

Summary of the main findings

The main conclusions of this thesis are: first, slowing of the oscillatory brain rhythms in both cortical and subcortical brain areas, measured with magnetoencephalography (MEG), of patients with Alzheimer's disease (AD) as well as a changed directional connectivity pattern. Second, hub locations were shifted in both conventional as multiplex networks. Third, several of these techniques were evaluated in multiple dementia types as diagnostic markers to aid diagnosis in memory clinics.

Since a wide variety of analyses techniques have been studied in this thesis, a summary of the main findings, ordered by increasing complexity of the techniques, is as follows:

- Review of the literature. First, we reviewed all studies concerning MEG and AD to give an overview about the state of the art knowledge and identify topics that still need to be elucidated. We identified three future directions, which were investigated in the following chapters and summarized below: 1) Information with higher spatial resolution than at the level of brain lobes (chapters 3, 4, 5 and 7); 2) Directionality of functional connectivity (chapter 4); 3) new network approaches like the minimum spanning tree ([MST], chapter 5 and 6) and multilayer network (chapter 7).
- Power spectrum analyses. We studied MEG signals of AD patients in both cortical structures and the hippocampus using 'virtual electrodes' and found a global slowing of brain oscillatory activity. Furthermore, the average cortical relative power in the theta band was shown to be the best diagnostic discriminator between AD patients and healthy control subjects. Interestingly, spectral measures in the hippocampus specifically had the strongest association with cognitive dysfunction compared to neocortical brain regions. With this novel approach using virtual electrodes, we have shown that measuring hippocampal activity is possible and can be useful in distinguishing AD from healthy controls.
- Functional and directional connectivity analyses. We analyzed MEG and EEG functional and directional connectivity changes in two patient groups (AD patients in a mild cognitive impairment stage [MCI], and AD in the dementia stage) compared to controls:
 - MCI patients showed both increases and decreases in functional connectivity of the alpha band compared to healthy controls in an MEG study. This dual pattern of disconnection will be discussed in more detail later in this chapter.
 - In AD patients, we found decreased functional connectivity compared to subjective memory complainers in an EEG study. Furthermore, we studied the directional connectivity in an MEG study of AD patients and found a disruption of the dominant posterior-to-anterior pattern in the beta band.
- Network analyses. Networks have again been studied in two different patients groups versus controls:
 - In MCI patients, we studied brain networks by calculating the "small-worldness" of the networks and by using a centrality measure of the minimum spanning tree. Although the global small-worldness was not different between MCI and healthy controls, we again found a dual pattern of increases and decreases of hubs in the MST networks between the groups.
 - In AD patients, we found decreases of hubness of the MST networks in the posterior region in an EEG study compared to subjective memory complainers
 - In AD patients, we found that several hub regions, particularly left hippocampus, posterior parts of the default mode network and occipital regions, were vulnerable in patients compared to control subjects in a multiplex network framework. Of note,

these detected vulnerable hubs in Alzheimer's disease were absent in each individual frequency-specific network, thus showing the value of integrating the networks.

- Classification of individual patients using machine learning. We studied whether the above described analyses could classify individual patients with five different forms of dementia by entering them as features in machine learning algorithms. We found that DLB patients were best discriminated from all other groups. Especially the differentiation from healthy controls and DLB patients was extraordinary high in the theta band (98% between DLB and controls and 93% between DLB and FTD). Adding directional connectivity and network features to the classifiers, in addition to spectral features, did not significantly increase classification accuracy.

The three main research questions of this thesis will be systematically discussed in this section. Additionally, we will discuss additional questions that were formulated as future directions in the review paper of Chapter 2 as well as methodological considerations and future directions.

How does AD affect the patterns of oscillatory brain activity and connectivity?

In the previous chapters, we described slowing of oscillatory brain activity in a spatially detailed manner, as well as changes in brain connectivity. Here, we will first discuss the oscillatory slowing, followed by functional connectivity and lastly, directional connectivity findings of this thesis.

Patterns of global cortical slowing of oscillatory brain activity have been widely described in multiple neurodegenerative diseases including AD (e.g. Klimesch, 1997; Jeong, 2004). While the results are regionally specified in lobes (de Waal et al., 2012; de Haan et al., 2008), a spatially detailed description of MEG data was lacking. We showed that the regions with the most severe slowing, as reflected by the lowest peak frequency, were mostly located in cortical association areas of the brain, whereas primary cortical areas (sensorimotor cortex and primary visual cortex) were spared. This pattern mimics the pattern of atrophy- and amyloid distribution across the brain (Buckner et al., 2005), as well as the pattern of disturbed glucose metabolism (Jack and Holtzman, 2013). This is not coincidental, since a relationship between neuronal activity and brain deterioration has been shown in a model by de Haan et al., 2012a. This model will be discussed in more detail in the next section. Since the brain oscillatory changes are region dependent, the relationship between neuronal activity and brain atrophy might be causal while the direction has yet to be discovered.

In the hypothetical biomarker model by Jack et al., (2010) a time-line in which different biomarkers for AD become abnormal during the disease is presented. The authors hypothesize that amyloid-beta deposition is the first neuropathological feature to occur, followed by Tau-mediated neuronal injury and dysfunction, and subsequent changes in brain structure that eventually lead to the cognitive deterioration seen in AD patients. Markers of brain oscillatory activity (slowing on EEG or MEG) are lacking in the above described model by Jack et al. (2010), although research showed evidence of a relationship with the described biomarkers. It is to be elucidated what the position of oscillatory brain activity is in this hypothetical time-line. Since cellular damage occurs within neurons and synapses, their function is inevitably altered. As both MEG and EEG measure synaptic activity of groups of neurons directly, one could argue that the brain functional changes (as captured by MEG measurements) are altered due to the cellular damage. However, there is some evidence that this causal relationship is opposite: the function is already disturbed before notable amyloid deposition can be detected (Sheline et al., 2010; Drzezga et al., 2011). In either case, measures of functional brain changes, like MEG, are

therefore an important topic for the early detection of AD. Furthermore, by placing virtual MEG electrodes in the hippocampus, we reported slowing of oscillatory brain activity that showed a better correlation with cognition in AD than the electrodes placed in more superficial cortical areas.

Patients with MCI that have positive biomarker for AD are presumed to have an early stage of AD, already show functional connectivity alterations. Although causal assumptions cannot be made in this group (since the clinical symptoms are already becoming apparent), it gives more insight into the disease mechanism. These patients show a mild cognitive deterioration while the symptoms are not severe enough (yet) to be called dementia. And, since all included MCI patients show signs of neuronal injury, the chances of developing AD are high (Shah et al., 2000; Farias et al., 2005). The functional alterations found in this group of patients were not uniformly pointing towards the same direction: we found both increases in functional connectivity as well as decreases (chapter 5). Although decreases of functional connectivity in the case of brain atrophy is easily explained, the increases of functional connectivity are more challenging to understand. In an attempt to explain this dual pattern in this early stage of AD, two hypotheses have been formulated: compensatory mechanism and activity dependent degeneration.

The idea of a compensatory mechanism that explains the increased functional connectivity in early stages of AD, is based on the idea that the brain overcomes the damage caused by the disease in the networks involved in cognitive functioning (see Grady, 2012; Scheller et al., 2014 for reviews). This means that the brain “overconnects” when the disease is in its early phase, before the disconnection initiates. In the case of healthy controls, this mechanism would not be needed while AD patients cannot not compensate any more due to the severity of the disease.

A more plausible explanation, in contrast to the above described compensatory mechanism, is that the increased connectivity is a pathological characteristic of MCI patients caused by the (selective) deterioration of inhibitory neurons in early stages while excitatory neurons only become affected in a later stage of the disease (de Haan et al., 2012a; López et al., 2014a,b). More specifically, the initial loss of inhibitory interneurons in the cortex would induce increasing brain activity/connectivity in MCI patients, leading to an aberrant functioning (disinhibition). During the course of the disease, there is a loss of GABAergic synapses caused by the accumulation amyloid (Garcia-Marin et al., 2009), producing an inhibitory deficit until the breakdown of the system, which is what occurs in AD.

Besides changes in functional connectivity, we also reported altered directional connectivity in patients with AD. Directional connectivity, also sometimes also referred to as effective connectivity, differs from functional connectivity by referring to the influence that one neural system exerts over another (Friston 2011). In terms of MEG connectivity: whether the information flow throughout the brain goes from region A to region B or the other way around. In healthy controls, a posterior-to-anterior information flow has been reported in the higher frequency bands both in this thesis (chapter 4) and in other independent MEG studies (Hillebrand et al., 2016; Boon et al., 2017) and EEG studies (Dauwan et al., 2016a). Using other measures of directional connectivity, the results have been reported to be in an opposite direction (for instance, Stam and van Straaten, 2012; Moon et al., 2017). We reported a disruption of posterior-to-anterior information flow in the higher frequency bands in AD. Because of the disruption of posterior regions in AD patients, the information flow from central regions to posterior and anterior regions in AD patients became more apparent than in the healthy controls. In addition, the

changes observed for the AD in the posterior-to-anterior beta band pattern were in line with two previous studies based on EEG (Babiloni et al., 2009; Dauwan et al., 2016a). Direction of information flow can give rise to new insights into disease pathologies and disease mechanisms, but the methodological issues are important to take into account and research into this topic has to be explored further. For instance, especially using EEG, the reference electrode can be of major influence by determining causal relationship between phases. On the other hand, the changes we reported in the information flow of AD patients, especially those that were found in regions known to be influenced by the disease (like the precuneus) is in need of thorough research.

How does AD, in both the dementia and the pre-dementia state, alter hubs in brain networks?

Hub regions are regions in the brain that serve an important role in either the structure or the function of brain networks. Although “hubs” do not have a clear definition in terms of brain networks (dependent on the research questions and aims of the study), the general consensus is that a hub region has many connections with other regions and is therefore highly connected and central in the network. If a hub-region gets disrupted, the topology of the brain network changes significantly, as compared to a peripheral region (de Haan et al., 2012; Tijms et al., 2013; Barabasi and Albert, 1999). As explained in the introduction of this thesis, a functional brain network can be formed by regarding the brain regions as network nodes and the functional interactions between brain regions as the connections between nodes. Quantification of functional networks is an important step in the understanding of brain networks as underlying feature of cognitive functioning.

In chapter 5 of this thesis, we found that “conventional” global network measures were not able to detect differences in network topology in MCI patients compared to controls, while hub-measures based on the minimum spanning tree (MST) were able to capture differences between the groups. The conventional network measures, i.e. clustering coefficient and path length, together give information about the “small-worldness” of the network. However, the network quantification and comparison of network topology has methodological issues that are thoroughly described in a paper by van Wijk et al. (van Wijk et al., 2011). The authors describe that network comparisons are hindered by differences in network size, network density and average connectivity. The MST was demonstrated to be able to overcome many of these methodological issues, except for network size (Tewarie et al., 2015). These contradictory findings could be caused by the fact that conventional measures mix information about network topology and connectivity (edge density). The MST, in contrast, is insensitive to these confounders which might explain the results of chapter 4 in which the MST measures were more sensitive to the brain network changes in MCI.

In chapter 6 of this thesis, the reported shift of the hub region (from posterior brain areas in healthy controls, towards central brain areas in AD patients) may have an indirect link towards the slowing of oscillatory activity (reported in chapter 3) and the disruption of the posterior-to-anterior information flow (reported in chapter 4). It appears that, in all these studies (in which the same dataset of AD patients and healthy controls were analyzed), the posterior region is one of the most affected areas. This suggests a connection between these findings. Although the slowing was a more wide-spread phenomenon than the information flow and hub-status, the question rises whether there is a causal relationship between these three findings. Here, the

model by de Haan et al., 2012a presents a possible explanation of how the brain as a network deteriorates in patients with AD. As briefly introduced before, the paper explored the possibility of an “activity dependent degeneration”. Two models that both induced damage to functional brain network: the first model damaged brain regions in a random order, the second model damaged specifically the brain regions with many connections towards- and from that region, the so called ‘hubs’ (Stam et al., 2009). The latter model, “targeted attack”, mimicked the functional alterations found in AD patients. This means that if the model was modified such that the chance of nodes getting disrupted is increased when it’s number of connections to other nodes is large, the AD disease stages as mimicked. This was a particularly relevant finding because it gives rise to the idea that more active nodes are more prone to disease pathology.

Can MEG-based oscillatory- connectivity and network measures function as diagnostic biomarkers to aid the clinician in memory clinics?

In previous chapters, AD has been studied using EEG and MEG analyses of increasing complexity. These chapters yielded new knowledge on pathophysiological mechanisms of the disease. However, an important goal of the MEG-research in AD is to find a clinical tool that can aid in the diagnosis of memory clinic patients. Importantly, in memory clinics patients are diagnosed with a variety of dementia types rather than only AD, so ideally a diagnostic tool should be able to distinguish several diseases. This has been the topic of chapter 8. We entered the MEG-based measures that showed group differences in the previous chapters into machine learning algorithms and assessed the accuracy of classification between each pair of diagnoses.

The main outcome was that dementia with Lewy bodies (DLB) patients were best discriminated from all other groups. Therefore, the possibility for a clinical tool that aid the clinician in identifying DLB (and differentiate DLB from, for instance, AD) is within reach. Clinically, DLB is characterized by fluctuation of cognitive abilities alongside distinctive psychopathological symptoms, including recurrent, regular visual hallucinations and delusions (Barber et al., 2001). In the clinical setting DLB is commonly misdiagnosed as AD or Parkinson’s Disease (PD) due to their overlapping clinical symptoms (Noe et al., 2004). Also, the clinical distinction between frontotemporal dementia (FTD) and DLB can sometimes be challenging (Claassen et al., 2008). Previously, a similar classifier has been used to distinguish DLB and AD with features including clinical characteristics, CSF biomarkers, EEG visual ratings, and EEG quantitative measures like power and network measures (Dauwan et al., 2016b), which also showed high diagnostic accuracies. Therefore, the findings of the classifier show that, even by only including MEG features, the aid to clinicians can be of relevance, in particular in differentiating AD from DLB.

Methodological considerations

There are several methodological considerations that have to be addressed to make progress in dementia research using EEG and MEG. Some have already been pointed out in this discussion and the remaining considerations are discussed below.

First of all, we need to stress that definitive dementia diagnosis can only be established by performing postmortem examination of brain tissue (Dubois et al., 2014). However, in chapter 3, 4, 7 and 8, we studied a group of AD patients in which amyloid pathology was present. This was achieved by using the National Institute of Aging-Alzheimer’s Association (NIA-AA) criteria for probable AD with a high likelihood of AD pathophysiology, based on the combination of a positive biomarker reflecting A β deposition (in either cerebrospinal fluid (CSF) or by positron

emission tomography (PET) scanning) and/or a positive biomarker for neuronal injury (tau or phosphorylated tau in CSF). In chapter 6 however, all AD patients were diagnosed using the clinical criteria for AD (McKhan et al., 1984; 2011) while biomarkers were not available for all these patients. In chapter 5, the MCI patients all showed signs of neuronal injury, which increased the likelihood for developing AD in this group.

Secondly, the control groups used in this thesis were selected carefully to match the patients groups. The controls and DLB patients in chapter 8 significantly differed in age, which may cause challenges in the interpretation of the results. The matching with AD, FTD and PSP was good for age, and gender (similar as in chapter 3, 4, 6, and 7). In these chapters, the healthy controls were not tested in a clinical setting, and therefore, clinical information about disease history (besides dementia or other neurological diseases) was not available. Therefore, confounding effects of concomitant illnesses could not be assessed. Also, the healthy controls did not have known amyloid status so it is possible that amyloid pathology was present in some of the participants in this group. The control group in chapter 6 presented at the clinic with subjective memory complaints and can therefore not strictly be considered healthy (Gouw et al., 2017). However, this group is clinically relevant since they represent daily practice in the memory clinic.

Future directions

Brain physiological measurements reveal the aberrant brain activity that accompanies dementia. This thesis, together with other work presented above as well as in the discussion sections of the different chapters, form a basis in the understanding of brain physiology in dementia. However, there are future perspectives that may increase the current knowledge and aid both the diagnostics and the pathophysiological understanding of diseases.

In this thesis we placed virtual MEG electrodes that increased the spatial resolution of the data as well as the accuracy of the spatial localization of oscillatory brain activity. Expansion of the number of virtual electrodes increases the spatial resolution and enables the possibility to focus on specific, disease related areas. For AD patients, we studied the frequency spectra in both cortical areas as in the hippocampus, while the functional interactions between hippocampus and cortical areas are likely to be affected as well. Moreover, by placing virtual MEG electrodes in deeper cortical structures, brain activity can be measured in brain structures that are highly relevant in AD, like the hippocampus.

Furthermore, in this thesis we only considered the static brain oscillatory activity and functional connectivity networks using a fixed epoch length. However, the brain is considered as a dynamically functional system and rather than static system (Hutchison et al., 2013). Studying the dynamics of functional interactions between brain areas may give more insight into disease mechanisms.

The clinical implementation of machine learning methods can be evaluated with more spatial detail and more features aiding for more diagnostic accuracies. Also the inclusion of biomarker or clinical features may increase the diagnostic accuracy. The ultimate goal of the machine learning frameworks is to build a diagnostic tool that clinicians can use in their clinical practice.