SUMMARY, DISCUSSION AND PERSPECTIVES

The present thesis aims at being an aid in clarifying the pathophysiology of Alzheimer’s disease by means of functional radionuclide imaging, this in order to reveal certain aspects of its disease process and finally aid its therapeutic management.

The option of functional radionuclide imaging being either positron emission tomography (PET) or single photon emission computed tomography (SPECT) was chosen because of its properties of allowing the non-invasive detection of specific cells and tissues involved in the pathophysiological process all depending of the radioligand of choice. This whereas the capability of structural imaging lies in visualising (mostly non-specific) structural changes and describing their detailed spatial relationship. As such, in general, the disease process must be already at an advanced stage before it can be depicted by structural imaging modalities, giving rise to its poor pathophysiological sensitivity at the early stages (when anatomical changes are not detectable yet). Also, for chronic processes like Alzheimer’s disease, structural changes may be detectable that do not reflect the actual state of disease activity.

As for the newer structural or metabolic imaging tools however, for example proton MR spectroscopy seems more specific in assessing the pathophysiological process by means of for example measuring neurocellular viability or neuroaxonal integrity (N-acetyl aspartate) and glial (choline and especially myo-inositol) markers which, according to some authors, could be able to predict and monitor the response to therapy, giving rise to an individual optimised treatment. Likewise, magnetisation transfer imaging (MTI) seems valuable in measuring brain involvement and provides information about brain damage with increased pathological specificity detecting subtle microscopic abnormalities in brain tissue, which go undetected with conventional scanning. As such, a low MTI ratio indicates a reduced capacity of the macromolecules in brain tissue membranes to exchange magnetisation with the surrounding water molecules, reflecting damage to these membranes. Also, coefficients of water diffusion can be measured with diffusion tensor MRI where an increased water diffusivity reflects a disintegration of brain tissue compartments. As for functional magnetic resonance imaging, some studies suggest that fMRI can be used to diagnose Alzheimer’s disease in its earliest stage, detecting subclinical deterioration of the memory function and may be useful to predict the future decline of memory in people with genetic or other risk factors.

This thesis is divided in two parts. One part (chapter two to six) deals with the neuroinflammatory hypothesis while the second part (chapter seven) deals with the serotonergic hypothesis in Alzheimer’s disease.

In chapter two, in an introductory section, the epidemiology, clinical features, pathophysiology, and treatment of Alzheimer’s disease is reviewed. A second section discusses three topics. Firstly, the basic neuroinflammatory mechanisms and its biochemical characteristics, the potential
neurotoxic mediators during activation of microglia (i.e. the brain resident macrophages), and the current status of inflammation with respect to neurodegeneration is reviewed. Indeed, in the past decades, our understanding of the central nervous system has evolved from one of an immune-privileged site, to one where inflammation is pathognomonic for some of the most prevalent and tragic neurodegenerative diseases. Inflammation, whether in the brain or periphery, is almost always a secondary response to a primary pathogen. In head trauma, for example, the blow to the head is the primary event. What typically concerns the neurologist and neurosurgeon more, however, is the secondary inflammatory response that will ensue and likely cause more neuron loss than the initial injury. Current research indicates that diseases as diverse as multiple sclerosis, stroke and Alzheimer’s disease exhibit inflammatory processes that contribute to cellular dysfunction or loss.

Secondly, the immune-related pathogenesis of Alzheimer’s disease is thoroughly reviewed. It is clearly shown that inflammation contributes to neurodegeneration in Alzheimer’s disease, not simply as a secondary phenomenon, but primarily as a significant source of pathology. As such, visualising this neuroinflammation would be of interest, firstly for clarifying the pathophysiology, secondly for selecting patient subgroups that are more eligible for anti-inflammatory treatment(s) and finally, for monitoring patients during trials with these anti-inflammatory agents.

Thirdly, the currently available neuroinflammatory imaging modalities, both structural and mainly functional, are critically reviewed and discussed. It is shown that, whereas structural imaging shows merely late anatomical consequences of an inflammatory response, functional imaging is a strong potential candidate to bridge the gap between in vitro and in vivo knowledge. In this perspective, a number of radioligands have been recently explored which allow the early in vivo visualisation of inflammatory responses, and, as such, open a promising window on both understanding as well as possible clinical management of inflammatory neurodegenerative disorders.

In the light of the aforementioned goals of neuroinflammatory imaging, a first attempt is made with $^{57}$Co and SPECT. Indeed, previous studies showed that $^{57}$Co SPECT is able to visualise inflammatory lesions, probably by means of the final common pathway of the Ca$^{2+}$-homeostasis disturbance in both neuronal degeneration and inflammation. Chapter three describes this attempt to visualise inflammation in vivo in Alzheimer’s disease patients by detecting $^{57}$Co SPECT changes and investigates whether $^{57}$Co SPECT can generate additional information which cannot be obtained with conventional neuroimaging techniques or neuropsychological testing (NPT). As such, $^{57}$Co SPECT data are compared with data obtained from Magnetic Resonance Imaging (MRI), perfusion SPECT, and neuropsychological testing, and finally findings in Alzheimer’s disease patients are compared with patients suffering from vascular dementia and frontal lobe-type dementia. It is shown that $^{57}$Co SPECT scans were not able to show any regional raised uptake and in this way ongoing tissue decay or inflammation, irrespective of the type of dementia, depth or extent of perfusion defects, presence of atrophy on MRI, or the results of NPT. In an attempt to explain these results it is concluded that the limitations of $^{57}$Co SPECT are manifold. Due to the long physical half-life of 270 days, only a limited dose can be injected which is responsible for the low count rate and the resulting low statistics. Moreover, whether $^{57}$Co visualises specific aspects of neuronal damage or blood-brain barrier integrity
is still uncertain. To what extent $^{57}$Co really visualises calcium-mediated processes (in vitro and more importantly in vivo), and therefore resembles identical molecular uptake mechanisms, has yet to be determined, although the cerebral uptake of intravenously administered radioactive $^{45}$Ca and $^{60}$Co in neuronal damage is highly similar. Finally, its exact cellular site of accumulation of radioactivity is, as yet, not known.

After this, the development and validation of another radioligand for the visualisation of neuroinflammatory lesions is described. Indeed, the peripheral benzodiazepine receptor (PBR) was reported to reflect neuro-inflammation damage by an upregulation on activated microglia. As such, radiolabelled PK11195, a high affinity ligand for the PBR, could be an ideal candidate. In chapter four, the biodistribution and dosimetry of $^{123}$Iiodo-PK11195, a potential SPECT radioligand is described in humans. For this, sequential whole body scans were performed up to 72 hours post-injection of $^{123}$I labelled iodo-PK11195. Multiple blood samples were taken, and urine was collected to measure the fraction voided by the renal system. Decay corrected regions of interest of the whole-body images were analysed, and geometric mean count rates were used to determine organ activity. Organ absorbed doses and effective dose were calculated using the MIRD method. It was seen that $^{123}$Iiodo-PK11195 was rapidly cleared from the blood, mainly by the hepatobiliary system. Approximately 22% was voided in urine after 48 hours. Average organ residence times were 0.74, 0.44, and 0.29 hrs for the liver, upper large intestine, and lower large intestine respectively. The testes received the highest dose, 109.4 $\mu$Gy/MBq. All other organs investigated received doses of less than 50 $\mu$Gy/MBq. The effective dose was 40.3 $\mu$Sv/MBq. It was concluded that $^{123}$I labelled iodo-PK11195 is a suitable agent for the visualisation of the PBR and indirectly for the imaging of neuroinflammatory lesions. Taking into account the radiation burden of 7.46 mSv following an administration of 185 MBq, a $^{123}$I labelled iodo-PK11195 investigation should as such be considered as an ICRP risk category IIb investigation.

Thereafter, in chapter five, the validation of radiolabelled PK11195 as a neuroinflammatory tracer is described, this by applying the radiolabelled ligand in multiple sclerosis, the prototype neuroinflammatory disorder with its pathophysiological involvement of microglia. In an introductory section, the history, epidemiology, pathophysiology, clinical features and disease course, and treatment of multiple sclerosis is reviewed. In a second section, the visualisation of activated microglia in multiple sclerosis patients using $^{11}$C)PK11195 and PET is studied. For this, semiquantitative $^{11}$C)PK11195 uptake values were assessed for multiple sclerosis patients compared to healthy controls with a normalisation on cortical gray matter. It was found that the radioligand uptake in Gadolinium-lesions was significantly increased compared to normal white matter. Uptake in T2-lesions was generally decreased, suggesting a PBR downregulation. However, uptake values for T2-lesions increased whenever a clinical or MR-relapse was present, all suggestive for a dynamic process with a transient PBR upregulation. During disease progression, an increase of normal-appearing white matter (NAWM) radioligand uptake was found, propagating NAWM as the possible real burden of disease. It was concluded that $^{11}$C)PK11195 PET imaging has an additional value over MRI concerning the immuno-pathophysiological process and is able
to demonstrate inflammatory processes with microglial involvement in multiple sclerosis. In a third section of this chapter, brain atrophy and microglial activation was assessed in multiple sclerosis patients during different disease stages and the relationship between inflammation, atrophy, and clinical measures was investigated. It was found that atrophy was significantly greater in multiple sclerosis patients compared to age-matched controls. Moreover, a significant correlation was found between brain atrophy and both disease duration and disability, as measured with the expanded disability status scale (EDSS). For NAWM, microglial activation as measured with $[^{11}]\text{C}\text{PK11195}$ uptake increased with the amount of atrophy, while $T_2$-lesional $[^{11}]\text{C}\text{PK11195}$ uptake values decreased according to increasing brain atrophy. It was concluded that brain atrophy, correlating with disease duration and disability, is directly related to NAWM and $T_2$-lesional inflammation as measured by this PET probe for microglial activation.

Chapter six describes the assessment of microglial activation using radiolabelled PK11195 for SPECT in Alzheimer’s disease and its relation with findings obtained from perfusion SPECT and neuropsychological testing. For this, $^{[123]}\text{I}\text{iodo-PK11195}$ SPECT images were realigned into stereotactic space where binding indices, normalized on cerebellar uptake, were calculated. It was found that the mean $^{[123]}\text{I}\text{iodo-PK11195}$ uptake was increased in Alzheimer’s disease compared to controls in nearly all neocortical regions, however, statistical significance was only reached in the frontal and right mesotemporal regions. Significant correlations were found between regional increased $^{[123]}\text{I}\text{iodo-PK11195}$ uptake values and specific cognitive deficits as assessed with neuropsychological testing. It is concluded that $^{[123]}\text{I}\text{iodo-PK11195}$ is a cellular disease activity marker allowing the in vivo assessment of microglial inflammation in Alzheimer’s disease. Future optional work could be both at a technical and clinical level. Technically, a direct comparison between the PET and SPECT radioligand should be accomplished whereas feasible methods for absolute quantification of radioligand uptake should be further validated and implemented. From the clinical level, the inclusion of more patients at different stages of the disease, the comparison with proton MR spectroscopy for the measurement of glial markers, or the assessment of radioligand uptake changes during (anti-inflammatory) therapy could be very useful. Also, new ligands could be developed for the neuroinflammatory visualisation depending on the eventual development of the current anti-inflammatory treatment of choice. Ideally, this new radioligand should be rapidly cleared from blood and other non-target tissues and moreover, the interaction of the ligand and its receptor should be characterized by a high binding affinity and specificity (shown by autoradiography, receptor blockade, or studies with control agents). For neuroimaging studies in pathologies without blood-brain barrier breakdown (like Alzheimer’s disease), the ligand should be able to easily penetrate this blood-brain barrier.

In a second part of this thesis (chapter seven) dealing with the serotonergic hypothesis of Alzheimer’s disease, the role and distribution of serotonin (5-HT) type 2A receptors in healthy controls and Alzheimer’s disease patients is described. In an introductory section the role of serotonin in general and serotonin type 2A receptors specifically in cognitive and non-cognitive behaviour and its relation with Alzheimer’s disease in general and the inflammatory hypothesis more specifically is briefly
reviewed. In a second section, the imaging of the 5-HT$_{2A}$ system in healthy controls and Alzheimer’s disease patients is performed. Using $^{123}$I-5-I-R91150, a selective radio-iodinated 5-HT$_{2A}$ receptor antagonist and SPECT, the 5-HT$_{2A}$ binding potential (BP) in healthy volunteers and Alzheimer’s disease patients is calculated. For this, a semiquantitative analysis with normalisation on cerebellar uptake provided estimates of BP for 26 cortical regions of interest. An age-related decline of neocortical BP was found in healthy controls (11.6 % per decade). Compared to age-matched controls, a generally decreased neocortical BP in Alzheimer’s disease was seen with a significant regional reduction in the orbitofrontal, prefrontal, lateral frontal, cingulate, sensorimotor, parietal inferior, and occipital region. These results are in line with previous postmortem, in vitro, and PET findings. As such, the age-related decline highlights the necessity for matched advanced age study samples. The fact that the 5-HT$_{2A}$ receptor is differentially affected in Alzheimer’s disease patients compared to controls has implications for both its etiological basis and therapeutic management.

As presented in the aforementioned manuscripts, both neuroinflammatory and serotonergic imaging yield different specific regional changes in binding potential for Alzheimer’s disease patients, which raises the question of their (inter)relationship in terms of the Alzheimer’s disease pathophysiology. Unfortunately, for reasons of logistics, no study was performed where both radiolabelled PK11195 and $^{123}$I-5-I-R91150 was used in the same patients, however, one can assume that both populations included in these two studies are, however small, representative for a, if existing, true Alzheimer’s disease population making the differences in regional binding potential changes in fact true differences reflecting as such not differences in the characteristics of the patients included, but reflecting a different point of view or part regarding the Alzheimer’s disease pathophysiology. In fact, there are three options. Firstly, there is the notion of disease heterogeneity where questions have been raised about the existence of Alzheimer’s disease as a single nosological entity. Although many researchers have tried to unify the different hypotheses concerning the aetiology and pathophysiology of Alzheimer’s disease, many scientific arguments exist pointing to the possibility that there may not be just one Alzheimer’s disease we are looking at. Indeed, many authors have raised the option of Alzheimer’s disease patients with for example different neuropsychological or clinical profiles or different perfusion disturbances, not to mention the overlap both concerning risk factors and concerning clinical presentation between Alzheimer’s disease and vascular dementia. Secondly, there is the option of a chronology in the pathophysiology of Alzheimer’s disease in which different brain systems are more or less affected during the disease time course. In the light of the present thesis, the series of events presented here would be firstly the inflammatory response following a primary event (amyloid burden, …) followed by functional neurotransmitter or more specifically serotonergic changes finally followed by structural changes like atrophy. Thirdly, there is the option of a co-existence of pathophysiological changes at the same time with a different regional sensitivity explaining these differences in regional binding potential changes. Indeed, one can speculate why the sensorimotor cortex is spared regarding perfusion but heavily affected concerning the 5-HT$_{2A}$ system or why the mesotemporal area is largely affected by tissue loss and relatively spared concerning the serotonergic system.
To answer these and other questions it would be worthy to study in the same patients several pathophysiological points of view for example amyloidogenic, neuroinflammatory, perfusion, cholinergic, serotonergic and structural changes and this on different time points in order to label different pathophysiological mechanisms to different time courses of the disease. Ideally, this should also be done making use of different markers for measuring the same aspect of its pathophysiology, for example both neuroimaging (structural, functional, and metabolic), laboratory (serum, CSF, …) as post-mortem neuropathological markers. This certainly, in an era where more and more treatment is available in order to be of any help in deciding when to apply which kind of treatment.