the spatial registration with a structural investigation, Fig. 7. The localizations of the markers were chosen since they can be detected in the CT investigation without markers. The fiducial markers were used in study I.

Fig. 7. The face mask and fiducial markers, indicated by arrows.

**Radiopharmaceutical and dosimetry**

In all studies the radiopharmaceutical $^{99m}$Tc-HMPAO has been used and prepared on the day of examination by eluting $^{99m}$TcO$_4^-$ from a $^{99}$Mo/$^{99m}$Tc generator just before adding it to a freeze-dried kit (CeretecTM, Nycomed Amersham plc, Buckinghampshire, UK) according to the instructions of the manufacturer. The amount of administered dose was according to ethical and radiation safety committee guidelines for each study. The tracer was administered intravenously after 30 minutes rest with eyes closed in a dim-lighted room in all studies [181]. In study V the subjects also received the tracer during a memory task. All examinations with the scintillation camera have been performed at rest 10 to 40 minute after injection.

In study I-IV the administered dose was 1000 MBq to each patient, which is the standard dose in clinical routine. All healthy controls received 675 MBq. In study V the administered activity was 675 MBq to all subjects at each examination. In this study the rest and test investigations were performed with a 48 h interval and half of the subjects started with the rest followed by the test situation, and vice versa.
**SPECT acquisition, image pre- and post-processing**

All SPECT studies were performed in a 360° stepwise rotation, with 90 seconds acquisition time in 64 equally spaced angles. The image matrices were 128 x 128 pixels and the spatial resolution of the system expressed as ‘Full Width at half Maximum’ (FWHM) at a 100 mm distance from the camera is 6.9 mm and approximately 12-13 mm in the centre of the field of view.

Reconstruction with FBP was used in study III and IV. In study I, II, and IV the images were reconstructed into transaxial slices with the iterative ordered-subsets (maximum-likelihood expectation maximization (ML-EM), OSEM) [182] algorithm using 8 iterations and a subset of 4 images. The images were pre-filtered with a 2D Hanning filter with a cut-off frequency of, 0.9 in study III, and IV and 1.0 cm⁻¹ in study I, II, and V.

In study III and IV the attenuation correction was performed according to the Chang algorithm [183]. The outline was automatically defined at 10% of maximum count with an ellipse in each slice. The attenuation coefficient was set to the standard value of 0.12 cm⁻¹. In study II, a homogeneous attenuation map was used as an input to the iterative reconstruction, where the outline of the homogeneous attenuation map was defined from a preceding step with an iterative reconstruction without attenuation correction. The map was constructed of all voxels with a value above zero after dilation and erosion processes. The attenuation coefficient was set to the value of water for 140 keV photons, 0.154 cm⁻¹. In study V, the same technique was used, but the outline was automatically defined at 10% of the maximum count with an ellipse in each slice. In study I, an inhomogeneous CT-based attenuation map was used as an input to the iterative reconstruction.

The transmission dependent convolution subtraction method with one iteration [184], was used for scatter correction before reconstruction in study I, II and V.

The pixel size was 2.0 x 2.0 mm in reconstructed images. The reconstructed image data was exported in Interfile format from the GE Genie station (General Electric, Milwaukee, WI, US) in all studies prior to analysis. In study II, III and V the image data was converted to ANALYZE format before further analysis.

An incomplete rCBF SPECT scan, with more than 50% of the cerebellum not being investigated has been used as an exclusion criterion in all studies

**CT acquisition,**

In study I, CT images were acquired with a LightSpeed QX/i (General Electric Medical Systems, WI, US) or a Somatom Plus 4 Power (Siemens, Erlangen, Germany) at 120 kVp. The slice thickness according to the brain protocol for the LightSpeed was 2.5 mm in the skull base region and 7.5 mm for the rest of the skull. On the Somatom the corresponding slice thicknesses are 4.1 mm and
8.0 mm, respectively. The thinner slices in the skull base region are used to reduce artefacts in that part of the image volume. The images were acquired in 512x512 matrices with a pixel size of 0.40–0.45 mm. The CT image matrix size was reduced to the same as the SPECT matrix. A linear interpolation between the CT slices was performed to make the slice thickness in the two image volumes to match.

**Neuroimaging analysis of rCBF-SPECT**

Two different software (SW), based on different analytic principles, have been used in the evaluation of rCBF-SPECT image data.

Computerized Brain Atlas (CBA) (Applied Medical Imaging, Uppsala, Sweden) is a SW for matching, viewing, and interpretation of brain scans from different modalities [185]. It is based on a detailed 3D atlas derived from a single post-mortem cryo-sectioned brain in which brain surface, gyri, sulci, central structures, and BAs have been defined [186]. The atlas can be transformed to fit SPECT, PET, MRI, and CT images using translations, rotations and linear scaling as well as second order, non-linear deformations. Registration of the atlas to the image data can either be made manually, semi automatically or fully automatically [187].

Statistical parametric mapping (SPM) is a free SW from the Wellcome Department of Imaging Neuroscience at University College London for the analysis of functional neuroimaging data. It is written in MATLAB (Mathworks Inv., Sherborn, Mass., US) and some of the main features are the possibility to realign, spatially normalize image data into a standard space and spatial smooth image data prior to a voxel based statistical analysis. Statistical parametric models are assumed at each voxel, using the General Linear Model, (GLM) to describe the data in terms of experimental and confounding effects, and residual variability. Classical statistical inference is used to test hypotheses that are expressed in terms of GLM parameters. This uses an image whose voxel values are statistics, a Statistic Image, or Statistical Parametric Map. Two SPM versions, SPM99 (Study III) and SPM2 (Study II and V) were used to study differences in rCBF between groups and conditions. Calculations were performed with MATLAB version 6.5.1.

**Intensity normalization**

To correct for interindividual differences in total brain counts, two strategies for normalization of the intensity were used. In study I and IV where CBA was used, the intensity normalization was made in each rCBF-SPECT in order to approximately normalize to healthy brain tissue and avoid normalization to pathologically low rCBF regions. This was made by averaging brain voxels and setting the global brain average to a predefined value. In order to avoid an
overestimation of uptake in cases with pathological low rCBF, a histogram analysis was performed to exclude voxels with a low rCBF. All reformatted 3D-images were intensity normalized to the average value of 13% of the voxels with the highest values in each image data, which was set to 50 "uptake units". The level is based on the size of the atlas brain and the relative amount of grey matter normally present in a human brain [188-191]. The method is specially designed for examinations of images with pathologically reduced blood flow.

SPM does not provide a standardized method to normalize to a proportion of the voxels with the highest values. In study II, III and V, the recommended [192] default method for intensity normalization in SPM was used, the ‘proportional scaling’, setting a global value of 50 ml/min per 100 g. This was followed by a grey matter threshold of 0.8 which is the standard procedure in SPM to exclude voxels from the analysis which is of no further interest in the analysis i.e. extracranial regions. The threshold specifies a fraction of the global mean signal where only voxels with signal intensities above this threshold are analyzed.

**Registration to brain atlas templates and other modalities**

In study I, a semiautomatic registration method was developed and used to register CT and SPECT images. The positions of the three markers in the SPECT image volumes were determined semiautomatically by pointing in the close proximity to each marker. The corresponding anatomical landmarks in the CT volume were manually pointed out by the staff. This was followed by an automatic registration of the SPECT and the CT image volumes in MATLAB, based on these point co-ordinates using a non-iterative least squares method of singular value decomposition in a 3x3 covariance matrix [193]. All point co-ordinates were recorded and the standard deviation for each of these located landmark positions in the CT image volumes was calculated. In study I a phantom was used to estimate the consequences of the semimanual misregistration. The reconstructed phantom image data were imported to CBA and an experienced operator fitted the atlas to the correctly positioned attenuation map. The parameters controlling the atlas deformation and position were saved and used for all reconstructed SPECT image volumes.

In study I and IV, CBA was used for the registration of patient image data to the integrated brain atlas. The rCBF-SPECT images were realigned to the default SPECT-template in CBA by using the non-linear transformation and the fully automatic registration. The method of similarity measure was cross-correlation.

When using SPM the rCBF-SPECT data were normalized to the MNI SPECT template using a non-linear anatomical standardization with 12 affine parameters, and bi-linear interpolation.
In all studies the results of the automatic and the semiautomatic registration have been visually checked to verify the accuracy of the registration.

**Statistical methods**

The rCBF was evaluated by Volumes of interests (VOIs) automatically drawn with CBA in both study I and IV. To test the effect of a translated attenuation map reflecting the mismatch seen in the semiautomatic registration, 27 clinically relevant VOIs from both hemispheres were chosen in study I. The following VOIs were drawn and evaluated in the reconstructed phantom images: all five lobes, thalamus, putamen, nucleus caudatus, pallidum, fornix, amygdala, hippocampus, cerebellum and the mesencephalon.

AD related VOIs in the temporal lobe and in the posterior parietal lobe were chosen in study IV. The BAs 20-22, 37 and hippocampus were evaluated in the temporal lobe and BA 39, 40 and 7 in the parietal lobe, Fig. 8. Furthermore, BA 10 in the frontal lobe was included since episodic memory retrieval and encoding is associated with the prefrontal region [194]. Since no rCBF-changes were expected in BA 17 in the occipital lobe or thalamus these were set as reference regions [36, 195-198]. Individual mean values of rCBF-SPECT measurements were extracted in each of the chosen regions.

![Fig. 8. Study IV - Chosen VOIs in transaxial (A), sagittal (B) and coronal (C) slices from an AD patient with the investigated VOIs marked and visualized by the CBA. The VOIs on the slices are marked with BA numbers.](image)

In study IV, the individual mean rCBF values from the chosen VOIs of the AD and healthy subjects were compared using ANOVA statistics. The normal rCBF was calculated as the 95% confidence interval for each BA in the healthy control group. BAs with reduction below this interval were considered significant in the AD patients. Regions with significantly reduced rCBF ratio in the AD group were correlated with the results from each memory test using Pearson’s correlation (no memory test data were available for the healthy control group). The results from the memory tests for AD patients were
compared with norm-group data using the 95% confidence interval and one sample t-test. The Statistical Package for the Social Sciences (SPSS) SW for windows version 10.0.5 was used for all statistical analysis (SPSS Inc., Chicago, US).

SPM was used to estimate differences in rCBF between groups in study II, III and V after smoothing with a 12 mm FWHM 3D Gaussian filter kernel before the statistical analysis [155]. The “compare-populations” design has been used to compare different patient groups (study III, V, two sample t-test ) or groups based on variables with significant group differences (study II, AnCova). The evaluation of positive and negative correlations with SPM in study II used the SPM model, “Single-subject, covariates only”. In order to evaluate the rCBF changes between rest and test situations in study V the “population main effect” model (1scan/cond, paired t-test) was used.

In SPM the level of significance has to be chosen based on the height of the p values of the voxels in the statistical parametric map. An uncorrected level with a threshold of p=0.001 would give a 0.1% chance for a voxel to be detected whether or not this is real or just random noise. Since the sum of all voxels in the brain is high there is a need for correction for multiple non-independent comparisons. This is traditionally done with the Bonferroni correction, i.e. the Family-wise Error (FWE) method. However in many cases, this method is too conservative because most functional imaging data have a correlation between neighbouring statistic values. That is why the less conservative False Discovery Rate (FDR) method has been developed for SPM and incorporated in the SPM2 version. In FDR a certain amount of noise is allowed in favour of not excluding voxels that is incorrectly interpreted as noise with FWE. The FDR method is a correction based on the expected proportion of false positives of the detected voxels [199] (www.fil.ion.ucl.ac.uk/spm/doc/books/hbf2/pdfs/Ch14.pdf) “An Introduction to Random Field Theory” (M. Brett, W. Penny and S. Kiebel).

In case of a priori knowledge the uncorrected level of 0.001 or even lower height thresholds can be used and if it concerns a discrete anatomical area, the SPM option of “small volume correction” can be used. In the SPM99 version that was used for study III, the FWE method was used and in study II and V, the FDR method in SPM2 was used, both at a p-value below 0.05. In all SPM studies peaks with an uncorrected p-value of 0.001 have also been reported if the result could be related to previous findings. The extent threshold has been 10 voxels (II, III) or 5 voxels (V).

Anatomical localization and visualization
The Montreal Neurological Institute (MNI) coordinates were converted to the Talairach brain atlas using a nonlinear function as described at the CBU
Imaging web site (www.mrc-cbu.cam.ac.uk/Imaging/mnispace.html) in order to determine the anatomical localization of the SPM data. The SPM add on MNI Space utility (MSU) and Talairach Space utility (TSU) developed at the PET Lab of Institute of the Human Brain (www.ihb.spb.ru/~pet_lab/MSU/MSUMain.html) was used to visualize and describe the anatomical extension of SPM data in terms of anatomical region labels used by the Talairach Daemon (http://ric.uthscsa.edu/ric_resources/resources.html). Cluster extension can then be described in terms of hemispheres, brain lobes, gyri-sulci, white- and grey matter as well as BAs. Another visualization method was superimposed data on MRI-templates in SPM.

**Neuropsychological assessment**

An experienced clinical neuropsychologist performed all memory tests in the patient group in study IV as well as all neuropsychological tests in study V. In study IV, age-matched normative data for each test served as control data and in study V the results were compared to the healthy control group.

In study IV the test session lasted for 1 h and was part of the clinical evaluation. A battery of memory tests was chosen with the purpose of evaluating a range of memory aspects corresponding to different regions in the brain applicable for patients with early AD. The episodic memory was tested by short sentences with (subject performed task, (SPT)) and without encoding enactment (verbal task, (VT)) [180], word recall [200], Rey memory test (RMT) [201] and face recognition [180]. Semantic memory was tested with word fluency [180] and a word comprehension test [202], Table 4.

In study V, all participants (patients and controls) were individually tested for about 2 h each. The cognitive domains tested in study V were verbal and non-verbal episodic memory, semantic memory, working memory, attention and visuospatial function. The tests for episodic memory were: Buschke selective reminding test [203], Rey Complex Figure Test (RCFT) [204], Paragraph recall Test [205], and face recognition [180]. Semantic memory was tested with Controlled Oral word association (FAS and categories) [206]. Working memory was tested with Digit span from Wechsler adult intelligence scale-revised (WAIS-R) [207] and Spatial span from WAIS-R, neuropsychological instrument (WAIS-R-NI) [208]. Attention was tested with Digit symbol from WAIS-R [207] and Trail making test (TMT) [209]. Visuospatial function was tested with Block design from WAIS-R [207]. The neuropsychological testing and the rCBF 99mTc-HMPAO SPECT investigations were conducted within 2 months. The cognitive and functional level was estimated, including MMSE [84] and CDR [83], Table 4.
Table 4. Neuropsychological test used in this thesis.

<table>
<thead>
<tr>
<th>Neuropsychological tests</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Episodic memory</strong></td>
<td></td>
</tr>
<tr>
<td>Sentence performance test (SPT) – Short sentences, with enactment</td>
<td>IV</td>
</tr>
<tr>
<td>Verbal test (VT) – Short sentences, without enactment</td>
<td>IV</td>
</tr>
<tr>
<td>Rey memory test (RMT)</td>
<td>IV</td>
</tr>
<tr>
<td>Face recognition</td>
<td>IV</td>
</tr>
<tr>
<td>Word recall</td>
<td>IV</td>
</tr>
<tr>
<td>Buschke free recall</td>
<td>V</td>
</tr>
<tr>
<td>Buschke total recall</td>
<td>V</td>
</tr>
<tr>
<td>RCFT delayed recall</td>
<td>V</td>
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<tr>
<td>Paragraph recall test</td>
<td>V</td>
</tr>
<tr>
<td><strong>Semantic memory</strong></td>
<td></td>
</tr>
<tr>
<td>Verbal fluency: FAS</td>
<td>V</td>
</tr>
<tr>
<td>Verbal fluency: Categories</td>
<td>V</td>
</tr>
<tr>
<td>Word fluency</td>
<td>IV</td>
</tr>
<tr>
<td>Word Comprehension</td>
<td>IV</td>
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<td><strong>Short-term memory</strong></td>
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<tr>
<td>Digit span</td>
<td>V</td>
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<tr>
<td><strong>Attention</strong></td>
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</tr>
<tr>
<td>Digit symbol</td>
<td>V</td>
</tr>
<tr>
<td>TMT A</td>
<td>V</td>
</tr>
<tr>
<td>TMT B</td>
<td>V</td>
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<tr>
<td><strong>Visuospatial function</strong></td>
<td></td>
</tr>
<tr>
<td>Block design</td>
<td>V</td>
</tr>
</tbody>
</table>
RESULTS

Study I

A registration method of the CT and SPECT images to a common space using three fiducial markers that only needed to be present during the SPECT study was developed and evaluated, Fig. 7. The average standard deviations of the registration method were approximately 2 mm in all three directions (lateral, anterio-posterior and craniocaudal), and the general spread was 1–3 mm. The total average standard deviation was calculated to 3.4 mm in each landmark position. For most of the patients the largest spread was found to be in the cranio-caudal direction related to the landmark on the root of the nose. The consequences of a changed attenuation correction due to a translated attenuation map (+/- 95% CI or 2 pixels = 4.0 mm) in each direction was estimated on a phantom study. The highest deviations from the reconstruction of the phantom study with the nontranslated map occurred in the structures involving the contour of the brain represented by the VOIs of the lobes in this study. The maximum deviation was 6.3%. The deviation was very symmetrical and therefore only results from the right side were presented. A visual comparison of reconstructed images with a translated attenuation map in the left, dorsal or caudal direction resulted in an increased activity concentration in these directions.

Quantitative data from the reconstruction with the homogeneous attenuation map corresponded fairly well with the data obtained with the non-homogeneous attenuation map. Images obtained without scatter correction was presented and had a lower contrast with a large deviations in the small central structures.

Study II

In this rCBF-SPECT study of Swedish healthy elderly the influence on rCBF of age, gender, education, MMSE, ache, and smoking was evaluated. Based on previous studies, a focus was on education in relation to the notion of a ‘cognitive reserve’.

There were age-related decreases in uptake in interhemispheric and interlobar regions. No gender differences were detected. An effect of education on rCBF could be seen in a matched (gender, age) group comparison, involving regions in the left temporal lobe. These temporal regions were not revealed in a correlation analysis, but a region in the inferior frontal lobe showed a positive rCBF correlation with education (p=0.05 corrected).

Significant differences in rCBF were detected between the two age groups of 50-60 years and 64-75 years. The older group had extensive regions with a
decreased uptake in both hemispheres. All regions were located in close relation to interhemispheric or interlobar space. The largest cluster extends from a region including the right Sylvian fissure towards the midline passing the cisterna vallecula cerebri and continues to bilateral parts of lamina terminalis. A second region is broadly symmetric around the midline and includes the gyrus cinguli and interhemispheric space between the limbic and frontal lobes. A third region involves a dorsal part of left Sylvian fissure extending through the temporal lobe to the brain surface, and finally the fourth region includes frontal parts of the Sylvian fissure on the left side. No regions with a significantly lower uptake were detected in the younger group compared to the older group. No significant differences were seen at the comparison between men and women.

No significant rCBF differences were seen between the groups with high versus low length of education, but when the subjects were matched regarding age and gender into 11 pairs there was a significantly higher uptake in the left temporal lobe in the high education group.

No significant differences were seen in the comparison between subjects with high and low MMSE performance, smokers vs. non-smokers, or groups with or without ache.

In the correlation analysis there were three significant extensive clusters with a negative rCBF correlation with age. All three regions were partly overlapping with the result from the group analysis, and localized in the left temporal and bilateral frontal regions including interhemispheric and interlobar regions. The SPM correlation analysis also showed a significant cluster with positive rCBF correlation with age in the right visual cortex with a mirrored non-significant cluster on the opposite side. The length of education, correlated to the rCBF activity in a cluster in the inferior frontal gyrus (p=0.05 FDR corrected). This regional effect was not observed in the group comparison.

**Study III**

There was a higher degree of complaints such as headache, forgetfulness and concentration problems in the whiplash patient group compared to the non-traumatic pain patient group. The SPM analysis of rCBF detected an altered blood flow in the non-traumatic patients compared to the healthy controls. There was a relatively lower rCBF in non-traumatic pain patients compared to the healthy controls (SPM contrast: healthy > nontraumatic) with two significant clusters in the right hemisphere and right cerebellum (Fig. 9A). The major cluster with reduced rCBF in the right hemisphere (11 ml, 1323 voxels) mainly involved the temporal lobe and the limbic lobe. The minor cluster (7 ml, 894 voxels) was located in the centre of cerebellum. There was no region with a significantly higher rCBF in non-traumatic pain patients than in healthy
controls. The comparison between whiplash patients and healthy controls did not detect any significant regions with a lower rCBF in whiplash patients (healthy > whiplash). However, two non-significant small regional differences; one in the right temporal region and one in the left temporoparietal region (Fig. 9B), were detected at an uncorrected voxel level of p = 0.001. The reversed contrast to detect an increased rCBF in whiplash compared to healthy controls did not reveal any significant differences. In the comparison between the two chronic neck pain patient groups there was a region with a significant lower rCBF (whiplash > non-traumatic) in an extensive cluster (22 mL, 2700 voxels) located in the right hemisphere extending into the right side of the brainstem. Half of the cluster was located in the right temporal lobe, one-fifth in the limbic lobe, and one tenth in pons. The cluster involved both hippocampus and the parahippocampal gyrus (Fig. 9C). The contrast to detect a relatively higher rCBF in non-traumatic pain patients compared to whiplash patients (non-traumatic > whiplash) showed a non-significant small region in the parietal lobe involving precuneus. In addition, there was also an rCBF difference in the anterior part of left insula (uncorrected p=0.001), Fig. 9D. In this three way comparison the non-traumatic chronic neck pain group showed extensive rCBF reductions in the temporal and limbic lobes towards both the whiplash and the healthy control group.

**Study IV**

In this study of memory and rCBF in early AD patients and healthy elderly, the AD group showed reductions in rCBF in temporoparietal regions as well as a decline in memory performance. The detected rCBF reductions were more pronounced in the right hemisphere of the evaluated BAAs and especially in the temporal lobe with sparing of anterior and inferior parts of the lobe. There was also a reduction in the posterior part of the left parietal lobe adjacent to the temporal lobe. There were a significantly reduced rCBF in AD compared with
the healthy controls, in the right BAs 21, 22, 37 and 39 and left BA 37, as well as in the left hippocampus. The reduction was most pronounced in right BAs 37 and 39 (P<0.001 for both), with a mean reduction of 6.5% and 6.2%, respectively. A colour-coded scheme of VOIs with significantly (P<0.05) reduced rCBF painted in blue among all selected VOIs (BA7, 10, 17, 20–22 and 37–40, thalamus and hippocampus are not shown) is presented in Fig. 10.

An evaluation of the diagnostic accuracy to diagnose AD based on VOI reductions below a 95% confidence interval of the healthy control persons was made. The highest sensitivity, 86%, and specificity, 93%, was obtained when a combination of a reduction in either right BA 37 or right BA 39 was used. The highest sensitivity, 64% and, specificity, 100% when using only a single BA was obtained when using right BA 39.

The AD patients performed at a lower level in all memory tests compared to age-matched normative data. As expected, the decrease was more pronounced for episodic than for semantic tests, Fig. 11.

The VOIs with significantly reduced rCBF in AD patients were used for analysis of correlation with the results from the individual memory tests. Significant correlations between rCBF in one or more of the following VOIs, BAs 21, 22, 39 and hippocampus, and performance on one or more of three episodic memory tests, SPT, face recognition or word recall, were observed among the AD patients. A reduction in rCBF in right BA 22 had a strong significant correlation with the scoring of all these three episodic memory tests (P<0.01). Face recognition
had a strong correlation (P<0.01) with rCBF ratio in right BA 22 and left hippocampus and a less significant correlation (P<0.05) with that in right BAs 21 and 39. Furthermore, a reduction in SPT performance was correlated with a reduction in rCBF ratio in left hippocampus. Word recall had a significant correlation with rCBF ratio in right BA 21 (P<0.05) and a non-significant correlation (P=0.07) in left hippocampus.

Study V

Significant increased rCBF during memory encoding was seen in extensive regions in bilateral occipital cortex, right temporal cortex (including fusiform gyrus, i.e. the “face area”) and bilateral inferior parietal cortex (BA 7, 37, 40) in the healthy elderly group. There were bilateral activations in medial caudal parts, including the inferior and middle frontal gyri (BA 47, 11) in the frontal lobes. More laterally there were small bilateral activations in the frontopolar cortex, including BA 10. This is illustrated by the SPM maximum intensity projection (MIP) images in Fig. 12A.

Like the healthy, the AD group had a similar but less pronounced and less dispersed activation pattern in the occipital region. In this group only one small frontotemporal region (BA 38, 47) in the right forebrain was activated. No increased rCBF was seen in the parietal cortex, Fig. 12B.

During the memory encoding, the healthy elderly, in comparison with AD patients, had a significantly increased rCBF in the left frontoparietal region (98% parietal, 2% frontal), mainly localized to the postcentral gyrus (33%) (BA 2, 3, 5) and the inferior parietal lobule (28%) (BA

![Fig. 12. In the upper two rows the SPM MIP images show significant memory-provoked rCBF activations in healthy elderly (A) and AD patients (B). There were significant rCBF reductions in the AD group compared with healthy elderly (C) at memory provocation. There were no significant group differences at rest. However, when the threshold was set at a more liberal level (*; 0.001 uncorrected), it was found that patterns of rCBF changes were similar under the rest and activation conditions (D, E)]
7, 40) extending over the central sulcus to the precentral gyrus (2%) (BA 4). The SPM MIP is presented in Fig. 12C, and D. At rest, there were no significant rCBF differences between the groups. An evaluation at a lower statistical threshold (voxel level of 0.001 uncorrected for multiple comparisons) is presented as SPM MIPS in Fig. 12E, and shows several peaks mainly in the temporoparietal and middle frontal regions in both conditions, i.e. rest and memory provocation. The AD patient group had significantly lower test performance in all cognitive tests except one compared to the healthy control group. The response time was significantly longer (1.4 vs. 1.7 s $p<0.001$) and the number of failures matching faces and names was significantly higher (3 vs. 1 out of 20 faces) in the AD group.
The functional tomographic imaging techniques SPECT and PET are sensitive methods for visualization of human brain function in a three-dimensional space, by the use of radio-labelled compounds. These techniques have been used to study dementia for over 20 years, and one of the most consistent finding in AD is a reduction of metabolism and perfusion in posterior temporoparietal region seen at rest. PET is considered being a superior method due to better spatial resolution, better properties for quantitative assessment and a higher temporal resolution allowing dynamic studies, although the method have not been as easy as SPECT to implement in the clinical routine. The availability is still very limited. However, PET has now overcome some of the initial problems with logistics and high overhead costs at the production of the radiopharmaceuticals, although its cost benefit profile compared to SPECT will still be unfavourable. Tracer specific for detection of amyloid proteins in the brain is in clinical testing with high expectations of a high diagnostic accuracy [210]. SPECT on the other hand, has the advantage of being highly available at a comparatively low cost and is proven to be useful to diagnose dementia.

In this thesis efforts have been made to further develop the method of rCBF-SPECT. Furthermore it has been used to study healthy elderly and patients with memory and pain related diseases, in order to gain more knowledge of brain functions.

The used evaluation SW, CBA and SPM are designed to detect regions with significant rCBF differences between groups or conditions in neuroimaging data. Both SW can map the images into a standard reference volume, i.e., spatial standardization, followed by smoothing and computation of statistics. A comparison between CBA and SPM has shown that they both are robust and produce similar results in an analysis of simulated activations. However, the analysis demonstrated that each step of the analysis must be optimized for a reliable final result [211].

There are however some principal differences between these SW that have to be taken into consideration when evaluating processed data. The typical evaluation in CBA is done with predefined VOIs while a voxel based analysis of the entire image data volume is the standard procedure in SPM. In CBA the VOIs can be selected from the integrated atlas and visualized as thin lines in individual data or result images, Fig. 8. This facilitates the evaluation of the spatial normalization and localization of significant VOIs. It is also favourable to be able to reduce the search volume and the number of variables in the statistical evaluation, avoiding the effect of multiple non-independent comparisons. In SPM this can be made by masking the sample data. The atlas
in CBA can be transformed to the image data and vice versa. This is not the case in SPM where image data is transformed to the atlas although the default transformation in SPM preserves the activity concentration [212]. The major disadvantage of CBA has been that it is relatively unstable SW, making the use troublesome since the SW has to be restarted frequently. The support is limited, especially in comparison to SPM. SPM has the major advantage of being very versatile and is used by many in the community of human brain research. There is a high degree of support by websites and e-mail search lists. SPM uses statistical maps which represent the statistical information in image data included in the analysis. These result images are troublesome to relate to actual differences in group data and even harder to diagnostic sensitivity at an individual level. It is therefore difficult to relate the results to rCBF reductions seen in the clinical evaluation. However, since SPM processes the entire image data on a voxel based method, it is very useful in the statistical comparison of group data to detect significant differences without a prior hypothesis of the location of the expected findings.

Irrespectively of SW, a reference has to be chosen in the intensity normalization step of the reconstruction. A simple way of doing this is to choose the global mean of the image data. Another way is to choose cerebellum, which is considered to be a robust reference region, often without pathological involvement [213]. There might however be a technical problem choosing cerebellum since due to the anatomy of the head and neck, parts of the cerebellum might not be included in the SPECT. This is the reason why it has not been chosen as a reference region in this thesis. In CBA the most appropriate way to normalize image data is to choose a reference thought to represent the grey matter, which is done by selecting 13% of the voxels with the highest counts [188-191]. This has the advantage of excluding regions with potentially pathologically reduced rCBF in the reference region which would otherwise result in an overestimation of the normalized image data. SPM does not provide a method to normalize to a proportion of the voxels with the highest values, although there are several other options of normalization in SPM such as proportional scaling and AnCova based normalization. In case of segmented image there is an option to normalize to grey matter. The proportional scaling method is recommended when SPECT without coregistered data from another modality is used and this method has been used in SPM in this thesis. Proportional scaling uses a reference region which is simply a mean of all the voxels above 1/8 of the mean of all voxels in the entire image dataset. This is the threshold likely to cut-off voxels being outside the brain. A problem with this method is that the reference level may be affected if there are extensive regions with pathologically changed rCBF as in moderate to severe AD.
To compare image data between different time points or between individuals there is a need to map the image data to a common space i.e. brain atlas. The uptake concentration is conserved at the spatial registration in SPM and theoretically this will compensate for difference in brain size. This is convenient in matter of size differences i.e. gender-based differences. However, it should be considered that this warping with preserved concentration might mask global size differences based on neuronal loss in the hemispheres. Due to the relatively poor spatial resolution in SPECT and PET images, each voxel can consists of data from different types of tissues, which is called the partial volume effect (PVE). This can cause an apparent rCBF decrease in small or atrophic structures and especially close to wide interlobar or interhemispheric space. This effect can be a confounding factor especially in the analysis of age and disease processes [23]. Furthermore, the spatial normalization will probably be affected in regions with high neuroanatomic variance such as in regions around the lateral ventricle of the temporal lobe [28, 214] or where the interlobar space is not well defined. It is also likely that extensive changes, i.e. lesion or manifest atrophy, would result in unwanted deformations in the spatial normalization [215]. A model of the PVE at atrophy with widening of the sulcies (voxels with brain and cerebrospinal fluid) and PVE effects on shrinking white matter (voxels with both grey and white matter) has recently been suggested [22]. The suggested PVE in shrinking white matter may arise if it becomes thinner than the resolution of the detector system. The detected rCBF will then be influenced by surrounding grey matter which has a higher rCBF [22]. This might explain the often reported preservation of white matter. Another preservation theory is an effect of two opposing factors on the volume of white matter where the age related loss of myelin [216] locally can be overridden by an age related expansion of the capillary network and extra cellular space [217]. A negative correlation between age and uptake in interlobar regions has been shown in two recent papers studying age related changes in brain function at rest [24, 218]. This is in line with findings in this thesis. Both studies used a three compartment segmentation method [219] to compensate for PVE, however with difference level of success. In the study by Matsuda using PVE correction on rCBF-SPECT the negative age correlation remained in a greater extent than in a study by Yanase et al using FDG-PET, which can be due to the differences in resolution and methodology. Since PVE affects the results of rCBF-SPECT evaluated in SPM, further research is needed to optimize methods of PVE correction. In case a structural imaging, i.e. CT or MR is obtained it could be used to optimize the spatial normalization and also to correct for PVE. In all studies in this thesis the default option of spatial normalization has been used followed by a visual evaluation. If the registration was considered to be inappropriate the registration was redone after a manual correction. This was
necessary in only a few cases. Correction for PVE based on structural modalities has not been performed.

Two version of SPM have been used, and the reason for this is that the new method for correction of multiple comparisons, i.e. the False Discovery Rate (FDR) [199] was fully incorporated in SPM2. The correction for multiple comparisons in SPM99 is based on a calculation of the chance of any false positives (Bonferroni or random field methods), while FDR in SPM2 corrects for expected proportion of false positives among the significant regions detected above the chosen significance level. This is considered being a more sensitive method than traditional statistics and is recommended in “Guidelines for Presenting Neuroimaging” (www.sph.umich.edu/~nichols/NIpub/), which is an extraction of guideline discussions on the SPM e-mail list for authors and reviewers of neuroimaging publications. It is also recommended to use a smoothing level close to the FWHM of the camera system before the analysis with GLM statistics [220]. A smoothing level of 12 mm (FWHM 3D Gaussian filter kernel) has been used in SPM analysis in this thesis, which is fairly close to the resolution of the scintillation camera system used.

In the first study, a method to manually register SPECT and CT image data to a common space was developed with help of three fiducial markers applied only in the SPECT study. The position of the external markers were chosen for being possible to apply with a minimum of deviation and fixed between different investigations. Furthermore, the location should be possible to identify on CT without markers present. The average standard deviation was approximately 3 mm which resulted in rCBF changes up to 6% in peripheral VOIs of the brain which might not be acceptable in the individual evaluation. A subvoxel (<2 mm) accuracy of the registration is a more appropriate level for a registration in clinical routine [221]. The developed registration method in combination with modern multi channel CT technique using thinner slices would probably result in less misalignment. The method would be most useful in dopamine transporter and receptor studies of the brain, where the anatomical structure and the outline of the brain can be impossible to define for a correct attenuation correction and an automatic registration. Especially studies of the dopamine system with a low background have a high degree of misalignment in automatic registration [221]. The differences compared to a linear attenuation correction in ordinary rCBF-SPECT images were low and the need for this rather complex procedure in this aspect is therefore restricted.

A normal material of healthy elderly was recruited to rCBF-SPECT. Effect on rCBF by age and education among other parameters was studied with SPM. The main findings were age related changes in rCBF in regions close to interlobar and interhemispheric space. It was important though that there were 46
no age related findings in regions affected in early AD, i.e. the posterior temporal or the parietal lobe. However it should be considered that age-related rCBF changes might disguise the AD changes in the anterior temporal lobe which might explain the discrepancy of reported changes in the anterior and medial temporal lobe and the findings at visual assessment. There seems to be several confounding factors involved in the sensitivity of detection of rCBF changes in the temporal lobe i.e. age, PVE and a high neuroanatomic variation. It has been reported that there is a difference in affected regions between early and late onset of AD [164]. One contributing explanation to this phenomenon might be these confounding factors. In other studies of aging similar rCBF changes in interlobar interhemispheric regions have been seen and compared to structural imaging. The rCBF changes corresponded mainly to structural changes of the brain [24, 218, 219]. The confounding effect of age should always be considered in clinical routine and especially close to interlobar and interhemispheric regions such as the medial temporal lobe where there can be a PVE. In cases where structural imaging (CT, MRI) is present, apparent rCBF reductions should be related to the width of the subaracnoid space.

Education seems to be another confounding variable in the evaluation of AD related rCBF changes [222, 223]. In the comparison of age and gender matched subgroups with high and low length of formal education, the more educated elderly had a significantly higher rCBF in the left temporal lobe. A similar connection of education length and rCBF in the left temporal lobe has recently been reported in a SPECT study of AD patients [222]. The region could not be seen in the correlation analysis. However, the correlation analysis of the whole sample showed a cluster with a positive correlation to rCBF in the right inferior frontal lobe. The cognitive reserve in its nature is an ability to perform on a higher level at a demand for an active brain process. The subjects in the study were investigated at rest, which should be considered. Nonetheless, our findings give further support for the theory of cognitive reserve [224] and the view that education length influences the basal rCBF. Results like those presented in this thesis are in line with some epidemiological and clinical data [225-227] which support the theory of education as being protective against the development of AD. In a recent twin study it was shown that longer education was more supportive against AD than genetic factors [80]. Studies showing pathophysiologic or functional support for the theory are sparse and the results in this thesis are of great interest in the ongoing discussion [78]. In the memory-provocation study of this thesis, there were also an influence of education after a correction (AnCova) for educational length in basal frontal and temporal regions (unpublished data) in the comparison between healthy and AD at the memory provocation. The findings of reduced rCBF in AD in the parietal region appear to be unrelated to the duration of education in both rest and provocation.
The findings in the study of chronic neck pain are very interesting and since whiplash is a disputable syndrome [140, 228] there might be a number of suggested interpretations. The most important interpretation is that this finding supports differences in pain processing in chronic neck pain patient with and without whiplash trauma. Since this is the first study suggesting a disturbed pain processing in WAD further research has to be performed to reveal the underlying cerebral pain processes in chronic pain. It should be kept in mind that both patient groups that were investigated in this study suffered significantly from the pain and further research of cerebral chronic pain processes should aim on subjects that seem to cope with chronic pain. Results of such studies would be complementary to the findings in this thesis, since they might reveal a more adequate cerebral response to chronic pain. The findings of extensive rCBF changes in non-traumatic chronic neck pain patients will not contribute to a higher diagnostic accuracy and should not be interpreted as an indication for doing rCBF-SPECT in these patient groups. The findings are of great interest in the basic research of brain processes during disabling chronic pain and hopefully there will be an explanation of the findings in future studies.

The two last studies were performed with focus on memory function and the diagnostic possibility to detect altered rCBF changes in early AD. Previous studies have shown that the most consistent rCBF changes in mild to moderate AD are a reduction in the temporoparietal regions [2–6]. In study IV it was shown that a simple observer-independent sub-lobar VOI analysis has the potential of being an aid in the clinical assessment of SPECT investigation. This should be further investigated since there might be fruitful to adjust the size and shape of the predefined VOIs to get a better representation of the region affected in AD which might pay off with a higher diagnostic accuracy. Studies in this thesis have found AD related pathology in BA 2-5, 21-22, 37, 39-40 and these regions should be included in such an attempt of finding the optimal VOI. In study V, rCBF in mild AD was investigated during rest and episodic memory provocation, i.e. face recognition. An increased diagnostic sensitivity of AD at the provocation was shown. This indicates that the memory provocation served to magnify group differences in AD-affected regions in the parietal lobe. It is important to consider that the evaluation was done at group level. If a higher sensitivity for AD at the individual level is verified in future studies, a single memory provoked SPECT might be sufficient in the clinical setting. In order to determine the accuracy of a memory provoked SPECT, further analysis at the individual patient level is needed in a larger patient population. This could be done with simple VOI analysis in critical regions. It is also important to consider that the rCBF was assessed during encoding conditions only and that the injection time might not be optimal. A memory provocation based on retrieval of episodic memory
during a longer time than one minute would be a natural next step. This open a new field of future research, to find the optical combination of, memory provocation, time of injection and VOI selection in order to raise the diagnostic sensitivity for AD with rCBF-SPECT.

This thesis confirms that there are typical findings in early AD that can be detected by advanced research imaging tools. However, the evaluation with these SW is time consuming and is not designed to be implemented in clinical routine as a support to verify suspected neuropathology. There is an increasing need in the clinical routine to receive SW that can easily and with high accuracy compare each rCBF-SPECT with a computerized data base of normal investigations. Today the normal database is in the hands or heads of experienced radiologists/nuclear medicine specialists. There are normal databases that could be used such as the normal database for SPECT brain perfusion at The Society of Nuclear Medicine Brain Imaging Council (http://brainscans.indd.org/brncncl4.htm). One obstacle is, however, the different characteristics of each gamma camera system that have to be compensated for and the vast variation in reconstruction techniques used at different departments. A study using the Hoffman phantom (Data Spectrum Corporation, Chapel Hill, NC) to characterize the accuracy of different SPECT systems, conclude that normal databases should be acquired and processed on the same system as the subject, since each system demands optimized parameters for the most accurate clinical reconstructions [229]. There is therefore also a need of SW allowing each department to store and create normal rCBF-SPECT databases with their own camera-specific reconstruction parameters, which can be used also in clinical routine. Demands from clinical imaging departments will drive the development of such SW. An example of a public database and SW is the ‘Ictal-Interictal SPECT Analysis by SPM’, ISAS (http://spect.yale.edu/) that can be used in the localization of epileptic foci.

The experience of the radiologist or nuclear medicine specialist can affect the decision making in AD diagnosis and the information given at the clinical setting is a often crucial to make a correct diagnosis. This might also in some extent lead to a confirmation bias. There is work in progress with development of SW as decision support, to be used as adjunct to visual interpretation. For instance a computerized 3-dimensional analysis of the SPECT images has shown to be superior to ordinary visual inspection to discriminate AD from healthy controls [230].
CONCLUSIONS

This thesis shows that the rCBF-SPECT method still can be improved per se as well as for a higher diagnostic accuracy of AD in many aspects. A new semimanual registration method, based on fiducial marker, suitable for investigations with low spatial resolution was developed. The differences compared to a linear attenuation correction were low and the need for this rather complex procedure is therefore restricted in ordinary rCBF-SPECT reconstructions.

Furthermore, the thesis shows that rCBF-SPECT can be used for other basic research purposes such as increasing the knowledge of underlying cerebral pain processes in chronic pain patients.

The diagnostic sensitivity of traditional rCBF-SPECT performed at rest in AD is high and there is a correlation between episodic memory performance and rCBF changes. At group level, a memory provoked rCBF-SPECT has an even higher sensitivity than traditional rCBF-SPECT. Age have a large influence of rCBF uptake in regions close to interlobar and interhemispheric space which should be considered. However, typical AD related regions is not affected by chronological age. These results support the use of visual interpretation which is usually performed in clinical routine.

Education seems to have an influence on rCBF in frontal and temporal regions in healthy elderly. This can be interpreted as further support for the theory of ‘cognitive reserve’.
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REFERENCES


141. Polyak S. The vertebrate visual system. 1957.


204. Meyers JE, Meyers KR. Rey Complex Figure Test under four different administration procedures. Clinical-Neuropsychologist 1995:63-67 JN: Clinical-Neu.


