Dissertation

Structural Brain Networks as Predictors of Cognitive Decline in Alzheimer's Disease – A Longitudinal Study

submitted by

Mag.rer.nat. Fabian WAGNER

for the Academic Degree of

Doctor of Medical Science

(Dr. scient. med.)

at the

Medical University of Graz

Department of Neurology

Under the Supervision of

Assoz. Prof.in PD Mag.a Dr.in Marisa KOINI

2024

Declaration

Hereby I declare that this thesis is my own original work and that I have fully acknowledged by name all those individuals and organizations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the “Standards of Good Scientific Practice and Ombuds Committee at the Medical University of Graz“.
Date: 12.05.2024

Disclosures

Part of this thesis has been published as a journal article and may therefore resemble in context and syntax:

Wagner F, Duering M, Gesierich BG, Enzinger C, Ropele S, Dal-Bianco P, Mayer F., Schmidt R and Koini M. Gray Matter Covariance Networks as Classifiers and Predictors of Cognitive Function in Alzheimer's Disease. Front Psychiatry. 2020 May 5;11:360.

All co-authors agreed to the use of their data in this thesis. Part of the mentioned article is reprinted with permission from Frontiers in Psychiatry.

Acknowledgements

I would like to thank my main supervisor, Assoz. Prof. Marisa Koini, who gave me the opportunity to work on this interesting project. This thesis would not have been possible without her constant technical and psychological support.
Furthermore, I am sincerely thankful to Prof. Dr. Reinhold Schmidt, my second supervisor. His feedback based on a vast amount of experience and knowledge in the field of dementia enhanced the content of my work and his help was crucial in the dissemination of my results.
Lastly, I want to thank my third supervisor, Priv.-Doz. Dr. Christian Fazekas, for his advice and feedback during the work on my project.

Much appreciated is the contribution of all the co-authors of the study. Their insight and contributions were invaluable.
Special thanks also go out to Dipl.Ing. Lukas Pirpamer PhD and Dr. Anna Damulina for their support and technical knowledge. They provided me access to a lot of necessary information.

I also want to thank the whole University Clinic for Neurology, especially the Division of Neurogeriatrics and the research group of biomedical engineers. I found a lot of support and made friends on the way. The daily dose of coffee at breaks were one of the things that kept me sane.

The doctoral student Fabian Wagner received funding from the Austrian Science Fund (KLI 564-BBL) and the Medical University of Graz through the Doctoral School for Sustainable Health Research.
The study work would have not been possible without funding from a D-A-CH grand (German Research DF DU1626/1-1) and an Austrian Funds (FWF 12889-B31).

Table of Contents

Abbreviations and Definitions

AC…Acetylcholine

AD… Alzheimer's Disease

ADAS-Cog… Alzheimer's Disease Assessment Scale - Cognitive Subscale

AHS… Allgemeinbildende höhere Schule (German)

ASPSF… Austrian Stroke Prevention Family Study

AUC… Area under the curve

BHS… Berufsbildende höhere Schule (German)

BV… Brain volume

bvFTD… behavioural variant Fronto-Temporal Dementia

CSF… cerebrospinal fluid

DMN… Default mode network

DWI… Diffusion weighted imaging

DV… Dependent variable

FCN… Functional connectivity networks

FINGER… The Finnish geriatric Intervention study to prevent cognitive impairment and disability

GM… Grey matter

HC… Healthy controls

HV… hippocampal volume

IV… Independent variable

LBA… Lehrerbildungsanstalt (German)

MCI… Mild cognitive impairment

ML… Machine learning

MMSE… Mini Mental State Examination

MNI… Montreal Neurological Institute

MoCA… Montreal Cognitive Assessment

MRI… Magnetic resonance imaging

MTA… Medial temporal (lobe) atrophy

PRODEM… PROspective Registry on DEMentia

RF… Random forest

ROC… Receiver operating characteristic

ROI… Region of interest

RSN… Resting state network

SCN… Structural covariance networks

SN… Salience network

VI… Variable importance

WM… White matter

Zusammenfassung

Strukturelle Netzwerke basierend auf Kovarianz in der grauen Substanz sind vielversprechende neue Biomarker, die unser Verständnis für die Alzheimer Krankheit vertiefen können. Die Erforschung dieser Netzwerke könnte unseren Wissenstand über die Alzheimerkrankheit stärker erweitern, als durch einen Blick auf abgegrenzte Gehirnareale möglich wäre. In dieser Studie wollen wir (1) strukturelle Netzwerke finden, die zwischen Alzheimer PatientInnen und gesunden Kontrollen unterscheiden können, (2) diese Netzwerke mit bereits etablierten klinischen Biomarkern vergleichen (Gehirnvolumen, Hippocampus Volumen, Atrophiescore im medialen Temporallappen) und (3) herausfinden ob diese Netzwerke mit kognitiven Fähigkeiten zusammenhängen, bzw. die Veränderung der kognitiven Fähigkeiten vorhersagen können. Für die Unterscheidung haben wir einen Random Forest Algorithmus verwendet, anhand dessen wir an einer Stichprobe von 104 Alzheimer Patient*innen und 104 alters- und geschlechts-gematchten gesunden Kontrollen 20 strukturelle Kovarianznetzwerke untersucht haben. Anhand hoher Unterscheidungsfähigkeit erkannte Kovarianznetzwerke wurden anhand eines erweiterten Random Forest Models mit oben genannten etablierten klinischen Biomarkern verglichen. Alle Modelle wurden danach an einer unabhängigen Kohorte mit 28 Alzheimer Patient*innen und 28 alters- und geschlechts-gematchten gesunden Kontrollen getestet. Zusammenhang des Atrophiescores mit kognitiven Fähigkeiten, bzw. deren Veränderung, wurde anhand multipler linearer Regression getestet.

Zwei der 20 strukturellen Netzwerke haben signifikant zur Unterscheidung zwischen Alzheimer PatientInnen und gesunden Kontrollen beigetragen (ein temporales Netzwerk sowie ein sekundäres somatosensorisches Netzwerk). Die beiden Netzwerke konnten bei der Klassifikation einen Wert von 0.81 (gemessen an der Fläche unter der Kurve) erreichen. In der Kontroll-kohorte wurden in der Klassifikation ein Wert von 0.86 erreicht. Verglichen mit den etablierten Markern (Gehirnvolumen, Hippocampus Volumen, Atrophiescore im medialen Temporallappen), zeigten die beiden Netzwerke allerdings geringere Präzision bei der Zuteilung. Im gemeinsamen Model konnten die beiden Netzwerke die Präzision der etablierten Marker nicht verbessern. Das temporale Netzwerk korrelierte positiv mit verbaler Gedächtnisleistung, wurde jedoch insignifikant, wenn für Alter, Geschlecht und schulische Ausbildung kontrolliert wurde. Zusammenfassend lässt sich sagen, dass strukturelle Netzwerke zwar Potential für die Unterscheidung aufweisen, dieses jedoch nicht über das bereits etablierter Marker hinausreicht.

Abstract

Studying grey matter structural covariance networks (SCNs) may enhance our knowledge about Alzheimer's disease (AD) beyond the information based on the assessment of isolated grey matter areas. We, therefore, aim to (1) identify networks with diagnostic power for the differentiation between AD patients and healthy controls (HC), (2) compare their diagnostic power to established markers (brain volume, hippocampus volume, medial temporal lobe atrophy-score) and (3) determine the networks association to cognitive ability and cognitive decline.

To identify networks with diagnostic power, we trained a random forest model on a sample of 104 AD patients and 104 age- and sex-matched HC and calculated the variable importances for a set of 20 SCNs. To prevent overfitting, we validated the model on an independent sample of 28 AD patients from another centre and 28 HC. Resulting networks were compared against above mentioned established markers by combining them in an additional random forest model, again, by training and validating in independent cohorts. To determine the networks association to cognitive ability and cognitive decline, multiple linear regression models were used.

Two of the 20 SCNs showed significant contribution to the discrimination between AD and HC. These two networks comprise a temporal SCN and a secondary somatosensory SCN showing diagnostic accuracy (measured by area under the curve) of 0.81 in the training set and 0.86 in the validation set. When compared with the already established markers, the SCN performed worse and did not add any further information in a combined model.

We found the temporal SCN to be associated with verbal memory at baseline, but this effect vanished after controlling for age, sex and education.

We conclude that SCNs have diagnostic potential, but they do not provide information beyond established clinical tools.

Introduction

Alzheimer's Disease

Dementia is a clinical syndrome and constitutes an umbrella term for a group of progressive disorders that have detrimental effects on the brain and consequently on the individuals and their families. All variants show impairment of cognitive functions with effects on mental and physical health (1). Typical types of dementia are associated to older age, showing a prevalence of 0.9-1% for the age-group of 65-69 years and increasing to 31.2-32,2% in the age group of > 90 year-olds in Europe (2,3). In total numbers, current estimations say that over 50 million people worldwide suffer from any type of dementia (4,5).

The most common type of dementia is Alzheimer's disease (AD) causing up to 60 - 80% of dementia (1,6,7). This means that approximately 28 – 38 million people worldwide suffer from AD, with numbers rising due to the rise of life expectancy around the world. Vascular dementia and dementia with Lewy bodies are other common causes, accounting for up to 23% each (6). Other forms of dementia, such as fronto-temporal dementia and its subtypes, are collectively related to up to 8% of dementias (6). However, in many cases dementia patients show a mixed type of dementia, where especially AD and vascular dementia are encountered together (1,8).

The current view of AD suggests it to be a cascading disconnection disease of the brain. The communication between parts of the brain decreases due to Alzheimer-related pathology. This is further leading to a loss of connections (decrease in structure) and/or desynchronization of activity (decrease in functional processes), resulting in decrease and loss of performance in multiple cognitive abilities (9–12). This understanding is based on achievements in brain imaging techniques providing insight to the brains working processes, i.e. different distant brain areas communicating with each other, rather than the prior view of exclusively one-area per-skill functionality. The understanding on how the brain functions changed tremendously.

Pathology of AD

The pathogenesis of AD, how the disease starts and what exactly drives its propagation throughout the brain, is still not entirely clear. The molecular processes involved in structural changes such as brain atrophy, as a cardinal symptom, have been described and led to three hypotheses of disease progressing drivers: the amyloid cascade hypothesis (13–16), the 'tau and tangle' hypothesis (15), and the cholinergic hypothesis (17–19).

In the amyloid cascade hypothesis, pathogenesis is prominently described by a build-up of extracellular deposits. These deposits are amyloid beta plaques which in AD increasingly form and cling to the outside of neurons, disrupting communication between them (15,20). These plaques are typically first found in the basal areas of frontal, temporal and occipital lobes and then spread to primary sensory areas (16).

The 'tau and tangles' hypothesis is about tau proteins that are important for regulation of the cytoskeleton in brain cells. In AD, the proteins become phosphorylated and aggregate into neurofibrillary tangles (16,20). The tangles accumulate within the neuron, blocking normal function, transport processes and communication at the synaptic level (15). The tangles are typically beginning to accumulate at the limbic area, from there on spreading to paralimbic areas, the prefrontal-parietal areas and finally to the primary sensory and motor areas (21). Both, the amyloid beta plaques as well as the tau tangles result in cell death, leading to the aforementioned atrophy of grey matter (GM).

The cholinergic hypothesis is about the role of acetylcholine (AC) as an important neurotransmitter involved in the formation of memory. It was found that inducing anticholinergic drugs in young adults produced dementia like deficits in memory performance (17,18). This lead to the idea of age and AD related cognitive decline also being caused by a deficient cholinergic system and progression of AD has been linked to loss of cholinergic transmitter function (17–19,22). Often misunderstood as such, the cholinergic hypothesis, in contrast to the former mentioned hypotheses, is argued to not be a causative model of AD (17). While often critiqued, it is still relevant in the course of the disease and its effects on cognition (17,19).

Other hypotheses about the pathogenesis of AD have been developed over time, including oxidative stress, inflammation and even dietary factors (23). Especially interesting, in the explanation of disease progression, is the idea of cascading network failure (10,12,24). This idea is based on the possibility that detrimental functional changes in the brain, due to AD, lead to overactivation to compensate for detrimental changes. This overactivation then leads to cell death and further progression of the disease leading to functional changes in the associated areas (12). Still, it is likely that AD as a syndrome is not attributable to one single cause but to multiple factors (20).

From a macroscopic view, grey matter atrophy due to cell loss occurs at disease onset primarily in the limbic areas and the cortex (25). The propagation of this atrophy shows a very stereotypical course which typically begins at the hippocampus, entorhinal cortex and posterior cingulate cortex and at later stages the atrophy progresses to the temporal, parietal and frontal cortices (26,27).

Subtypes of AD

AD can be divided into multiple entities by different criteria. Clinically, two subtypes are described, a typical and an atypical variant (28–30). The effects of typical AD are manyfold, but the most prominent symptom is decline in episodic memory. Clinical symptoms may begin to arise in the consolidation of memories of recent events. Described as “forgetfulness”, people with AD, for example, may not remember common daily events or have problems in naming the correct current weekday. With further progression of the disease, the extend of memory loss reaches further back in time and eventually even earlier life memories are lost (31). Besides memory, language and problem solving are also affected (32,33).
Executive functions including attention may also be diminished. Tasks that exceed single and simple steps become difficult, limiting the quality of daily living severely. Besides cognitive impairment, psychiatric symptoms such as depression have also been described to be associated with AD (34).

Atypical AD differs from the typical form by beginning with a non-amnestic clinical course. Over time the cognitive effects of both are similar, but instead of episodic memory, atypical AD starts with problems in either one or more of the following domains: visual, language, executive, behavioural and motor (28–30). Deficiencies in these domains are usually accompanied with atrophy in corresponding brain areas and often the hippocampus is spared in early stages (30).

Based on the patients age at onset of the disease, a further distinction is made between early-onset AD (EOAD) and late-onset AD (LOAD).
The more common type is LOAD which is defined by an onset at the age of 65 or older (35). EOAD is currently defined by an onset at an age less than 65, which affects 4-6% of AD patients (36). EOAD and LOAD have shown to differ based on an over proportion of atypical AD in EOAD as compared to LOAD (37). However, a differentiation based on clinical or neuropathological features is currently not recommended (38). The main focus of this thesis is the typical variant of LOAD. Therefore "AD" will refer to this if not otherwise mentioned.

Diagnosis

There are multiple sets of criteria for the diagnosis of AD, which can be broadly divided into clinically oriented sets of criteria and research-oriented sets of criteria. Clinically, the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision (DSM-V-TR) and the International Statistical Classification of Diseases and Related Health Problems, Eleventh Edition (ICD-11) provide criteria for the diagnosis of Dementia due to AD (39–41).
Two currently accepted research-oriented criteria sets for AD were developed from the National Institute on Aging and the Alzheimer's Association (NIA-AA) and the International Working Group (IWG) (42,43). All four sets show strong overlap in their syndromic description of AD. The basic criteria of dementia must be met, which include impairment in quality of daily living and a quantifiable decline in function from previous levels. Differential diagnosis must be done to exclude other likely sources of similar symptoms (e.g., stroke, other forms of dementia in case of AD diagnosis, substance abuse, delirium). Furthermore, cognitive and/or behavioural impairment must be quantifiable and include a minimum of two major domains (memory, executive function, visuospatial abilities, language, personality) (44). For diagnosis of AD, the

development of the symptoms must show a slow progression. EOAD and LOAD as well as typical and atypical forms of dementia are defined due to different criteria for diagnosis being met (e.g., first symptoms are amnestic vs. non-amnestic). The biggest difference is the consideration of biomarkers (tau, amyloid-beta) and brain-imaging (atrophy, functional changes) in research-oriented sets, which is currently not the case in the in ICD-11 (45) and only supported for research but not for clinical diagnosis in DSM-V-TR (41,46). The availability of positive AD biomarkers and AD related brain changes enhances the certainty of the diagnosis.

In daily clinical practice, AD diagnosis is a multi-step process (38) which is typically triggered by the patient suffering decrease in cognitive abilities or change in personality which effects their quality of living. The medical history is created with the help of the patient and family members and/or close acquaintances. The third party is especially crucial to provide reference to former quality of living and the time course of changes. Medical examination is provided additionally to the medical history to investigate alternative explanations for the current symptoms. This is a broad field and in case of AD, similar symptoms can emerge from multiple problems, including comparatively marginal things like hearing impairment (47,48).
A short screening like the Mini Mental State Examination (MMSE) is performed with the patient to provide a basis for a dementia diagnosis and its likely severity. In case of AD, the severity ranges from mild to moderate to severe dementia due to AD, which can be rated based on the reached MMSE score (38,49). If dementia is found to be a likely reason for the patient's ailments, or even if it cannot reasonably be eliminated as a reason, more specific tests and examinations will be performed. Extensive neuropsychological tests and questionnaires (behavioural changes, limitation in execution of activities of daily living), biomarkers, multiple imaging methods and genetic analysis are used to find an etiological explanation for the patient's state, which can include or be based on AD. In cases where AD is not (yet) diagnosed, (amnestic) mild cognitive impairment (MCI) might be alternatively diagnosed (38).
Amnestic MCI is conceptualized as a prodromal stage of clinical dementia due to AD, which can be described as showing similar but weaker symptoms. However, the patient still is independent in daily living and does not meet the diagnosis criteria for dementia (42,50,51). It is important to point out that there is no clear demarcation between MCI and early dementia, and it is ultimately a clinical judgement made by experts. Although, statistical cut-offs are often

used in practice. Studies report a yearly incidence of 5% to 15% for individuals with MCI diagnosis to further proceed to the clinical stage of dementia due to AD, but some MCI cases never proceed (50,52–55).

Accuracy of clinical diagnosis is reported to have a wide range, with sensitivity ranging of 41% to 100% and specificity ranging of 37% to 100% (56). Diagnostics and measurement of AD are an ongoing area of research. Most diagnostic tools are not strictly negative or positive for AD. Biomarkers, clinical estimates, and neurocognitive tools present information on continuums. Simple cut off points for the demarcation between AD and other forms of dementia or even healthy aging are hard to come by and need strict scientific testing (57).

Treatment and Prognosis

There is no cure for AD yet. Currently established provided treatments aim at the alleviation of AD's primary symptoms and possible accompanying problems (e.g., depression) or slowing down the progression of the disease.
Donezepil, Galantamin and Rivastigmin are cholinergic drugs with the aim to inhibit acetylcholinesterase, which is the primary enzyme responsible for the breakdown of acetylcholine. They were shown to enhance cognitive abilities and performance of activities of daily living in patients with mild to moderate dementia due to AD (38).
Memantin is an N-Methyl-D-aspartate (NMDA) antagonist shown to have positive effects on cognition, behavioural problems and performance of activities of daily living in moderate to severe dementia due to AD (58,59). It works by blocking NMDA-receptors, which are assumed to show abnormally high activation under pathological conditions like AD, which can lead to neuronal death (60). Gingko based drugs like Cerebokan are also prescribed in cases of mild to moderate dementia due to AD. It has been found to improve cognition and the performance of activities of daily living (61).

Besides above mentioned drugs, multiple other types of pharmacological treatments have been and are still researched, their effects on clinical outcome range from non-existent to promising, but are often overshadowed by side-effects (20,62–64).

Based on the former mentioned cholinergic hypothesis, different treatments were tested to increase the amount of acetylcholine (17,18). Therapeutics by agonistic activation of muscarinic receptors have not been very promising based on insignificant and adverse effects. Treatment aimed at stimulating nicotinic receptors had shown promising effects of slowing the decrease of cognition but have also shown adverse effects. The inhibition of cholinesterase, an enzyme that blocks indirectly the breakdown of acetylcholine, is approved, and applied as treatment (17).

Generally, cholinergic treatment is often seen as symptomatic treatment not affecting the course of AD itself, but newer studies imply that this view may be a downplay of the cholinergic treatment, which showed association to a reduced rate of atrophy in the GM (17).

Medical research within the amyloid cascade hypothesis led to multiple main strategies of AD treatment. Reduction of amyloid beta load in the brain is the main goal and theoretically this can be achieved by decreasing formation of amyloid beta plaques or removing them, for example by enhancing clearance (20,65). Similar to cholinergic treatment, both approaches had problems showing effects on the clinical course of the disease and in case of effectiveness, side effects were found (65).
In 2021, Aducanumab has been approved as treatment of AD (64,66). Aducanumab belongs to the group of anti-amyloid beta monoclonal antibodies, or "mabs" and targets and removes amyloid beta in the brain, leading to a decrease in AD (63). Another approved mab, Lecanemab, also shows promising results in slowing down AD progression as measured with biomarkers as well as decelerated cognitive decline (67–69). Research of mabs as treatment represent a breakthrough, still, adverse effects like brain bleeds were reported and there is much criticism on the evidence of effectiveness (63,64,66,69–71).

A therapeutic approach to reduce accumulation of neurofibrillary tangles, the main culprit in the tau and tangles hypothesis, was based on immunization via antibodies. Unfortunately they were shown to induce AD like pathologies as well as other severe side effects (65,72).
Multiple complementary non-pharmacological treatments are also used, which are combined under the umbrella term psycho-social-interventions (38). Cognitive training takes a big part in this and aims at preserving cognitive abilities but also activities of daily living as long as

possible. Broadly speaking, there are two types of cognitive training, restorative and compensatory (73). Restorative cognitive training is aimed at restoring cognitive abilities to higher functioning by training specific abilities. In contrast, compensatory training aims at learning alternative strategies to perform tasks to circumvent or support existing deficits. Both have been found to show effects on cognition in early AD phases, but restorative training seems to outperform compensatory training (73).

Occupational therapy was found to be an effective treatment for patients with dementia (74,75). Occupational therapy engages the patient in activities that provide purpose and self-efficacy to reduce behavioural disturbances and agitation while enhancing life quality. A further protective factor in AD might be physical activity which shows positive effects on psyche, behaviour and activities of daily living besides more obvious outcomes like agility and mobility (76).
Art based forms of therapy like music and dancing have been found to provide relief for different problems associated to AD. Music, for example, can have a calming effect and reduce agitation and fear in patients (77).

To optimize a patient's care, it is likely best to provide a multi-modal approach of treatment including all or many of the above-mentioned treatments. Especially the non-pharmacological treatments are low risk and therefore worth a try even with smaller reported effect sizes. A study researching this multi-modal treatment is the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER). The multi-modal treatment consisted of four components, including nutritional intervention by nutritionists, physical exercise training programmes by physiotherapists, cognitive training by psychologists and monitoring of vascular and metabolic risk factors in cooperation with a nurse and a physician. They found that the combined treatment to provides protective effects on general and specific cognitive domains as well as other areas of health (BMI, physical activity) (78,79).

Still, prognosis of AD after diagnosis is dire, as the disease only shows to be moderately decelerated by some treatments. We are far from halting it and we need more research to gain a deeper understanding of the disease to reach this goal. Currently, the average life expectancy is of 3 to 7 years after diagnosis (80,81). Depending on multiple factors, life expectancy may vary. The average life expectancy after diagnosis is higher for younger people, women, for

specific genotypes, healthier dietary habits, better general health status as well as people in relationships (80,81).

Cognitive Tests and the Problem with Reserve

The effects of AD on cognitive abilities are vast and devastating. Impairment in memory, executive functions, language, visuospatial skills and attentional control are primary symptoms (51,82), with the first often being the trigger for a first doctor's appointment and a following diagnosis. Measuring these abilities are important for AD diagnosis and in AD research. The tools specially developed for the task of measuring cognitive abilities are standardized psychological tests.

The MMSE, which was shortly mentioned in the diagnosis chapter, is a fast screening tool for global cognitive functioning and is constructed of 30 items, addressing each of the above mentioned abilities. Despite vast psychological criticism regarding the MMSEs insensitivity to detect mild cognitive impairment, it is internationally the mostly used screening tool for the estimate of progression of AD (83). The Montreal Cognitive Assessment (MoCA), a screening tool developed for detection of cognitive impairment in context of dementia, provided greater ability to detect cognitive impairment as compared to the MMSE (84–86).

There also exist tests specialized for specific domains. The ability to learn and retain information (memory) can be measured by testing immediate and delayed recall of information, which is most often done in form of word lists (verbal memory) or drawings (non-verbal memory). Executive abilities are a set of abilities including attentional control, working memory and cognitive flexibility. An example of a specialized test for executive function would be the Wisconsin Card Sorting Test (WCST) (87). As its name implies, a participant would have to sort cards, but the sorting rules are (a) unknown to the participant and are only revealed by the examiners feedback ("right" or "wrong") and (b) change after a certain number of cards.

Multiple tests can be found for the cognitive abilities affected by AD and often they are combined as "subtests" into a whole test battery like the Consortium to Establish a Registry for

Alzheimer's Disease (CERAD) Plus or the Alzheimer's Disease Assessment Scale Cognitive Behavior Section (ADAS-Cog) (88–93). The test-batteries tend to outperform the screening tools in measurement reliability of individual cognitive abilities (94).
Reliability is an important term in test theory and describes the accuracy of a measurement which is often equated with the expectation of getting similar or the same results after performing a test multiple times. A challenge for cognitive testing and reaching high measurement accuracy is that the measured underlying constructs are also dependent on the current state of a patient. On a bad day due to stress, emotional problems or whatever may be the reason, the test result may deviate strongly from the ability a patient shows on a good day. One can imagine that this has a problematic effect on diagnostics, but even more so if one aims to observe the progress of AD over time. Decreasing ability may be masked or overestimated, which is one reason why studies about treatment need big sample sizes to reveal true treatment effects.

A further challenge is that AD is not simply mirrored by its manifestation in clinical symptoms. The same cognitive impairment in two people can conclude from very different stages of atrophy in the underlying areas associated to AD and in some cases, profound atrophy due to AD never is indicated clinically. This difference in withstanding the brain pathology due to AD is researched and yielded different theoretical constructs. These are brain reserve, cognitive reserve, brain maintenance and compensation (95,96). Brain reserve describes the brain substrate (brain size, neuron count, structural integrity of some form) as the primary factor for time of clinical onset and the assumption that a higher brain capacity has protective effects. This is due to the idea that there might be some amount of healthy brain structure needed for optimal cognitive functioning and below that, cognitive impairment arises (95). It is considered a passive form of reserve. Cognitive reserve on the other side is considered an active form of reserve (95,97). It is grounded on the hypothesis that higher IQ, the partaking in late life leisure activities and live long acquired cognitive skills and abilities (education) deliver a protective factor (95). One explanation for this is that some brains are, on average, more efficient and can therefore provide normal function for longer even after detrimental changes in the brain structure. Brain maintenance is described by the findings of patients showing less GM atrophy or loss of function as compared to others within similar time frames (96,98). Compensation is

about the active bypassing of failing brain areas and integrating other areas to uphold normal functioning (95).

Studies of comparatively strong cognitive impairment due to AD found on average more advanced AD pathology within patients with higher scores of cognitive reserve measures (99,100) indicating that one ore multiple of the above mentioned forms of reserve exist. A disadvantage of approaches in cognitive reserve is that they are mostly limited to proxy measures like education and IQ. The construct of cognitive reserve itself, is called a "latent" construct and there is still much discussion about the methodical approach to take to reach valid and reliable measurements (101).

Without a valid and reliable measurement of cognitive reserve, a mediating link might be missing between cognitive change and the underlying changes due to AD pathology (95). As mentioned above, there are also implications to find the brain correlate of cognitive reserve, but relevant areas and markers are hard to pinpoint without something to compare against.

While very costly, one possibility to handle the challenges regarding cognition as a measurement tool for AD is to include biomarkers, which we will discuss in the next chapter.

Biomarkers and Imaging

Biomarkers are quantitatively measurable indices of the body which mirror biological processes that may be useful for diagnosis and monitoring (7,102). The field of biomarker research includes a host of approaches, including genetics, inflammation markers, metabolomics, and imaging methods.

The most prominent genetic marker for AD is the apolipoprotein E gene (APOE gene), which is strongly associated to late onset AD (103). APOE can be present in different alleles from ε2, to ε3 and ε4, of which ε2 is reported to reduce risk of AD and ε4 is reported to highly enhance the risk of developing AD (from-3 fold in heterozygosity and 12-fold in homozygosis) (103–105). Detection of APOE4, which is the ε4 type of APOE, in a patients genotype is currently not regarded as a diagnostic tool due to the low sensitivity (65%) and specificity (68%) (106). Furthermore, due to its binary nature of being part of an individual's genotype or not, other markers might be more suitable for measuring the course of AD.

There are more fitting candidates in the group of fluid-based biomarkers for AD. As mentioned in the chapter about pathology, neurofibrillary tangles and amyloid-β plaques are protein residues highly associated with AD and the progression of cognitive impairment (107). Their occurrence due to pathological processes are best mirrored in the cerebrospinal fluid (CSF) which is the preferred medium for measurement of these markers. However, the extraction of CSF is highly invasive and painful for the patient and routine checks are problematic. Alternative and less invasive fluids to measure neurofibrillary tangles and amyloid beta plaques are blood and oral fluids. The blood brain barrier is a limiting factor on the movement of these markers and therefore their load is much lower in other fluids as compared to CSF. This causes sensitivity challenges for current measuring technologies, which may be a factor within studies showing no or small effects of drug treatment on AD (108). Another challenge in AD treatment research using amyloid beta plaques from fluids are findings that amyloid beta shows ceiling effects at early clinical stages of AD due to saturation effects of accumulation (109). Tau as compared to amyloid beta, was found to show association to clinical outcomes of AD but this is also decreased at progressed stages of clinical AD (109).

Besides being measured in fluids, amyloid in the brain can be displayed by positron emission tomography (PET). Newer development of tracers made it possible to use PET also to investigate tau distribution in the brain, which showed promising associations with cognitive decline (110,111). PET also is used to depict hypometabolism in the brain which is linked to atrophy due to AD. The hypometabolism can be measured by using F-fluorodeoxyglucose (F-FDG) as a tracer for glucose consumption, which shows decrease in brain areas characteristic for AD induced dysfunction (112).

High resolution images of brain structures can be taken by structural magnetic resonance imaging (sMRI). T1-weighted sMRI is a well-established tool to depict GM, white matter (WM), CSF as well as remaining structures around the brain (skull, fat, skin, air). This method shows changes in GM volume due to AD-related neuronal loss, which mirrors the typical change in cognitive abilities (26). Beginning with deficits in memory, GM atrophy is found in the entorhinal cortex, hippocampus, and posterior cingulate cortex. At later stages, when executive functions, language, and behaviour are affected, atrophy is typically found to spread

to the temporal, parietal and frontal areas (26). Rating of atrophy, and hence AD progression, is still challenging. It is either done by trained clinicians performing visual ratings on the images or by computationally intensive computer analysis extracting measures of interest from the images.

Measures of interest that were found to correlate with AD progression are the hippocampal volume (HV), the whole brain volume (BV) and cortical thickness patterns, with some reduction years before clinical symptoms are noticeable (26).
An indirect marker of interest for GM atrophy is the increase in size of ventricles. It is one of the criteria in the medial temporal lobe atrophy (MTA) score, which is based on a visually rated score assessing structures within the medial temporal lobe (hippocampus proper, dentate gyrus, subiculum, parahippocampal gyrus) as well as the surrounding area of CSF fluid within the ventricle (113,114). Due to the high contrast between CSF filled areas and GM, automated analysis tools were also developed to assess the hippocampal volume and ventricular enlargement, of which the latter show associations to decline in cognition and AD progression (115).

A steppingstone between structure and clinical outcome is functional MRI (fMRI). It provides an insight into the activity patterns of the brain by measuring its oxygen consumption (116). The method of fMRI is particularly interesting in studies looking at "task-based brain activity", with tasks being solved while fMRI scans are performed and the tasks themselves being based on cognitive abilities that are affected by AD (117–120). Akin to findings in GM atrophy, AD patients as compared to healthy controls, showed changes in the activity of the medial temporal lobe (associated to memory) (117–119), lateral temporal lobe (associated to memory) (121), occipital lobe (associated to visuospatial processing) (122), left inferior frontal gyrus (associated to executive function) (120,122) and bilateral frontoparietal regions (associated to memory) (122). Interestingly, brain activity was not found to be uniformly decreased but was found to show both, increase, and decrease at different stages of the disease (117,122). In a study including AD patients, elderly with MCI and elderly without memory complaints, increased activity was found in the hippocampal area for elderly with MCI as compared to healthy elderly without complaints. AD patients showed reduced activity in the hippocampal area and entorhinal cortex areas as compared to both groups (117).

This pattern of increased activity was discussed as compensatory function due to effects of AD. At a later stage a breaking point may be reached and the activity decreases due to a loss of neurons (95,96,121,122). This was also suggested as a possible form of cognitive reserve, which could present in (a) higher activation in affected areas of the brain in intention to maintain levels of function (117,121,122). Another discussed idea was higher activation overall due to diffuse activation in the need to recruit more area(s) for the compensation of lost functioning (96,122,123).

The knowledge gain based on fMRI imaging, that the brain works on a network basis rather than distinct areas, shifted the focus of research towards the brain understood as a network phenomenon.

Brain Networks and the disconnection hypothesis

Moving away from a modular view of the brain, which associates different functions to distinct areas, the arising of these functions can be seen as the result of different brain areas communicating and interacting. Basically, the brain can be seen as a hierarchical structure with networks of neurons forming functional entities from the micro level to the macro level. This view was formulated in connection with AD where the disease was partly explained by clinical symptoms following the disturbance of brain connectivity due to neuronal loss (11).

Support for this disconnection hypothesis was delivered by investigation of the spatial spreading of neurofibrillary tangles which were found to distribute along cortical connection pathways, disrupting normal functioning (124). Disconnection as an effect of AD has also been found in studies investigating white matter connectivity in AD patients as compared to healthy controls (125,126). Based on diffusion weighted imaging (DWI), the integrity of 18 major WM tracts were examined (125). All 18 tracts showed degeneration due to AD, including the fornix, which is part of the limbic system and the main WM pathway emerging from the hippocampus and connecting it to the corpus mamillare. Another study also reported decreased WM fibre count and loss of connective efficiency within and between AD relevant brain areas like the hippocampus, precuneus and temporal gyrus (126).

Disconnection due to AD has also been found in task-based fMRI studies. These studies focus on functional networks which are researched through temporal patterns of activity in the brain while solving standardised tasks as presented by the researchers. Greicius and colleagues contributed studies focused on the default mode network (DMN) (127,128). The DMN consists of the bilateral medial and lateral parietal cortices, bilateral medial and lateral temporal cortices, and the bilateral medial prefrontal cortices and was found to be activated at rest but to be deactivated at task processing (129). It also showed functional connection to the hippocampus (128,130). This functional connectivity was found to be decreased in AD patients while performing attention tasks, as compared to healthy controls (128). Another study focused on associative memory tasks and showed a de-synchronization between hippocampus and DMN activity that changed over the course of AD (130). At earlier stages of AD with less memory impairment, it was found that hippocampus activity increased while DMN activity decreased, which changed at later stages of AD where hippocampus activation and the deactivation of the DMN where both reduced.

Another method to research functional networks with fMRI works by examining the temporal patterns of activity in the brain while the subject is at rest without solving any tasks (131). This state of "deactivation" makes it possible to measure the baseline activity of the brain and its associations to cognition and AD. As mentioned above, the DMN shows higher activation in these situations of "doing nothing in particular".
Fittingly, these networks are called resting state networks (RSN) and the method resting state fMRI (rsfMRI) or sometimes functional connectivity MRI (fcMRI). RSNs showed promising results for AD research. In healthy subjects it was found that the strength of connectivity within the RSN DMN is associated with memory retrieval, a cognitive ability affected in AD patients (132). Another very interesting finding in healthy subjects was that connectivity within RSNs decreased with increasing age but the connectivity between RSNs enhanced with increasing age. This was interpreted as dedifferentiation, followed by compensating recruitment of other areas due to loss of efficiency within networks (96,133).

In early AD patients, as compared to healthy subjects, multiple RSNs were found to be different in connectivity either within RSNs or between them (134). Measuring positive connectivity as

defined by synchronisation between networks, they found (a) decreased positive connectivity between prefrontal regions and parietal regions, but also (b) increased positive connectivity between prefrontal regions and multiple other areas. Prefrontal regions were also found to have higher connectivity within themselves. The increase in connectivity within and between above mentioned areas in AD patients was suggested as a compensatory strategy of the brain to account for decreases in other areas (134). The authors also found multiple decreases in negative associations with regions attributed to the DMN such as the posterior cingulate gyrus, the precuneus, the angular gyrus and the parahippocampal gyrus, results already seen in prior studies (134).

Multiple other studies found decreased connectivity within the DMN of AD patients (135–137) but changes were also found in other RSNs, dependent on the study specific conventions. This included salience, control, dorsal attention and sensomotoric RSNs (135) a frontal-parietal and executive RSNs (137). Although, the latter study did not find differences in connectivity for the salience networks between AD and healthy control subjects (137).

Besides connectivity within the RSNs, other studies also reported change in connectivity between networks, especially between the DMN and other networks (135,138). Associations between the DMN and dorsal attention RSN and sensomotoric RSN were reported to change from negative correlations to near zero (135). Interestingly, RSNs were also suggested as a pathway for transmission of the amyloid precursor protein resulting in accumulation of amyloid beta and providing an explanation for AD progress mechanisms (139).

As a structural equivalent to RSNs, structural networks can be found by analysing distinct GM covariation patterns in remote cortical areas. These networks are termed structural covariance networks (SCN) and were found to be partly visually analogical to functional networks (140). They are an attempt in uncovering the “cortical disconnection” as a structural basis for network changes in the brain (141).

Montembeault and co-workers predefined SCNs by regions of interest (ROIs) and found that in healthy aging subjects, the cognitive domains language, executive functions, working memory and mentalization show decreased SCN connectivity with increasing age (141). Similar results were found in studies using model-free approaches (without pre-defined areas), reporting negative association between age and SCN volume. The SCNs were found to be negatively associated with age included subcortical structures (lateral occipital, posterior and

anterior cingulate cortex) as well as the thalamus, nucleus accumbens, caudate nucleus and hippocampus. Furthermore they showed positive association to declarative memory (142,143). These findings were supported and extended by a study by Koini and co-workers investigating the association of SCNs with age and cognitive functions. In total, fourteen SCNs were shown to be negatively associated to age, of which eight were also positively associated to cognitive functions or motor functions. The eight SCNs were a secondary somatosensory SCN, a temporal SCN, sensorimotor SCN, a limbic SCN, a fronto-parietal SCN, a cerebellar SCN, a fronto-occipital SCN and a cuneal SCN) (144).
Decreased SCN connectivity in association with worse cognitive abilities in memory and psychomotor speed were even found when controlled for other influences like small vessels disease (145).

These results implied promising applicability in AD research, since the included brain structures and associated cognitive abilities are strongly affected by the disease. Seeley and colleagues compared structural network based atrophy between different neurodegenerative diseases and found increased atrophy due to AD in areas including the precuneal cortex, posterior cingulate cortex, lateral occipital cortex and lingual gyrus, areas often associated with AD and the DMN (140).
This difference in DMN SCN connectivity between AD patients and healthy elderly was replicated by Montembeault and colleagues (146). Multiple other SCNs, including a salience SCN and an executive control SCN were found to be associated with AD progression (147). The DMN SCN was also found to be associated to MMSE scores and to show faster decline in connectivity in AD patients as compared to MCI and healthy elderly (148).
Another study by Hafkemeijer and co-workers investigated possible differential diagnostic abilities by comparing multiple SCNs between healthy elderly, AD patients and patients with behavioural variant fronto-temporal dementia (bvFTD) (149). They found different degeneration patterns for the diseases. In bvFTD, as compared to AD and healthy elderly, decreased network integrity was found in a SCN containing the anterior cingulate cortex, insular cortex, paracingulate gyrus and frontal medial cortex. For AD, as compared to bvFTD and healthy elderly, decreased network integrity was found for a precuneal SCN visually resembling the DMN found in other SCN studies (140). The network integrity of the precuneal SCN also showed a positive association to MMSE score in AD patients.

Furthermore, another SCN, namely a hippocampal SCN, showed decreased network integrity in AD as compared to healthy elderly. Besides the hippocampus, the SCN contained the temporal fusiform cortex, occipital pole and the temporal pole, areas shown to be associated to AD (132,140). SCNs were also shown to be predictive in cognitive change in MCI patients (150). SCNs may provide a new method of measure the progression of AD and its detrimental effect on GM in the brain on a network basis i.e. with a focus on higher level systems as compared to focal changes in independent areas.

Research Question

In this work, our aim is to extend former research by investigating the ability of 20 SCNs, identified in an earlier work of our group (144), to classify between AD patients and a healthy control group of cognitively healthy elderly. We expect that SCNs with spatial similarity to the DMN and SCNs containing medial temporal areas like the hippocampus will show higher diagnostic power than other SCNs. To validate the diagnostic power of potentially significant identified SCNs, we applied them on an independent sample. Furthermore, we wanted to test if SCNs add further diagnostic information to already available markers, therefore we will compare SCNs with high diagnostic power against established markers including the HV, BV and MTA score.

Thereafter, we want to examine their ability to predict impairment of global cognition and memory in AD patients, as well as their ability to predict decrease of global cognition and memory over time due to AD.

Methods

Hypotheses

1a. We hypothesize that SCNs which are spatially similar to the DMN as well as SCNs containing medial temporal lobe areas, show diagnostic power to classify between AD and healthy controls.

1b. We hypothesize that the SCNs with diagnostic power will perform with similar diagnostic power in an independent sample.

2. We hypothesize that the SCNs with diagnostic power add diagnostic accuracy to established markers (HV, BV and MTA score) used for comparison in this study.

3a. We expect that SCNs with diagnostic power for AD diagnosis are associated to impairment in memory and global cognition, in a sense that patients with less SCN integrity show, on average, higher impairment on memory and global cognition.

3b. We expect that SCNs important for AD diagnosis are associated with change in impairment of memory and global cognition over time. This means that we expect patients with lower SCN integrity at baseline to show, on average, a steeper increase of impairment in memory and global cognition.

Participants

The AD patient's data are retrieved from the Prospective Registry on Dementia (PRODEM), a longitudinal multi-center study on the disease-progression of dementia in Austria. The inclusion criteria were as follows: (1) a diagnosis of dementia (not only AD) according to DSM-IV criteria (2) non-institutionalization and no need for 24-h care, (3) availability of a caregiver who can provide additional information on the patient. Due to the longitudinal nature of the study, one exclusion criteria were co-morbidities that are likely to lead to early termination from the

study. Another exclusion criteria was if a patient was not able to sign an informed consent at the beginning of the study (151)**.** The data from the healthy controls are retrieved from the Austrian Stroke Prevention Family Study (ASPSF). The ASPSF is a single-center, prospective study on cerebral effects of vascular risk factors for the aging brain in the normal elderly. Exclusion criteria was any history of neuropsychiatric disease, including cerebrovascular attacks and dementia (152).

For our study all PRODEM patients with (a) either possible or probable AD defined by the NINCDS-ADRDA Alzheimer's criteria, (b) at least one 3T T1-weighted 3D MRI scan, and (c) a cognitive assessment within 3 months of the imaging examination were included. From the healthy control sample of the ASPSF we extracted all subjects with a 3T T1-weighted 3D MRI scan.

ASPSF was approved by the ethics committee of the Medical University of Graz (Approval number: 17-088 ex 05/06). PRODEM was approved by the ethics committees of the Medical Universities of Graz (Approval number: 19-135 ex07/08), Vienna (Approval number: 176/2008) and Innsbruck (Approval number: UN3259/2009), as well as the state of Upper Austria (Vote code: 2008.04.25). Informed consent was obtained from all subjects and/or caregivers included in the study.

MRI Protocol

Structural data were assessed using a three-dimensional, T1-weighted, magnetization prepared rapid gradient echo sequence (MPRAGE) in Graz as well as in Vienna. Both centres had the same model of scanner, a TRIO Tim 3.0T, manufactured by Siemens Healthcare in Erlangen, Germany. The scans were all with whole brain coverage. The selected sequence parameters for PRODEM were as follows: Repetition time: 1.900/2.000 ms, inversion time: 900 ms, echo time: 2.19 ms, flip angle: 9° and a resolution of 1mm * 1mm * 1mm/1.2mm. For ASPSF the selected parameters were as follows: Repetition time: 1.900ms, inversion time: 900ms, echo time: 2.19 ms, flip angle: 9° with an isotropic resolution of 1mm.

Image Analysis

Structural Covariance Networks

To extract the SCNs from the structural data we used the above mentioned T1 weighted 3T images. To ensure that the data set from Vienna is treated independently, all pre-processing steps were done separately. Preprocessing analysis was done with FMRIB's Software Library (FSL) Version 5.0.9 (153). All T1 weighted images were visually checked by the researchers to ensure consistent image quality for analysis. The processing steps are according to the workflow in the work of Hafkemeijer et al. (142). First, a semi-automated brain extraction tool (BET) implemented in FSL (154) was used to remove non-brain tissue (e.g. skull, scalp). Thereafter, all images were visually checked and remaining non-brain tissue was removed by hand. Then, tissue-type segmentation in WM, GM and CSF was performed with voxel-based morphometric analysis (155) based on partial volume extraction. Partial volume extraction provides estimated proportions of tissue types for every voxel (156). The resulting GM images were visually checked and then linearly aligned to GM Montreal Neurological Institute (MNI) 152 standard space image (157), followed by nonlinear registration (158). The registered images of all participants (AD and healthy control) were then averaged to create a study-specific template, to which the native GM segmented images were nonlinearly re-registered. A further modulation step was included to correct the images for local expansion or shrinkage (155,159), which implements the amount and direction of expansion or shrinkage for every voxel. Finally, the resulting images were spatially smoothed with an isotropic Gaussian kernel with a sigma of 3 mm and concatenated to a 4D data set. To extract SCN integrity scores, this 4D data set was spatially regressed onto 20 network masks obtained by Koini et al (144) from a healthy aging sample. The spatial regression is the first step of the dual regression function included in FSL and described in detail in Filippini et al (160). The results are beta scores for each subject and network, representing a measure for network integrity at an individual basis. The networks included can be seen in Figure 1 and are described in detail in Table 1.

Visual and Automated Atrophy Marker

To acquire the medial temporal lobe atrophy score (MTA - score), all patients were visually rated by trained specialists. The MTA-score is a five-point rating scale for assessment of

atrophy, ranging from 0 (no atrophy) to 5 (maximum atrophy) (114). The rating is done for both hemispheres. For our statistical analysis we aggregated both scores to a mean value. Brain volume (BV) and hippocampus volume (HV) were obtained from the native T1-weighted images using FreeSurfer (Version 5.3) (161). Each, BV and HV volume values were normalized for intracranial volume. HV of both hemispheres were again aggregated by calculating the mean.

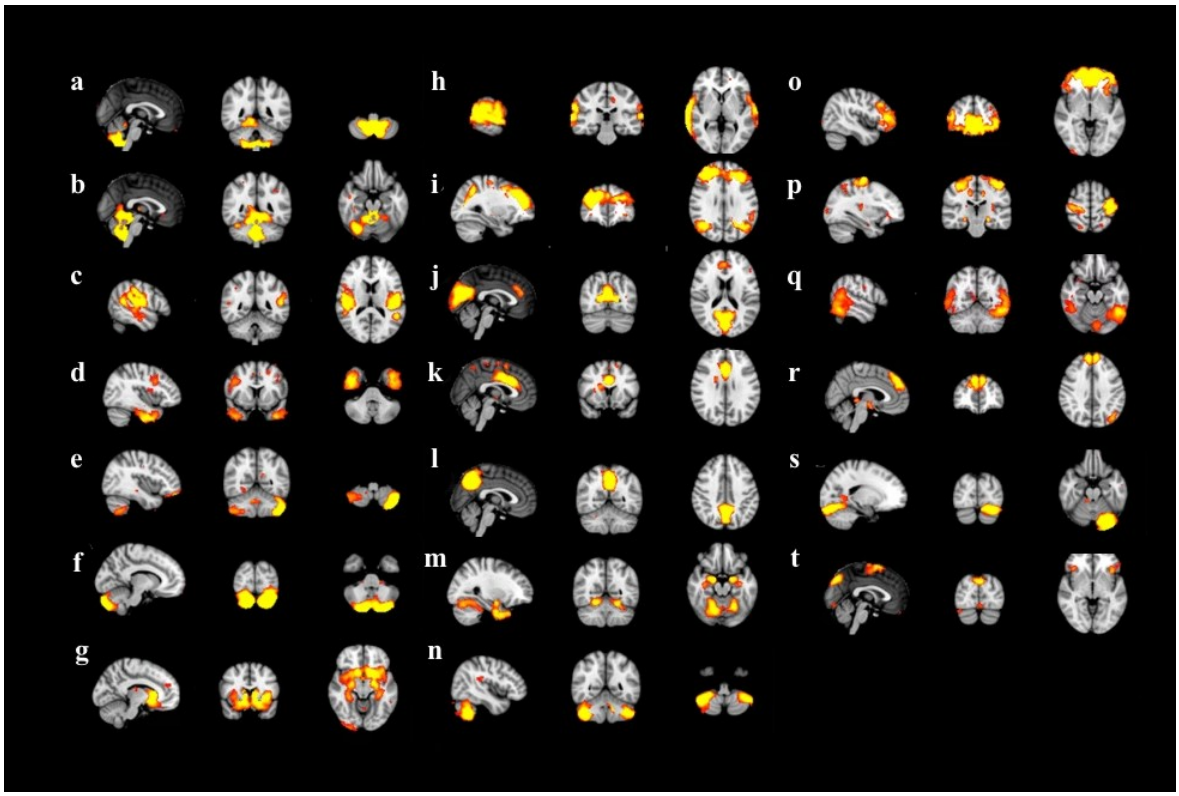

Figure 1: 20 SCN masks included in this study as identified by Koini and colleagues (2018). Figure adapted from "Grey-matter network disintegration as predictor of cognitive and motor function with aging" by M.Koini et al. 2018, Brain Structure and Function, 223(5), 2475-2487 (https://doi.org/10.1007/s00429-018-1642-0), CC-BY 4.0. Original network names can be found in Table 1.

Table 1: Adapted from "Grey-matter network disintegration as predictor of cognitive and motor function with aging" by M.Koini et al. 2018, Brain Structure and Function, 223(5), 2475-2487 (https://doi.org/10.1007/s00429-018-1642-0), CC-BY 4.0

SCN		Voxels	MNI coordinates			Location	Hem.
Koini	This work		x	y	z		
a	j	9488	2	-70	22	Cuneal cortex	R
		1682				Paracingulate gyrus	R
b	k	7872	0	16	28	Cingulate gyrus, anterior division	R
		1683	-34	-62	-4	Occipital fusiform gyrus	L
		1139	-34	-6	2	Insular cortex	L
c	l	6392	-2	-62	28	Precuneus cortex	L
		1095	12	-66	-26	Cerebellum (VI)	R
d	g	15,580	10	14	-12	Subcallosal cortex	R
		1484	28	-100	8	Occipital pole	R
e	d	10,442	40	-4	-40	Inferior temporal gyrus, anterior division	R
		5355	-38	8	-38	Temporal pole	L
		1466	0	20	40	Paracingulate gyrus	R
		1313	10	38	-4	Cingulate gyrus, anterior division	R
		1106	-32	22	-4	Insular cortex	L
f	m	10,911	18	-4	-20	Amygdala	R
		3841	-20	-4	-22	Amygdala	L
		1036	42	-22	56	Postcentral gyrus	R
g	i	17,295	30	46	20	Frontal pole	R
		3753	-42	-72	24	Lateral occipital	L
		3366	34	-66	20	cortex, superior division	R
						Lateral occipital cortex, superior division	
h	c	9650	-48	-26	16	Parietal operculum cortex	L
		7478	50	-28	16	Parietal operculum cortex	R
		1028	10	-26	34	Cingulate gyrus, posterior division	R
i	r	5332	8	50	34	Superior frontal gyrus	R
		4809	-8	-32	0	Thalamus	L
		1958	-42	-66	40	Lateral occipital cortex, superior division	L
j	p	6772	36	-24	56	Postcentral gyrus	R
		5894	-40	-18	58	Precentral gyrus	L
		1687	30	-56	-32	Cerebellum (VI)	R
		1183	24	-34	-2	Hippocampus	R
		1016	-24	-22	-12	Hippocampus	L
k	h	8071	66	-34	10	Superior temporal gyrus	R
		5038	-70	-26	8	Superior temporal gyrus	L
		2810	-54	-70	-36	Cerebellum (crus I)	L
		2500	-14	36	22	Paracingulate gyrus	L
l	o	23,648	2	44	-10	Paracingulate gyrus	R
		1581	34	-98	-2	Occipital pole	R

SCN		Voxels	MNI coordinates			Location	Hem.
Koini	This work		x	y	z		
m	q	11,130	-48	-54	-18	Inferior temporal gyrus, temporooccipital part	L
		5891	44	-40	-32	Cerebellum (crus I)	R
n	t	4451	2	2	66	Supplementary motor cortex	R
		4433	-38	22	-2	Insular cortex	L
		2532	2	-76	40	Precuneus cortex	R
		1843	-40	-58	-22	Temporal occipital fusiform cortex	L
		1115	40	-72	-20	Cerebellum (crus I)	R
u	b	15,844	2	-48	-50	Cerebellum (right IX)	R
		1247	12	-38	48	Precuneus	R
v	e	4741	-42	-72	-44	Cerebellum (crus II)	L
		2314	4	52	-28	Frontal medial cortex	R
		2022	44	-58	-52	Cerebellum (VIIb)	R
w	f	9773	-26	-88	-34	Cerebellum (crus II)	L
x	n	15,801	42	-54	-42	Cerebellum (crus I)	R
y	a	4618	10	-58	-54	Cerebellum (right IX)	R
		2411	12	-42	-16	Cerebellum (right I-IV)	R
z	s	6105	-28	-78	-22	Cerebellum (crus I)	L
		2357	16	-106	0	Occipital pole	R

Cognitive Assessment

The PRODEM study protocol includes the CERAD-Plus (Consortium to Establish a Registry for Alzheimer's Disease) test battery (88). It includes the following subtests: verbal fluency (s-words and animals), the Boston Naming Test, the Mini Mental State Examination, word lists (learning, recall, recognition), figures (drawing, recall) and Trail Making Test A and B. For statistical analysis we used the two subtests related to memory (verbal and figural) as well as a global, composite score based on the battery (Chandler score) (162). For figural memory and verbal memory, we calculated the savings scores, which describes the ratio of correctly recalled items to formerly learned items. This can be seen as a more direct measurement of memory retention over a short time, with a higher savings score describing higher retention of items.
As a general score for cognitive abilities we used the Chandler- score, a composite score of the Verbal fluency test (animals), Boston Naming Tests, word list learned, word list recalled, word list recognised and figural abilities (recalled) of the CERAD Plus (162). A higher Chandler-score depicts higher cognitive abilities and vice versa.

Covariates

The PRODEM study cohort data also contains the socio-economic variables age, sex and education, which will be included as covariates in this work. Education is comprised of elementary and secondary school (German: Volks-, Hauptschule), apprenticeship (German: Lehre), academic secondary school (German: allgemeinbildene höhere Schule - AHS), higher vocational school (German: berufsbildende höhere Schule - BHS), college of education (German: Lehrerbildungsanstalt - LBA), University (German: Hochschule).

Statistical Analysis

The statistical programming software R (on MacOS) in RStudio was used for all statistical analyses done in this thesis (163).

Classification

Regarding Hypothesis 1a and 1b, we used random forest (RF) models for the classification between AD and healthy controls (164). The algorithm "cforest" used is included in the "party" package (165,166). We split the available data into two independent groups, to use one for training (PRODEM and ASPSF from Graz, N = 104 each; Hypothesis 1) and a final subset to test the models (PRODEM from Vienna, ASPSF from Graz, N = 28 each ; Hypothesis 2). For this reason, the training of our algorithm is only based on the Graz sample of the PRODEM study and the matched ASPSF subjects. Validation is done with the method of "out of bag" prediction, a characteristic of RF algorithms, which resamples automatically within the training set. As an independent test set for diagnostic power, the patient sample of Vienna and their matched ASPSF sample was used. Diagnostic power will be reported with the area under the curve (AUC) of the receiver operating characteristic (ROC), a measurement of diagnostic accuracy ranging from 0.5 (random guessing) to 1 (absolute correct classification) in case of two groups (167). This differs from accuracy, sensitivity and specificity as measures for diagnostic power used in journal article this dissertation is based on (168). The AUC depicts

sensitivity and specificity at different decision thresholds for a classification algorithm, providing a summary measure to compare models against each other (169).
The parameters for the cforest algorithm were chosen as follows: ntree = 1001 (1001 trees for every random forest), “mtry” (number of predictors randomly chosen for every single tree) was left at the default, resulting in 5 predictors. Variable Importance (VI) was calculated for the model containing all SCNs. VI is a measure of loss in diagnostic power if an individual predictor (SCN) is excluded from the model (166,170). We set a cutoff of VI > 1 to choose significant SCNs for diagnostic power. This cutoff was chosen somewhat arbitrarily but based on an expected (a) moderate to high diagnostic power of the full random forest model (including all SCNs) and (b) correlation between the SCNs which lessens the likelihood of strong losses in diagnostic measures due to removal of single predictors.

Established visual and automated marker

Regarding hypothesis 2, we used the same analysis workflow as mentioned in the last subchapter. Random forest models containing the SCNs with VI > 1 will be extended with the features BV, HV and MTA-score to compare their classification abilities against each other. VI will also be calculated for the model containing diagnostically significant SCNs and established markers. AUCs analysis will again be reported to compare and validate the models in the independent sample.

Cross-sectional association of SCNs with cognition

Regarding hypothesis 3a, all SCNs with VI > 1 were chosen for further analyses of the association with cognitive abilities based on N=82 PRODEM patients. Integrity scores of SCNs were used in three multiple linear regression models (“lm” function in base R) to predict performance in the Chandler aggregated cognitive ability score, verbal memory savings score and figural memory savings score. Models were corrected for age (as metric variable), sex (as binary variable) and education (as dummy variable).

Regarding hypothesis 3b, all SCNs with VI > 1 were chosen for further analysis of the association with change of cognitive abilities of N=82 PRODEM patients. To measure change, baseline and follow-up clinical measurements were aggregated by subtracting the baseline score from the follow-up score (negative values show a decrease in cognitive ability) and divide the result by the amount of time between measurements in months. The SCN integrity scores were used in three multiple linear regression models ("lm" function in base R) to predict change in the Chandler aggregated cognitive ability score, verbal memory savings score and figural memory savings score. Models were corrected for age (as metric variable), sex (as binary variable) and education (as dummy variable).

Results

Participants

Overall, 104 AD patients ($mean_{age}$ = 71.45 ± 7.97 years, $range_{age}$ = 51–87 years, 59 females) from the Medical University of Graz, Austria, and 28 AD patients ($mean_{age}$ = 73.79 ± 6.17 years, $range_{age}$ = 58–82 years, 14 females) from the Medical University of Vienna, Austria, were included, as described in (168). In the statistical analysis, the sample of Graz was used for statistical modelling, while the sample of Vienna served as the independent validation cohort. For all AD patients, healthy elderly controls from the ASPS were matched. Matching was done for age (+/− 3 years) and sex (Graz: N = 104, $mean_{age}$ = 71.09 ± 7.38 years; $range_{age}$ = 53–86 years, 59 females; Vienna: = 28, $mean_{age}$ = 72.44 ± 7.18 years; $range_{age}$ = 56–85 years, 14 female).

Eighty-two AD patients from Graz had a follow-up clinical and cognitive assessment with time between assessments ranging between 6 and 37 months ($mean_{timedifference}$ = 18 months; $mean_{age}$ = 70.73 ± 8.26; $range_{age}$ = 51-87; 46 females). At follow-up, 3 patients had no data point for the Chandler score, and 5 patients had no data point for wordlist savings (overall 7 patients with one of them having no data points in both cases). Figures savings data at follow up was available for all patients. Patients who dropped out from the study did not differ from subjects with follow-up in terms of the Mini Mental State Examination scores at baseline, age, gender or education. Median and IQR of cognitive functions at baseline and follow-up can be found in Table 2 and a visual representation of the change from baseline to follow-up in Figure 2.

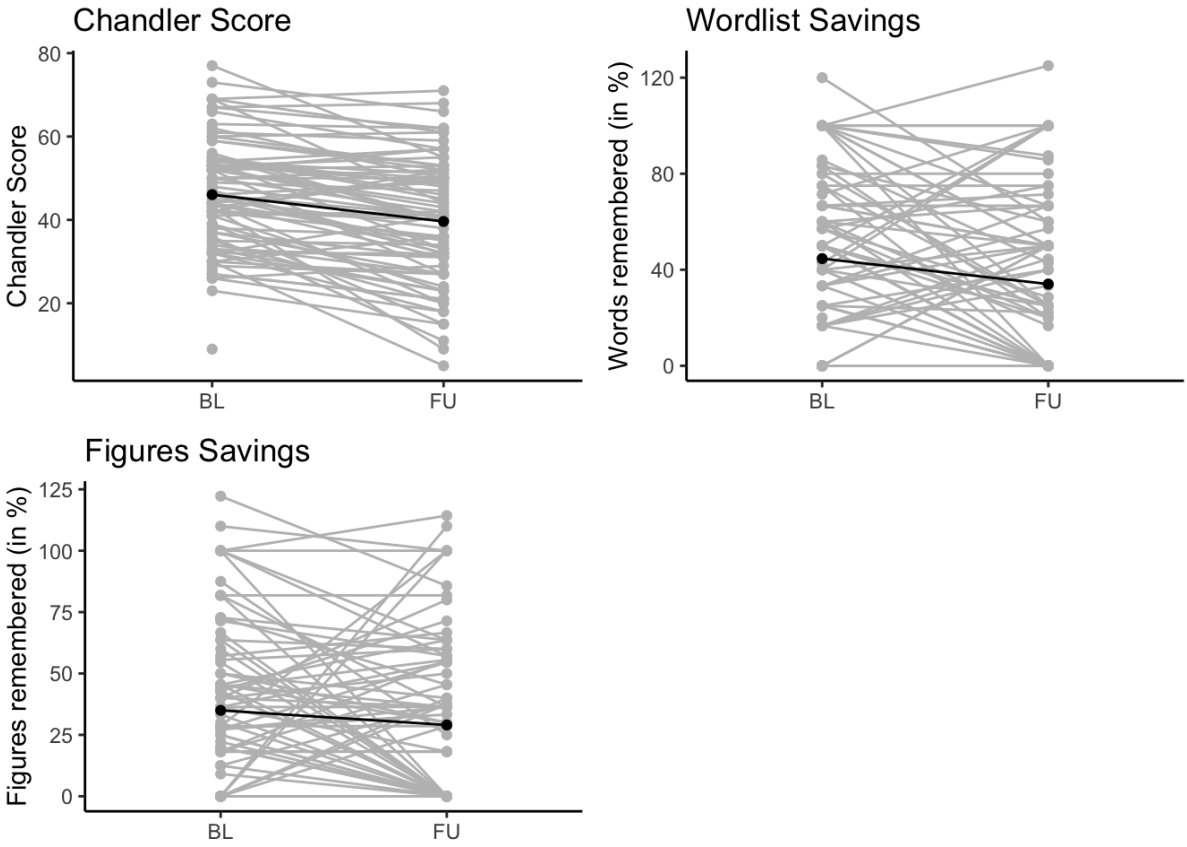

Figure 2: Change of cognitive abilities from baseline (BL) to follow-up (FU). Grey lines depict individual change between timepoints and the black line depicts change between the mean at baseline and the mean at follow-up. Individual points with no connected lines show BL measurements without FU. In all three cases, higher scores are associated with higher cognitive abilities (N=82 for all BL measurements, N=79 for Chandler Score FU, N = 77 for Wordlist Savings FU, N = 82 for Figures Savings FU).

Table 2: Median and IQR (in brackets) for the three cognitive measures used in this work. Savings scores describe the percent of recalled information at a later point in time (N = 82 for all BL measurements, N = 79 for Chandler Score FU, N = 77 for Wordlist Savings FU, N = 82 for Figures Savings FU).

	Baseline	Follow-up
Chandler score (global cognition)	46.00 (36.25-54.00)	41.00 (28.00-50.50)
Wordlist savings (memory)	50.00 (16.66-66.66)	25.00 (0.00-57.14)
Figure savings (memory)	28.57 (0.00-53.40)	18.18 (0.00-54.54)

Classification

To investigate hypothesis 1a and 1b, we evaluated the diagnostic power of 20 SCNs (144) for the classification between AD patients and healthy elderly, using random forest algorithms. Figure 3 shows the VIs for all SCNs based on this random forest model. Only two SCNs showed VI > 1 (SCN c and d). SCNs c and d were identified as a secondary somatosensory SCN and a temporal SCN, respectively (144). Most SCNs scored around VI=0, meaning that their individual exclusion from the model would not lead to loss in classification ability.

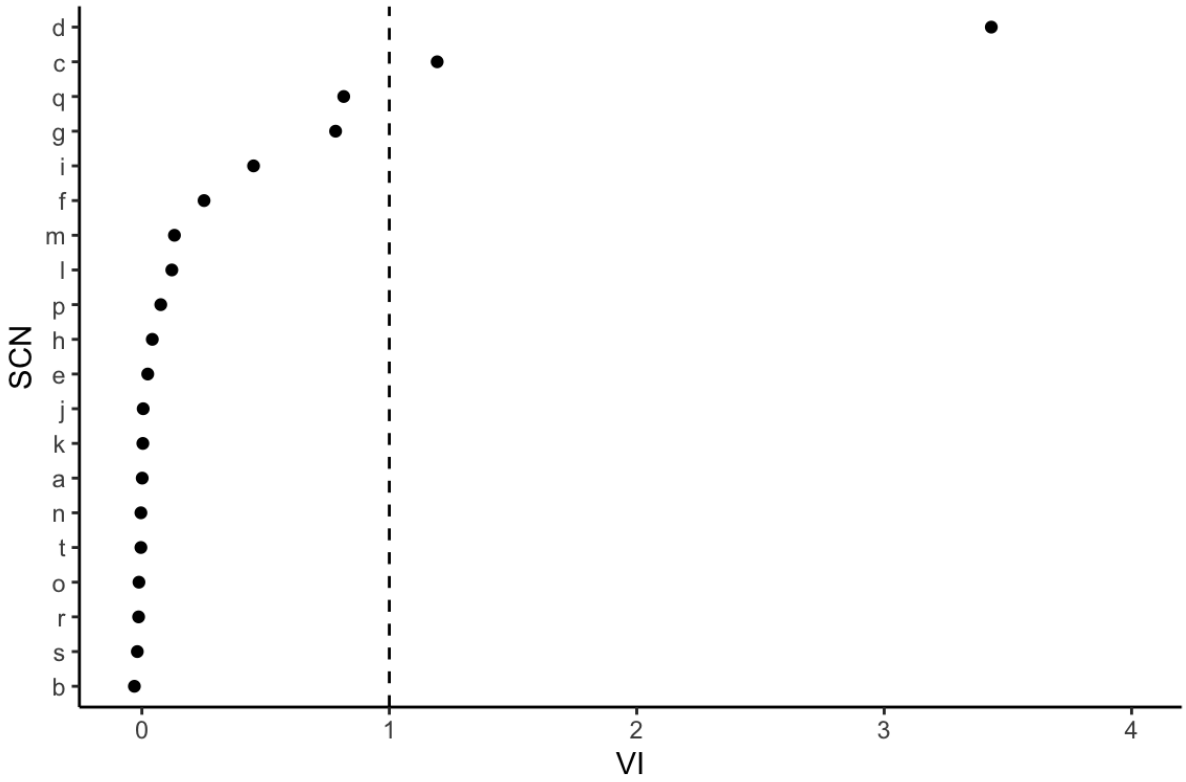

Figure 3: VI for all 20 SCNs used in this work. The vertical line marks the VI cut-off as decided by the researchers of this work.

AUC Analysis results can be found in Figure 4. The random forest model containing all SCNs showed an AUC of 0.87. We also performed a random forest analysis for the two SCNs with VI > 1 and a random forest analysis with the other SCNs. The model containing the two SCNs resulted in an AUC of 0.81, while the model containing the other eighteen SCN performed

similar with an AUC of 0.81. A similar pattern was found in the independent sample ($AUC_{All\ SCN}$: 0.88, $AUC_{two\ significant\ SCN}$: 0.86, $AUC_{other\ eightten\ SCN}$: 0.85).

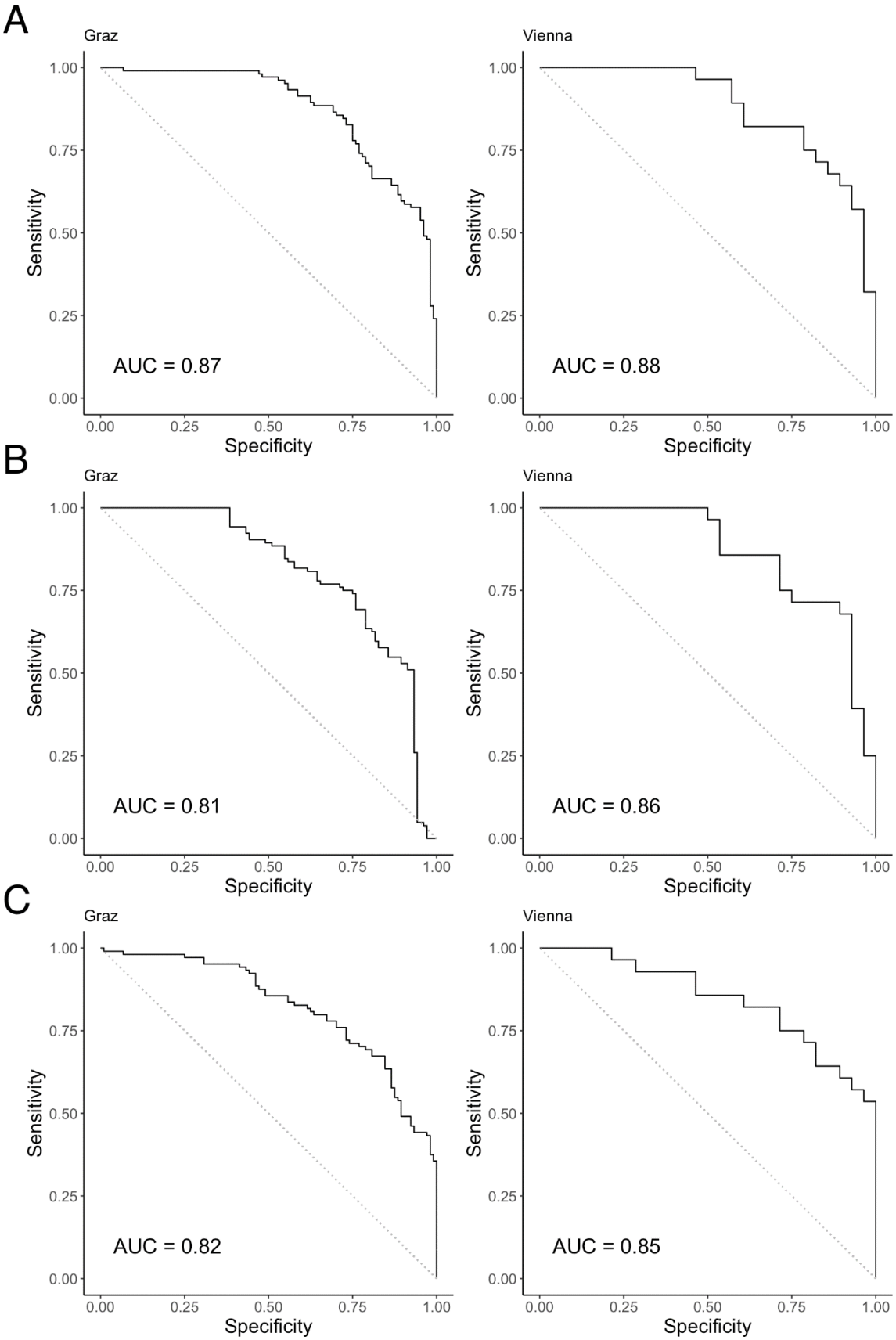

Figure 4: ROC AUC curves for random forest models. A: All 20 SCNs in one analysis. B: The temporal SCN and the secondary somatosensory SCN only. C: The other remaining 18 SCN only. Left side shows curve for the model trained on the Graz subset. Right side shows curve, when trained model is validated on Vienna subset.

The ridgeline plot in Figure 5 provides further information on the above reported results. The plot shows SCN connectivity distributions compared between AD patients and healthy elderly from the Graz sub-cohort for all 20 SCNs. All SCNs showed a strong overlap of network integrity distributions between AD and healthy elderly. SCN c and d (the ones with VI > 1) showed the comparatively lowest overlap.

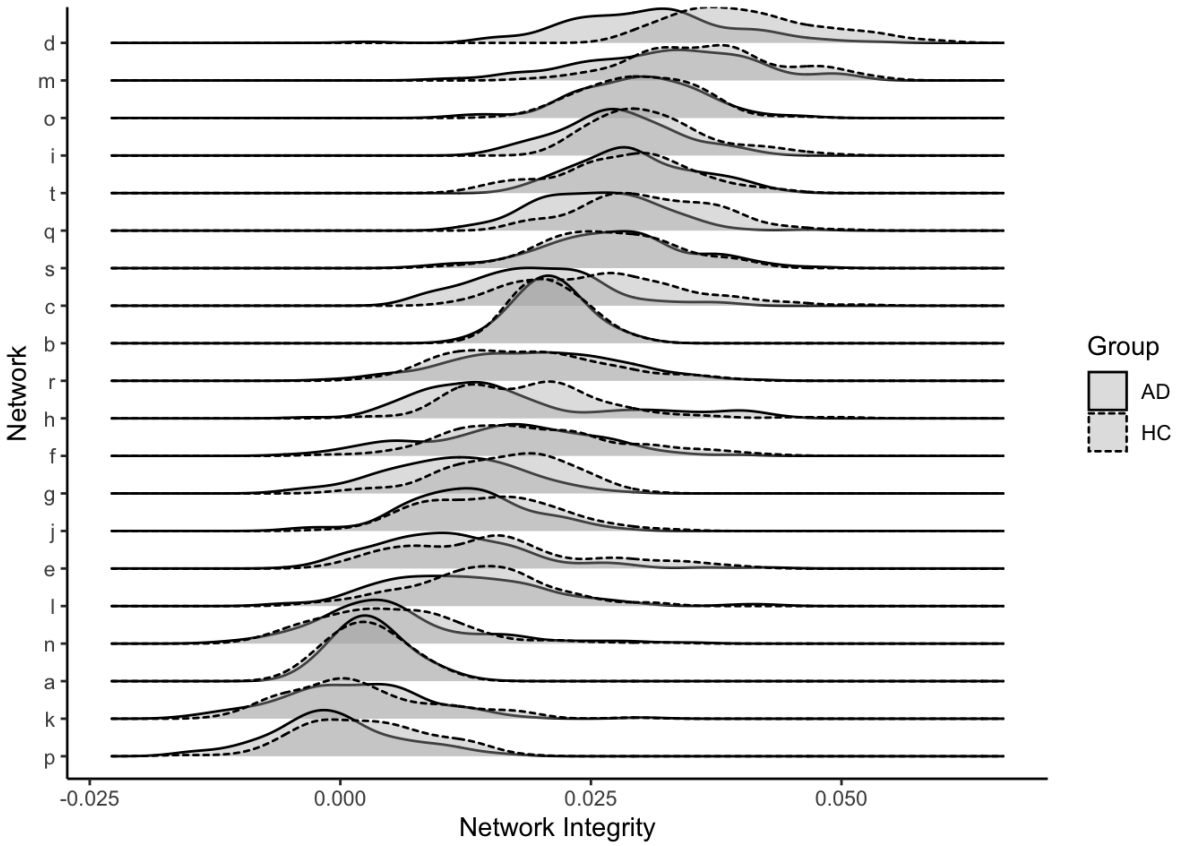

Figure 5: Ridgeline plot showing integrity distributions for all SCNs grouped by AD and HC. Integrity is based on individual beta scores as provided by dual regression analysis. The plots show the Graz subset only. The two SCNs that showed significant diagnostic power were c (secondary somatosensory) and d (temporal).

Comparison of established marker and SCNs

According to our hypothesis 2, we examined the diagnostic power of the significant SCNs compared to the already established markers BV, HV and MTA score. We calculated a random forest model containing both SCNs (secondary somatosensory - c and temporal - d) and the three established markers. Figure 6 shows the extracted VI scores for those 5 markers. In this

model, the BV and the MTA Score outperformed the others, including the SCNs, which in this case do not reach the determined cutoff of VI>1.

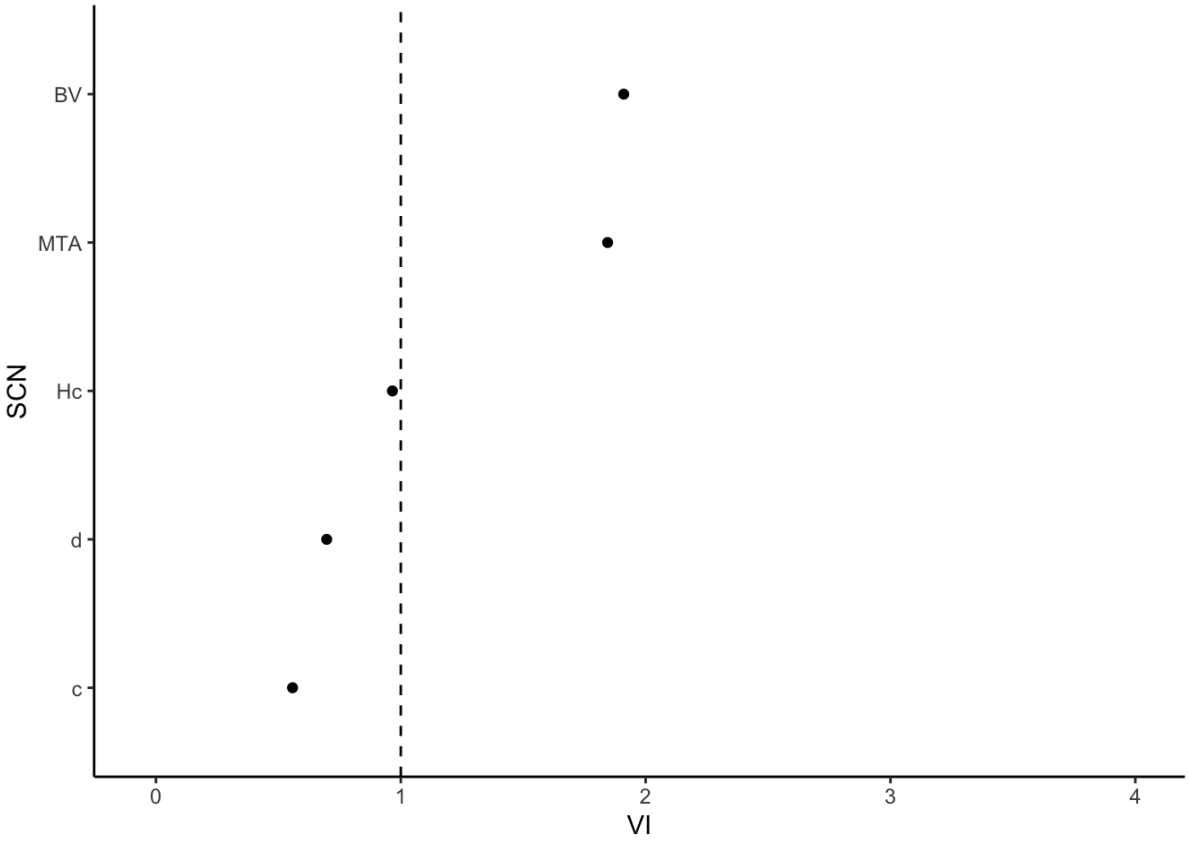

Figure 6: VI shown for the temporal SCN and the secondary somatosensory SCN in comparison to the established markers MTA-score, brain volume (BV) and hippocampus volume (HV). All three established markers perform better than the SCNs and the added information provided by the SCN is less than 1 in all cases.

The model containing the secondary somatosensory SCN (c), temporal SCN (d), BV, HV and MTA score resulted in an AUC of 0.89, which is equivalent for a model only containing the three established markers (AUC = 0.89). Compared to that, the AUC containing the secondary somatosensory SCN (c) and the temporal SCN (d) only reaches an AUC of 0.81. A similar pattern was found in the independent sample ($AUC_{sigSCNandestablished}$: 0.92, $AUC_{established}$: 0.92). The AUCs can be found in Figure 7.

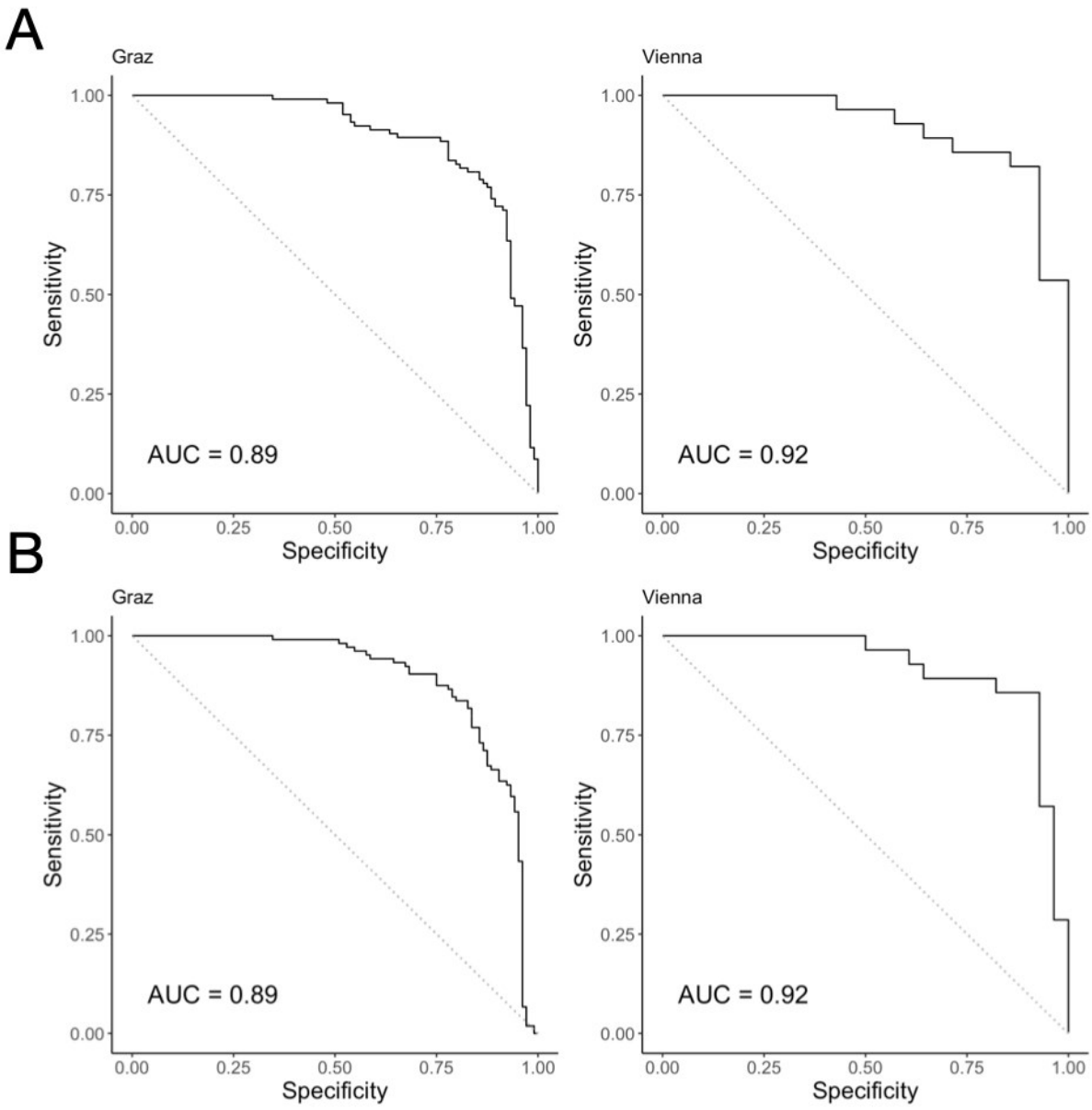

Figure 7: ROC AUC curves for random forest models. A: the temporal (d) and secondary somatosensory (c) SCNs and established markers are included in the model. B: Only the established markers were included. Left side shows curve for the model trained on the subset of Graz. Right side shows curve, when trained model is validated on the subset of Vienna.

SCNs cross-sectional Association to Cognition

For the investigation of hypothesis 3a, three multiple linear regression models were calculated to determine the association of the secondary somatosensory and the temporal SCN integrity

with cognitive ability in AD patients at baseline. We found the temporal network to be predictive of verbal memory, but this effect did not survive after controlling for age, sex and education. None of the other two models showed statistically significant associations to the networks. Details can be seen in Figure 8, Table 3, Table 4 and Table 5.

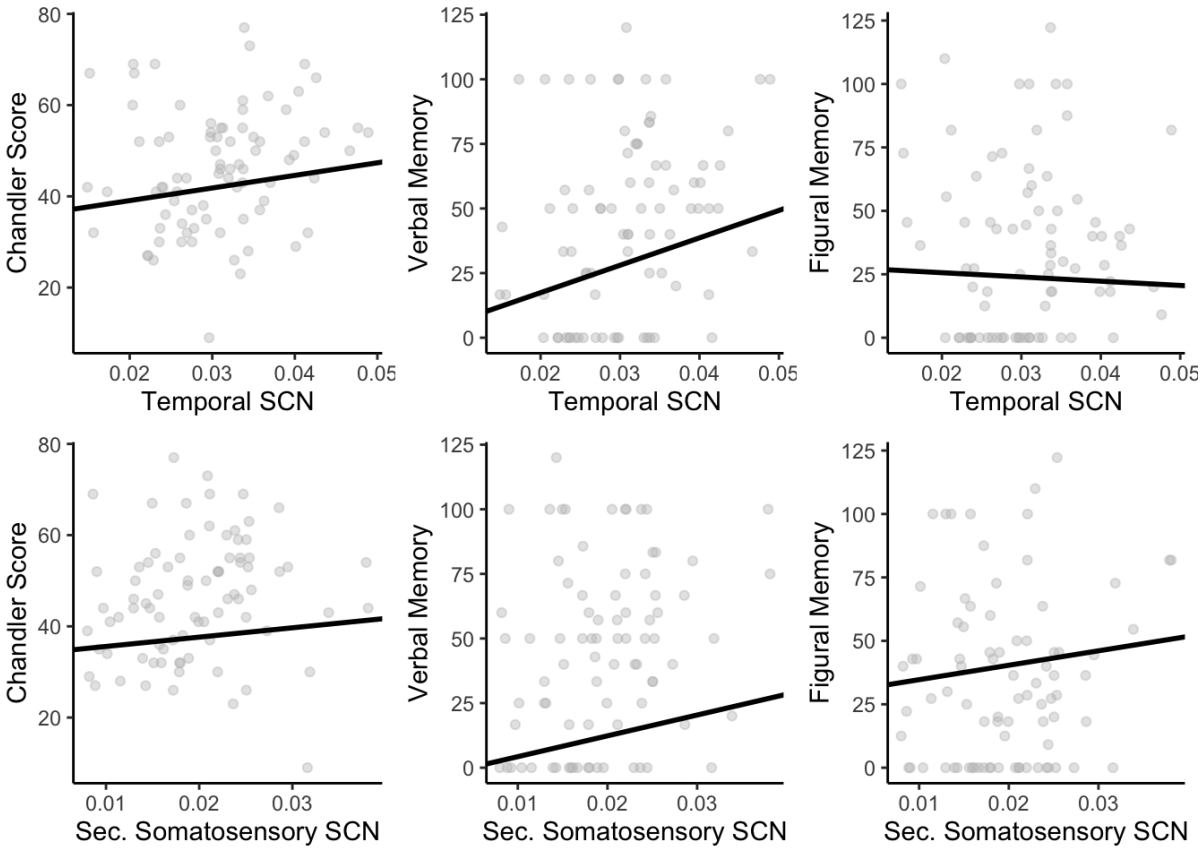

Figure 8: Slopes and intercepts for the influence of the temporal SCN and secondary somatosensory SCN on the cognitive abilities at baseline. Slopes and intercepts were drawn from the linear regression models (N =82). Corresponding Model parameters can be found in Table 3, Table 4 and Table 5.

Table 3: Multiple linear regression results of the model investigating association between SCN integrity (independent variables) and Chandler score (dependent variable). Results are shown after controlling for age, sex and education (N = 82).

Chandler score at Baseline				
	B estimate	std.error	t-statistic	p.value
Intercept	17.237	17.907	0.962	0.338
Temporal SCN	299.208	209.189	1.430	0.156
Secondary Somatosensory SCN	308.111	242.457	1.270	0.207
Age	0.134	0.189	0.706	0.481
Sex	4.281	3.178	1.347	0.182
Education (Apprenticeship)	1.114	3.666	0.303	0.762
Education (AHS)	0.457	6.094	0.075	0.940
Education (BHS)	-6.798	8.115	-0.837	0.404
Education (LBA)	3.705	6.440	0.575	0.566
Education (University)	10.205	5.120	1.993	0.050
				$R^2 = .128$, $R^2_{adj} = .019$, $F_{(9,72)} = 1.176$, $p = .323$

Table 4: Multiple linear regression results of the model investigating association between SCN integrity (independent variables) and verbal memory (dependent variable). Results are shown after controlling for age, sex and education (N = 82).

Verbal savings score at Baseline				
	B estimate	std.error	t-statistic	p.value
Intercept	-4.682	46.321	-0.101	0.919
Temporal SCN	967.612	541.117	1.788	0.077
Secondary Somatosensory SCN	1002.231	627.172	1.598	0.114
Age	-0.022	0.490	-0.045	0.963
Sex	1.329	8.221	0.161	0.871
Education (Apprenticeship)	1.083	9.484	0.114	0.909
Education (AHS)	-8.786	15.765	-0.557	0.579
Education (BHS)	-26.944	20.991	-1.283	0.203
Education (LBA)	1.496	16.660	0.089	0.928
Education (University)	15.228	13.244	1.149	0.254
				$R^2 = .144$, $R^2_{adj} = .037$, $F_{(9,72)} = 1.35$, $p = .227$

Table 5: Multiple linear regression results of the model investigating association between SCN integrity (independent variables) and figural memory (dependent variable). Results are shown after controlling for age, sex and education (N = 82).

Figural savings score at Baseline				
	B estimate	std.error	t-statistic	p.value
Intercept	78.874	44.020	1.791	0.077
Temporal SCN	-338.540	514.242	-0.658	0.512
Secondary Somatosensory SCN	537.803	596.023	0.902	0.369
Age	-0.517	0.466	-1.110	0.270
Sex	-17.585	7.813	-2.250	0.027*
Education (Apprenticeship)	0.941	9.013	0.104	0.917
Education (AHS)	-5.544	14.982	-0.370	0.712
Education (BHS)	22.946	19.948	1.150	0.253
Education (LBA)	-3.505	15.832	-0.221	0.825
Education (University)	15.594	12.587	1.238	0.219
				$R^2 = .154$, $R^2_{adj} = .048$, $F_{(9,72)} = 1.458$, $p = .180$

SCNs longitudinal Association to Cognition

Regarding hypothesis 3b, three multiple linear regression models were calculated to investigate the association of SCN integrity to change in cognitive ability in AD patients. Sample sizes for the three models differed due to data availability at follow up (N = 79 for Chandler Score, N = 77 for Wordlist Savings, N = 82 for Figures Savings). Change over time of the Chandler score,

wordlist savings and figures savings can be seen in Figure 2. Neither the temporal nor the secondary somatosensory SCN had predictive value for cognitive functions. Details can be found in Figure 9, Table 6, Table 7 and Table 8.

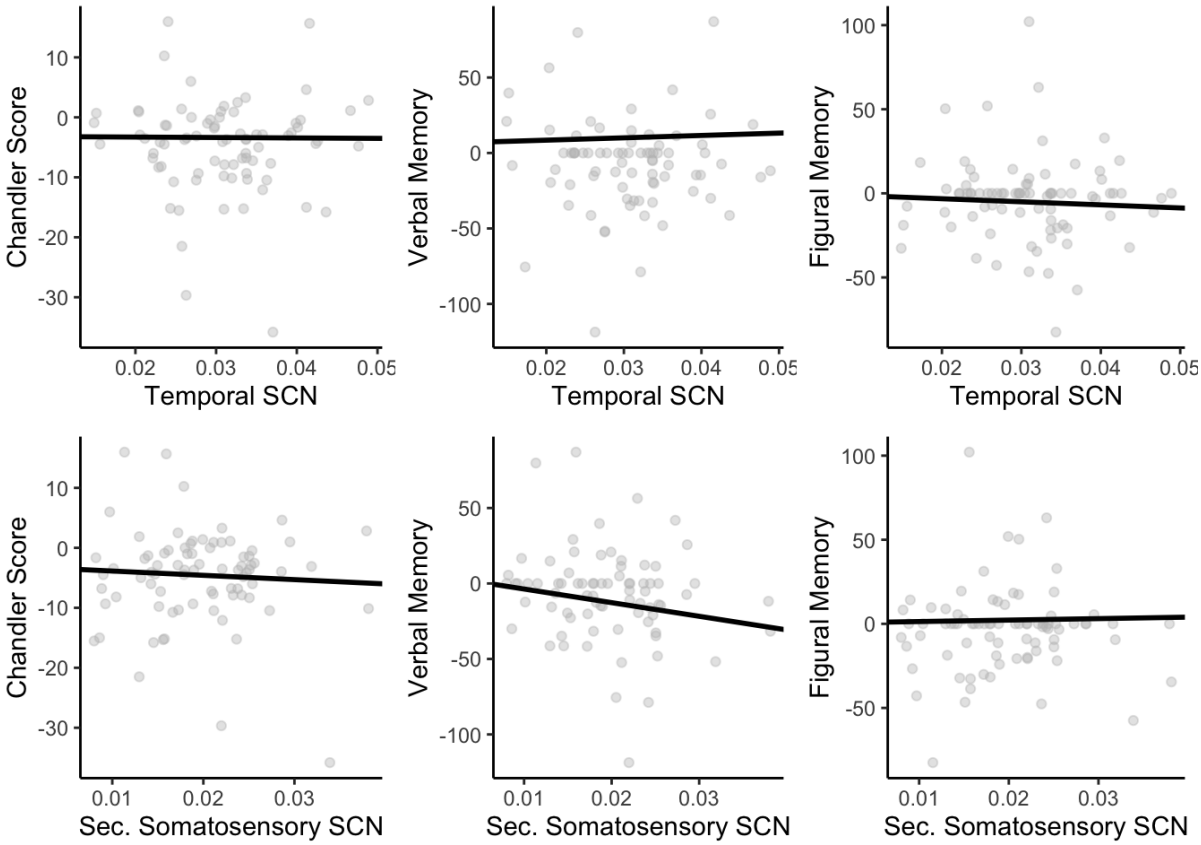

Figure 9:Slopes and intercepts for the influence of the temporal SCN and secondary somatosensory SCN on the change of cognitive abilities over time. Slopes and intercepts were drawn from the linear regression models (N=79 for Chandler Score FU, N = 77 for Wordlist Savings FU, N = 82 for Figures Savings FU). Corresponding model parameters can be found in Table 6, Table 7 and Table 8.

Table 6: Multiple linear regression results of the model investigating association between SCN integrity (independent variables) and longitudinal change in Chandler score (dependent variable). Results are shown after controlling for age, sex and education (N = 79).

Chandler score - change over time

	B estimate	std. error	t-statistic	p-value
Intercept	-9,898	11,094	-0,892	0,375
Temporal SCN	18,531	131,283	0,141	0,888
Secondary Somatosensory SCN	-50,576	148,76	-0,34	0,735
Age	0,117	0,116	1,01	0,316
Sex	-2,664	1,916	-1,39	0,169
Education (Apprenticeship)	-1,234	2,252	-0,548	0,585
Education (AHS)	2,15	3,71	0,579	0,564
Education (BHS)	-7,869	4,888	-1,61	0,112
Education (LBA)	-7,497	3,884	-1,93	0,058
Education (University)	-1,858	3,068	-0,606	0,547

$R^2 = .145$, $R^2_{adj} = .034$, $F_{(9,69)} = 1.306$, $p = .249$

Table 7: Multiple linear regression results of the model investigating association between SCN integrity (independent variables) and longitudinal change in verbal memory (dependent variable). Results are shown after controlling for age, sex and education (N = 77).

Verbal savings score - change over time

	B estimate	std. error	t-statistic	p-value
Intercept	17,685	42,799	0,413	0,681
Temporal SCN	287,633	505,414	0,569	0,571
Secondary Somatosensory SCN	-1129,893	609,674	-1,853	0,068
Age	0,001	0,459	0,001	0,999
Sex	-16,343	7,588	-2,154	0,035*
Education (Apprenticeship)	-6,151	8,739	-0,704	0,484
Education (AHS)	16,525	14,394	1,148	0,255
Education (BHS)	13,756	22,711	0,606	0,547
Education (LBA)	-17,483	15,114	-1,157	0,251
Education (University)	-8,038	11,977	-0,671	0,504

$R^2 = 0.136$, $R^2_{adj} = 0.020$, $F_{(9,67)} = 1.176$, $p = .324$

Table 8: Multiple linear regression results of the model investigating association between SCN integrity (independent variables) and longitudinal change in figural memory (dependent variable). Results are shown after controlling for age, sex and education (N = 82).

Figural savings score - change over time

	B estimate	std. error	t-statistic	p-value
Intercept	-38,218	35,133	-1,088	0,28
Temporal SCN	-175,323	410,413	-0,427	0,671
Secondary Somatosensory SCN	367,257	475,682	0,772	0,443
Age	0,448	0,372	1,205	0,232
Sex	8,421	6,236	1,35	0,181
Education (Apprenticeship)	-2,132	7,193	-0,296	0,768
Education (AHS)	-11,149	11,957	-0,932	0,354
Education (BHS)	-17,581	15,921	-1,104	0,273
Education (LBA)	-12,743	12,636	-1,008	0,317
Education (University)	-3,166	10,046	-0,315	0,754

$R^2 = 0.084$, $R^2_{adj} = -0.029$, $F_{(9,72)} = 0.741$, $p = .669$

Discussion

Summary of the main results

The aim in this thesis was to (a) investigate classification abilities of SCNs for the differentiation between AD patients and a control group of cognitively healthy aging elderly in contrast to established biomarkers and (b) to examine the SCNs ability to associate and predict impairment of cognition and future cognitive decline due to AD.
SCNs are GM networks identified by means of population-based covariation in anatomy, revealing even region overarching associations (171) and have been shown in the past to be distinctively affected by different forms of dementia (140,149). Furthermore, they were found to be predictors of memory ability in the elderly, a result persisting even when age was statistically accounted for (144).

Twenty SCNs were examined, highlighting two SCNs with diagnostic power. These two SCNs visually correspond to a temporal network (network d) and a secondary somatosensory network (network c) (144), which spatially overlap with SCNs found in a study by Hafkemeijer and colleagues (149). The temporal SCN connects the right inferior temporal gyrus (anterior part), the left temporal pole, the right paracingulate gyrus, the right cingulate gyrus (anterior part) and the left insular cortex (144,168). These brain areas have been found to be associated with AD in the past (172–175) and were also found to be associated to aging in a study by Hafkemeijer and co-workers (142) using an equivalent methodological approach. The secondary somatosensory SCN (network c) contains the parietal operculum and the posterior cingulate cortex, a functional part of the DMN associated to AD (129). The DMN has been shown in AD to be particularly relevant due to its vulnerability to atrophy and its AD typical pathogenic changes (128,130,176). Functional connectivity within the DMN was found to change over the course of the disease, especially connectivity between the posterior cingular cortex and other structures, including the medial prefrontal cortex, the precuneus and the hippocampus was found to decrease with progression of AD (176).
When examining the diagnostic power of these networks in contrast to established markers, such as the hippocampal volume, brain volume and the MTA-score, the SCNs failed to outperform available biomarker. Also, the predictive performance of the SCNs for cognitive

status (memory and global cognitive functioning) and cognitive change over time are suboptimal and warrant further investigation.

A more detailed reflection of why we did not find significant results

In the following we want to discuss the results in further detail and try to give an explanation why our results deviate from published literature. The classification analyses of the SCNs in both independent cohorts (Graz and Vienna) revealed acceptable results (AUC > .8), with the temporal SCN and the secondary somatosensory SCN showing higher diagnostic power than the other SCNs. However, while they may have performed well compared to other SCNs, they were outperformed by all three established markers, the BV, HV and the MTA-score. Additionally, in a combined classification model with the established markers, neither the temporal nor the secondary somatosensory SCN reached the cut-off for VI as chosen in this work. This might be due to the three established markers already containing most of the diagnostic information provided by the temporal and secondary somatosensory SCN, with the SCNs adding no further diagnostic power to a combined model. In our analyses, the BV and the MTA-score provided VI higher than 1, which might imply that the combined information based on a global (BV) and local (MTA-score) marker already provides considerable value for classification.

Moreover, we examined the association and the prognostic power of these SCNs for verbal and visual memory and global cognitive functions. However, the results of these analyses also were below our expectations, not showing any association when controlling for confounding variables such as age, sex and education. Overall, our findings with respect to diagnostic accuracy could not replicate the results of studies which argue that structural networks in the brain, mirroring synchronous degeneration by detrimental effects of AD, contain diagnostic information exceeding volumetric assessment of isolated areas (9,140,177,178).

Groundwork for the network hypothesis of degeneration is the paramount study by Seeley et al. (140). Seeley and colleagues argued that network measures provide advantages for differential diagnosis of dementia, by showing different types of dementia to be (dis-) connected to different patterns of simultaneous degeneration in GM. Most prominent in the case of AD was the DMN, which was identified as a promising marker in multiple other studies

and expected to outperform isolated areas as markers for AD progression (140,146–149). However, our results do not support this assumption. In our study, the SCNs used were not able to exceed the diagnostic accuracy of those isolated volumetric markers and more surprising, could not add diagnostic information of interest either. There are multiple potential reasons why our study could not meet the expected diagnostic power. For one, most of the above-mentioned studies investigated differences in SCNs between groups of interest, like AD, healthy elderly, and other neurodegenerative diseases. While differences in SCN connectivity between AD and healthy elderly were found repeatedly in studies with sample size ranges of N_{AD} = 24-109 patients and N_{HC} = 30-150 subjects (140,146–149), methodical differences and/or lack of effect sizes leave doubt on the clinical usability of SCN connectivity to classify individuals with high degrees of certainty (179).

To examine this issue within our own data, we added additional analyses in the supplementary material of this thesis. In Table 9 (Supplementary) we show the results of t-tests, comparing the SCN integrity as measured by beta Scores for all individual networks between AD and healthy elderly from the Graz cohort. After Bonferroni correction, eight of the SCNs showed statistically significant differences in their connectivity. This validates established studies that found differences in SCN integrity between AD patients and healthy controls (140,149). Still, the strong overlap of SCN integrity distributions between AD and healthy elderly as shown in Figure 5 introduces a high degree of uncertainty for an algorithm assigning individuals to a group. In Figure 10 (Supplementary), we add the integrity scores of one randomly chosen individual from our sample to the corresponding network distributions (based on set.seed(12345) and the sample() function in R). Taking the simplest random forest model of this work, the assignment of this individual to the group of AD patients or healthy controls is only based on the network integrities of the temporal SCN and the secondary somatosensory SCN. In this particular case, the integrity scores of the temporal SCN and the secondary somatosensory SCN provide rather inconclusive information for group assignment. The individual was assigned to healthy controls with a certainty of 50.77%. This also means that the model calculated a 49.23% probability that the individual belongs to the AD patients. (To the interested reader: the individual is part of the PRODEM cohort and was incorrectly assigned to the ASPSF cohort – by the random forest model containing the temporal and secondary somatosensory SCN). The AUCs reported in this work are acceptable, but cases like this

individual may be frequent within our cohort due to the large overlap in network integrity between AD patients and healthy controls.
In conclusion, while we consider the statistical differences of network integrity between groups as reliable, outperforming already established diagnostics based on the connectivity may still not be feasible.

Furthermore, while we found two diagnostically valuable SCNs, they did not contain all areas as expected in our hypotheses. Multiple studies have implied DMN, a hippocampal network, a salience network and an executive control network as potential markers, which can only limitedly be corroborated in our study (140,146,147,149). The SCNs containing the hippocampal structures (p and h) performed near zero at the VI analysis, implying that the networks individually added no further diagnostic accuracy between AD and healthy elderly. The SCN containing the precuneus (l), a functional area of the DMN (180), was also found to add nearly no diagnostic power compared to other SCNs. Montembeault and colleagues (146) defined the salience network and executive control networks as ROIs anchored at the right fronto-insular cortex and right dorsolateral prefrontal cortex as important for AD. Our masks, containing the right fronto-insular cortex or right dorsolateral prefrontal cortex, show visual differences to the ones reported by Montembeault. These differences might be expected due to methodical differences between ROI based network identification, which allows for non-allocated areas in the brain structure compared to SCN identification based on ICA, which must segment the complete GM structure into a pre-determined number of components. Randomness due to different samples also might play a factor, especially when network identification is based on only healthy subjects as compared to a mixed group (healthy and AD patients).
It is important to consider that a near zero VI in our study does not conclude zero diagnostic power. Our SCNs showed strong multicollinearity, implying that they might contain similar information about structural atrophy. As we have seen in our analyses, exclusion of the temporal and secondary somatosensory SCN still lead to a model performing comparably well. This might indicate diagnostic value for the remaining SCNs, just to a lesser individual degree compared to the temporal and secondary somatosensory SCNs.

Another aim of this work was to investigate if the connectivity of the SCNs with predictive diagnostic power also could predict individual scores for global cognition (Chandler score) and memory (verbal and figural) in AD patients. Only the temporal SCN was found to be a predictor for verbal memory, where loss of integrity was associated with decreased memory ability. However, this effect vanished after statistically controlling for age, sex and education. We could not reveal other significant associations between the integrity of the temporal SCN or the secondary somatosensory SCN with the change of global cognition or memory over time. This contrasts with multiple studies that found associations between network integrity and cognition as well as network integrity and the decline of cognition (143–145,148,150,181).

Koini and co-workers (144) found multiple associations between cognition and SCN integrity with the same imaging masks used in this current work. They found SCN integrity in healthy adults (N = 216 with neuropsychological test data, age ranged from 41 to 87 years) to be independently associated to memory (144,182) even when corrected for covariates like age, sex and education. This is true for the temporal, the secondary somatosensory, the limbic, the fronto-parietal, the fronto-occipital and the cuneal SCNs. Furthermore, they also found associations between the temporal, the secondary-somatosensory and multiple other SCNs with executive function. However, the methods for analysis of association are different: Koini and co-workers used random forest regression to identify associations to cognitive abilities (144). In this work random forest classification was used for differentiation between AD and healthy controls and linear regression was used to identify associations to cognitive abilities, which might be a reason why we could not reproduce their findings. Another reason might be strength of the association between SCN integrity and memory. Compared to the work of Koini and co-workers, our study included fewer subjects (82 AD patients vs. 216 healthy adults and elderly) and had a slightly less extensive test setup for memory performance (CERAD verbal and figural memory subtest vs. the Bäumler's Lern- und Gedächtnistest (182)), which might not be enough to reproduce the association found in their work (144).

Brickman and colleagues found SCNs to be associated to verbal list learning even after controlling for age (143). Steffener and colleagues used similar methods as Brickman et al. and could show associations between global structural covariance and verbal memory in different

age groups (181). Spreng and Turner (148) found association between the DMN SCN and global cognition (as measured by the MMSE) in healthy adults as well as in MCI and AD patients. Those associations remained significant after correction for age, gender, years of education, handedness, estimated total intracranial volume (eTIV) and estimated whole brain volume (eWBV). Foster-Dingley et al. found a temporal SCN which showed positive association to memory function measured by the 15-Word Verbal Learning Test (15-WVLT). Furthermore, they found an association between either, cerebellar-occipital SCN and occipital-precuneus SCN, and psychomotor speed (145). Dicks and colleagues investigated SCN connectivity in MCI patients and found that decreased network connectivity in temporal lobes and prefrontal areas showed an association to decline in memory. The research question was similar to ours, using baseline network integrity as predictor of cognitive decline over time, although investigated in MCI patients. They also found that lower structural connection in the right precuneus was associated to faster decline in memory. Other areas, including right supramarginal, middle occipital, superior parietal, middle temporal, parahippocampal gyrus and bilateral inferior temporal gyri, showed decreased connectivity which was positively associated to decline in memory. Lower connectivity associated to lower global cognitive abilities were found for left superior medial orbito-frontal, bilateral precentral gyrus, left anterior cingulate, right lingual gyrus, right fusiform gyrus, right hippocampus, left parahippocampal gyrus and right thalamus (150).

There may be multiple other reasons why we could not reproduce above-mentioned associations between SCN integrity and cognition. Some of the above-mentioned studies used other methods for SCN identification (143,148,150,181). The structural covariance of SCNs can be analysed by methods like network analysis based on graph theory, simple correlation analysis or like this work uses, independent component analysis. Methodical differences are a complication in context of comparability and might be a reason why we were not able to replicate the associations between SCNs and cognitive abilities. The differences in sample size of different studies (N = 61 to 434) for SCN identification might also have effects on the resulting SCNs (143,148,181). The study of Foster-Dingley et al. was methodically similar to this work but had a bigger sample size (N = 219), which might be the reason why they were able to identify an association between temporal SCN integrity and memory performance (145). Still, their group of interest was elderly persons with mild cognitive deficits due to small vessel

disease, while our work focuses on the effects of AD on SCNs, which might also be the basis for differences in findings (145).

Also, not all AD patients in our study showed decline in cognitive performance over the observational period (Figure 2). All three measures visually showed a decrease from baseline to follow-up on average, but 19.5% of patients showed an increase in Chandler score, 23.2% of patients showed an increase in word list savings and 25.6% of patients showed an increase in figure savings. It is important to mention that these percentages are not based on clinically accepted cut-off values of increase/decrease in cognitive abilities, they simply depict mathematically "positive" changes over time (>0) and are therefore likely not the best representation. Furthermore, it is possible that deviations from an overall decrease might be expected and are likely due to factors of confounding variables. Multiple factors might have influenced the measurement of cognitive abilities, including poor sleep quality, side effects of medications, bad mood, stress of any kind. Simply put, having a good or bad day introduces considerable within person variability of test results and might impact the ability to identify statistical effects (183–185). While the CERAD is a well-established test battery in AD research, more extensive memory tests to increase the reliability of measurement may be considered in the future.

Another influence to increase the variability in measurements may be the time between assessments of cognitive abilities. On average, the time difference between baseline and follow-up cognitive measurement was 1.5 years, but some were retested as early as 6 months after baseline (2 AD patients) and in 20% of AD patients, the follow up was less than a year later. Considering above mentioned influences for cognitive testing, shorter timeframes amplify the noise to signal ratio (confounding variables vs decline due to AD) masking possible effects. In the study of Dicks et al (150), follow-up was on average 2.3 years after baseline measurement. Their focus was on patients with MCI, but nearly 38% of their MCI patients received a diagnosis of probable or possible AD at follow-up, so we cannot exclude the, on average, shorter time between measurements as a factor why we could not replicate their findings.

Still, a study by Lim et al (186) investigated cognitive decline in an 18 month study period and found that even for mild AD cases, decline of verbal memory can already be shown after 3 to 6 months follow-up period, implying that the follow-up period in our study should be long

enough to exclude confounding variables as the driver for better performance in cognitive functions.

Limitations due to methodical choices

Our study includes multiple non-predetermined methodical steps in image and statistical analysis. For these steps, the researchers of this work had to make decisions which also may have influenced our outcomes.

One of these methodical steps includes the choice of network templates to extract information about individual network integrity. To our knowledge, two reasonable approaches for template selection exist. Either, one creates templates based on the available study sample, part of the study sample, or uses already created templates. Both of these approaches present advantages and disadvantages that are discussed in more detail in Bijsterbosch et al. (187).

Briefly summarised, Bijsterbosch et al. (187) report that the first mentioned approach of creating study specific templates based on all experimental groups provides higher statistical power as compared to the other options. The downside to this is approach is that the templates likely present hybrid networks of the experimental groups, which provides problems for interpretability.

In this work, we used network templates from another study of our work group which were constructed from an adequate sample size of non-demented aging individuals (144). These network templates provide comparability with previous work and should also be representative of healthy elderly networks. Still, loss of statistical power cannot be ruled out and may have influenced the outcome of this study.

Associated to the methodical choice above is the number of networks used to investigate our cohorts. The number of networks to decompose the brain into is an open discussion in the field (144,149,187,188). It is related to different levels in brain hierarchy. Decomposing into fewer networks results in big overarching networks and decomposing into a higher number of networks might result in split up sub-networks of these overarching networks. Hafkemeijer and colleagues (142) chose nine networks and found four of them (a subcortical network, a lateral occipital network, a posterior network and an anterior cingulate network) to be associated to age, atrophying over time. In case of the work of Koini and colleagues (144), the data was

decomposed into twenty networks, six of them were found to be associated to age (cuneal network, precuneus network, anterior-posterior network, a temporal network, a fronto-polar network and a motor network). These differences in the choice of number of networks may cause different results and interpretations. Still, a very interesting result in both studies was the visual similarity of multiple networks, despite the different methodical approach in the chosen number of networks. Nonetheless, we cannot rule out that a different number of networks and the associated different hierarchical level may have shown impact on our results.

Another reason for low diagnostic power and insignificant associations may have been the use of the MNI152 template for co-registration of the individual GM images to standard space. MNI152 may not be ideal, since the template is based on a healthy cohort of young adults (189), which might not be representative for elderly brains (190,191). Hence, especially with AD patients, there might, on average, exist strong spatial differences to the template, leading to distortion of information, masking effects of AD.

A general limitation factor in our study may be the limited number of cognitive domains analysed. There are multiple cognitive abilities affected over the course of AD and as a comprehensive battery, the CERAD+ battery measures memory, attention, language, praxis, and executive functions (88,92). However, for several reasons we decided to limit our analyses to a global score and measures for memory, since, global cognitive functions and memory are affected at very early stages of the disease and show highest sensitivity to AD related decline of ability (148,150,192).

A last weakness in our study was the absence of imaging data at follow-up. While the predictive ability of baseline network integrity would be of strong interest for clinical use, longitudinal change in integrity over time likely better mirrors the decline in cognitive ability and might be a better marker for AD diagnosis. While we did not find associations between SCNs integrity at baseline and the progression of cognitive decline, the decline might still be paralleled by atrophy of SCNs integrity over time. This is still an interesting question for future research and may provide valuable insights in the endeavor to treat AD.

Strengths

A strength of our study was the considerable sample size of our cohort as compared to other studies researching the effects of AD on SCNs (140,146–149). For the assessment of the diagnostic potential, it included 264 subjects of 132 AD patients and 132 age and sex matched healthy controls. Furthermore, imaging data of 28 AD patients were provided from a different centre, enabling us to use this part of the cohort (and matched healthy controls) as an independent sample for model validation, similar to established train/test split methods for machine learning based algorithms (193). Comparable results were found in training and validation context, which might imply robustness of our models even over different MRI sites.

Future directions

As mentioned above, in our study we tried to determine the association of baseline SCN integrity with cognitive change over time. The change of SCN integrity itself over time, and its possible association with decline of cognitive abilities will have to be determined in future studies. Furthermore, potential diagnostic value in different rates of SCN change between cognitively healthy elderly compared to patients with MCI, AD or other forms of dementia might also be of interest for future studies.

Still, Alzheimer's disease is a multifaceted disease. Multiple competing hypotheses for the diseases pathology have been formulated, resulting in many biomarkers that have been tested and found to be predictive of AD progression or to be valuable for diagnostic purposes. Due to its multifaceted nature, it is possible that markers for AD diagnosis and measurement will need to be integrated together into diagnostic processes, comparable to neuropsychological test batteries. Utilizing newer technologies in machine learning, Schouten and co-workers showed that the combined information of MRI markers, including anatomical, diffusion and resting state functional imaging, outperformed the single use of each one of them in diagnosing AD patients (N = 77) against healthy controls (N = 173). The combined model reached an AUC of 0.95 in comparison to grey matter density, the single anatomical marker with the highest AUC of 0.90 (194).

This was also found to be valid even if there were only anatomical markers combined into a single model. Classifying between AD (N = 21) and healthy controls (N = 21), the anatomical

markers cortical thickness, cortical area, cortical curvature, grey matter density, subcortical volumes and hippocampal shape together improved diagnostic power for AD over each single method. The whole model provided an AUC of 0.95-0.98 against the best single anatomical marker grey matter density with an AUC of 0.94 (195).

Furthermore, these types of multidimensional models, containing information from different sources, were also found to enhance diagnostic differentiation between AD and other causes for dementia. In a study by Bouts et al., classification models were built using a sample of AD patients (N = 30), bvFTD patients (N = 23) and healthy controls (N = 35) (196). For differentiation between AD and bvFTD, a combined model using mean diffusivity, functional connectivity and fractional anisotropy (AUC of 0.81) outperformed the best single marker mean diffusivity (AUC of 0.70). Addition of other markers did not enhance the AUC. For the differentiation between bvFTD and healthy controls, the best combined model was comprised of fractional anisotropy, grey matter density and functional connectivity (AUC of 0.92). It outperformed the best single marker fractional anisotropy (AUC of 0.86). For AD vs healthy controls the best model included grey matter density, axial diffusivity, fractional anisotropy, radial diffusivity, mean diffusivity (AUC = 0.94) and outperformed the best single marker grey matter density (AUC = 0.94) (196).

While these examples are based on imaging markers, the inclusion of CSF based markers, blood-based markers or cognitive scores might even enhance diagnostic power. And SCNs might be another building block to strengthen these diagnostic models.

Conclusion

In conclusion, we found that established volumetric markers and the visual MTA score show higher performance in the differentiation of AD from healthy controls as compared to SCNs, which need extensive image postprocessing. We also found that the SCNs with the highest diagnostic power show only weak or no association to cognition.

As to whether SCNs are helpful in discriminating between different forms of dementia syndromes needs to be determined and it is likely that longitudinal assessment of SCNs can increase our understanding of the spatial and timely evolution of neurodegenerative processes.

Bibliography

1. National Collaborating Centre for Mental Health (UK). DEMENTIA. British Psychological Society; 2007.

2. Bacigalupo I, Mayer F, Lacorte E, Di Pucchio A, Marzolini F, Canevelli M, et al. A Systematic Review and Meta-Analysis on the Prevalence of Dementia in Europe: Estimates from the Highest-Quality Studies Adopting the DSM IV Diagnostic Criteria. J Alzheimers Dis. 2018;66(4):1471–81.

3. Hofman A, Rocca WA, Brayne C, Breteler MM, Clarke M, Cooper B, et al. The prevalence of dementia in Europe: a collaborative study of 1980-1990 findings. Eurodem Prevalence Research Group. Int J Epidemiol. 1991 Sep;20(3):736–48.

4. GBD 2019 Dementia Forecasting Collaborators. Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. Lancet Public Health. 2022 Feb;7(2):e105–25.

5. Prince M, Wimo A, Guerchet M, Ali G-C, Wu Y-T, Prina M. World Alzheimer Report 2015 - The Global Impact of Dementia: An analysis of prevalence, incidence, cost and trends. 2015 Sep 21; Available from: https://kclpure.kcl.ac.uk/portal/en/publications/world-alzheimer-report-2015-the-global-impact-of-dementia-an-anal

6. Brunnström H, Gustafson L, Passant U, Englund E. Prevalence of dementia subtypes: a 30-year retrospective survey of neuropathological reports. Arch Gerontol Geriatr. 2009 Jul;49(1):146–9.

7. Alzheimer's Association. 2013 Alzheimer's disease facts and figures. Alzheimers Dement. 2013 Mar;9(2):208–45.

8. Zekry D, Hauw J-J, Gold G. Mixed dementia: epidemiology, diagnosis, and treatment. J Am Geriatr Soc. 2002 Aug;50(8):1431–8.

9. Brier MR, Thomas JB, Ances BM. Network dysfunction in Alzheimer's disease: refining the disconnection hypothesis. Brain Connect. 2014 Jun;4(5):299–311.

10. Palop JJ, Mucke L. Network abnormalities and interneuron dysfunction in Alzheimer disease. Nat Rev Neurosci. 2016 Dec;17(12):777–92.

11. Delbeuck X, Van der Linden M, Collette F. Alzheimer's disease as a disconnection syndrome? Neuropsychol Rev. 2003 Jun;13(2):79–92.

12. Jones DT, Knopman DS, Gunter JL, Graff-Radford J, Vemuri P, Boeve BF, et al. Cascading network failure across the Alzheimer's disease spectrum. Brain. 2016 Feb;139(Pt 2):547–62.

13. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. Science. 1992 Apr 10;256(5054):184–5.

14. Hardy J, Allsop D. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. Trends Pharmacol Sci. 1991 Oct;12(10):383–8.

15. Mudher A, Lovestone S. Alzheimer's disease-do tauists and baptists finally shake hands? Trends Neurosci. 2002 Jan;25(1):22–6.

16. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol. 1991;82(4):239–59.

17. Hampel H, Mesulam M-M, Cuello AC, Khachaturian AS, Vergallo A, Farlow MR, et al. Revisiting the Cholinergic Hypothesis in Alzheimer's Disease: Emerging Evidence from Translational and Clinical Research. J Prev Alzheimers Dis. 2019;6(1):2–15.

18. Francis PT, Palmer AM, Snape M, Wilcock GK. The cholinergic hypothesis of Alzheimer's disease: a review of progress. J Neurol Neurosurg Psychiatry. 1999 Feb;66(2):137–47.

19. Mesulam M. The cholinergic lesion of Alzheimer's disease: pivotal factor or side show? Learn Mem. 2004 Jan;11(1):43–9.

20. Huang Y, Mucke L. Alzheimer mechanisms and therapeutic strategies. Cell. 2012 Mar 16;148(6):1204–22.

21. Braak H, Braak E. Evolution of the neuropathology of Alzheimer's disease. Acta Neurol Scand Suppl. 1996;165:3–12.

22. Bartus RT, Dean RL, Pontecorvo MJ, Flicker C. The cholinergic hypothesis: a historical overview, current perspective, and future directions. Ann N Y Acad Sci. 1985;444:332–58.

23. Castellani RJ, Perry G. Pathogenesis and disease-modifying therapy in Alzheimer's disease: the flat line of progress. Arch Med Res. 2012 Nov;43(8):694–8.

24. Palop JJ, Mucke L. Amyloid-beta-induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks. Nat Neurosci. 2010 Jul;13(7):812–8.

25. Román G, Pascual B. Contribution of neuroimaging to the diagnosis of Alzheimer's disease and vascular dementia. Arch Med Res. 2012 Nov;43(8):671–6.

26. Frisoni GB, Fox NC, Jack CR Jr, Scheltens P, Thompson PM. The clinical use of structural MRI in Alzheimer disease. Nat Rev Neurol. 2010 Feb;6(2):67–77.

27. Braak E, Griffing K, Arai K, Bohl J, Bratzke H, Braak H. Neuropathology of Alzheimer's disease: what is new since A. Alzheimer? Eur Arch Psychiatry Clin Neurosci. 1999;249 Suppl 3:14–22.

28. Ferreira D, Nordberg A, Westman E. Biological subtypes of Alzheimer disease: A systematic review and meta-analysis. Neurology. 2020 Mar 10;94(10):436–48.

29. Dickerson BC, McGinnis SM, Xia C, Price BH, Atri A, Murray ME, et al. Approach to atypical Alzheimer's disease and case studies of the major subtypes. CNS Spectr. 2017 Dec;22(6):439–49.

30. Graff-Radford J, Yong KXX, Apostolova LG, Bouwman FH, Carrillo M, Dickerson BC, et al. New insights into atypical Alzheimer's disease in the era of biomarkers. Lancet Neurol. 2021 Mar;20(3):222–34.

31. Ropper AH, Samuels MA, Klein J, Prasad S. Adams and Victor's Principles of Neurology 11th Edition. McGraw-Hill Education; 2019. 1664 p.

32. Taler V, Phillips NA. Language performance in Alzheimer's disease and mild cognitive impairment: a comparative review. J Clin Exp Neuropsychol. 2008 Jul;30(5):501–56.

33. Willis SL, Allen-Burge R, Dolan MM, Bertrand RM, Yesavage J, Taylor JL. Everyday problem solving among individuals with Alzheimer's disease. Gerontologist. 1998 Oct;38(5):569–77.

34. Royall DR, Lauterbach EC, Kaufer D, Malloy P, Coburn KL, Black KJ, et al. The cognitive correlates of functional status: a review from the Committee on Research of the American Neuropsychiatric Association. J Neuropsychiatry Clin Neurosci. 2007 Summer;19(3):249–65.

35. Tellechea P, Pujol N, Esteve-Belloch P, Echeveste B, García-Eulate MR, Arbizu J, et al. Early- and late-onset Alzheimer disease: Are they the same entity? Neurología (English Edition). 2018 May 1;33(4):244–53.

36. Zhu X-C, Tan L, Wang H-F, Jiang T, Cao L, Wang C, et al. Rate of early onset Alzheimer's disease: a systematic review and meta-analysis. Ann Transl Med. 2015 Mar;3(3):38.

37. Barnes J, Dickerson BC, Frost C, Jiskoot LC, Wolk D, van der Flier WM. Alzheimer's disease first symptoms are age dependent: Evidence from the NACC dataset. Alzheimers Dement. 2015 Nov;11(11):1349–57.

38. Deuschl, Günther and Mayer, Wolfang and others. "S3-Leitlinie Demenzen." Deutsche Gesellschaft fuer Neurologie, Hrsg Leitlinien fuer Diagnostik und Therapie in der Neurologie. 2016;

39. Dilling H, Mombour W, Schmidt MH, World Health Organization. Internationale Klassifikation psychischer Störungen : ICD-10, Kapitel V (F, klinisch-diagnostische Leitlinien / hrsg. von H. Dilling, W. Mombour und M. H. Schmidt. In: Internationale Klassifikation psychischer Störungen : ICD-10, Kapitel V (F, klinisch-diagnostische Leitlinien / hrsg von H Dilling, W Mombour und M H Schmidt. 1991.

40. Williams JBW, First M. Diagnostic and statistical manual of mental disorders. In: Encyclopedia of social work. 2013.

41. First MB, Yousif LH, Clarke DE, Wang PS, Gogtay N, Appelbaum PS. DSM-5-TR: overview of what's new and what's changed. World Psychiatry. 2022 Jun;21(2):218–9.

42. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology. 1984 Jul;34(7):939–44.

43. Dubois B, Feldman HH, Jacova C, Hampel H, Molinuevo JL, Blennow K, et al. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. Lancet Neurol. 2014 Jun;13(6):614–29.

44. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011 May;7(3):263–9.

45. Jessen F, Frölich L. [ICD-11: Neurocognitive Disorders]. Fortschr Neurol Psychiatr. 2018 Mar;86(3):172–7.

46. Sachdev PS, Blacker D, Blazer DG, Ganguli M, Jeste DV, Paulsen JS, et al. Classifying neurocognitive disorders: the DSM-5 approach. Nat Rev Neurol. 2014 Nov;10(11):634–42.

47. Cutler SJ, Grams AE. Correlates of self-reported everyday memory problems. J Gerontol. 1988 May;43(3):S82-90.

48. Liyanage SI, Santos C, Weaver DF. The hidden variables problem in Alzheimer's disease clinical trial design. Alzheimers Dement. 2018 Nov 13;4:628–35.

49. Guideline N. National Collaborating Center for Mental Health commissioned by the Social Care Institute for Excellence. National Institute for Health and Clinical Excellence A NICE-SCIE Guideline on supporting people with dementia and their carers in health and social care National Clinical Practice Guideline. (42).

50. DeCarli C. Mild cognitive impairment: prevalence, prognosis, aetiology, and treatment. Lancet Neurol. 2003 Jan;2(1):15–21.

51. Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011 May;7(3):270–9.

52. Ritchie K, Artero S, Touchon J. Classification criteria for mild cognitive impairment: a population-based validation study. Neurology. 2001 Jan 9;56(1):37–42.

53. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. Arch Neurol. 1999 Mar;56(3):303–8.

54. Bozoki A, Giordani B, Heidebrink JL, Berent S, Foster NL. Mild cognitive impairments predict dementia in nondemented elderly patients with memory loss. Arch Neurol. 2001 Mar;58(3):411–6.

55. Fleisher AS, Sowell BB, Taylor C, Gamst AC, Petersen RC, Thal LJ, et al. Clinical predictors of progression to Alzheimer disease in amnestic mild cognitive impairment. Neurology. 2007 May 8;68(19):1588–95.

56. Beach TG, Monsell SE, Phillips LE, Kukull W. Accuracy of the clinical diagnosis of Alzheimer disease at National Institute on Aging Alzheimer Disease Centers, 2005-2010. J Neuropathol Exp Neurol. 2012 Apr;71(4):266–73.

57. Jack CR Jr, Wiste HJ, Weigand SD, Therneau TM, Lowe VJ, Knopman DS, et al. Defining imaging biomarker cut points for brain aging and Alzheimer's disease. Alzheimers Dement. 2017 Mar;13(3):205–16.

58. Winblad B, Jones RW, Wirth Y, Stöffler A, Möbius HJ. Memantine in moderate to severe Alzheimer's disease: a meta-analysis of randomised clinical trials. Dement Geriatr Cogn Disord. 2007 May 10;24(1):20–7.

59. McShane R, Westby MJ, Roberts E, Minakaran N, Schneider L, Farrimond LE, et al. Memantine for dementia. Cochrane Database Syst Rev. 2019 Mar 20;3:CD003154.

60. Olivares D, Deshpande VK, Shi Y, Lahiri DK, Greig NH, Rogers JT, et al. N-methyl D-aspartate (NMDA) receptor antagonists and memantine treatment for Alzheimer's

disease, vascular dementia and Parkinson's disease. Curr Alzheimer Res. 2012 Jul;9(6):746–58.

61. Gauthier S, Schlaefke S. Efficacy and tolerability of Ginkgo biloba extract EGb 761® in dementia: a systematic review and meta-analysis of randomized placebo-controlled trials. Clin Interv Aging. 2014 Nov 28;9:2065–77.

62. Becker RE, Greig NH. Why so few drugs for Alzheimer's disease? Are methods failing drugs? Curr Alzheimer Res. 2010 Nov;7(7):642–51.

63. Shi M, Chu F, Zhu F, Zhu J. Impact of Anti-amyloid-β Monoclonal Antibodies on the Pathology and Clinical Profile of Alzheimer's Disease: A Focus on Aducanumab and Lecanemab. Front Aging Neurosci. 2022 Apr 12;14:870517.

64. Wojtunik-Kulesza K, Rudkowska M, Orzeł-Sajdłowska A. Aducanumab-Hope or Disappointment for Alzheimer's Disease. Int J Mol Sci [Internet]. 2023 Feb 22;24(5). Available from: http://dx.doi.org/10.3390/ijms24054367

65. Giacobini E, Gold G. Alzheimer disease therapy--moving from amyloid-β to tau. Nat Rev Neurol. 2013 Dec;9(12):677–86.

66. Nisticò R, Borg JJ. Aducanumab for Alzheimer's disease: A regulatory perspective. Pharmacol Res. 2021 Sep;171:105754.

67. Cummings J, Apostolova L, Rabinovici GD, Atri A, Aisen P, Greenberg S, et al. Lecanemab: Appropriate Use Recommendations. J Prev Alzheimers Dis. 2023;10(3):362–77.

68. Mead S, Fox NC. Lecanemab slows Alzheimer's disease: hope and challenges. Lancet Neurol. 2023 Feb;22(2):106–8.

69. van Dyck CH, Swanson CJ, Aisen P, Bateman RJ, Chen C, Gee M, et al. Lecanemab in Early Alzheimer's Disease. N Engl J Med. 2023 Jan 5;388(1):9–21.

70. Karlawish J, Grill JD. The approval of Aduhelm risks eroding public trust in Alzheimer research and the FDA. Nat Rev Neurol. 2021 Sep;17(9):523–4.

71. Alexander GC, Karlawish J. The Problem of Aducanumab for the Treatment of Alzheimer Disease. Ann Intern Med. 2021 Sep;174(9):1303–4.

72. Rosenmann H, Grigoriadis N, Karussis D, Boimel M, Touloumi O, Ovadia H, et al. Tauopathy-like abnormalities and neurologic deficits in mice immunized with neuronal tau protein. Arch Neurol. 2006 Oct;63(10):1459–67.

73. Sitzer DI, Twamley EW, Jeste DV. Cognitive training in Alzheimer's disease: a meta-analysis of the literature. Acta Psychiatr Scand. 2006 Aug;114(2):75–90.

74. Gitlin LN, Winter L, Burke J, Chernett N, Dennis MP, Hauck WW. Tailored Activities to Manage Neuropsychiatric Behaviors in Persons With Dementia and Reduce Caregiver Burden: A Randomized Pilot Study. Am J Geriatr Psychiatry. 2008 Mar 1;16(3):229–39.

75. Bennett S, Laver K, Voigt-Radloff S, Letts L, Clemson L, Graff M, et al. Occupational therapy for people with dementia and their family carers provided at home: a systematic review and meta-analysis. BMJ Open. 2019 Nov 11;9(11):e026308.

76. Pitkälä K, Savikko N, Poysti M, Strandberg T, Laakkonen M-L. Efficacy of physical exercise intervention on mobility and physical functioning in older people with dementia: a systematic review. Exp Gerontol. 2013 Jan;48(1):85–93.

77. Ueda T, Suzukamo Y, Sato M, Izumi S-I. Effects of music therapy on behavioral and psychological symptoms of dementia: a systematic review and meta-analysis. Ageing Res Rev. 2013 Mar;12(2):628–41.

78. Ngandu T, Lehtisalo J, Solomon A, Levälahti E, Ahtiluoto S, Antikainen R, et al. A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): a randomised controlled trial. Lancet. 2015 Jun 6;385(9984):2255–63.

79. Kivipelto M, Solomon A, Ahtiluoto S, Ngandu T, Lehtisalo J, Antikainen R, et al. The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER): study design and progress. Alzheimers Dement. 2013 Nov;9(6):657–65.

80. Zanetti O, Solerte SB, Cantoni F. Life expectancy in Alzheimer's disease (AD). Arch Gerontol Geriatr. 2009;49 Suppl 1:237–43.

81. Larson EB, Shadlen M-F, Wang L, McCormick WC, Bowen JD, Teri L, et al. Survival after initial diagnosis of Alzheimer disease. Ann Intern Med. 2004 Apr 6;140(7):501–9.

82. Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011 May;7(3):280–92.

83. Tombaugh TN, McIntyre NJ. The mini-mental state examination: a comprehensive review. J Am Geriatr Soc. 1992 Sep;40(9):922–35.

84. Aggarwal A, Kean E. Comparison of the folstein mini mental state examination (MMSE) to the Montreal cognitive assessment (MoCA) as a cognitive screening tool in an inpatient rehabilitation setting. Neurosci Med. 2010;01(02):39–42.

85. Gill DJ, Freshman A, Blender JA, Ravina B. The Montreal cognitive assessment as a screening tool for cognitive impairment in Parkinson's disease. Mov Disord. 2008 May 15;23(7):1043–6.

86. Hoops S, Nazem S, Siderowf AD, Duda JE, Xie SX, Stern MB, et al. Validity of the MoCA and MMSE in the detection of MCI and dementia in Parkinson disease. Neurology. 2009 Nov 24;73(21):1738–45.

87. Grant DA, Berg EA. Wisconsin Card Sorting Test. J Exp Psychol [Internet]. Available from: https://psycnet.apa.org/fulltext/9999-31298-000.pdf

88. Morris JC, Heyman A, Mohs RC, Hughes JP, van Belle G, Fillenbaum G, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. Neurology. 1989 Sep;39(9):1159–65.

89. Mohs RC, Rosen WG, Davis KL. The Alzheimer's disease assessment scale: an instrument for assessing treatment efficacy. Psychopharmacol Bull. 1983;19(3):448–50.

90. Rosen WG, Mohs RC, Davis KL. A new rating scale for Alzheimer's disease. Am J Psychiatry. 1984 Nov;141(11):1356–64.

91. Cano SJ, Posner HB, Moline ML, Hurt SW, Swartz J, Hsu T, et al. The ADAS-cog in Alzheimer's disease clinical trials: psychometric evaluation of the sum and its parts. J Neurol Neurosurg Psychiatry. 2010 Dec;81(12):1363–8.

92. Schmid NS, Ehrensperger MM, Berres M, Beck IR, Monsch AU. The Extension of the German CERAD Neuropsychological Assessment Battery with Tests Assessing Subcortical, Executive and Frontal Functions Improves Accuracy in Dementia Diagnosis. Dement Geriatr Cogn Dis Extra. 2014 May;4(2):322–34.

93. Harrison J, Minassian SL, Jenkins L, Black RS, Koller M, Grundman M. A neuropsychological test battery for use in Alzheimer disease clinical trials. Arch Neurol. 2007 Sep;64(9):1323–9.

94. Balsis S, Benge JF, Lowe DA, Geraci L, Doody RS. How Do Scores on the ADAS-Cog, MMSE, and CDR-SOB Correspond? Clin Neuropsychol. 2015 Nov 30;29(7):1002–9.

95. Stern Y. Cognitive reserve. Neuropsychologia. 2009 Aug;47(10):2015–28.

96. Stern Y, Barnes CA, Grady C, Jones RN, Raz N. Brain reserve, cognitive reserve, compensation, and maintenance: operationalization, validity, and mechanisms of cognitive resilience. Neurobiol Aging. 2019 Nov;83:124–9.

97. Stern Y. What is cognitive reserve? Theory and research application of the reserve concept. J Int Neuropsychol Soc. 2002 Mar;8(3):448–60.

98. Nyberg L, Lövdén M, Riklund K, Lindenberger U, Bäckman L. Memory aging and brain maintenance. Trends Cogn Sci. 2012 May;16(5):292–305.

99. Bennett DA, Wilson RS, Schneider JA, Evans DA, Mendes de Leon CF, Arnold SE, et al. Education modifies the relation of AD pathology to level of cognitive function in older persons. Neurology. 2003 Jun 24;60(12):1909–15.

100. Ewers M, Insel PS, Stern Y, Weiner MW, Alzheimer's Disease Neuroimaging Initiative (ADNI). Cognitive reserve associated with FDG-PET in preclinical Alzheimer disease. Neurology. 2013 Mar 26;80(13):1194–201.

101. Jones RN, Manly J, Glymour MM, Rentz DM, Jefferson AL, Stern Y. Conceptual and measurement challenges in research on cognitive reserve. J Int Neuropsychol Soc. 2011 Jul;17(4):593–601.

102. Craig-Schapiro R, Fagan AM, Holtzman DM. Biomarkers of Alzheimer's disease. Neurobiol Dis. 2009 Aug;35(2):128–40.

103. Silva MVF, Loures C de MG, Alves LCV, de Souza LC, Borges KBG, Carvalho M das G. Alzheimer's disease: risk factors and potentially protective measures. J Biomed Sci. 2019 May 9;26(1):33.

104. Karch CM, Goate AM. Alzheimer's disease risk genes and mechanisms of disease pathogenesis. Biol Psychiatry. 2015 Jan 1;77(1):43–51.

105. Mahley RW. Apolipoprotein E: from cardiovascular disease to neurodegenerative disorders. J Mol Med . 2016 Jul;94(7):739–46.

106. Mayeux R, Saunders AM, Shea S, Mirra S, Evans D, Roses AD, et al. Utility of the apolipoprotein E genotype in the diagnosis of Alzheimer's disease. Alzheimer's Disease Centers Consortium on Apolipoprotein E and Alzheimer's Disease. N Engl J Med. 1998 Feb 19;338(8):506–11.

107. Lee JC, Kim SJ, Hong S, Kim Y. Diagnosis of Alzheimer's disease utilizing amyloid and tau as fluid biomarkers. Exp Mol Med. 2019 May 9;51(5):1–10.

108. Lue L-F, Guerra A, Walker DG. Amyloid Beta and Tau as Alzheimer's Disease Blood Biomarkers: Promise From New Technologies. Neurol Ther. 2017 Jul;6(Suppl 1):25–36.

109. Park SA, Han SM, Kim CE. New fluid biomarkers tracking non-amyloid-β and non-tau pathology in Alzheimer's disease. Exp Mol Med. 2020 Apr;52(4):556–68.

110. Bucci M, Chiotis K, Nordberg A, Alzheimer's Disease Neuroimaging Initiative. Alzheimer's disease profiled by fluid and imaging markers: tau PET best predicts cognitive decline. Mol Psychiatry. 2021 Oct;26(10):5888–98.

111. Brier MR, Gordon B, Friedrichsen K, McCarthy J, Stern A, Christensen J, et al. Tau and Aβ imaging, CSF measures, and cognition in Alzheimer's disease. Sci Transl Med. 2016 May 11;8(338):338ra66.

112. Chételat G, Arbizu J, Barthel H, Garibotto V, Law I, Morbelli S, et al. Amyloid-PET and 18F-FDG-PET in the diagnostic investigation of Alzheimer's disease and other dementias. Lancet Neurol. 2020 Nov;19(11):951–62.

113. Visser PJ, Verhey FRJ, Hofman PAM, Scheltens P, Jolles J. Medial temporal lobe atrophy predicts Alzheimer's disease in patients with minor cognitive impairment. J Neurol Neurosurg Psychiatry. 2002 Apr;72(4):491–7.

114. Scheltens P, Leys D, Barkhof F, Huglo D, Weinstein HC, Vermersch P, et al. Atrophy of medial temporal lobes on MRI in "probable" Alzheimer's disease and normal ageing: diagnostic value and neuropsychological correlates. J Neurol Neurosurg Psychiatry. 1992 Oct;55(10):967–72.

115. Thompson PM, Hayashi KM, De Zubicaray GI, Janke AL, Rose SE, Semple J, et al. Mapping hippocampal and ventricular change in Alzheimer disease. Neuroimage. 2004 Aug;22(4):1754–66.

116. Matthews PM, Jezzard P. Functional magnetic resonance imaging. J Neurol Neurosurg Psychiatry. 2004 Jan;75(1):6–12.

117. Dickerson BC, Salat DH, Greve DN, Chua EF, Rand-Giovannetti E, Rentz DM, et al. Increased hippocampal activation in mild cognitive impairment compared to normal aging and AD. Neurology. 2005 Aug 9;65(3):404–11.

118. Small SA, Perera GM, DeLaPaz R, Mayeux R, Stern Y. Differential regional dysfunction of the hippocampal formation among elderly with memory decline and Alzheimer's disease. Ann Neurol. 1999 Apr;45(4):466–72.

119. Rombouts SA, Barkhof F, Veltman DJ, Machielsen WC, Witter MP, Bierlaagh MA, et al. Functional MR imaging in Alzheimer's disease during memory encoding. AJNR Am J Neuroradiol. 2000 Nov-Dec;21(10):1869–75.

120. Johnson SC, Saykin AJ, Baxter LC, Flashman LA, Santulli RB, McAllister TW, et al. The relationship between fMRI activation and cerebral atrophy: comparison of normal aging and alzheimer disease. Neuroimage. 2000 Mar;11(3):179–87.

121. Masdeu JC, Zubieta JL, Arbizu J. Neuroimaging as a marker of the onset and progression of Alzheimer's disease. J Neurol Sci. 2005 Sep 15;236(1–2):55–64.

122. Wierenga CE, Bondi MW. Use of functional magnetic resonance imaging in the early identification of Alzheimer's disease. Neuropsychol Rev. 2007 Jun;17(2):127–43.

123. Bookheimer Susan Y., Strojwas Magdalena H., Cohen Mark S., Saunders Ann M., Pericak-Vance Margaret A., Mazziotta John C., et al. Patterns of Brain Activation in People at Risk for Alzheimer's Disease. N Engl J Med. 2000 Aug 17;343(7):450–6.

124. Arriagada PV, Growdon JH, Hedley-Whyte ET, Hyman BT. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. Neurology. 1992 Mar;42(3 Pt 1):631–9.

125. Jin Y, Huang C, Daianu M, Zhan L, Dennis EL, Reid RI, et al. 3D tract-specific local and global analysis of white matter integrity in Alzheimer's disease. Hum Brain Mapp. 2017 Mar;38(3):1191–207.

126. Wang T, Shi F, Jin Y, Yap P-T, Wee C-Y, Zhang J, et al. Multilevel Deficiency of White Matter Connectivity Networks in Alzheimer's Disease: A Diffusion MRI Study with DTI and HARDI Models. Neural Plast. 2016 Jan 13;2016:2947136.

127. Greicius MD, Menon V. Default-mode activity during a passive sensory task: uncoupled from deactivation but impacting activation. J Cogn Neurosci. 2004 Nov;16(9):1484–92.

128. Greicius MD, Srivastava G, Reiss AL, Menon V. Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. Proc Natl Acad Sci U S A. 2004 Mar 30;101(13):4637–42.

129. Raichle ME. The brain's default mode network. Annu Rev Neurosci. 2015 Jul 8;38:433–47.

130. Celone KA, Calhoun VD, Dickerson BC, Atri A, Chua EF, Miller SL, et al. Alterations in memory networks in mild cognitive impairment and Alzheimer's disease: an independent component analysis. J Neurosci. 2006 Oct 4;26(40):10222–31.

131. Friston KJ, Jezzard P, Turner R. Analysis of functional MRI time-series. Hum Brain Mapp. 1994;1(2):153–71.

132. Buckner RL, Snyder AZ, Shannon BJ, LaRossa G, Sachs R, Fotenos AF, et al. Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. J Neurosci. 2005 Aug 24;25(34):7709–17.

133. Chan MY, Park DC, Savalia NK, Petersen SE, Wig GS. Decreased segregation of brain systems across the healthy adult lifespan. Proc Natl Acad Sci U S A. 2014 Nov 18;111(46):E4997-5006.

134. Wang K, Liang M, Wang L, Tian L, Zhang X, Li K, et al. Altered functional connectivity in early Alzheimer's disease: a resting-state fMRI study. Hum Brain Mapp. 2007 Oct;28(10):967–78.

135. Brier MR, Thomas JB, Snyder AZ, Benzinger TL, Zhang D, Raichle ME, et al. Loss of intranetwork and internetwork resting state functional connections with Alzheimer's disease progression. J Neurosci. 2012 Jun 27;32(26):8890–9.

136. Klaassens BL, van Gerven JMA, van der Grond J, de Vos F, Möller C, Rombouts SARB. Diminished Posterior Precuneus Connectivity with the Default Mode Network Differentiates Normal Aging from Alzheimer's Disease. Front Aging Neurosci. 2017 Apr 19;9:97.

137. Agosta F, Pievani M, Geroldi C, Copetti M, Frisoni GB, Filippi M. Resting state fMRI in Alzheimer's disease: beyond the default mode network. Neurobiol Aging. 2012 Aug;33(8):1564–78.

138. Hafkemeijer A, Möller C, Dopper EGP, Jiskoot LC, Schouten TM, van Swieten JC, et al. Resting state functional connectivity differences between behavioral variant

frontotemporal dementia and Alzheimer's disease. Front Hum Neurosci. 2015 Sep 8;9:474.

139. Buckner RL, Sepulcre J, Talukdar T, Krienen FM, Liu H, Hedden T, et al. Cortical hubs revealed by intrinsic functional connectivity: mapping, assessment of stability, and relation to Alzheimer's disease. J Neurosci. 2009 Feb 11;29(6):1860–73.

140. Seeley WW, Crawford RK, Zhou J, Miller BL, Greicius MD. Neurodegenerative diseases target large-scale human brain networks. Neuron. 2009 Apr 16;62(1):42–52.

141. Montembeault M, Joubert S, Doyon J, Carrier J, Gagnon J-F, Monchi O, et al. The impact of aging on gray matter structural covariance networks. Neuroimage. 2012 Nov 1;63(2):754–9.

142. Hafkemeijer A, Altmann-Schneider I, de Craen AJM, Slagboom PE, van der Grond J, Rombouts SARB. Associations between age and gray matter volume in anatomical brain networks in middle-aged to older adults. Aging Cell. 2014 Dec;13(6):1068–74.

143. Brickman AM, Habeck C, Zarahn E, Flynn J, Stern Y. Structural MRI covariance patterns associated with normal aging and neuropsychological functioning. Neurobiol Aging. 2007 Feb;28(2):284–95.

144. Koini M, Duering M, Gesierich BG, Rombouts SARB, Ropele S, Wagner F, et al. Grey-matter network disintegration as predictor of cognitive and motor function with aging. Brain Struct Funct. 2018 Jun;223(5):2475–87.

145. Foster-Dingley JC, Hafkemeijer A, van den Berg-Huysmans AA, Moonen JEF, de Ruijter W, de Craen AJM, et al. Structural Covariance Networks and Their Association with Age, Features of Cerebral Small-Vessel Disease, and Cognitive Functioning in Older Persons. Brain Connect. 2016 Nov;6(9):681–90.

146. Montembeault M, Rouleau I, Provost J-S, Brambati SM, Alzheimer's Disease Neuroimaging Initiative. Altered Gray Matter Structural Covariance Networks in Early Stages of Alzheimer's Disease. Cereb Cortex. 2016 Jun;26(6):2650–62.

147. Li K, Luo X, Zeng Q, Huang P, Shen Z, Xu X, et al. Gray matter structural covariance networks changes along the Alzheimer's disease continuum. Neuroimage Clin. 2019 Apr 17;23:101828.

148. Spreng RN, Turner GR. Structural covariance of the default network in healthy and pathological aging. J Neurosci. 2013 Sep 18;33(38):15226–34.

149. Hafkemeijer A, Möller C, Dopper EGP, Jiskoot LC, van den Berg-Huysmans AA, van Swieten JC, et al. Differences in structural covariance brain networks between behavioral variant frontotemporal dementia and Alzheimer's disease. Hum Brain Mapp. 2016 Mar;37(3):978–88.

150. Dicks E, Tijms BM, Ten Kate M, Gouw AA, Benedictus MR, Teunissen CE, et al. Gray matter network measures are associated with cognitive decline in mild cognitive impairment. Neurobiol Aging. 2018 Jan;61:198–206.

151. Seiler S, Schmidt H, Lechner A, Benke T, Sanin G, Ransmayr G, et al. Driving cessation and dementia: results of the prospective registry on dementia in Austria (PRODEM). PLoS One. 2012 Dec 26;7(12):e52710.

152. Schmidt R, Ropele S, Enzinger C, Petrovic K, Smith S, Schmidt H, et al. White matter lesion progression, brain atrophy, and cognitive decline: the Austrian stroke prevention study. Ann Neurol. 2005 Oct;58(4):610–6.

153. Jenkinson M, Beckmann CF, Behrens TEJ, Woolrich MW, Smith SM. FSL. Neuroimage. 2012 Aug 15;62(2):782–90.

154. Smith SM. Fast robust automated brain extraction. Hum Brain Mapp. 2002 Nov;17(3):143–55.

155. Ashburner J, Friston KJ. Voxel-based morphometry--the methods. Neuroimage. 2000 Jun;11(6 Pt 1):805–21.

156. Zhang Y, Michael Brady J, Smith SM. An HMRF-EM algorithm for partial volume segmentation of brain MRI [Internet]. [cited 2021 Jan 23]. Available from: https://www.fmrib.ox.ac.uk/datasets/techrep/tr01yz1/tr01yz1.pdf

157. Jenkinson M, Bannister P, Brady M, Smith S. Improved Optimization for the Robust and Accurate Linear Registration and Motion Correction of Brain Images. Neuroimage. 2002 Oct 1;17(2):825–41.

158. Andersson JLR, Jenkinson M, Smith S. Non-linear registration aka Spatial normalisation FMRIB Technial Report TR07JA2.

159. Good CD, Johnsrude IS, Ashburner J, Henson RN, Friston KJ, Frackowiak RS. A voxel-based morphometric study of ageing in 465 normal adult human brains. Neuroimage. 2001 Jul;14(1 Pt 1):21–36.

160. Filippini N, MacIntosh BJ, Hough MG, Goodwin GM, Frisoni GB, Smith SM, et al. Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. Proc Natl Acad Sci U S A. 2009 Apr 28;106(17):7209–14.

161. Fischl B. FreeSurfer. Neuroimage. 2012 Aug 15;62(2):774–81.

162. Chandler MJ, Lacritz LH, Hynan LS, Barnard HD, Allen G, Deschner M, et al. A total score for the CERAD neuropsychological battery. Neurology. 2005 Jul 12;65(1):102–6.

163. Team RC, Others. R: A language and environment for statistical computing [Internet]. Vienna, Austria; 2013. Available from: http://cran.univ-paris1.fr/web/packages/dplR/vignettes/intro-dplR.pdf

164. Breiman L. Random Forests. Mach Learn. 2001 Oct 1;45(1):5–32.

165. Strobl C, Boulesteix A-L, Augustin T. Unbiased split selection for classification trees based on the Gini Index. Comput Stat Data Anal. 2007 Sep;52(1):483–501.

166. Strobl C, Boulesteix A-L, Kneib T, Augustin T, Zeileis A. Conditional variable importance for random forests. BMC Bioinformatics. 2008 Jul 11;9:307.

167. Bradley AP. The use of the area under the ROC curve in the evaluation of machine learning algorithms. Pattern Recognit. 1997 Jul 1;30(7):1145–59.

168. Wagner F, Duering M, Gesierich BG, Enzinger C, Ropele S, Dal-Bianco P, et al. Gray Matter Covariance Networks as Classifiers and Predictors of Cognitive Function in Alzheimer's Disease. Front Psychiatry. 2020 May 5;11:360.

169. de Hond AAH, Steyerberg EW, van Calster B. Interpreting area under the receiver operating characteristic curve. Lancet Digit Health. 2022 Dec;4(12):e853–5.

170. Hapfelmeier A, Hothorn T, Ulm K, Strobl C. A new variable importance measure for random forests with missing data. Stat Comput. 2014 Jan;24(1):21–34.

171. Alexander-Bloch A, Raznahan A, Bullmore E, Giedd J. The convergence of maturational change and structural covariance in human cortical networks. J Neurosci. 2013 Feb 13;33(7):2889–99.

172. Arnold SE, Hyman BT, Van Hoesen GW. Neuropathologic changes of the temporal pole in Alzheimer's disease and Pick's disease. Arch Neurol. 1994 Feb;51(2):145–50.

173. Frisoni GB, Testa C, Zorzan A, Sabattoli F, Beltramello A, Soininen H, et al. Detection of grey matter loss in mild Alzheimer's disease with voxel based morphometry. J Neurol Neurosurg Psychiatry. 2002 Dec;73(6):657–64.

174. Jones BF, Barnes J, Uylings HBM, Fox NC, Frost C, Witter MP, et al. Differential Regional Atrophy of the Cingulate Gyrus in Alzheimer Disease: A Volumetric MRI Study. Cereb Cortex. 2006 Jan 4;16(12):1701–8.

175. Scheff SW, Price DA, Schmitt FA, Scheff MA, Mufson EJ. Synaptic loss in the inferior temporal gyrus in mild cognitive impairment and Alzheimer's disease. J Alzheimers Dis. 2011;24(3):547–57.

176. Hafkemeijer A, van der Grond J, Rombouts SARB. Imaging the default mode network in aging and dementia. Biochim Biophys Acta. 2012 Mar;1822(3):431–41.

177. Reid AT, Evans AC. Structural networks in Alzheimer's disease. Eur Neuropsychopharmacol. 2013 Jan;23(1):63–77.

178. Zhou L, Wang Y, Li Y, Yap P-T, Shen D, Alzheimer's Disease Neuroimaging Initiative (ADNI). Hierarchical anatomical brain networks for MCI prediction: revisiting volumetric measures. PLoS One. 2011 Jul 19;6(7):e21935.

179. Li JJ, Tong X. Statistical Hypothesis Testing versus Machine Learning Binary Classification: Distinctions and Guidelines. Patterns (N Y). 2020 Oct 9;1(7):100115.

180. Utevsky AV, Smith DV, Huettel SA. Precuneus is a functional core of the default-mode network. J Neurosci. 2014 Jan 15;34(3):932–40.

181. Steffener J, Brickman AM, Habeck CG, Salthouse TA, Stern Y. Cerebral blood flow and gray matter volume covariance patterns of cognition in aging. Hum Brain Mapp. 2013 Dec;34(12):3267–79.

182. Bäumler G. Lern-und gedächtnistest LGT-3. Hogrefe; 1974.

183. Miyata S, Noda A, Iwamoto K, Kawano N, Okuda M, Ozaki N. Poor sleep quality impairs cognitive performance in older adults. J Sleep Res. 2013 Oct;22(5):535–41.

184. Salthouse TA, Nesselroade JR, Berish DE. Short-term variability in cognitive performance and the calibration of longitudinal change. J Gerontol B Psychol Sci Soc Sci. 2006 May;61(3):P144-51.

185. Benedict RH, Dobraski M, Goldstein MZ. A preliminary study of the association between changes in mood and cognition in a mixed geriatric psychiatry sample. J Gerontol B Psychol Sci Soc Sci. 1999 Mar;54(2):P94-9.

186. Lim YY, Kong J, Maruff P, Jaeger J, Huang E, Ratti E. Longitudinal Cognitive Decline in Patients With Mild Cognitive Impairment or Dementia Due to Alzheimer's Disease. J Prev Alzheimers Dis. 2022;9(1):178–83.

187. Bijsterbosch J, Smith SM, Beckmann CF. Introduction to Resting State fMRI Functional Connectivity. Oxford University Press; 2017. 200 p.

188. Smith SM, Fox PT, Miller KL, Glahn DC, Fox PM, Mackay CE, et al. Correspondence of the brain's functional architecture during activation and rest. Proc Natl Acad Sci U S A. 2009 Aug 4;106(31):13040–5.

189. Grabner G, Janke AL, Budge MM, Smith D, Pruessner J, Collins DL. Symmetric Atlasing and Model Based Segmentation: An Application to the Hippocampus in Older Adults. In: Medical Image Computing and Computer-Assisted Intervention – MICCAI 2006. Springer Berlin Heidelberg; 2006. p. 58–66.

190. Fillmore PT, Phillips-Meek MC, Richards JE. Age-specific MRI brain and head templates for healthy adults from 20 through 89 years of age [Internet]. Vol. 7, Frontiers in Aging Neuroscience. 2015. Available from: http://dx.doi.org/10.3389/fnagi.2015.00044

191. Huang C-M, Lee S-H, Hsiao I-T, Kuan W-C, Wai Y-Y, Ko H-J, et al. Study-specific EPI template improves group analysis in functional MRI of young and older adults. J Neurosci Methods. 2010 Jun 15;189(2):257–66.

192. Peña-Casanova J, Sánchez-Benavides G, de Sola S, Manero-Borrás RM, Casals-Coll M. Neuropsychology of Alzheimer's disease. Arch Med Res. 2012 Nov;43(8):686–93.

193. Vabalas A, Gowen E, Poliakoff E, Casson AJ. Machine learning algorithm validation with a limited sample size. PLoS One. 2019 Nov 7;14(11):e0224365.

194. Schouten TM, Koini M, de Vos F, Seiler S, van der Grond J, Lechner A, et al. Combining anatomical, diffusion, and resting state functional magnetic resonance imaging for individual classification of mild and moderate Alzheimer's disease. Neuroimage Clin. 2016 Jan 9;11:46–51.

195. de Vos F, Schouten TM, Hafkemeijer A, Dopper EGP, van Swieten JC, de Rooij M, et al. Combining multiple anatomical MRI measures improves Alzheimer's disease classification. Hum Brain Mapp. 2016 May;37(5):1920–9.

196. Bouts MJRJ, Möller C, Hafkemeijer A, van Swieten JC, Dopper E, van der Flier WM, et al. Single Subject Classification of Alzheimer's Disease and Behavioral Variant Frontotemporal Dementia Using Anatomical, Diffusion Tensor, and Resting-State Functional Magnetic Resonance Imaging. J Alzheimers Dis. 2018;62(4):1827–39.

Supplementary Material

Additional Figures

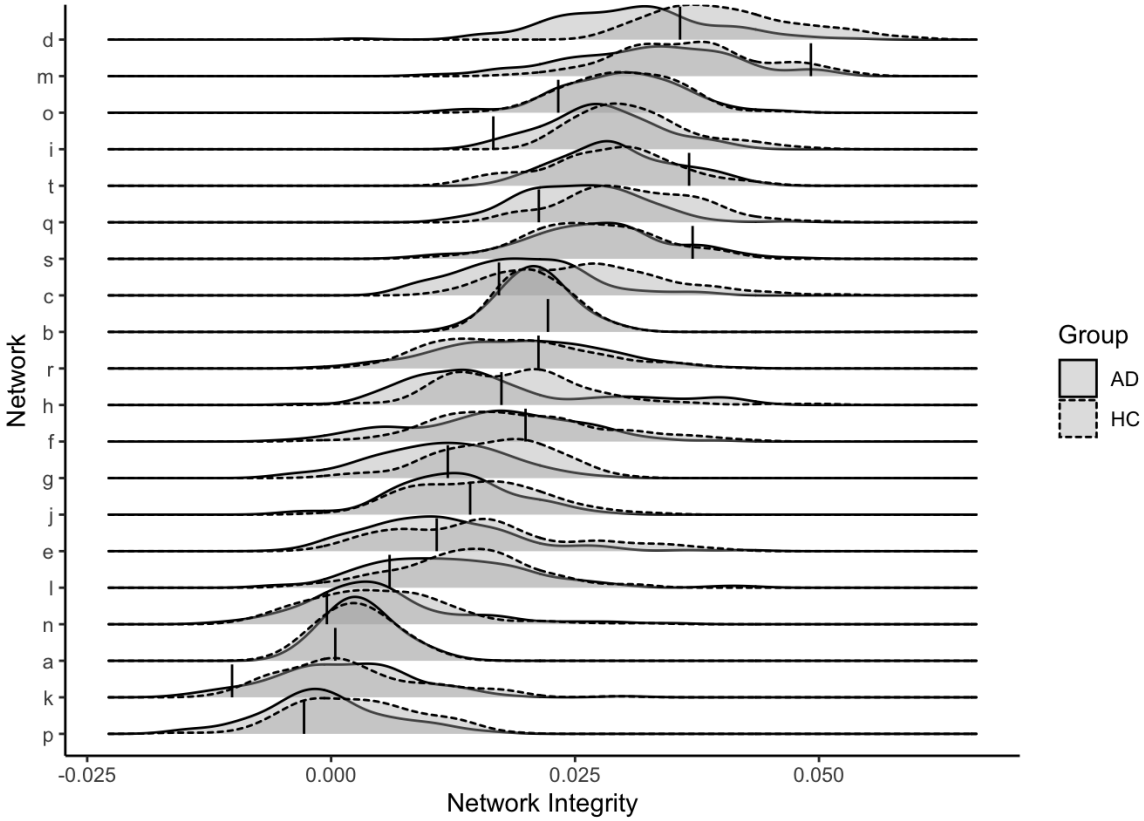

Figure 10: Ridgeline plot showing integrity distributions for all SCNs grouped by AD and HC. The vertical lines show network integrity scores for the corresponding networks of one randomly chosen subject from our sample. The plots show the Graz subset only.

Table 9: T-test results for all 20 SCNs as compared between AD patients and healthy controls. In this analysis, only the Graz cohort was included (N = 208). Network c is the secondary somatosensory SCN and network d is the temporal SCN.

network	p-value	p-value_bonf	Mean diff	t statistic	df
a	0,4881	1	0,0003	0,6946	205,0705
b	0,8475	1	-0,0001	-0,1925	205,8734
c	<0.000	<0.000*	-0,007	-6,9272	197,1186
d	<0.000	<0.000*	-0,0099	-9,2078	202,3693
e	0,0003	0,006*	-0,0045	-3,7124	199,7606
f	0,0138	0,276	-0,003	-2,4843	205,9973
g	<0.000	<0.000*	-0,0055	-6,1837	203,6142
h	0,1022	1	-0,0021	-1,6423	190,3164
i	<0.000	<0.000*	-0,0034	-4,316	205,9963
j	0,013	0,26	-0,0022	-2,505	204,4368
k	0,502	1	-0,0007	-0,6725	205,8605
l	0,1455	1	-0,0016	-1,4612	198,0986
m	0,0002	0,004*	-0,0041	-3,7565	201,0066
n	0,9615	1	0,0001	0,0483	203,5285
o	0,6553	1	-0,0004	-0,4471	202,1726
p	0,0003	0,006*	-0,0032	-3,6635	205,9819
q	<0.0000	<0.000*	-0,0053	-6,014	204,002
r	0,3611	1	0,001	0,9154	205,2593
s	0,6777	1	0,0004	0,4162	202,6384
t	0,1943	1	0,0011	1,3024	201,6049