1.

Introduction

Underlying mechanisms of Alzheimer's disease not completely understood

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive impairment in daily life and cognitive deterioration. For the clinical diagnosis of AD cognitive problems are most important; at least two cognitive domains should be impaired which cause problems in daily functioning [1]. The stage before AD, when problems within only one cognitive domain occur but do not interfere with daily life, is referred to as Mild Cognitive Impairment (MCI) [2]. MCI patients have a high risk (~50%) of developing AD [3]. Age is an important risk factor for developing AD [4]; most patients (90-98%) are diagnosed with AD after the age of 65. In such late-onset AD patients, memory problems are most prominent and predate decline in other cognitive domains [5]. In early-onset AD patients (age of onset <65 years old), memory problems appear less frequent [5,6]. At present, there is no cure for AD and current pharmacological treatment at best reduces the rate of decline [7]. Even though many studies have investigated the underlying mechanisms of AD, it is not exactly clear how neuropathological changes occur. The various neuropathological changes underlying AD seem to develop progressively [8] although the order of the changes is still a matter of debate and may differ between the several subtypes of AD. Several biomarkers reflecting these pathological changes can be used for AD research, both for diagnosis and for monitoring the disease. According to the commonly accepted amyloid hypothesis, amyloid pathology is the main factor driving AD pathogenesis [9]. This is followed by the formation of neurofibrilliary-tangles leading to neuronal dysfunction and eventually neuronal loss. Although it is evident that amyloid pathology is an important early marker of AD, its effect on brain function is not completely understood. In about 25% of healthy controls over 65, amyloid-plaque formation is already present, without accompanying apparent neuronal loss or cognitive problems [10]. Interestingly, these 'amyloid-positive' elderly subjects already show changes in neuronal function; synchronized brain activity is altered [11,12]. Some even found evidence that neuronal dysfunction precedes amyloid-plaque formation [13]. Insight into these early neuronal changes is crucial for understanding the mechanisms underlying AD, and ultimately, for improving early diagnosis of AD. For visualization of AD biomarkers several brain imaging techniques are available. Two techniques will be discussed in this thesis: Positron emission tomography (PET) and magnetic resonance imaging (MRI), including functional MRI (fMRI).

Positron emission tomography in Alzheimer's disease

With the PET tracer carbon-11 labelled Pittsburgh Compound-B ([^{11}C]PIB) [14], or the relatively new [^{18}F]-Flutemetamol tracer [15], it is possible to measure amyloid burden in-vivo. PET allows for visualization and quantification of a wide range of (patho)physiological processes, using different radioligands (PET-tracers) [16,17]. A PET-tracer is designed to target a specific site or mechanism of interest. Patients are injected with a small amount of radioactive labeled PET-tracer which is then visualized with a PET camera. With the amyloid PET tracers, it was found that amyloid-plaque accumulation increases in the preclinical phases of AD and stagnates in the clinical phase of the disease [18,19,20], and may therefore be useful as early biomarker. With the fluorine-18 labelled fluorodeoxyglucose ([^{18}F]FDG) PET-tracer, glucose metabolism can be measured [21]. Glucose metabolism of the brain

provides information on neuronal functioning in AD. Typically, reduced metabolism (hypometabolism) is observed in AD in the parietal cortex and in the precuneus/ posterior cingulate gurus, the latter is already present in the preclinical stage of AD [22].

Magnetic resonance imaging in Alzheimer's disease

Neuronal loss, or atrophy, can be visualized using structural MRI and is well validated in AD research [23]. Loss of brain tissue is most pronounced in the medial temporal lobe and parietal cortex in AD patients [24,25]. Atrophy of the brain follows a similar temporal pattern as hypometabolism, and continues to change substantially in the clinical phase of AD [8]. Indeed, volume loss has been found to correlate strongly with disease severity even at the later stages of the disease [26,23] and is considered a late biomarker [27]. Besides structural MRI, functional MRI (fMRI) is informative in AD, since it provides information on neuronal (dys)function. Changes in brain function measured with fMRI functional connectivity, and the association with other AD biomarkers, such as amyloid and glucose metabolism measured with PET, is the main focus of this thesis.

Functional connectivity in Alzheimer's disease

The blood-oxygen-level dependent (BOLD) contrast used in fMRI reflects synaptic activity through changes in blood flow and the oxygen-fraction in blood [28]. Changes in the BOLD signal over time are measured, in order to map brain activity associated with a certain task or during rest (resting-state fMRI). Resting-state fMRI has the advantage that it is noninvasive and does not require subject participation in cognitive demanding tasks [29]. Besides these practical advantages, functional connectivity has received increasing attention in the past few years since it provides important information about functional integrity of the brain. Spatially independent brain areas that show temporally correlated activity are considered to be functionally connected [30] (Figure 1).

These correlated patterns of BOLD signal between brain areas indicate consistent neuronal activity underlying communication of these regions [31]. Abnormal functional connectivity in AD might, at least in part, explain the cognitive decline seen in these patients [32,33]. Sets of brain regions that are functionally connected are regarded as intrinsic connectivity networks of the brain; so-called resting-state networks (RSNs) [34]. Several robust RSNs are consistently found and are linked to higher-order and more basic cognitive functions; such as executive control and visuo-constructive functioning, and sensory-motor and auditory processing [35; Figure 2].

Figure 1; Functional connectivity. Spatially distinct brain regions (top image in blue and green) with co-varying resting state BOLD fMRI signal (bottom image in blue and green) are considered to be functionally connected.

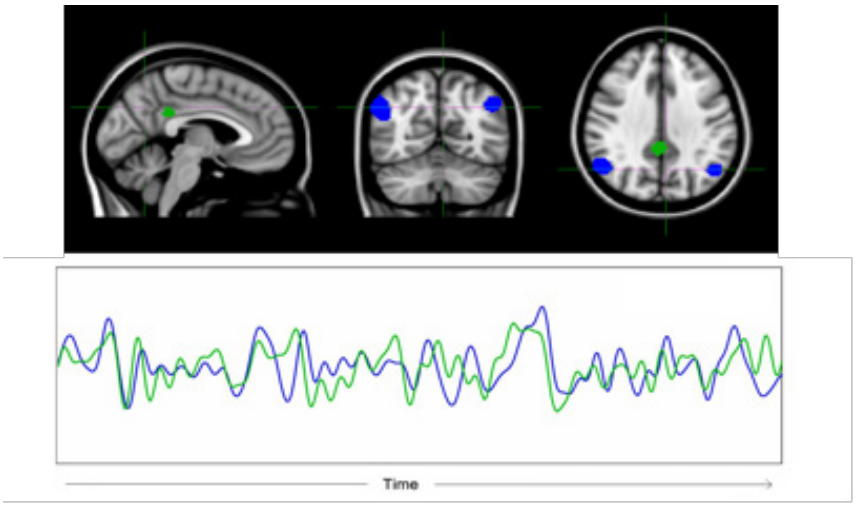
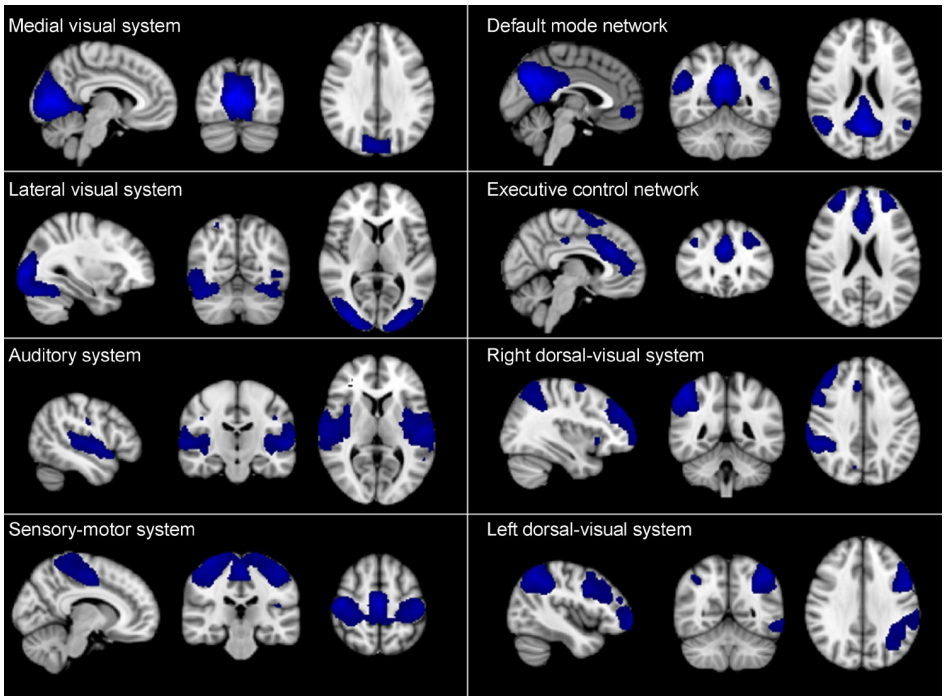


Figure 2; Resting-state network (RSN) maps.



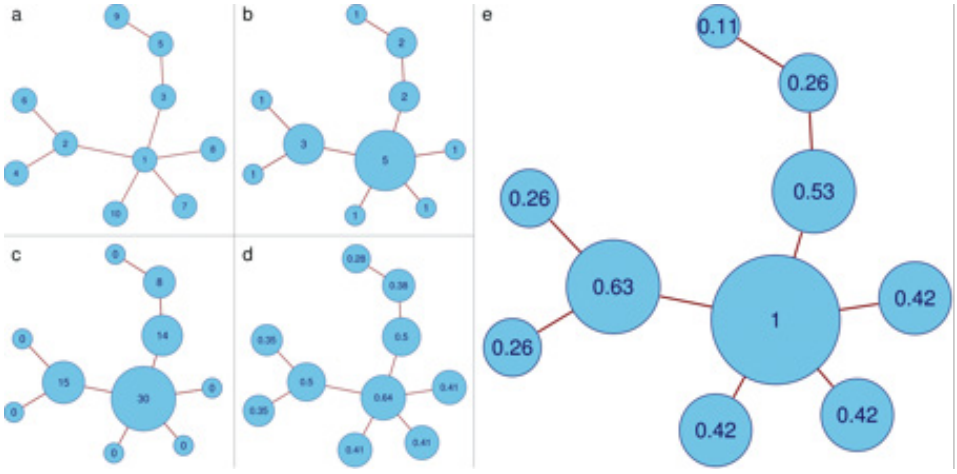
Vulnerability of the default mode network in Alzheimer's disease

A commonly investigated RSN in AD research is the default mode network (DMN). Activity of the DMN has been hypothesized to be implicated in episodic-memory [36,37], and is therefore of interest in AD. Many studies have found impaired functional connectivity of regions belonging to the DMN; especially the precuneus and posterior cingulate cortex seem vulnerable in AD [38,39,40]. Interestingly, the areas composing the DMN show a striking overlap with regions with high amyloid depositions. It has been shown that healthy subjects with high amyloid load showed lower functional connectivity in the DMN at rest [11,12] compared to subjects with low amyloid burden. Interestingly, functional connectivity of the DMN has been found already impaired in healthy young subjects at risk of developing AD developing AD in later life [13]. These changes occur decades before the time measurable amyloid-beta deposits have been reported. Based on these observations, it has been proposed that there might be a direct association between amyloid pathology and decreased functional connectivity in AD [41]. Either amyloid-plaque formation leads to neuronal dysfunction or neuronal activity leads to amyloid formation [42]. It has been shown that neuronal activity will increase the presence of amyloid [43]. This would explain why the regions of the DMN are especially vulnerable to amyloid depositions; the DMN is composed of very active and highly connected brain regions that continuously mediate information throughout the entire brain [41]. However, the exact mechanism underlying vulnerability of highly connected brain regions in AD is still unknown.

Default mode network vs. whole brain connectivity

It is clear that the DMN plays an important role in AD. However, examining only the DMN ignores functional integrity of other brain regions. Local disturbances due to AD pathology can have widespread effects. Examining functional connectivity of RSNs other than the DMN provides additional information on functional integrity of the AD brain. In addition, communication *between* RSNs is crucial for integrating information. It is therefore of importance to investigate different levels of functional organization. Studying the brain as a whole shows us the effects of local changes on global functional organization. Graph analytic methods can be used to investigate global organization of the brain, by looking at the brain as a network. Network efficiency and robustness, or specifically, importance of specific regions in the network can then be investigated. Importance, for example the number of functional connections of a brain region, can be expressed by a measure called centrality, which has recently been introduced to functional neuroimaging. There are several measures of centrality (Figure 3); degree centrality, closeness centrality, betweenness centrality and eigenvector centrality (EC). An important advantage of EC is its sensitivity to different layers in the network hierarchy since it takes into account the centralities of the neighbouring brain regions or voxels. In addition, EC has shown to be easily computable, robust against global physiological effects and requires no selection of (sub)networks of interest [44]. At present, it is not known how EC measured with fMRI changes in AD patients. A study using magneto-encephalography (MEG) reported interesting results in AD with; decreased centrality in temporal areas which was related to global cognitive performance [45].

Figure 3: Differences between common centrality measures. a) Example of a simple graph. b) Degree centrality counts the number of edges attached to each vertex. c) Betweenness centrality counts how often a vertex occurs on the shortest path between two other vertices. d) Closeness centrality computes the average distance from a vertex to all other vertices, and differentiates between both central vertices and end vertices. e) EC counts the neighbors of each vertex, weighted by their centralities.



Technical advances – integrating functional and molecular imaging

At present, measures of functional connectivity do not seem sensitive enough for diagnostic purposes. Combining information from multiple biomarkers might improve the use of (f)MRI for diagnostic purposes. Automatic classifications using pattern recognition approaches are especially advantageous for this purpose. Current pattern classification methods often focus on single-modality classifications [46]. For example, using structural brain damage in pattern classification methods shows high diagnostic accuracy [47,48], at the end of the AD cascade. Integrated analyses that directly combine multi-modality information in the classification itself might be promising for automatic classification in early AD. This is expected to yield earlier and more accurate detection based on a more complete picture of the pathological processes in AD. In addition, integrated PET/MR systems have become recently available enabling acquisition of both MR and PET images in a single scanning session [49,50]. In future, such integrated systems will present us with exciting opportunities in multi-modal imaging of AD. However, at present, PET/MR is not yet applicable for quantitative imaging in AD. This is mainly because there is no perfect solution available for attenuation correction (AC) on PET/MR yet [51]. AC is essential for accurate quantitative measurements of radiotracer concentrations [52]. In order to use PET/MR systems for brain imaging in AD, the effect of MR-AC should first be explored.

Aim of the thesis

The aim of this thesis was to explore functional connectivity changes in AD and reflect this against other AD biomarkers. For this purpose, both (f)MRI and PET data will be examined. Functional connectivity fMRI will be explored using various analysis techniques, in order to understand changes in brain organization at different hierarchical levels.

Outline of the thesis

In order to understand how commonly used AD biomarkers are related to each other, chapter 2 describes the effect of amyloid and glucose metabolism on ongoing neuronal loss. Then, chapter 3 reports the direct association of amyloid and functional connectivity of the DMN. Chapter 4 describes functional connectivity in two subtypes of AD and discusses whether these differences in functional connectivity can explain cognitive problems. Then, in chapter 5, the use of a new graph analytical approach, eigenvector centrality, is examined in AD patients. The agreement of both fMRI and ASL with [^{18}F]FDG will be explored in chapter 6 and 7; exploring possibilities of replacing FDG-PET as diagnostic tool in future. Finally, the last two chapters will discuss future implications of integrated imaging biomarkers in automatic classification of AD (chapter 8) and technical considerations when using PET/MR in AD patients on amyloid-imaging (chapter 9).

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