Title: Effect of cholinergic treatment depends on cholinergic integrity in early Alzheimer's disease

Running Title: Cholinergic stimulation in early Alzheimer's

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Abstract

In early Alzheimer's disease, which initially presents with progressive loss of short-term memory, neurodegeneration especially affects cholinergic neurons of the basal forebrain. Pharmacotherapy of Alzheimer's disease therefore often targets the cholinergic system. In contrast, cholinergic pharmacotherapy of mild cognitive impairment is debated since its efficacy to date remains controversial. We here investigated the relationship between cholinergic treatment effects and the integrity of the cholinergic system in mild cognitive impairment due to Alzheimer's disease.

Fourteen patients with high likelihood of mild cognitive impairment due to Alzheimer's disease and 16 age-matched cognitively normal adults performed an episodic memory task during functional magnetic resonance imaging under three conditions: (1) without pharmacotherapy, (2) with placebo, and (3) with a single dose of rivastigmine (3mg). Cortical acetylcholinesterase activity was measured using positron emission tomography with the tracer [11C]N-methyl-4-piperidyl acetate.

Cortical acetylcholinesterase activity was significantly decreased in patients relative to controls, especially in the lateral temporal lobes. Without pharmacotherapy, mild cognitive impairment was associated with less memory-related neural activation in the fusiform gyrus and impaired deactivation in the posterior cingulate cortex, relative to controls. These differences were attenuated under cholinergic stimulation with rivastigmine: patients showed increased neural activation in the right fusiform gyrus but enhanced deactivation of the posterior cingulate cortex under rivastigmine, compared to placebo. Conversely, controls showed reduced activation of the fusiform gyrus and reduced deactivation of the posterior cingulate under rivastigmine, compared to placebo. In both groups, the change in neural activation in response to rivastigmine was negatively associated with local acetylcholinesterase activity.

On the behavioral level, an analysis of covariance revealed a significant group-by-treatment interaction in episodic memory performance when accounting for hippocampal grey matter atrophy and function.

Our results indicate that rivastigmine differentially affects memory-related neural activity in patients with mild cognitive impairment and cognitively normal, age-matched adults, depending on acetylcholinesterase activity as a marker for the integrity of the cortical cholinergic system. Furthermore, hippocampal integrity showed an independent association with the response of memory performance to acetylcholinesterase inhibition.

Key Words (4/5): acetylcholinesterase, MP4A, subsequent memory effect, fMRI

Abbreviations

AChE – acetylcholinesterase

ChAT – choline acetyltransferase

EPI – gradient echo-planar imaging

fMRI – functional magnetic resonance imaging

FWE – family wise error

FWHM – full width at half maximum

GLM – general linear model

MCI – mild cognitive impairment

MMSE – Mini-Mental status exam

MNI – Montreal Neurological Institute

MP4A – [^{11}C]N-methyl-4-piperidyl acetate

PCC – posterior cingulate cortex

ROI – region of interest

SME – subsequent memory effect

TE – echo time

TI – inversion time

TR – repetition time

VBM – voxel based morphometry

VLMT – verbal learning and memory test

Introduction

In Alzheimer's disease, which presents with the cardinal symptom of progressive short-term memory loss, neurodegeneration especially affects cholinergic basal forebrain neurons (Mesulam, 2004; Schliebs and Arendt, 2006). Most pharmacological therapies of dementia due to Alzheimer's disease therefore target the cholinergic system (Lahiri *et al.*, 2004). In the clinical course of Alzheimer's disease, dementia is preceded by the stage of MCI. MCI is characterized by measurable neuropsychological deficits, which are not yet severe enough to affect significantly a person's daily functioning (Albert *et al.*, 2011). Compared to cognitively normal aging, MCI is associated with signs of altered cholinergic neurotransmission (Herholz, 2008; Haense *et al.*, 2012; Richter *et al.*, 2017). Nevertheless, it remains controversial, whether patients with MCI due to Alzheimer's disease also benefit from cholinergic pharmacotherapy. While some studies suggested that AChE inhibitors may lower the rate of progression from MCI to dementia (Petersen *et al.*, 2005; Diniz *et al.*, 2009), meta-analyses did not confirm this finding (Birks and Flicker, 2006; Loy and Schneider, 2006; Raschetti *et al.*, 2007). This negative outcome of meta-analyses is

surprising in light of basic research indicating a positive effect of AChE inhibitors on memory-related brain activation in MCI and even cognitively normal older individuals (Goekoop *et al.*, 2006; Grön *et al.*, 2006; Kukolja *et al.*, 2009; Pa *et al.*, 2013).

One possible explanation for this discrepancy is that the response to cholinergic stimulation may depend on the integrity of the cholinergic system in analogy to the Yerkes-Dodson law (Yerkes and Dodson, 1908), stating that task performance first improves and then deteriorates with increasing arousal. It has been observed in cognitively normal aging, that individuals with low baseline memory performance benefit from physostigmine treatment, while those with high baseline performance show no such benefit and, at least in part, even poorer memory (Kukolja *et al.*, 2009). Furthermore, previous studies on the effect of AChE inhibitors in MCI selected patients due to clinical criteria only (Petersen *et al.*, 2005; Grön *et al.*, 2006), resulting in a degree of uncertainty regarding the presence of Alzheimer's pathology in these patients.

Accordingly, the goal of this study was to investigate whether the effect of cholinergic stimulation on memory-related neural activation differs between cognitively normal older individuals and patients with a high likelihood of MCI due to Alzheimer's disease. Furthermore, we aimed at linking the response to cholinergic stimulation to the integrity of the cholinergic system. In light of previous research (Haense *et al.*, 2012; Richter *et al.*, 2017), we expected decreased cortical AChE activity in MCI due to Alzheimer's disease. We hypothesized that a change in neural activation in response to rivastigmine would be related to local intrinsic AChE levels: Specifically, we hypothesized that in MCI with Alzheimer's pathology, neural activation in task-related areas would normalize under rivastigmine (Rombouts *et al.*, 2002; Kircher *et al.*, 2005; Pa *et al.*, 2013; Risacher *et al.*, 2013), consequently improving memory performance. Conversely, we expected that controls would not benefit from rivastigmine or might

even perform worse (Kukolja *et al.*, 2009). In light of ambiguous previous findings regarding the cholinergic system in MCI due to Alzheimer's disease (Petersen *et al.*, 2005; Birks and Flicker, 2006; Raschetti *et al.*, 2007; Diniz *et al.*, 2009), we expected that the cholinergic deficit is not the only factor determining the behavioral response to AChE inhibition. We hypothesized that an additional predictor of the response to rivastigmine is the integrity of task-related cerebral structures, i.e., in the case of recent episodic memory: the hippocampus. Using functional magnetic resonance imaging (fMRI) of an episodic memory task and *non-invasive in-vivo* imaging of cortical AChE activity with MP4A-PET, we tested these hypotheses in cognitively normal older subjects and patients with a high likelihood of MCI due to Alzheimer's disease (Albert *et al.*, 2011).

Methods

Participants

Twenty patients recently diagnosed with MCI in the Memory Clinic Cologne-Jülich (11 male, aged 54 to 80, mean 68.7 ± 6.8 years), without neurological or psychiatric comorbidities, were recruited as part of the MACS study (Memory, Aging and the Cholinergic System, EudraCT No. 2008-008896-32). The study was approved by the ethics committee of the medical faculty of the University of Cologne, as well as local and federal authorities. Written informed consent was obtained from all participants prior to the study.

Five patients had to be excluded from further analysis because of excessive head movement during fMRI scanning (3), side-effects (nausea and vomiting) during fMRI scanning (2), and one subsequent withdrawal of consent. The remaining 14 patients had positive CSF biomarkers indicative of Alzheimer pathology and signs of neuronal

injury, thus meeting the criteria for a high likelihood of MCI due to Alzheimer's disease described by Albert and colleagues (Albert *et al.*, 2011). Evidence of neuronal injury was operationalized as i) medial temporal atrophy according to the medial temporal atrophy scale (Scheltens *et al.*, 1992) on high-resolution T1-weighted images, ii) temporo-parietal and precuneal hypometabolism in fluorodeoxyglucose-PET quantified with 3D-SSP (Minoshima *et al.*, 1995), or iii) elevated Tau-protein in CSF (> 375pg/ml). Positive CSF amyloid pathology was defined as Amyloid $ß_{1-42}$ < 550pg/ml or a Tau/Amyloid-$ß_{1-42}$ ratio > 0.52 (Duits *et al.*, 2014). CSF was obtained via lumbar puncture (for details on processing see Supplementary Materials).

As control group, 22 age-matched cognitively normal adults (14 male, aged 53 to 80, mean 66.6 ± 7.1 years) without history of neurological or psychiatric disease and without cholinergic, anticholinergic, or other psychoactive drugs were recruited from the community. Six cognitively normal older subjects were not eligible for further analyses due to an incidental pathological finding (1; severe cerebral atrophy), side-effects of rivastigmine during fMRI scanning (2), severe dental metal artifacts interfering with MRI data acquisition (2), and PET acquisition failure due to a technical issue (1).

Thus, the data of 16 controls and 14 MCI patients entered the analysis. All participants underwent a physical and neurological examination by a neurologist and a comprehensive neuropsychological assessment. The latter consisted of the MMSE, Beck's Depression Inventory V (Schmitt *et al.*, 2006), the Bayer Activities of Daily Living (Hindmarch *et al.*, 1998), the Trail-Making-Test A and B (Berres *et al.*, 2000), Brief Test of Attention (Schretlen *et al.*, 1996), the Leistungsprüfsystem part 4 (logical thinking) (Horn, 1983), the VLMT (the German version of the Rey Auditory Learning Test (Helmstaedter *et al.*, 2001)), and the Rey-Osterrieth Complex Figure Test (Rey, 1964). MCI was defined as performance more than 1.5 standard deviations below the

norm in the delayed recall of the VLMT, and a score greater than 24 points in the MMSE, normal scores in tests of attention and logical thinking, as well as normal ratings on the Bayer Activities of Daily Living questionnaire. Furthermore, the patients' functional independence was corroborated by the history obtained from the family (spouse/children) and behavior during the course of the study.

Procedure

Clinical, neuropsychological and MRI data were acquired in four sessions over the course of two months. In the first MRI session, anatomical and functional scans without pharmacological stimulation were acquired. In the second and third MRI sessions, participants received capsules with either 3mg of rivastigmine or placebo in a double-blind cross-over design, randomized with respect to order of treatment. This rivastigmine dose has been shown to significantly reduce AChE activity in CSF in healthy adults (Kennedy *et al.*, 1999) and patients with Alzheimer's disease (Cutler *et al.*, 1998). Furthermore, a single oral application has been demonstrated to modulate neural activation in both populations (Di Lazzaro *et al.*, 2002; Rombouts *et al.*, 2002; Langguth *et al.*, 2007). Medication was administered 90 minutes prior to MR scanning to ensure peak plasma concentrations of rivastigmine (Lefèvre *et al.*, 2008). To account for side-effects of cholinergic stimulation (i.e., nausea, dizziness, headache), subjective well-being was assessed before administration of the trial medication and after MR acquisition using a semiquantitative rating scale of complaints (von Zerssen, 1976). Both MR sessions with pharmacological stimulation took place within four weeks. PET measurement took place within an average of 31 days (standard deviation 35 days) of the initial MRI session. In order to exclude effects on AChE activity due to treatment with AChE inhibitors (Bohnen *et al.*, 2005), PET measurement took place before, or at least seven days after pharmacological stimulation.

During fMRI scanning, participants performed a visual item memory task consisting of an encoding and a retrieval session spaced seven minutes apart. During each visit, the task was rehearsed outside and inside the MR scanner prior to scanning, using a shortened version of the paradigm. For a detailed description of the task, please cf. Supplementary Materials.

Behavioral data analysis

Trials with reaction times less than 400ms or more than two standard deviations greater than the mean were considered outliers and classified as invalid trials together with missed trials. The analysis focused on items that were correctly classified during the retrieval session. Performance was assessed using the sensitivity index *d'* (for details see Supplementary Methods). Behavioral data were analysed with repeated measures ANCOVAs with treatment (placebo, rivastigmine) as within subject factor. Parameters entered in ANCOVAs were normally distributed within groups as tested with Shapiro-Wilks tests. If the cholinergic deficit is a central aspect of the group difference and hence essential for the group-by-treatment interaction, the interaction should not be significant when accounting for the cholinergic deficit. To test this, the cholinergic deficit was operationalized as the k_3 averaged across voxels showing a significant group difference in AChE activity (FWE-corrected $p < 0.05$) (**Figure 1, Table 2**, **Supplementary Table 3**) and included in the ANCOVA as covariate of no interest.

To account for mild side-effects of rivastigmine that did not require discontinuation of measurement, changes in subjective well-being were incorporated into the analysis as covariate of no interest. To assess the relevance of hippocampal integrity, average grey matter density within the hippocampus was used to reflect hippocampal structure, and verbal episodic memory performance was used to reflect hippocampal function (Kim,

2011; Wolk *et al.*, 2011). All statistical analyses of non-imaging data were conducted with SPSS (Version 23.0, IBM Corp., Armonk, NY).

MR and PET acquisition

MP4A was synthesized as previously described (Herholz *et al.*, 2000; Haense *et al.*, 2012), with minor modifications. Between 192 and 586 MBq (mean 469.27 MBq, standard deviation 84.21MBq) of MP4A were injected intravenously as bolus. The wide range of injected activities is mainly attributable to two control subjects with injected doses of 192.37 MBq and 278.17 MBq. Despite this relatively large range of injected activities, a consequent bias on results is unlikely, since scatter correction was performed during reconstruction of PET images (see below), and results of statistical analyses were essentially unchanged when removing these two participants.

A detailed summary of injected activities (**Supplementary Table 1**) and a discussion of their variability is provided in the Supplementary materials. PET-scanning was performed using an ECAT HRRT scanner (CPS Innovations, Knoxville TN, USA) using the protocol described by Haense et al. (2012). Images were reconstructed using 3-dimensional ordered subset expectation maximization, including correction for random coincidences, attenuation and scatter. Moreover, putative effects of scattered activities would have been minimal, since these mainly affect the amplitude of time activity curves, while the shape, which governs the estimation of k_3, is barely affected (Häggström *et al.*, 2014). High-resolution T1-weighted images were acquired using a 3T Trio scanner (Siemens, Erlangen, Germany). fMRI scans were acquired using an EPI sequence. The field of view was angulated to parallel the medial tentorium cerebelli to reduce susceptibility artefacts in the medial temporal lobe (Deichmann *et al.*, 2003; Weiskopf *et al.*, 2006). A detailed description of MRI sequences is provided in the Supplementary Methods.

PET processing

AChE activity was assessed by quantifying the hydrolysis rate of MP4A, i.e., k_3, at the voxel level. Images were processed as previously described (Richter *et al.*, 2017) for more details please see Supplementary Methods). k_3-values were extracted from neocortical and hippocampal grey matter, as k_3-estimation in other subcortical structures is often unreliable (Herholz *et al.*, 2001; Herholz *et al.*, 2004).

Processing of functional MRI data

Preprocessing of fMRI data was performed with standard procedures employing different modules of the FSL-software package (FMRIB's Software Library, Version 5.0, http://www.fmrib.ox.ac.uk/fsl). A detailed description is provided in the Supplementary Methods.

Neural activation underlying successful encoding can be operationalized as fMRI signal that is greater for stimuli that were later remembered than for those later forgotten (the subsequent memory effect (SME)). To analyze the SME, encoding trials were divided into two types of events: items correctly identified as "old" (OC) during the retrieval phase and invalid trials (INV; i.e., misclassification during retrieval, misses, and outliers). For each event type, a constant epoch regressor consisting of a boxcar function beginning at stimulus onset and lasting 3.5 s (stimulus duration) was defined. Additionally, for each event type, a variable epoch regressor with the identical time-course as the respective constant epoch regressor, but variable boxcar width defined by the response time of the respective trial was constructed. The variable epoch regressor was orthogonalized to the constant epoch regressor to account for effects of response time variability, which can significantly influence the amplitude of the

hemodynamic response (Grinband *et al.*, 2008; Richter *et al.*, 2013). The resulting four regressors were convolved with a double gamma hemodynamic response function and entered into a first level GLM analysis, together with the covariates generated during the preprocessing steps. Resulting statistical maps were normalized to standard space using the parameters generated in the VBM analysis and entered into a second level analysis using non-parametric testing as implemented in the FSL module *randomise* (Nichols and Holmes, 2002; Winkler *et al.*, 2014). The non-parametric approach was chosen because of its statistical robustness in small sample sizes and because the FSL module *randomise* allows the inclusion of voxel-wise covariates, in the present analysis the grey matter density maps generated with VBM.

Second level analyses were constrained to the medial temporal lobe (hippocampus and amygdala), the fusiform gyrus, and the posterior cingulate cortex, based on previous studies of memory-related neural activation in cognitively normal subjects (Garoff *et al.*, 2005; Kim, 2011), patients with Alzheimer's disease (Golby *et al.*, 2005; Huijbers *et al.*, 2012), and patients with Alzheimer's disease under cholinergic stimulation (Rombouts *et al.*, 2002). ROIs were defined using the Harvard-Oxford cortical and subcortical structural atlases (Desikan *et al.*, 2006).

The SME was computed as the difference between OC and INV. Due to the nature of the non-parametric approach all second level analyses were performed as T-tests. To test for a placebo effect, the data from both groups were pooled and a paired-T-test was computed across groups. Since no significant difference in the SME was observed at the ROI or the whole brain level, data from the baseline and placebo scans were pooled when testing for a group average SME and when computing for group differences in the SME. Furthermore, we tested whether there is a differential response to pharmacological stimulation in the two groups. For this purpose, the parameter

estimates from the placebo condition were subtracted from those of the rivastigmine condition and the resulting difference maps were subjected to a two sample T-test to compare between groups. VBM maps were incorporated as covariate of no interest in all second level analyses to correct for effects of grey matter atrophy. To correct for multiple testing, threshold-free cluster enhancement (Smith and Nichols, 2009) and FWE-correction were employed. Voxels with $p < 0.05$ are reported as significant.

Voxel-based morphometry. Structural data were analysed using VBM as implemented in the software FSL-VBM (Good *et al.*, 2001; Smith *et al.*, 2004; Douaud *et al.*, 2007); http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM). Grey matter images modulated for nonlinear normalization were smoothed with an isotropic Gaussian kernel of 8.55mm (FWHM) resulting in the same final resolution as the functional images.

Results

Demographics and neuropsychological characteristics

Groups did not differ with respect to age ($t_{(28)}$= -1.035, p = 0.31), gender distribution ($x^{2(1)}$ = 0.117, p = 0.732), or depressive symptoms assessed using the BDI ($t_{(28)}$ = -0.261, p = 0.8). All participants had normal ratings of activities of daily living, but there was a trend for poorer ratings in the patient group. However, this was not significant when accounting for multiple comparisons **(Table 1)**.

Patients performed significantly poorer on all neuropsychological tests except for the TMT-A. All patients performed within normal ranges on tests of attention (the Brief Test of Attention) and logical thinking (part 4 of the Leistungsprüfungssystem; **Table 1)**. However, nine patients had pathological scores on the TMT-B and five patients also on the TMT-A. While the predominant impairment in all patients was in the memory domain, five had a purely amnestic MCI subtype, and nine could be classified as multi-domain MCI. There was no difference between placebo and rivastigmine with respect to subjective complaints at the beginning of the session ($t_{(29)}$ = -0.643, p = 0.525). However, there was a non-significant difference between placebo and rivastigmine regarding the increase of complaints after administration relative to baseline ($t_{(29)}$ = 1.84, p = 0.076). Groups did not differ with respect to an increased number of complaints under rivastigmine (controls 0.875, SD 5.464; MCI 3.286, SD 6.426, $t_{(28)}$ = -1.111, p = .276).

Behavioral performance

Performance on the item memory task, quantified as d', was normally distributed and differed significantly between groups without pharmacological stimulation (controls 2.651, SD 0.561, patients 1.416 SD 0.617, $t_{(28)}$ = 5.746, p < 0.001), after administration

of placebo (controls 2.714, SD 0.584, MCI 1.379, SD 0.677, $t_{(28)} = 5.797$, $p < 0.001$), and after administration of rivastigmine (controls 2.53, SD 0.536, MCI 1.459, SD 0.789, $t_{(28)} = 4.397$, $p < 0.001$). There was a non-significant group difference of the effect of rivastigmine on d' (controls -0.184, SD 0.444; MCI 0.08 SD = 0.439, $t_{(28)} = -1.632$, $p = 0.114$).

Behavioral data: cortical cholinergic system integrity and the effect of rivastigmine

AChE activity, quantified as k_3 of MP4A, was significantly decreased in MCI compared to controls (controls M = 0.064, SD 0.006, MCI M = 0.057, SD 0.007, $t_{(28)} = 3.105$, $p = 0.004$). This effect was most pronounced in the lateral temporal, parietal and occipital lobes, but also encompassed the hippocampi, adjacent medial temporal structures, and frontal= areas (**Figure 1, Table 2**) in two clusters of 39,720 and 26 voxels, respectively. For a detailed list of the structures comprising the clusters and the number of significant voxels within each structure please cf. **Supplementary Table 3**. A voxel-wise analysis revealed no areas where AChE activity was greater in MCI than in controls.

A two-way mixed ANCOVA including changes in well-being across treatments as covariate of no interest revealed a group-by-treatment interaction which missed significance ($F_{(1,27)} = 4.228$, $p = 0.05$). To operationalize the cholinergic deficit in MCI, k_3 was averaged across voxels where it was significantly lower in patients than in controls. When including the resulting values as covariate in the ANCOVA, the group by treatment interaction was not significant ($F_{(1,26)} = 2.874$, $p = 0.102$). However, when including hippocampal grey matter density as a covariate of no interest, there was a significant group-by-treatment interaction ($F_{(1,26)} = 5.581$, $p =$

0.026). Including episodic memory performance at delayed recall, i.e., a measure of hippocampal function, as a covariate of no interest yielded an even stronger effect ($F_{(1,26)}$ = 10.787, p = 0.003). This finding was independent of age and white matter lesion load (please see Supplementary Materials for details).

fMRI: SME across groups

Across groups, increased neural activation on subsequently remembered items was observed in the left anterior hippocampus (maximum at MNI-coordinates x = -30, y = -8, z = -22, **Figure 2, Table 3**). Mainly in the left but also in the right fusiform gyri, greater neural activity for subsequently remembered items was observed **(Figure 2, Table 3).** In the PCC, decreased neural activation was seen for subsequently remembered items (**Figure 2**, **Table 3**).

fMRI: Group differences in the SME

No group difference in the SME was observed in the hippocampus. In the left fusiform gyrus, controls showed greater neural activation on subsequently remembered items than MCI patients (**Figure 3, Table 3**). In the PCC, greater deactivation on subsequently remembered items was observed in controls than in MCI patients (**Figure 3, Table 3)**.

fMRI: group differences in the effect of rivastigmine on neural activation

In response to rivastigmine, MCI patients showed an increase and controls showed a decrease of neural activation in the right fusiform gyrus (**Figure 4, Table 3**). In the PCC, patients showed a decrease in neural activation, while controls exhibited an

increase **(Figure 4, Table 3)**. No difference between groups in the pharmacological effect on the SME was observed in the hippocampus.

fMRI: effect of rivastigmine on neural activation and its relationship to local AChE activity

Average parameter estimates extracted from the areas showing a significant group difference in the pharmacological effect on neural activity were highly correlated with local cortical k_3 (fusiform gyrus: Spearman's rho = -0.476, p = 0.008, PCC: Spearman's rho = 0.481, p = 0.007), as assessed with a correlation analysis including both groups. This effect remained when accounting for local grey matter density and group as covariates of no interest in a multiple regression analysis (fusiform gyrus: ß = -0.394, p = 0.019; PCC: ß = 0.359, p = 0.035; **Figure 4**). Within the control group, a multiple regression showed the same pattern with a stronger positive association in PCC (ß = 0.784, p = 0.01), but a negative trend in the fusiform gyrus, which missed significance (ß = -0.499, p = 0.056). In the patient group, the associations had the same direction but were not significant in either region (PCC: ß = 0.273, p = 0.380; fusiform gyrus: ß = -0.341, p = 0.174). This finding was independent of age and white matter lesion load (please see Supplementary Materials for details).

Discussion

We observed that neural activation underlying episodic memory encoding depends on local AChE activity as a marker for the integrity of the cortical cholinergic system. AChE activity was significantly reduced in MCI due to Alzheimer's disease. Characteristic neural effects of the SME – i.e., activation of the fusiform gyrus and

deactivation in the PCC – were attenuated in patients, but partially normalized under cholinergic stimulation. This positive pharmacological effect of rivastigmine on neural activation was significantly correlated with independently assessed local AChE activity. In response to rivastigmine, greater deactivation in the PCC and activation in the fusiform gyrus were associated with lower AChE activity in the respective regions. The effect of rivastigmine on memory also depended on the cortical cholinergic deficit in MCI. There was a significant group-by-treatment interaction effect of rivastigmine on memory performance, which was not present when accounting for the group difference in AChE activity. Interestingly, this interaction was especially significant when accounting for hippocampal structure (hippocampal grey matter density) and function (baseline verbal memory performance). The integrity of the hippocampus and the reduction of cortical AChE activity thus appear to have separate influences on the effect of rivastigmine on memory.

Group Difference in AChE activity: The cortical cholinergic deficit in MCI

As previously described (Haense *et al.*, 2012; Marcone *et al.*, 2012), the most significant decrease in AChE activity was observed in the lateral temporal lobes (i.e., the middle and inferior temporal gyrus as well as the planum polare). AChE reduction was less pronounced in the hippocampal formation. This may be attributable to the different origins of cholinergic neurons providing efferents to neocortical regions and the hippocampal formation: while the neocortex and amygdala are supplied by cholinergic neurons in the posterior basal nucleus of Meynert, the hippocampus receives its cholinergic input from the medial septal nuclei and the diagonal band of Broca (Mesulam and Geula, 1988b). Since Alzheimer pathology initially and most severely affects cholinergic neurons in the posterior basal nucleus of Meynert (Sassin *et al.*, 2000) a more pronounced AChE decrease in the lateral temporal cortex

compared to the hippocampal formation is likely to reflect differential effects of Alzheimer's disease related degeneration of separate cholinergic nuclei.

In the neocortex and the hippocampus, AChE is found in the axons of cholinergic neurons that originate from the basal forebrain (Mesulam and Geula, 1988b), and to a lesser extent in AChE-rich pyramidal neurons, both of which show a significant reduction in Alzheimer's disease (Mesulam and Geula, 1988a). Since the majority of the cholinergic axons in the cortex originate from the basal forebrain, cortical AChE activity also provides information about the integrity of cholinergic tracts (Mesulam and Geula, 1988b).

Other measures of cholinergic neurotransmission, i.e., the presynaptic vesicular acetylcholine transporter (VChAT) and nicotinic acetylcholine receptors, show similar alterations to AChE: their levels are decreased in PET studies of MCI and early dementia due to Alzheimer's disease, albeit to a lesser extent than in later stages (Kuhl *et al.*, 1996; Nordberg, 2001; Rinne *et al.*, 2003; Mazère *et al.*, 2008; Haense *et al.*, 2012; Marcone *et al.*, 2012). Choline acetyltransferase (ChAT), another key enzyme in cholinergic neurotransmission, is an exception to this pattern. While it is generally co-localized with AChE in cholinergic neurons in normal aging and Alzheimer's disease (Mesulam and Geula, 1992; Geula and Mesulam, 1996), it has been found to be increased in MCI due to Alzheimer's disease in the hippocampus and frontal lobe (DeKosky *et al.*, 2002; Ikonomovic *et al.*, 2003). This increase may be due to reactive synaptogenesis arising from cholinergic neurons in the medial septal nuclei following a loss of glutamatergic input from the entorhinal cortex to the hippocampus (Cotman *et al.*, 1973; Savaskan and Nitsch, 2001). Importantly, an increase of ChAT is not necessarily at odds with a degeneration of the cortical cholinergic system, as cholinergic fiber abnormalities observed in aging and Alzheimer's disease include an enlargement of nerve terminals immunoreactive to ChAT (Geula *et al.*, 2008).

Interestingly, neuronal counts (Gilmor *et al.*, 1999) and AChE activity (Herholz *et al.*, 2004) in the basal forebrain remain normal in MCI and mild dementia, although there is evidence from macroscopic MRI studies indicating a progressive atrophy of the basal forebrain in aging and Alzheimer's disease (Grothe *et al.*, 2012). In summary, AChE and most other cholinergic markers provide a relatively consistent picture of a progressive degeneration of the cholinergic inputs to the cortex and the hippocampus.

The pharmacological modulation of the SME depends on local AChE activity

In patients and controls, cholinergic stimulation induced opposite effects on the SME. In the fusiform gyrus, activation increased in MCI but decreased in controls. Conversely, in the PCC, a region typically deactivated during episodic memory encoding, rivastigmine administration elicited a greater deactivation in MCI but reduced the deactivation in controls. These results are in line with previous studies, reporting increased activation in the fusiform gyrus in Alzheimer's disease in response to acute (Rombouts *et al.*, 2002; Kircher *et al.*, 2005) and chronic cholinergic stimulation (Pa *et al.*, 2013) and improved deactivation in postero-medial regions under chronic administration of donepezil in MCI (Risacher *et al.*, 2013). Crucially, in our data the direction and size of the effect of cholinergic stimulation on neural activation depended on local AChE activity: low local AChE activity was associated with increased neural activation in the fusiform gyrus under rivastigmine. In the PCC, by contrast, reduced AChE activity was associated with greater deactivation under rivastigmine. In other words, data suggest that cholinergic stimulation may only exert its beneficial effects on neural activation in the presence of a local cholinergic deficit. Likewise, detrimental effects of rivastigmine on neural activation and behavioral performance in controls (in contrast to the beneficial effects in MCI) can best be explained by an "inverted-U" response, often observed in pharmacotherapy (Yerkes

and Dodson, 1908; Diamond *et al.*, 2007): While controls, who already operated close to optimal levels at baseline, performed poorer after drug administration, performance in patients with a cholinergic deficit was enhanced (Kukolja *et al.*, 2009; Bentley *et al.*, 2011).

Generally, cholinergic input to the cerebral cortex is of a modulatory nature, reducing background activity, increasing the responsiveness of cortical neurons to sensory input (Hasselmo *et al.*, 1992; Patil and Hasselmo, 1999; Mesulam, 2004), and facilitating long-term potentiation (Huerta and Lisman, 1993; Dringenberg *et al.*, 2007). The observed changes in neural activation are in line with findings that cholinergic stimulation optimizes processing of task-relevant stimuli (Sarter *et al.*, 2005) and facilitates a shift from an internally focused state with an active default-mode network to the processing of external stimuli (Hahn *et al.*, 2007; Bentley *et al.*, 2011). During initial episodic memory formation, neural resources are redistributed, enhancing activity in regions relevant for encoding at the cost of activity in other regions (Bentley *et al.*, 2009; Kukolja *et al.*, 2009). Cholinergic stimulation supports this redistribution by enhancing activations in the fusiform gyrus, part of the encoding network (Cabeza and Nyberg, 2000; Kim, 2011), and deactivations in the PCC.

We did not observe an effect of rivastigmine on neural activation in the medial temporal lobe. The SME in the hippocampus has been shown to increase after chronic, but not acute administration of the AChE inhibitor galantamine (Goekoop *et al.*, 2004). The most parsimonious explanation for the discrepancy of our finding of no rivastigmine effect on neural activation in the medial temporal lobe and studies which showed increased hippocampal activation after acute administration is that those studies employed tasks emphasizing spatial context association (Kukolja *et al.*, 2009), or navigation (Grön *et al.*, 2006), placing a higher demand on hippocampal function.

The neural SME differs between groups

Across groups a positive SME was observed in the left anterior hippocampus and the fusiform gyrus bilaterally. This lateralization has been demonstrated in several studies for item memory (Spaniol *et al.*, 2009; Kim, 2011). In accordance with the literature, in the PCC the SME was negative (Kim, 2011). In the fusiform gyrus, the SME was greater in controls than in patients as has been reported in early Alzheimer's disease (Golby *et al.*, 2005; Kircher *et al.*, 2005). In the PCC on the other hand, a less negative SME was observed in MCI than in controls, which is a consistent finding in studies examining episodic memory in MCI (Petrella *et al.*, 2007; Pihlajamäki *et al.*, 2010; Risacher *et al.*, 2013; Nellessen *et al.*, 2015).

The SME in the hippocampal formation did not differ between groups. Previous studies have found both increased (Trivedi *et al.*, 2008) and decreased (Pariente *et al.*, 2005) SMEs in MCI compared to controls. Findings are inconsistent regarding changes in memory-related hippocampal activation in MCI, which has been attributed to different patterns depending on disease stage. Compensatory hyperactivation may precede decreased activation at later stages (Nellessen *et al.*, 2015).

The fact that hippocampal activation did not differ between groups may also reflect load dependency of neural activation. At low loads, patients with MCI have been shown to exhibit greater neural activation than controls in the hippocampus, while the opposite pattern was observed at high cognitive loads (de Rover *et al.*, 2011). In analogy to the 'compensation-related utilization of neural circuits hypothesis' (CRUNCH)(Reuter-Lorenz and Cappell, 2008) of cognitive aging, it has been argued that in MCI additional neural resources are recruited as a compensatory mechanism, which breaks down when cognitive load passes a threshold. Hence, when neural reserves are exhausted activation is lower in MCI than in cognitively normal individuals (Grady, 2012).

Factors influencing the behavioral response to rivastigmine

As expected, baseline performance on the item memory task was significantly worse in MCI patients, but also after application of placebo and rivastigmine. There was a weak trend for a group difference in the change in memory performance, but no group-by-treatment interaction. While participants with severe adverse reactions were excluded from analysis a priori, milder side-effects, which are common under chronic (Birks and Flicker, 2006) and acute cholinergic pharmacotherapy (Bentley *et al.*, 2008), were not accounted for in this analysis. When correcting for such subtle changes in well-being, the group-by-treatment interaction barely missed significance, suggesting an association with the effect of rivastigmine on performance. In other words, even mild side-effects of AChE inhibition may interfere with its effects on memory performance.

To confirm the relevance of the cortical cholinergic deficit in MCI, i.e., the reduction of cortical AChE especially in lateral temporal lobes, AChE values extracted from these areas were included in the ANCOVA as a covariate of no interest. This decreased the strength of the group-by-treatment interaction, leaving it insignificant. This pattern is consistent with a close association of the cortical cholinergic deficit and the presence of Alzheimer's disease.

As damage to memory relevant medial temporal lobe structures can already be observed at the MCI stage of Alzheimer's disease (Nickl-Jockschat *et al.*, 2012; Wolk *et al.*, 2017), we examined whether the integrity of the hippocampus has a separate influence on the behavioral effect of rivastigmine. When including baseline episodic memory performance as measure of hippocampal function, the group-by-treatment interaction was highly significant. A similar effect was observed when replacing verbal episodic memory performance with hippocampal grey matter density to reflect

hippocampal structure. It thus appears that the cortical cholinergic deficit and hippocampal integrity are separately associated with the response of memory performance to cholinergic stimulation: A benefit of AChE inhibition was only observed if cortical AChE activity was reduced but hippocampal structures were relatively intact. This could be of prognostic value when deciding to treat MCI due to Alzheimer's disease with AChE inhibitors: a patient with little hippocampal atrophy, which can be assessed on a clinical MRI scan, may be more likely to benefit from AChE inhibition than one with more pronounced medial temporal lobe atrophy.

There is also the possibility that the change in performance is not a consequence of enhanced memory-related neural activation, but improved stimulus processing. This is corroborated by the pattern of the pharmacological effect on neural activation. Activation increased in the fusiform gyrus, which plays a central role in the processing of visual stimuli (Grill-Spector *et al.*, 2001; Garoff *et al.*, 2005) and decreased in the PCC as part of the default-mode network (Fox and Raichle, 2007), but remained unaltered in the hippocampal formation, a key structure in episodic memory (Bird and Burgess, 2008). The notion that AChE inhibition mainly affects stimulus processing and attention, thereby only indirectly improving memory, could explain why large clinical trials consistently report a significant improvement of global cognition under AChE inhibitors in Alzheimer's dementia, but not specifically of episodic memory (Birks, 2006).

Limitations

Our study sample size was comparatively small due to a significant number of dropouts, which were largely attributable to the complex study design. However, a consecutive bias on the results is unlikely, as the total number of dropouts (controls: six, patients: six) due to side-effects (controls: two, patients: two) was identical in both

groups. Although the sample size studied is relatively small, our results are consistent with previous studies of the cholinergic system using PET or pharmacological challenges. Another limitation lies in the fact that an acute single rivastigmine challenge was applied only. Therefore, it remains to be elucidated whether our results regarding cortical AChE activity and the response to chronic cholinergic treatment in MCI due to Alzheimer's disease extend to chronic application of rivastigmine, which warrants future inverstigations.

Conclusions

The present data demonstrate that a cortical cholinergic deficit is already present at the MCI stage of Alzheimer's disease. It affects mainly lateral temporal and parietal cortices and predicts the response to cholinergic stimulation at both the neural and behavioral levels. However, our data also indicate that the cholinergic deficit is one of several factors that influence the response to cholinergic stimulation. We provide evidence that improvement of memory performance depends on the presence of a local cholinergic deficit in task-relevant structures, treatment side-effects, and the functional and structural integrity of the hippocampus. Data suggest that the presence of a cortical cholinergic deficit and relatively intact hippocampal function may be prognostic factors for the response of memory to AChE inhibitors, a clinical hypothesis which warrants further investigation.

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Tables

Table 1. Neuropsychological performance

	Cognitively normal participants		**MCI patients**		**p-value**
	n = 16		**n = 14**		
	Mean	**SD**	**mean**	**SD**	
MMSE	29.56	0.51	25.79	1.25	< 0.001
$VLMT_{DR}$ (words)	11.25	2.18	2.71	2.02	< 0.001
$ROCF_{DR}$	23.00	3.83	5.32	3.23	< 0.001
TMT-A (seconds)	42.25	10.67	67.29	30.52	0.010*
TMT-B (seconds)	97.56	43.58	248.79	65.70	< 0.001
LPS4	25.50	3.16	16.57	4.67	< 0.001
BTA	18.06	1.12	14.07	2.73	< 0.001
BDI	18.00	12.26	19.07	10.22	0.796
B-ADL	1.50	0.58	2.24	1.10	0.036*

MMSE = Mini-Mental-Status Exam; $VLMT_{DR}$ = Verbal Learning Memory Test delayed recall; BTA = Brief Test of Attention; $ROCF_{DR}$ = Rey Osterrieth Complex Figure Test delayed recall; TMT = Trail Making Test; BTA = Brief Test of Attention; LPS4 = Leistungsprüfungssystem 4 (logical thinking); BDI = Beck's Depression Inventory; B-ADL = Bayer Activities of Daily Life. * = not significant when accounting for multiple comparisons.

Table 2. Cortical acetylcholinesterase activity is reduced in MCI due to Alzheimer's disease

Structure	Local maxima				
	Side	p (FWE-corr.)	x	y	z
Inferior Temporal Gyrus	R	< 0.001	62	-24	-24
Middle Temporal Gyrus	R	< 0.001	56	-14	-16
Inferior Temporal Gyrus	R	< 0.001	60	-36	-20
	R	< 0.001	62	-40	-18
	R	< 0.001	56	-58	-16
Planum Polare	R	< 0.001	40	-22	0
Middle Temporal Gyrus	L	< 0.001	-54	-26	-8
Planum Polare	L	< 0.001	-42	-20	-6
Middle Temporal Gyrus	L	< 0.001	-54	-30	-8
	L	< 0.001	-52	-12	-14
	L	< 0.001	-54	-16	-12
	L	< 0.001	-48	-24	-10

p < 0.05, FWE-corrected with cluster free threshold enhancement. Side: left (L) or right (R) hemisphere.

Table 3. Local maxima of the subsequent memory effect

Anatomical Structure	Contrast	Side	Voxels	Local maxima p (FWE-corr.)	x	Y	z	Effect
Amygdala and hippocampus	Subsequent memory effect	L	110	0.015	-30	-8	-22	positive
Fusiform gyrus	Subsequent memory effect	L	161	0.01	-42	-24	-26	positive
		L	95	0.028	-36	-58	-16	positive
		R	46	0.021	34	-32	-22	positive
	Subsequent memory effect (group difference HC > MCI)	L	188	0.003	-36	-58	-18	HC > MCI
	Pharmacological effect (group difference – HC < MCI)	R	11	0.038	34	-36	-24	HC < MCI
Posterior cingulate cortex	Subsequent memory effect	L	445	< 0.001	2	-22	40	negative
		L	17	0.042	0	-44	20	negative
		L	14	0.042	8	-52	32	negative
		L	13	0.045	-10	-48	34	negative
	Subsequent memory effect (group difference HC < MCI)		35	0.042	0	-42	32	HC < MCI
	Pharmacological effect (group difference – HC > MCI)	R	14	0.045	8	-42	36	HC > MCI

p < 0.05, FWE-corrected with cluster free threshold enhancement. Side: left (L) or right (R) hemisphere.

Figure Legends

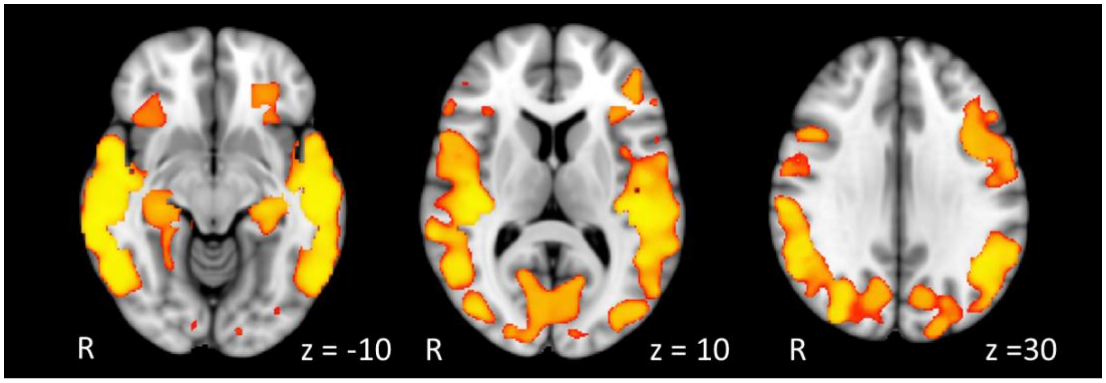
Figure 1. Cortical cholinergic deficit in MCI due to Alzheimer's disease. Cortical acetylcholinesterase was significantly decreased in patients with MCI due to Alzheimer's disease, compared to controls. This is effect was most pronounced in the lateral temporal lobes. $p < 0.05$, FWE-corrected with cluster free threshold enhancement. R = right. Slice labels indicate MNI coordinates.

Figure 2. The subsequent memory effect. Across groups, successful encoding of visual stimuli was associated with activation of temporal lobe structures and deactivation in the posterior cingulate cortex. $p < 0.05$, FWE-corrected with cluster free threshold enhancement. R = right, A = anterior, HC = hippocampus, FusG = fusiform gyrus, PCC = posterior cingulate cortex, PE = parameter estimates. Slice labels indicate MNI coordinates.

Figure 3. Neural activation for successfully encoded content differs between patients with MCI and cognitively normal individuals. In the PCC, patients did not show deactivation. Conversely, in the fusiform gyrus neural activation was greater in controls than in the patient group. $p < 0.05$, FWE-corrected with cluster free threshold enhancement. R = right, A = anterior, CN = cognitively normal, MCI = mild cognitive impairment, FusG = fusiform gyrus, PCC = posterior cingulate cortex, PE = parameter estimates. Slice labels indicate MNI coordinates.

Figure 4. Cholinergic stimulation differentially affects the neural subsequent memory effect in MCI patients and cognitively normal individuals. (A) Group difference in

treatment effect ($control_{rivastigmine}$ - $control_{placebo}$) - ($MCI_{rivastigmine}$ - $MCI_{placebo}$) in the right fusiform gyrus. (B) The bar graph depicts the change in neural activation under cholinergic stimulation (Δ rivastigmine - placebo) in the fusiform gyrus, illustrating that activation increased in patients and decreased in controls. (C) In the fusiform gyrus this change in neural activation was negatively associated with local acetylcholinesterase activity. (D) Group difference in treatment effect ($control_{rivastigmine}$ - $control_{placebo}$) - ($MCI_{rivastigmine}$ - $MCI_{placebo}$) in the posterior cingulate cortex (PCC). (E) This bar graph depicts the change in neural activation under cholinergic stimulation (Δ rivastigmine - placebo) in the PCC: an increase in controls and a decrease in MCI patients. (F) In the PCC, the change in neural activation under cholinergic stimulation was positively associated with local acetylcholinesterase activity. Statistical maps were FWE-corrected with cluster free threshold enhancement ($p < 0.05$). R = right, CN = cognitively normal, MCI = mild cognitive impairment, FusG = fusiform gyrus, PCC = posterior cingulate cortex, PE = parameter estimates, k3 = hydrolysis rate constant of PET-tracer MP4A. Slice labels indicate MNI coordinates.



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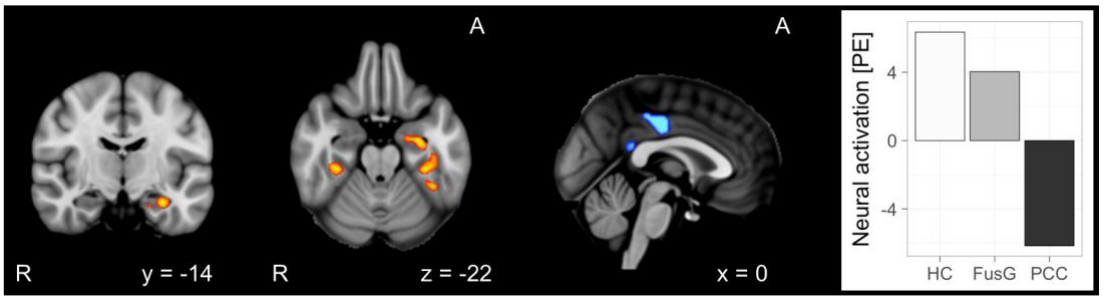

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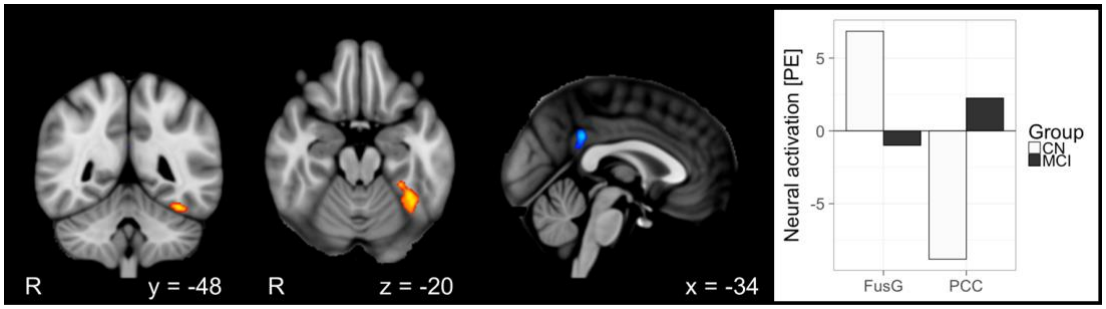

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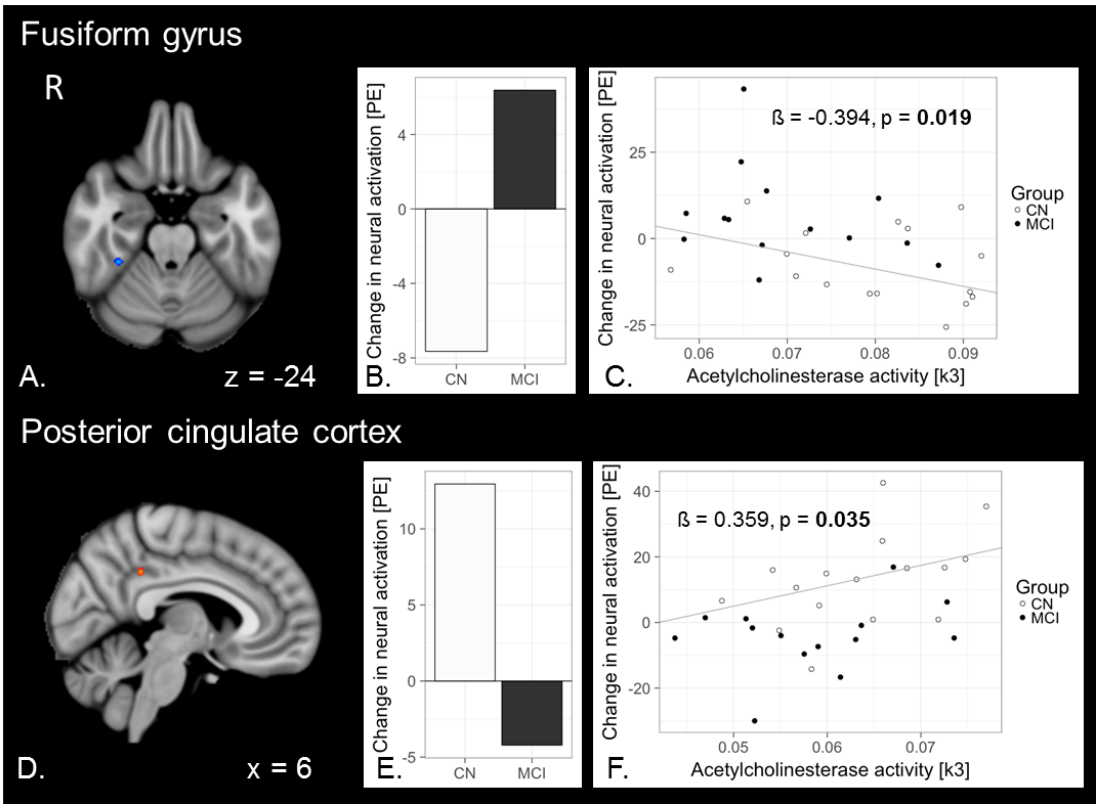

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