PET and the Multitracer Concept: An Approach to Neuroimaging Pathology

HENRY ENGLER
Dissertation presented at Uppsala University to be publicly examined in Roberg salen, University Hospital, Ingång 40, 4tr, Uppsala, Saturday, May 10, 2008 at 13:00 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in English.

Abstract

Patients suffering from different forms of neurodegenerative diseases, such as: Creutzfeldt Jacob Disease (CJD), Alzheimer disease (AD), mild cognitive impairment (MCI), frontotemporal dementia and Parkinson’s disease (PD) were examined with Positron Emission Tomography (PET) and the combination of different radiotracers: $^{15}$O-water, N-[1$^{13}$C-methyl]-L-deuterodoprenyl (DED), $^{[1]}$F-2-fluorodeoxyglucose: (FDG), N-methyl-$^{[13]}$C2-(4-methylaminophenyl)-6-hydroxybenzothiazole (PIB) and L-$^{[13]}$C-3,4-dihydroxiphenyl-alanine (DOPA). The radiotracers and the combinations of different radiotracers were selected with the intention to detect, in the brain, patterns of neuronal dysfunction, astrocytosis, axon degeneration or protein aggregation (amyloid), in the brain which are pathognomonic for specific diseases and may contribute to improve clinical differential diagnoses. Examinations in healthy volunteers were performed to allow comparisons with patients. In addition, animal studies were conducted to complement the information. In some cases, the PET findings could be compared with the results of autopsies.

In contrast to the micropathology, in which only a limited part of a tissue (obtained post-mortem or by biopsy) is inspected, one PET acquisition provides an image of the whole system (e.g.: the brain and the cerebellum). This form of imaging pathology is “in vivo”, where the examination is innocuous for the patient.

This thesis is an attempt to stimulate the development of new tracers, new tracer combinations and methods that directly or indirectly describe the anatomo-physiopathological changes produced in the brain in neurodegenerative diseases. A better description of different diseases can be obtained, confirming or questioning the clinical diagnoses and widening our understanding of the mechanisms underlying neurodegeneration. Different pathologies can produce similar symptoms and thus causing confusion regarding clinical diagnosis. The used PET combinations improved the accuracy of the diagnoses. The incipient knowledge emerging from a new neuroimaging pathology in combination with other disciplines may open the way to new classifications of dementias and neurodegenerative diseases based on an “in vivo” pathology.

Keywords: PET (Positron Emission Tomography), multitracer, neuroimaging pathology, astrocytes, microglia, neurodegeneration, amyloid, AD, CJD, PD

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urn:nbn:se:uu:diva-8687 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-8687)
“Un jour nous saurons la physiologie lorsque nous pourrons suivre pas à pas un molécule de carbon ou d’azote, faire son histoire, raconter son voyage dans le corps d’un chien, depuis son entrée jusqu’à sa sortie”

“One day we shall know physiology when we are able to follow step by step a molecule of carbon or nitrogen, to make its history, to tell its trip in the body of a dog, from its entrance to its exit”

_Claude Bernard_

A Inger, mi compañera, porque es “mi cómplice y todo” y porque juntos, somos mucho más que dos.
To Inger my wife, because she is my “accomplice and everything” and because together, we are much more than two.

To my mother “The Peregrine” for giving me the life many times and to my fader, the German contractor who taught me honesty and love to work.

To Jah, the Jewish carpenter who taught me that wisdom is more important than science.

To my Uruguayan and Swedish brothers and sisters.

To my Uruguayan and Swedish children.

To the Tupamaros who died fighting for a better world.

Thank you all for supporting me so many years.
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Jakob disease: Case report with pathological findings”. Manuscript.

The author’s contribution to the papers:

In all studies:  Participated in planning all the studies with other researchers. Contributed in writing applications to ethical committees (the part concerning PET investigations) and presented the studies to the radiation safety ethic committee. Prepared the protocols for all human examinations in collaboration with Gunnar Blomqvist. Assumed the responsibility for the examinations of all patients and healthy controls. Designed the sets of ROIs to be used and participated in deciding with Anders Wall how to reslice images. Participated in processing the images and drawing ROIs or controlling that they were well placed when other researcher performed this work. Participated in the evaluation, discussion and interpretation of the results. Wrote or participated in writing the articles, particularly the parts concerning the PET examinations and interpreting the results.
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<td>Alzheimer’s disease</td>
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<tr>
<td>BBB</td>
<td>Blood Brain Barrier</td>
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<td>CJD</td>
<td>Creutzfeldt-Jakob disease</td>
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<tr>
<td>CMRglc</td>
<td>Cerebral metabolic rate for glucose</td>
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<tr>
<td>DED</td>
<td>N-[(^{11})C-methyl]-L-deuterodeprenyl</td>
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<td>DOPA</td>
<td>L-[^11C]dihydroxyphenylalanine</td>
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<td>FDG</td>
<td>[^{18}\text{F}]2-fluorodeoxyglucose</td>
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<td>FTD</td>
<td>Frontotemporal dementia</td>
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<td>GFAP</td>
<td>Glial fibrillary acidic protein</td>
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<td>MCI</td>
<td>Mild cognitive impairment</td>
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<td>PET</td>
<td>Positron emission tomography</td>
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<td>PIB</td>
<td>N-methyl[^11C] 2-(4-methylaminophenyl)-6-hydroxybenzothiazole</td>
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<td>PD</td>
<td>Parkinson’s disease</td>
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<tr>
<td>ROI</td>
<td>Region of interest</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SUV</td>
<td>Standard uptake value</td>
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<tr>
<td>WAT</td>
<td>(^{15}\text{O}-labeled) water.</td>
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Introduction

Historical background.

Modern knowledge on the structure and function of the central nervous system (CNS) began with the neuron doctrine based on the work of Santiago Ramón y Cajal [1852-1934] and the staining method developed by Camilo Golgi [1843-1926], (de Felipe, 1998).

Before the work of Cajal and Golgi, very little was known about the components of the nervous system. Appropriate methods to visualize the brain tissue were not available. The early methods of staining only permitted the visualization of neuronal cell bodies and a small portion of their proximal processes (de Felipe, 1998).

Histological techniques, such as fixation procedures and tissue staining (hematoxylin or carmine) had been introduced in the middle of the 19th century. However, these procedures were inadequate and hence unsatisfactory for the investigation of the structure of the nervous system (Bentivoglio, 1998).

In 1869, Golgi published an article in which he stated that mental diseases could be due to organic lesions of the neural centers. Convinced that theory had to be supported by facts, Golgi abandoned psychiatry and concentrated on the experimental study of the structure of the nervous system (Bentivoglio, 1998).

In 1873 Golgi published an abstract: (“On the structure of the brain grey matter”) in the Gazzetta Medica Italiana, in which he described that he could observe the elements of the nervous tissue "studying metallic impregnations... after a long series of attempts". This was the discovery of the “black reaction” (reazione nera), which is based on nervous tissue hardening in potassium bichromate and impregnation with silver nitrate (Bentivoglio, 1998).

This staining permitted for the first time a clear visualization of a nerve cell body with all its processes in its entirety, which ultimately led to their cha-
racterization and classification, as well as to the study of their connections. (Bentivoglio, 1998).

Santiago Ramón y Cajal was introduced to Golgi’s method in 1887, where he recalled that the sight of the silver-impregnated nerve cells was the turning point that led him to abandon general anatomy and concentrate on neurohistology (Fernandez and Breathnach, 2001).

The descriptions of the nervous system performed by Cajal that were based on his observations using the Golgi method were so accurate that his book “Histologie” is still a reference in most neuroscience laboratories (de Felipe, 1998).

In 1906, Golgi was awarded the Nobel Prize for Physiology or Medicine for discovering this technique: Cajal shared this Prize for his interpretations of the Golgi preparations he had prepared.

One hundred years ago: the microscope reveals the micropathology of brain tissue.

Cajal, Golgi, Del Río Hortega, Nissl, Alzheimer and many others opened a window to the understanding and knowledge of the cellular and molecular components of the normal and pathologic brain tissue (de Felipe, 1998; Del Rio Hortega, 1919; Nissl, 1892; Alzheimer, 1907).

Using the microscope and different dying techniques the researchers could first detect the neurons, the microglia, the astrocytes and then, the presence of abnormal substances or changes in the normal histology, which were pathognomonic of certain diseases, (e.g. amyloid plaques and neurofibrillary tangles in Alzheimer’s disease).

The pathologists gradually paved the way for a new era in the development of the medical sciences. They made possible the comparison of the diagnosis set by the clinicians with the results of the autopsies. They extracted the brain, cut it into slices and prepared small samples using different dyes. They could describe the normal anatomy as well as the pathological changes present in different diseases.

Characteristic changes affecting one or more components of the brain system underlay clinical symptoms in most diseases. Diverse patterns containing variable proportions of neuronal loss, reactive astrocytosis, microgliosis,
oligoastrocytes, intracellular inclusions or extra cellular aggregations could be detected. This strongly contributed to the classification of brain diseases.

Fig. 1- The use of a microscope and staining techniques applied to biopsies and tissues from post mortem material has strongly contributed to our knowledge of brain histology as well as to the histopathology of different diseases. To the left: neurons drawn by Franz Nissl in 1892 (Nissl, 1892). To the right: Astrocytosis in CJD. Imuno-staining for glial fibrillary acidic protein (GFAP) with hematoxylin counterstain.

Fig. 2- Left: Neuritic plaques with prominent amyloid cores (Bielschowsky). Right: immunofluorescence technique, confocal micrography of a senile plaque. Amyloid-beta (green) and phosphorilated tau (red). From “Greenfield’s neuropathology” by Graham and Lantos, 7th edition, 2002.

Today and for the future: from the microscope to the macroscope
The technologic development has given us different “macrosopes”. One of the most important is the positron emission tomography (PET).
With the help of a cyclotron (particle accelerator), it is possible to produce radionuclides, radioactive elements that allow us to label organic molecules converting them into positron emitting tracers. With the PET scanner we can “observe” the distribution of these substances in the body. This modern “staining” technique allows us to follow the steps of Cajal, Golgi and other researchers. Just like Claude Bernard envisioned, we can follow-up today, thanks to PET scanners and special tracers, the way a molecule wanders in the body and tell its story (Taine; 1891).

When the brain suffers the aggression of a “noxa” (injury of an unspecific agent), the brain cells react in different ways. In the acute period the neurons often respond with a hypermetabolic reaction (Kreutzberg and Emmert, 1980; Smith et al., 1984; Woolf et al., 1984) and if the “noxa” persists, the result may be hypometabolism (Alavi et al., 1986), reflecting dysfunction that could eventually lead to neuronal death.

Astrocytosis and microgliosis (Kreutzberg, 1996) are common forms of reaction that the pathologists have described in post-mortem samples (Eng et al., 1992; Raivich et al., 1996).

Another form of change in the brain is the presence of protein aggregations: amyloid, tau-protein, prions, etc., (Bockman et al., 1985; Braak and Braak, 1991a; Braak and Braak, 1991b; Prusiner, 1985; Prusiner et al., 1985; Prusiner and Kingsbury, 1985; Thal et al., 2000).

These different biological or molecular expressions at the cell level can be present with a varying distribution in different diseases building typical patterns.
All these changes are directly or indirectly detectable using PET and proper radiotracer combinations.
Fig. 3- The “noxa” triggers a series of interconnected reactions tending to protect the tissue and repair the damage caused by the injury. The magnitude of these changes might have different proportions in different diseases. PET has the feasibility to detect these changes revealing characteristic patterns. FDG: fluorodeoxyglucose; PBR: peripheric benzodiazepine receptors; DED: deuterodeprenyl; PIB: Pittsburgh compound B; NST-ML10: new tracer for detecting apoptosis.

Various types of tracers have been used to detect diseases in the brain:

**Endogenous labeled tracers:**

**[^15O]Oxygen** is used to determine the cerebral oxygen extraction ratio and metabolism (Wessen et al., 1997).

**[^15O]Carbon monoxide** is used to determine brain blood volume (Leenders et al., 1990).

**[^15O] labeled water (WAT)** is used to determine cerebral blood flow (Wessen et al., 1997).

**[^11C]-Choline** is a marker for the biosynthesis of phospholipids (Spence et al., 2003).

**[^11C]-Acetyl carnitine (ACM)** is the smallest fatty acid acetate on the carnitine, the fatty acid carrier. It might reflect turnover to excitatory amino acids (Kuratsune et al., 2002; Yamaguti et al., 1996)
Amino acids

$[^{11}\text{C}]-\text{Methionine}$, is a marker for the turnover of the amino acid methionine. It is useful in detecting tumors in the brain (Bergstrom et al., 1991). This tracer has been utilized in other body applications as well (Nettelbladt et al., 1998; Sundin et al., 1996).

Neurotransmitter substrates

$[^{11}\text{C}]-\text{L-DOPA}$ is decarboxylated to dopamine by the enzyme dopadecarboxylase, giving a measure of the enzyme activity in the distal part of the proximal axons of the striatonigral pathway, detecting indirectly, axonal degeneration (Hartvig et al., 1992b; Tedroff et al., 1992).

5-Hydroxy-L-tryptophan (HTP) labeled with $[^{11}\text{C}]$ is a tracer for the in vivo assessment of brain serotonin synthesis (Hartvig et al., 1992a).

$[^{18}\text{F}]-\text{L-DOPA}$ gives a measure of the enzyme activity in the distal part of proximal axons of the striatonigral pathway, detecting indirectly, axonal degeneration (Hartvig et al., 1992b; Tedroff et al., 1992).

Tracers for measuring enzyme concentration

$N[^{11}\text{C}-\text{methyl}]-\text{L-deuterodeprenyl (DED)}$ binds irreversibly to the enzyme Mono-Amino-oxydase -B (MAO-B), which is expressed by reactive astrocytes and can be used as a marker for reactive astrocytosis (Ekblom et al., 1993; Engler et al., 2003; Fowler et al., 1998; Jossan et al., 1991; Orelend and Gottfries, 1986; Orelend et al., 1990).

In a multitracer combination with $[^{11}\text{C}]-\text{Methionine}$, DED has been used to differentiate meningiomas from pituitary adenomas (Bergstrom et al., 1992).

Tracers for reuptake systems
[\textsuperscript{11}C]-Nomifensine has been utilized to detect striatal dopamine reuptake sites (Aquilonius et al., 1987; Hagglund et al., 1987).

The cocaine analog 2 beta-carbomethoxy-3 beta-[4-iodophenyl]tropane (beta-CIT) labeled with \textsuperscript{11}C and analoges bind to the presynapic plasma membrane dopamine and other monoamines reuptake sites. It is used to measure axon degeneration in the presynaptic striatonigral pathway (Gunther et al., 1997; van Dyck et al., 1995).

**Tracers for various receptor systems**

\textsuperscript{11}C]-N-Methylspiperone is the first dopamine (D2) serotonin (HT2) receptor ligand (Hartvig et al., 1988; Muhr et al., 1986; Wagner et al., 1983)

\textsuperscript{11}C]-Raclopride, a D2 receptor ligand, has been used to detect signs of striatonigral degeneration (Rinne et al., 1990a; Rinne et al., 1993; Rinne et al., 1990b; Rinne et al., 1990c) and in prolactinomas predicting treatment efficacy(Bergstrom et al., 1991).

\textsuperscript{11}C]-SCH 23390 has been used to investigate striatal dopamine D-1 receptor binding in patients with early Parkinson's disease (PD) (Rinne et al., 1990a).

\textsuperscript{11}C]-Flumazenil is a benzodiazepine antagonist that competitively binds to benzodiazepine receptors expressed by neurons. The absence of binding in the brain is interpreted as neuronal death. This tracer is used to detect abnormalities in neuron expression in the cortex of patients with epilepsy and other diseases when neuronal death is suspected (Savic et al., 1993; Savic et al., 1988; Savic et al., 1995).

\textsuperscript{11}C]- and \textsuperscript{18}F]-PBR ligands, e.g.: \textsuperscript{11}C](R)-PK11195, bind to peripheral benzodiazepine receptors and can detect microgliosis (Cagnin et al., 2001).

\textsuperscript{11}C]-diprenorphine is a weak opioid agonist with equal high affinities for mu-, kappa- and delta-opioid receptors that has been used in the investigation of pain systems (Jones et al., 1991) and epilepsy but also to differentiate between PD, striatonigral degeneration (SNA) and Steele-Richardson- Olszewski syndrome (SRO), (Burn et al., 1995).

**Tracers for protein aggregation**

\textit{N}-methyl-[\textsuperscript{11}C]2-(4_-methylaminophenyl)-6-hydroxybenzothiazole
(PIB) binds to amyloid and is used to demonstrate the presence of brain amyloidosis (Engler et al., 2006; Engler et al., 2008; Forsberg et al., 2007; Klunk et al., 2004).

FDDPN (Agdeppa et al., 2001) and $[^{11}\text{C}]$SB-13 (Cai et al., 2007) are used to detect neurofibrillary tangles and amyloidosis.

**Apoptosis tracers**

NST-ML10 has been used to detect signs of apoptosis in stroke patients (Sirvan et al., 2008)

These are only some examples of the possible tracers that are known today.

In this thesis the following combinations of tracers were selected:

<table>
<thead>
<tr>
<th>Disease</th>
<th>Tracer combination</th>
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<tr>
<td>CJD, Creutzfeldt-Jacob disease</td>
<td>DED + FDG + WAT</td>
</tr>
<tr>
<td>AD, Alzheimer’s disease</td>
<td>PIB + FDG</td>
</tr>
<tr>
<td>MCI, mild cognitive impairment</td>
<td>PIB + FDG</td>
</tr>
<tr>
<td>FTD, frontotemporal dementia</td>
<td>PIB + FDG</td>
</tr>
<tr>
<td>Parkinson/parkinsonism</td>
<td>DOPA + PIB</td>
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**General considerations of tracer combinations and applications.**

The combination of different tracers affords the feasibility to observe the disease from different angles and therefore detecting changes in more than one system. This can be compared with observing a building from the front but also from the backside. Like a two- or three-dimensional, optical inspection, the result is a more detailed impression. Antomo-physiopathological changes occurring in the living brain and often at early stages of a disease can be detected and described. The dynamic progress of a disease can be captured at different “time windows”, obtaining patterns of the pre-symptomatic phases, images at the clinical debut of a disease and later imaging the sub-acute and chronic phases.
There are tracers that act on different parts of a system but indicate the same neuronal alteration. For example, $[^{11}\text{C}]-\text{DOPA}$ and $[^{18}\text{F}]-\text{DOPA}$ measure dopadecarboxylase activity, whereas $[^{11}\text{C}]\beta\text{-CIT}$ or $[^{11}\text{C}]\beta\text{-CFT}$ measures proximal re-uptake of monoamines, including dopamine. Both physiological processes occur in the terminal part of the axons in the presynaptic nigrostriatal pathway, indicating directly or indirectly, axonal degeneration.

Because the reuptake occurs in the pre-synaptic membranes, the decrease of $[^{11}\text{C}]-\beta\text{-CIT}$ binding expresses more directly axon degeneration.

In the case of $[^{11}\text{C}]-\text{DOPA}$, the velocity of dopamine production is dependent on the enzyme activity (dopadecarboxylase) and might be calculated as the accumulation rate of $[^{11}\text{C}]-\text{DOPA}$ derived radioactivity. In the incipient axon degeneration, DOPA radioactivity uptake might be increased by a compensatory “up-regulation” of the enzyme, whereas the $[^{11}\text{C}]\beta\text{-CIT}$ binding is decreased together with the diminution of the number of axons.

Both tracers may be used to confirm presynaptic degeneration because the symptoms appear when the damage has overcome the compensatory mechanisms. However, to find preclinical signs of the disease, $[^{11}\text{C}]\beta\text{-CIT}$, should be more sensible to axon degeneration than DOPA.

With progression of the disease, both tracers will indicate axon degeneration when the compensatory mechanisms are not sufficient. These two tracers reveal different alterations in the same system (the presynaptic pathway), indicating PD or parkinsonism.

**Parkinson’s disease and parkinsonism**

Neuroimaging studies with PET may help in the differential diagnosis of PD and parkinsonism, detecting postsynaptic degenerations that are not present in PD.

The term “parkinsonism” is applied to a group of neurodegenerative diseases that, in the onset, can be difficult to separate from PD. Multiple system atrophy (MSA) is a condition characterized clinically by variable combinations of rigidity, bradykinesia and tremor with poor response to L-dopa, autonomic failure and ataxia (Quinn, 1989). It includes conditions such as striattonigral degeneration (SND), olivopontocerebellar atrophy (OPCA) and the Shy-Drager syndrome (Shy and Drager, 1960).
It is known that up to 25% of those patients clinically diagnosed with PD have evidence of other degenerative disorders at post-mortem evaluation (Gibb, 1992; Rajput et al., 1984).

Different tracers can be utilized to find signs of postsynaptic degeneration. Raclopride, which binds to D2 receptors, is one of them. Because the D2 receptors diminish with the progression of the atrophy of the striatum, the decrease binding of the tracer may indicate postsynaptic degeneration (Mochizuki and Shimizu, 1997).

An increase in D2 receptor binding has been observed in patients with early PD (Rinne et al., 1993; Rinne et al., 1995; Rinne et al., 1990c) but after levodopa treatment, a reduction of raclopride binding has been reported (Tedroff et al., 1996). There is evidence from animal work that both L-dopa and dopamine agonists may down-regulate striatal D2 sites (Fuxe et al., 1981; Mishra et al., 1978). Other studies however, have noted normal or raised levels of striatal D2 sites after L-dopa administration (Hall et al., 1984).

The problem in clinical routine is that many of the patients with parkinsonism respond to the Parkinson treatment in the beginning of the disease. When they became non-responders, they have been or are treated with L-dopa during a more or less extensive period. Exogen supply, which increases the amount of dopamine or D2 agonists in the synaptic cleft, may originate a “down-regulation” of D2 receptors. The risk is that the down-regulation could be interpreted as a sign of degeneration, confusing the diagnosis.

At the beginning of the disease in untreated patients, a compensatory “up-regulation” of D2 receptors may occur and the examination with raclopride will not reveal signs of degeneration. Mild diffuse loss of striatal D2 sites was noted in patients with SND, but some of the patients who failed to respond to L-dopa had normal levels of striatal D2 receptors (Brooks et al., 1992).

In a study of PD with non-treated patients dopamine D2 receptor binding potential (BP) was measured by \([^{11}\text{C}]-\text{N-methylspiperone}\) (NMSP). The BP was slightly elevated in these patients, which was interpreted as reflecting denervation hypersensitivity in the postsynaptic site (Momose and Sasaki, 1997).
Fig. 4- Schematic representation of presynaptic neurons (substantia nigra), synaptic cleft and postsynaptic connections (putamen). 1) Normal condition (normal FDG uptake, dopadecarboxylase activity and raclopride binding). Horizontals arrows represent normal condition; down arrows and up arrows indicate decreased and increased function. 2) Untreated PD. Representation of neurodegeneration in the substantia nigra. Compensatory “up-regulation” or increased number of D2 receptors as a consequence of decreased dopamine delivery to the synaptic cleft and increased glucose uptake (high energy demanding process). 3) PD after treatment. Compensatory “down-regulation” or reduction of D2 receptors. Low raclopride binding with normal FDG uptake (low energy-demanding process). 4) striatonigral degeneration. Decrease in uptake or binding for all tracers. It might be difficult to achieve a correct diagnosis by only using raclopride. FDG provides information not only of the metabolism in the striatum but also of the whole cerebral cortex, brain stem and cerebellum.

Significantly higher blood flow and oxygen metabolism in the caudate head and putamen was found contralateral to the patient’s symptoms (Tanaka et al., 1997).

The asymmetry in the perfusion and metabolism is observed in the early stage of the disease or in patients not treated with L-dopa therapy. These findings suggest that the striatal dysfunction may be caused by uninhibited activities, supersensitivities or compensatory hyperactivities of the striatum (Tanaka et al., 1997).

FDG offers better possibilities to find signs of post synaptic degeneration than tracers that bind to D2 receptors. D2 receptor binding characteristics do not reliably separate PD patients from controls or from patients with other extrapyramidal symptoms such as the Steele-Richardson-Olzewski syndrome or multiple system atrophy (Brooks et al., 1992; Rinne et al., 1995).
Fig. 5- Upper row: patient with PD. FDG indicates high uptake in the striatum, DO-
PA shows low dopadecarboxylase activity, indicating presynaptic degeneration and
[11C]- raclopride shows normal binding. Lower row: patient with SND. Low FDG
uptake, low DOPA activity and decreased [11C]- raclopride binding are observed in
the striatum.

A relative increased metabolism in the middle caudate, ventral striatum and
inferior thalamus on the side contralateral to the predominant symptoms side
has been found in patients with PD (Dethy et al., 1998).

Many researchers have reported normal (Eidelberg et al., 1994; Kobayashi
and Sakuragawa, 1993; Mochizuki and Shimizu, 1997) or increased (Tanaka
et al., 1997) glucose uptake in the striatum.

This phenomenon of normal or increased glucose uptake have been observed
as well in the striatum of patients with PD in our clinical examinations (un-
published material).

The enhanced metabolism may be the result of a compensatory energy-
demanding mechanism leading to an increase in the number or activity of the
D2 receptors stimulated by the decreased delivery of dopamine to the synap-
tic cleft.

FDG can not only detect impaired metabolism in the striatum but also in the
pons, cerebellum and cerebral cortex, opening the possibility to differentiate
between forms of parkinsonism: SND, OPCA, Shy Drager syndrome, cortico
basal degeneration, etc., (Gilman et al., 1988; Otsuka et al., 1996).
To work with tracer combinations in clinical applications signifies not only to understanding the mechanisms of action of each tracer. It also demands a dynamic interpretation of the interactions of the tracers with different drugs used in treatment. This interaction may modify receptor kinetics creating a distortion in binding or uptake that disturbs the diagnosis.

Furthermore, the disease is not a static entity but rather a dynamic process changing over time with different pathological substrates. To think static is probably the most common error that physicians commit. With PET, we can see windows in the progress of the disease that will change from one examination to the next. PET images contain a lot of information that must be extracted and this is possible only with a dynamic interpretation.

Alzheimer’s disease

Studies in Alzheimer disease performed with PET and the combination of the tracers FDG and PIB illustrate the concepts discussed above. We found a relation between the progression of the amyloid deposition, the neuronal dysfunction and the clinically evaluated cognitive deterioration. The tracers provide information at different times of the disease contributing to a better understanding of the in vivo pathology (Engler et al., 2006; Klunk et al., 2004).
Fig. 7- The amyloid deposition seems to begin early in the course of the disease eventually reaching a “plateau”. Glucose uptake is not affected during this period. There is possibly a critical point in which the capacity of the brain to compensate damage is overcome with the consequence that symptoms appear. Deterioration in cognition follows the decrease in glucose uptake but not the increase of amyloid deposition. FDG is a useful tracer to confirm the diagnosis of AD, but often when damage of brain tissue is pronounced. PIB may detect the presence of the protein before the neurodegenerative changes cause symptoms because the amyloid deposition occurs early in the course of the disease.
Aim of the thesis

In this thesis the results of clinical PET investigations using different tracer combinations and performed on patients suffering from different neurodegenerative diseases are presented with the following aims:

1) To describe, directly or indirectly, *in vivo*, the anatomical and physiopathological changes produced in neurodegeneration with the intention to increase our understanding of brain diseases.

2) To find characteristic patterns for brain diseases presenting with similar symptoms but having different pathological substrate. The combinations of tracers have been selected with the intention to detect in the brain patterns of neuronal dysfunction, astrocytosis, axon degeneration or protein aggregation (amyloid) in the brain.

3) To investigate if the presence of characteristic patterns can contribute in improving clinical differential diagnoses. This is fundamental not only for the treatment of the patient but also for relatives and physicians.

4) To stimulate the development of new methods, new tracers and combinations of tracers in neuroimaging pathology.
Material and methods

PET and the combination of various tracers have been applied to examine patients suffering from different forms of neurodegenerative diseases: Creutzfeldt Jacob Disease (CJD), AD, mild cognitive impairment (MCI), Frontotemporal dementia and PD. We even performed examinations in healthy volunteers for the purpose of comparison. Animals were also examined in order to obtain additional information. In some cases, it was also possible to compare the PET findings with the results from autopsy.

Positron Emission Tomography (PET).

The PET technique allows the possibility to detect, with a scanner, positron decay produced from radiolabeled molecules administrated to animals and humans.

The radionuclides are produced by a particle accelerator (cyclotron) and incorporated by organic synthesis into molecules of biological interest (such as carbohydrates, fatty acids, amino acids and even drugs). The most used positron emitters are $^{18}$F, $^{11}$C, $^{15}$O and $^{13}$N (half life: 110, 20, 2 and 10 min, respectively).

Carbon, oxygen and nitrogen are present in most of the biological molecules and the biochemical properties will not be dramatically altered after introduction of these radionuclides in a compound.

There is a significant interest in positron emitting metals like cyclotron produced copper isotopes e.g., $^{62}$Cu and $^{64}$Cu (half life: 10 min and 13h respectively), (Vavere and Lewis, 2007) and the generator produced $^{68}$Ga (half life: 68 min), (Velikyan et al., 2005).

The emitted positrons travel 1-2 millimeters in the tissue before they collide with an electron. The result of the collision is the annihilation of the pair positron-electron. Two photons with a constant high energy of 511 keV are generated and wander in opposite directions (180 degrees).
The double photon emission may be detected by special crystal detectors placed in pairs in a ring contained in the gantry of the PET scanner. The scanner detects the coincident emissions filtering the decay emissions reaching only one crystal. Electronic devices can detect the place where the annihilation occurs and the different density of events allows the mapping of the distribution of the positron emitter compound.

The coincidence measurement of photon pairs allows the administration of low amounts of positron emitters and because of the short life of the radionuclides, it is possible to combine different tracers and thus perform multiple examinations on the same object (patient or animal) during the same day.

The limitation of the technique is expressed theoretically by the distance of the wandering positrons before they collide with an electron, which limits the theoretical resolution to approximately 2 mm. Other disadvantages are that the detectors cannot distinguish whether the photons are generated from positrons emitted by the original tracer or its metabolites and the short half-life of the radionuclides, which demands rapid and efficient methods in organic chemistry and careful quality control analyses.

In the first study of this thesis (I), the PET examinations were performed in a GE 2048-15B (GE Electronics, USA) brain PET scanner that simultaneously recorded 15 tomographic slices covering an axial field of 10 cm with a slice separation of 6.5 mm and a planar resolution of 5 mm [15].

All the other studies were performed in Siemens ECAT HR+ cameras with an axial field of view of 155 mm, providing 63 contiguous 2.46 mm slices with a 5.6 mm transaxial and a 5.4 mm axial resolution. The orbito-meatal line was always used to center the subject’s head.

The radionuclides were produced using a MC17 cyclotron (Scanditronix, Uppsala, Sweden).

PET acquisitions

PET brain studies may be performed in two modes: the static acquisition in which the brain is scanned under a defined time or the dynamic acquisition in which the brain is scanned sequentially at different time points or frames, generating dynamic data sets. In all studies (I-VII) dynamic acquisitions were used.

Data acquisition in 2D mode (extended inter-plane septa) using a rod source of the long-lived positron emitter $^{68}$Ge provided data for attenuation correction. This procedure is termed transmission scan and is used in conjunction with the emission.
In 2D acquisition mode, the detection of coincidences is limited by an interplane septa extended between the rings. This causes lower sampling density and lower count efficiency. In 3D mode the acquisition is performed with retracted interplane septa.

All the emission scans in this thesis (I-VII) were acquired in 3D mode. FDG, PIB, DED, DOPA and \(^{15}\)O-water were produced according to the standard operating procedures at the Uppsala University PET Centre/Uppsala Imanet.

Tracer modeling

The information contained in a PET acquisition is not only a static image. Reconstruction of the images at different acquisition times provides quantitative kinetic information of the tracer distribution in the organs.

Regions of interest (ROIs) drawn by hand were used to measure the activity of tracers over time. Different models to interpret the compartment distribution of the tracer were used.

It must be recalled that the models applied are simplifications of the real complex distribution, metabolism, elimination, unspecific or specific binding, uptake or retention in the body of the traces inoculated and thus they can only give a rough idea of the biological processes studied.

A widely used method for quantification of tracer distribution is to utilize the radioactivity in plasma as input function for compartment analysis of metabolic processes in the brain (Patlak et al., 1983; Sokoloff et al., 1977). These methods demand blood samples to analyze the radioactivity corrected for metabolites in plasma derived from injected radiotracer during the PET study.

Further, it is necessary to investigate whether the radiotracer forms labeled metabolites that can penetrate the blood brain barrier (BBB) interfering with the measures.

For tracer validation, the samples must be obtained from arterial blood and a catheter must be inserted in the arteria radialis, complicating the examination for both the patient and the physician. In some cases (such when using FDG) the arterial line can be replaced by a line in the dorsal veins of the hand wormed to 43° C (degrees Celsius) with the intention to provoke a vasodilation allowing a higher proportion of arterial component in the distal system (“arterialized blood”).

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The use of a reference region, that lacks specific uptake of the radiolabeled tracer is a more simple way to generate quantitative information (Patlak and Blasberg, 1985).

With the reference tissue model, the analysis of metabolites in plasma is no longer necessary and blood samples can be avoided simplifying the examination.

However, when a tracer is used for the first time, the value obtained of a reference region must be demonstrated comparing the values with those obtained using blood or plasma as “input function” (Lundquist et al., 2006).

Concerning the FDG studies performed in this work, parametric maps of the glucose metabolic rate (rCMRglc) were generated by the Patlak technique using the time course of the tracer in arterialized-venous plasma as the input function (Gjedde, 1981; Patlak et al., 1983). Taking advantage of the 20-minute half-life of $^{11}$C, most of the FDG scans could be performed 120 min after the injection of the $^{11}$C-tracers.

For the PIB tracer, arterial plasma data were available for seven of the twenty five subjects examined in (II). Arterial lines also were used in (III), but in the rest of the studies the reference tissue modeling using the cerebellum was applied.

To avoid possible unspecific contamination from the cerebellar medulla the reference region was based on an early summation image. This image gave a good anatomical representation of the cortical areas because the early frames seem to show the flow component of the tracer. On top of the early summation image, a late summation image was placed showing the non-specific binding in whiter matter. The reference region was then drawn in the cortical area outside the whiter matter seen in the late summation image.

Recently, a method to detect automatically the reference region applying principal component analysis (PCA) has been developed (Razifar et al., 2008)

Quantitative estimates of DED binding to MAO-B and initial tracer distribution were generated by the Patlak method (Patlak et al., 1983), with proposed modifications (Bergstrom et al., 1998). The tracer uptake on a pixel-by-pixel basis was divided by the cerebellar cortex tracer concentration and plotted against normalized time, although the cerebellar time-activity data were first multiplied by an exponential. The procedure has been shown to linearize the Patlak graph slope and allows separate generation of images representing the Patlak slope and intercept. This slope has also been found to be proportional to MAO-B enzyme expression (Bergstrom et al., 1998).
Regions of interest (ROIs)

All PET investigations were analyzed using standardized regions of interest (ROIs) in the brain. The reference slice was that where the thalami were most clearly visible. In this plane both thalami were defined with an area of 1.5 cm$^2$, and a whole brain ROI was formed at this level. Ventrices were not included. Cortical ROIs with a width of 1 cm and a length of 3 cm were placed in the frontal (three slices) and parietal (four slices) cortices. ROIs for the putamen were placed at the level with the highest uptake. Other cortical ROIs were placed in the anterior cingulate cortex (three slices), the temporoparietal area (one slice, two slices below the level of the thalamus), the occipital and cerebellar cortices at the level of highest radioactivity uptake and the temporal lobe (five coronal slices), including the following regions: temporomedial, lateral and inferior. These regions were divided into anterior and posterior. Two ROIs each 1.5 cm in diameter were located in the pons and linked, and the white matter was defined with a free-hand ROI at the location of the centrum semiovale. A ROI representing cingulum posterior was included in (III).

Ethics

All the studies were approved by the Ethics Committee of the Faculty of Medicine at Uppsala University and by the Radiation Safety Ethics Committee of Uppsala University Hospital. All subjects gave their written informed consent to participate. In the case of patients with suspect CJD unable to communicate, the consent was obtained from close relatives. The Rhesus monkeys (I) were maintained and handled in accordance with recommendations of the US National Institutes of Health (NIH) and the guidelines of the Central Research Laboratory, Hamamatsu Photonics, Japan.
Paper I. Multitracer study with PET in Creutzfeldt- Jakob disease.

**Background**

Sporadic CJD (spCJD) is neuropathologically characterized by neuronal loss, astrocytosis, spongiform changes and deposits in the brain of a protease-resistant prion-protein (called PrPres or PrPSc). Clinical diagnosis might be difficult because the symptoms are not always specific. Suspicion of the disease may emerge in patients with rapidly progressive dementia and multifocal signs. A large number of neurological disorders might be mistaken for CJD. These include other neurodegenerative disorders such as Alzheimer’s disease, Lewy body disorders, parkinsonism, Pick’s disease, Huntington disease, cerebellar disorders and amyotrophic lateral sclerosis; primary and metastatic brain neoplasmas; multi-infarct dementia and intracerebral hematomas (Morita et al., 1987; Salazar et al., 1983).

It is of utmost importance to obtain a diagnosis as early as possible because some types of rapidly progressive dementia may also be associated with treatable diseases (autoimmune and paraneoplastic encephalitis).

The study was initiated based on the assumption that reactive astrocytosis and neuronal death in these patients could be revealed with the combination of DED and FDG. Furthermore, the cerebral blood flow was studied with $^{15}$O-water and the relation between blood flow and the intercept in the Patlak plot was investigated.

**Patients and methods**

Fifteen patients with clinical symptoms of “suspect” CJD were examined with PET.
The examinations were performed in one session starting with oxygen-15 labeled water to measure regional cerebral blood flow, followed by imaging with the monoamine oxidase B inhibitor \( N\)\(^{[11]C}\)-[11C-methyl]-L-deuterodeprenyl (DED) to assess astrocytosis in the brain and finally imaging with FDG to assess regional cerebral glucose metabolism (rCMRglu).

Nine of the patients fulfilled the clinical criteria of “probable” CJD.

Anesthesia was necessary in these severely demented patients in order to perform the PET examinations. This raised the question of the effects of propofol administration on rCMRglu and DED binding. A study using Rhesus monkeys in awake and sedated states was thus performed to evaluate the propofol-induced changes on FDG uptake and DED binding.

**Results**

No significant effect of propofol anaesthesia on DED binding was seen in the examined monkeys, whereas there were pronounced dose-dependent decreases in rCMRglu without a symmetrical difference.

Seven of the nine patients who fulfilled the WHO/EU criteria of probable CJD (WHO 1996) were available for neuropathological examination of the brain. Six of them were found to have definite CJD. In a seventh patient who fulfilled the clinical criteria for CJD, PrPres could not be demonstrated. In two of the patients, autopsy was not performed.

In eight of the nine patients with probable CJD (DED production failed in one of these patients), FDG and DED imaging revealed, in comparison with normal controls, a typical pattern characterized by a pronounced regional decrease (<2SD) in glucose brain metabolism, indicative of neuronal dysfunction. This decrease was accompanied by a similar increase (>2SD) in DED binding indicating reactive astrocytosis. These changes were most pronounced in the cerebellum and the frontal, occipital and parietal cortices, whereas this pattern was not found in the temporal cortex, pons, thalami, and putamen.
Fig. 8- Bar graph showing the mean change from normal (in standard deviations) for each region of interest in patients with CJD. (Ant = anterior; inf = inferior; post = posterior; ctx = cortex, CBL = cerebellum; CBR WhM = cerebral white matter; Cing = cingulum; Fr = frontal; Frass = frontal association; Par = parietal; ParTmp = parieto-temporal; PrVis = primary visual; Put = putamen; SM = sensory-motor; Th = thalamus; tmp = temporal)

PET findings obtained using DED and FDG paralleled neuropathological findings, indicating neuronal dysfunction and astrocytosis, changes that are found in CJD.

The glucose hypometabolism and the cerebral blood flow (CBF) decrease were also correlated in different brain regions and the DED intercept values of the Patlak plot showed a direct correlation with the CBF values (R² = 0.82)

Fig. 9- Normalized CBF and DED intercept values for each region for all CJD patients that had been examined with ¹⁵O-labeled water (R² = 0.82).
Fig. 10- Left: normal DED binding at the level of pons and cerebellum and at the level of thalami and binding in a CJD patient. Right: patient with the “Heidenhain” type of CJD. Areas with high DED-binding and low CMR$_{\text{glu}}$ are indicated. The occipital area and the right thalamus show less decrease in glucose uptake whereas the binding of DED is highly increased.
Background

AD, which is the most common form of dementia, is characterized by progressive impairment in cognitive function and behavior. The pathological features of AD include the presence in the brain of amyloid plaques composed of amyloid-peptide (A-beta) fibrils, neurofibrillary tangles of hyperphosphorylated tau and neurotransmitter deficits. Increases in the concentration of A-beta in the course of the disease lead to a gradual increase in the load of amyloid plaques and progression in cognitive impairment.

This report was the first human study using a novel amyloid-imaging PET tracer, termed Pittsburgh Compound-B (PIB). Modification of the amyloid binding histological dye, thioflavin-T, led to the finding that benzothiazoles bound to amyloid with high affinity and crossed the BBB well.

The human use of PIB was preceded by a toxicity assessment using the PET-microdosing concept (Bergstrom et al., 2003). Toxicological studies included genotoxicity (chromosomal aberration, mouse lymphoma mutagenesis, bacterial reverse mutation assay, and mouse micronucleus assay), single dose toxicity in rats and cardiopulmonary physiology in the rhesus monkey. No toxic effects of PIB were observed.

Patients and methods

Sixteen patients with the diagnosis of probable AD according to the National Institute of Neurological and Communication Disorders Alzheimer’s Disease and Related Disorders Association criteria and nine controls were examined.

Results

In comparison with controls, AD patients typically showed marked retention of PIB in areas of association cortex known to contain large amounts of amyloid deposits in AD. In the AD patient group PIB retention was most prominently increased in the frontal cortex. Large increases were also observed in the parietal, temporal and occipital cortices as well as the striatum. PIB retention was equivalent in AD patients and controls in areas known to be relatively unaffected by amyloid deposition (such as subcortical white matter, pons, and cerebellum).

In cortical areas PIB retention correlated inversely with cerebral glucose metabolism as determined with FDG. This relationship was most robust in
the parietal cortex. PIB could discriminate better than FDG between AD patients and controls. The results suggest that PET imaging with the novel tracer, PIB, might provide quantitative information on amyloid deposits in living brains.

This “proof-of-concept” study represents the first evaluation of a benzothiazole compound as an in vivo radiotracer for imaging amyloid deposition in human brain.

Direct imaging of amyloid load in patients with AD in vivo might be very useful for the early diagnosis of AD and the development and assessment of new treatment strategies.

Fig. 11- Upper row: Healthy control (HC). Lower row: AD patient. Left: FDG examinations. Right: PIB examinations. Regions containing gray matter are delimited by white lines. The retention of PIB in the HC is exclusively restricted to the white matter. High retention of PIB is observed in the frontal and temporoparietal cortices in the AD patient.
Paper III. Two-year follow-up of amyloid deposition in patients with Alzheimer’s disease

Background
This study includes a “test-retest” study with 4 AD patients and a follow-up study after two years of the 16 AD patients examined in (II). The interval change in amyloid deposition and regional cerebral metabolic rate for glucose (rCMRGlc) at follow-up are described.

Patients and methods.
To study the intra-individual variability in PIB retention four additional Alzheimer patients (T1–T4), three females and one male (58-79 years) with a MMSE range 9-28, underwent repeated PET investigations with PIB. For three of the AD patients, the two PIB studies were performed within 12 hs and for the fourth patient, after a 20-day interval.
The sixteen patients were the same we examined previously (II).

Results
Concerning the test-retest, only small variations in PIB retention were observed in the cerebral cortex (3-7%), indicating low variability of the tracer when injected in different occasions in the same person.

Concerning the examination of the sixteen patients, there was no increase in amyloid depositions over two-year period, whereas the glucose uptake was decreased particularly in the parietal region and cingulum posterior.

At the follow-up, cognitive testing showed a non-significant decrease in MMSE score of 1.6 points as compared with base-line.

Five of the sixteen patients however, had very clear clinical deterioration with a decrease of 3 points or more (3-9) in the MMSE.

In these five Alzheimer patients the mean MMSE score was significantly lower (P < 0.01) at follow-up [15.6 ± 3.9 (SD)] as compared with baseline (21.4 ± 3.5). This group of patients was considered to comprise those who had clinically deteriorated (AD-P; progression). The change in the MMSE score in the other eleven patients was <3 (-2 to +3) at follow-up versus base-line. This group of Alzheimer patients was considered clinically relatively stable at follow-up (AD-S).
Tab. 1- Mean values for the PIB ratio ROI/reference +/- standard deviations; probabilities were calculated by using the Student paired t-test; S = stable/normally progressing Alzheimer patients; P = faster progressing Alzheimer patients; *significant difference between the AD-S and AD-P group (P < 0.05). There are missing data for one patient because of an error in the scanning procedure at baseline.

The results of the present study reveal relatively stable PIB retention in patients with mild AD over a mean period of two years (1.5–2.5 years) despite progressive deterioration in rCMRGlc as well as a clear decrease in cognitive function in some cases. This relatively stable PIB retention, with only minor variations (increase/decrease), might reflect a dynamic process in amyloid deposition reaching equilibrium. The results are in agreement with those in earlier human post-mortem studies showing a dynamic balance between amyloid deposition and resolution in senile plaques, or amyloid burden (Hyman et al., 1993).

Fig. 12- Correlation between rCMRGlc (ROI/ref) and PIB retention (ROI/ref) in the parietal cortex of individual Alzheimer patients at baseline (A) and follow-up (B). Dotted lines indicate mean values for the healthy controls plus 1 SD for PIB (mean = 1.35) and minus 1 SD for rCMRGlc (mean = 1.47). Open circles represent Alzheimer patients cognitively relatively stable, with changes in the MMSE score of <3.0 at follow-up (AD-S). Filled circles represent Alzheimer patients who deteriorated cognitively with a decrease in the MMSE score of >3.0 at follow-up (AD-P). Only SUV rCMRGlc values were available for one patient (09), which is indicated with filled squares. Measured radioactivity in the parietal cortex was normalized to that in the pons as regards glucose uptake.
Fig. 13- Parametric images concerning PIB (ROI/ref) and rCMRGlc (ROI/ref). The Alzheimer patient showed deterioration in cognition at follow-up (7 points in MMSE). The images show only slightly increased PIB retention (upper) but a pronounced decrease in glucose uptake (lower).
Paper IV. PET imaging of amyloid deposition in patients with mild cognitive impairment (MCI)

Background

MCI represents a transitional phase between normal aging and dementia disorders, especially AD. Patients with MCI have an increased risk of developing AD (Petersen et al., 1999). At present there is great interest in finding diagnostic tools for detecting an increased risk of developing AD. The aims of this study were to measure, with PET, PIB retention in the brains of MCI patients and analyze its relationship with cerebral glucose metabolism, cognitive function, CSF biomarkers (Aβ1-42, total Tau, phosphorylated Tau) and investigate the conversion of these patients to AD.

Patients and methods

Twenty-one patients diagnosed with MCI underwent PET studies with PIB and FDG to measure amyloid deposition, cerebral glucose metabolism, as well as to assess cognitive function and CSF sampling. Reference group data from twenty seven AD patients and six healthy controls were used for comparison. To account for global differences between patients the CMRglc data were normalized to the pons (ROI/ref). For PIB, the ROI/reference model utilized previously (II, III) was applied.

Results

The MCI patients showed a significant higher PIB amyloid retention in cortical brain regions than healthy controls (p<0.05). Eleven MCI patients within the MCI group showed high PIB retention that was comparable to AD patients. Ten MCI patients showed PIB retentions comparable to healthy controls. Seven MCI patients who have converted to AD showed all high PIB retention as MCI patients. Significant correlations were obtained between CSF Aβ1-42, total Tau, phosphorylated Tau and PIB retention whereas no significant correlation was observed between cognition and PIB.

High amyloid load in the brain can be detected in MCI patients. PIB amyloid imaging in MCI patients might therefore be a promising diagnostic marker for discriminating incipient AD enabling early drug intervention.
Fig. 14- PIB retention (ROI/ref) in the posterior cingulum in MCI patients compared with healthy controls (HCs) (n = 6) and AD patients (n = 27). Long horizontal lines indicate mean, short horizontal lines indicate standard deviation (SD). Filled circles indicate MCI patients that after the PET scans converted to AD. The small letters indicate the patients shown in Fig. 2: a, HC; b, MCI converter; c, MCI non-converter; d, AD. One healthy control (77 years of age) with normal cognitive function had high PIB retention as earlier reported at baseline and at the two-year follow-up studies (Engler et al., 2006; Klunk et al., 2004).

Fig. 15- PIB retention in one MCI converter, one MCI non-converter, one AD patient, and one healthy control. The PET scans show PIB retention at a sagital and longitudinal section at the level of the basal ganglia. Red indicates high, yellow medium and blue low PIB retention.
Paper V. In vivo amyloid imaging with PET in frontotemporal dementia

**Background**

To evaluate the potential clinical use of PIB with PET in differential diagnosis it is necessary to determine the uptake patterns in dementia disorders other than AD.

Clinically, FTD is a syndrome characterized by emotional blunting, a breakdown of social conduct, loss of empathy, and impaired illness awareness. Typically, the patient acts in an impulsive manner without being able to consider the consequences.

Amyloid pathology is not present in FTD. The current opinion is that several types of neuropathological changes underlie the clinical syndrome of FTD (McKhann et al., 2001). These are referred to as frontotemporal lobar degenerations (FTLD) to separate them from the clinical syndrome of FTD (McKhann et al., 2001). All patients have in common the lobar atrophy, neuronal loss, and gliosis.

The purpose of this study was to investigate the presence or absence of PIB retention in FTD by comparison with PIB retention positive AD patients and PIB negative HCs.

**Patients and methods**

Ten patients with FTD participated in the study. The results were compared with PIB retention data previously obtained from seventeen AD patients with positive PIB retention and eight HCs with negative PIB retention.

**Results**

Eight of the FTD patients did not have amyloid depositions but instead showed images similar to those found in the HCs. Two of ten FTD patients had PIB PET scans similar to those in the AD patients. Studies with larger and more naturalistic patient samples with post-mortem pathology will be necessary to determine the potential of PIB PET as a clinical differential diagnostic tool. New multi-tracer approaches detecting other neuropathological changes such as astrocytosis, microgliosis and the presence of tau protein will contribute to improve the accuracy of differential diagnoses. PIB may become an important tool in this approach.
Fig. 16- PIB standardized uptake values (SUVs) of four representative subjects. FTD, PIB-negative. FTD, PIB-positive. AD: scan of a typical patient with Alzheimer's disease. SUVs were obtained using the time interval 40–60 min.

Fig. 17- PIB retention in FTD (n = 10), AD (n = 17) and HC (n = 8) expressed as ROI/reference region (cerebellum) as means ± SD. *Statistically significant differences between FTD and AD (p < 0.00625). There was no statistically significant difference between FTD and HC. Occ = occipital cortex, Front = frontal cortex, Par = parietal cortex, Temp = temporal cortex, Put = putamina, Thal = thalami, WhM = cerebral white matter.
Paper VI. PIB imaging in patients with Parkinson’s disease: Preliminary results.

Background

PIB is a sensitive marker of amyloid in AD, but its specificity has not been fully evaluated. Vascular amyloid-beta deposition is common in PD and alpha-synuclein, the major component of the Lewy bodies in PD, forms amyloid fibrils. The predominant protein of the Lewy bodies that characterize PD, alpha-synuclein, has a high propensity to aggregate into beta-sheet-rich fibrils forming typical amyloid (Conway et al., 2000). Binding of Thioflavin T to alpha-synuclein fibrils enhances its fluorescence emission intensity similarly to A-beta fibril binding. The specificity of PIB binding has not been studied in detail. Thus, PIB PET images may include alpha-synuclein pathology. Lewy bodies are present in most AD cases in postmortem studies (Hamilton, 2000) and alpha-synuclein pathology can be widespread in PD without cognitive impairment. This study, the first using PIB in PD, is a case report of five patients.

Patients and methods

We investigated five apparently cognitively normal PD patients with DOPA and PIB PET to confirm:

1) The presynaptic degeneration of the striatonigral pathway typical for PD and
2) To detect signs of amyloidosis.

The PIB PET results of PD patients were compared with those from six HCs: three women and three men, mean age 70 years, (range 63–76 years) and sixteen patients with AD (five women and eleven men), mean age 68 years (range 53–83 years) from the follow-up group in (III).

Results

All PD patients had reduced DOPA uptake in the putamen consistent with PD. PIB retention differed significantly between groups in all regions except white matter, pons and cerebellum. None of the PD patients had increased PIB retention. PIB retention in PD was lower than in controls in the cingulus posterior, frontal and parietal cortex.
In conclusion, we found no increase in PIB retention in early stage PD when compared with healthy controls.

Fig. 18- Parametric images of PIB (ROI/ref) displaying similar retention in PD and controls (HCs) but increased retention in AD. To the bottom right, DOPA: Patlak slope image of one of the patients with Parkinson’s disease (PD) showing decreased dopa-decarboxylase activity in the putamen.
Paper VII. Imaging astrocytosis with PET in Creutzfeldt-Jakob disease: Case rapport with histopathological findings.

**Background**

In a previous study (I), patients with suspect CJD were examined with PET combining DED and FDG in an attempt to detect astrocytosis and neuronal dysfunction, two of the hallmarks in CJD. Increased DED uptake with pronounced hypometabolism matching the areas with high DED retention was found in the fronto-parieto-occipital areas and cerebellum of patients with confirmed CJD. However, the temporal lobes did not present such a pattern. In six of the fifteen examined patients the autopsy was performed but a strict comparison between the PET results and the histopathology could not be performed.

In this study (VII), a patient with suspect CJD was examined with PET using DED and FDG. Shortly after the examination, the patient died and an autopsy could be performed.

**The patient**

The patient was a 64-year-old man with symptoms of rapid onset dementia and myoclonic jerks. Examination of the cerebrospinal fluid was normal with regard to cells and protein. MR of the brain was unremarkable. Repeated EEG investigations were characterized by bilateral symmetric triphasic waves with a frequency of 1 Hz with slow background activity of delta type 2-2, 8 Hz. The condition rapidly deteriorated and five weeks after taking ill the patient died.

The autopsy was performed and the brain was put in fixative material and frozen in $-80^\circ$C. After about seven weeks of fixation in 4 % formaldehyde ten different regions of the brain were sampled according to the results in the PET-study where prominent radioactivity for the DED tracer had been found. For most of these regions the contralateral area was also collected.

Immunostaining with antibodies against glial fibrillary acidic protein (GFAP, from DAKO) was also performed for the separately sampled material.

Analyses in a piece of frozen brain tissue were performed at the National CJD Surveillance Unit (University of Edinburgh, Western General Hospital) to detect the presence of protease resistant PrP.
Results

The results of the examinations with DED and FDG in this patient showed a pattern similar to that found in the brain of the CJD patients from the first study (I).

Increased DED with decrease FDG uptake were found in the cerebellum. Low glucose uptake with concomitant increased DED binding was observed in the left hemisphere. In spite of the low glucose uptake, this pattern was not found in the right hemisphere. Like in the cases from study (I) low FDG and high DED was found in the frontal and parietal cortices and the cerebellum. The pattern with high DED-FDG ratio was not found neither in the left temporal lobe or the right cerebral cortex.

The regions with increased DED activity in the PET study correlated very well with prominent gliosis microscopically. In general, the immunoreactivity for GFAP in the cerebral cortex seemed to be more pronounced in the left hemisphere than in the right. The Western blot analysis confirmed the presence of protease-resistant PrP with a Type 1 isotype, characteristic of sporadic CJD.

The relation between enhanced DED binding and the presence of reactive astrocytes as revealed by the GFAP immunostaining was evident.

![PET image of the brain of a patient with CJD obtained with DED. Histopathology indicates clear correlation between high DED binding and reactive astrocytosis revealed by GFAP immunostaining.](image)

Fig. 19- PET image of the brain of a patient with CJD obtained with DED. Histopathology indicates clear correlation between high DED binding and reactive astrocytosis revealed by GFAP immunostaining.
Closing remarks

Discussion

**FDG and DED**

The combination of DED, indicating reactive astrocytosis and FDG, showing changes in the neuronal function, allows us to differentiate between diseases with simultaneous astrocytosis and neuronal death from diseases with astrocytosis and concomitant increased glucose uptake indicating inflammation.

This is important because today there is no cure for CJD but the other diseases can be treated. Inflammation can be produced by an exogenous (bacteria, virus, etc.) or an endogenous agent (autoimmune diseases or paraneoplastic limbic encephalitis) (Corsellis et al., 1968) that is caused by anti-neuronal antibodies produced by body tumors. In these patients the clinical symptoms can appear as dementia of rapid onset. Some times the laboratory tests can help in the diagnosis, but in other cases they cannot help. The different patterns obtained with PET and appropriated tracer combinations allow a differential diagnosis. It is important to not only exclude CJD but also suggest other diagnoses.

In one patient with rapid dementia onset and symptoms similar to those found in CJD high FDG uptake was observed in the limbic system. Especially affected were the regions of the medial temporal lobes including the amygdala, the hippocampus and the parahippocampus (Engler et al., 2003). This uptake was different to the pattern obtained with FDG found in patients with confirmed CJD (Engler et al., 2003). Paraneoplastic limbic encephalitis was suspected, a whole body PET was performed and a tumor was found in the left lung.

An autopsy of the patient revealed an adenocarcinoma of the lung and tumoral antibodies against the brain were demonstrated. The PET examination could quickly distinguish this patient from the other patients with CJD.
In this patient, the quantitative FDG examination proved sufficient to make the differentiation.

![Fig. 20- Patient with suspect CJD. The patient however, had paraneoplastic limbic encephalitis. Left, coronal view: high FDG uptake bilaterally in the medial temporal cortex. Center and right: transaxial view. The FDG pattern is different to that seen in confirmed cases of CJD.](image)

Other patient with suspect CJD had a glucose uptake pattern similar to that found in AD. The metabolism was bilaterally decreased in the temporoparietal regions, but it was conserved in the central parts of the brain and the occipital cortex.

The FDG examination indicating hypometabolism in the parietotemporal areas with conserved metabolism in basal ganglia, cerebellum and sensory motor cortex suggested the possibility that the patient had AD instead of CJD, but further investigations (biopsy of a salivary gland) revealed Sjögren’s disease.

Treatment with corticoids had a dramatic effect in the patient’s symptoms.

Parieto-occipital hypometabolism is a conspicuous finding observed mainly in MRI-negative neuropsychiatric systemic lupus erythematosus (SLE). As this cerebral region is located at the boundary of blood supply of all three major arteries, it could be the most vulnerable zone of the cerebrum and may be affected at early stages of the cerebrovascular disease (Otte et al., 1997). An autoimmune disease, producing inflammatory meningo-encephalitis or a perivascular inflammation that affect this region might create a pattern of glucose uptake which is not possible to differentiate with PET from AD. Patients with Sjögren’s disease can present with symptoms of AD (Caselli et al., 1993).

It is important to differentiate between these diseases because a dementia with an onset corresponding to CJD or AD may be caused by immunological mechanisms (SLE, Sjögren disease, paraneoplastic phenomenon) and can be treated. The result, particularly in the case of Sjögren’s disease or SLE, can be dramatic, reversing a dementia condition in hours by administration of corticoids and cyclophosphamide (Caselli et al., 1993). Even dementias pro-
duced by antineuronal tumor antibodies can be reversed by the extirpation of the tumor (Provenzale et al., 1998).

In paper VII in a case of CJD, we demonstrate the congruence between pathological findings and PET results as expressed by the combination DED/FDG.

In the case of rapid onset dementias, a brain investigation with FDG could be followed by a scan of the whole body to quickly eliminate the possibility of the presence of an unknown primary tumor producing antineuronal antibodies.

If a brain scan shows hypermetabolism and the body scan is negative, autoimmune diseases or infectious diseases may be suspected. If the brain scan indicates asymmetric hypometabolism, the examination with DED can reveal high ratio DED/FDG suggesting CJD. Hypometabolism similar to that found in AD, but negative to PIB retention, may be caused by a disease affecting the vascular system.

**FDG and PIB**

The combination of FDG and PIB applied to our studies has helped us to understand better the dynamic process in the *in vivo* amyloid formation.

The deterioration in cognition seems related to the decrease in the brain’s glucose metabolism but not directly to the increase in amyloid depositions. The accumulation of amyloid in the brain seems to be an early process in the development of AD that increases to a certain level (possibly many years before the debut of the symptoms) and then reaches equilibrium between aggregation and degradation (Engler et al., 2006).

The glucose uptake decreases slightly in the beginning of the disease, reaching a critical point in which it becomes pronounced enough to be detected by PET FDG.

Because the metabolic changes occur later in the development of the disease, FDG can be used to confirm a diagnosis when the symptoms indicate a dementia disease. The tracer however, is not able to discriminate between healthy persons and AD patients in the early stage of the disease in asymptomatic individuals, whereas PIB offers the possibility to detect amyloid depositions when the symptoms are not evident.

FDG in combination with PIB increases the possibility to perform differential diagnoses. If the PIB examination is negative and FDG reveals a pattern
similar to that found in AD, the possibility is that a disease with vascular anatomopathological substratum (autoimmune diseases?) underlies the symptoms.

Several questions arise from the results presented in this thesis.

1) Is the presence of amyloid in the brain a process that can be related to aging without consequences for normal functions?
   It have been suggested a commonality in the pathologic processes that lead to neurofibrillary tangles and amyloid plaques in both aging and AD (Arriagada et al., 1992).

In a study performed in 1999, neurofibrillary tangles were found in the brain of all 39 nondemented persons examined, especially in the hippocampal and parahippocampal areas. The average tangle concentration was found to increase exponentially with age. In contrast, plaques were absent in some of the patient brains up to 88 years of age (Price and Morris, 1999). Other nondemented patients presented with widely distributed neuritic as well as diffuse plaques throughout the neocortex and limbic structures. It has been proposed that they represent "preclinical" AD (Price and Morris, 1999). We suggest that the presence of amyloid is always a sign of degeneration and the depositions will disturb and injure the brain, causing dementia.

Like a city in which the recollection of refuse has been stopped and the garbage is blocking the streets preventing cars from circulating, the amyloid depositions and neurofibrillary tangles block interneuronal communication.

The brain has a highly developed capacity to compensate damage produced by the presence of strange substances accumulating in cytoplasm and interstitium and perturbing functions. This capacity to compensate is not exclusive to the brain. All the organs in the human body have a reserve that allows normal functions when the tissue is affected by a disease.

We have seen in PET clinical routine extensive tumors invading a whole brain hemisphere before they cause symptoms. Symptoms of PD appear when the degeneration of the presynaptic pathway has reach 30-50% of the striatonigral component. The diagnosis with PET can often be made because the damage is extended enough.

We suggest that the brain compensates the slow and progressive biochemical and mechanic damage originated for the increasing presence of amyloid depositions and neurofibrillary tangles until a critical point when the collapse of the function is a fact without the possibility of return.
If the person having amyloid depositions could live long enough she or he should develop AD.

Education, and aspects of occupational experience have been indicated as factors that may delay the clinical manifestation of AD (Stern et al., 1995). Other researchers suggest that the effect of education is modest (Fritsch et al., 2001).

The mechanism underlying such a delay could be the presence in educated or trained people of more “activated” neurons from the “reserve pool”, which could supply the effects of neuronal death.

2) Not all patients fulfilling international criteria for AD show the presence of amyloid depositions.

A discussion concerning new classifications of dementia diseases seems necessary.

Frontotemporal dementia is a syndrome comprising many different entities with a different anatomopathological background.

We suggest that the diagnosis AD must be changed to “Alzheimer’s syndrome” or “Cognitive Deficit of Alzheimer’s type” (CDAT) because there are many diseases expressing the same symptoms and producing similar changes in glucose uptake.

The diagnosis AD must be established in patients with “Alzheimer’s syndrome” having positive PET examinations with PIB or other well-tried amyloid markers. The absence of amyloid depositions is the most questioning result to the diagnosis AD.

We have examined patients with “Alzheimer’s syndrome” without amyloid depositions and it is possible that these patients have, for example, a disease with a different pathological basis (vasculitis?) that is necessary to investigate.

Physicians do not treat only symptoms. We need to clarify the underlying pathology to find the proper treatment for patients expressing similar symptoms.

MCI is considered a transitional stage between normal aging and dementia, especially in early AD.

It is known that MCI may have a multitude of causes, including AD and other forms of dementia as well as depression and various physical disorders. Because MCI is a common syndrome, there is a great need to establish methods for predicting progression to AD.
In paper IV we demonstrate that PIB retention in MCI patients is intermediate between HCs and AD patients and that there are MCI patients with high or low PIB retention. Eleven of twenty-one MCI patients showed high PIB retention in the frontal, parietal and temporal cortices.

The seven MCI patients that at follow-up converted to AD all showed significant high levels of amyloid in the brain as compared with MCI non-converters and HCs.

These seven converting MCI patients significantly differed as a group from the non-converters regarding a higher proportion of ApoE-4 carriers, impairment in episodic memory, lower CSF A-beta 1-42 and higher PIB retention.

In general, we did not detect in this study a decreased glucose metabolism in cortical brain areas as earlier reported in MCI patients (Anchisi et al., 2005; Chetelat et al., 2003); Nevertheless, in some individual MCI patients, decreased rCMRglc was found in such areas as the parietal and temporal cortices.

In the table below it is possible to observe that the non-converters in the MCI group had significant higher glucose uptake than AD patients whereas for the converters the difference was less.

<table>
<thead>
<tr>
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<th>Fronta</th>
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<tbody>
<tr>
<td>AD</td>
<td>1.4 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.2</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>MCI</td>
<td>1.7 ± 0.2 *</td>
<td>1.7 ± 0.3 *</td>
<td>1.3 ± 0.2 *</td>
<td>1.7 ± 0.3 *</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>MCI-nc</td>
<td>1.5 ± 0.3</td>
<td>1.5 ± 0.6</td>
<td>1.2 ± 0.2</td>
<td>1.5 ± 0.5</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>MCI-c</td>
<td>1.5 ± 0.5</td>
<td>1.7 ± 0.2 *</td>
<td>1.2 ± 0.1 *</td>
<td>1.7 ± 0.2 *</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>HC</td>
<td>1.5 ± 0.3</td>
<td>1.5 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>1.5 ± 0.3</td>
<td>0.6 ± 0.1</td>
</tr>
</tbody>
</table>

* Statistical significance between MCI and MCI non-converters compared to AD patients (p<0.01), corrected for multiple comparisons with Bonferroni correction.

Tab. 2.

In clinical routine we have observed increased glucose uptake in some of the patients with the diagnosis MCI. We have previously discussed that the reaction of neurons to an unspecific injury was hypermetabolism. Injured neurons increase the glucose uptake by an enhanced glycogenesis, possibly as a form to economize energy necessary to repair the damages (Kreutzberg and Emmert, 1980; Smith et al., 1984).

Hypermetabolism in patients with MCI may indicate some inflammatory process that is affecting the brain. These patients, however, might be negative for PIB deposition.

Diseases such as autoimmune reactions may produce a perivascular disease or a vasculitis, which, in the acute period, may course with slight hypometabolism, but in the chronic period, produce hypometabolism. Because the more sensible part of the brain is the boundary of blood supply between the cerebral arteries, the temporoparietal and the frontotemporal regions will be
affected. In a more advanced stadium the patient may have symptoms of AD and the FDG may indicate a pattern similar to that found in this disease.

The MCI patients classified as converters had amyloid depositions but the FDG uptake was similar to the controls. The possibilities are that they had increased glucose uptake in the beginning of the amyloid depositions that normalized later; however, we suggest that amyloid depositions do not increase the glucose uptake because they occur slowly under years of progress as a silently, devastating chronic deterioration.

More studies are needed to confirm or question these interpretations. Based on our findings we suggest that diseases with cognitive impairment causing hypermetabolism in the brain and without amyloid depositions are of a more acute nature, affecting the corridors between arteria cerebri anterior media and posterior, possibly caused by an autoimmune or inflammatory base. The symptoms may be reverted spontaneously or by treatment.

The AD probably courses with normal metabolism until the damage, provoked by the accumulation of plaques and tangles, reaches the critical point in which the neurons can no longer compensate the damage, at which time the symptoms appear.

**PIB and DOPA**

Amyloid-containing deposits are a defining neuropathological feature of a wide range of dementias and movement disorders. In (VI) we concluded that he normal PIB results in PD do not exclude the possibility that PIB binds to Lewy bodies, Lewy neurites or Amyloid-beta in PD.

Recently, ten patients with Parkinson's disease with dementia (PDD) were examined (Maetzler et al., 2008). Only two PDD patients displayed increased PIB binding to cortical amyloid comparable to AD patients. The other eight patients showed control-like cortical findings but elevated PIB binding in the pons and mesencephalon. It has been suggested that in addition to nonspecific binding, PIB uptake in the brainstem may also reflect PDD-related amyloid (Maetzler et al., 2008).

Another recent study demonstrated the presence of a high affinity binding site for benzothiazole derivatives, including [3H]-PIB, on alpha-synuclein (AS) filaments generated in vitro (Ye et al., 2008). However, no discernible interaction of [3H]-PIB was detected with amygdala sections from PD cases containing frequent AS-immunoreactive Lewy bodies and related neurities. These findings suggest that the density and/or
accessibility of AS binding sites in vivo is significantly less than those associated with amyloid-beta peptide lesions. Lewy bodies pathology is therefore unlikely to contribute significantly to the retention of PIB in PET imaging studies (Ye et al., 2008).

**PIB and CBF**

In previous studies we have used FDG images based on a 40-60-minute summation as a template to realign PIB images because they give a good anatomic subtract allowing the definition of different brain regions, (Engler et al., 2006; Engler et al., 2003; Engler et al., 2008; Forsberg et al., 2007; Klunk et al., 2004). An automatic procedure has been applied to transfer the set of ROI from FDG images to PIB images(Andersson and Thurfjell, 1997).

In the case of PIB, it is difficult to define the brain cortex in HCs in a late PIB summation (40-60 min) because the tracer is retained only in the white matter disturbing the automatic realignment of images.

In the first study performed in Uppsala (Klunk, 2004), it was found that an early summation of the PIB activity frames (6 min) gave images that could be compared with later images of the FDG summation. The early PIB summation images were realigned to the late FDG images and the rest of the PIB activity frames (7-60 min) were “co-resliced” using the realigned early summation as template. In this way the ROIs drawn in the late FDG activity image could be transferred automatically to the later (40-60 min) PIB summation.

The similarity of the FDG images with the images of the early PIB summation suggested a positive relationship between the cumulative uptake of PIB in a short time interval following bolus administration and CBF.

A kinetic model with one reversible and one irreversible tissue compartment and three rate constants was used to investigate the PIB net accumulation (K_{acc}) and unidirectional influx (K_1) across the BBB in HC and AD-patients (Blomqvist et al.).

This is in contrast to previous studies in which measures of PIB retention were obtained from reversible kinetic models (Lopresti et al., 2005; Price et al., 2005). The blood input function was determined with arterial sampling in 4 HC-subjects and 4 AD-patients. Parametric maps of K_{acc} = K_1k_3/(k_2+k_3) and K_1 were created using a linear algorithm. The results were compared with the ratio between the uptake in a target and a reference region in a late interval, in HC and AD patients from studies (II
and III). The parameter $K_{acc}$ and the late uptake ratio were found to have similar regional distributions.

The rate constant $K_1$ for PIB was found to be comparatively large, demonstrating high extraction of PIB into the brain tissue and indicating that this parameter might reflect CBF. The possibility to replace $K_1$ by a simple index of the PIB uptake in an early time interval following tracer administration was explored but needs further validation.

The regional $K_1$ values were lower in the AD patients than in the HCs. Most AD patients had higher $K_{acc}$ values than the HCs in cortical areas, but some patients had values similar to HCs (fig. 21). However, these patients had lower $K_1$ than the HCs and did not differ from the other patients in this respect.

Furthermore, the effect on $K_1$ and $K_{acc}$ of CBF changes were investigated in an animal study using an anesthetized monkey and the following study design. PET scans were performed at baseline and 2 h later by after increasing PaCO$_2$ with the aid of respiratory control.
At both the experimental conditions 2 tracers $[^{15}\text{O}]{\text{H}_2}\text{O}$ and PIB was performed within a short time interval. The results are summarised in Figure 22.

In conclusion, the unidirectional influx rate constant $K_1$ of PIB was found to be a good index of CBF. The results indicate that the combined information of unidirectional influx and net accumulation of PIB might differentiate between groups of patients with an AD diagnosis (Blomqvist et al.).

![Fig. 22- Parametric maps of CBF, $K_1$ and $K_{acc}$ in one slice of a monkey brain under baseline conditions and after increased PaCO2. Changes in PaCO2 increased the CBF and $K_1$ in values of the same magnitude. These results indicate that $K_1$ for PIB to a largely reflects CBF. The increase in $K_{acc}$ as an effect of the increase in CBF was detectable, but small compared with the increase in $K_1$.](image)

Conclusions

In this thesis we have described anatomical and physiopathological changes produced in vivo in neurodegenerative diseases using PET imaging technology based on the concept of multi-tracers.

Contrary to the micropathology analysing a limited post-mortem or biopsy tissue, one PET acquisition gives an image of the whole system (e.g., the brain and the cerebellum) in vivo.

Signs of neuronal dysfunction, astrocytosis, axon degeneration and protein aggregation (amyloid) have been found in patients with different neurodege-
neurodegenerative diseases. Different pathologies can produce similar symptoms which can confuse clinical diagnosis.

PET and the multitracer combinations have revealed different patterns in diseases presenting with similar symptoms. An increasing understanding of these characteristic changes may contribute to improve the accuracy of clinical diagnoses.

The results contribute to a better understanding of the mechanisms underlying brain diseases, bringing up discussions around the use of the PET technology to improve patient management.

Consequently, the knowledge emerging of neuroimaging pathology, in combination with other disciplines, can open ways to new classifications of neurodegenerative diseases. The detection of specific pathological changes with PET may contribute to follow-up treatments in a more effective way.

Future work

This thesis is based on the idea to develop new methods that directly or indirectly describe in vivo, the anatomo-physiopathological changes produced in the brain in neurodegenerative diseases. With this approach, we can obtain a better description of different diseases, confirming or questioning the clinical diagnoses and widening our understanding of the mechanisms underlying neurodegeneration. The incipient knowledge emerging from the new neuroimaging pathology in combination with other disciplines can help bring about a new classification of dementias and neurodegenerative diseases based on an “in vivo” pathology.

The challenge is to search and label new molecules that can demonstrate underlying pathological changes. The use of selected tracers and combination with other biomarkers will most likely improve the sensitivity and the specificity in the diagnoses.

The microdosing concept will play an important role in the development of new tracer tools for imaging pathology as was shown in paper II. It also opens up the possibility to explore toxicological effects of drugs from bacteria to a human being, allowing a fast translation from simple to more complex biological forms and speeding up drug development in many aspects: understanding drug mechanisms, finding correct doses, etc. It is clear that
regulatory bodies are being increasingly aware of the importance of performing risk by differentiating between clinical science and clinical use (Bergstrom et al., 2003)

The combination of PET with other imaging technologies including computerized tomography (CT) and magnetic resonance imaging (MRI) will increase the diagnostic power of the imaging technology.

The aim of the imaging pathology will be to find new ways to learn about the mechanisms underlying normal and pathological biology and translate this knowledge to the clinical field in a cost effective way in order to improve management of patients in a more individual form.
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Denna avhandling består av studier där man har undersökt patienter med olika former av neurodegenerativa sjukdomar: Creutzfeldt Jacob sjukdom (CJD), Alzheimer sjukdom (AD), mild kognitive svikt (MCI), frontotemporal demens and Parkinsons sjukdom (PD). Undersökningarna genomfördes med Positron Emission Tomography (PET) och en kombination av olika markörer: $^{15}$O-water, $N$-[11C-methyl]-L-deuterodeprenyl (DED), $[^{18}$F]$ 2$-fluorodeoxyglucose: (FDG), $N$-methyl-$[^{11}$C]2-(4-methylaminophenyl)-6-hydroxybenzothiazole (PIB) och $L[^{11}$C]-3,4-dihydroxiphenylalanine (DOPA).

De markörer vi har använt och deras kombinationer valdes i syfte att i hjärnan hitta tecken på neuronal dysfunktion, astrocytos, axondegeneration eller protein anhopningar (amyloid) som tyder på en specifik sjukdom och kan bidra till att ställa differentialdiagnoser. Patienternas resultat från PET-undersökningarna har jämförts med resultaten från friska frivilliga. Studier på djur gjordes för att komplettera informationen. I några fall har resultaten från PET-undersökningarna jämförts med resultat från obduktioner.

Till skillnad från mikropatologi, som visar en begränsad del av vävnaden tagen via en biopsi eller vid en obduktion ger en PET undersökning en bild av hela hjärnan. Denna form av ”imaging” patologi med PET görs “in vivo”, d.v.s. hos levande människor, men undersökningen är ofarlig för patienten.

Denna avhandling försöker uppmuntra utvecklingen av nya markörer, nya kombinationer av markörer och metoder som direkt eller indirekt beskriver in vivo, de anatomiska och fysiopatologiska förändringarna som sker vid neurodegenerativa sjukdomar.

En bättre beskrivning av olika sjukdomar kan göras i syfte att bekräfta eller ifrågasätta kliniska diagnoser och utvidga vår kunskap om mekanismerna bakom neurodegeneration.
Olika patologier kan orsaka likartade symptomen och förvirra kliniska diagnoser. De använda radiotracer kombinationerna har gett skärpt diagnostisk styrka och givit ny förståelse för sjukdomsförloppet.

Den begynnande kunskapen från en ny ”neuroimaging” patologi kombinerad med andra discipliner kan öppna vägar till nya klassifikationer av demens och neurodegenerativa sjukdomar baserad på en ”in vivo” patologi.
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