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Relationship Between Grey Matter Volume in Subregions of the Basal Forebrain and the Cortical Acetylcholinesterase Activity in Mild Cognitive Impairment Patients due to Alzheimer's disease

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Lara-Sophia David

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Dekan: Universitätsprofessor Dr. med. G. R. Fink

1. Gutachter: Professor Dr. med. J. Kukolja

2. Gutachterin: Privatdozentin Dr. rer. nat. A. K. L. Wevers

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Der dieser Arbeit zugrunde liegenden Datensatz (MACS, Memory, Ageing and the Cholinergic System, EudraCT No. 2008-008896-32) wurde ohne meine Mitarbeit in der Zusammenarbeit zwischen dem neurologischen Institut der Universität zu Köln und dem Forschungszentrum Jülich zwischen Mai 2012 und März 2014 durchgeführt. Die Daten wurden von Herrn Professor Dr. med. Juraj Kukolja zur Verfügung gestellt. Die in dieser Arbeit durchgeführten Analysen und Berechnungen wurden nach entsprechender Anleitung durch Herrn Dr. med. Nils Richter von mir selbst ausgeführt.

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Ich widme diese Dissertationsschrift meinem Opa, Pito (*Joachim Buff*).

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List of Abbreviations

ACh	Acetylcholine
AChE	Acetylcholinesterase
AD	Alzheimer's disease
Aβ	Amyloid-β
BADL	Bayer activities of daily life
BDI	Beck depression inventory
BF	Basal forebrain
BfArM	Bundesamt für Arzneimittel und Medizinprodukte
BfS	Bundesamt für Strahlenschutz
bpm	Beats per minute
BTA	Brief test of attention
ChAT	Choline acetyltransferase
C	Cognitively healthy control subjects
Ch1/2	Medial septal nuclei and vertical limb of the diagonal band of Broca
Ch3	Horizontal limb of the diagonal band of Broca
Ch4a-i	Anterior medial and intermediate nucleus basalis of Meynert
Ch4al	Anterior lateral nucleus basalis of Meynert
Ch4p	Posterior nucleus basalis of Meynert
CSF	Cerebrospinal fluid
DR	Delayed recall
FLAIR	Fluid-attenuated inversion recovery
GM	Grey matter
k_3	Hydrolysis rate of AChE
LPS4	Leistungs-Prüf-System für 50-90 Jährige, Teil 4
M	MCI due to AD patients
MAC-Q	Memory assessment clinics questionnaire
MACS	Memory, ageing and the cholinergic system
MCI	Mild cognitive impairment
MMSE	Mini-mental state exam
mmHg	Millimetres of mercury
MNI	Montreal Neurological Institute of McGill University Health Center
MP4A	^{11}C-N-methyl-4-piperidyl acetate
MRI	Magnetic resonance imaging
n	Group size

NIA-AA	National Institute on Aging and Alzheimer's Association
NBM	Nucleus basalis of Meynert
NSP	Nucleus subputaminalis
p	Significance
PET	Positron emission tomography
[^{11}C]P4OH	N-methyl-4-piperidinol
ROCF	Rey-Osterrieth-complex-figure exam
ROI	Region of interest
SD	Standard deviation
SI	Substantia innominata
Sig. (2-tailed)	Sigma (2-tailed)
tfce	Threshold free cluster enhancement
TMTA	Trail making test A
TMTB	Trail making test B
TIV	Total intracranial volume
VBM	Voxel based morphometry
VLMT	Verbal learning and memory test
vx	Voxel
WM	White matter

1. Summary

Two thirds of all dementia cases worldwide are due to Alzheimer's disease (AD). With rising life expectancy AD cases are increasing. Therefore, it has become a focus of research in the past decades. This thesis focuses on the symptomatic "pre-dementia stage" mild cognitive impairment (MCI). AD patients show pathophysiological changes in the brain, such as accumulation of toxic species of amyloid-β (Aβ) and development of neurofibrillary tangles. The basal forebrain (BF) cholinergic neurons, more specifically the nucleus basalis of Meynert (NBM), provide the main source of cholinergic innervation which is essential for different cognitive processes. These neurons have been found to be vulnerable to neuropathologic changes due to AD.

Using the existing memory, ageing and the cholinergic system (MACS) dataset 17 MCI due to AD patients (M) and 18 cognitively healthy control subjects (C) were included in the analyses of this project. Each participant received a neuropsychological assessment, a cranial magnetic resonance imaging (MRI) scan and a ^{11}C-N-methyl-4-piperidyl acetate (MP4A) positron emission tomography (PET) scan for quantification of cerebral Acetylcholinesterase (AChE) activity. After processing the MRI and PET images I completed various statistical analyses. The grey matter (GM) volume was calculated using the MRI scans. The hypothesis, based on previous studies is that the degree of atrophy in the BF differs between M and C. I therefore hypothesised that there would be a positive correlation between the GM volume in the NBM and cortical AChE activity.

M scored significantly worse on all neuropsychological tests compared to C. The results of this thesis show differences in GM volume between M and C in the posterior nucleus basalis of Meynert (Ch4p), anterior medial and intermediate nucleus basalis of Meynert (Ch4a-i) and the hippocampus. Furthermore, there was a significant positive correlation between GM volume of Ch4p and cortical AChE activity in the hippocampus as well as the temporal lobe without the hippocampus. The atrophic regions in the BF show a significant positive correlation with AChE activity in the temporal and occipital lobe. A significant negative correlation was present between the GM of Ch4p and the "cholinergic deficit" across both groups. Interestingly, there was positive correlation between the GM in the medial septal nuclei and vertical limb of the diagonal band of Broca (Ch1/Ch2) and the "cholinergic deficit".

In conclusion, there is no significant association between the GM in the Ch4p and the cholinergic deficit within the patient group. However, larger Ch1/Ch2 volume was associated with larger "cholinergic deficit" within the M group. The results may help create predictions for

the efficacy of the pharmacological treatment of AD patients. The next goal would be to create an individualized therapy plan based on the patients' "cholinergic deficit".

2. German Summary (Deutsche Zusammenfassung)

Zwei Drittel aller Demenzfälle weltweit werden der Alzheimer-Demenz (AD) zugeschrieben. Mit zunehmender Lebenserwartung steigt die Zahl der AD-Fälle – ein Grund für eine steigende Prävalenz in der Gesellschaft. Daher hat die Erforschung der AD und dessen Vorstufen in den letzten Jahrzenten zugenommen. Diese Arbeit widmet sich dem symptomatischen prädemenziellen Stadium der leichten kognitiven Beeinträchtigung (MCI). AD-Patienten zeigen pathophysiologische Veränderungen im Gehirn, wie die Ansammlung toxischer Spezies von Amyloid-β (Aβ) und die Bildung von Neurofibrillenbündeln. Die cholinergen Neuronen im basalen Vorderhirn (BF), insbesondere im Nucleus Basalis Meynert (NBM), stellen die Hauptquelle der cholinergen Innervation des Cortex dar. Diese sind für verschiedene kognitive Prozesse entscheidend. Diese Neurone sind anfällig für AD-bedingte neuropathologische Veränderungen.

In diese Studie wurden 17 MCI-Patienten aufgrund von AD (M) und 18 kognitiv gesunde Kontrollprobanden (C) einbezogen, unter Verwendung des vorhandenen „Memory, Ageing and the Cholinergic System“ (MACS) Datensatzes. Jeder Teilnehmer unterzog sich einer neuropsychologischen Testung sowie einer Schädel-Magnetresonanztomographie (MRI) und einer Positronen-Emissions-Tomographie (PET) mit ^{11}C-N-Methyl-4-Piperidyl Acetat (MP4A) zur Quantifizierung der Acetylcholinesterase (AChE)-Aktivität im Gehirn. Nach der Bildverarbeitung wurden verschiedene statistische Analysen durchgeführt. Das Volumen der grauen Substanz (GM) wurde aus den MRI-Scans berechnet. Auf Grundlage früherer Studien wurde die Hypothese aufgestellt, dass der Grad der Atrophie im BF zwischen M und C variiert. Weiter wird vorhergesagt, dass es eine positive Korrelation zwischen dem GM-Volumen im NBM und der kortikalen AChE-Aktivität gibt.

Die M-Gruppe schnitt in allen neuropsychologischen Tests signifikant schlechter ab als die C Gruppe. Die Ergebnisse dieser Arbeit zeigen Unterschiede im GM-Volumen zwischen C und M im posterioren Nucleus Basalis Meynert (Ch4p), anteriorer medialen und intermediären Nucleus Basalis Meynert (Ch4a-i) sowie im Hippocampus. Darüber hinaus gab es eine signifikante positive Korrelation zwischen dem GM-Volumen des Ch4p und der kortikalen AChE-Aktivität im Hippocampus sowie im Schläfenlappen ohne Hippocampus. Die atrophen Regionen im BF zeigten eine signifikante positive Korrelation mit der AChE-Aktivität im Schläfen- und Okzipitallappen. Es bestand eine signifikante negative Korrelation zwischen dem GM des Ch4p und dem „cholinergen Defizit“ in beiden Gruppen. Interessanterweise gab es eine positive Korrelation zwischen dem GM in medialen Septumkern und dem vertikal Schenkel des diagonalen Bandes von Broca (Ch1/Ch2) und dem „cholinergen Defizit“.

Zusammenfassend lässt sich festhalten, dass keine signifikante Verbindung zwischen dem GM im Ch4p und dem cholinergen Defizit innerhalb der Patientengruppe besteht. Ein größeres Volumen von Ch1/Ch2 hingegen war mit einem größeren „cholinergen Defizit“ in der M Gruppe verbunden. Die Ergebnisse könnten dazu beitragen, Vorhersagen zur Wirksamkeit der pharmakologischen Behandlung von AD-Patienten zu treffen. Das nächste Ziel wäre die Erstellung eines individualisierten Therapieplans basierend auf dem „cholinergen Defizit“ der Patienten.

3. Introduction

3.1 MCI, Dementia and Alzheimer's Disease

In our constantly growing, ageing society, dementia is becoming an increasingly apparent illness. As life expectancy is continuously increasing, especially in developed countries, the incidence of dementia is on the rise. Two thirds of all dementia cases are due to AD.[46] In 2005, around 1 to 1.5 million citizens in Germany, aged 65 and above, lived with dementia. By 2050, the number is expected to rise to between 1.5 and 3.5 million cases in the age group of 65 and above. The overall incidence ranges from under 2% in the age group of 65-69 years to over 30% in the age group of over 90 years.[58] Currently, life expectancy of a new-born girl in Germany is 83.3 years and for boys 78.5 years.[69]

The rising number of dementia cases has a severe social as well as economic impact. In the early stages, the impact of dementia on daily life may be minor and relatively easy to compensate. In later stages, the patients require extensive help with their everyday activities and may need to be nursed full time. In Germany around 75% of dementia patients are cared for/nursed at home by family members.[46] Therefore, caregiving family members are an important pillar in care.[46] In 2016, the total cost of patients with dementia (from a payer's perspective) was around 34 billion. By 2060, these costs are expected to rise to 49 billion. This is up to 15% of all costs that are associated with the elderly population. In comparison, the societal cost was around 73 billion in 2016. This number is expected to rise to around 194 billion in 2060.[46] These numbers give an idea of the economic and social burden of dementia diseases. Because dementia (especially AD) has an impact on the lives of so many individuals' and their families and because it produces growing costs for the economy and health care systems, it has become a focus of research.

An increasing focus has been put on the symptomatic "pre-dementia stage" MCI. During this stage, patients suffer an objective decline in cognitive abilities, but usually do not need help with their daily activities.[39] This group of individuals are at higher risk to developing AD when compared to a non-MCI population.[47] This early stage of dementia due to AD is the focus of the present study.

AD is prevalent throughout the world and is the leading cause of cognitive impairment and dementia in older individuals (≥ 65 years).[70] AD patients show pathophysiological changes in the brain. These changes often develop years before clinical manifestations are observed in the patients. Changes include an accumulation of toxic species of Aβ as well as the

development of neurofibrillary tangles of hyperphosphorylated tau protein. Neurodegeneration in patients may result from an uncontrolled activation of microglia. This activation can lead to secretion of neurotoxins and inflammatory factors.[60,67] Patients exhibiting such pathophysiological changes may experience different stages of memory loss and cognitive function. Over time, different neuropsychiatric symptoms can manifest themselves. Hallucination and delusion are considered symptoms of the later stages.[70]

The 2011 National Institute on Aging and Alzheimer's Association (NIA-AA) guidelines defined three phases of AD. The first phase is defined as preclinical AD. This is an asymptomatic stage, where early pathological changes take place in the brain of cognitively healthy individuals. The second phase is MCI, being the symptomatic predementia stage. The third phase is the symptomatic dementia phase.[1,70]

3.2 Cholinergic Transmission, Basal Forebrain and the Cholinergic Hypothesis

The BF cholinergic neurons provide the main source of cholinergic innervation of the human cerebral cortex.[24] The cholinergic innervation is essential for different cognitive processes such as learning, memory and attention. It is specifically these neurons in the BF that are vulnerable in neuropathologic changes that cause dementia. In AD these changes being accumulation of Aβ and neurofibrillary tangles.[24] The progressive degeneration of cholinergic neurons and consequently the cognitive impairment in AD is a key characteristic of the disease. This relationship is supported by the clinical efficacy of pharmacotherapy which targets the cholinergic system.[25]

The enzyme choline acetyltransferase (ChAT) synthesizes acetylcholine (ACh). As described above, ACh plays an essential role in cognitive function. Aβ is believed to cause reduction in the choline uptake and release of ACh. Various studies have shown that the accumulation of Aβ may lead to cholinergic synaptic loss.[8] The loss of cholinergic activity has been the focus of research for decades. The loss in cholinergic activity initially led to the cholinergic hypothesis.

During the process of aging, our brain goes through adaptions and changes. One of these changes take place in the cholinergic system. First postulated by Bartus et al. in 1982, the "cholinergic hypothesis" suggested that AD mainly develops due to a loss of cholinergic activity, especially in the BF.[3] The hypothesis was based on previous findings showing the loss of cholinergic neurons in the NBM.[17,75] The ongoing observations, that a severe and early neuronal degeneration takes place (especially in the BF region) in AD patients was the reason

that the cholinergic system became the main target of AD pharmacotherapy.[61] Today, the majority of AD treatment (pharmacological) still have the cholinergic system as a target.[38] As previously suggested, the cognitive decline in MCI patients seems to correlate with a dysfunction of the cholinergic system.[30]

The NBM is located within the substantia innominata (SI) which can be found at the ventral surface of the BF.[73] The region was first named SI by Theodore Meynert.[45] Retrograde horseradish peroxidase tracer experiments were done on primates in the 1970s which helped to identify that cortical cholinergic innervation mainly has its origins in the NBM. With the help of various histochemical methods, Mesulam and his colleagues managed to identify different cholinergic loci within the BF. They then introduced the nomenclature Ch1-Ch4, which is still used today. Regions in the BF include Ch1/2 as well as the horizontal limb of the diagonal band of Broca (Ch3). The Ch4 region was described as the NBM, known as the cholinergic component of the BF.[41,43] The Ch4 region is further divided into Ch4a-i, the anterior lateral NBM (Ch4al) and Ch4p. Today the SI is often described as a group of magnocellular neurons in the BF, synonymous with the NBM.[76]

See *figure 1* for a projectory map of where the various cholinergic Ch4 regions project to-based on identification of ACh rich fibers (taken from Liu et al.).[41] The use of the figure shown below is explicitly permitted by the authors. The article was distributed under the terms of the Creative Commons Attribution License permitting the use, distribution and reproduction in any medium, provided the original author(s) and source are credited.[41]

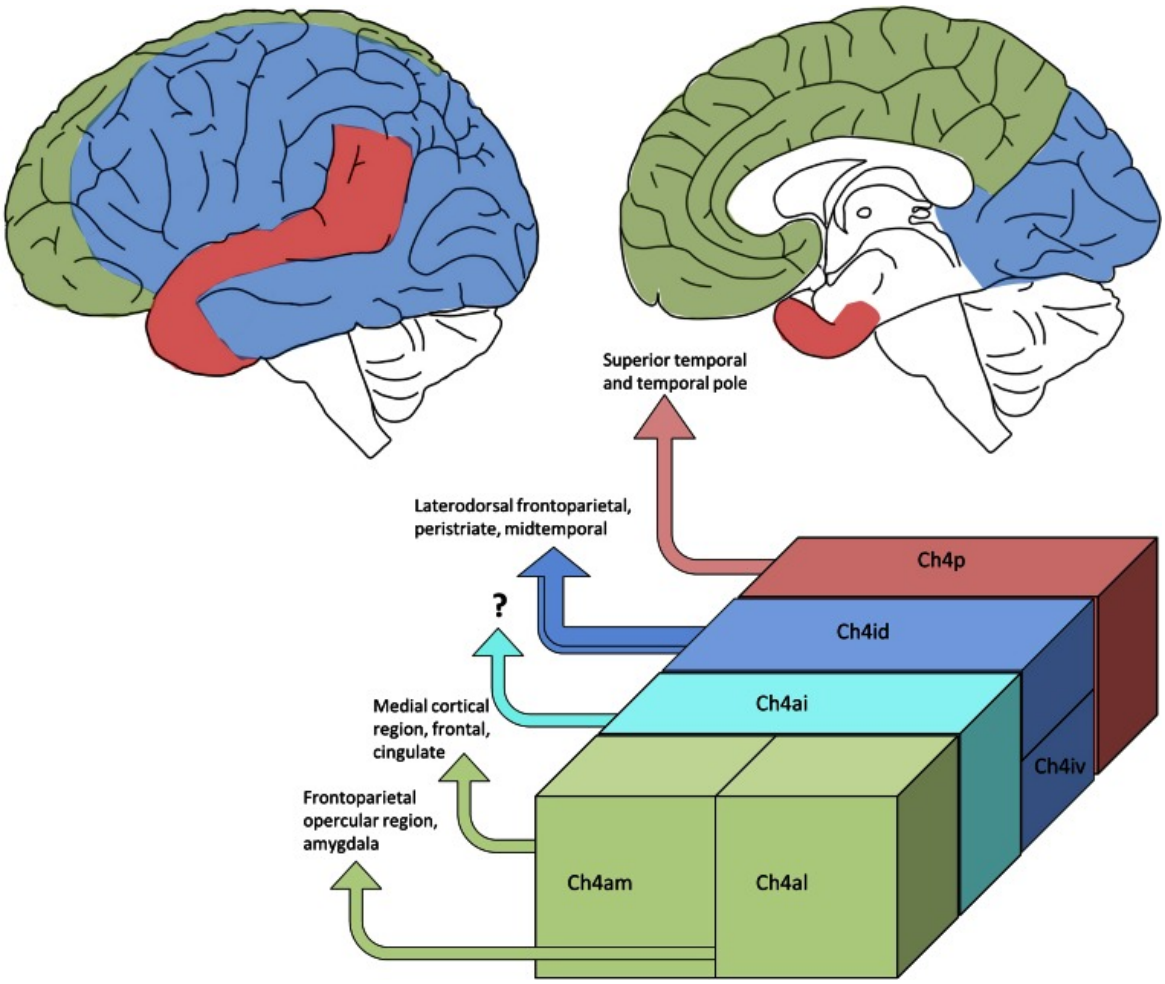


Figure 1: Projected innervation map of the different Ch4 regions (taken from Liu et al.).[41] *Based on the findings of Mesulam et al., 1983 and 1988).*[42,43]

Today, AChE inhibitors are the standard pharmacotherapy options used for the treatment of AD. AChE inhibitors such as galantamin, donepezil and rivastigmin increase the concentration of ACh in the synaptic cleft by blocking AChE, thus inhibiting the degradation of ACh.[13] They have shown to be effective in improving cognitive function in patients with mild to moderate AD.[6] Using AChE inhibitors in MCI patients continues to be controversial. In a meta-analysis, the positive effect of AChE inhibitors to help to prevent the transition from MCI to AD could not be confirmed.[7] One possible explanation for the incongruent findings in MCI patients is that the "cholinergic deficit" in these patients is not equally present. This notion was supported by previous studies which have shown a positive effect of AChE inhibitors on memory performance and the related neural activation in MCI (due to AD) patients. The potential effect depended mainly on the integrity of the patients' cholinergic system.[52]

3.3 Imaging

Using the tracer MP4A in PET we were able to assess the integrity of the cholinergic system in vivo. This method allows the measurement of the activity rate of AChE (the enzyme which breaks down ACh). Using this information, the integrity of the cholinergic system can be assessed. This technique has been frequently used in previous studies and has previously shown a reduction of cortical AChE activity in AD patients.[32,66] In MCI due to AD patients a decline in cortical AChE activity has been observed, which was most apparent in temporal regions. This led to the conclusion that a dysfunction of the cholinergic system is an early event in MCI due to AD and later AD stages.[30]

Using magnetic MRI, it is possible to assess the GM volume of the BF nuclei. As one of the first studies in this field, Teipel and his colleagues suggested in 2005 that a signal decrease in the BF area could be identified, measured by an MRI technique.[73] Changes in BF nuclei, detected by MRI, could later be confirmed.[26,37] Current literature suggests that GM volume of the BF nuclei (and of the hippocampus) could be a predictive factor for the conversion of MCI to AD.[9] In 2004, Herholz and his colleagues postulated that changes in the amygdaloid and cortical cholinergic system are an early event in AD, instead of a consequence of neurodegeneration as previously proposed.[33] Schmitz and colleagues found that pathological changes in the BF were detectable before changes in the entorhinal cortex and therefore suggested that BF pathology precedes and predicts cortical (entorhinal) changes.[63] Using MRI in this study, it was possible to quantify the GM volume and assess the difference in volume between MCI due to AD patients (M) and cognitively healthy control subjects (C).

3.4 Hypotheses and Predictions

The intention of this study was to find a predictor of cholinergic impairment, which could be used for an individual adaption of cholinergic therapy. The study investigated whether the cholinergic impairment could be predicted by measuring the volume of the BF nuclei. A group of C were compared to a group of M. Since it has been previously discovered that there is a difference in cholinergic neurotransmission between MCI and cognitively healthy participants,[66] the hypothesis is that M will show a lower activity of AChE, which will be measured using MP4A PET imaging.[30,56]

The main hypothesis is that the BF volume is decreased in M, compared to C, and that the degree of atrophy is associated with the activity of cortical AChE, measured with MP4A PET imaging. The hypothesis is that the GM volume of BF nuclei negatively correlates with the

activity of AChE. Thus, the prediction is a positive correlation between GM volume in the NBM and cortical AChE activity.

A further hypothesis is that the GM atrophy can be mainly found in the Ch4p region of the Mesulam nomenclature, thus in the posterior parts of the NBM. This hypothesis is based on studies showing that the main degeneration of cholinergic neurons is present in the posterior part of the basal forebrain, the NBM (See section above: *The Basal Forebrain and the Cholinergic Hypothesis*).[73] I also hypothesise that there is a correlation of the NBM GM volume and the AChE activity within the patient group.

The intention of this study is to help further understand the lack of efficacy of AChE inhibitors in MCI patients and suggest improvements for pharmacotherapy, especially in early stages of AD patients. Please note that parts of this thesis have previously been published as a paper.[54] This thesis goes beyond the analyses published in the paper and addresses different research questions.

4. Material and Methods

4.1 Participants

Existing data sets from the larger Memory, Ageing and the Cholinergic System (MACS) study (EudraCT No. 2008-008896-32) Cologne-Jülich were analysed. Between May 2012 and March 2014, a total of 20 patients, diagnosed with MCI due to AD, were recruited. As a control group, 22 age-matched C were recruited. The MACS study was approved by local and federal authorities such as the Bundesamt für Arzneimittel und Medizinprodukte (BfArM) and the Bundesamt für Strahlenschutz (BfS). The study was approved by the ethics committee of the medical faculty of the University of Cologne under the application number 09-035. In accordance with the declaration of Helsinki, written consent was obtained prior to the study from all participants.

All 20 MCI patients had no neurologic or psychiatric comorbidities at the time of recruitment (9 female, 11 male, aged 54-80). Two patients were excluded due to severe subcortical white matter lesions. A third patient withdrew consent during the study. Thus, 17 M were included in the final data set. The 17 M had positive cerebrospinal fluid (CSF) biomarkers of AD pathology[54], therefore completing the criteria for a high likelihood of MCI due to AD, as it has been previously described by Albert et al.[1] CSF was obtained via lumbar puncture. Positive CSF amyloid pathology was defined as $A\beta_{1-42}$<550 pg/ml or a tau/$A\beta_{1-42}$ ratio >0.52.[52] See 4.1.1 for criteria regarding inclusion or exclusion of healthy and control participants.

22 C were recruited from the general community (8 female, 14 male, aged 53-80). Within the healthy control group, 18 participants were included for analysis in this project. Two of the participants had severe metal dental artefacts in their MRI and thus had to be excluded. One participant was excluded due to severe cerebral atrophy and another due to technical difficulties and consequently failure of PET acquisition. Finally, 17 M and 18 C entered the analyses for this project.

The MACS study consisted of an image characterization stage, which included MRI and a PET scan using a cholinergic marker (MP4A – see 4.3.2). Subsequently, the study consisted of a pharmacological challenge during which patients received 3mg rivastigmine or placebo as a single dose for oral administration in a double-blind cross-over design. The results of the pharmacological stage have been reported in Richter et al. (2018).[52] In this study, only the results from the image characterization stage were of interest. All patients received a physical neurological exam, which was performed by a trained neurologist (N.R., J.K.). In addition, all

participants received a neuropsychological assessment, where a variety of previously tested exams were conducted (see 4.2). The neuropsychological testing and MRI data were collected over a time period of two months. The PET exam was conducted within an average of 31 days of the MRI exam (with a standard deviation (SD) of 35 days).

4.1.1 Criteria for Inclusion/Exclusion in MACS Study

The participants (both M and C) had to be between 50 and 80 years of age in order to be included. In addition, the participants were required to have normal findings in the neurological and physical exam. For example, instable heart or vascular diseases, chronic neurological or psychiatric conditions led to exclusion. During the physical examination, the heart rate had to be between 50 and 100 beats per minute (bpm), systolic blood pressure between 100 and 160 millimetres of mercury (mmHg) and diastolic blood pressure between 50 and 100 mmHg. Another exclusion factor was medication acting in the central nervous system. Finally, written consent had to be at hand.

4.2 Neuropsychological Testing

The neuropsychological testing was completed by members of the lab group “Cognitive Ageing and Dementia”, which at the time was under the leadership of Prof. Dr. med. Juraj Kukolja. The neuropsychological testing consisted of a comprehensive composition of exams. All neuropsychological tests were performed in the German language. See *table 1* (Section 2.2.10) for the standard values of the neuropsychological tests.

4.2.1 Mini-Mental State Examination (MMSE)

The mini-mental state examination (MMSE) is a well-established test which requires only 5-10 minutes to perform.[22] The test is divided into two sections, the first of which only requires verbal responses. The questions cover and address the participants’ orientation, memory and attention. The second part focuses on the participants’ ability to follow commands (written as well as verbal), name items, write a spontaneous sentence and copy a polygon structure. The maximum score is 30. In our study MCI was defined as a score greater than 24 in the MMSE.

4.2.2 Verbal Learning and Memory Test (VLMT)

The verbal learning and memory test (VLMT) is the German version of the Rey auditory verbal learning test.[31] It is a word learning neuropsychological test, which consists of two 15-word lists. The participant receives five presentations of list A (15 words) and is immediately asked to recall as many words as possible. A second list consisting of 15 words (list B) follows with the subsequent recall phase. Then the patient is asked to recall list A. Delayed recall (DR) as well as recognition are tested. For our study, MCI was defined as a result of over 1.5 SD under the norm in the section “DR” of the VLMT. Particularly interesting for our analyses is the DG5 (fifth repetition of list A) and the DG7 (delayed recall of list A after about 30 minutes).

4.2.3 Beck Depression Inventory (BDI)

Beck depression inventory (BDI) consists of 21 multiple-choice questions and is widely used to measure the severity of depression. The items address typical symptoms of a depression such as hopelessness, guilt feelings, as well as physical symptoms such as weight loss. The inventory can be used for self-rating as well as interviewer rating. The BDI was first introduced in 1961.[4] In this study the BDI-5 was used, a simplified version of the original BDI.[62]

4.2.4 Leistungs-Prüf-System für 50-90 Jährige, Teil 4 (LPS-4)

The Leistungs-Prüf-System für 50-90 Jährige, Teil 4 (LPS-4) is a German, multipart test system, designed to capture the intelligence performance via different primary skills.[36] For this study, the LPS-4 was mainly used to analyse the logical thinking skills by recognizing patterns. The examinees were shown 40 rows of letters and/or numbers. In each row, there was one mistake. The goal was to identify the mistake(maximum number of points = 40).

4.2.5 Brief Test of Attention (BTA)

The brief test of attention (BTA) is designed to examine and measure the auditory divided attention.[64] The test included two different forms. The first one includes 10 lists of letters and numbers (increasing from 4 to 18 items) which are read aloud to the examinee. Participants had to count the amount of numbers read aloud. The second list is similarly constructed, but now participants had to count the number of letters read aloud. The number of correctly monitored lists are added together. The maximum score was 20. Subjects are not asked to recall which number or letters were said, only how many.

4.2.6 Rey-Osterrieth-Complex-Figure Exam (ROCF)

The Rey-Osterrieth-Complex-Figure exam (ROCF) was first described in 1941, by Rey, further developed by Osterrieth in 1944. Translated from French to English in 1993 this test asks the examinee to reproduce a complex line drawing.[14] First, the examinee is asked to copy the figure free hand. Next, he/she is asked to immediately recall the figure and draw it from memory. After a time span of 20-30 minutes, the examinee is asked to draw the figure from memory, in form of a delayed recall. As many different cognitive functions are needed to complete this test, the ROCF test can be used to assess memory, visuospatial abilities, attention as well as executive functions.

4.2.7 Memory Assessment Clinics Questionnaire (MAC-Q)

The memory assessment clinics questionnaire (MAC-Q) can be used to evaluate an age-dependent decrease in memory function through self-evaluation. The examinee is asked to evaluate 6 different memory performances from everyday life (the 6th of which is counted twice). The examinee has to evaluate whether the memory performance is a lot better today (1 point) than compared to the time of fourth grade or a lot worse than during that time (5 points). All points are added (max. 35) and then evaluated.[15]

4.2.8 Bayer Activities of Daily Life (BADL)

The Bayer activities of daily life (B-ADL) questionnaire was designed on an international level to identify whether patients have difficulties with daily activities based on cognitive impairment.[34] The questionnaire has 25 questions which are meant to be answered with a scale ranging from 1 (never) to 10 (always). The sum is divided by the number of questions (25). The final value is between 1 and 10. The questionnaire can be used for self-evaluation or can be filled out by relatives or people close to the patient.[19]

4.2.9 Trail Making Test A/B

The trail making test A/B (TMT A/B) is a test used to evaluate the eye-hand coordination as well as attention and cognitive flexibility. During test A participants are asked to quickly join the dots from number 1 to 25 an A4 paper. They are not supposed to take the pen off the paper. During test B patients are asked to join the dots between numbers 1-13 and letters A to L (alternating sequence), also not taking the pen off the paper and as quickly as possible. The

time taken to complete the task in seconds is recorded as the result of the exam.[74] Several patients were not able to complete the TMT B due to cognitive impairment.

4.2.10 Standard Values of the Neuropsychological Tests

Source	Test	Age	Normal	MCI	Pathological
Lezak et al., 2004.[40]	MMSE		27-30	24-27	< 24
Helmstaedter et al., 2001.[31]	VLMT		≥ 7	< 7	
Schmitt et al., 2006.[62]	BDI-V		0-35	0-35	> 35
Horn, 1983.[36]	LPS4	> 50	≥ 12	≥ 12	< 12
Schretlen et al., 1996.[64]	BTA	50-59 60-69 70-80	12 11 10	12 11 10	< 12 < 11 < 10
Fastenau et al., 1999.[20]	ROCF	50-59 60-69 70-80	10 9 8		< 10 < 9 < 8
	MAC-Q				Not specified
Erzigkeit et al., 2001.[19]	BADL		≤ 5	> 5	> 5
Derived from the standardisation data from Tombaugh et al. 2004.[74]	TMT A according to CERAD+	F < 70y F ≥ 70 y M < 70 y M ≥ 70 y	≤ 64 sec ≤ 71 sec ≤ 65 sec ≤ 74 sec		> 64 sec > 71 sec > 65 sec > 74 sec
See TMT A	TMT B according to CERAD+	F < 70y F ≥ 70 y M < 70 y M ≥ 70 y	≤ 163 sec ≤ 226 sec ≤ 194 sec ≤ 239 sec		> 163 sec > 226 sec > 194 sec > 239 sec

Table 1: Standard values of the neuropsychological tests (F = female, M = male, sec = seconds)

4.3 Data Acquisition

4.3.1 MR-Imaging

High-resolution T1-weighted images (MDEFT3D; repetition time (TR) 1930 ms; inversion time (TI) 650 ms; echo time (TE) 5.8 ms; flip angle 18°; 128 sagittal slices; resolution 1.0 × 1.0 × 1.25 mm^3), T2-weighted images (TR 3200 ms; TE 458 ms; 176 sagittal slices; resolution 1.0 × 1.0 × 1.0 mm^3), and fluid-attenuated inversion recovery (FLAIR) images (TR 8040 ms; TI 2400 ms; TE 121 ms; flip angle 150°; 36 axial slices; slice thickness 4 mm, in-plane resolution, 0.46875 × 0.46875 mm^2) were acquired using a 3T Trio (Siemens, Erlangen, Germany) scanner.[55]

4.3.2 PET-Imaging (MP4A PET)

MP4A

MP4A is a radio activated marked lipophilic ACh-analogon which has the ability to pass the blood-brain barrier.[51] Inside the brain tissue, a part of the MP4A is specifically hydrolysed by AChE into N-methyl-4-piperidinol ([^{11}C]P4OH). The metabolite MP4OH, which is hydrophilic, cannot easily pass the blood brain barrier and accumulates in the region where the hydrolysation process took place. This enables the measurement of cerebral AChE activity. Another part of the MP4A is washed out back into the blood. The process described above can be explained using a 3-compartment model. K_1 describes the rate at which the tracer passes through the blood-brain barrier, k_2 describes the wash out rate at which the tracer leaves the brain again and k_3 describes the hydrolysis rate of AChE (k_3). Therefore, k_3 is the rate at which the enzyme is active.[32,49]

MP4A

Figure 2: Chemical structure of MP4A.[49]

Production and Administration of the Tracer

The tracer MP4A was similarly synthesized as described in previous studies and protocols, with some minor adjustments.[30,32] MP4A was produced by the Radiochemistry group at the Max-Planck-Institut for Metabolism Research under the leadership of Prof. Dr. rer. nat. Bernd Neumaier. The administration was performed via intravenous injection – the examinees received a bolus between 192 and 556MBq of [^{11}C]MP4A.[55]

PET Scanning

PET-scanning was conducted using an ECAT HRRT scanner (CPS Innovations, Knoxville TN, USA) with a maximum of 2.4mm transaxial and 2.0mm axial.[52] The scanning was done in a supine position with the examinee's eyes being closed and dimmed light. The actual scanning started with the injection of the tracer and took 60 minutes. The PET-scans were obtained by use of the protocol as described by Haense and her colleagues.[30]

4.4 Processing MRI and PET Images

4.4.1 Processing T1 MRI Images

By processing the MRI Images using voxel-based morphometry (VBM) we were able to assess GM volume. VBM was used as implemented in the CAT12 toolbox in the SPM12 programme.[23,68] The first step was to divide the T1 MRI images into different tissue classes: GM, white matter (WM) and CSF. Next, we spatially normalized the images to Montreal Neurological Institute (MNI) of McGill University Health Center (MNI) 152 space using DARTEL, an algorithm for high-dimensional registration.[2,35] The next step consisted of correcting the GM maps for non-linear normalisation (volume change) which essentially meant applying a Jacobian determinant. The total intracranial volume (TIV) was not included in subsequent analyses using the GM values which we obtained using VBM. I used pre-processed T1 MRI images in this study to complete my analyses. For further information of pre-processing please see the paper published by Richter et. al.[54]

4.4.2 Processing PET Images

PET imaging was performed to assess regional AChE activity. The activity can be assessed by quantifying the hydrolysis rate of MP4A (k3) at the individual voxel (vx) level. This method has been previously described.[52,55] Please see the paper published by Richter et al. in 2018

for detailed information on the processing of the PET images.[52] A 3-parameter compartment model, as described under 4.3.2, implemented in the VINCI software, was used to estimate k3 of MP4a and therefore assess AChE activity.[32] The k3-maps which were created by using this model were then coregistered to the MRI T1 images and subsequently normalised to the MNI space. See 4.5 below for further information of how the regions of interest (ROI) were further defined.

Since the study was interested in the "cholinergic deficit" of the MCI due to AD patients, the inter-individual differences in the spatial pattern of the MP4a hydrolysis rate (k3) were asessed. In order to compare the reduction in AChE activity, the "cholinergic deficit" was determined by transforming the AChE maps of each patient (z-transformation). The mean of the control group was subtracted from the map as well as dividing by the SD of the control group:

$$Z = \frac{(AChE\ activity_{participant} - mean\ AChE\ activity_{control\ group})}{SD\ of\ AChE\ activity_{control\ group}}$$

The product of the average z-score (of vx where AChE activity was more than two SD from the mean of the control group) and the volume was defined as the measure of the cholinergic deficit.[54]

4.5 Regions of Interest (ROI)

Using a cytoarchitectonic map in MNI standard space, GM values of different subareas of the BF were extracted. The division of the BF into ROI was completed as described by Kilimann et al.[37] Ch1 and Ch2 were grouped together into a single ROI. Ch3 represents another ROI. Ch4a-i, Ch4al and Ch4p were separated into individual ROIs.[37] The hippocampal ROI was derived from the Jülich Histological Atlas.[18] The different subregions of the hippocampus were combined (except the entorhinal cortex), creating a single ROI. For further information see the paper prepublished to this thesis by Richter et al. in 2022.[54] Similarly the cortical ROI for each of the cerebral lobes were defined using the MNI structural atlas.[54]

Analyses with the cortical k3 values used the same divisions as described above, using the six subregions of the Kilimann mask from 2014, based on the nomenclature of Mesulam.[37,43]

4.6 Statistical Analyses

All statistical analyses on non-imaging data were performed using SPSS (Version 24.0, IBM Corp., Armonk, NY).

4.6.1 Group Statistics

In order to analyse the distribution of the parameters, Shapiro-Wilks tests were used. For data that showed a normal distribution, group differences were analysed using independent samples T-tests and Pearson correlations. For data that did not show a normal distribution, Spearman coefficients and Mann-Whitney were used to assess group differences. Since the sample-size was relatively small, the process of bootstrapping was used as implemented in SPSS. This is a process to test the reliability as well as stability of models. Bootstrapping relies on random sampling with replacement. In our analyses, it was performed with 5000 permutations (rearranging the data set). The results are presented with 95%-confidence intervals.

4.7 Manually Tracing BF masks

In order to validate the GM values obtained (using the cytoarchitectonic map) for the Ch 1/2 region, the BF masks were manually traced according to the protocol as described by Butler et al.[11] FSLview was used to manually trace masks on T_1-images using different anatomical landmarks which were derived from a histology-based protocol.[11] The masks were created independently once by me and once by Nils Richter.

5. Results

5.1 Demographic Characteristics

To analyse the age distribution, a Shapiro-Wilks-Test was performed.[65] Since the data for age of the participants showed a normal distribution an independent t-test for group comparisons was used. The two groups did not differ with respect to age ($t_{(33)}$= -1.014, p = 0.318, *table 2*). Within the C group, 11 of the participants were male and 7 female. Of the 17 M included in the study, 9 were male and 8 female. The mean average age of C was 65.2 years with a SD of 6.7 years (range was from 53 to 80 years). The M group showed an average age of 67.5 years with a range from 54 to 80 years (*Table 2)*. The group size is represented by n.

	Group	**n**	**Mean**	**SD**	**Standard Error Mean**
Age	C	18	65.22222	6.673526	1.572965
	M	17	67.47059	6.423761	1.557991

Table 2: Demographic characteristics of the study population (n = Group Size)

5.2 Neuropsychological Testing

The M group (n=17) performed significantly worse than the C group (n=18). See *Table 3* below for details. Groups did not differ in terms of depressive symptoms, assessed using the BDI ($t_{(33)}$ = -0.112, p = 0.911). All participants scored below 35 points on the BDI and could therefore be included in the study. Over 35 points would have been a pathological score, equivalent to a moderate depression. The TMT-B was excluded from analyses, as a number of patients who were not able to complete the task.

	C		M		p-value
	Mean	SD	Mean	SD	
MMSE	29.50	0.62	25.94	1.60	**<0.001**
VLMT (DR)	11.11	2.19	2.35	2.03	**<0.001**
LPS4	24.78	4.01	16.24	4.45	**<0.001**
BTA	17.94	1.16	14.59	2.67	**<0.001**
ROCF (DR)	22.83	3.67	5.53	3.37	**<0.001**
B-ADL	1.44	0.78	2.29	1.10	**0.013**
TMT-A (sec)	42.83	11.09	71.12	29.05	**0.001**

Table 3: Neuropsychological testing results (significant results are shown in bold)

5.3 Group Differences in GM und AChE Activity

5.3.1 Group Differences in GM Volume

First, the degree of GM atrophy in C and M was analysed and group differences were compared. *Figure 3* below shows the significant group differences in GM volume between the C and M. The largest group differences in GM volume could be found in the mesial and medial temporal lobe, as well as the inferior frontal gyrus and the precuneus (parietal lobe).

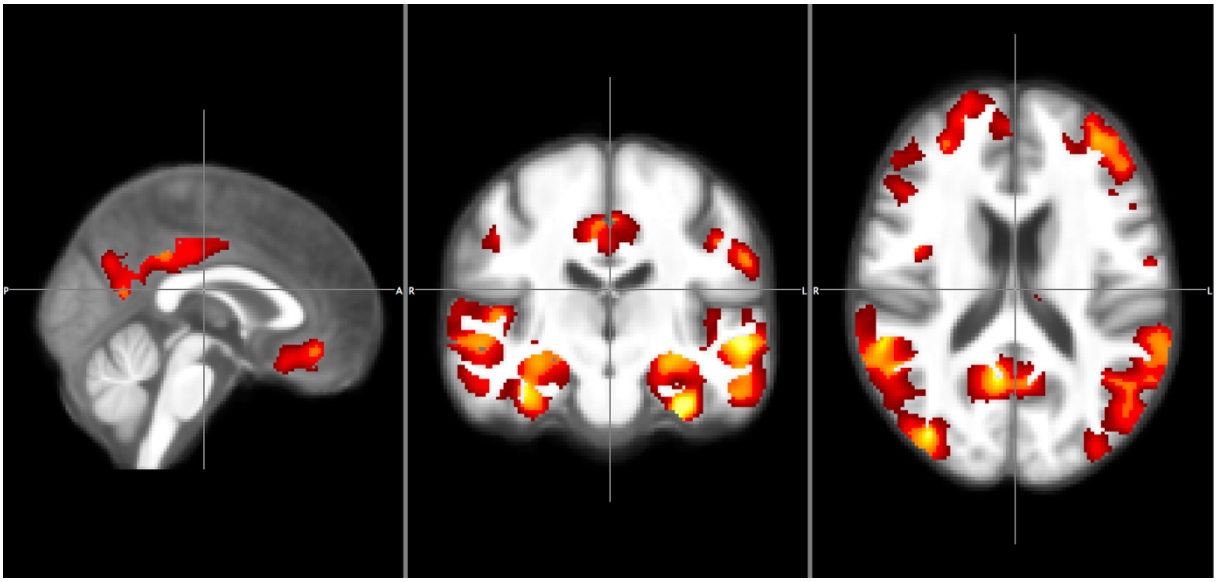


Figure 3: Group differences in GM volume (age as a Covariate, 8mm smoothing and threshold free cluster enhancement (tfce) corrected $p < 0.05$)

Figure 4 below shows the group differences in GM within the BF mask.[37] The blue shading shows the entire BF and the red shading shows the significant differences in GM volume within the mask. Significant GM volume reductions in M compared to C were found in the posterior BF (NBM).

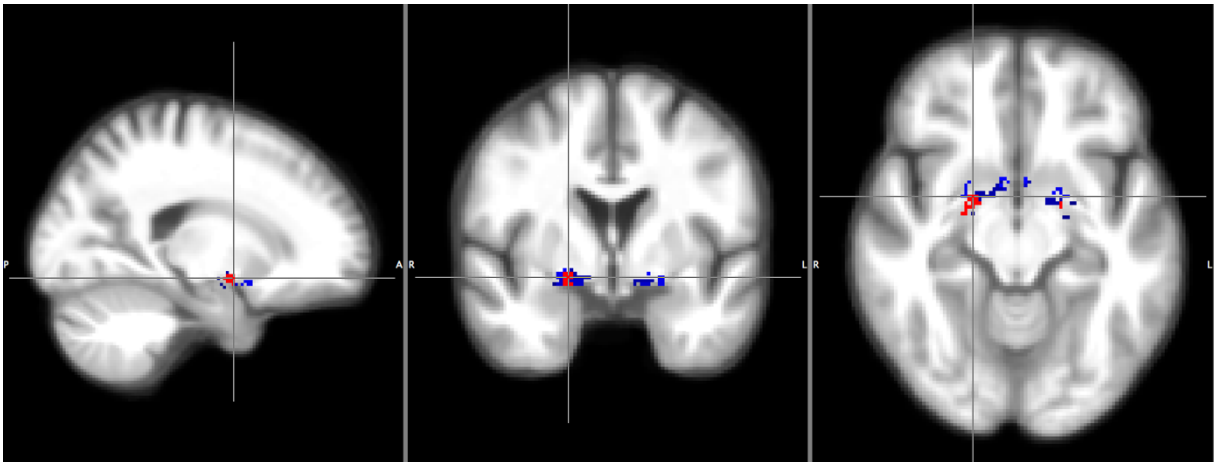


Figure 4: Significant group differences (red) in GM volume within the entire BF (blue) mask (age as a covariate, 4mm smoothing and tfce corrected $p < 0.05$)

Next, the group differences in GM volume within the BF were analysed. *Table 4* shows the results of the t-tests for independent samples. The Ch4p showed a significant difference in GM volume between M and C. GM volume was also significantly decreased in M compared to C in the Ch4a-i as well as in the hippocampus.

ROI	C (n = 18)		M (n = 17)		p-Value
	Mean	SD	Mean	SD	
Ch4p	0.76276	0.066448	0.6343	0.100118	**< 0.001**
Ch4a-i	0.55934	0.047814	0.51049	0.075302	**0.031**
Ch3	0.69836	0.061266	0.65963	0.105259	0.198
Nucleus subputaminalis (NSP) and Ch4al	0.74663	0.093078	0.68957	0.121294	0.127
Interstitial nuclei	0.30579	0.051216	0.27335	0.069878	0.125
Ch1/Ch2	0.83898	0.086595	0.80452	0.108901	0.306
Hippocampus	0.619	0.049	0.539	0.067	**<0.001**

Table 4: Group differences in GM volume (significant results are shown in bold)

5.3.2 Group Differences in AChE Activity

There were significant between group differences in AChE activity in the temporal, occipital and parietal lobes with M showing less activity than C (see *table 5* and *figure* 5).

Cortical k3	C (n = 18)		M (n = 17)		p-Value
	Mean	SD	Mean	SD	
Frontal Lobe	0.089	0.010	0.084	0.008	0.085
Temporal Lobe	0.094	0.010	0.078	0.007	**< 0.001**
Parietal Lobe	0.076	0.008	0.067	0.008	**0.005**
Occipital Lobe	0.072	0.009	0.062	0.007	**0.001**

Table 5: Group differences in AChE activity (significant results are shown in bold)

Figure 5 below shows regions with significant differences in AChE activity between the two groups. The largest differences were seen in the temporal and occipital lobes as M showed less AChE activity than C.

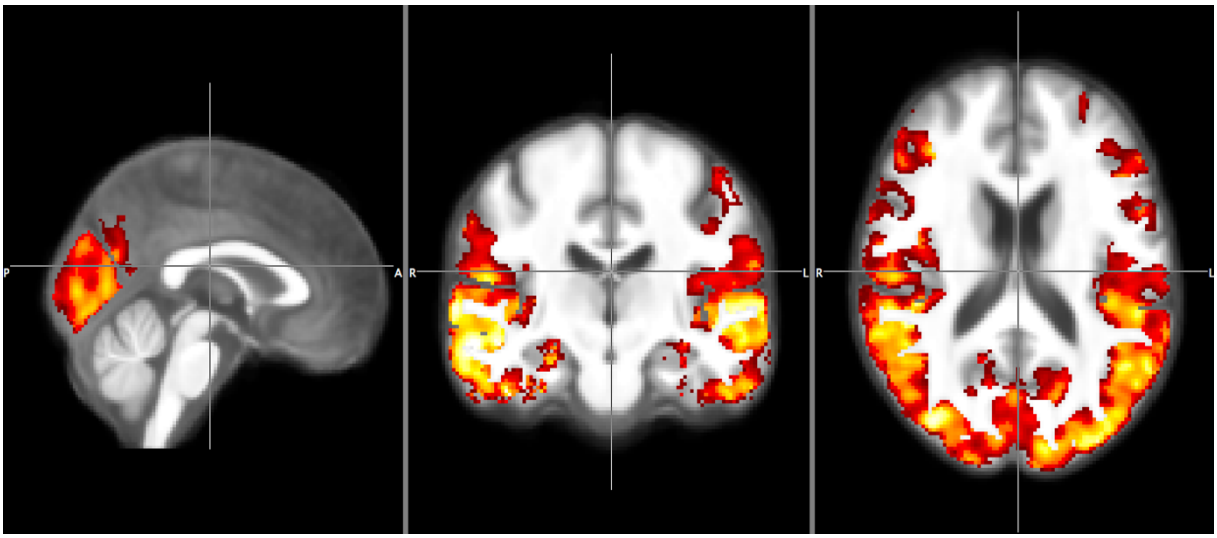


Figure 5: Group differences in AChE activity (age as a covariate, 8mm smoothing and tfce corrected $p < 0.05$)

5.4 Association Between GM and Cortical AChE Activity

5.4.1 Association Between BF GM Volume and Cortical AChE Activity

The association between GM volume in the BF (the entire ROI), as well as subregions that show signs of grey matter atrophy within the BF, with cortical AChE activity was investigated. *Table 6* below shows the results. There was a significant correlation, across both groups, between GM volume in atrophic regions within the BF and k3 in the temporal lobe excluding the hippocampus, the hippocampus and the occipital lobe (significant results are in bold). There was no significant association between GM volume of the entire BF (without considering which areas are atrophic) and the k3 of the different lobes.

GM	Correlation	Cortical k3				
		Temp NoHippocampus	**Temp Hippocampus**	**Parietal**	**Frontal**	**Occipital**
GM in entire BF	Correlation Coefficent	0.191	0.036	-0.018	-0.092	0.129
	Sig. (2-tailed)	0.271	0.838	0.917	0.597	0.46
	n	35	35	35	35	35
GM in atrophic regions of BF	Correlation Coefficient	0.459	0.345	0.128	-0.04	0.336
	Sig. (2-tailed)	**0.006**	**0.043**	0.465	0.818	**0.049**
	n	35	35	35	35	35

Table 6: Results of the correlation between GM volume of the BF and cortical k3 Activity (TempNoHippocampus = Temporal lobe without the Hippocampus, TempHippocampus = Hippocampus. Significant results are shown in bold)

Next the study analysed the correlation between Ch4p volume and cortical AChE activity, Ch4p being the cholinergic center of the NBM.[41,43] *Figure 6* below shows areas with significant correlations of GM volume in atrophic parts of the Ch4p with cortical k3 activity. Significant correlations between GM volume from atrophic parts of Ch4p and cortical k3 were found in the right medial temporal gyrus.

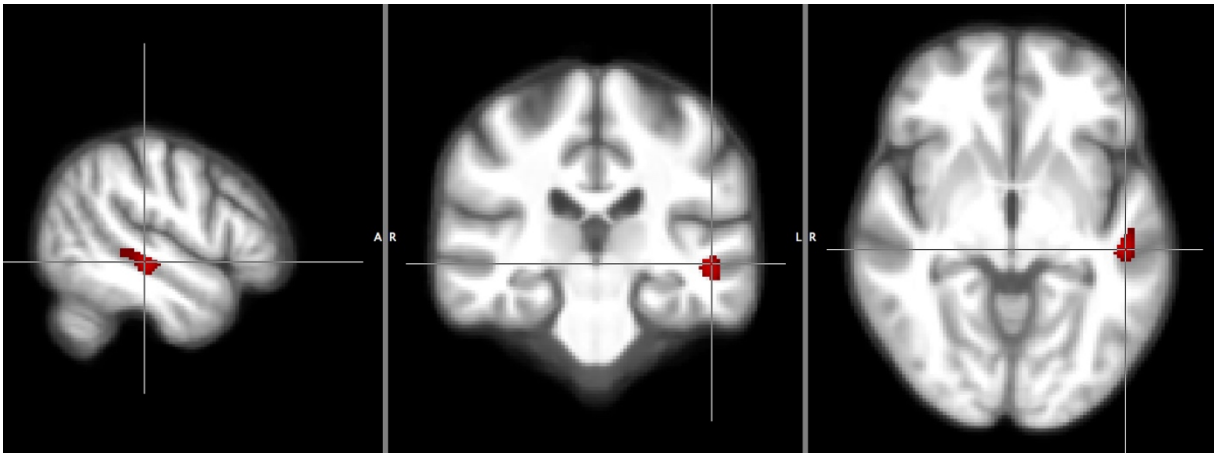


Figure 6: Significant areas where the GM volume from atrophic parts of Ch4p correlates with cortical k3 (age as a covariate, 8mm smoothing and tfce corrected p<0.05).

5.4.2 Association Between GM in Subregions of the BF and Cortical AChE Activity

In the next step, the association between different subregions within the BF across both groups and the cortical AChE activity was analysed. See *table 7* below for the results. There was a significant positive correlation between Ch4p and the cortical AChE activity in the hippocampus ($r_{(35)} = 0.386$, $p = 0.022$) as well as in the temporal lobe without the hippocampus ($r_{(35)} = 0.418$, $p = 0.012$). The GM volume in Ch1/2 did not show a correlation with cortical k3 activity across the groups. See *table 7* below for a detailed list of all results.

BF GM	Correlation	Cortical k3 Activity				
		Temp. (NoHippocampus)	Temp. (Hippocampus)	Parietal	Frontal	Occipital
Ch4p	Correlation Coefficient	0.418	0.386	0.041	-0.041	0.251
	Sig. (2-tailed)	**0.012**	**0.022**	0.814	0.816	0.146
	n	35	35	35	35	35
Ch4a-i	Correlation Coefficient	0.22	0.011	0.091	0.014	0.176
	Sig. (2-tailed)	0.204	0.949	0.603	0.936	0.313
	n	35	35	35	35	35
Ch3	Correlation Coefficient	0.166	-0.02	-0.012	-0.043	0.106
	Sig. (2-tailed)	0.341	0.911	0.945	0.808	0.546
	n	35	35	35	35	35
Ch4al/NSP	Correlation Coefficient	0.153	-0.099	-0.01	-0.072	0.11
	Sig. (2-tailed)	0.38	0.57	0.955	0.681	0.528
	n	35	35	35	35	35
Interstitial nuclei	Correlation Coefficient	0.134	-0.06	0.02	0.057	0.0989
	Sig. (2-tailed)	0.444	0.732	0.911	0.746	0.611
	n	35	35	35	35	35
Ch1 /Ch2	Correlation Coefficient	-0.012	-0.069	-108	-0.127	-0.069
	Sig. (2-tailed)	0.944	0.692	0.536	0.468	0.694
	n	35	35	35	35	35

Table 7: Results of the correlation between GM volume of subregions of the BF and the cortical k3 activity (TempNoHippocampus = Temporal lobe without the Hippocampus, TempHippocampus = Hippocampus, significant results are shown in bold).

5.5 The Cholinergic Deficit

5.5.1 Quantifying the Cholinergic Deficit

As described in 4.4.2 the “cholinergic deficit” was quantified for each patient. The Z-values of the k3 in the M group were compared to the Z-values of the C group (the difference). Using the Z-values, it was possible to determine the individual deviation in AChE activity in each patient from the mean of the control group. *Figure 7* shows five representative examples of the cholinergic deficit in the M group. While there was substantial inter-individual variation in the spatial patterns, the “cholinergic deficit” in MCI often involved temporal and parietal regions.[54]

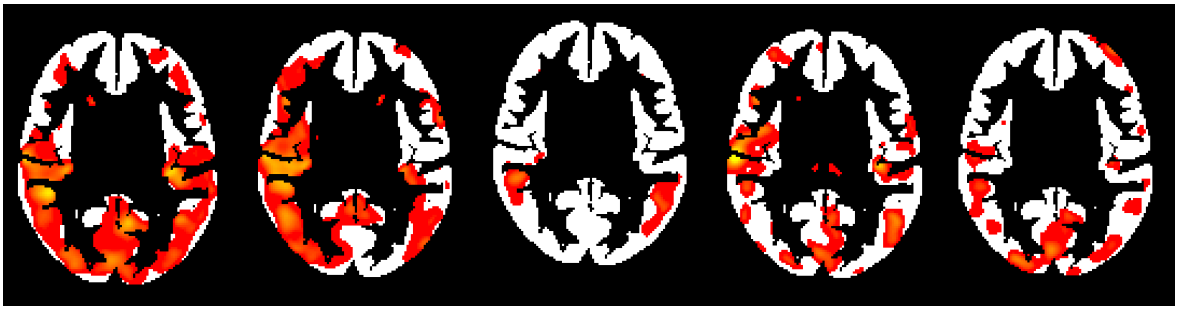
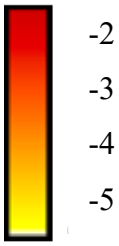


Figure 7: Representative examples of the “cholinergic deficit” in the M group. The colour scale depicts the deviation in SD from the mean of the group. Below is the scale: Left is the colour and the right column shows the Z-value (deviation from the Z-value from the C group).



5.5.2 Correlation Between the Cholinergic Deficit and GM in the NBM

Next, the correlation between the cholinergic deficit (see 4.4.2) and GM volume in the NBM was analysed. The cholinergic deficit was calculated for each individual separately. Across groups, there was a significant negative correlation ($r_{(35)}$ = -0.476, p = 0.004) between GM volume of the Ch4p and the “cholinergic deficit” (*Table 8*). Lower GM volume (greater atrophy) in the Ch4p was associated with a larger “cholinergic deficit”.

Correlations				
			Ch1/Ch2	**Ch4p**
Spearman's rho	Vx_Z2_k3_crt	Correlation Coefficient	-0.064	-0.476
		Sig. (2-tailed)	0.717	**0.004**
		n	35	35

Table 8: Correlation between the cholinergic deficit and the GM volume of the NBM (Vx_Z2_k3_crt = The number of subthreshold Z-values (Z < -2) in the cortex that were subtracted. Significant results are shown in bold).

Next, the cholinergic deficit of the two groups was separately compared with GM volume in the NBM. In the C group, t*here was* no significant correlation of the cholinergic deficit and different areas of the NBM (Table 9, Figure 9). By contrast, there was a significant positive correlation between the cholinergic deficit and GM volume in Ch1/Ch2 in the M group (Table 10, Figure 8).

Correlation			**GM in NBM**	
			Ch1/Ch2	Ch4p
Spearman's rho	Vx_Z2_k3_crt	Correlation Coefficient	-0.201	-0.082
		Sig. (2-tailed)	0.423	0.748
		n	18	18

Table 9: Correlation between the cholinergic deficit of C and GM volume of the NBM (Vx_Z2_k3_crt = The number of subthreshold Z-values (Z < -2) in the cortex that were subtracted)

Correlation			**GM in NBM**	
			Ch1/Ch2	Ch4p
Spearman's rho	Vx_Z2_k3_crt	Correlation Coefficient	0.561	0.118
		Sig. (2-tailed)	**0.019**	0.653
		n	17	17

Table 10: Correlation between the cholinergic deficit of M and GM volume of the NBM (Vx_Z2_k3_crt = The number of subthreshold Z-values (Z < -2) in the cortex that were subtracted. Significant results are shown in bold.)

Figure 8 below shows a scatter plot with the correlation between the cholinergic deficit and the GM volume in Ch1/Ch2 of the NBM.

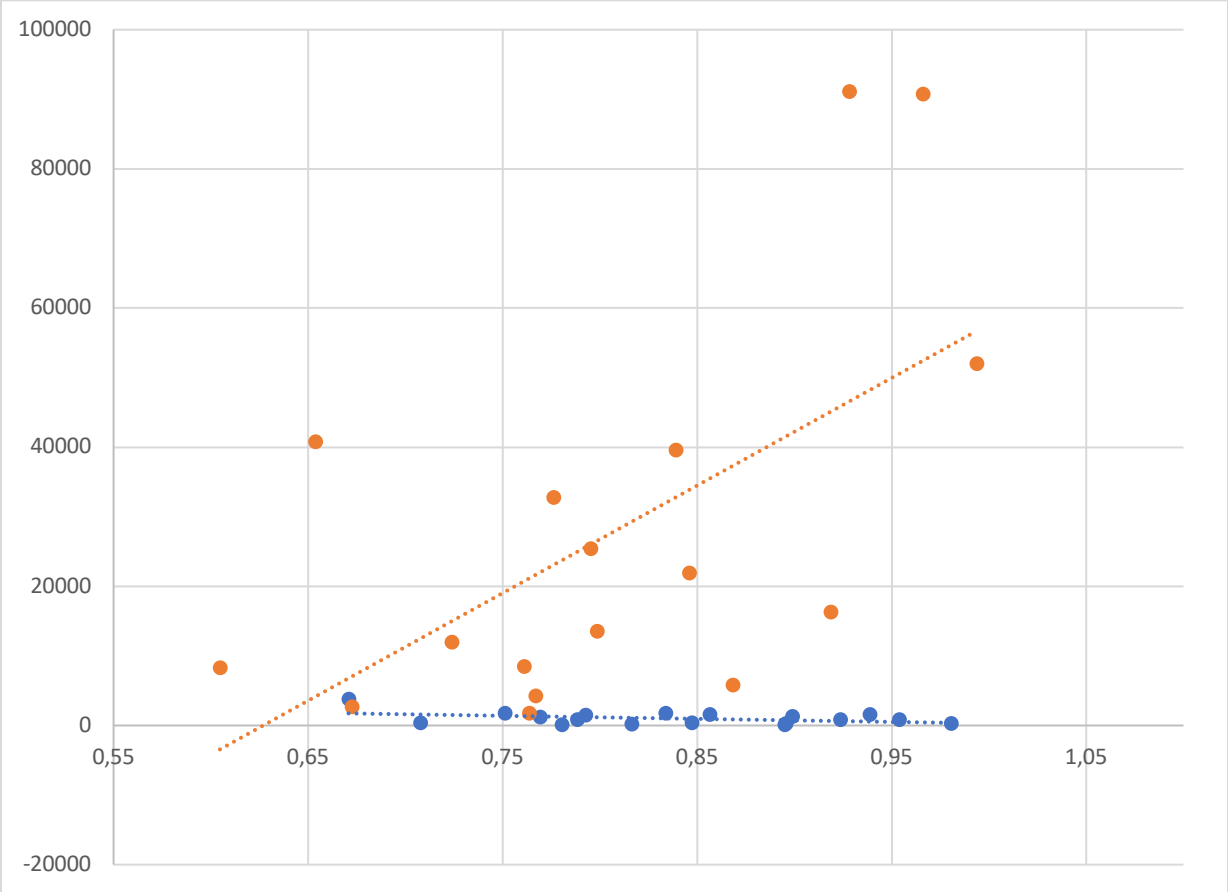


Figure 8: Graph showing the correlation between the cholinergic deficit (number of vx with Z < -2, shown on y-axis) and GM volume in Ch1/2 (shown on x axis). Red dots are M and blue dots C.
GM – Rel. units
Z – Number of vx

Figure 8 above clearly shows the difference in the correlation between the two groups. Here the positive correlation between the cholinergic deficit and the GM volume in the Ch1/Ch2 area of the NBM is demonstrated. The blue dots, the C group, show a different distribution.

5.5.3 Correlation Between the Cholinergic Deficit and Neuropsychological Data

Next, the correlation between the individual cholinergic deficit and the neuropsychological test results (see 3.2.) was analysed. There was a significant negative correlation between the cholinergic deficit and the results of the MMSE and the LPS4 exam (see *table 11* below for the results).

Correlations			**Neuropsychological Test**					
			VLMT7	MMSE	LPS4	BTA	RO_CF	TMT-A
Spearman's rho	Vx_Z2_k3_crt	Correlation coefficient	-0.133	-0.608	-0.493	-0.254	0.17	0.272
		Sig. (2-tailed)	0.624	**0.01**	**0.044**	0.324	0.515	0.29
		n	16	17	17	17	17	17

Table 11: Correlation between the cholinergic deficit of the M group and different neuropsychological tests (Vx_Z2_k3_crt = the number of subthreshold Z-values (Z < -2) in the cortex that were subtracted. Significant results are shown in bold.)

6. Discussion

As predicted, the M group scored significantly worse on all neuropsychological tests than the C group. Furthermore, this study was able to demonstrate that M compared to C show different degrees of GM atrophy within the BF nuclei. The Ch4p, Ch4a-i and the hippocampus showed significantly less GM volume in M when compared to C. Also, the data show a significant between-group difference in cortical AChE activity in the temporal, occipital and parietal lobes with M showing less activity than C.

This study was able to show a significant positive correlation between Ch4p GM volume and the cortical AChE activity in the temporal lobe (with and without the hippocampus). In addition, the GM volume in atrophic areas of the BF correlated with AChE activity in the temporal (with and without the hippocampus) and occipital lobe.

This study was able to demonstrate that the “cholinergic deficit” in MCI involves temporal and parietal regions. There was a significant negative correlation between GM volume in the Ch4p and the “cholinergic deficit” across both groups. By contrast, there was a positive correlation between the GM volume in Ch1/Ch2 and the “cholinergic deficit”, indicating that larger GM volume in these regions was associated with less cortical AChE activity.

6.1 Discussion of the Results

This study found significantly lower GM volume in the CH4p, Ch4a-i and the hippocampus in the M group compared to the C group. This is congruent with prior MRI-based studies, which have shown that there is a GM volume decline of the cholinergic BF nuclei from prodromal to clinical stages of AD.[26,37] Furthermore, it has been shown that especially the Ch4p shows early volume reductions, already in the MCI due to AD stage.[27,37]

The significant positive correlation between the GM volume in Ch4p and the AChE activity in the temporal lobe further undermines the hypothesis that GM volume predicts the AChE activity. This idea supported by previous data, showing that Ch4p neurons are the main source of cholinergic innervation of the cortex.[41-43] Thus, the conclusion is that GM volume (the degree of atrophy in the BF) can be used to make a statement about the integrity of the cholinergic system.
Similarly, previous studies using Fluordesoxyglucose-PET imaging have shown that atrophy in the BF, identified using MRI, is associated with a reduction in cortical metabolic activity in MCI patients.[28] In patients with subjective cognitive decline, a significant association was found

between the volume of the Ch4p and the right precuneal glucose hypometabolism detected by Fluordesoxyglucose-PET.[59] The precuneus is a region that shows alterations in early stages of AD in terms of decreasing glucose metabolism.[10] These data together with our findings corroborate the suggestion that changes in BF GM volume affect neural function in distant cortical areas.

As mentioned above, the data suggests that BF GM volume can be a predictor of cortical AChE activity. Furthermore, it could potentially be used to predict the development of early stages of AD, maybe even stages prior to MCI. This suggestion is supported by previous studies, which have shown that GM atrophy in the hippocampus and BF (as well as other AD-related regions) can be predictive of an increased risk for conversion from MCI to AD.[9] This technique may potentially be implemented in clinical routine, as VBM can be used with regular T1 weighted MRI images. This potential predictor could be combined with other early disease-relevant changes such as the transcriptomatic signature sensitive to prodromal AD.[5] Together, it may be possible to identify an individual risk-profile. This may help develop potential therapeutic targets at the MCI stage.[5]

Previous studies analysing predictors of the efficacy of AChE inhibitors have suggested that hippocampal volume as well as an indirect measure of BF volume are able to predict the efficacy of Donepezil in patients with AD.[16,71] This further supports the theory, that BF volume can predict cortical AChE activity and thus, the efficacy of pharmacotherapy in MCI due to AD patients. In contrast, a previous study with MCI due to AD patients demonstrated that neither the volume of the hippocampus nor the BF volume were able to predict the response to Donepezil.[72] These contradictory results may be explained by the burden and presence of non-Alzheimer's pathologies, which increase with age.[50,54]

Within M the study found a significant association between the GM volume of the Ch1/Ch2 region of the NBM and the cholinergic deficit.[54] At first sight, this finding may be counterintuitive. However, previous histopathological studies have shown that septal cholinergic cell bodies are mostly preserved in early staged of AD.[48] Butler and colleagues have even demonstrated a larger volume in the Ch1/Ch2 area in cognitively healthy individuals that were to develop MCI in the following years, compared to cognitively healthy individuals that showed no changes in cognition.[11] Therefore, the positive correlation between Ch1/Ch2 GM volume and the cholinergic deficit may be a consequence of a compensatory hypertrophy.

The prepublished paper to this study (Richter et. al. 2022) further examines the role that age at disease onset plays in association between Ch1/2 volume and cholinergic deficit.[54] The

patients were divided into an early-onset and a late-onset MCI group. The early-onset subgroup had a larger cholinergic deficit than late-onset MCI patients.[54] This further suggests the necessity of including age in future studies.

6.2 Discussion of the Methods

In this study, the MACS data set was used which incorporated two imaging techniques MRI and PET. The GM volume was analysed using VBM, which requires high resolution T1 images. These are comparable to the images used in standard clinical setting. This means, the technique for analysing GM volume is not only operable in research settings, but can be implemented in a regular clinical setting, maybe even as a standard procedure.

One could argue that the volume of the subregions of the BF are too small to be assessed correctly using VBM. This seems unlikely since it has been demonstrated that the volumes in the Ch1/2 region generated by VBM correlate well with results from manually tracing the region.[12] See the prepublished paper (Richter et al. 2022) where the same protocol was used to manually trace the Ch1/2 region.[54] Similar results were found to the VBM method.[54] When considering these factors, using the Ch1/2 volume as a predictor for the cholinergic deficit seems promising. This technique could then help predict the efficacy of pharmacological treatment.

Something to keep in mind is that AChE activity may not fully capture the degeneration of the cholinergic system in AD.[54] Even though previous studies have shown a greater response to rivastigmine in patients with lower AChE activity, it must must not be forgotten that AChE is only one of many biomarkers of the cholinergic neurotransmission system.[52] Literature suggests that other markers, such as nicotinic ACh receptors are also reduced in MCI due to AD patients.[30]

Neurofibrillary degeneration plays an important role in the NBM, especially during the earlier stages of the disease.[44,57] Neurofibrillary degeneration is not necessarily reflected in loss of GM. This may help to explain why none of the GM volumes in the BF nuclei ROIs correlated with the cortical AChE activity. Thus, in future studies, we may have to focus on and try to further understand the role that neurofibrillary degeneration may play.[53]

The MP4a PET imaging data which was acquired for the MACS study is costly and requires time, equipment that is currently found mainly in specialized centers. Thus, the method of finding the individual cholinergic deficit is not yet ready to be implemented as a clinical routine.

As suggested above, in the future it could become an option for developing an individual pharmacotherapy option for each patient, designed for their cholinergic needs.

7. References

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8. Appendix

8.1 List of Figures

8.2 List of Tables

9. Vorabveröffentlichungen von Ergebnissen

Mit Einverständnis des Betreuers, Prof. Dr. med. Juraj Kukolja, wurde vorab folgendes Paper veröffentlicht:

1. Richter N, David L-S, Grothe MJ, *et al.* Age and anterior basal forebrain volume predict the cholinergic deficit in patients with mild cognitive impairment due to Alzheimer's disease. *Journal of Alzheimer's Disease* 2022; **86**: 425–40.