Understanding heterogeneity in Alzheimer's disease:

de Waal, H.

2014

document version
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Summary

The aims of this thesis were to study the neurophysiological background of heterogeneity in AD in order to elucidate differences in disease mechanism that lead to variations in clinical presentation. In addition we aimed to study the usefulness of EEG as an outcome measure in a clinical trial. The main findings are briefly summarized below followed by a general discussion to put the findings in a broader perspective, some methodological issues and finally recommendations for further research.

In **chapter 2**, we compared differences in severity and type of EEG abnormalities between a large group of AD patients and subjects with subjective memory complaints, serving as a control group, and we assessed the influence of APOE genotype on this association. Subjects were categorized into a younger (≤65 years) and older (> 65 years) group based on age at time of diagnosis. EEG's were visually scored on severity, ranging from normal to slightly abnormal to (moderately) severe and on type of abnormality, characterized as focal abnormalities, diffuse abnormalities or both focal and diffuse abnormalities. We found that younger AD patients more often had a combination of focal and diffuse EEG abnormalities than older AD patients. They also had more severe EEG abnormalities than older AD patients. In controls we found the opposite effect: more frequent and more severe EEG abnormalities occurred in older rather than younger controls. In APOE e4 negative AD patients, severe EEG abnormalities occurred more often than in APOE e4 positive patients. This effect was independent of the effect of age. These findings provide evidence for the existence of biological differences between early and late onset AD and for an independent influence of APOE genotype on brain function.

In **Chapter 3** we used quantitative EEG to study the association between location and severity of changes in oscillatory brain dynamics and determinants of heterogeneity in AD. In **chapter 3.1** we focused on age at onset of AD and we studied differences in relative power in a large group of AD patients and controls, both categorized according to age into a young and old group, to gain more insight on the heterogeneity of pathophysiological differences within AD. We found that young AD patients present with more severe slowing of oscillatory activity than old AD patients, with the most pronounced differences in

the parieto-occipital region. From these findings we concluded that there may be different pathways of disease in young and old AD patients, supporting the findings of chapter 2.

In **chapter 3.2** the role of APOE genotype in oscillatory brain dynamics is further explored. Differences in relative power according to APOE genotype were studied in AD patients and controls. Subjects were categorized into carriers (at least one e4 allele) and non-carriers (no e4 allele). In control subjects, we found no difference in peak frequency and subtle differences in regional distribution of alpha power according to APOE genotype. In AD patients differences were more pronounced. Non-carriers had a slower peak frequency than carriers, more power in the slower frequency bands and less power in the faster frequency bands compared to APOE e4 carrying AD patients. These differences were most pronounced in the parieto-occipital region. The findings were independent of age at onset and seem to point to a different role of APOE genotype on direct brain activity than it has on amyloid deposition. In other modalities than EEG, the effect of healthy aging on functional connectivity has received quite some attention and it has been found that there is a decrease of connectivity according to age especially in the Default Mode Network. In **chapter 4** we wanted to study the combined effect of AD diagnosis and age on functional connectivity, with a focus on early onset AD. We found evidence for a relationship between diagnosis of AD and decreased alpha band connectivity, a relationship between higher age and decreased beta connectivity and independent relationships between both AD diagnosis and higher age and increased theta band connectivity. These findings show an association between increasing age and changes in functional connectivity and confirm the notion of AD as a disconnection syndrome, independently of age-related changes.

Chapter 5 extends on the study described in chapter 4 by exploring brain network topology in AD patients and controls. Aim of this study was to investigate the hypothesis that young AD patients have a greater loss of hubs (highly connected brain areas) than old AD patients, since the parietal cortex, a part of the brain that is well connected to other parts of the brain, is preferentially affected in early onset AD. We studied this with a novel method of network analysis, the Minimum Spanning Tree (MST). We found less optimal network integration in AD patients compared to controls, reflecting a loss of hubs, but no association between age at onset with loss of hubs. The spatial distribution of hub loss was

different between young and old AD patients however, with more connected areas in the left temporal areas in young AD patients and left occipital in old AD patients. This points in the direction of a different pathophysiological mechanism dependent upon age of onset.

Since brain network topology is thought of as a reflection of neuronal communication between brain regions, we hypothesized that EEG network measures could form a reliable outcome measure for clinical trials. In **chapter 6** we describe a randomized controlled trial studying the effect of an intervention with a medical food (Souvenaid) intended to enhance synapse formation and function in mild AD patients on large-scale functional brain network organization. In the control group we found a decrease in network measures, while in the Souvenaid group they remained stable.

General discussion

Association between age at onset and AD

It has become increasingly clear that Alzheimer's disease (AD) with an early onset (before 65 years) presents rather differently than AD with an onset after 65 years. Clinically, young AD patients more often present with problems in visuo-spatial functioning, executive functioning, attention and praxis,[1-4] as compared to AD patients with a late onset. It has also been shown that patients with early onset experience a faster disease progression and also a higher mortality.[5,6] In clinical practice at our memory clinic it has been noted that young AD patients seemed to have also more abnormal EEG's than old AD patients, but before this thesis, hardly any studies had been done to confirm this.[7-10] The studies described in this thesis show, for the first time in a large group of patients, that early onset patients had indeed more severe EEG abnormalities than late onset AD, which were both focal and diffuse. In additional studies we were able to further explore these differences. We looked at oscillatory brain dynamics and found more slowing in young AD patients. This is in line with the few studies that have been done on EEG differences between young and old AD patients.[8-11] As has been described in studies in other modalities, we found

the largest differences between young and old patients in the posterior brain regions. This implies a different regional vulnerability in young AD patients compared to old patients. Our finding of more slowing of EEG background activity in posterior parts of the brain in AD patients coincides with studies in other modalities that describe most pronounced abnormalities in posterior brain regions. In structural MRI studies it has been shown that patients with early disease onset have more pronounced atrophy in parietal and precuneus regions, while in old AD patients medial temporal lobe atrophy is more prominent.[12-14] PET studies have shown different patterns of hypometabolism in young and old AD patients, with more severe hypometabolism in parietal, posterior cingulate, precuneus, frontal, subcortical and occipital regions in young AD patients.[15-18] Studies on the regional distribution of amyloid deposition show incongruent results. One study reports no difference in distribution of amyloid between young and old AD patients,[15] while in another study parietal hypometabolism coincides with increased amyloid burden.[18]

The role of APOE genotype in brain dynamics of AD patients

We found more EEG abnormalities, a lower peak frequency and more slowing in especially the parieto-occipital regions in ε4 non-carriers. These findings were all independent of age. They are in contrast to previous studies on the association between APOE genotype and EEG abnormalities, which found more slowing in ε4 carriers.19-21 However these studies all had small sample sizes, or the effect of age was not taken into account.

The association between APOE genotype and AD (endo)phenotype is even less straightforward than that of age at onset and AD. Some studies have shown that AD patients not carrying the ε4 allele have a faster disease progression and more rapid cognitive decline than ε4 carriers, with more global atrophy, a different structural connectivity loss and a lower gradient of regional changes of neuritique plaque deposition.5,[22-25] An interaction between APOE genotype and age at onset of disease has been described,[26] but in the studies in this thesis effects of age-at-onset and APOE genotype were independent, with no hint of interaction.

The independent role of APOE genotype on age at onset and on direct neuronal activity could be due to different properties of APOE. The APOE ε4 genotype generally leads to an earlier age at onset, which has been attributed to the role of APOE in amyloid deposition.[27] Since we found no interactions between age at onset and APOE genotype on direct brain activity, measured by EEG, the role of APOE on synaptic activity might be completely independent and could be related to the association of APOE with cholesterol, which has an important role in synaptogenesis and neurotransmission or on its role in neuritic outgrowth.[28,29] A better understanding of these different pathways leading to AD provides important targets in the development of disease specific interventions.

Aging in normal cognition and in AD

The role of aging on neurophysiology has not been studied quite extensively.[30] For oscillatory brain dynamics it was shown that with aging there is a decrease in alpha frequency,[31] for functional connectivity even less studies have been performed. A study on functional connectivity across the lifespan showed an increase of alpha and beta band connectivity during maturation with a peak at the fifth decade and a decrease after 55 years of age.[32] Specific changes in connectivity after this age were not further specified. Howwever, to study the association between age at onset and functional connectivity in AD, it is indispensable to know the effect of normal aging on functional connectivity. In other modalities, like fMRI, it has consistently been shown that there is a decrease of connectivity with age, especially in the DMN.[33,34] EEG studies on normal aging and functional connectivity are inconsistent. Due to methodological differences the results of different studies are not directly comparable.[32,35,36] In a large sample of both AD patients and controls we studied functional connectivity in alpha, beta, theta and delta band and found a specific association between aging and functional connectivity in the beta band, with a higher age associated with a lower beta band functional connectivity. An increased theta band connectivity in older controls coincided with an increased theta band connectivity in AD patients. A specific association between diagnosis of AD and functional connectivity was found in the alpha band. Together, this seems to point to a different effect of aging on EEG functional connectivity than the effect that AD has on EEG functional connectivity. In future neurophysiological studies using

EEG and MEG, on functional connectivity in AD it is important to take the role of normal aging in functional connectivity as shown in this thesis into account.

AD as a disconnection syndrome

In an EEG study using a novel method of network characterization, the minimum spanning tree (MST), we found loss of hub regions in both young and old patients. When studying the regional differences, we found that the locations of loss of connections were different for young and old AD patients, whereas in controls we found no difference according to age. The difference between young and old AD patients were mainly found in the left hemisphere, with more connected brain areas in young AD patients located in the temporal regions and more connected brain areas in old patients located in the occipital area. In AD highly connected regions, hubs, are especially at risk for deterioration, but how these changes are modulated by age at onset had not been studied before.[37,38]

In the following paragraph we will try to put the results so far together and give a first step towards a model that explains the observed heterogeneity in AD. A picture emerges of partially specific disease pathways leading to variations in clinical presentation of AD and partially an overlap of disease pathway. A hypothetical model of disease pathways in different clinical profiles of AD is proposed below (see **figure 1** for a schematic overview).

The now famous amyloid cascade hypothesis proposed by Hardy and Selkoe puts amyloid deposition at the start of the process of degeneration.[39] However, a process called Activity Dependent Degeneration (ADD) might precede the first step in the model and gives an explanation why some brain regions are highly vulnerable to amyloid deposition. It states that specific hub regions show an excessively high level of neuronal activity, are damaged more rapidly and severely, and thereby attract more amyloid deposition than less active regions. Different roles can be assigned to nodes within a network:[40,41] hubs can be divided into provincial hubs, which are highly connected to other nodes within the same region, and connector hubs, which connect different areas of high connectivity to each other. It could be perceived that both kinds of hubs will suffer from ADD, since they will both have a high level of neuronal activity, but

that the next step in the cascade, namely the tau-meditated neuronal injury, will follow a different course. In two recent modelling studies using graph theoretical analysis it has been shown that a possible way of spread of (tau) pathology is transneuronally through neural networks.[42-44] When the disease starts from a provincial hub and spreads transneuronally, within the specific module it starts in, it probably follows a different course than when the disease starts from a connector hub, which is connected to important regions spread over the entire brain. A start of disease from a connector hub might lead to a much faster disease progression and also a different clinical presentation. This could explain the difference in disease course between early and late onset AD. Another factor that we found to influence clinical presentation in AD is the APOE genotype. We presumed that, besides the function of APOE genotype in amyloid deposition, the role of APOE in brain activity measured by EEG might be through its relation with cholesterol, which has a role in synaptogenesis and neurotransmission. This could have a modifying effect on the step of transneuronal spread of tau pathology through the neural network and thereby modifying the clinical outcome, independent of the effect of age. The model proposed here is a first step, but it generates new hypotheses that are ready to be tested.

Measuring an intervention effect on network topology

Although EEG is a relatively inexpensive, fast and non-invasive method to measure brain activity directly, it has not been used as outcome measure in clinical trials in AD very often.[45,46] Our study is the first to use functional connectivity analysis and modern network theory to study the effect of an intervention on synaptic activity and it proves to be a feasible method to not only demonstrate the intervention effect, but also the natural disease progression over a course of 24 weeks. We found a deterioration of network measures towards a more random configuration in patients who took placebo over a relatively short follow up time, while in the patients on Souvenaid network measures remained stable over the same period. This partly coincides with an fMRI study that looked at a longer follow up duration of about to 2-4 years and found a decrease of functional connectivity in several resting state networks.

Methodological issues

Five of the six studies described in this thesis are derived from the same large dataset of EEG data and clinical characteristics. This allowed us to use a step-by-step method of EEG analysis, ranging from a crude method of visual EEG analysis to a novel method of complex brain network analysis, based upon the phase lag index and the minimum spanning tree, within the same dataset. All data are collected in a standardized manner in a clinical setting. A possible limitation is the use of a group of subjects with subjective memory complaints as a control group, since they are known to have an increased risk of progression to AD.[47] However, these subjects do not fulfil the criteria for MCI and do not have major depressive disorder, so are likely to have normal cognition. The prevalence of an AD biomarker profile in subjects with subjective complaints from our Amsterdam Dementia Cohort is comparable to that of a population based sample.48 Furthermore, if the subjective complaints group would indeed form a preclinical stage of AD, this would have made the difference from the AD group only smaller, so the actual differences might be even larger.

EEG has several advantages and disadvantages. First of all it has the major advantage of being a measure with excellent temporal resolution. The spatial resolution of EEG however is quite poor, especially in a 21 channel EEG as it is used in the studies described in this thesis. To combine the advantage of high temporal resolution with a higher spatial resolution, magnetoencephalography (MEG) would be the method of choice. The use of MEG makes it possible to study brain network topology more extensively and gives a possibility to study network characteristics that are not as easy to study with EEG, like modularity.[41]

Another issue in connectivity and network studies in general is the large number of measures one can choose from.[49,50] In this thesis we have used the Phase Lag Index (PLI) as a measure of functional connectivity. The PLI is a measure that is relatively insensitive to volume conduction, but thereby rendering it a conservative measure. When neighbouring electrodes pick up a signal from a common source it could get interpreted as an interaction between brain regions, since there will be an almost zero phase lag between the signals. The PLI discards (near)zero phase differences. However, it could be that with discarding this in-phase activity, some true connections are also left out, making this measure quite conservative . Discarding the (near)zero phase differences might also

impose a bias towards long-distance connections. The main advantage however is that the connections that are left, are quite certain to be genuine, and not biased by volume conduction or active reference electrode effects.

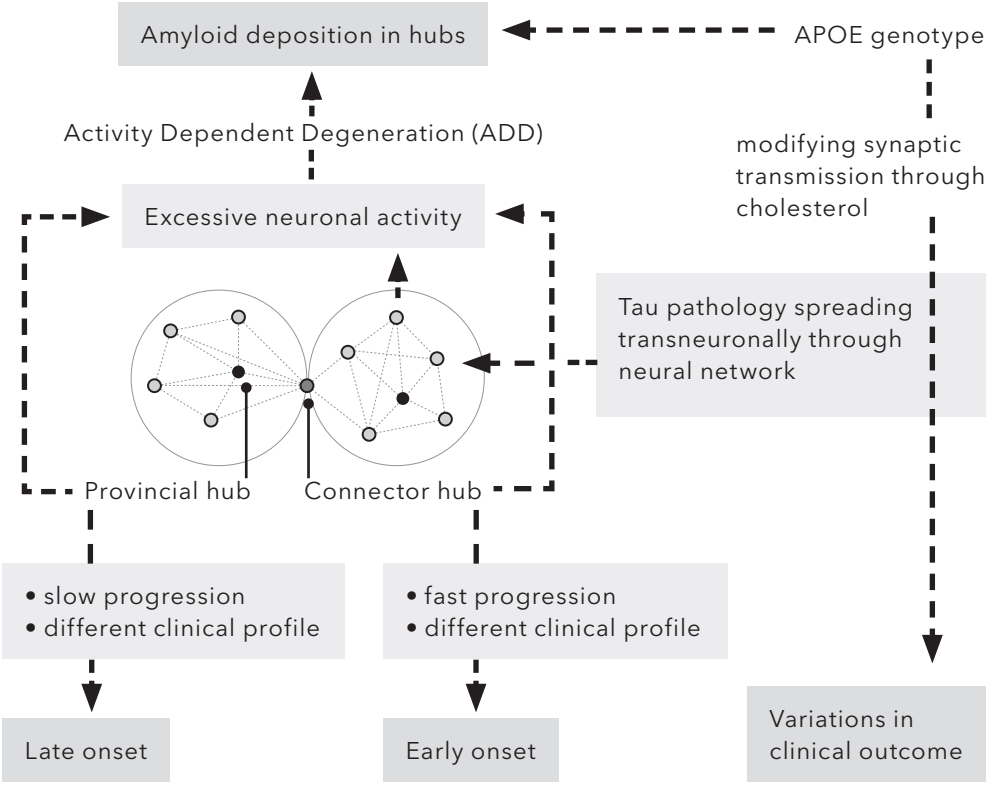
Also for network topology many choices can be made. In this thesis we describe two methods of characterizing networks. In the study described in chapter 5 we use a fairly novel method of network characterization, the minimum spanning tree (MST). In many graph theoretical studies it has proven to be difficult to compare networks of different sizes or between different groups. Often a normalization step is necessary, commonly performed through thresholding or the observed networks are compared to randomized networks. These methods however do not provide an ultimate solution.[51] In contrast, the MST is a unique subgraph within the network that connects all the nodes in the network with, in our case, the maximum connection strength, without forming cycles. This surpasses the need to choose a threshold or to compare the network to random networks. Since some connections are left out to prevent forming cycles, this might mean that some important connections are left out, possibly resulting in a loss of clinically relevant information. The resulting MST consists of the strongest links in the network, making it a rather consistent representation of the functional brain network. From the MST several different network characteristics like leaf number, eccentricity and betweenness centrality can be derived. This gives a broader perspective on the organization of a network and allows us to gain information about hubs and efficient information transfer within the network. In chapter 6 we chose a more conservative method of network analysis, a graph theoretical approach. This method is consistent with previous EEG studies in AD[52,53] and therefore more useful to establish an effect of an intervention on brain network organization. The MST could become relevant as a outcome measure in future clinical studies.

Future studies

Now that we have an account of what happens in AD neurophysiologically, also in relation to age at onset and APOE genotype, it becomes clear that the next step in unravelling the different pathways of pathophysiology is to get a more profound understanding of the role of hubs. As has been hypothesized in the model described above, hub locations may fulfil different roles in the brain and

thereby modify the clinical outcome of AD. With EEG we were only able to investigate the role of hubs in a rather global manner, since the spatial resolution of EEG is relatively low. In future studies this needs to be further specified with measures with high spatial resolution like MEG and fMRI. To create an overall framework of developing pathology in AD it is also important to combine different measures that all presume to measure brain activity and to relate them to measures of structural connectivity, the physical pathways within the brain. Especially when studying heterogeneity in AD it is important to clarify what and where the onset of disease is, which calls for studies very early in the disease process. To elucidate more exactly where in the disease pathway disconnection of brain activity takes place will help us to get closer to finding targets for therapy. Another important step in this approach is to use longitudinal studies to map exactly from health to disease what happens with functional brain networks.

Figure 1



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