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Functional connectivity increase in the default-mode network of patients with Alzheimer's disease after long-term treatment with Galantamine

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Abstract
Acetylcholinesterase inhibitors (AChEIs) are efficacious for the treatment of mild to moderate forms of Alzheimer's dementia (AD). Default-mode network (DMN) connectivity is considered to be early impaired in AD. Long-term effects of AChEIs on the DMN in AD have not yet been investigated.

Twenty-eight AD patients and 11 age-matched healthy volunteers (HC) participated in the prospective study. AD patients were randomly assigned to either a pharmacotherapy arm (Galantamine, AD G) or to a placebo arm (AD P+G) for the period of 6 months followed by open-label Galantamine therapy from month 7–12. All subjects underwent neuropsychological testing, resting-state functional and structural MRI at baseline and after 12 months, AD patients additionally in between after 6 months.

Thirteen AD patients completed the treatment trial and underwent all functional MRI follow-up sequences of good quality. Functional connectivity significantly increased within the AD G group in the posterior cingulate cortex and in the Precuneus between baseline and 12 months follow-up ($p_{corr} < 0.05$). Between-group analyses demonstrated that functional connectivity in the AD G group significantly increased in the posterior cingulate cortex as well as in the Precuneus compared to the HC group and in the anteromedial aspect of the temporal lobes compared to

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the AD P+G group, respectively, at 12 months follow-up ($p_{corr}<0.05$). Cognitive performance remained stable within groups over time indicating that resting-state fMRI may be sensitive for the detection of pharmacologically induced effects on brain function of AD patients.

1. Introduction

Resting-state functional magnetic resonance imaging (fMRI) has evolved into a powerful tool for mapping functional connectivity in large-scale neural networks (Biswal et al., 2010). These resting-state networks (RSNs) represent coherent patterns of spontaneous low-frequency (0.01–0.1 Hz) fluctuations in the blood-oxygen level dependent (BOLD) signal and strongly overlap with cortical regions known to be involved in primary and higher order cognitive functions (Laird et al., 2011; Smith et al., 2009). One of the most frequently studied RSNs is referred to as the default-mode network (DMN) (Greicius et al., 2003; Raichle et al., 2001).

Functionally, the DMN has been discussed to support – among other functions – especially internally directed mental activity (Buckner et al., 2008). Structurally, the network consists of several core brain regions including the posterior cingulate and the adjacent precuneus cortex, the medial prefrontal cortex, the anterior cingulate cortex, bilaterally the inferior parietal lobules expanding to posterior temporal areas around the temporo-parietal junction as well as the hippocampal formation and the lateral temporal lobe (Buckner et al., 2008).

Numerous studies have consistently reported an association between disruptions of the functional DMN architecture and Alzheimer's disease (AD) (Greicius et al., 2004; Koch et al., 2010; Seeley et al., 2009). In that context, specific alteration patterns of the DMN have been shown to allow the differentiation between healthy aging and AD (Greicius et al., 2004; Rombouts et al., 2005) as well as between different stages of AD (Damoiseaux et al., 2012; Zhang et al., 2010) suggesting the possibility of applying that network as a non-invasive, readily available and radiation exposure free imaging marker for the detection of incipient AD or as an early objective marker of treatment response (Greicius et al., 2004).

Acetylcholinesterase inhibitors (AChEIs), in general, enhance the cerebral cholinergic system (Zhang et al., 2004), which due to a reduction in the activity of cholinergic neurons is altered in AD (Geula and Mesulam, 1995). Consistent with their pharmacological properties, AChEIs have been demonstrated to be efficacious for the treatment of mild to moderate forms of AD producing improvements in cognitive function (Birks, 2006). Imaging studies have shown that AChEIs slower the progression of hippocampal atrophy (Hashimoto et al., 2005), enhance the regional cerebral blood flow (Venneri et al., 2002), preserve or slightly increase the cortical glucose metabolism (Teipel et al., 2006; Tune et al., 2003), and slower the regional decline of fiber tract integrity as measured by fractional anisotropy (Likitjaroen et al., 2012) in AD patients. Task-related fMRI approaches revealed AChEI induced modulations of cortical networks not only in healthy volunteers (Furey et al., 2000), but also in AD patients (Bokde et al., 2009; McGeown et al., 2008).

Given the effects of AChEIs on various cerebral parameters it is obvious to assume that these drugs may also influence functional connectivity within the DMN of AD patients. Studies investigating the effects of cholinergic enhancement on resting-state functional connectivity in AD patients are still rare, in particular those including placebo or control groups. One recently published study applying resting-state fMRI demonstrated increased functional connectivity in areas of the DMN in AD patients after 3 months of treatment with an AChEI in comparison with AD patients undergoing a placebo treatment suggesting a drug-induced stabilization of that system (Sole-Padulles et al., 2013). Longer-term effects of AChEIs on the DMN of AD patients have not yet been investigated in placebo controlled trials. Moreover, it is unknown how AChEIs over the long-term modulate functional DMN architecture of AD patients in comparison with untreated healthy subjects.

Here, we investigate over the period of one year the longitudinal effects of a pharmacotherapy with the competitive and reversible AChEI Galantamine on the DMN of two AD groups (verum-group & placebo followed by verum-group) using a prospective, double-blind, and randomized study design. In addition, we perform between-group analyses comparing possible relative DMN changes over time within both AD groups with those of age-matched healthy volunteers not receiving any psychopharmaceutical treatment.

2. Experimental procedures

The study was carried out in accordance with the Declaration of Helsinki and approved by the local ethics committee. The treatment trial was registered at Clinicaltrials.gov (Identifier: NCT00523666).

2.1. Participants

Twenty-eight patients with AD and 11 healthy volunteers were included into the prospective study after giving their written informed consent. Patients were recruited from the university's Memory Clinic and met the NINCDS-ADRDA criteria for clinically probable AD (McKhann et al., 1984). Healthy volunteers were recruited among the spouses of patients. Neuropsychological assessment included the Mini-Mental-Status Examination (MMSE) (Folstein et al., 1975), the Clinical Dementia Rating scale (CDR) (Fillenbaum et al., 1996), and the Consortium to Establish a Registry for AD (CERAD) cognitive battery (Berres et al., 2000). Healthy volunteers had no subjective memory complaints and scored 0 in the CDR.

2.2. Study design

The study was a randomized controlled trial with AD patients assigned to either Galantamine or placebo treatment for a

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period of 6 months. In the following extension phase of the study, all AD patients underwent an open-label Galantamine treatment for further 6 months. Healthy volunteers did not take any psychopharmaceutical drugs during the course of the study. All subjects regularly underwent clinical examination and laboratory testing.

Administration of both Galantamine and placebo was started at a daily single dose of 8 mg for 4 weeks, then increased to 16 mg daily for 4 weeks, and continued with a daily dose of 24 mg.

White matter hyperintensities as shown on T2w FLAIR imaging exceeding 10 mm in diameter or more than 3 in number were defined as exclusion criteria to avoid participation of subjects with significant cerebrovascular lesions. Also, medication side effects, lack of compliance as well as strong head motions causing imaging artifacts led to the exclusion of participants resulting in a final study population of $n=24$ (Figure 1).

Data were taken only from the AD patients who completed the trial and were able to undergo all fMRI scans of good quality. This sample consisted of six patients undergoing a Galantamine treatment from month 1-12 (AD G group; 5 female; mean age=70.7±8.4 years; mean years of education 10.33±1.11) and seven patients receiving a placebo from month 1-6 followed by Galantamine from month 7-12 (AD P+G group; 4 female; mean age=73.6±7.4 years; mean years of education 9.86±1.25). In addition, 11 healthy volunteers (4 female; mean age=67.5±7.2 years; mean years of education 12.27±2.89) were included into further analyses.

2.3. Neuropsychological testing and magnetic resonance imaging

Both neuropsychological testing and MR imaging including resting-state fMRI were performed on the same day at the beginning and at the end of the study after 12 months; in case of the AD patients, neuropsychological testing and MR imaging were additionally conducted in between after 6 months.

2.3.1. Neuropsychological testing

At all sessions, neuropsychological testing included the CERAD battery sub-tests (Mini-Mental State Examination (MMSE), Verbal Fluency, Boston Naming Test, Word List Learning, Word List Recall, Word List Recognition, Constructional Praxis, and Recall of Constructional Praxis). Inter-session changes in neuropsychological data were analyzed using paired t-tests and inter-group differences were analyzed with independent t-tests as implemented in SPSS version 20.0. To adjust for multiple comparisons (8 neuropsychological tests), the significance threshold of $p<0.05$ was Bonferroni-corrected resulting in $p_{corr}<0.00625$ (Abdi, 2007).

2.3.2. Magnetic resonance imaging

Imaging was performed using a 3.0 T Magnetom (TRIO, Siemens, Erlangen, Germany) with a 12-element head coil. For anatomical reference, a sagittal high-resolution magnetization-prepared rapid gradient-echo sequence (MPRAGE) was acquired with the following

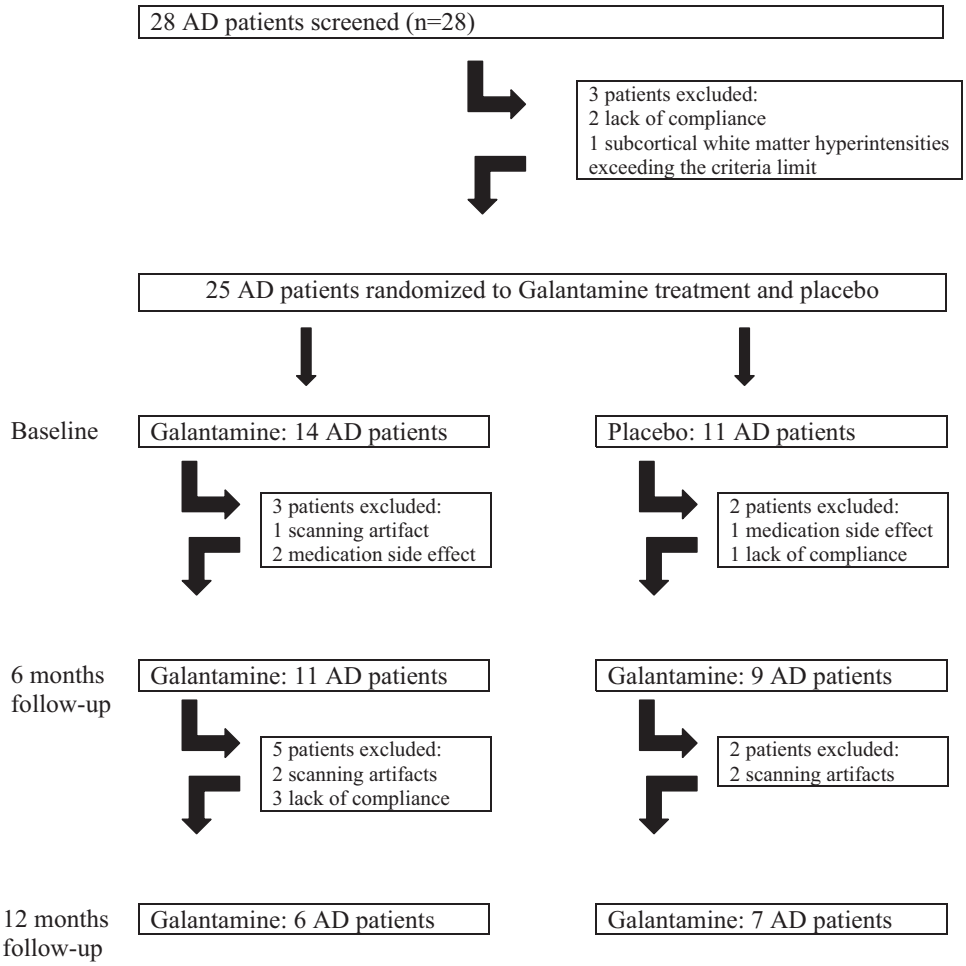

Figure 1 Flow chart of the intervention study in AD patients illustrating the numbers of subjects screened and randomized to the study, drop-outs and completers.

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imaging parameters: field of view (FoV): 250 mm; voxel size: $0.8 \times 0.8 \times 0.8$ mm^3; time of repetition (TR): 1400 ms; time of echo (TE): 7.61 ms; flip angle (FA): 20°; number of slices: 160.

Functional data were recorded by means of a BOLD sensitive echo-planar gradient-echo sequence in axial orientation (120 volumes; FoV: 192 mm; voxel size: $3 \times 3 \times 4$ mm^3; slice gap: 0.4 mm; imaging matrix: 64×64; TR: 3000 ms; TE: 30 ms; FA: 80°; number of slices: 36). Before starting imaging, 3D-field shimming was performed using automated shimming algorithms implemented in the scanner.

2.4. Functional MRI data preprocessing

Functional MRI data preprocessing was carried out using FSL (FMRIB Software Library, http://www.fmrib.ox.ac.uk/fsl/index.html) version 5.0.7 and AFNI (Analysis of Functional NeuroImages, http://afni.nimh.nih.gov/afni) according to the procedure described by Biswal and colleagues (2010) (see also www.NITRC.org for further informa tion). In detail, the steps included: (1) skull removing of individual high-resolution T1-weighted images; (2) motion correction, skull stripping, spatial smoothing applying a 6 mm FWHM Gaussian kernel, band-pass filtering using a high-pass filter of 0.01 Hz and a low-pass filter of 0.1 Hz as well as removing of linear and quadratic trends of 4D functional data sets after discarding the initial five volumes of each 4D functional data set to account for T1 saturation effects; (3) registration and normalization of anatomical and functional data sets to the MNI152 standard space applying a linear and a non-linear transformation (FLIRT & FNIRT) (Jenkinson et al., 2002; Jenkinson and Smith, 2001); (4) regression of the functional 4D data sets on global signal, signals derived from cerebrospinal fluid and white matter, and six motion parameters.

2.5. Independent component analysis

The preprocessed functional data sets were further analyzed using MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components) software, version 3.10, in combination with a dual-regression analysis as implemented in FSL 5.0.7.

Time-courses of all participants and occasions – a total of 61 scans – were spatially concatenated in the MNI152 standard space for allowing the estimation of a covariance matrix, which was used for reduction of individual fMRI data sets via Probabilistic Independent Component Analysis. Reduced individual data sets of all participants and scans were further processed with Temporal Concatenation Group ICA (TC-GICA) to calculate multi-subject multi-sessions group-level independent components (ICs). We allowed the MELODIC 3.10 software to estimate the number of ICs using an automatic dimensionality estimation tool as implemented in FSL.

2.6. Within- and between-group analyses

The TC-GICA derived group-level ICs containing the DMN sub-components were reconstructed into individual ICs for each participant and session applying a dual regression approach (Filippini et al., 2009). Based on these individual ICs, we calculated longitudinal contrast maps for each subject (AD patients: baseline session vs. 6 months follow-up and 6 months follow-up vs. 12 months follow-up; all participants: baseline session vs. 12 months follow-up). The resulting individual contrast maps were used to calculate voxel-wise within-group and (relative) between-group differences with non-parametric testing as implemented in FSL's Randomize (Winkler et al., 2014); note that we used longitudinal contrast maps for between-group analyses in order to control for the effect of (possibly existing) initial differences in the fMRI datasets (i.e., between groups at baseline). Age and gender were included into the analysis as covariates. The within- and between-group analyses both were performed within network-derived templates generated from group-level ICs using a z-score threshold of 5.0 (Figure 2 in the Results section). Given the explorative character of the study, we considered effects significant at a family-wise error (FWE) corrected $p_{corr} < 0.05$.

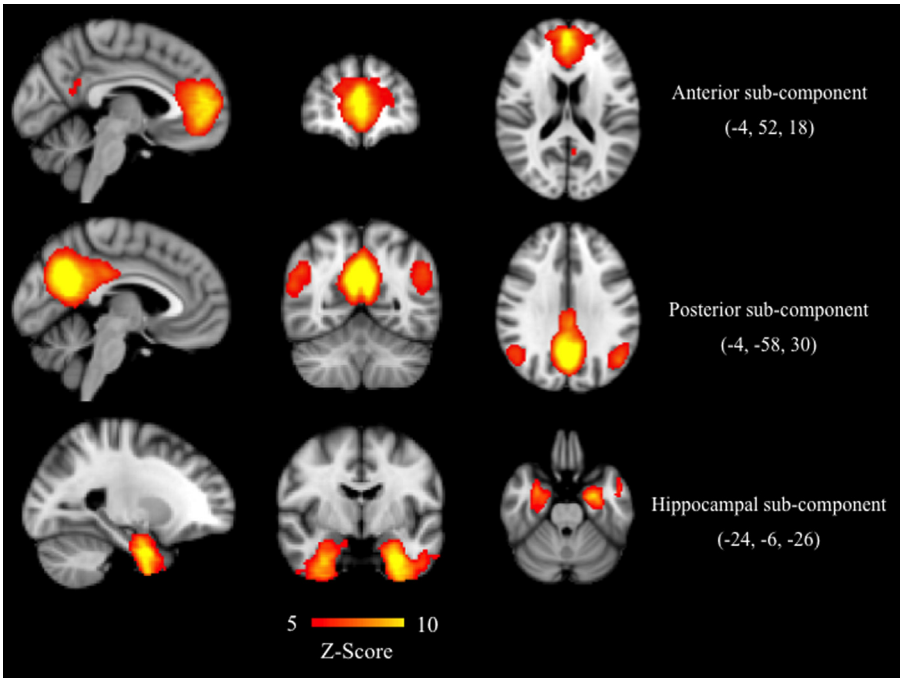

Figure 2 Group-level component maps averaged across all subjects and sessions as generated by the TC-GICA approach representing the anterior (upper row), the posterior (middle row), and the hippocampal (lower row) sub-component of the default-mode network.
Sagittal, coronal, and axial images are displayed in radiological convention (x-, y-, and z-coordinates of each slice in the MNI152 standard space – in mm – are given in parenthesis). The networks presented in this Figure (using a lower z-score threshold of 5.0) were used as templates for further within- and between-group analyses.
TC-GICA: temporal concatenation group independent component analysis; DMN: Default-mode network.

2.7. Structural MRI data analysis

In order to consider group specific structural changes over time accounting for potential differences in functional data, we additionally conducted structural data analysis using the VBM tool implemented in FSL 4.1.7. T1-weighted images were brain-extracted using BET. Tissue-type segmentation was carried out using FMRIB's Automated Segmentation Tool (FAST). The resulting gray matter partial volume images were normalized to the MNI152 standard space using an initial affine (FLIRT) and a subsequent nonlinear registration (FNIRT). The resulting warped gray matter images were averaged to create a study-specific template and the native gray matter images were then non-linearly re-registered to this template. In order to correct for local expansion or contraction the registered partial volume images were divided into the Jacobian of the warp fields (modulation). The modulated normalized gray matter images were smoothed with an isotropic Gaussian kernel with a sigma of 4 mm. Each individual's total gray matter was used as a covariate. Within-group differences in gray matter volume were determined with non-parametric testing (FSL's Randomize) using the threshold-free cluster enhancement (TFCE) approach (Smith and Nichols, 2009). Effects were considered significant at a family-wise error (FWE) corrected $p_{corr}<0.05$.

3. Results

Both AD groups did not significantly differ in mean age ($p=0.56$) and years of education ($p=0.51$). There was also no significant age difference between healthy volunteers and the AD G group ($p=0.48$) as well as between healthy volunteers and the AD P+G group ($p=0.13$). A trend towards longer education was present in the healthy control group in comparison with the AD G group ($p=0.08$) and education has been received slightly longer in healthy volunteers compared to the AD P+G group ($p=0.04$).

3.1. Neuropsychological testing

According to the CERAD test battery there were no significant differences in cognitive performance within either AD group in the longitudinal run nor between both AD groups at either of the 3 sessions ($p_{corr}>0.00625$; Tables 1-3).

Also, no significant changes in cognitive performance could be detected in healthy volunteers during the course of the study ($p_{corr}>0.00625$; Tables 1 and 2).

As expected both AD groups achieved significantly worse results in almost all CERAD tests compared to healthy volunteers at baseline and at one-year follow-up ($p_{corr}<0.00625$; Tables 1 and 3).

3.2. Functional MRI data

The TC-GICA approach generated 34 ICs. The classic DMN was detected in form of several sub-components: An anterior DMN sub-component combined the medial prefrontal cortex with the anterior cingulate cortex, the posterior cingulate cortex, the precuneus cortex, and the caudate (Figure 2,

Table 1 Cognitive performance of the three groups (AD G, AD P+G, & HC) measured by means of the CERAD test battery at baseline and follow-up examinations after 6 and 12 months.

CERAD test	Session	Mean ± standard deviation		
		AD G	AD P+G	HC
MMSE	Baseline	20.33 ± 1.89	22.00 ± 3.46	29.18 ± 0.72
	Follow-up 6 months	18.17 ± 4.06	21.00 ± 3.63	-
	Follow-up 12 months	18.50 ± 4.61	20.00 ± 5.42	28.55 ± 1.23
Verbal fluency	Baseline	11.17 ± 5.05	12.00 ± 4.00	24.64 ± 6.94
	Follow-up 6 months	11.67 ± 5.34	10.14 ± 3.44	-
	Follow-up 12 months	11.17 ± 4.14	9.00 ± 2.20	22.82 ± 5.46
Boston naming	Baseline	11.83 ± 3.02	11.29 ± 2.37	14.36 ± 1.43
	Follow-up 6 months	11.50 ± 2.57	10.00 ± 2.73	-
	Follow-up 12 months	12.50 ± 3.04	11.57 ± 1.84	14.91 ± 0.29
Word list learning	Baseline	10.00 ± 3.61	7.57 ± 4.44	22.09 ± 3.55
	Follow-up 6 months	10.33 ± 3.94	10.43 ± 1.99	-
	Follow-up 12 months	9.00 ± 3.06	9.43 ± 2.03	21.91 ± 4.52
Constructional praxis	Baseline	8.50 ± 1.50	6.86 ± 2.10	10.91 ± 0.29
	Follow-up 6 months	10.17 ± 0.90	7.57 ± 3.11	-
	Follow-up 12 months	9.17 ± 1.21	7.57 ± 2.32	10.73 ± 0.45
Word list recall	Baseline	0.67 ± 1.11	1.43 ± 1.68	8.09 ± 1.73
	Follow-up 6 months	0.83 ± 1.07	0.29 ± 0.70	-
	Follow-up 12 months	0.00 ± 0.03	1.00 ± 1.07	7.55 ± 1.97
Word list recognition	Baseline	6.50 ± 0.96	5.29 ± 1.03	9.73 ± 0.62
	Follow-up 6 months	6.17 ± 1.57	6.00 ± 2.07	-
	Follow-up 12 months	3.83 ± 3.24	3.29 ± 1.67	9.82 ± 0.57
Recall of constructional praxis	Baseline	1.83 ± 1.46	2.83 ± 2.34	10.45 ± 0.99
	Follow-up 6 months	2.17 ± 1.34	1.14 ± 1.36	-
	Follow-up 12 months	1.17 ± 1.21	0.29 ± 0.70	10.18 ± 1.34

AD G (3rd row): patients with Alzheimer's disease treated continuously with Galantamine for 12 months; AD P + G (4th row): patients with Alzheimer's disease treated with a placebo from month 1-6 followed by Galantamine treatment from month 7-12. HC (5th row): Healthy volunteers. MMSE: Mini-Mental State Examination.

Table 2 Intra-group changes in cognitive performance.

CERAD test	Group	*p*-value		
		Baseline vs. follow-up 6 months	Follow-up 6 months vs. follow-up 12 months	Baseline vs. follow-up 12 months
MMSE	AD G	0.2063	0.8013	0.3992
	AD P+G	0.3947	0.4274	0.2508
	HC	-	-	0.0261
Verbal fluency	AD G	0.4150	0.8258	1.0000
	AD P+G	0.4639	0.2797	0.1975
	HC	-	-	0.2605
Boston naming	AD G	0.4650	0.2031	0.3939
	AD P+G	0.3684	0.1659	0.6540
	HC	-	-	0.2767
Word list learning	AD G	0.4650	0.4206	0.5111
	AD P+G	0.1566	0.3216	0.3228
	HC	-	-	0.8909
Constructional praxis	AD G	0.0199	0.0117	0.3276
	AD P+G	0.3758	1.0000	0.3100
	HC	-	-	0.3409
Word list recall	AD G	0.3632	0.2354	0.1412
	AD P+G	0.1879	0.0465	0.6394
	HC	-	-	0.3109
Word list recognition	AD G	0.7646	0.0778	0.1829
	AD P+G	0.4779	0.0371	0.0679
	HC	-	-	0.7560
Recall of constructional praxis	AD G	0.6606	0.2752	0.5160
	AD P+G	0.2031	0.2695	0.0369
	HC	-	-	0.5884

Numbers in Table 2 show the results of the paired *t*-tests. No significant differences ($p_{corr} < 0.00625$, Bonferroni-corrected for multiple comparisons) are present. AD G: patients with Alzheimer's disease treated continuously with Galantamine for 12 months; AD P+G: patients with Alzheimer's disease treated with a placebo from month 1–6 followed by Galantamine treatment from month 7–12. HC: Healthy volunteers. MMSE: Mini-Mental State Examination.

upper row); a posterior DMN sub-component involved the precuneus cortex, the posterior cingulate cortex, and bilaterally the inferior parietal lobules extending to posterior temporal areas around the temporo-parietal junction (Figure 2, middle row). In addition, a network extending mainly over anterior parts of the temporal lobe including the (para-) hippocampal gyrus, the amygdala and the pole region was detected; given that the hippocampal formation is commonly attributed to the DMN we included this connectivity pattern as another potential DMN sub-component into our analysis (Figure 2, lower row).

3.2.1. Anterior DMN sub-component

In case of functional connectivity within the anterior DMN sub-component there were neither significant changes within groups in the longitudinal run nor between groups at the first follow-up session after 6 months as well as at the second follow-up session after 12 months ($p_{corr} > 0.05$).

3.2.2. Posterior DMN sub-component

In the longitudinal run, functional connectivity significantly decreased within the AD P+G group in the left-hemispheric precuneus cortex between baseline and the first follow-up session after 6 months ($p_{corr} < 0.05$; Figure 3, upper row). No significant changes were detectable during this period in the AD G group and in either AD group between the 6 months and the 12 months follow-up sessions ($p_{corr} > 0.05$).

With regard to the one year period (12 months follow-up vs. baseline), we observed a significant increase of functional connectivity within the AD G group in the posterior cingulate cortex, pronounced in the left hemisphere, as well as in the left-hemispheric Precuneus ($p_{corr} < 0.05$; Figure 3, middle row); no significant longitudinal changes in functional connectivity were present in the AD P+G group and in HC during this period.

In the between-group comparisons, a significant increase of functional connectivity was observed in the left posterior cingulate cortex as well as in the precuneus cortex of both hemispheres, accentuated on the left side, in the AD G group compared to the HC group at one-year follow-up ($p_{corr} < 0.05$; Figure 3, lower row). There were no further significant between-group changes at either session ($p_{corr} > 0.05$).

3.2.3. Hippocampal sub-component

In the longitudinal run, there were no statistically significant changes of functional connectivity in any of the groups. Between-group analyses demonstrated an increase of

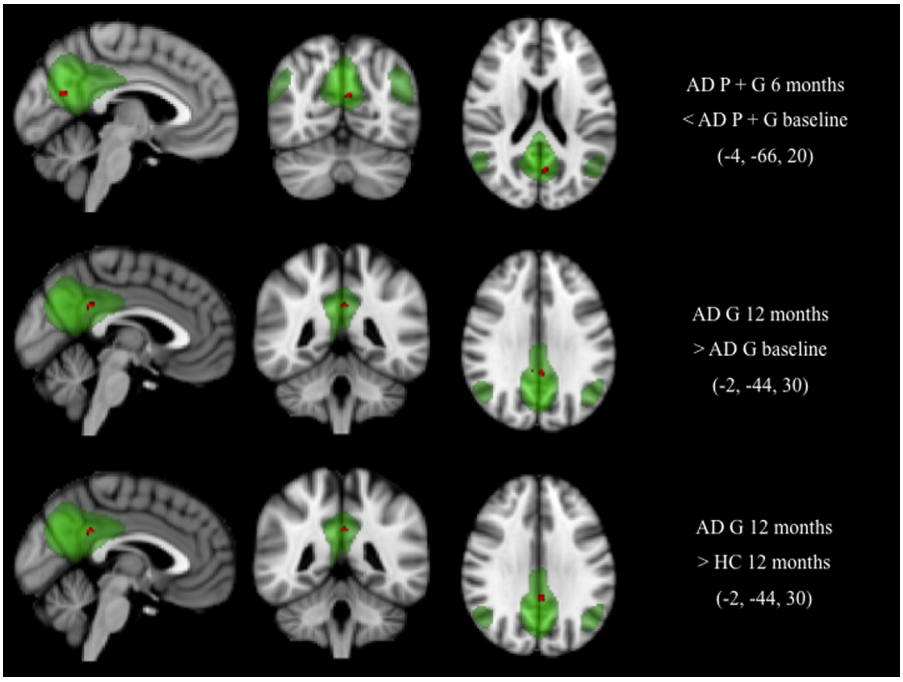

Figure 3 Contrast images depicting significant changes of functional connectivity (red) within the posterior DMN sub-component (green) of the AD placebo arm (AD P+G; upper row) and the AD Galantamine arm (AD G; middle row); significant differences between the AD Galantamine arm (AD G) and healthy controls (HC) are shown in the lower row. Data are FWE corrected at $p_{corr} < 0.05$.
Sagittal, coronal, and axial images are displayed in radiological convention (x-, y-, and z-coordinates of each slice in the MNI152 standard space – in mm –are given in parenthesis). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

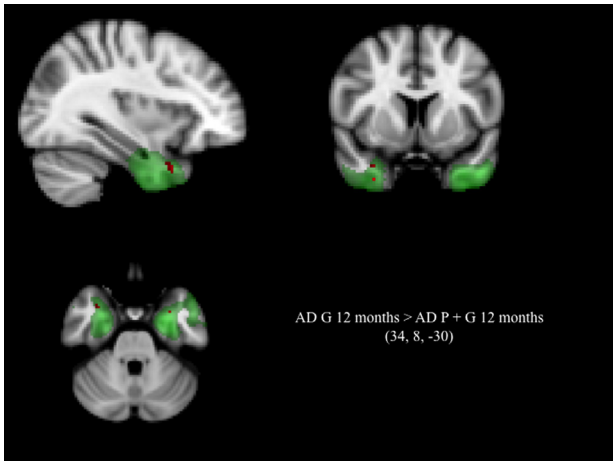

Figure 4 Contrast images depicting significant differences of functional connectivity (red) within the hippocampal sub-component (green) between the AD Galantamine arm (AD G) and the AD placebo arm (AD P+G) at one-year follow-up. Data are FWE corrected at $p_{corr} < 0.05$.
Sagittal, coronal, and axial images are displayed in radiological convention (x-, y-, and z-coordinates of each slice in the MNI152 standard space – in mm – are given in parenthesis).

functional connectivity in the AD G group in medial aspects of the temporal pole extending into the anterior division of the parahippocampal gyrus bilaterally, right > left, compared to the AD P+G group at one-year follow-up (Figure 4). No further statistically significant between-group differences were present.

3.3. VBM analysis

No significant changes in gray matter volume were detectable within either group (baseline vs. follow-up session after 6 months within the AD groups; baseline vs. follow-up session after 12 months within the AD groups and within the HC group; $p_{corr} > 0.05$).

4. Discussion

Here, we present the results of a randomized controlled trial demonstrating an increase of functional connectivity within the default-mode network (DMN) of patients with Alzheimer's disease (AD) after the long-term treatment with the acetylcholinesterase inhibitor (AChEI) Galantamine.

Two components were found in the Independent Component Analysis (ICA) based approach considered to represent an anterior and a posterior DMN sub-component; the cortical regions presented within these sub-systems correspond to core DMN nodes consistently found in resting-state fMRI studies (Buckner et al., 2008; Laird et al., 2009). In addition, a connectivity pattern extending bilaterally over parts of the temporal lobe including the hippocampal formation was detected; given that these regions are also considered parts of the DMN (Buckner et al., 2008), we included this pattern as another potential sub-component of

Table 3 Inter-group differences in cognitive performance at baseline and follow-up examinations.

CERAD test	Session	*p*-value		
		AD G vs. AD P+G	AD G vs. HC	AD P+G vs. HC
MMSE	Baseline	0.3363	*0.0001*	*0.0021*
	Follow-up 6 months	0.2538	-	-
	Follow-up 12 months	0.6298	*0.0041*	0.0080
Verbal fluency	Baseline	0.7713	*0.0009*	*0.0003*
	Follow-up 6 months	0.5971	-	-
	Follow-up 12 months	0.3261	*0.0005*	*0.0000*
Boston naming	Baseline	0.7492	0.1251	0.0190
	Follow-up 6 months	0.3685	-	-
	Follow-up 12 months	0.5668	0.1369	*0.0042*
Word list learning	Baseline	0.3381	*0.0001*	*0.0000*
	Follow-up 6 months	0.9622	-	-
	Follow-up 12 months	0.7958	*0.0000*	*0.0000*
Constructional praxis	Baseline	0.1600	0.0153	*0.0031*
	Follow-up 6 months	0.0913	-	-
	Follow-up 12 months	0.1768	0.0337	0.0155
Word list recall	Baseline	0.4917	*0.0000*	*0.0000*
	Follow-up 6 months	0.5231	-	-
	Follow-up 12 months	0.0618	*0.0000*	*0.0000*
Word list recognition	Baseline	0.0683	*0.0002*	*0.0000*
	Follow-up 6 months	0.8823	-	-
	Follow-up 12 months	0.7418	0.0087	*0.0000*
Recall of constructional Praxis	Baseline	0.4400	*0.0000*	*0.0005*
	Follow-up 6 months	0.2368	-	-
	Follow-up 12 months	0.1904	*0.0000*	*0.0000*

Numbers in Table 3 demonstrate the results of the independent *t*-tests. Significant differences ($p_{corr} < 0.00625$, Bonferroni-corrected for multiple comparisons) are italicized. AD G: patients with Alzheimer's disease treated continuously with Galantamine for 12 months; AD P + G: patients with Alzheimer's disease treated with a placebo from month 1–6 followed by Galantamine treatment from month 7–12. HC: Healthy volunteers. MMSE: Mini-Mental State Examination.

the network. The combination of these three connectivity patterns is similar to that recently described as DMN sub-components by others (Salami et al., 2014). The decomposition of the classic DMN previously described by Raichle and colleagues (Raichle et al., 2001) into several sub-components is consistent with more recent reports using ICA methods (Allen et al., 2011; Damoiseaux et al., 2012; Otti et al., 2013; Zuo et al., 2010), especially when applying a higher model order, i.e., the decomposition of an fMRI data set into a higher number of Independent Components (ICs) (Kiviniemi et al., 2009). In our case, the fMRI data set was segregated by the automatic dimensionality estimation tool as implemented in FSL into 34 ICs which is higher than the low model order of fixed 20 ICs often applied in the field (e.g., Biswal et al., 2010) but within the range reported by others also applying automatic dimensionality estimation methods (Allen et al., 2011; Otti et al., 2013). One reason for the observed decomposition of the DMN, at least in case of the anterior and posterior sub-component, may constitute the heterogeneity within this network (Uddin et al., 2009) that is more likely to be identified with a higher model order approach (Kiviniemi et al., 2009).

With regard to the functional architecture of the anterior DMN sub-component, there were no significant longitudinal changes within any of the three groups, *i.e.*, functional connectivity remained stable over time in patients as well as in healthy controls. In line with these findings, there were also no significant changes in functional connectivity between groups at the first follow-up session after 6 months as well as at the second follow-up session after 12 months demonstrating that the anterior regions of the DMN were not significantly affected by Galantamine therapy or the course of disease within the study period.

Different results were obtained for the posterior DMN sub-component. Here, a significant longitudinal increase of functional connectivity was present in the posterior cingulate cortex (both hemispheres, pronounced on the left side) as well as in the left-hemispheric Precuneus in the AD G group that has been treated with Galantamine from the very beginning over the course of one year; in contrast, functional connectivity remained stable in the same period in the AD P +G group – treated with verum only for the period of 6 months in the second phase of the study – indicating a modulation of posterior DMN nodes in AD patients only in case of a longer-term Galantamine treatment.

Interestingly, we observed a significant decrease of functional connectivity in the left-hemispheric precuneus cortex in the AD P+G group after 6 months (baseline vs. first follow-up session) although no significant changes could be detected in this group over the course of one year. Possibly this – at first glance conflicting – finding may be explained by a decrease of functional connectivity known to occur

inevitably during progression of AD (Damoiseaux et al., 2012; Dennis and Thompson, 2014; Zhang et al., 2010), which may have been partly reversed by Galantamine during the following 6 months resulting in a comparable degree of functional connectivity between baseline and 12 months follow-up. It has to be noted, however, that no significant longitudinal increase could be detected in this group between the first and the second follow-up session.

No longitudinal changes in gray matter volume could be detected within either group suggesting that the detected augmentation of functional connectivity within the posterior DMN sub-component was not primarily caused by underlying structural alterations.

The between-group comparisons were comparable with the findings derived from the longitudinal analyses in the sense that a one year long treatment with Galantamine was associated with increases of functional connectivity in the left posterior cingulate cortex and the precuneus cortex bilaterally in the AD G group as compared to healthy controls. No other between-group comparison – both at the first and the second follow-up examination – was statistically significant, which is interesting as one also may have expected an increase of functional connectivity in the AD G group in comparison with the AD P+G group. We speculate that the time delay of 6 months in verum treatment obviously was insufficient to produce (statistically) significant between-group effects, potentially due to insufficient statistical power given the limited number of AD patients that were included in the final analysis. However, the fact that there were also no significant changes in functional connectivity between the AD P+G group and the HC group at 12 months follow-up as well as no longitudinal changes within the AD P+G group in the course of one year (given the decrease at 6 months follow-up) indicates that the shorter-term Galantamine medication potentially had an initial – although statistically non-significant – effect that was sufficient to prevent a decrease of functional connectivity in this group.

The increase of functional connectivity particularly within the posterior cingulate cortex is important as it has been shown that this area exhibits extensive connectivity with other DMN nodes and therefore can be considered to act as a "functional hub" within this network (Laird et al., 2009). This functional hub is known to exhibit connectivity alterations in AD patients (Greicius et al., 2004), especially early in the disease (Damoiseaux et al., 2012), emphasizing its role for this disease.

The findings presented in the current work regarding increases of functional connectivity in the posterior DMN sub-component may be linked to those previously presented by Likitjaroen and colleagues who demonstrated an impact of Galantamine on the posterior body of the corpus callosum in AD patients using diffusion tensor imaging (Likitjaroen et al., 2012). As this area is known to contain fibers projecting into parietal regions (Hofer and Frahm, 2006), it can be assumed that changes in fiber tract integrity may have contributed to the observed increases of functional connectivity within the posterior cingulate cortex and the Precuneus.

We also observed significant effects of a one year long Galantamine treatment on functional connectivity within the hippocampal sub-component. Although no significant changes could have been found in the longitudinal run in any of the groups, there was an increase of functional connectivity at 12 months follow-up in the medial aspect of the temporal pole extending into the anterior parahippocampal gyrus bilaterally in the AD G group compared to patients attributed to the AD P+G group. In contrast to the findings observed in case of the posterior DMN sub-component, the relative increase of functional connectivity within the hippocampal sub-component was obviously too subtle to generate a statistically significant effect size within the group in the longitudinal run. One reason for this may constitute the small number of subjects examined; another the advanced neurodegeneration and – as a consequence – a possibly reduced response of functional connectivity to cholinergic enhancement within this region given that medial aspects of the temporal lobe atrophy among the first brain regions in the course of AD (Scheltens et al., 1992). We therefore think that the subtle effect may be interpreted as stabilization of functional connectivity in the hippocampal sub-component of patients from the AD G group which is induced by the longer-term (or earlier beginning of) Galantamine treatment (*i.e.*, 12 months vs. 6 months in the AD P+G group).

In contrast to the results presented in the current work, some prior fMRI studies investigating the effects of AChEIs on resting-state functional connectivity of AD patients have reported significant longitudinal changes after treatment periods of only 8-12 weeks (Goveas et al., 2011; Li et al., 2012; Sole-Padulles et al., 2013; Zaidel et al., 2012). However, there is a difference in the approach of data analysis between the majority of these prior studies and our work that must be taken into account as we used ICA for the extraction of DMN sub-components from fMRI data sets – i.e., a data-driven approach – whereas others mostly applied seed-based analyses, i.e., an approach where the functional structure of a detected network – and therefore a possible network change over time – is dependent upon a predefined voxel or cluster of voxels. Given this difference in methods, the comparability of our results with those previously reported on brain regions that are positively influenced by cholinergic enhancement is limited, in particular as studies using seed-based analyses have reported heterogeneous observations dependent upon the seed region selected for analysis. For instance, Goveas and colleagues reported an increase of functional connectivity in a hippocampal functional connectivity network particularly in the precentral gyrus but also in the left Parahippocampus and in the right posterior cingulate cortex (Goveas et al., 2011), Li and colleagues demonstrated an increase in cortical regions similar to DMN nodes including the ventral anterior cingulate cortex, the right Precuneus, and the left parahippocampal gyrus among others (Li et al., 2012), and Zaidel and co-workers reported an increase of interhemispheric functional connectivity only between dorsolateral prefrontal cortices (Zaidel et al., 2012). Despite the limitations in comparability, the findings presented in the current work are in line with these previous reports with respect to AChEI induced effects on the posterior cingulate cortex, the Precuneus, and the medial aspect of the temporal lobe.

To our knowledge only Sole-Padulles and co-workers used an ICA based approach; similar to our results they could not find any significant changes in the DMN in the longitudinal run

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after a shorter-term period (12 weeks) of Donepezil treatment; however, they report a stabilization of functional connectivity in Donepezil treated patients compared to untreated AD controls particularly in the right-hemispheric parahippocampal gyrus (Sole-Padulles et al., 2013). Our findings are in line with their result insofar as the stabilization of functional connectivity in the temporal lobes as observed in the current study was more pronounced on the right hemisphere. However, it has to be noted that the exact location of effects on functional connectivity differs between both studies (temporal pole extending into the anterior division of the parahippocampal gyrus in our case vs. posterior division of the parahippocampal gyrus according to their Figure 1); this divergence may be caused by differences in the period between sessions (12 months vs. 12 weeks), the small number of patients participating in either study, and the fact that our hippocampal sub-component extended mainly over the anterior aspects of the medial temporal lobe. Also, the use of different AChEIs in either study (Galantamine vs. Donezepil) may have contributed to the results. Although there is no evidence that one of these drugs is superior to the other in terms of clinical efficacy, they have slightly different pharmacological properties (Colovic et al., 2013) that may be reflected in a slightly different modulation of the brain's functional architecture.

There were no significant changes in the cognitive performance in either AD group over time which is inconsistent with the longitudinal increase of functional connectivity in the posterior DMN sub-component and the stabilization of the hippocampal sub-component in the AD G group. In that context, we suggest that resting-state fMRI may be more sensitive for the detection of pharmacologically induced effects on brain function of AD patients than clinically established neuropsychological examinations.

In summary, long-term treatment with the AChEI Galantamine was associated with an increase of functional connectivity within the posterior cingulate cortex and the Precuneus as well as with a stabilization of functional connectivity within the anteromedial aspect of the temporal lobes of AD patients. A shorter-term Galantamine treatment produced no significant increases, but possibly prevented a decline of functional connectivity. Based on our findings we conclude that a long-term (or early starting of) AChEI therapy may be beneficial for the functional DMN architecture by positively influencing functional connectivity within that network. However, future studies may further clarify the impact of different methodological approaches used for resting-state fMRI data analysis as well as enlighten the potentially different effects of different AChEIs on the brain's functional architecture prior to applying resting-state fMRI as a novel, objective, and sensitive marker of treatment response in AD patients.

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Contributors

J.B. wrote the manuscript and undertook the statistical analysis. D. K., M.P., V.K., & A.B. managed the literature searches and data analyses. U.C. was involved in data acquisition and preparation. M. R., S.J.T., & T.M. designed the study and wrote the protocol. All authors contributed to and have approved the final manuscript.

Conflict of interest

None of the authors has an actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three (3) years of beginning the work submitted that could inappropriately influence, or be perceived to influence, their work.

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