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Elevated serum cortisol levels are associated with cerebral grey matter atrophy, hippocampal volumes and verbal memory performance in healthy aging and the Alzheimer's disease continuum

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Für Mama, die Ihre Doktorarbeit auf Diskette „gespeichert“ hat.

Contents

ABBREVIATIONS

A	Amyloid pathology
AA	Alzheimer's Association
AAL	Automated anatomical labeling
AChEi	Acetylcholinesterase inhibitor
ACTH	Adrenocorticotropic hormone
AD	Alzheimer's disease
ANS	Autonomic nervous system
APOE	Apolipoprotein E
Aβ	Amyloid-beta
BALM	Basic Language Morningness scale
BCa	Bias-corrected and accelerated
BMI	Body mass index
CAMDEX	Cambridge examination for mental disorders of the elderly
CAT	Computational Anatomy Toolbox
CNS	Central nervous system
CONN	Functional connectivity toolbox
COPCAD	Connectivity of the Posterior Cingulate in Alzheimer's Disease
CORT	Cortisol
CRF	Corticotropin releasing factor
CRFr	Corticotropin releasing factor receptor
CSF	Cerebrospinal fluid
DARTEL	Diffeomorphic Anatomical Registration Through Lie Algebra
DESC	Rasch-based Depression Screening
df	degrees of freedom
DR	Delayed recall
EM	Episodic memory
FA	Flip angle
FLAIR	Fluid-attenuated inversion recovery
FOV	Field of view
FWE	Familywise Error Rate
FWHM	Full width at half maximum
GC	Glucocorticoid
GDS	Geriatric Depression Scale
GM	Grey matter
GR	Glucocorticoid receptor
HAM-D	Hamilton depression rating scale

HC	Hippocampus
HPA	Hypothalamic pituitary adrenal (axis)
HS	Healthy Seniors
IR	Immediate recall
IWG-2	International working group 2
MCI	Mild cognitive impairment
MD	Mean difference
MMSE	Mini-Mental State Examination
MNI	Montreal Neurological Institute
MPRAGE	Magnetisation-prepared rapid gradient echo
MR	Mineralcorticoid receptor
MRI	Magnetic resonance imaging
MTL	Medial temporal lobe
N	Neurodegeneration / neuronal injury
NFT	Neurofibrillary tangle
NIA	National Institute on Aging
NMDA	N-methyl-D-aspartate
PET	Positron emission tomography
PIB	Pittsburgh compound B
PSQ	Perceived stress questionnaire
p-tau	phosphorylated Tau protein
r	Pearson correlation coefficient
REC	Recognition
ROI	Region of interest
r_{pb}	Point-biserial correlation coefficient
r_s	Spearman correlation coefficient
SD	Standard deviation
SES	Socio-economic status
SNAP	Suspected non-Alzheimer's pathophysiology
SP	Senile plaques (Amyloid-beta plaques)
SPM	Statistical Parametric Mapping
SPSS	Statistical Package for the Social Sciences
T	pathological Tau
TA	Acquisition time
TE	Echo time
TICS	Trier inventory for the assessment of chronic stress
TIV	Total intracranial volume

TP	Temporal pole
TR	Repetition time
U	Mann-Whitney U test statistic
VBM	Voxel-based morphometry
VLMT	Verbal learning and memory test
VLMT A1-5 total	Verbal learning and memory test, list A, learning
VLMT A6 IR	Verbal learning and memory test, list A, immediate recall
VLMT A7 DR	Verbal learning and memory test, list A, delayed recall
VLMT B1	Verbal learning and memory test, list B, interference
VLMT REC	Verbal learning and memory test, recognition
WAIS	Wechsler adult intelligence scale
WM	White matter
WMS	Wechsler memory scale
WS	Whole sample

1. SUMMARY

Background: Elevated levels of cortisol are commonly observed in Alzheimer's disease (AD) patients. Hypothalamic-pituitary-adrenal axis dysregulation, resulting in elevated cortisol levels, is associated with grey matter atrophy, memory impairment and elevated risk for AD in otherwise healthy individuals. However, in most of the studies patients were not characterized by biomarkers and AD diagnoses were vague. Imaging findings were commonly based on CT imaging and voxel-wise analysis of MR-imaging was used sparsely.

Methods: Morning pre-scan serum cortisol levels, structural neuroimaging data and verbal memory performance were evaluated in patients with positive biomarkers (CSF, amyloid/tau-PET) suggestive of AD (n=29), and age-matched cognitively healthy seniors (n=29). Verbal memory performance was evaluated by a composite recall score derived from the verbal learning and memory test. The relationship between serum cortisol levels and grey matter volume (derived by CAT12) were assessed via whole brain voxel-wise analysis ($pFWE < 0.05$). In addition, a ROI-based approach using hippocampal volumes corrected for age, education and intracranial volume was applied.

Results: Cortisol levels were significantly higher in AD patients than in healthy seniors. On the voxel level, cortisol levels were negatively correlated with left-hemispheric grey matter volumes of the hippocampus, fusiform gyrus, temporal pole and angular gyrus across the whole sample and in the diagnostic subgroups. In the ROI-based analysis elevated cortisol levels were negatively correlated with left and right hippocampal volumes in the whole sample and left hippocampal volumes in healthy seniors. Verbal memory performance was negatively correlated with serum cortisol levels across the whole sample and in AD patients.

Conclusion: Elevated serum cortisol levels are associated with grey matter atrophy and lower hippocampal volumes not only in AD, but also in cognitively healthy seniors and thereby may increase the risk for cognitive decline. In AD, higher serum cortisol levels are associated with verbal memory impairment. To clarify a causal relationship longitudinal studies are needed. However, serum cortisol levels might serve as an early biomarker and could play a relevant role as a target for preventive measures and therapeutic approaches.

2. ZUSAMMENFASSUNG DER DISSERTATIONSSCHRIFT IN DEUTSCHER SPRACHE

Erhöhte Serumcortisolspiegel sind mit Atrophie zerebraler grauer Substanz, hippocampalen Volumina und verbaler Gedächtnisperformance bei gesundem Altern und dem Alzheimer-Kontinuum

Der Beitrag von chronischem Stress und einer konsekutiven Erhöhung der Cortisol-Spiegel im Serum auf die kognitive Leistung, insbesondere im Kontext neurodegenerativer Erkrankungen wie der Alzheimer-Erkrankung, war zuletzt vielfach Gegenstand neurowissenschaftlicher Forschung. Unter anderem wird ein Zusammenhang mit atrophen Veränderungen der grauen zerebralen Substanz, insbesondere der Hippocampi, und damit einhergehende kognitive Beeinträchtigungen, sowie eine erhöhte Vulnerabilität für Neurodegeneration postuliert. Eine Erhöhung der Cortisol-Spiegel als Ausdruck einer Überaktivität der Hypothalamus-Hypophysen-Nebennierenrinden Achse wird bereits in präklinischen Stadien der Alzheimer-Erkrankung angenommen.

Zur Untersuchung des Zusammenhangs erhöhter Cortisol-Spiegel im Serum mit atrophen Veränderungen der grauen Substanz und der Hippocampi sowie der Gedächtnisfunktion bei Patient*innen mit Biomarker-positiver Alzheimer Erkrankung (n = 29) im Kontrast zu einer kognitiv unbeeinträchtigten Vergleichspopulation (n = 29) wurden morgendliche Serum-Cortisol-Werte, strukturelle MRT-Daten sowie die verbale Gedächtnisfunktion analysiert.
Hierzu wurde die Leistungsfähigkeit des verbalen Gedächtnisses testpsychologisch anhand eines aus dem verbalen Lern- und Gedächtnistest abgeleiteten zusammengesetzten Recall-Scores bewertet. Der Zusammenhang zwischen den Serum-Cortisolspiegeln und dem Volumen der grauen Substanz wurde mittels einer voxel-weisen Analyse des gesamten Gehirns untersucht. Darüber hinaus wurde ein *region of interest* (ROI)-basierter Ansatz angewandt, bei dem die Hippocampus-Volumina für Alter, Bildung und intrakranielles Volumen korrigiert wurden.

Die Cortisolspiegel waren bei Patient*innen des Alzheimer-Kontinuums signifikant höher als bei gesunden Senioren. Auf Voxel-Ebene waren die Cortisolspiegel negativ mit den Volumina der grauen Substanz in der linken Hemisphäre des Hippocampus, des Gyrus fusiformis, des Temporalpols und des Gyrus angularis in der gesamten Stichprobe und in den diagnostischen Untergruppen korreliert. In der ROI-basierten Analyse waren erhöhte Cortisolspiegel negativ mit dem linken und rechten Hippocampusvolumen in der gesamten Stichprobe und dem linken Hippocampusvolumen bei gesunden Senioren korreliert. Die verbale Gedächtnisleistung war

in der gesamten Stichprobe und bei Alzheimer-Patient*innen negativ mit den Serum-Cortisolspiegeln korreliert. Die vorliegende Arbeit weist darauf hin, dass erhöhte Serum-Cortisolspiegel nicht nur im Kontext der Alzheimer-Erkrankung, sondern auch bei kognitiv gesunden Kontrollproband*innen mit einer Atrophie der grauen Substanz und einem geringeren Hippocampus-Volumen mit konsekutiver Beeinträchtigung der kognitiven Leistungsfähigkeit in Zusammenhang stehen. Bei Patienten mit Biomarker-positiver Alzheimer-Erkrankung korrelieren erhöhte Werte des Serum-Cortisols positiv mit einer Beeinträchtigung des verbalen Gedächtnisses.

Um aus diesen Ergebnissen einen kausalen Zusammenhang mit der Pathogenese der Alzheimer-Erkrankung abzuleiten, sind weiterführende longitudinale Studien erforderlich. Weitergehende Untersuchungen des Einflusses des Serum-Cortisols auf die Pathogenese sowie den Verlauf der Alzheimer-Erkrankung erscheinen, unter anderem basierend auf den vorliegenden Ergebnissen, im Sinne eines möglichen frühzeitigen diagnostischen Serum-Biomarkers sowie einem potentiellen Angriffspunkt für präventive, medikamentöse und therapeutische Maßnahmen angebracht.

3. INTRODUCTION

Stress and hypothalamic-pituitary-adrenal (HPA) axis overactivation with elevated levels of glucocorticoids have been proposed as a risk factor for the development of Alzheimer's disease (AD). The main function of cortisol, the glucocorticoid which is released in humans via activation of the HPA axis, is to restore homeostasis following the exposure to stress. A chronic increase of cortisol levels due to HPA axis dysregulations causes grey matter volume reductions in different regions of the brain and increases the vulnerability to neurodegenerative diseases, e.g. AD.

Cortisol levels are increased in patients with AD compared to cognitive healthy controls, and there are findings that strongly suggests that these changes in cortisol levels occur at a rather early stage of the disorder.[1,2] Elevated cortisol levels are also correlated with grey matter atrophy in AD patients and cognitive healthy seniors. These changes may contribute to cognitive deficits, especially hippocampal-dependent cognitive tasks, and clinical progression. Due to the assumption that the elevation of cortisol levels causes negative effects on cognition, atrophy and disease progression, a possible intervention seems to be in reducing cortisol levels by clinical and lifestyle interventions during the course of Alzheimer's disease.

This study aimed to determine the relationship between serum cortisol levels, grey matter volume and verbal memory performance in a sample of healthy seniors and AD patients. We hypothesize that elevated serum cortisol levels are negatively correlated with both grey matter volume, especially in the hippocampus, and episodic memory performance.

3.1. Alzheimer's disease

Alzheimer's disease is a progressive neurodegenerative disorder, first described by Alois Alzheimer in 1907[3] which is marked by cognitive and behavioural impairment that later in its course has a significant impact on the social and occupational functioning of an individual. AD is primarily a disease of older age[4] and its incidence and prevalence increase exponentially with age[5–7] Most cases of AD are sporadic (> 95 %)[8] but familial forms of AD also exist.[9] Aside from age,[10] the most established risk factors are a family history of dementia,[11] mutations in genes that alter amyloid beta protein production, aggregation and clearance in the brain,[12,13] e.g. presenilin-1 and presenilin-2,[14] amyloid precursor protein gene[15] and the apolipoprotein E (APOE) ε4 allele,[16] which is correlated with an increased risk and earlier onset of AD.[17] For other risk factors see *Table 1*.

Table 1: Intrinsic and extrinsic risk factors for Alzheimer's disease

Intrinsic AD risk factors	Extrinsic AD risk factors
- Age[10,13] - Family history / genetics[11,13] - Down Syndrome[18–21] - History of traumatic brain injury[22–24] Neurotic personality[25]	- Stress[26] - Poor sleep[27–29] - Hypertension[30,31], other vascular risk factors, e.g. intracranial artery stenosis[32] - Obesity[33,34] - Dyslipidemia[35] - Diabetes mellitus type 2[36,37] - Metabolic syndrome[38,39] - Elevated levels of homocysteine[40] - Physical inactivity[41,42] - Social isolation[41,43] - Low level of education[44] - Depression[45] - Environmental exposures, e.g. pesticides[46], aluminium[47,48], air pollution[49] - Vitamin D deficiency[50] - Hearing loss[51] - Smoking[41] - Alcohol[52]

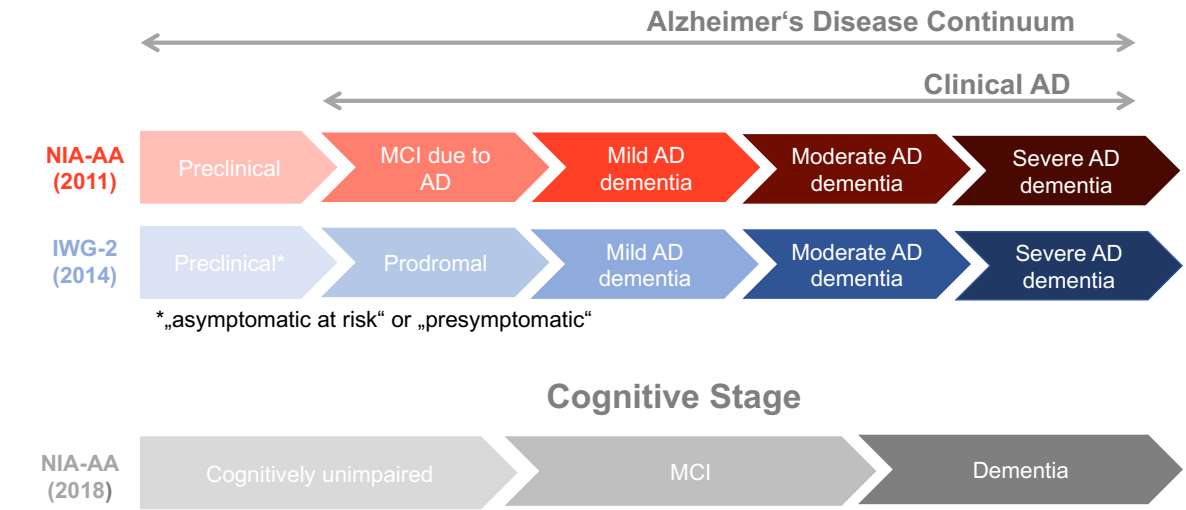
AD is the leading cause of dementia[16] and approximately 70% of dementia cases are due to AD[53] Globally, dementia is estimated to affect 47 million people.[54] In Germany about 1.6 million people are affected.[54] AD inevitably results in the need of high-level care and ultimately in death, mostly due to secondary complications such as pneumonia, which is the most common cause of death in demented patients.[55,56] Whereas deaths from other major causes are decreasing, the number of deaths related to AD is increasing steadily.[57] AD is one of the leading causes of morbidity and mortality in the elderly population.[58] The social and economic burden of AD is high.[59–61] Due to rising healthcare costs and the public burden of the disease, AD is an immense public health problem in the industrialised world.[62]

The pathogenesis of AD remains not fully understood. The neuropathological hallmarks of the disorder are extracellular amyloid-beta (Aβ) plaques[63] and intracellular neurofibrillary tangles (NFT) of hyperphosphorylated tau (p-tau) protein.[64] These lead to neuronal dysfunction and ultimately cell death, resulting in cortical atrophy.[65]

The Braak staging system for intraneuronal lesions was introduced in 1991.[64] AD-related pathological processes develop over decades and the distribution pattern at predisposed cortical and subcortical sites and the developmental sequence of the lesions are anticipated.[64,66–72] The AD related neurofibrillary changes allow to differentiate between six stages of intraneuronal lesions (stage I-VI), which can be subdivided into three groups: abnormal tau protein is detectable in the transentorhinal and entorhinal regions (stages I-II), in the limbic allocortex and adjoining neocortex (stages III-IV) or in the neocortex (stages V-VI).[64] Atrophy first develops in in the medial temporal lobe (MTL), e.g. the entorhinal cortex and the hippocampus (HC).[73] Further progression of the disease is associated with neocortical atrophy.[74] Histopathological[75] structural imaging[76] and functional imaging[77] studies demonstrated, that the hippocampal complex is implicated long before the clinical diagnosis of AD.

There are several different clinical and research criteria for the diagnosis of Alzheimer's disease (see *Figure 1*). The National Institute on Aging / Alzheimer's Association (NIA / AA) criteria[78] for the diagnosis of AD require the finding of a slowly progressive memory loss with a lingering onset. Dementia is diagnosed when there are progressive cognitive or behavioural symptoms that "interfere with the ability to function at work or usual activities" and which "represent a decline from previous levels of functioning and are not caused by delirium or other major neurological or psychiatric disorders".[78] The NIA-AA diagnostic criteria stage AD into asymptomatic "preclinical AD",[79] "MCI due to AD" (predementia)[80] and "dementia due to AD".[78] In the MCI stage, mild cognitive deficits can be present but activities of daily living are mostly preserved,[81] and approximately 50 % of the affected MCI patients evolve to AD pathology in a few years.[82] The International Working Group 2 (IWG-2) criteria[83] differentiate between "preclinical AD", "prodromal AD", "atypical AD" and "mixed AD". The preclinical stage is divided into two subgroups: "pre-symptomatic AD" (carriers of autosomal dominant monogenetic mutation who will eventually develop AD) and "asymptomatic at-risk for AD" (asymptomatic individuals with in vivo evidence of beta-amyloid accumulation in the brain). The most recent criteria by *Jack et al.* is an update of the NIA / AA research criteria and aims to "shift the definition of AD in living people from a syndromal to a biological construct"[84] by assessment of in vivo biomarkers like Aβ, pathologic tau and neurodegeneration using biofluids and different imaging modalities, e.g. structural magnetic resonance imaging (MRI) and positron emission tomography (PET). The term "Alzheimer's pathologic change" is used for individuals with evidence of Aβ deposition with normal tau biomarkers and the term "Alzheimer's disease" is applied, if both Aβ and pathologic tau are present in an individuum.[84] The revised research criteria do not regard "Alzheimer's pathologic change" and "Alzheimer's dementia" as separate entities but as different stages of the "Alzheimer's continuum".[84] There is a long pre-

symptomatic period between the onset of biochemical changes in the brain and the development of clinical symptoms.[84]



Biomarker category	Cognitively unimpaired	MCI	Dementia
A-T-(N)-	Normal AD biomarkers, no cognitive impairment	Normal AD biomarkers with MCI	Normal AD biomarkers with dementia
A+T-(N)-	Preclinical Alzheimer's pathologic change	Alzheimer's pathologic change with MCI	Alzheimer's pathologic change with dementia
A+T+(N)-	Preclinical Alzheimer's disease	Alzheimers disease with MCI (prodromal AD)	Alzheimer's disease with dementia
A+T+(N)+			
A+T-(N)+	Alzheimer's and concomitant SNAP, no cognitive impairment	Alzheimer's and concomitant SNAP with MCI	Alzheimer's and concomitant SNAP with dementia
A-T+(N)+	Non-Alzheimer's pathologic change, no cognitive impairment	Non-Alzheimer's pathologic change with MCI	Non-Alzheimer's pathologic change with dementia
A-T-(N)+			
A-T+(N)+			

Biomarker Grouping		
	A	Aggregated Aβ or associated pathologic state, CSF $A\beta_{42}$, CSF $A\beta_{42}/A\beta_{40}$ ratio, Amyloid PET
	T	Aggregated tau (NFT) or associated pathologic state, CSF pTau, Tau PET
	N	Neurodegeneration or neuronal injury, Structural MRI, FDG PET, CSF total Tau

Figure 1: Modified Figure adapted from Rubino et al.[85] of the different diagnostic criteria of the Alzheimer's disease continuum

Diagnostic criteria of the NIA-AA[78] are based on clinical symptoms. The diagnostic criteria of the IWG-2[83] and the NIA-AA diagnostic framework for research[84] combine clinical criteria and biomarkers. In the research framework by *Jack et al.* biomarker profiles refer to the three different pathologic processes of AD: amyloid pathology (A+/-), pathological pTau (T+/-) and neurodegeneration / neuronal injury (N+/-). Binarizing of the three biomarker groups results in eight possible biomarker profiles and assigns the individuum to one of the three biomarker categories: "normal AD biomarkers", "Alzheimer's continuum" and "suspected non-Alzheimer's pathophysiology" (SNAP). + indicates positive and – indicates negative.

Abbreviations: NIA-AA: National Institute on Aging and the Alzheimer's Association, IWG-2: International Working Group 2, MCI: mild cognitive impairment, AD: Alzheimer's dementia, SNAP: suspected non-Alzheimer's pathophysiology, A: amyloid pathology, T: pathological tau, N: neurodegeneration / neuronal injury

Neuroradiological imaging is important to rule out potentially treatable causes of cognitive impairment (e.g. normal pressure hydrocephalus) and may be able to detect typical patterns of atrophy.[78,86,87] A lumbar puncture can exclude infectious and inflammatory processes and aid the diagnosis through assessment of cerebrospinal fluid (CSF) biomarkers.[78,87]
Research has shown that AD causes changes in CSF levels of tau and Aβ. Patients with AD show a decrease in $A\beta_{1-42}$ concentration.[88] Increased CSF levels of tau and pTau are thought to occur after its release from damaged and dead neurons, which contained NFT's.[89] The combination of decreased CSF concentrations of $A\beta_{1-42}$ and increased CSF pTau concentrations is considered to be a pathological CSF biomarker that is diagnostic for AD.[90,91]

AD is characterized by cognitive deficits that gradually affect multiple domains.[92,93] Cognitive impairment in persons who will develop AD occurs years[94,95] and in some cases decades[96,97] before the clinical AD diagnosis. Memory impairment, especially impairment of declarative episodic memory, is the most common initial symptom of AD.[98] Deficits of episodic memory reflect pathological changes in the medial temporal lobe, particularly in the hippocampal region.[98] The episodic memory system is clinically most relevant for patients with AD, as impairment of episodic memory causes a disturbance in memory encoding and retrieving of recent events which leads to functional deficits.[99–101] These episodic memory deficits occur at the early stages of the disease, and serve as the hallmark feature of AD.[78,95,102,103] AD patients typically have difficulties learning lists of words, but they also have difficulties recalling the learned words and recognizing the learned words among distractors.[104–106] Executive dysfunction and visuospatial impairment also often manifest in earlier stages, while deficits in language and behavioural symptoms occur later in the progression of the disease.[78] Deficits in attention and working memory are associated with damage to frontal subcortical circuits and cause impaired planning and problem-solving in AD.[107] Neuropsychiatric and behavioural symptoms (e.g. apathy, social disengagement, irritability, depression, psychosis, agitation and aggression) are also common.[108–110] Other possible symptoms are apraxia,[111,112] sleep disturbances[27,28] and seizures.[113,114] Pyramidal and extrapyramidal motor signs, primitive reflexes (e.g. grasp reflex and palmomental reflex) and incontinence may manifest in late stages of the disease.[115–117]

Until recently, treatment options for Alzheimer's disease were limited to symptom management. The standard medical treatments are cholinesterase inhibitors (rivastigmine, donepezil and galantamine), and the partial N-methyl-D-aspartate (NMDA) antagonist memantine.[118,119] Although cholinesterase inhibitors are indicated for the symptomatic treatment of AD,[120] their positive effects on cognition is modest and of limited duration.[121a] Meta-analysis has demonstrated that these drugs have no effect on the rate of conversion

from MCI to AD.[122] Secondary symptoms of AD, e.g. depression, agitation, aggression, delusions, hallucinations and sleep disorders, are treated with psychotropic medications e.g. antidepressants and neuroleptics. Recently, disease-modifying therapies targeting amyloid pathology have emerged. These monoclonal antibodies like lecanemab and donanemab target amyloid-beta and promote the clearance of amyloid plaques in the brain and are approved for patients with early Alzheimer's disease (MCI and mild dementia). The long-term efficacy and safety of these new drugs needs further validation. [121b, 121c]

3.2. Stress, hypothalamic-pituitary-adrenal axis and cortisol

The stress response is triggered by a stressor and includes the activation of the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis.[123] The ANS is responsible for the rapid onset of the stress response via secretion of catecholamines epinephrine and norepinephrine by the adrenal medulla and controls a wide range of functions.[124]

The HPA axis is responsible for the slower onset of the stress response via secretion of glucocorticoids.[125] The activation of the stress response and its different target systems are important to prepare the individuum for a "fight-or-flight" response,[126] which was first described by Walter Cannon.[127,128] Both, activation and termination of the stress response, are crucial for the survival of an individual.[129–132] A critical point of the stress response is its self-termination after "the stressor has ended or is no longer perceived as a threat",[133] to maintain physiological homeostasis.[134,135] A persistent stress response (i.e. chronic stress) can have severe negative effects on health.[136–139]

HPA axis activation begins with release of the neuropeptide corticotropin releasing factor (CRF) by neurons from the paraventricular nucleus of the hypothalamus[392] into the hypophyseal portal system in response to different somatic stimuli e.g. hunger[140] and inflammation,[141] and to perceived psychological stress.[142] The primary function of CRF is to promote the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary into the bloodstream, where it stimulates the release of glucocorticoids from the adrenal cortex.[143–145] Glucocorticoids regulate their own secretion via a negative feedback inhibition.[146,147] This feedback inhibition occurs at multiple levels, i.e. the hypothalamus by inhibition of CRF release,[148,149] the pituitary by inhibition of ACTH secretion[150] and the hippocampus.[151] See *Figure 2*.

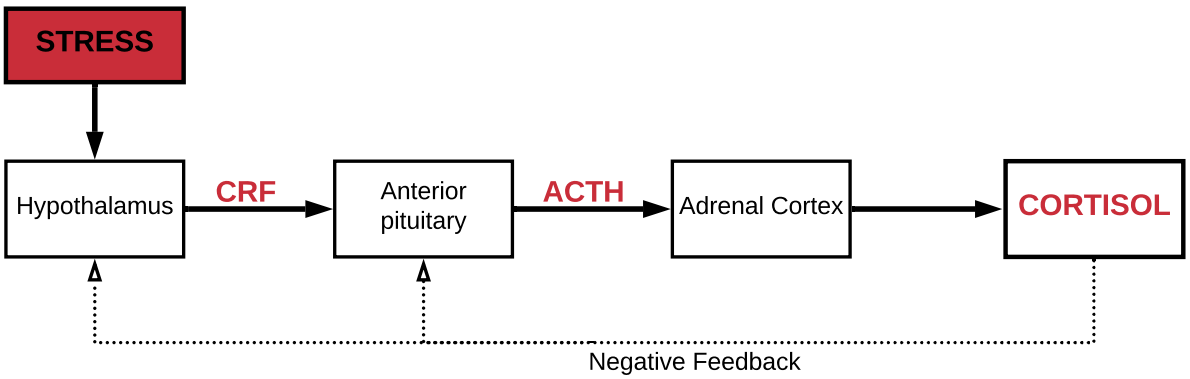


Figure 2: Schematic of the HPA axis

Stress triggers activation of PVN neurons, causing the release of CRF, which reaches the anterior pituitary via the portal blood, where it induces the secretion of ACTH. ACTH travels to the adrenal cortex via the systemic circulation, and causes the secretion of GCs.

Abbreviations: HPA: hypothalamic-pituitary-adrenal axis, CRF: corticotropin releasing factor, ACTH: adrenocorticotropic hormone, PVN: paraventricular nucleus of the thalamus, GC: glucocorticoid

Cortisol is a glucocorticoid hormone synthesized from cholesterol by enzymes of the cytochrome P450 family in the zona fasciculata of the adrenal cortex.[152] Regulated via the HPA axis, cortisol is the primary hormone responsible for the stress response in humans. Glucocorticoids are released in a circadian rhythm, reaching a peak before the onset of the active phase in the early morning.[153–156] Cortisol's main function is to restore homeostasis following exposure to stress,[157] which is achieved by energy mobilization i.e. increasing blood glucose levels by inducing gluconeogenesis, glycogenolysis and multiple aspects of glucose homeostasis.[158–162] Cortisol has both stimulatory and inhibitory effects on the immune response,[132] but the suppressive effects i.e. anti-inflammatory and immunosuppressive properties of cortisol are predominating.[132,163–166]

Furthermore, cortisol has multiple effects on memory and cognition in situations of acute and chronic stress.[167–169] Acute stress can cause impairing effects[170–172] and enhancing effects[173–176] on cognition and memory, depending on the time of GC secretion/administration, sex and age of the subject and the specific memory task. Chronic stress, i.e. prolonged elevation of glucocorticoids, mostly has impairing effects on cognition and memory.[177–180]

3.3. Cortisol effects on brain structure and function in health, aging and disease

Glucocorticoids are among the hormones with the most important and complex effects on the central nervous system, cognition and memory.[132,181–183]

Acute stress causes a rapid rise in circulating cortisol that, because of its lipophilic character,[184] readily passes the blood-brain barrier and binds to specific intracellular receptors, especially in regions implicated in cognitive functions, e.g. the hippocampal formation.[185–187]

The hippocampus is part of the limbic system and plays an important role in the consolidation of information from short-term memory to long-term memory (episodic memory, autobiographic memory), spatial memory and navigation.[188,189] It is located sub-cortically in the medial temporal lobe and consists of the hippocampus, which can be subdivided into the cornu ammonis, the dentate gyrus (DG) and the subiculum.[190] The DG receives afferent neurons which are projected from the entorhinal cortex, located in the gyrus parahippocampalis, via the tractus perforans.[191]

Glucocorticoids bind with a low affinity to glucocorticoid receptors (GR, type II), and with a high affinity to the mineralocorticoid receptors (MR, type I), which are highly expressed in limbic areas,[192] and make the hippocampus vulnerable to elevated glucocorticoid levels caused by chronic stress or exogenous administration.[133,182,183]

In 1986 *Sapolsky et al.* proposed the "glucocorticoid cascade hypothesis", which describes the dynamic relationship between glucocorticoids and the hippocampus in the course of aging.[193] See *Figure 3*.

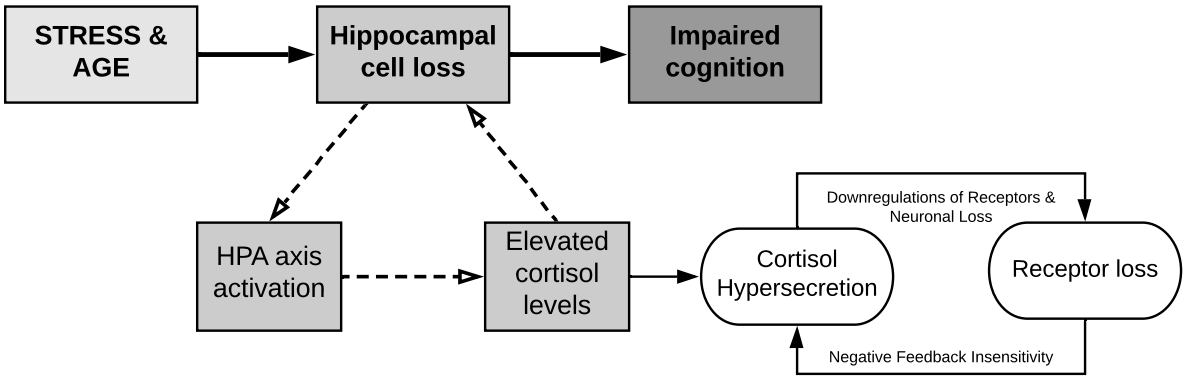


Figure 3: Schematic representation of the "glucocorticoid cascade hypothesis"

Glucocorticoids secreted during periods of stress cause reversible downregulation of glucocorticoid receptors in the hippocampus, to desensitize the hippocampus to further glucocorticoid exposure. High glucocorticoid levels and simultaneously occurring metabolic challenges i.e. stroke, ischemia, hyperglycemia, or hypoxia, lead to irreversible hippocampal cell loss,[193,194] which causes reduced disinhibition of the HPA axis, leading to a further increase in glucocorticoid levels and therefore more hippocampal damage, leading to a cascade effect.[151,195,196]

The “glucocorticoid cascade hypothesis” has been proposed as one of the pathogenic mechanisms of AD, and several clinical and experimental studies identified chronic stress as a risk factor for the development of AD.[26,197,198]

The “glucocorticoid vulnerability hypothesis” suggests that glucocorticoids are not directly responsible for the hippocampal damage, but that they are a contributing factor to hippocampus vulnerability to damage.[133] Chronic stress and therefore chronic elevation of glucocorticoids prolong the timespan in which the hippocampus is susceptible to damage.[133] The biggest difference to the “glucocorticoid cascade hypothesis” is, that *Conrad et al.* propose that glucocorticoids do not need to be elevated during the “time of a metabolic challenge”, because prolonged exposure to elevated glucocorticoids leaves some kind of “imprint” on the hippocampus, which makes it more vulnerable to a metabolic insult.[133]

In vitro experimental and animal studies demonstrated various negative effects of cortisol on the hippocampus. Stress reduces the excitability of hippocampal neurons,[199] negatively affects synaptic plasticity,[200,201] decreases neurogenesis,[202–206] and causes dendritic retraction.[207–209] This has implications for age-related elevated cortisol levels[210–214] and for a variety of conditions associated with elevated cortisol levels in humans e.g. Cushing’s syndrome,[215–218] major depressive disorder[219] and post-traumatic stress disorder,[220] all of which are linked to reduced hippocampal volumes and dysfunction,[133,221,222] resulting in cognitive deficits.[217,223,224]

As mentioned earlier, the hippocampus participates in the inhibitory feedback loop of the HPA axis. Hippocampal atrophy results in a reduced disinhibition of the HPA axis and ultimately increased cortisol levels, which further potentiate the hippocampal damage, leading to a vicious circle[223] as described in *Figure 4*.

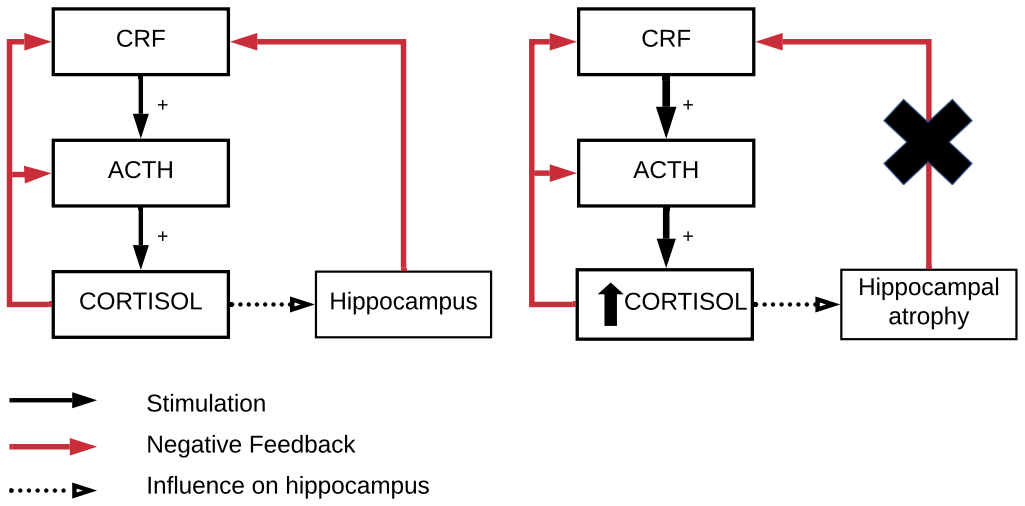


Figure 4: Modified scheme of the inhibitory effects of the hippocampi on the HPA axis

The hypothalamus releases CRF, which stimulates the release of ACTH by the pituitary gland, which stimulates the adrenal glands to secrete cortisol. Cortisol inhibits its own secretion via a negative feedback loop. The hippocampus inhibits the HPA axis. Elevated levels of cortisol can induce hippocampal atrophy, which leads to disinhibition of the HPA axis. The resulting elevation of cortisol induces further hippocampal atrophy, resulting in a vicious circle[223].

Abbreviations: CRF: corticotropin releasing factor, ACTH: adrenocorticotrophic hormone, HPA: hypothalamus-pituitary-adrenal

Patients with dementia and MCI due to AD have higher cortisol levels than cognitively healthy controls. Different studies demonstrated elevated cortisol levels in different modalities in patients of the Alzheimer spectrum i.e. CSF cortisol,[2] serum cortisol,[225] plasma cortisol,[226–228] urinary cortisol[229] and saliva cortisol.[230]

High cortisol levels have been linked to decreased volume – in particular grey matter volume – of different brain regions, e.g. the hippocampus, in cognitive healthy older adults, MCI and AD patients.[148,231–234] These reductions of grey matter volumes may contribute to cognitive deficits[148,182,232] especially hippocampal-dependent cognitive tasks.[233]

Hippocampal atrophy could be the consequence of the exposure to increased cortisol levels, but the atrophy itself could also participate in the elevation of the cortisol levels.[223] High cortisol levels in MCI subjects compared to cognitive healthy controls strongly suggests, that the increase in cortisol and its negative impact on the pathogenesis of AD occur at an rather early stage in the development of AD, and therefore is less likely to be caused by brain atrophy, e.g. hippocampal atrophy.[1,2] However, this finding is not in agreement with previous studies, which

did not find a difference between MCI subjects and cognitively healthy controls,[235,236] but overall these studies were limited by a small sample size.

Furthermore, elevated cortisol levels seem to be associated with an accelerated clinical progression[2,198,228,229,237] and a more severe cognitive decline,[238,239] and are positively correlated with brain Aβ burden in AD patients.[240]

In healthy subjects, higher cortisol levels have been associated with an increased risk of cognitive decline and dysfunction, especially in hippocampus dependent memory tasks, hippocampal volume loss and the development of AD.[223,233,237]

Furthermore, elevated glucocorticoid levels promote oxidative stress and increase amyloid beta toxicity in hippocampal neurons.[241] They have been linked to increased amyloid beta accumulation and tau pathology[242] and increased $A\beta_{1-42}/A\beta_{1-40}$ ratio and therefore an increased production of the more brain toxic $A\beta_{1-42}$.[243]

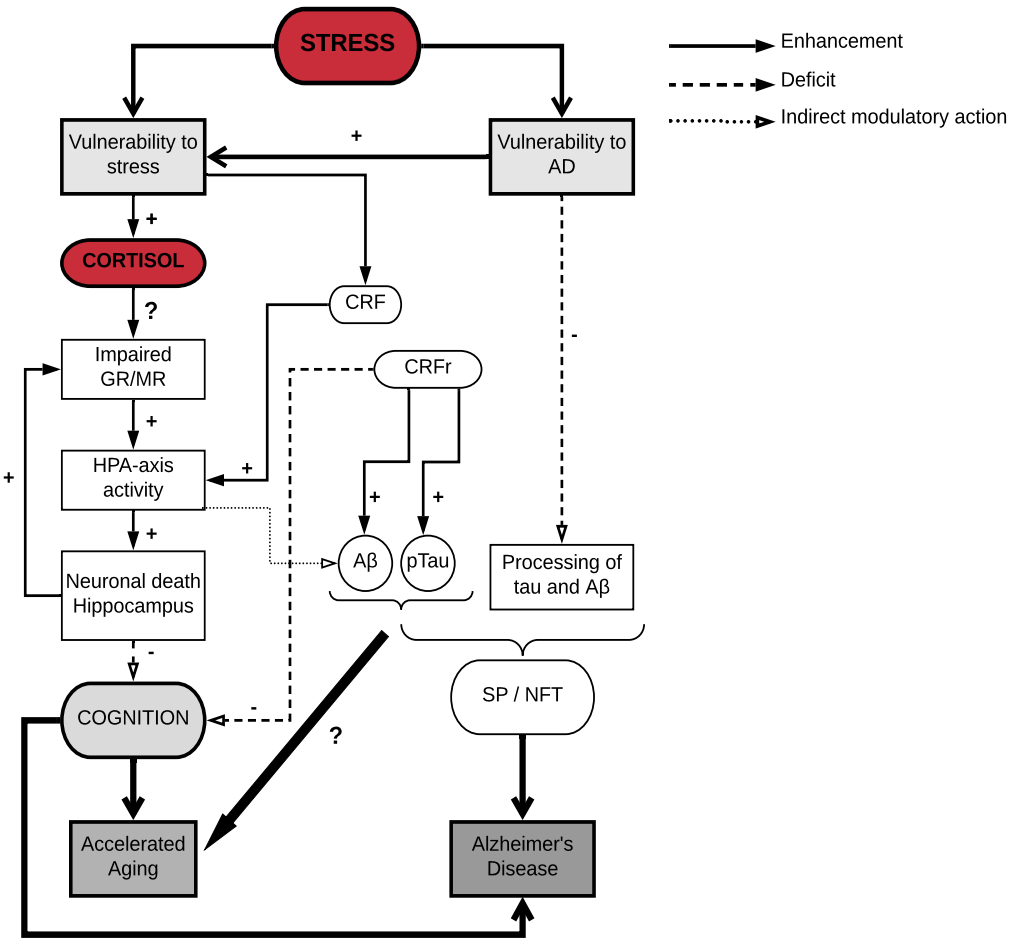


Figure 5: Modified hypothetical scheme by Pardon and Rattray linking stress with aging and AD pathology[244]

Stress increases age-related cognitive dysfunction in stress susceptible individuals via dysregulation of the HPA axis, GC-mediated neuronal death and overactivation of the CRH pathways. CRH (directly) and GC (indirectly) cause elevated Aβ levels and tau phosphorylation. Vulnerability to stress in subjects at risk of developing AD will result in increased cognitive decline and possibly elevate the incidence of AD.[244]The thin dotted line indicates indirect modulatory action of the HPA-axis on Aβ and pTau. (+) indicates enhancement, (-) indicates deficits and (?) indicates unknown influence.

Abbreviations: AD: Alzheimer's disease, CORT: cortisol, CRF: corticotropin releasing factor, CRFr: corticotropin releasing factor receptor, GR: glucocorticoid receptor, MR: mineralcorticoid receptor, Aβ: amyloid beta, pTau: phosphorylated Tau, SP: senile plaques, NFT: neurofibrillary tangles

3.4. Hypothesis

The role of glucocorticoids in the pathogenesis of AD remains unclear and a topic of controversy amongst scientists. In this study we aimed to reproduce the findings of several studies, that AD patients have elevated serum cortisol levels compared to cognitive healthy controls.[2,225–229,245]

Furthermore, our goal was to correlate cortisol levels with structural neuroimaging data and neuropsychological tests that assess hippocampus-dependent memory processes. We selected the verbal learning and memory test (VLMT, described in *Chapter 2 - Material and Methods, Section 2.2.3*), because it assesses encoding (learning), consolidation and retrieval (recall, recognition).[246] The hippocampal formation is highly involved in those episodic memory processes,[247] which are all affected at an early stage of the AD continuum.[98]

The relationship between serum cortisol levels and grey matter volumes were assessed via a whole brain voxel-wise analysis, thereby using an approach unrestricted to a priori hypotheses. Additionally, we performed ROI-based hippocampal analyses of hippocampal volumes for a more hypothesis-directed evaluation of the relation between grey matter volumes and elevated serum cortisol.

We included a collective across the AD continuum with biomarkers (CSF, neuroimaging) positive for AD pathology, which is according to the *Jack et al.* research criteria,[84] and an age-matched control group of cognitive healthy seniors. It is notable, that a lot of earlier studies did not assess biomarkers to support the clinical AD diagnosis and therefore the samples possibly included AD mimics. Some recent studies even used old diagnostic criteria to diagnose AD (Wirth et al. 2019 – McKhann 2011, “clinical probable AD”; Ennis et al. 2017 – DSM-III-R, Popp 2015 – McKhann 1984 diagnostic criteria, Umegaki et al. 2000 – DSM-IV).

Another shortcoming of many earlier studies is a CT-based, rough estimation of hippocampal volumes.[230,232] MRI-based, voxel-wise hippocampal analysis allows for a more accurate assessment of the hippocampal grey matter volumes with high regional specificity.

To our best knowledge, there are only three other studies (Toledo 2013, Echouffo-Tcheugui 2018, Wirth 2019) that assessed the relationship of grey matter volume and cortisol levels using voxel-based analysis. The recent study of *Wirth et al.*[248a] demonstrated that higher plasma cortisol levels were associated with lower left hemispheric grey matter volume in patients of the AD continuum and cognitively healthy controls. Total hippocampal volume was only significantly correlated with cortisol in the entire cohort and the MCI subgroup, but not in the AD or the cognitively healthy group.[248] *Echouffo-Tcheugui et al*.[231] showed that higher serum cortisol levels were associated with lower total parietal and frontal grey matter volume, white matter volume in multiple locations and impaired memory in cognitive healthy middle-aged adults. However, no significant voxel-wise relationship between grey matter volume and

cortisol levels could be demonstrated. In addition, there was no correlation between hippocampal volume and cortisol levels detectable (Echouffo-Tcheugui 2018, Neurology).[231] To the best of our knowledge, there is no study that assessed the relationship of serum cortisol levels and episodic memory function using the verbal learning and memory test.

Our hypotheses are:

- Serum cortisol levels are significantly higher in the AD group than in the HS group.
- Serum cortisol levels are negatively correlated with verbal memory performance in HS and AD.
- Serum cortisol levels are negatively correlated with hippocampal volumes and voxel-wise grey matter volumes in HS and AD.

4. MATERIAL AND METHODS

4.1. Participants

The current study reports analyses and results of data, which was obtained within two larger related studies COPCAD and COPCAD-TAU ("Connectivity of the Posterior Cingulate Cortex in Alzheimer's Disease"), which were approved by the local ethics committee, and performed in accordance with the declaration of Helsinki. Participants signed a written informed consent. All participants were between 50 and 80 years old (study sample: 50-73 years, mean 64.4 years, SD 6.2 years) and were either native German speakers or fluent in German and underwent a comprehensive study protocol including multimodal imaging, blood sampling, neuropsychological testing and clinical assessments.[248b]

Healthy older adults were recruited from the general population and the research facility in Jülich (Forschungszentrum Jülich), and received compensation for their involvement in the study. The healthy seniors had no deficits on neuropsychological testing and no depression.
Patients with Alzheimer's disease (AD) were recruited from the Memory Clinic at the University Hospital of Cologne and data acquisition was part of their clinical and diagnostic evaluation regarding memory complaints.[248b] Patients with prodromal AD (i.e. "MCI with AD biomarkers") and patients with AD dementia were identified based on the presence of objective memory impairment at least 1.5 standard deviations below the normative mean of a healthy older adult population.[248b] Prodromal AD patients had a Mini-Mental State Examination (MMSE)[249] score > 23 and preserved activities of daily living and AD dementia patients had MMSE scores between 15 and 22 and the activities of daily living were impaired.[248b] The status of the activities of daily living was confirmed by a knowledgeable informant. For all analysis both patient groups were combined and are referred to as "patients" or "AD group" in the following text, consistent with recent research criteria[84] that classify MCI and dementia due to AD as stages of the same disease continuum. The diagnosis was set according to standard diagnostic criteria.[78,83] In addition, "all patients had biomarker profiles indicative of Alzheimer's disease", as determined by CSF analysis or PET.[248b] CSF biomarker positivity was defined by a CSF tau/$A\beta_{1\text{-}42}$ ratio greater than 0.52.[250] Thirteen patients underwent [^{11}C]PIB or [^{18}F]Florbetapir amyloid PET and [^{18}F]AV-1451 tau PET additionally to (12 patients) or instead of (1 patient) CSF analysis.[248b] "Patients had a pattern of amyloid and tau deposition typical of AD as determined by a nuclear medicine specialist".[248b] Two patients had a CSF tau/$A\beta_{1\text{-}42}$ ratio, which was slightly below the cut-off of (< 0.52), but were included in the study based on AD-typical patterns of tau and amyloid PET tracer distribution.[248b]

Subjects were excluded when they showed symptoms of (other) neurological or psychiatric disorders, with exception of mild depression in the patient group, or took medication affecting

the central nervous system or cognitive abilities, except for acetylcholinesterase inhibitors, memantine or antidepressants in the patient group.[248b] Seventeen patients of the AD group were taking antidementive or antidepressive medication or both (only acetylcholinesterase inhibitor [n = 11], acetylcholinesterase inhibitor and memantine [n = 1], acetylcholinesterase inhibitor and antidepressant [n = 4], only antidepressant [n = 1]).[248b]

Other exclusion criteria were structural brain abnormalities, movement artefacts, cortisone-containing medications and general contraindications for undergoing MRI (e.g. cardiac pacemaker, metal implants etc.). Mild clinical depression, which is a well-known co-morbid condition of AD, was not considered an exclusion criterion for AD subjects, provided it was not the primary cause of cognitive deficits. Depressive symptoms were assessed using different scales (see *Section 4.2.1*). Subjects with confluent white matter lesions (Fazekas score > 2)[251] [n=6] or other structural abnormalities [n=3] were excluded from further analyses.[248b]

To address the significant age difference and unequal sample sizes, AD patients were age-matched to healthy seniors using propensity score matching, implemented via a custom R-based dialog for SPSS 25,[252] resulting in a final sample size of 58 subjects[248b] (healthy seniors [n=29], AD [n=29]). For demographics see *Table 3, Section 5.1.*.

4.2. Neuropsychological assessment

All measures were administered and scored according to standard protocols. Patients who received neuropsychological testing as part of the clinical routine at the Memory Clinic Cologne of the University Hospital of Cologne were only tested again if the tests were administered three months prior to study participation. The subjects underwent extensive neuropsychological testing, a detailed description of the neuropsychological testing protocol can be attained in the publications by *Dillen et al.*.[262,263] We focused our analysis on the verbal language and memory test (VLMT, see *Section 4.2.3.*), which assesses memory encoding, consolidation and retrieval, because those processes of episodic memory are affected at early stages of AD.[98]

4.2.1. Depression score

Depressive symptoms were assessed with the Hamilton Depression Rating Scale (HAM-D),[264] the Geriatric Depression Scale (GDS)[265] or the Rasch-based Depression Screening version 1 and 2 (DESC-I and DESC-II).[266,267]

The HAM-D consists of 21 items, of which 17 items are used to give a score of severity of depression. As proposed by *Zimmermann et al.* the resulting score of the HAM-D indicates no depression [score 0–7], mild depression [score 8–16], moderate depression [score 17–23] or

severe depression [score > 23].[268] The GDS contains 30 items with a yes/no option. For 20 items a "yes" response and for 10 items a "no" response indicates depressive symptoms. A total score is calculated (1 point per item) and indicates no depression [score 0–9], mild depression [score 10–19] or severe depression [score 20–30].[269] The DESC-I and DESC-II are 10 item depression questionnaires. Each item is scored from 0 to 4 [0: never, 1: rare, 2: sometimes, 3: mostly, 4: always], considering the last 14 days. The total score can range from 0 to 40. A score ≥ 12 indicates depressive symptoms. Healthy seniors required a HAM-D score < 8.[268] AD patients required a HAM-D score < 17,[268] a GDS score < 20[269] or a DESC-I / DESC-II score < 12.[266] Because different tests to assess depressive symptoms were used in the AD group, scores of the HAM-D, GDS, DESC-I and DESC-II where categorized into "no depression", "mild depression" and "moderate/severe depression" (see *Table 2*). Accordingly, three AD patients reported mild depressive symptoms (HAM-D score of 16, GDS scores of 6 and 8).

Table 2: Depression Score

Depression score		**HAM-D**	**GDS**	**DESC-I/DESC-II**
0	no depression	0 - 7	0 - 10	0 - 11
1	mild depression	8 - 16	10-19	12 - 40
2	moderate - severe depression	17 - 68	20-30	
Transformation of the different tests to assess depressive symptoms into a unified depression score. Abbreviations: HAM-D: Hamilton depression rating scale, GDS: Geriatric Depression Scale, DESC-I/DESC-II: Rasch-based Depression Screening version 1 and 2				

4.2.2. Mini Mental State Examination

The Mini Mental State Examination (MMSE) is extensively used as a screening test for cognitive impairment. It is divided into two parts. The first section requires vocal responses only and covers orientation, memory and attention. The second part tests the ability to name objects (e.g. pencil, watch, glasses), follow verbal and written commands, write a sentence and copy a complex polygon figure.[249,270] Scores can range from 0 to 30. A score ≥ 24 indicates normal cognition. Lower scores indicate mild [score 19–23], moderate [score 10–18] or severe cognitive impairment [score ≤ 9].

4.2.3. Verbal Learning Memory Test

The Verbal Learning and Memory Test (VLMT) is the German equivalent of the Rey Auditory Verbal Learning Test (RAVLT). The VLMT is a verbal learning list procedure and a valid measure of verbal episodic declarative memory.[271] The test consists of a list of 15 words (A,

learn list), which is read aloud by the examiner at fixed intervals and in a fixed order. During five runs the list is repeated and each time needs to be recalled by the participant (VLMT A1–5, max. 75 points). This is followed by the presentation and immediate free recall of a second interfering list B, also consisting of 15 words (VLMT B1). Subsequently, the content of list A has to be recalled again after this interference (VLMT A6 IR, recall after interference, max. 15 points). Memory is again assessed after a 20 to 30 minutes break with a free recall of list A (VLMT A7 DR, delayed recall, max. 15 points) and a following recognition test (VLMT REC), where words from list A, list B and 20 "new" words are presented and the participant has to identify the correct words from list A. The number of recalled words and the number and type of errors (e.g. false positive, perseveration or interference errors) for each attempt are evaluated. The VLMT assesses the modalities of immediate, consolidated and long-term memory.[272,273] We focused our analyses to the immediate recall after interference (VLMT A6 IR) and the delayed recall (VLMT A7 DR), as impaired recall of recent memory is the most prominent and initial symptom of AD[274–276] and the ability to retain and recall episodic memories is highly dependent on the hippocampal formation.[277]

All statistical analyses were performed using IBM SPSS Statistics (Version 25, Chicago, IL, USA). Neuropsychological testing of one AD subject had to be terminated due to mental exhaustion before the final assessment of the delayed recall of the VLMT. The subject was subsequently assigned a score of 0 for delayed recall, which also would have been expected based on prior performance in this test. One AD subject had missing values for the immediate recall after interference due to reporting issues, although the subtest had been performed. Because of this missing value, Little's MCAR Test[278] was performed and it demonstrated that the data was missing completely at random ($p = 0.72$). Afterwards the data was completed via expectation maximization n SPSS missing values module and the estimated value aligned with expectations established by results observed in other domains.

We composed a composite "verbal memory recall score" of the standardized scores of the immediate recall (VLMT 6 IR) and the delayed recall (VLMT 7 DR) of the VLMT through simple averaging z-scores.[248b] Composite scores reduce the number of statistical comparisons to evaluate cognitive outcomes, and therefore controls the Type I error rate for multiple comparisons.[279] See *Figure 6* for a schematic illustration of the VLMT.

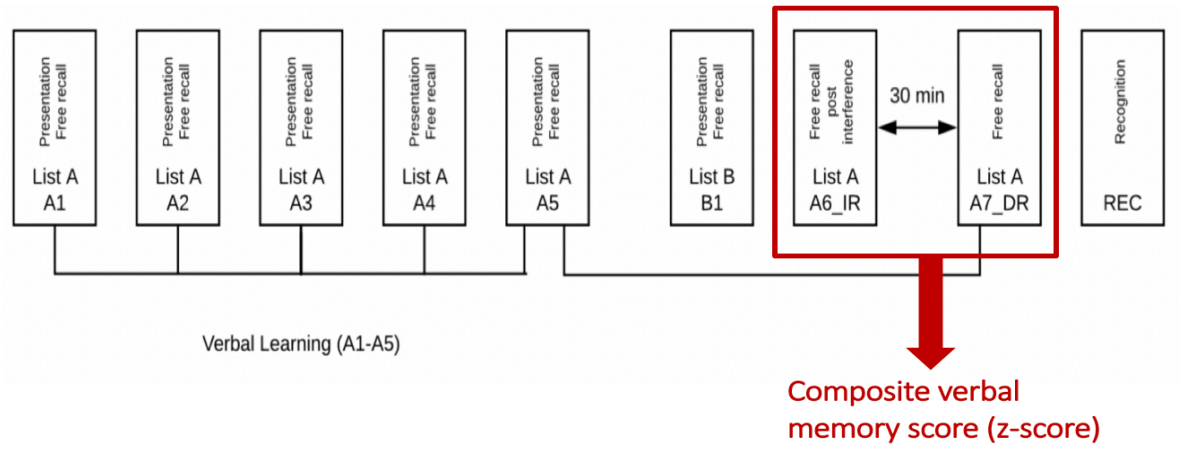


Figure 6: Illustration of the Verbal Learning and Memory Test (VLMT)

The VLMT is a verbal learning list procedure and a valid measure of verbal episodic declarative memory[246] and assesses the modalities of learning and immediate, consolidated and long-term memory.[272,273] A composite "verbal memory recall score" of the standardized scores of the immediate recall (VLMT 6 IR) and the delayed recall (VLMT 7 DR) of the VLMT through simple averaging z-scores was composed to reduce the number of statistical comparisons to evaluate cognitive outcomes.[279, 248b]

Abbreviations: IR: immediate recall, DR: delayed recall, REC: recognition

4.3. Cortisol

Morning serum cortisol levels were assessed by whole blood sampling before the MRI examination. Samples were stored in the fridge before analysing. As blood collection system the S-Monovette® 4.7 ml Z-Gel was used (Sarstedt, Serum Gel with Clotting Activator). Serum cortisol samples were analysed using a commercial competitive electrochemiluminesence immunoassay (ECLIA, Elecsys® II Cortisol, Roche Diagnostics), which was performed according to the manufacturer instructions.[280]

4.4. Magnetic resonance imaging

4.4.1. MRI data acquisition

Imaging data were acquired using a 3.0 Tesla MAGNETOM Trio Tim syngo MR B13 MRI scanner (Siemens, Erlangen) with a transmit-receive and 8-channel receive coil at the Jülich research centre. Vacuum cushions were used to minimize head movements during scanning of the participants. Automated and manual shimming was applied prior to data acquisition to account for potential field inhomogeneities. Anatomical high-resolution T1-weighted images were acquired as part of a broader imaging protocol using a magnetisation-prepared rapid gradient echo (MPRAGE) sequence. Scan parameters were as follows: 176 sagittal slices; no gap; interleaved; acquisition time (TA) = 5:14 min; repetition time (TR) = 2250 ms; echo time (TE) = 3.03 ms; flip angle = 9°; field of view (FOV) = 256 x 256 mm; voxel size 1.0 x 1.0 x 1.0 mm.[248b]

4.4.2. MRI data pre-processing

All structural images, fluid-attenuated inversion recovery (FLAIR) and T1 sequences, underwent a visual quality assessment by an interdisciplinary team of neurologists and neuroscientists with expertise in dementia research. Subjects with confluent white matter lesions (Fazekas score > 2)[251] or significant structural abnormalities (n = 3; one arachnoid cyst, one extensive post-ischemic lesion, one severe hydrocephalus) were excluded from further analysis.[248b] Brain scans were aligned to Talairach space using SPM 12 to ensure that the anterior and posterior commissures were aligned horizontally.[281] All data pre-processing and analysis steps were performed in Matlab (Version R2012b, 8.0.0.783, The Mathworks, Natick, USA). The Voxel-based morphometry (VBM) analyses were performed using the Computational Anatomy Toolbox (CAT12, Version 12.1, http://dbm.neuro.uni-jena.de/cat/), an extension toolbox of the Statistical Parametric Mapping software (SPM12, https://www.fil.ion.ucl.ac.uk/spm/software/spm12). CAT12 was operated in expert mode with primarily default settings as described in detail in the official CAT12 manual (http://www.neuro.uni-jena.de/cat12/CAT12-Manual.pdf). Structural T1 images were bias-corrected and segmented into grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF).[248b] Grey matter probability maps were spatially normalized to Montreal Neurological Institute (MNI) space using Diffeomorphic Anatomical Registration Through Lie Algebra (DARTEL) with the provided IXI555 DARTEL template and resampled to a voxel size of 1.5 x 1.5 x 1.5 mm^3.[282, 248b] To preserve tissue volumes, the normalized grey matter images were modulated by their Jacobian determinants derived during normalization.[248b] Then the normalized grey matter probability maps were spatially smoothed using a 6 mm full width at half maximum (FWHM) isotropic Gaussian kernel.[248b] Visual quality control was performed by

individual inspection of each subject's data in an axial plane at the level of the basal ganglia and thalamus to detect failures in tissue segmentation or spatial normalization.[283]
See *Figure 7* for the schematic depiction of the MRI data pre-processing steps.

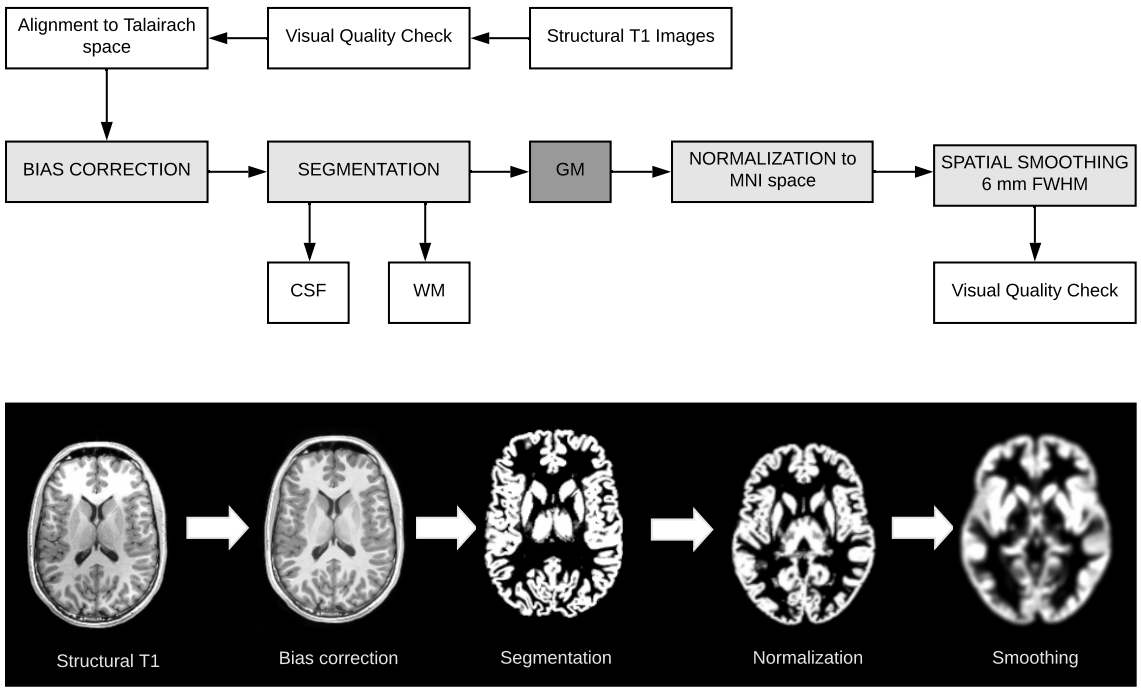


Figure 7: Schematic depiction of the MRI data pre-processing steps

Structural T1 images are bias corrected, segmented into GM, WM and CSF. Then the segmented GM images are normalized to MNI space and smoothed with a 6 mm FWHM Gaussian kernel.[248b]

Abbreviations: WM: white matter, GM: grey matter, CSF: cerebrospinal fluid, MNI: Montreal Neurological Institute, FWHM: full width at half maximum

4.4.3. Structural MRI data analysis

Analysis of the structural MRI data consisted of region-of-interest (ROI) of the left and right hippocampal volumes and voxel-wise whole brain analysis.

4.4.3.1. Hippocampal volumes analysis

Hippocampal volumes were extracted using the CAT12 Toolbox,[248b] which offers the possibility to estimate raw tissue volumes (in mm^3) in each subject's native space prior to spatial

normalization for different volume-based atlas maps implemented into the toolbox. For all ROI-based analyses, we used the Automated Anatomical Labelling (AAL) atlas[248b], a widely used and well-established anatomical atlas map.[284] Volumes of the left and right hippocampus were extracted and corrected for the total intracranial volume (TIV) using a simple ratio method and the resulting volumes were expressed as a percentage of TIV.[248b] Correlation analyses between left and right hippocampal volumes and serum cortisol levels were performed across the whole sample and within each diagnostic group.[248b]

4.4.3.2. Voxel-wise whole brain analysis

Whole-brain voxel-wise analyses were performed using SPM12 across the whole sample and in the individual groups.[248b] Significant results are reported at a family-wise error (FWE) corrected cluster threshold of $p < 0.05$ using a cluster forming threshold of $p < 0.001$.[248b]

To account for potential confounds, exploratory analyses of preliminary relationships between the covariates of no interest (i.e. age, gender, education) and variables of interest (i.e. serum cortisol levels, verbal memory performance, grey matter volume) were performed across the whole sample and in the individual groups. Covariates with significant associations with the primary variables of interest were included in the analyses.[248b] Although, body mass index (BMI)[285–288] and depression[289–291] have previously been reported to relate to cortisol levels, neither BMI nor the presence of mild depression in the AD group, showed significant associations with serum cortisol levels in our sample,[248b] and were therefore excluded from further analysis. Evaluation of the relationship between serum cortisol levels and grey matter volume was performed using a voxel-wise multiple regression model with age, education, and TIV as covariates of no interest, due to significant correlations of these variables with serum cortisol levels (age, education) and grey matter volume (age).[248b] The inclusion of TIV as a covariate is generally recommended by the developers of the CAT12 Toolbox (CAT12 Manual, p.13, http://www.neuro.uni-jena.de/cat12/CAT12-Manual.pdf). Mean grey matter volume values were extracted from significant clusters for further analysis in SPSS and corrected for TIV using a simple ratio method, therefore the cluster values are expressed as percentage of TIV.[248b] Anatomical locations of the significant clusters were determined by the SPM Anatomy Toolbox Version 2.2b.[292]

To further explore the relationship between serum cortisol levels and grey matter volume of the clusters (i.e. “cluster volume”), hippocampal volumes and verbal memory performance, we conducted correlation and partial correlation analyses in SPSS across the whole sample and within diagnostic subgroups.[248b]

4.5. Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics (Version 25, Chicago, IL, USA). Descriptive analysis of demographic, clinical, and neuropsychological characteristics of the whole sample and the two diagnostic groups was performed. Group comparisons were carried out to examine differences between the diagnostic groups. All tests were two-tailed. Normality in the data was assessed using the Shapiro-Wilk test. Normally distributed data (education, BMI, cortisol) was compared by the two-sample t-tests. Non-normally distributed data (age, MMSE, verbal memory composite score) was assessed using the independent samples Mann-Whitney U test. Categorical variables (sex, depression) were compared using the Chi-square test.

Correlation analysis was performed to identify preliminary relationships between variables of interest (serum cortisol, grey matter volume of the clusters and verbal memory performance) and variables of no interest (BMI, education, age, sex and depression). Correlation between dependent variables and independent variables was evaluated using Pearson's r for normally distributed data. For non-normally distributed data, we applied nonparametric correlation analysis using Spearman's rho (Spearman correlation coefficient, ρ). If one of the variables was discrete dichotomous, point-biserial correlation analysis was performed (point-biserial correlation coefficient, r_{pb}). In case of a considerable correlation ($p < 0.05$) of any covariate with the dependent variable of interest, partial correlation analyses were performed, controlling for the respective covariates.

5. RESULTS

5.1. Descriptive statistics

Descriptive demographic, clinical and neuropsychological characteristics are provided for the entire sample and the two diagnostic groups in *Table 3*.

The data of the variables education, serum cortisol and BMI were normally distributed and therefore compared by two-sample t-test, and Levene's test for equality of variances was performed. The variances of the variables of BMI were significantly different in the two groups, but for education and serum cortisol homogeneity of variance could be assumed. The variables age, VLMT scores and MMSE scores were not normally distributed and assessed using the independent samples Mann-Whitney U test. The categorical variables sex and depression were compared by Chi-square test.

The results of the normality assessment using the Shapiro-Wilk test and the results of the different tests are reported in *Table 4-7.* The whole sample consisted of 58 subjects and there were no significant differences in age, sex, education and depression in the two diagnostic subgroups.[248b] There were significant differences in BMI ($p = 0.011$), serum cortisol ($p < 0.001$), MMSE ($p < 0.001$) and verbal memory recall score ($p < 0.001$) in the subgroups.[248b]

Table 3: *Sample characteristics and neuropsychological test results*

Characteristics	Whole sample	HS	AD	p-value
N [total]	58	29	29	
Age [years]	64.62 ± 6.21	63.17 ± 6.50	66.07 ± 5.65	0.090[2]
Sex [f:m]	23:35	9:20	14:15	0.180[3]
Education [years]	14.62 ± 3.89	15.17 ± 4.27	14.17 ± 3.47	0.332[1]
BMI [kg/m^2]	24.31 ± 3.45	25.46 ± 3.84	23.17 ± 2.61	**0.011**[1]
Medication [n]	17	0	17	
AChEi	11	0	11	
AChEi + Memantine	1	0	1	
AChEi + Antidepressant	4	0	4	
Antidepressant	1	0	1	
Serum cortisol [µg/l]	157.98 ± 59.81	130.00 ± 43.36	185.97 ± 61.48	**<0.001**[1]
Mild depression [n]	3	0	3	0.075[3]
MMSE [/30]	26.81 ± 3.55	29.52 ± 0.95	24.10 ± 3.10	**<0.001**[2]
Verbal memory [z-score]	0.00 ± 0.99	0.85 ± 0.58	-0.85 ± 0.40	**<0.001**[2]

Data is given, if applicable, as mean and standard deviation. P-values in bold represent significant group differences ($p < 0.05$).

[1]Independent samples t-test was performed.

[2]Independent samples Mann-Whitney U test was performed.

[3]Chi-Square test was performed.

Abbreviations: HS: healthy seniors, AD: participants with Alzheimer's dementia, N: number of subjects, BMI: body mass index, AChEi: acetylcholinesterase inhibitor, MMSE: Mini-Mental State Examination, VLMT: Verbal Learning Memory Test

Table 4: *Shapiro Wilk test for normal distribution*

Variable	Shapiro Wilk test								
	Whole sample			HS			AD		
	Statistic	df	p	Statistic	df	p	Statistic	df	p
Education	0.976	58	0.293	0.947	29	0.149	0.919	29	**0.028**
BMI	0.972	58	0.192	0.969	29	0.529	0.975	29	0.699
Sex	0.621	58	**<0.001**	0.584	29	**<0.001**	0.638	29	**<0.001**
Age	0.982	58	**0.002**	0.934	29	0.071	0.892	29	**0.006**
Depression	0.232	58	**<0.001**	[a]	[a]	[a]	0.354	29	**<0.001**
Cortisol	0.972	58	0.208	0.964	29	0.401	0.983	29	0.911
MMST	0.846	58	**<0.001**	0.558	29	**<0.001**	0.930	29	0.055
Verbal memory	0.926	58	**0.002**	0.954	29	0.238	0.905	29	**0.013**
Cluster	0.986	58	0.759	0.959	29	0.317	0.976	29	0.723
Left HC	0.984	58	0.642	0.951	29	0.189	0.955	29	0.252
Right HC	0.983	58	0.594	0.968	29	0.497	0.975	29	0.706

[a] Variable is constant.

Abbreviations: HS: Healthy Seniors; AD: participants with Alzheimer's dementia, df: degrees of freedom, p: p-value, HC: hippocampus volume

Table 5: *Levene's Test for Equality of Variances and Independent sample t-test of the variables of the two diagnostic groups*

Variable	Levene's Test				Independent samples t-test				
	F	df1	df2	p	t	df	p	MD	95% CI
Education	0.64	1	57	0.428	0.98	56	0.332	1.000	[-1.05, 3.05]
BMI	4.48	1	57	0.039	2.65	49.25	**0.011**	2.284	[0.55, 4.02]
Cortisol	3.72	1	57	0.059	-4.01	56	**<0.001**	-55.966	[-83.95, 27.98]
Cluster	0.01	1	57	0.921	9.41	56	**<0.001**	0.007	[0.005 - 0.008]
Left HC	0.03	1	57	0.871	9.27	56	**<0.001**	0.070	[0.055 - 0.085]
Right HC	0.93	1	57	0.340	7.88	56	**<0.001**	0.058	[0.044 - 0.073]

Abbreviations: F: Levene statistic, df: degrees of freedom, p: p-value, t: t statistic, MD: Mean Difference, CI: Confidence Interval of the Difference, HC: hippocampus volume

Table 6: *Independent samples Mann-Whitney U Test*

Variable	Independent samples Mann-Whitney U Test			
	p	**U**	**z**	**r**
Age	0.090	529.000	1.693	0.222
MMSE	**<0.001**	14.500	-6.482	-0.851
Verbal memory [z-score]	**<0.001**	8.500	-6.411	-0.842
Abbreviations: p: p-value; U: Mann-Whitney U test statistic, r: effect size (r = z / √N), MMSE: mini mental state examination				

Table 7: *Chi-Square (χ^2) test for equal distribution*

Variable	Whole sample		HS		AD	
	χ^2	**p**	χ^2	**p**	χ^2	**p**
Sex	1.801	0.180	4.172	0.041	0.034	0.853
Depression	3.164	0.075	[a]	[a]	18.241	**<0.001**
[a] This variable is constant. Chi-square Test cannot be performed. Abbreviations: HS: Healthy Seniors; AD: participants with Alzheimer's dementia; p: p-value						

5.2. Cortisol group differences

Mean serum cortisol levels were 130 µg/l in the HS (SD 43, range 40 – 214 µg/l) and 186 µg/l in the AD (SD 61, range 69 – 311 µg/l) group.[248b] Serum cortisol levels were significantly higher in the AD group than in the HS group (MD = -55.97, 95% CI [-83.95, -27.98], t(56) = -4.006, p < 0.001, *Figure 8*).[248b] Across the whole sample serum cortisol levels were correlated with age (ρ = 0.348, p = 0.007) and education (r = -0.319, p = 0.015).[248b]

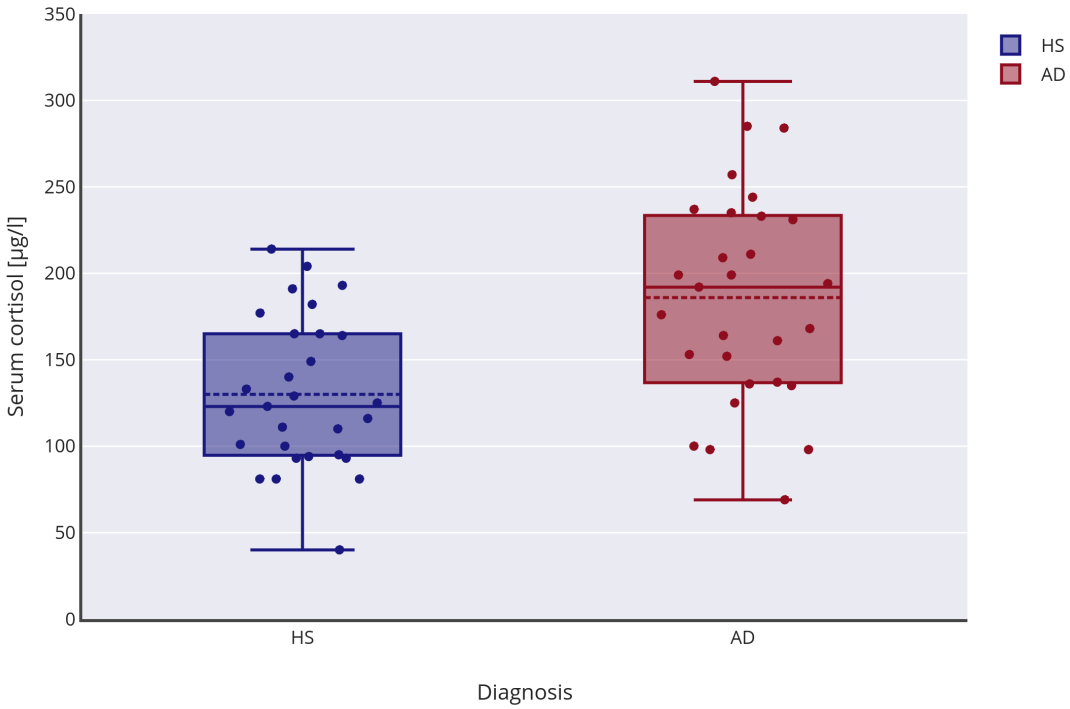


Figure 8: Serum cortisol levels of the two diagnostic groups (HS: blue, AD: red)

Dotted lines represent group mean, solid lines represent group median. Bold dots depict single subjects. AD > HS (p < 0.001)

Abbreviations: HS: healthy seniors, AD: participants with Alzheimer's dementia

5.3. Structural imaging results

5.3.1. Analysis of hippocampal volumes

The left and right hippocampal volumes were significantly lower in AD patients (left HC: M = 0.203, SD = 0.030; right HC: M = 0.204, SD = 0.030) compared to HS subjects (left HC: M = 0.273, SD = 0.028; right HC: M = 0.262, SD = 0.026).[248b] This difference was significant (left HC: MD = 0.070, 95% CI [0.055, 0.085], t(56)= 9.265, p < 0.001; right HC: MD = 0.058, 95% CI [0.044, 0.073], t(56)= 7.880, p < 0.001).[248b] See *Table 8* for the structural imaging results of hippocampal volumes.

Table 8: *Structural imaging results of hippocampal volumes*

Characteristics	Whole sample	HS	AD	p-value
Left HC volume [% of TIV]	0.238 ± 0.045	0.273 ± 0.028	0.203 ± 0.030	**<0.001**[1]
Right HC volume [% of TIV]	0.233 ± 0.041	0.262 ± 0.026	0.204 ± 0.030	**<0.001**[1]

Data is given, if applicable, as mean and standard deviation. P-values in bold represent significant group differences (p < 0.05).

[1]Independent samples t-test was performed.

Abbreviations: HS: healthy seniors, AD: participants with Alzheimer's dementia, HC: hippocampus

5.3.2. Voxel-wise whole brain analysis

As expected, there was a significant difference in grey matter volume between the two groups in regions, that are typically atrophic in Alzheimer's disease, between the two groups (p < 0.05), see *Figure 9* for the structural imaging results of the voxel-wise whole brain analysis. Across the whole sample, serum cortisol levels were negatively correlated with grey matter volume in the hippocampus, the fusiform gyrus, the temporal pole, the angular gyrus and the medial temporal lobe of the left cerebral hemisphere (pFWE < 0.05) corrected at cluster level and corrected for age, education and TIV (*Table 9-10 and Figure 10*).[248b] The resulting grey matter volume of those clusters is referred to as "cluster volume" or "volume of clusters of interest" in the following Chapters / Sections.

Table 9: *Structural imaging results of the voxel-wise whole brain analysis*

Characteristics	Entire sample	HS	AD	p-value
Cluster volume [% of TIV]	0.022 ± 0.004	0.025 ± 0.001	0.018 ± 0.003	**<0.001**[1]

Data is given, if applicable, as mean and standard deviation. P-values in bold represent significant group differences ($p < 0.05$). [1]

Independent samples t-test was performed.

Abbreviations: HS: healthy seniors, AD: participants with Alzheimer's dementia, TIV: total intracranial volume

Table 10: *Significant clusters resulting from voxel-wise analysis of serum cortisol against grey matter volume corrected for age and education [n = 58]*

Contrast	Cluster-size [voxels]	Cluster-level	MNI space	
		p	x,y,z [mm]	Structure*
negative	633	**0.002**	-46, -48, 18	Angular gyrus, MTL
	2248	**<0.001**	-42, -26, -10	Hippocampus
	927	**<0.001**	-36, -4, -32	Fusiform gyrus, TP
positive	*No suprathreshold< clusters.*			

Results are summarized for FWE adjusted $p < 0.05$. Only one representative local peak (highest t-value) per anatomical region and cluster is displayed. MNI peaks linked to anatomical structures of the clusters were labelled using the Hammersmith atlas provided by CAT12 toolbox.[248b]

*All clusters are located on the left hemisphere.

Abbreviations: MTL: medial temporal lobe, TP: temporal pole, FWE: familywise error rate, x,y,z [mm]: coordinates in MNI space in millimetres

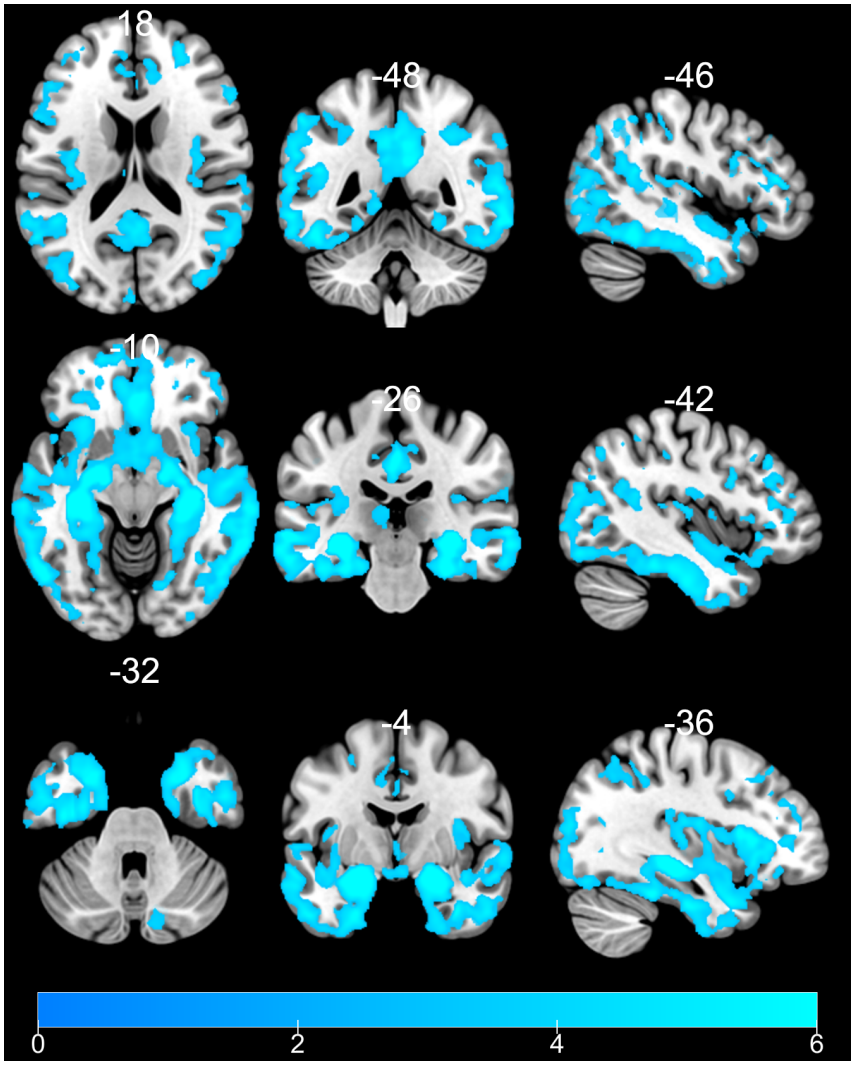


Figure 9: Grey matter volume HS > AD pFWE < 0.05 (blue)

MNI coordinates are given. The colour bar represents t-values starting at p < 0.05 and FWE-corrected. The figure was created with MRIcroGL (v1.2.20181114, University of South Carolina).

Abbreviations: HS: healthy seniors, AD: participants with Alzheimer's dementia, FWE: familywise error rate

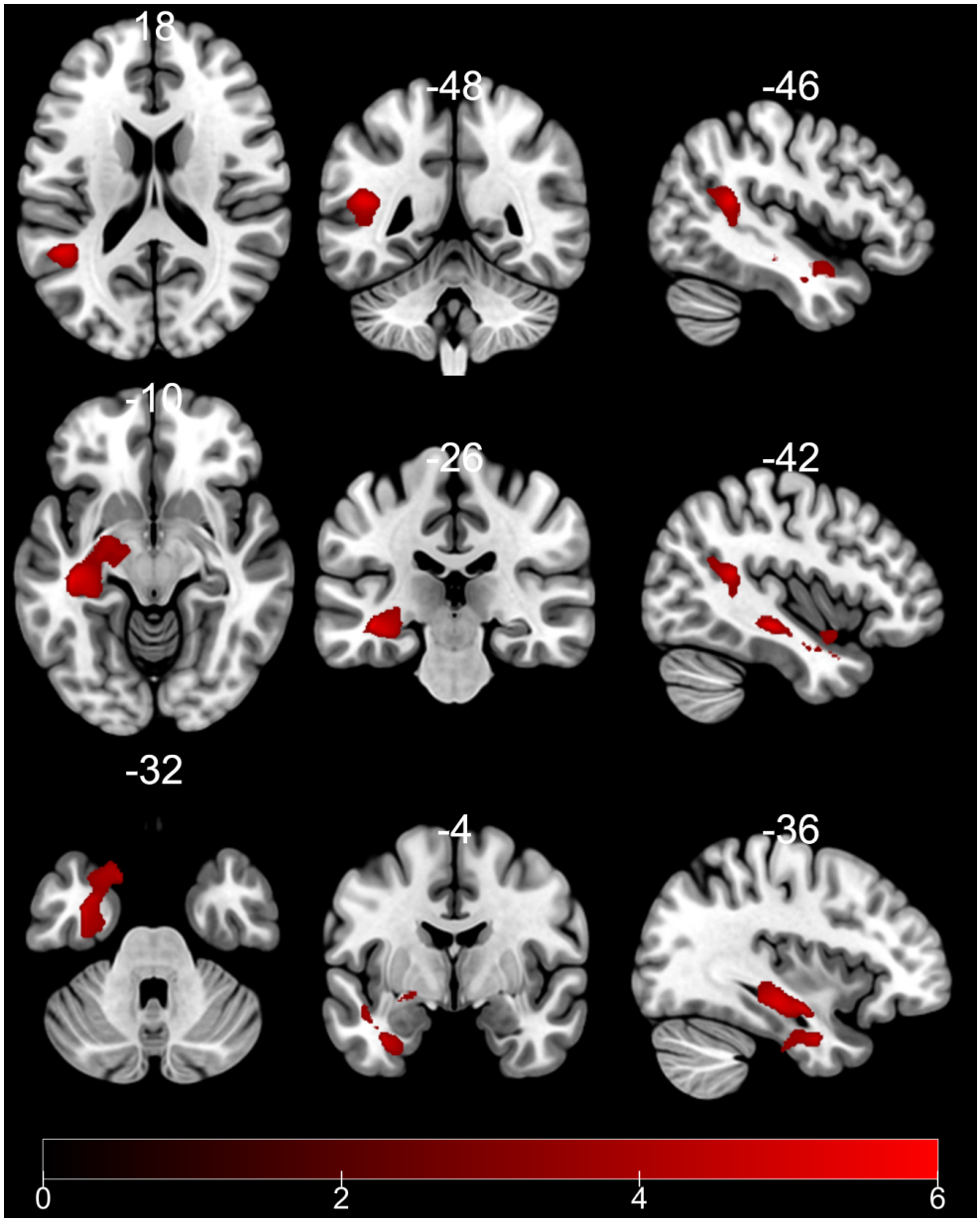


Figure 10: Negative correlation of serum cortisol with grey matter volume pFWE < 0.05 (red) of the two diagnostic groups HS and AD, corrected for age, education and TIV.

The first row depicts a red cluster in the left angular gyrus and medial temporal lobe, the second row shows a red cluster in the left hippocampus and the third row shows a red cluster in the left fusiform gyrus and temporal pole. The MNI coordinates are given. The colour bar represents t-values starting at p < 0.05 and FWE-corrected. The figure was created with MRIcroGL (v1.2.20181114, University of South Carolina).

Abbreviations: HS: healthy seniors, AD: participants with Alzheimer's dementia, FWE: familywise error rate

5.4. Results of the neuropsychological assessment

Mean MMSE scores were 29.52/30 (SD 0.95, range 27 – 30) in the HS and 24.10/30 (SD 3.10, range 15-29) in the AD group. Mean z-scores of verbal memory performance were 0.85 (SD 0.58, range -0.38 – 1.65) in the HS and -0.85 (SD 0.39, range -1.39 – -0.27) in the AD group See *Figure 11* for mean neuropsychological test scores in percent of the two diagnostic groups. As expected, there was a significant difference between the two diagnostic groups in MMSE scores ($U = 14.50$, $z = -6.48$, $p < 0.001$, $r = -0.85$) and verbal memory performance ($U = 8.50$, $z = -6.41$, $p < 0.001$, $r = -0.84$).

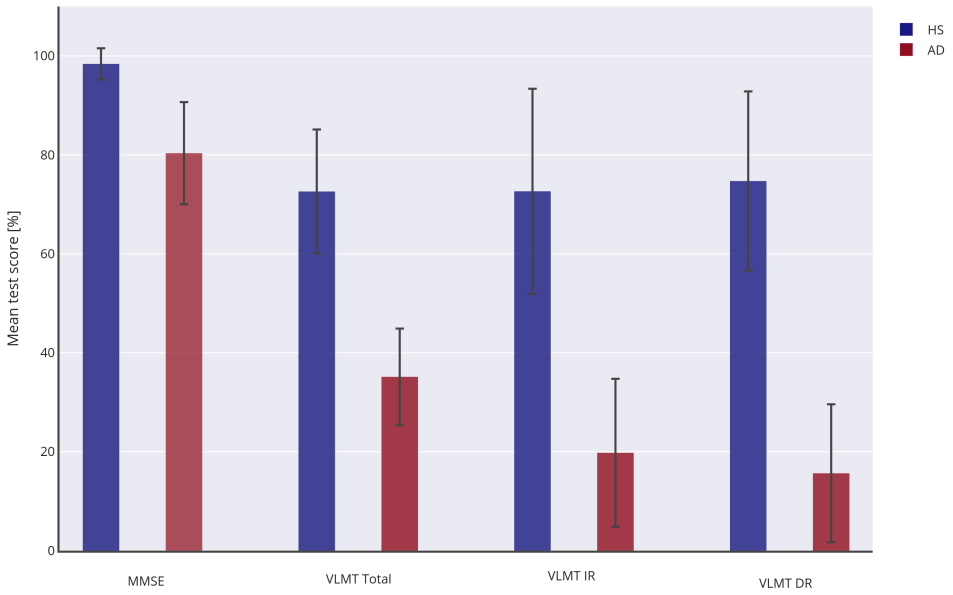


Figure 11: Mean neuropsychological test results of the two diagnostic groups

Bar chart showing overall memory performance (mean test score in percent) across MMSE and VLMT total, immediate recall and delayed recall for HS (blue) and AD (red) participants. Error bars represent standard error of the mean.

Abbreviations: HS: healthy seniors, AD: participants with Alzheimer's dementia, Mini-Mental State Examination, VLMT: Verbal Learning Memory Test, IR: immediate recall, DR: delayed recall

5.5. Correlation analyses

Serum cortisol was significantly correlated with education (r = -0.319, p = 0.015), age (ρ = 0.340, p = 0.009), MMSE (ρ = -0.468, p < 0.001), verbal memory (ρ = -0.595, p < 0.001), "volume of clusters of interest" (r = -0.656, p < 0.001), left hippocampal volume (r = -0.573, p < 0.001) and right hippocampal volume (r = -0.462, p < 0.001) in the whole sample. [248b] In the HS group cortisol was correlated with age (r = 0.406, p = 0.029), sex (r_{pb} = 0.411, p = 0.027), cluster volume (r = -0.603, p = 0.001) and left hippocampal volume (r = -0.444, p = 0.016).[248b] In the AD group cortisol was negatively correlated with education (ρ = -0.375, p = 0.045), verbal memory performance (ρ = -0.518, p = 0.004) and cluster volume (r = -0.048, p = 0.009). In the AD group, the relationship between serum cortisol and left hippocampal volume showed a non-significant trend.[248b] Results of the correlation analyses can be seen in *Table 11*.

There was a significant correlation of MMSE and verbal memory performance in the whole sample (ρ = 0.838, p < 0.001) and the AD group (ρ = 0.495, p = 0.006), but there was no significant correlation in the HS group (ρ = 0.267, p =0.162).

***Table 11**: Results of the correlations analyses*

Variable 1	Variable 2	Whole sample		HS		AD	
		CC	**p**	**CC**	**p**	**CC**	**p**
Serum cortisol [µg/l]	BMI	-0.245	0.64	-0.258	0.176	0.038	0.843
	Education	-0.319*	**0.015**	-0.176	0.362	-0.375*[a]	**0.045**
	Age	0.340**[a]	**0.009**	0.316	0.095	0.241[a]	0.207
	Sex	-0.100[b]	0.457	0.411*[b]	**0.027**	-0.300[b]	0.114
	Depression	-0.123[a]	0.357	[c]	[c]	-0.359[a]	0.056
	MMSE	-0.468**[a]	**<0.001**	-0.170[a]	0.378	-0.087	0.654
	Verbal memory	-0.595**[a]	**<0.001**	-0.323*	0.087	-0.518**[a]	**0.004**
	Cluster volume	-0.656**	**<0.001**	-0.603**	**<0.001**	-0.478**	**0.009**
	Left HC volume	-0.573**	**<0.001**	-0.444*	**0.016**	-0.329	0.082
	Right HC volume	-0.462**	**<0.001**	-0.328	0.082	-0.118	0.541
Verbal memory [z-score]	BMI	0.263*[a]	**0.046**	-0.073	0.708	-0.058[a]	0.763
	Education	0.272*[a]	**0.039**	0.369*	**0.049**	0.223[a]	0.245
	Age	-0.374**[a]	**0.004**	-0.613**	**<0.001**	-0.095[a]	0.625
	Sex	0.114[b]	0.395	-0.227[b]	0.237	0.082[b]	0.672
	Depression	-0.181[a]	0.173	[c]	[c]	0.061[a]	0.753
	MMSE	0.838**[a]	**<0.001**	0.267[a]	0.162	0.495**[a]	**0.006**

Table is continued on the following page.

Variable 1	Variable 2	Whole sample		HS		AD	
		CC	p	CC	p	CC	p
Cluster volume [% of TIV]	BMI	0.197	0.138	-0.143*	0.458	-0.073	0.708
	Education	0.123	0.357	-0.198	0.302	0.249[a]	0.193
	Age	-0.360**[a]	**0.005**	-0.473	**0.009**	-0.226[a]	0.239
	Sex	0.033[b]	0.804	-0.540**[b]	**0.002**	0.187[b]	0.331
	Depression	-0.146[a]	0.273	[c]	[c]	0.149[a]	0.441
	MMSE	0.751**[a]	**<0.001**	0.069[a]	0.722	0.136	0.482
	Verbal memory	0.785**[a]	**<0.001**	0.440*	**0.017**	0.199[a]	0.301
	Left HCV	0.944**[a]	**<0.001**	0.855**	**<0.001**	0.885**	**<0.001**
	Right HCV	0.865**[a]	**<0.001**	0.651**	**<0.001**	0.547**	**0.002**
Left HC volume [% of TIV]	BMI	0.163	0.223	-0.144	0.458	-0.202	0.293
	Education	0.097	0.469	-0.128	0.507	0.106[a]	0.583
	Age	-0.394**[a]	**0.002**	-0.519**	**0.004**	-0.293[a]	0.123
	Sex	0.086[b]	0.520	-0.345[b]	0.067	0.147[b]	0.448
	Depression	-0.193[a]	0.147	[c]	[c]	0.027[a]	0.889
	MMSE	0.714**[a]	**<0.001**	0.088[a]	0.650	-0.064	0.741
	Verbal memory	0.775**[a]	**<0.001**	0.540**	**0.002**	0.179[a]	0.354
	Right HCV	0.898**[a]	**<0.001**	0.856**	**<0.001**	0.681	**<0.001**
Right HC volume [% of TIV]	BMI	0.205	0.123	0.063	0.747	-0.216	0.260
	Education	0.146	0.273	0.060	0.757	0.093[a]	0.632
	Age	-0.413**[a]	**0.001**	-0.553**	**0.002**	-0.306[a]	0.106
	Sex	0.062[b]	0.642	-0.236[b]	0.218	0.016[b]	0.935
	Depression	-0.179[a]	0.179	[c]	[c]	-0.014[a]	0.944
	MMSE	0.672**[a]	**<0.001**	0.219[a]	0.255	-0.083	0.669
	Verbal memory	0.738**[a]	**<0.001**	0.510**	**0.005**	0.102[a]	0.598

The correlation between serum cortisol levels and the other variables were evaluated with the Pearson correlation for normal distributed data (Pearson correlation coefficient, r). If data was not normally distributed we applied non-parametric correlation analysis using Spearman's rho (Spearman correlation coefficient, ρ). If one of the variables was dichotomous, point-biserial correlation analysis was performed (point-biserial correlation coefficient, r_{pb}).

[a] Spearman correlation analysis was performed.

[b] Point-biserial correlation analysis was performed.

[c] Not computable due to a lack of variance (variable depression is constant in the HS group).

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

P-values in bold represent significant correlations ($p < 0.05$).

Abbreviations: HS: healthy seniors, AD: participants with Alzheimer's dementia, CC: correlation coefficient, BMI: body mass index, MMSE: Mini-Mental State Examination, HC: hippocampus, HCV: hippocampus volume

5.6. Partial correlation analyses

The relationship between serum cortisol levels and grey matter volume and episodic memory function in the whole sample and in the two diagnostic groups were evaluated using partial correlation analysis in SPSS. See *Table 12* for results.

We conducted a partial correlation of serum cortisol controlled for age, sex, BMI and education in both diagnostic groups. Serum cortisol, controlled for age, sex, BMI and education, was significantly negative correlated with cluster volume (CC = -0.636, p = 0.001, BCa 95% CI [0.80, 0.48]) and left hippocampal volume (CC = -0.426, p = 0.034, BCa 95% CI [-0.64, -0.19]) in the HS group and with verbal memory performance (CC = -0.481, p = 0.015, BCa 95% CI [-0.78, -0.15]) in the AD group. There was no significant correlation of serum cortisol and the cluster volume of interest in the AD group (CC = -0.375, p = 0.065, BCa 95% CI [-0.74, 0.14]). Because there was a positive correlation with serum cortisol and sex (r_{pb} = 0.411, p = 0.027) and age (r = 0.406, p = 0.029) in the HS group, and a positive correlation with serum cortisol and education (ρ = -0.420, p = 0.023) in the AD group. We performed partial correlation analysis with serum cortisol and the before stated variables as control variables. Partial correlation analysis, only controlled by age and sex was significantly correlated with the cluster volumes (CC = -0.437, p = 0.023, BCa 95% CI [-0.69, -0.12]) in the HS group. Partial correlation analysis, only controlled by education was significantly correlated with verbal memory performance (CC = -0.485, p = 0.009, BCa 95% CI [-0.75, -0.19]) and cluster volumes (CC = -0.408, p = 0.031, BCa 95% CI [-0.66, -0.05]) in the AD group.

Table 12: *Partial correlation analysis of cortisol of the two diagnostic groups*

Group	Var	Control variables		Verbal memory	Cluster Volume	Left HC volume	Right HC volume
HS	Cortisol	Age Sex BMI Education	CC	-0.187	-0.636	-0.426	-0.180
			p	0.371	**0.001**	**0.034**	0.389
			CI	[-0.52, 0.09]	[-0.80, 0.48]	[-0.64, -0.19]	[-0.50, 0.10]
		Age Sex	CC	-0.134	-0.437	-0.268	-0.150
			p	0.504	**0.023**	0.177	0.455
			CI	[-0.54, 0.25]	[-0.69, -0.12]	[-0.53, 0.01]	[-0.48, 0.16]
AD	Cortisol	Age Sex BMI Education	CC	-0.481	-0.375	-0.260	-0.034
			p	**0.015**	0.065	0.209	0.873
			CI	[-0.78, -0.15]	[-0.74, 0.14]	[-0.63, 0.20]	[-0.44, 0.48]
		Education	CC	-0.485	-0.408	-0.303	-0.086
			p	**0.009**	**0.031**	0.117	0.663
			CI	[-0.75, -0.19]	[-0.66, -0.05]	[-0.57, 0.01]	[-0.42, 0.24]

Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples. Confidence interval level 95 % and bias-corrected and accelerated (BCa). P-values in bold represent significant partial correlations ($p < 0.05$).

Abbreviations: Var: variable, BMI: body mass index, HS: healthy seniors, AD: participants with Alzheimer's dementia, CC: correlation coefficient, CI: confidence interval

5.7. Relationship between the different grey matter volumes and cortisol

There was a negative correlation between serum cortisol and hippocampal volumes in the whole sample (left HC: $r = -0.573$, $p < 0.001$; right HC: $r = -0.462$, $p < 0.001$). In the HS group, only the left hippocampus volume was significantly correlated with serum cortisol levels (left HC: $r = -0.444$, $p = 0.016$; right HC: $r = -0.328$, $p = 0.082$). In the AD group there was no significant relation between cortisol levels and hippocampal volumes (left HC: $r = -0.329$ $p = 0.082$; right HC: $r = -0.118$, $p = 0.541$). See *Figure 12* for the relationship of serum cortisol levels and left and right hippocampal volumes.

Furthermore, there was a significant negative correlation between serum cortisol and the extracted grey matter volume of the "clusters" detected in the VBM analysis in the whole sample ($r = -0.656$, $p < 0.001$) and in the two diagnostic groups HS ($r = -0.603$, $p < 0.001$) and AD ($r = -0.478$, $p = 0.009$). *See Figure 13* for the relationship of serum cortisol levels and the volumes of clusters of interest.

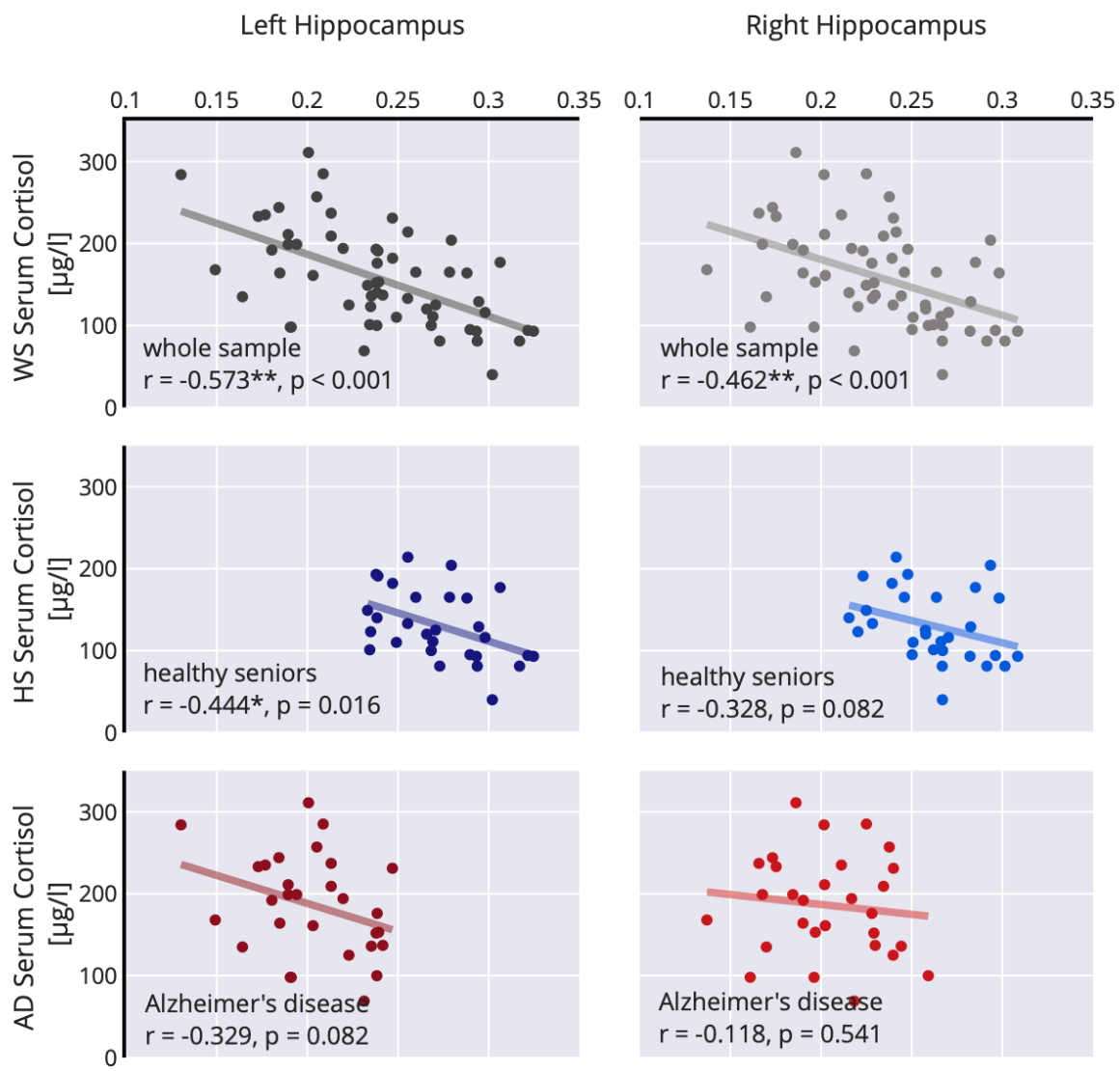


Figure 12: Relationship between serum cortisol and grey matter volume of the hippocampi

The scatter plot depicts the relationship between serum cortisol and grey matter volume of the left and right hippocampus of the whole sample (left HC: r = -0.573, p < 0.001; right HC: r = -0.462, p < 0.001) and the two diagnostic groups HS (left HC: r = -0.444, p = 0.016; right HC: r = -0.328, p = 0.082;) and AD (left HC: r = -0.329, p = 0.082; right HC: r = -0.118, p = 0.541). Dots represent individual data points (WS: black, HS: blue, AD: red). Lines indicate the linear trend.

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

Abbreviations: WS: whole sample, HS: healthy seniors, AD: participants with Alzheimer's dementia, TIV: total intracranial volume, HC: hippocampus, r: Pearson correlation coefficient

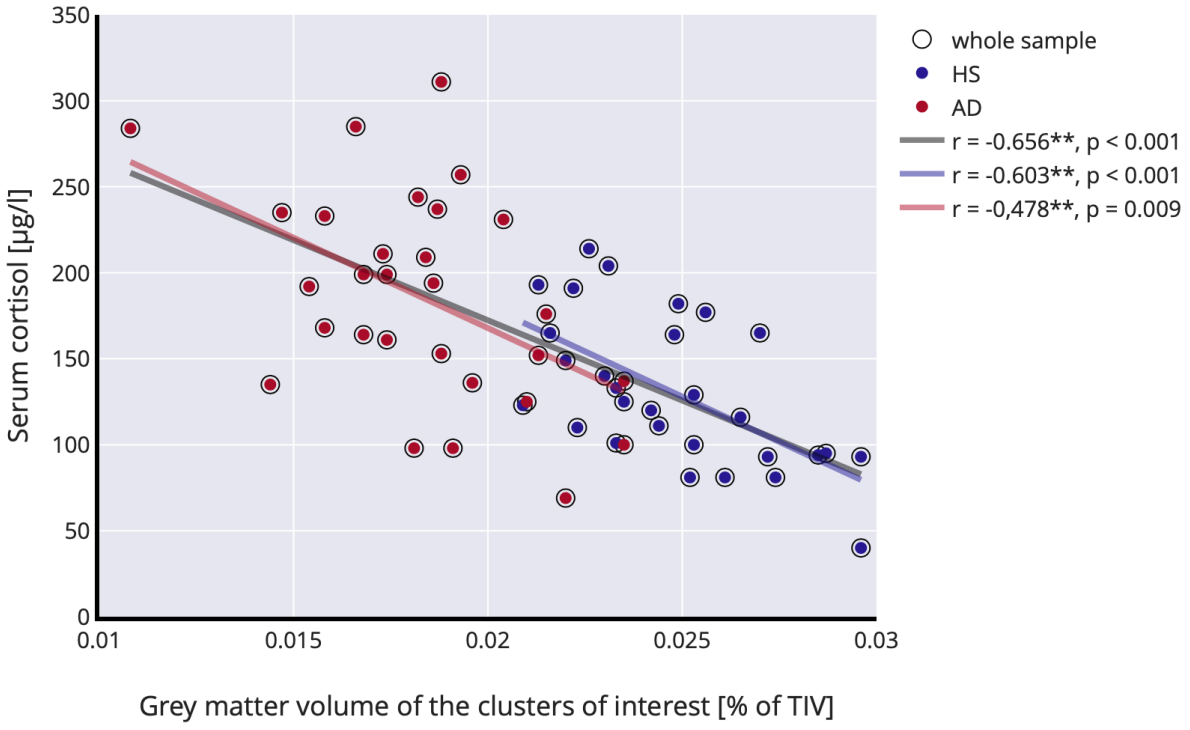


Figure 13: Relationship between serum cortisol and grey matter volume of the clusters

The scatterplot depicts the relationship between serum cortisol and grey matter volume and of the clusters of the whole sample ($r = -0.656$, $p < 0.001$) and the two diagnostic groups HS ($r = -0.603$, $p < 0.001$) and AD ($r = -0.478$, $p = 0.009$).

Dots represent individual data points (WS: black, HS: blue, AD: red). Lines indicate the linear trend.

**Correlation is significant at the 0.01 level (2-tailed).

Abbreviations: WS: whole sample, HS: healthy seniors, AD: participants with Alzheimer's dementia, TIV: total intracranial volume, r: Pearson correlation coefficient

5.8. Verbal memory performance and serum cortisol

Verbal memory performance was negatively correlated with serum cortisol levels across the whole sample (ρ = -0.595, p < 0.001) and the AD group (ρ = -0.518, p = 0.004), but there was no correlation in the HS group (r = -0.323, p = 0.087). See *Figure 14* for the correlation graph of serum cortisol levels and verbal memory performance of the whole sample and the two diagnostic groups.

The correlation of verbal memory performance and serum cortisol levels in the AD group was significant after controlling for age, sex, BMI and education (CC = -0.481, p = 0.015).

In the HS group the verbal memory performance was correlated with education (r = 0.369, p = 0.049) and highly negatively correlated with age (r = -0.613, p < 0.001).

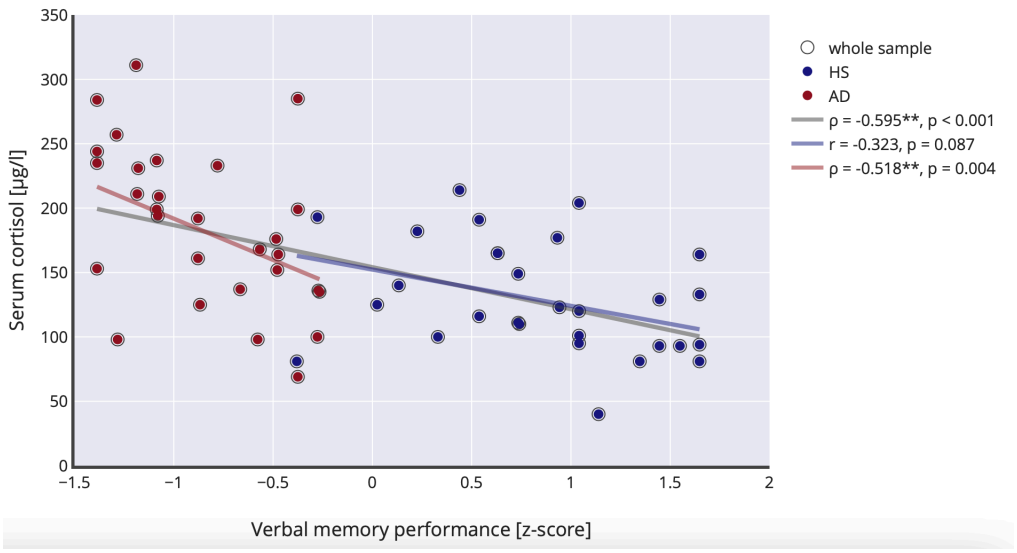


Figure 14: Relationship between serum cortisol and the composite verbal memory score in the whole sample and the two diagnostic groups

The scatter plot depicts the relationship between serum cortisol and verbal memory performance (z-score) in the whole sample (ρ = -0.595, p < 0.001) and in the two diagnostic groups (HS: r = -0.323, p= 0.087; AD: ρ = -0.518, p = 0.004). Dots represent individual data points (WS: black, HS: blue, AD: red). The lines indicate the linear trend.

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

Abbreviations: WS: whole sample, HS: healthy seniors, AD: participants with Alzheimer's dementia, r: Pearson correlation coefficient, ρ: Spearman correlation coefficient

5.9. Verbal memory performance and grey matter volumes

Hippocampal volumes were significantly positively correlated with verbal memory performance in the whole sample (left HC: ρ = 0.775, p < 0.001; right HC: ρ = 0.738, p < 0.001) and in HS (left HC: r = 0.540, p = 0.002; right HC: r = 0.510, p = 0.005). In AD hippocampal volumes were not correlated with verbal memory performance (left HC: ρ = 0.179, p = 0.354; right HC: ρ = 0.102, p = 0.598). See *Figure 15* for the relationship of verbal memory performance and the cluster volumes and the volumes of the hippocampi.

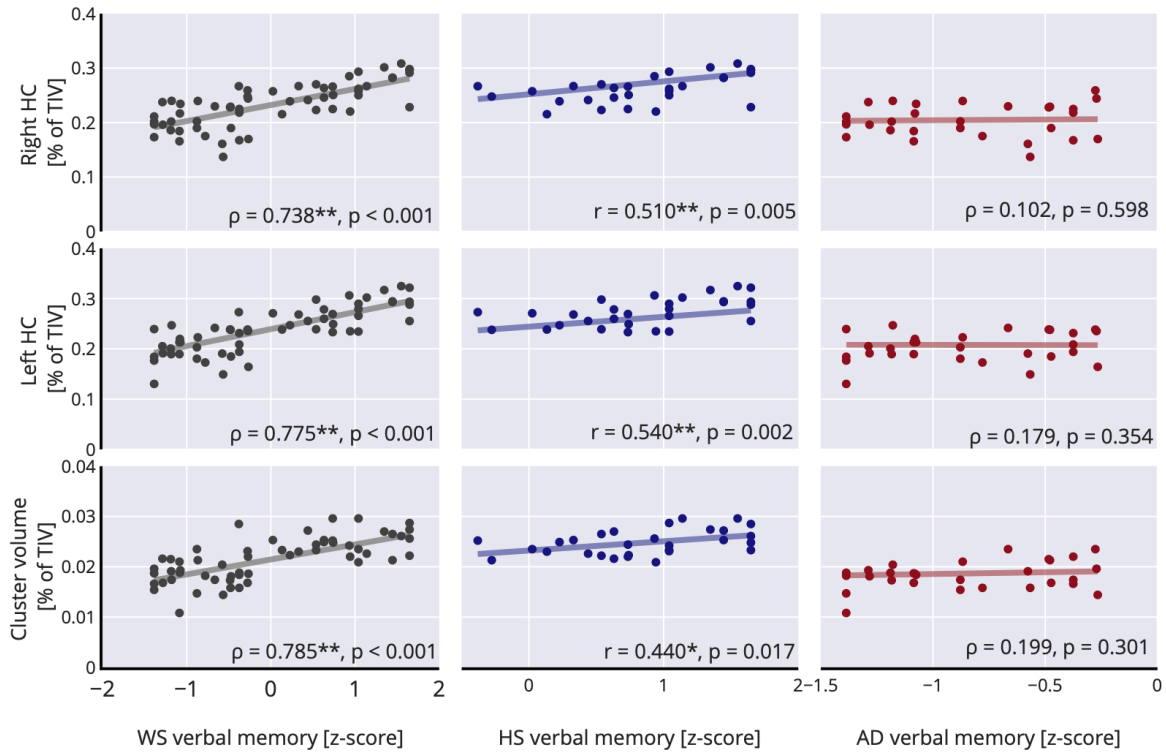


Figure 15: Relationship of the verbal memory and the cluster volume and the left and right hippocampal volumes of the whole sample and both diagnostic groups

Scatter plots depict the relationship between verbal memory performance and grey matter volume of the cluster and the left and right hippocampus of the two diagnostic groups.
Dots represent individual data points (WS: black, HS: blue, AD: red). Lines indicate the linear trend.
*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).
Abbreviations: WS: whole sample, HS: healthy seniors, AD: participants with Alzheimer's dementia, TIV: total intracranial volume, HC: hippocampus, ρ: Spearman correlation coefficient, r: Pearson correlation coefficient

6. DISCUSSION

Our study demonstrated significantly elevated serum cortisol levels in AD patients compared to cognitively healthy older adults, aligning with previous research.[2,223,229,235,294] Across the entire sample, as well as within each diagnostic group, cortisol levels were correlated with age, which is in line with other studies that showed an increase in cortisol levels in physiological aging. The circadian cortisol rhythm is maintained in aging, but cortisol levels show a significant age-related increase in men and women.[295,296] Advanced age is the most significant risk factor for the development of AD.[10] In the diagnostic group of AD, there was a negative correlation between serum cortisol levels and education[248b], assessed in total years of formal education, consistent with previous studies suggesting that a lower education may contribute to increased risk for the development of AD.[44,297–300]

6.1. Relationship of serum cortisol levels and grey matter volume of the clusters and the hippocampi

The voxel-wise whole brain analysis showed that serum cortisol levels were associated with lower grey matter volume in the hippocampus, the angular gyrus, the medial temporal lobe, the fusiform gyrus and the temporal pole of the left hemisphere in the entire sample and in each diagnostic group individually.[248b]

The application of a voxel-wise approach allowed for the identification of several different regions of grey matter volume, including non-limbic regions, correlated with cortisol levels. In the region-of-interest (ROI) analysis we could demonstrate a significant negative correlation between serum cortisol levels and left and right hippocampal volumes across the entire sample, and with left hippocampal volume in the cognitively healthy senior (HS) group.[248b] Serum cortisol levels and right hippocampal volume in the HS group and left and right hippocampal volumes in the AD group did not reach statistical significance at an alpha-level of 5 %, but a trend towards negative correlation between serum cortisol and the right hippocampus in the HS group and serum cortisol and the left hippocampus in the AD group was observed.[248b]

The medial temporal lobe structures, especially the hippocampus, are among the regions most susceptible to atrophic changes in Alzheimer's disease,[301,302] but other regions of the temporal lobe, e.g. the fusiform gyrus (medial occipitotemporal gyrus) also show volume reductions in AD patients compared to healthy controls.[303] Notably, our findings reveal associations between elevated cortisol levels and grey matter atrophy in regions affected primarily and most prominently in Alzheimer's disease.[248b]

Glucocorticoids regulate various physiological and neuronal processes and play a central role in mediating the stress response. Glucocorticoids regulate metabolism via multiple

mechanisms to achieve glucose homeostasis (gluconeogenesis, glycogenolysis),[162] and regulating protein and lipid metabolism. Furthermore, glucocorticoids are involved in regulating the inflammatory and immune response[304] and gene expression in the brain, in particular in the hippocampus. Cortisol exerts its multiple effects via binding to two different types of glucocorticoid receptors, the mineralcorticoid receptors (MRs) and the glucocorticoid receptors (GRs), which are expressed in different concentrations throughout the brain.[305] For example, the hippocampus expresses both GRs and MRs whereas the frontal cortex expresses mainly GRs.[181,185] Moderate levels of cortisol activate the glucocorticoid receptors with higher affinity first, i.e. MRs, but as the cortisol concentrations increase GRs are activated as well.

The circulating cortisol molecules might affect the cortical grey matter via direct neurotoxic effects or via indirect effects by increasing the vulnerability of neurons to other damaging effects of normal aging and disease.[306]

Glucocorticoids promote oxidative stress and increase the Aβ toxicity in cultured hippocampal neurons.[241] Additionally, elevated glucocorticoids have been associated with Aβ and tau pathology in the brain in an AD mouse model.[242] *Toledo et al.* also discovered an association of plasma cortisol and Aβ brain burden in humans, [240] which could be attributed to reduced clearance of Aβ as well as increased cleavage of Aβ into the more toxic compounds ($A\beta_{1-42}$).[243]

Cortisol's impact on hippocampal volume may be partially mediated by brain-derived neurotrophic factor (BDNF), a growth factor that stimulates and controls neurogenesis.[307] Activation of MRs seemed to increase, and activation of GRs are thought to decrease the expression of BDNF.[307] The formerly listed central nervous effects of glucocorticoids suggest that elevated cortisol levels may play a role in AD pathology by increasing Aβ burden, tau pathology, oxidative stress, neurotoxic effects and reduced neurogenesis resulting in neurodegeneration.

Our findings, that there is an association of elevated cortisol levels and cerebral grey matter volume reduction of the hippocampal area and other brain regions in patients of the AD spectrum and cognitively healthy aged adults,[248b] are in accordance with other studies.[148,231–234,248]

In our study, the significant negative correlation of serum cortisol levels and grey matter clusters, derived from whole brain voxel-wise analysis, was restricted to the left hemisphere of the brain in the entire sample and both diagnostic groups.[248b] Furthermore, the significant inverse correlation of serum cortisol levels and both hippocampal volumes was only present in the entire sample and restricted to the left hippocampus in the HS group (ROI analysis).[248b]

Wirth et al. similarly reported a left-hemispheric lateralization of the correlation of cortisol and reduced grey matter volumes of the hippocampus and other cortical temporo-parietal-occipital regions in a sample of cognitive healthy, MCI and AD patients.[248a]

Two studies suggested an increased vulnerability of the left hemisphere to glucocorticoids in animals and humans. [308,309] The underlying mechanisms that cause the accelerated susceptibility of the left cortex to stress and glucocorticoids remain unclear.[310] Rodent studies suggested a lateralization of cortical control of GC levels, with the left side showing a predominantly inhibitory influence.[212,311] Whether elevated GC levels cause left hemispheric atrophy or whether left hemispheric atrophy causes elevated GC levels, because of disinhibition of the HPA axis, remains unclear and needs to be evaluated in future studies.

The results of our study are consistent with the hypothesis that dysregulation of the HPA axis and the resulting elevation of cortisol levels appears to contribute to the pathophysiological and clinical disease progression of Alzheimer's disease.

Elevated glucocorticoid levels and their effects on the brain are suspected to occur prior to the development of AD or at early/preclinical stages of the disease,[1,2] but can also occur completely independent of AD pathology.[223,233,237,312] Due to the long prodromal (preclinical) period of the AD continuum, it is difficult to distinguish between causative factors, i.e. hippocampal atrophy occurs because of the elevation of cortisol, and prodromal changes, i.e. hippocampal atrophy causes dysregulation of the HPA axis as a result of reduced negative feedback inhibition. Nevertheless, the result is a vicious circle leading to hippocampal atrophy and further dysregulation of the HPA axis.

6.2. Serum cortisol levels and verbal memory performance

As expected, the two diagnostic groups differed significantly in the Mini-Mental State Examination (MMSE) and verbal memory performance.[248b]

Serum cortisol levels were significantly negatively correlated with verbal memory performance in the entire sample and in the AD group, corrected for age, sex, BMI and education, but not in the HS group.[248b] However, no significant correlation was observed in the HS group, which could be attributed to the limited sample size, a possible ceiling effect due to the inclusion of subjects with normal cognitive function, and the possibility, that the effect is more profound in patients with AD than in cognitive healthy elderly.

As previously noted, cortisol exerts its effect on cognition through its interaction with the two types of glucocorticoid receptors: MRs and GRs. The hippocampi, which are involved in episodic memory function, express both type of glucocorticoid receptors.[181,185] MRs have been linked to positive effects and GRs have been associated with negative inhibitory effects on cognitive performance. At moderate cortisol levels, MRs, which have a higher binding affinity, are activated. As cortisol concentrations increase, GRs are activated, which leads to an increase in impairing effects on cognition and memory.[223]

Previous studies demonstrated an association between elevated cortisol levels and impaired memory performance in healthy aging. These studies used different modalities of cortisol assessment: serum cortisol[312–314], urinary cortisol[237,315] and salivary cortisol.[316] In healthy elderly subjects, negative associations between cortisol levels and episodic memory performance and executive function tasks have been reported[317]. This relationship is consistent with the high prevalence of corticosteroid receptors in the hippocampus and the prefrontal cortex.[318]

Many studies that assessed the relationship between stress, elevated cortisol levels and memory performance, have been conducted in healthy subjects, but only a few studies assessed these associations in patients of the AD continuum. Two studies demonstrated an association between high cortisol levels and impaired recall[319] and impaired global cognition in AD patients.[239] Other studies failed to demonstrate an association between elevated cortisol levels and recall performance in patients of the AD continuum,[320] which could be explained by a floor effect due to the overall severely impaired episodic memory performance of the AD patients.

6.3. Relationship of grey matter volumes and verbal memory performance

There was a significant correlation between hippocampal grey matter volumes and the whole brain voxel-wise analysis derived clusters with verbal memory performance in the entire sample and the HS group.[248b] There was no significant correlation between verbal memory performance and the grey matter volumes of the hippocampi or the cluster in the AD group.[248b]

Structures of the medial temporal lobe (MTL), e.g. the hippocampal formation, are integral to memory encoding.[321] MTL atrophy and associated episodic memory impairment are defining characteristics of AD, [322, 391] which "progressively decline during the course of the disease".[391]

Functional neuroimaging studies demonstrated that memory function is lateralized,[323,324,391] and that structures of the left hemisphere, e.g. the left hippocampus, are implicated in verbal memory processing,[325–327] while right hemispheric structures are implicated in non-verbal and spatial memory.[328]

Previous studies proposed associations between hippocampal volumes and memory performance in cognitively healthy individuals and patients with AD.[324,326,329–335] Nevertheless, there seems to be a significant variability of this relationship and some studies could not find an association.[336,337] The inconsistent results of previous studies could suggest, that verbal memory deficits in Alzheimer's disease patients are not solely associated with reduced hippocampal volumes, which are affected at an early timepoint and most prominently in the course of AD, but may also be associated with damages to other limbic structures, e.g. the thalamus,[338] and other central nervous regions.[339]

6.4. Cortisol as a potential preclinical biomarker and risk factor for Alzheimer's disease

Early clinical diagnosis of AD can be challenging, as initial symptoms are often attributed to normal aging by patients. Established cerebrospinal fluid (CSF) and neuroimaging biomarkers have a high diagnostic accuracy, but the availability is limited to the clinical setting. Finding a blood-derived biomarker for AD with high sensitivity and specificity is urgently required for an early accurate AD diagnosis, as a blood biomarker would be a widely accessible, minimally invasive, time-effective and economic first line screening-tool.

Elevated cortisol levels are an established feature in patients of the AD continuum and are thought to occur at a preclinical or early clinical stage of the disease. Cortisol dysregulation is thought to act as a risk factor for the development of AD,[229] a potential diagnostic biomarker for preclinical AD,[239,340,341] and as a predictive biomarker for a more rapid progression from MCI to AD.[2] Therefore, elevated cortisol levels could play an important role as a target for preventive measures and therapeutic approaches aimed to prevent or delay irreversible neurodegeneration and cognitive decline and to significantly improve the outcome and quality of life of patients.

Plasma cortisol was one of six biomarkers capable to accurately predict progression from MCI to AD within 6 years in a cohort study of the Alzheimer Disease Neuroimaging Initiative.[340] *Laske et al.* identified a panel of three blood markers (cortisol, von Willebrand factor, oxidized LDL antibodies), which differentiated AD patients from healthy controls by using support vector machines, which is a supervised machine learning technique.[342] Support vector machines have been already used to distinguish AD patients from elderly control subjects, using MRI.[343] *Ennis et al.* conducted a prospective longitudinal study and discovered, that long-term exposure to elevated cortisol levels (urinary free cortisol/creatinine ratio) and intra-subject cortisol variability increased the risk for AD by a factor of 1.31 and 1.38 respectively.[229] These changes in cortisol levels and variability preceded AD onset on average by 6 years, therefore occurring in the preclinical phase of the disease when there are no apparent symptoms of the disease.[229]

6.5. Strength and limitation

The main strength of our study is, that compared to other studies, we included a collective across the AD continuum with positive biomarkers supporting the clinical diagnosis, which becomes more and more important in modern research criteria.[84] The use of objective biomarkers are objective enables the exclusion of individuals with non-Alzheimer progressive amnestic syndromes, e.g. limbic-predominant age-related TDP-43 encephalopathy (LATE), that mimick the clinical syndrome of Alzheimer's disease,[344] and thereby allow researchers to create a homogenous study population of patients of the AD continuum.

Our study was a cross-sectional investigation and we cannot infer on the temporal sequence between HPA axis dysregulation and changes in verbal memory performance and grey matter atrophy.

The effects of elevated cortisol levels on cerebral grey matter are believed to be chronic and not acute.[133] The single-timed assessment of serum cortisol levels is not able to reflect the circadian rhythm of cortisol secretion, the circadian fluctuations of glucocorticoid levels and may not adequately represent cortisol long-term exposure. Collecting cortisol samples over multiple days, assessing cortisol levels at multiple timepoints during the day, and calculation of the mean cortisol level may provide a more accurate representation of the dynamic nature of cortisol secretion.[345,346] Whilst a single assessment of serum cortisol may be a limitation of our study design, large epidemiologic studies have shown, that cortisol concentrations have a high degree of within-individual stability over a 2-year period.[347]

Another limitation is our exclusive use of serum cortisol levels, which may exhibit a higher level of intra-individual variation than other measures of cortisol.[2] The assessment of CSF cortisol levels may provide a more adequate reflection of the exposure of cortisol on the central nervous system,[2] because in blood, the unbound and biologically active form of cortisol is only a minor part of the total cortisol levels, but in CSF cortisol is mostly present in its unbound form.[348] Several studies demonstrated that there were significant but only moderate correlations of plasma cortisol and CSF cortisol[294,348,349] and suggests that single time serum cortisol levels do not accurately reflect cortisol levels in the brain.

Further studies are needed to investigate the correlations between different cortisol measurement modalities in healthy aging subjects and patients with AD.

6.6. Summary and outlook

Our findings are convergent with the idea that HPA axis dysregulation might influence and / or acceleratea AD pathogenesis and contributes to a more rapid clinical disease progression. We observed the presence of higher serum cortisol levels in patients of the Alzheimer's disease continuum compared to cognitively healthy seniors, and demonstrated the inverse relationship between serum cortisol levels and grey matter volume of brain regions that are prone to pathologic changes in the course of AD, e.g. the hippocampal region. Notably, this association was present not only in patients with AD, but also in cognitively healthy seniors, potentially indicating a links between elevated cortisol levels and increased risk of future cognitive decline. Furthermore, higher serum cortisol levels were associated with verbal memory impairment in AD patients.

To clarify a causal relationship of the previously mentioned associations of cortisol dysregulation, neurodegeneration and cognitive decline, longitudinal studies are essential.

Further investigations of glucocorticoid levels and their influence on cognitive decline and pathophysiological processes involved in the development of Alzheimer's disease are relevant, because HPA axis overactivation and the resulting elevation of glucocorticoid levels are thought to occur at preclinical stages of the disease, and therefore could function as a potential biomarker and play an important role as a potential target for preventive measures and early therapeutic interventions with the goal to reduce serum cortisol levels.

Because most of the harmful effects of cortisol are probably executed via GRs, therapeutic interventions with GR antagonists (e.g. mifepristone) have been conducted in AD mice models, which resulted in a decreased Aβ and tau brain load and an improvement of cognitive deficits.[350,351] However, a pilot study with mifepristone in AD patients was prematurely terminated before reaching the endpoints of the study,[352] and others were not published.[353] Preventative trials in cognitive healthy humans have not been reported so far. Another potential pharmacological intervention, is to inhibit the enzyme 11beta-hydroxysteroid dehydrogenase type 1, which plays a major role in cortisol synthesis.[354]

Non-pharmacological approaches, focused on stress reduction may also play a role in modulating cortisol levels. Cognitive-behavioural stress management (e.g. mindfulness, meditation, yoga, diet, music, exercise) at the preclinical stage of the disease could have a positive impact on disease progression and overall cognitive functioning. Several different studies pointed out positive effects of different lifestyle interventions for the reduction of cortisol levels in clinical and non-clinical populations, e.g. low-intensity physical exercise,[355–357] yoga,[358–360] mindfulness training,[361–363] meditation[364] and cognitive behavioural therapy.[365–367]

Future studies should assess cortisol in different settings (home, clinic) to be able to correct for the stress that is induced through the hospital environment and the anticipation of the clinical evaluation, and should assess different modalities of cortisol (i.e. serum, CSF, urine, saliva, hair). Furthermore, the cortisol levels should be assessed at different time points to have an accurate representation of the circadian rhythm of the diurnal cortisol secretion of the individual subjects. In blood samples, bound and free cortisol levels can be measured, in saliva only the free cortisol appears and is very similar to the amount of free blood cortisol levels.[368] Saliva cortisol also provides a non-invasive, i.e. no stress of venepuncture, method to obtain an index of the biologically active fraction of cortisol.

Sleep deprivation, shift work and jetlag disrupt the normal biological rhythms and have a major impact on health. Circadian disorganization is often observed in stress-related disorders, i.e. depression[369] and post-traumatic stress disorder.[370] In future studies, it would be useful to assess the "stress" of participants with questionnaires, e.g. stressful life events screening questionnaire (SLESQ),[371] perceived stress questionnaire (PSQ)[372] and Trier Inventory for the Assessment or Chronic Stress (TICS).[373] Furthermore, the circadian rhythm of the subjects

should be assessed with specific questionnaires, e.g. the Morningness Eveningness Questionnaire (MEQ)[374] or the Basic Language Morningness (BALM) scale.[375]

Individuals with decreased socio-economic status (SES), measured by income, education and occupation, are hypothesized to have both an increased exposure to stressful events in their lives, and reduced social and material resources to compensate the stress.[376,377] A literature review by *Dowd et al.* showed that SES is not consistently associated with cortisol levels.[378] Several studies reported an association of lower SES with higher cortisol levels,[379–381] and other studies reported the opposite relationship,[382,383] mixed results[384–386] or no relation of SES and cortisol levels.[387–389] In our study, there was a negative correlation between serum cortisol levels and education, assessed in total years of primary and secondary education and college, university or apprenticeship education, in the AD group. In the future, it would be interesting to create a SES composite score which is composed of different variables, e.g. income, education and occupation, to account for these potential confounding variables.

In conclusion, the investigation of cortisol metabolism and its effects on the central nervous system provides meaningful insight into the complex and still incompletely understood pathophysiology of Alzheimer's disease and may contribute to future preventive and therapeutic interventions.

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8. APPENDIX

8.1. Table directory

8.2. Figure directory

9. Veröffentlichung von Ergebnissen

Teilergebnisse der vorliegenden Dissertationsschrift haben zu der Veröffentlichung von Dronse et al. „Serum cortisol is negatively related to hippocampal volume, brain structure, and memory performance in healthy aging and Alzheimer's disease" veröffentlicht im Journal Frontiers in Aging Neuroscience im Mai 2023 beigetragen.

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