Aging and Alzheimer's disease have a diverse effect on restingstate EEG functional connectivity

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Submitted

Chapter 4

Abstract

Objective

Changes in communication between different brain regions, functional connectivity, may play an important in Alzheimer's disease (AD). Aim of this study was to examine the combined effect of AD diagnosis and age on functional connectivity measured by electroencephalography (EEG).

Methods

We examined resting state EEGs of 319 probable AD patients and 245 non-demented controls, categorized into young (≤65 years, age: AD 59(5); controls 55(7)) and old (>65 years, age: AD 75(5); controls 72(4)). Phase Lag Index (PLI) as a measure of functional connectivity was calculated in four frequency bands. The influence of diagnosis and age on PLI was examined by two-way analysis of variance (ANOVA) and linear regression analyses. All analyses were adjusted for sex and apolipoprotein E (APOE) genotype.

Results

ANOVA showed that compared to controls, AD patients had a lower PLI in the alpha band and higher PLI in the theta band. We also found a main effect of age with older subjects having a higher theta band PLI than younger subjects. Linear regression using age as continuous variable confirmed these results. In addition, a main effect of age in the beta band was shown, as a higher age was associated with a lower PLI.

Conclusions

Changes of resting-state EEG functional connectivity due to aging occurred in both AD patients and controls. Furthermore, we observed specific changes of functional connectivity in AD patients compared to controls.

Introduction

Functional connectivity refers to the idea that statistical correlations between neural activity recorded from different brain regions reflect functional associations responsible for cognitive processing.[1,2] Electroencephalography (EEG) is frequently used to measure functional connectivity as well as more local neural activation. Disruptions in communication between brain areas may lead to disorders like Alzheimer's disease (AD), which has therefore been considered a 'disconnection syndrome'.[3] EEG studies on functional connectivity have consistently shown that AD patients have lower alpha band connectivity.[4-10] In other frequency bands results are less clear-cut.[5,6,10,11] Functional connectivity can be characterized by various measures, each with their own qualities.[2] The Phase Lag Index (PLI) is a measure of functional connectivity that is hardly affected by spurious connections due to volume conduction, making it a reliable measure of functional connectivity.[12]
The influence of aging on functional connectivity in EEG and magnetoencephalography (MEG) has not been thoroughly explored yet.[13-15] From these studies it is hard to make inferences whether the effect of aging on EEG functional connectivity is similar to the effect that AD has on functional connectivity. In other modalities the effect of healthy aging on functional connectivity received more attention. Most work has been done in functional Magnetic Resonance Imaging (fMRI). A consistent finding in these studies is decreased functional connectivity especially within the Default Mode Network, a network also implicated in AD.[16-18]

The aim of this study was to explore the combined effect of AD diagnosis and age on functional connectivity, with a focus on early onset AD.

Methods

Subjects

We included 319 AD patients and 245 non-demented controls from the memory-clinic based Amsterdam Dementia Cohort, similar to the dataset used in our previous study on quantitative EEG analysis.19 All subjects have been referred to the Alzheimer center of the VU University Medical Center, Amsterdam, the Netherlands, between September 2003 and June 2009.

Standardised dementia screening included a history, and when available, an informant based history, a standard neurological examination, a cognitive examination including mini mental state examination (MMSE), neuropsychological examination, EEG, Magnetic Resonance Imaging (MRI) of the brain and screening laboratory tests. Patients were diagnosed with probable AD during a multidisciplinary consensus meeting, according to the NINCDS-ADRDA criteria.[20] All patients also met core clinical criteria of the National Institute on Aging-Alzheimer's Association (NIA-AA).[21] The control group consisted of patients who presented at our memory clinic with subjective complaints, but who had normal clinical investigations and did not have significant cognitive deficits (i.e. Mild Cognitive Impairment criteria were not fulfilled)[22] or major psychiatric disorder.

Both AD patients and controls were categorized according to age in a young group (65 years or younger at time of diagnosis) and an old group (older than 65 years at time of diagnosis). The ethical review board of the VU University Medical Center has approved the study. All subjects or their formal representatives gave written informed consent for the storage of the results of the examinations in a local database and for the use of the data for research purposes.

APOE genotyping

APOE genotyping was performed after DNA isolation from 10 ml ethylenediaminetetra-acetic blood, with the Light Cycler APOE mutation detection method (Roche Diagnostics GmbH, Mannheim, Germany). APOE ε4 carrier ship was dichotomized in APOE ε4 carriers (at least one ε4 allele) and non-carriers.

EEG recording: All EEGs were recorded using the OSG digital equipment (Brainlab®; OSG b.v., Rumst, Belgium) at the positions of the 10-20 system: Fp2, Fp1, F8 F7, F4, F3, A2, A1, T4, T3, C4, C3, T6, T5, P4, P3, O2, O1, Fz, Cz, Pz, with an average reference which included all electrodes, except Fp2, Fp1, A2 and A1. Sample frequency was 500 Hz. Electrode impedance was below 5kΩ. Initial filter settings were: time constant 1s; low pass filter, 70 Hz. Patients were seated in a slightly reclined chair in a sound attenuated room. Patients sat mainly with eyes closed, EEG technicians were alert on keeping patients awake by sound stimuli.

From each EEG 4 epochs of each 4096 samples of artefact free data (containing no eye blinks, slow eye movement, excess muscle activity, ECG artefacts, etc.) were selected.

Functional connectivity

Functional connectivity was assessed with the Phase Lag Index (PLI).[12] The PLI is a measure of statistical interdependencies between time series based upon the asymmetry in the distribution of instantaneous phase differences. It reflects the strength of the phase coupling, and has the advantage to be, due to discarding the (near-)zero phase differences, less sensitive to volume conduction and the influence of active reference electrodes than most other frequently used measures for functional connectivity, such as the Imaginary component of Coherency (IC) and Phase Coherence (PC).[12] The PLI ranges between 0 and 1, a PLI value of zero indicates no coupling and a PLI of 1 means perfect coupling. The PLI was calculated in Brainwave software version 0.9.76 (in house developed by CS; further information and free available software: http://home.kpn.nl/stam7883/brainwave.html), in four frequency bands (alpha band 8-13 Hz, beta band 13-30 Hz, theta band 4-8 Hz and delta band 0.5-4 Hz). For each epoch a PLI value for every electrode is calculated, representing the mean PLI of a single electrode with all other electrodes, and the mean PLI of all electrodes together is calculated. Since PLI values were not normally distributed, they were log transformed to obtain a more Gaussian distribution. These values were averaged over the four epochs for each subject.

Statistics

IBM SPSS Statistics version 20 for Mac was used for statistical analysis. Differences between groups in baseline characteristics were tested with χ^2-tests and

t-tests where appropriate. We performed two-way Analyses of Variance (ANOVA) to test the effect of diagnosis and age (young: age ≤ 65 years vs. old: age > 65 years) on PLI. Sex and APOE genotype (APOE ε4 carrier or non-carrier) were entered as covariates. Furthermore, to test the influence of age as a continuous variable, we used multiple linear regression analyses with age and diagnosis as independent variables, sex and APOE genotype as covariates and PLI in four frequency bands as dependent variables. As there were no significant interactions between age and diagnosis, we report models without the interaction term. We reported standardized betas. In general, a significance level of $\alpha < 0.05$ was used.

Results

Subject characteristics are summarized in table 1. Young AD patients were older than young controls and old AD patients were older than old controls (both $p < 0.05$). Gender distribution and MMSE score did not differ between young and old AD patients and also between young and old controls. Disease did not differ between young and old AD patients.

The results of the two-way ANOVA to assess the effect of age and diagnosis on PLI values are shown in **figure 1** (see table 2 for raw PLI values). In the alpha band we found a main effect of diagnosis as AD patients had a lower PLI than controls ($p < 0.001$). There was no main effect of age, or an interaction between age and diagnosis. In the beta band we found no significant effects of diagnosis or age on PLI. There was a trend towards an interaction between age and diagnosis ($p = 0.079$). Older controls had a lower beta band PLI than young controls, while both young and old AD patients had a PLI value similarly low as the old controls. In the theta band we found that AD patients and older subjects had a higher PLI (main effect diagnosis $p = 0.004$; main effect age $p < 0.001$). In addition, there was a trend towards an interaction between age and diagnosis ($p = 0.076$), reflecting that old AD patients and controls had a higher PLI than young AD patients and controls; the difference between young and old was highest in AD patients. Two-way ANOVA did not show any effects in the delta band.

Next, we performed linear regression analyses to assess the effects of diagnosis and age as continuous variable on PLI in four frequency bands (see table 3 for all standardized betas). In the alpha band we found a main effect of diagnosis on PLI ($p < 0.001$) (**figure 2A**). AD patients had a lower PLI than controls. There was no main effect of age. In the beta band we found a main effect of age ($p = 0.041$), but no main effect of diagnosis (**figure 2B**). With increasing age, there was a decrease of PLI for both patients and controls. In the theta band we found main effects of diagnosis and age on PLI ($p = 0.017$ and $p < 0.001$) (**figure 2C**). AD patients had a higher PLI than controls and in both groups PLI increased with increasing age. The delta band did not show any associations between age or diagnosis and PLI. There were no interactions between age and diagnosis in any of the frequency bands.

Discussion

Our results confirm the notion of AD as a disconnection syndrome and show an association between increasing age and changes in functional connectivity in both AD patients and controls. Taken together the results provide evidence for a relationship between a diagnosis of AD and decreased alpha band connectivity, a relationship between higher age and decreased beta band connectivity and independent relationships between both AD diagnosis and higher age and increased theta band connectivity.

This study extends on previous studies on functional connectivity in AD, by examining the association between age at onset and functional connectivity in a large sample of AD patients and controls and by using a reliable, non-linear measure of functional connectivity, the PLI. When interpreting EEG studies, one must take into account that neighbouring electrodes might pick up a signal from a common source, instead of an interaction between separate sources, so-called volume conduction. The PLI is a measure that is less sensitive to this volume conduction than other measures of connectivity.[12,23] Comparing AD patients with controls we found a lower alpha band connectivity in AD patients, which is in agreement with previous studies.[4-10] We further found an increased connectivity in the theta band, which coincides with findings of a recent EEG study in a small group of AD patients and controls.10 Studies using phase co-

herence as connectivity measure reported a decrease in theta band connectivity,[5,11] while a study using a non-linear measure found no effect in theta band connectivity.[6] Differences between these studies could be due to differences in sample size, to variable amounts of volume conduction of different connectivity measures or because linear measures of connectivity, like phase coherence, might capture the complex nature of interactions in brain activity differently. The increase in theta band connectivity in AD patients in this study was found with a relatively strict measure, which is less sensitive to volume conduction. Additionally, it takes into account not only linear but also non-linear interactions, which are likely to be present to a large extent in brain activity. This makes us more certain of the true nature of connectivity changes in AD patients.

Strengths of this study are the large sample size of both AD patients and controls and the use of PLI as measure of connectivity. A possible limitation of PLI is that it is quite a conservative measure. By discarding the near-zero phase differences, because they might be based on volume conduction, some connectivity might not be taken into account. The remaining connectivity is more certain to be genuine however. It has been suggested that AD influences not only connectivity strength but also network topology.[7,24] Further studies are needed to explore variability in AD patients on different network measures.

While the association between aging and functional connectivity in other modalities has been studied quite extensively,[16] there are only a few studies on this topic using EEG or MEG.[13-15] Results of these former studies are inconsistent and hard to summarize, due to vast differences in used methodology and relatively small sample sizes. In the current study including both controls and patients with AD we found that with aging, functional connectivity increased in the theta band and decreased in the beta band. When studying functional connectivity in AD it is important to take the influence of normal aging into account. Our study adds to the knowledge of EEG functional connectivity in normal aging and during AD and illustrates that although there is overlap in age- and diagnosis- related connectivity changes there are also specific changes related to these determinants.

In previous EEG studies we showed that young AD patients had more pronounced slowing of their background rhythm than old AD patients, with also a different regional distribution.[25] In this study we only founds a trend towards

significance for an interaction between diagnosis and age in the beta and theta band, contrary to our expectations. Perhaps in AD a decrease in functional connectivity precedes slowing of the background rhythm. This would explain why we did not find differences in functional connectivity according to age in AD patients, while in our former study we did find differences in background activity. Interactions between age and diagnosis in the beta and theta bands seemingly suggested that the difference in PLI between older AD patients and controls is smaller than the difference between younger AD patients and controls. In a neural mass modelling study it was shown that a higher neuronal activity level ultimately leads to disrupted functional connectivity.[26] Perhaps patients developing AD at a younger age have a higher vulnerability for this activity dependent degeneration. Alternatively they might, due to an as yet unknown reason, have an initially higher level of neuronal activity, causing activity dependent degeneration at a younger age.

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References

1. Aertsen AM, Gerstein GL, Habib MK, Palm G. Dynamics of neuronal firing correlation: modulation of "effective connectivity". J Neurophysiol 1989;61:900-917.

2. Stam CJ, van Straaten ECW. The organization of physiological brain networks. Clin Neurophysiol 2012;

3. Delbeuck X, Van der Linden M, Collette F. Alzheimer's disease as a disconnection syndrome? Neuropsychol Rev 2003;13:79-92.

4. Knott V, Mohr E, Mahoney C, Ilivitsky V. Electroencephalographic coherence in Alzheimer's disease: comparisons with a control group and population norms. J Geriatr Psychiatry Neurol 2000;13:1-8.

5. Adler G, Brassen S, Jajcevic A. EEG coherence in Alzheimer's dementia. J Neural Transm 2003;110:1051-1058.

6. Koenig T, Prichep L, Dierks T, et al.. Decreased EEG synchronization in Alzheimer's disease and mild cognitive impairment. Neurobiol Aging 2005;26:165-171.

7. Stam CJ, de Haan W, Daffertshofer A, et al.. Graph theoretical analysis of magnetoencephalographic functional connectivity in Alzheimer's disease. Brain 2009;132:213-224.

8. Pijnenburg YAL, v d Made Y, van Cappellen van Walsum AM, Knol DL, Scheltens P, Stam CJ. EEG synchronization likelihood in mild cognitive impairment and Alzheimer's disease during a working memory task. Clin Neurophysiol 2004;115:1332-1339.

9. Jelles B, Scheltens P, van der Flier WM, Jonkman EJ, da Silva FHL, Stam CJ. Global dynamical analysis of the EEG in Alzheimer's disease: frequency-specific changes of functional interactions. Clin Neurophysiol 2008;119:837-841.

10. Dubovik S, Bouzerda-Wahlen A, Nahum L, Gold G, Schnider A, Guggisberg AG. Adaptive reorganization of cortical networks in Alzheimer's disease. Clin Neurophysiol 2012;

11. Besthorn C, Förstl H, Geiger-Kabisch C, Sattel H, Gasser T, Schreiter-Gasser U. EEG coherence in Alzheimer disease. Electroencephalogr Clin Neurophysiol 1994;90:242-245.

12. Stam CJ, Nolte G, Daffertshofer A. Phase lag index: assessment of functional connectivity from multi channel EEG and MEG with diminished bias from common sources. Hum Brain Mapp 2007;28:1178-1193.

13. Smit DJA, Boersma M, Schnack HG, et al.. The brain matures with stronger functional connectivity and decreased randomness of its network. PLoS One 2012;7:e36896.

14. SHINOSAKI K, ISHII R, UKAI S, et al.. Effect of normal aging on functional connectivity of the brain: an EEG study Psychogeriatrics 2003;3:49-53.

15. Schlee W, Leirer V, Kolassa I-T, Weisz N, Elbert T. Age-related changes in neural functional connectivity and its behavioral relevance. BMC Neurosci 2012;13:16.

16. Ferreira LK, Busatto GF. Resting-state functional connectivity in normal brain aging. Neurosci Biobehav Rev 2013;37:384-400.

17. Damoiseaux JS, Beckmann CF, Arigita EJS, et al.. Reduced resting-state brain activity in the "default network" in normal aging. Cereb Cortex 2008;18:1856-1864.

18. Binnewijzend MA, Schoonheim MM, Sanz-Arigita E, et al.. Resting-state fMRI changes in Alzheimer's disease and mild cognitive impairment Neurobiol Aging 2012;33:2018-2028.

19. de Waal H, Stam CJ, Blankenstein MA, Pijnenburg YAL, Scheltens P, van der Flier WM. EEG abnormalities in early and late onset Alzheimer's disease: understanding heterogeneity. J Neurol Neurosurg Psychiatry 2011;82:67-71.

20. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939-944.

21. McKhann GM, Knopman DS, Chertkow H, et al.. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:263-269.

22. Petersen RC, Doody R, Kurz A, et al.. Current concepts in mild cognitive impairment. Arch Neurol 2001;58:1985-1992.

23. Peraza LR, Asghar AUR, Green G, Halliday DM. Volume conduction effects in brain network inference from electroencephalographic recordings using phase lag index. J Neurosci Methods 2012;207:189-199.

24. de Haan W, van der Flier WM, Koene T, Smits LL, Scheltens P, Stam CJ. Disrupted modular brain dynamics reflect cognitive dysfunction in Alzheimer's disease. Neuroimage 2012;59:3085-3093.

25. de Waal H, Stam CJ, de Haan W, van Straaten ECW, Scheltens P, van der Flier WM. Young Alzheimer patients show distinct regional changes of oscillatory brain dynamics. Neurobiol Aging 2012;33:1008.e25-1008.e31.

26. de Haan W, Mott K, van Straaten ECW, Scheltens P, Stam CJ. Activity dependent degeneration explains hub vulnerability in Alzheimer's disease. PLoS Comput Biol 2012;8:e1002582./n

Table 1. Subject characteristics

	Control		AD	
	Young	Old	Young	Old
n	172	73	113	206
Age, years	55(7)$_a$	72(4)$_b$	59(5)	75(5)
Sex, female	77(45%)	34(47%)	62(55%)	93(45%)
Disease duration, years	n.a.	n.a.	3.8(2)	3.5(2)
MMSE	28(2)	29(1)	20(5)	21(5)

Data are mean (SD) or n (%). aYoung controls vs. young AD: $p < 0.05$. bOld controls vs. old AD: $p < 0.05$

Table 2. Raw PLI values

	Frequency band			
	Alpha	Beta	Theta	Delta
Young control	0.213(0.10)	0.081(0.02)	0.138(0.04)	0.151(0.03)
Old control	0.210(0.09)	0.076(0.01)	0.160(0.07)	0.151(0.03)
Young AD	0.171(0.07)	0.076(0.01)	0.152(0.04)	0.152(0.03)
Old AD	0.191(0.08)	0.076(0.01)	0.162(0.05)	0.151(0.03)

Data are mean (SD). Please note that raw data are shown, while statistical analyses were performed on log-transformed data.

Table 3. Standardized betas

	Frequency band			
	Alpha	Beta	Theta	Delta
Diagnosis	-0.188**	-0.078	0.113*	-0.039
Age	0.012	-0.095*	0.181**	0.090

Results of linear regression analyses. Data are standardized betas. The model contains diagnosis and age as independent variables and APOE ε4 and sex as covariates. *p < 0.05, **p < 0.01

Figure 1

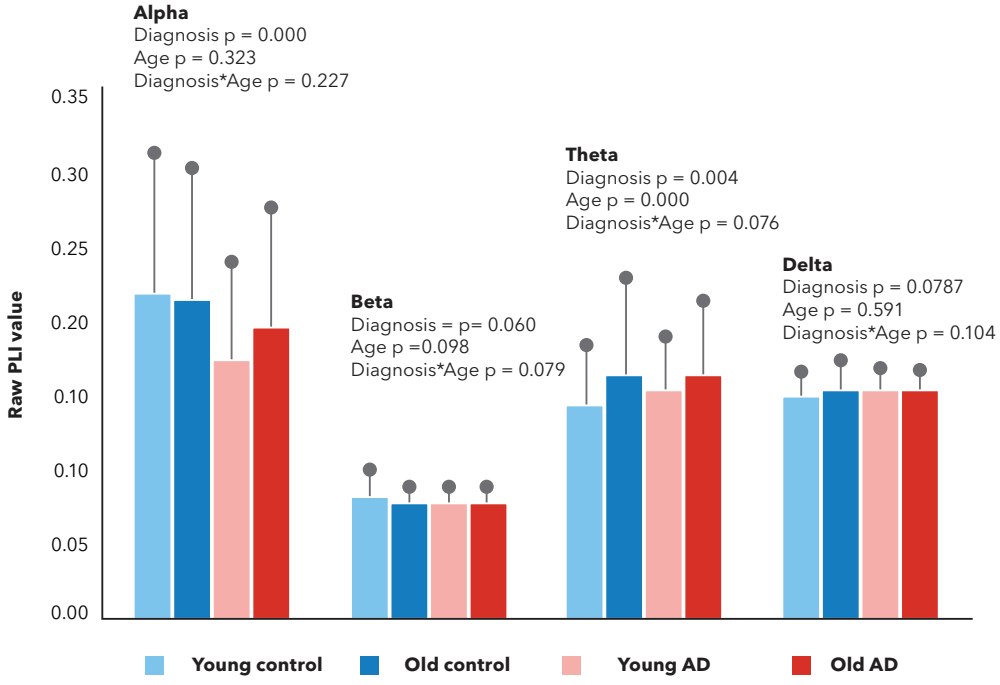

Figure 2

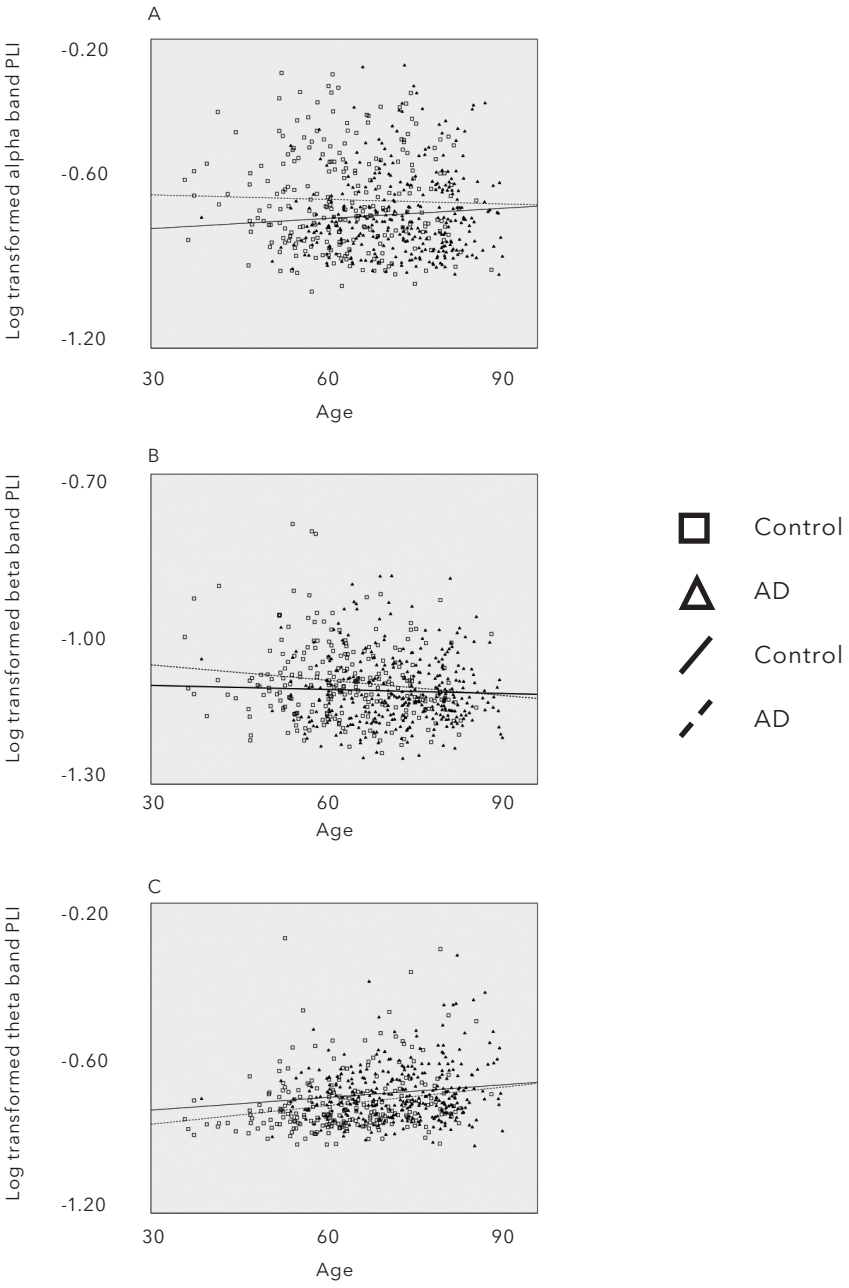
A
Log transformed alpha band PLI
-0.20
-0.60
-1.20
30
60
90
Age
B
Log transformed beta band PLI
-0.70
-1.00
-1.30
30
60
90
Age
C
Log transformed theta band PLI
-0.20
-0.60
-1.20
30
60
90
Age
Control
AD
Control
AD

Figure 1 (p. 91). Raw PLI values in four frequency bands for young and old controls and young and old AD patients.
Error bars are SD. Please note that raw data are shown, while statistical analyses were performed on log-transformed data. With two-way ANOVA we found a main effect of diagnosis in the alpha and theta band. We found an interaction between diagnosis and age in the alpha band and a main effect of age in the theta band.

Figure 2 (p. 92). Scatterplots of log transformed PLI values by age.
Squares and dotted line represent controls, triangles and straight line represent AD patients. **A**) Alpha band: AD patients had a lower PLI value than controls for all ages (main effect Diagnosis $p < 0.01$). **B**) Beta band: in both AD patients and controls there was a decrease of PLI with increasing age (main effect age $p < 0.05$). **C**) Theta band: AD patients had a higher PLI than controls and in both groups PLI increased with increasing age (main effect diagnosis $p < 0.05$; main effect age $p < 0.01$