A residual marker of cognitive reserve is associated with resting-state intrinsic functional connectivity along the Alzheimer's disease continuum

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Running title: Residual marker of CR captures network FC
Keywords: Cognitive reserve; Alzheimer's disease; cognition; fMRI; resting-state functional connectivity; intrinsic network connectivity

ABSTRACT

Background: Cognitive reserve (CR) explains inter-individual differences in the impact of the neurodegenerative burden on cognitive functioning. A residual model was proposed to estimate CR more accurately than previous measures. However, associations between residual CR markers (CRM) and functional connectivity (FC) remain unexplored.

Objective: To explore the associations between the CRM and intrinsic network connectivity (INC) in resting-state networks along the neuropathological-continuum of Alzheimer's disease (ADN).

Methods: Three hundred eighteen participants from the DELCODE cohort were stratified using CSF biomarkers according to the A(myloid-β)/T(au)/N(eurodegeneration) classification. CRM was calculated utilizing residuals obtained from a multilinear regression model predicting cognition from markers of disease burden. Using an independent component analysis in resting-state fMRI data, we measured INC of resting-state networks, i.e. default mode network (DMN), frontoparietal network (FPN), salience network (SAL) and dorsal attention network. The associations of INC with a composite memory score and CRM and the associations of CRM with the seed-to-voxel functional connectivity of memory-related were tested in general linear models.

Results: CRM was positively associated with INC in the DMN in the entire cohort. The A+T+N+ group revealed an anti-correlation between the SAL and the DMN. Furthermore, CRM was positively associated with anti-correlation between memory-related regions in FPN and DMN in ADN and A+T/N+.

Conclusion: Our results provide evidence that INC is associated with CRM in ADN defined as participants with amyloid pathology with or without cognitive symptoms, suggesting that the neural correlates of CR are mirrored in network FC in resting-state.

INTRODUCTION

The concept of cognitive reserve (CR) refers to the capacity and flexibility of cognitive and brain processes that help to attenuate the impact of brain aging or pathology on cognitive function or daily activities, for example, in Alzheimer's disease (AD) [1]. Key mechanisms underlying CR include the brain's ability to maintain neural functions, recruit compensatory networks, or use existing networks more efficiently [2]. The related concepts of brain reserve [3] and brain maintenance describe different, complementary aspects of resilience [2]. Years of formal education [1,4] and occupational complexity [5] are used frequently as CR proxy measures [6]. Still, they only reflect selected aspects of intellectual attainment. The residual estimation of cognitive reserve (i.e., residual CR marker (CRM)) has been obtained by quantifying the discrepancy between the observed cognitive performance and the performance estimated based on the neuropathological burden of a person using a multiple linear regression analysis. The neuropathological burden included demographical data, genetic predisposition and disease surrogates, i.e. cerebrospinal fluid biomarkers. This approach may offer more granular data resulting in more detailed insights into the nature of CR [2]. Residual approaches were shown to be relatively reliable and were studied in cross-sectional [7,8] and longitudinal studies [9–11]. A residual CR measure considers demographical and disease-related confounders multidimensionally and may be more comprehensive and informative at the individual level than traditional markers such as education [2].

Resting-state networks (RSNs) such as the default mode network (DMN), which is involved in cognition, self-reference, social cognition, or autobiographical memory (i.e., inwardly directed cognition) [12–14], and networks associated with externally directed cognitive processing, including the dorsal attention (DAN), salience (SAL), and frontoparietal network (FPN) [12,15–18], are affected by AD pathology and correlate with disease progression. Spatial links exist between AD pathology and functional connectivity (FC) changes, particularly in the posterior DMN and FPN [19]. Moreover, the inter-network connectivity among RSNs is also affected in AD, especially between DMN and DAN [12] as well as SAL [20]. As AD progresses, functional network changes affect predominantly intra-network connectivity and to a lesser extent inter-network connectivity [15].

The inter-individual variances in FC using various functional imaging approaches are increasingly the focus of studies of the neural implementation of CR that might contribute to functional neural processes to preserve a relatively better cognition [2,21]. Previous studies showed positive associations between residual markers of CR and the graph-theoretical measurement of network efficiency and FC [7,8]. FC was also associated positively with CR measured as educational attainment in DMN regions [22,23] and between DMN and FPN [24]. CR was associated with lower metabolic activity in the DMN and the DAN [25]. Furthermore, increasing evidence suggests a crucial role of the FPN in CR, including global connectivity of the left frontal cortex (LPC) in resting-state fMRI, a hub region within the FPN [26–28]. Unlike node-to-node connectivity analyses, whole network intrinsic

connectivity analysis following the data-driven independent component analysis [29] allows FC analysis more broadly within and across networks [15].

A biologically-based definition of the AD diagnosis has been proposed in a recent research framework using a binary biomarker status (presented or absent) for (A)myloid-β (Aβ), (T)au and (N)eurodegeneration (i.e., the ATN classification) as biomarker-based diagnostic profiles, prompting the switch from a symptom-based to a biological definition of AD [30]. Considering the various stages of AD, FC alterations appear already in the preclinical and early clinical stages of AD [31], showing meaningful effects of CR on the individual clinical progression trajectories [27]. Nevertheless, the characterization of a residual CRM is improving in individuals at-risk of dementia and dementia populations [32]; there is a need to operationalize them in early disease and explore their associations with intrinsic network connectivity (INC) of cognitive RSNs.

In this study, we aimed to examine the associations of a residual CRM with FC alterations within and between network connectivity, focusing on the disease-susceptible RSNs, in the AD neuropathological continuum (ADN), using a biomarker-based approach for diagnosis and staging. Furthermore, we defined memory function-related functional network connectivity of the DMN and the FPN to test their associations with the CRM to provide insights into the function of CRM in cognitive decline.

MATERIALS AND METHODS

Data from the prospective, observational German Center for Neurodegenerative Diseases (Deutsches Zentrum für Neurodegenerative Erkrankungen, DZNE)-Longitudinal Cognitive Impairment and Dementia Study (DELCODE) [33] was used for the present analyses, an observational brain imaging study initiated by the DZNE in 2014 (German Clinical Trials Register: DRKS00007966).

PARTICIPANTS

All eligible DELCODE participants were included if they had available clinical dementia rating (CDR), neuropsychological tests, cerebrospinal fluid (CSF) biomarker analyses and apolipoprotein E (*APOE*) genotyping results and relevant structural and functional MRI data. We classified each participant following the A/T/N classification scheme using binarized CSF biomarker measurements of Aβ for A, total tau (tTau) for N and phosphorylated-tau181 (pTau) for T [34]. To restrict the cohort to participants in the ADN and healthy controls, we excluded participants classified as A-T/N+ (A-T+N- and A-T-N+) (i.e., suspected non-AD pathology) and A-T-N- with a global CDR rating of higher than 0 (i.e. non-AD cognitive impairment). The final cohort of 318 participants included 112 A-T-N- individuals with global CDR=0 as healthy controls (HC, mean age 69 ± 6, 52 females) and 206 A+ patients as ADN regardless of the cognitive status or clinical diagnosis (mean age 72 ± 6, 101 females), encompassing 106 A+T-N-, 28 A+T/N+ (A+T+N- and A+T-N+) and 72 A+T+N+ individuals. The detailed inclusion and exclusion criteria and study procedures of the DELCODE study are reported elsewhere

[33]. The following CSF biomarker cut-off values were obtained by Gaussian mixture modeling using the R package flexmix (version 2.3-15) in the DELCODE dataset included 481 participants with CSF biomarker data (sampling rate among entire baseline cohort: 48%): Aβ42: <= 638.7 pg/ml, tTau: > 510.9 pg/ml and pTau181: >= 73.65 pg/ml, as reported elsewhere [35]. The maximum time lag between study visit with clinical and neuropsychological assessment and CSF draw and fMRI scan was four weeks.

MR IMAGE ACQUISITION AND PREPROCESSING

Imaging was performed at nine different DZNE sites on 3T MRI scanners (Siemens Healthineers, Erlangen, Germany; three Verio, three TimTrio, one Prisma and two Skyra) using synchronized acquisition parameters. T1-weighted anatomical imaging was acquired in a 5-minute magnetization-prepared rapid gradient echo (MPRAGE) scan with the following parameters: field of view (FOV) 256×256 mm, isotropic voxel size: 1 mm, echo time (TE) 4.37 ms, flip angle (FA) 7°, repetition time (TR) 2500 ms, number of slices 192. Resting-state functional MRI was acquired in a 7-minutes 54-seconds run (180 volumes, FOV: 224x224x165 mm, isotropic voxel size: 3.5 mm, TE: 30 ms, TR: 2580 ms, FA: 80, parallel imaging acceleration factor 2). In the DELCODE study, participants consistently in all study centers were instructed to keep their eyes closed and not fall asleep before the resting state scan. All scans were visually inspected for completeness, cuts, subject motion and other artifacts (such

as blurring, echoes and ghosting). Images were classified as usable, questionable, or unusable and only images that were classified as usable were included.

All T1-weighted images were processed in FreeSurfer (v6, http://surfer.nmr.mgh.harvard.edu/) using the recon-all pipeline, including registration to Montreal Neurological Institute (MNI) standard space, intensity normalization, brain extraction, tissue type classification, surface reconstruction and probabilistic anatomical labeling [36]. Cortical thickness was estimated in FreeSurfer (Desikan-Killiany) atlas segmentations. A mean cortical thickness score in a composite region comprising the most vulnerable regions to atrophy in AD was calculated. This composite score was used to adjust the subsequent analyses throughout the manuscript for inter-individual differences in the degree of cortical atrophy. The composite region included the entorhinal cortex, temporal pole, inferior and middle temporal gyri, inferior and superior parietal cortices, precuneus and posterior cingulate cortex [37].

Functional connectivity analysis was performed using the CONN-fMRI Functional Connectivity Toolbox (v17, www.nitrc.org/projects/conn) and SPM12 (www.fil.ion.ucl.ac.uk/spm/), implemented in MATLAB (Release2017b, https://de.mathworks.com/products/new_products/release2017b.html). The default preprocessing pipeline for volume-based analyses was used, comprising realignment, slice-time correction, segmentation and structural and functional normalization. The Artifact Detection Toolbox (ART)-based outlier detection (https://web.mit.edu/swg/software.htm) and

smoothing using a Gaussian kernel of 6 mm at FWHM [25] was applied. Denoising was performed using the default pipeline based on linear regression of potential confounding effects of white matter and CSF [38], estimated subject-motion parameters [39], outlier scans and scrubbing [40], followed by applying a band-pass filter (below 0.008 Hz or above 0.09 Hz) [41]. Afterward, the distribution of FC correlation values was directly compared to the null-hypothesis distribution that showed a 95.5% match with the null-hypothesis, indicating a lack of noticeable associations between quality control and FC [42].

CLINICAL CHARACTERISTICS, COGNITIVE TESTING AND ASSESSMENT OF CSF BIOMARKERS

The clinical severity of dementia symptoms was quantified using the CDR-sum of boxes (CDR-sb). Cognitive performance was assessed using the Mini-Mental State Examination (MMSE), given its clinical relevance. Moreover, cognitive domain-specific (learning and memory (MEM), executive functions and mental processing speed, visuo-spatial abilities, language ability and working memory) composite scores were derived by using confirmatory factor analysis of a larger neuropsychological assessment battery (DELCODE-NP), while the global cognitive composite score was calculated by averaging all five domain-specific cognitive composite scores [43]. DELCODE-NP comprised established neuropsychological tests such as MMSE, The Alzheimer's Disease Assessment Scale-Cognitive Subscale 13, the Free and Cued Selective Reminding Test and Wechsler Memory Scale revised version Logical Memory (Story A) and Digit Span, Boston Naming Test, two semantic fluency tasks (animals and groceries),

the Boston Naming Test, the oral form of the Symbol-Digit-Modalities Test, Trail Making Test A and B, Clock Drawing and Clock Copying, and a recall task of previously copied figures and two newly developed computerized tests, i.e. the Face Name Associative Recognition Test and a Flanker task to assess executive control of attention [43]. The details of the confirmatory factor analysis procedures are reported in the previous studies [35,43], while a complete overview of individual test scores that were assigned to the five different cognitive domains are reported in the supplementary material. A trained neuropsychologist performed the neuropsychological tests at all sites [33]. CSF biomarkers were assessed using established commercially available analysis kits: V-PLEX Aβ Peptide Panel 1 (6E10) Kit (K15200E), V-PLEX Human tTau Kit (K151LAE) (Meso Scale Diagnostics LLC, Rockville, MD, USA) and Innotest Phospho-Tau(181P) (Fujirebio Germany GmbH, Hannover, Germany) [33].

ASSESSMENT OF STATIC PARAMETERS OF COGNITIVE RESERVE

Individual lifestyle differences defined as CR proxies were assessed using the total years of formal education and a validated German version [44] of the Lifetime Experiences Questionnaire (LEQ) total score, reflecting activities across the lifespan (educational, occupational, managerial history, social and intellectual activities) [45]. The LEQ total score was derived as a mean score of three sub-scores for different stages of life (early adulthood (LEQ-e, age 13 to 30 years), mid-life (LEQ-m, age 30 to 65 years) and late-life (LEQ-l, age 65 and older)). Participants with missing data (also provided in **supplementary table 1)** of N_{All}=132 (N_{HC}=49, N_{ADN}=83) for LEQ total, N_{All}=63 (N_{HC}=23, N_{ADN}=40) for LEQ-e scores,

N_{All}=73 (N_{HC}=22, N_{ADN}=51) for LEQ-m scores and N_{All}=118 (N_{HC}=47, N_{ADN}=71) for LEQ-l scores were excluded from the analyses that had included these variables. Of note, one participant with an outlier LEQ-l value (z-score > 3) was excluded from the analyses included LEQ-l. Moreover, the mean values and standard deviations of LEQ subscores by study groups are provided in **supplementary table 1**.

QUANTITATIVE RESIDUAL COGNITIVE RESERVE MARKER

In order to estimate a residual CRM for each participant, we calculated a stepwise regression model including the global cognitive composite score as the dependent variable and demographic (age and sex), genetic risk and neurodegenerative burden as predictors [7], adjusting for study sites and estimated total intracranial volume. Estimates of neurodegenerative burden included binarized *APOE* ε4 allele carrier status, CSF biomarker levels (Aβ42, tTau and pTau181), mean cortical thickness of predefined brain regions vulnerable to atrophy in AD and mean bilateral hippocampal volume (**Supplementary Fig. 1-A**, adjusted R^2=0.54). While the variables male sex (b=0.04, p=0.85 and VIF=1.2), *APOE* ε2 allele carrier status (b=0.02, p=0.95 and VIF=1) and estimated total intracranial volume (b=0.04, p=0.85 and VIF=1) did not improve the model, p-tau levels showed high collinearity (b=-0.05, p=0.16 and VIF=6.4). The high multicollinearity for p-tau was considered to be caused by t-tau, as the p-tau related variance in global cognition is fully predicted by individual differences in t-tau, e.g. very high collinearity between t-tau and p-tau, which is explained in the **supplementary material** in detail. Of note, age revealed shared effects of 10% with total-

tau and 7% with phospho-tau on global cognition. Therefore, the stepwise method removed these variables from the multilinear regression model. We utilized a stepwise approach in the multilinear regression model, aiming for the best-fitted model and less subjectivity for covariables selection. Of note, the stepwise multilinear regression utilizes both forward selection and backward elimination methods according to the defined criteria (Probability-of-F-to-enter <= 0.05, Probability-of-F-to-remove >= 0.1) .

The linearity of the regression model was approved by the normally distributed residuals (Kolmogorov-Smirnov p>0.05 under Lilliefors Significance Correction).

INDEPENDENT COMPONENT ANALYSIS OF FUNCTIONAL MRI

We applied an independent component analysis (ICA) to determine the spatial extent of the RSNs [29] and to test the intrinsic network connectivity on preprocessed resting-state fMRI data using the CONN toolbox [46]. We calculated ICA-maps, representing a measure of different networks expression and connectivity at each voxel, following the group-ICA methodology implemented in CONN. The CONN toolbox uses a temporal concatenation of blood-oxygen-level-dependent (BOLD) signal data across participants, as described previously [47,48]. Following group-ICA, subject-specific independent component maps of the DMN, SAL, DAN and FPN were back-reconstructed using the GICA3 algorithm [49]. Participant-level spatial maps were estimated through back projection, which was attained by performing dual regression with univariate spatial regression and multivariate temporal-regression steps [48]. The number of independent components to extract was set a priori to 20 [50]. To identify

RSNs from the ICA components, the obtained group ICA components were spatially compared to templates derived from the resting state network templates of the network cortical ROIs defined by ICA in 497 healthy subject from the human connectome project (HCP) dataset including FPN, DMN, DAN and SAL (dice coefficients indicating a spatial overlap: 0.37, 0.49, 0.6 and 0.2, respectively) [46].

INTRINSIC NETWORK FUNCTIONAL CONNECTIVITY ANALYSIS OF RESTING-STATE NETWORKS

Using the first-level ICA data, a second-level analysis was performed using the identified resting-state functional connectivity networks on the subject level: Separate general linear models were calculated for MEM and CRM. The results were presented using the Harvard-Oxford Atlas labels [51]. Additionally, we identified the network regions using binarized masks as group component maps at an intensity threshold of >2 from ICA for each of the four RSNs, through which we identified overlapping network regions.

SEED-TO-VOXEL FUNCTIONAL CONNECTIVITY OF MEMORY-RELATED SEED REGIONS

Using the associations between FC and MEM (see below for statistical description), we identified regions of interest using binary masking based on regions with significant MEM-related FC changes for each RSN (identified as the significant associations of the corresponding network with MEM, as described above), separately. Masked regions were used as seed regions

for every voxel in the brain. Seed-based connectivity analyses were computed using the Fisher-z-transformed bivariate correlation coefficients between a seed region's BOLD time series and any individual voxel BOLD time-series.

STATISTICAL ANALYSES

All statistical analyses were performed using SPSS, version 25.0 (IBM Corp., Somers, NY). The Bonferroni method was used to adjust for multiple comparisons in the assessment of demographical and clinical data. False discovery rate (FDR) [52] correction was applied to FC data. Kruskal-Wallis and Chi-square tests were used to compare the study groups' baseline sociodemographic, clinical and genetic variables. Analysis of Covariance (ANCOVA) was used to compare cortical thickness composite scores and hippocampal volumes, INC of each cognitive RSN, CSF biomarkers and CRM between the groups, adjusting for age and sex (additional adjustments were made for years of education in comparisons of cognitive assessments and for imaging sites in comparisons of INC), as appropriate.

The associations of CRM with CR Proxies (i.e., years of education and LEQ-total) were tested using separate multilinear regression models, adjusting for age, sex, study sites and A/T/N diagnostic subgroups. Results were reported with standardized beta coefficients (b) considered significant when $p<0.05$, corresponding to the multiple testing corrected significance level for p-Bonferroni<0.05 (one-tailed).

The associations between FC and MEM as well as CRM were tested separately on voxel-level using general linear models (see above for ICA). Likewise, the associations between

CRM and any MEM-related connectivity seeds for each RSN were tested using general linear models (see above for Seed-to-voxel analysis). Statistical models were adjusted for age, sex, site, cortical thickness composite score and the A/T/N group. Results were considered significant when $p<0.05$ in Gaussian random field theory [53] for INC, indicating a significance when cluster-level FDR-corrected $p<0.05$ and voxel-level $p<0.001$.

RESULTS

The characteristics of the study groups are shown in **Table 1**. The severity of cognitive decline and the clinical status of the ADN group and the healthy controls is presented in terms of MMSE and CDR mean scores. The ADN group was defined using a biomarker-informed stratification approach in which all participants with underlying amyloid beta pathology were combined into one group consisting of a spectrum from cognitively normal participants to participants with early AD and revealed significantly low MMSE and CDR-sb compared to controls. Aβ positive individuals were more frequently APOE ε4 allele carriers and less frequently APOE ε2 allele, had lower mean hippocampal volumes and mean cortical thickness, lower CSF Aβ42, higher CSF tTau, and pTau181, higher global CDR-sb scores and lower MMSE as well as lower global cognitive composite scores, as expected. HC was younger than ADN participants, while the groups did not differ in years of education or CRM.

ASSOCIATIONS BETWEEN CRM AND EDUCATIONAL ATTAINMENT AND LIFETIME EXPERIENCES

CRM was predicted by years of education when analyzing the entire cohort (**Supplementary Fig. 1B**, b=0.28, p<0.001, adjusted-R^2=0.06) and ADN subgroup separately (b=0.34, p<0.001, adjusted-R^2= 0.09), but not in the HC (b=0.07, p=0.52, adjusted-R^2=0.04). Furthermore, higher CRM was associated with higher LEQ-total scores in the entire sample (**Supplementary Fig. 1-C**, b=0.26, p<0.001, adjusted-R^2=0.05) and ADN subgroup (b=0.29, p=0.002, adjusted-R^2=0.05) , but not in the HC (b=0.15, p=0.27, adjusted-R^2=0.03).

ASSOCIATIONS BETWEEN FUNCTIONAL NETWORK CONNECTIVITY AND MEM

We tested the associations between INC of each RSN and MEM. The whole cohort revealed a positive association between DMN INC and MEM. Furthermore, MEM score was positively associated with inter-network connectivity between DMN and SAL (**Table 2, Fig. 1-B and Supplementary Fig. 2-A**). However, in the FPN and the SAL, MEM scores were negatively associated with INC in frontal and parietal brain regions (**Table 2, Fig1-B and Supplementary Fig.2-B and 2-C**).

ASSOCIATIONS BETWEEN CRM AND INTRINSIC NETWORK CONNECTIVITY IN COGNITIVE NETWORKS

CRM was positively associated INC within the DMN, particularly in the posterior cingulate cortex and the precuneus (**Table 3, Fig. 1-C and Supplementary Fig. 3-A**). In a subgroup analysis, CRM was positively associated with anti-correlation between the FPN and DMN in the A+T-N- group (**Table 3, Fig. 1-C and Supplementary Fig. 3-C**) and between the SAL and

the DMN in the A+T/N+ group (**Table 3, Fig. 1-C and Supplementary Fig. 3-C**). We found a negative association of FPN with the frontal pole in the A+T/N- group and a negative association of SAL in occipital regions in the A+T/N+ group, showing no spatial overlap with the cognitive RSNs. Notably, no associations were found in the ADN.

To test the specific effects of CRM on network FC, we adjusted the models additionally for years of education. CRM revealed positive associations with INC of the DMN and FPN only in the entire cohort when accounted for years of education (**Supplementary Table 2 and Supplementary Fig. 4A-B**). Moreover, years of education revealed no association with INC of any RSN in the general linear model, even after CRM was excluded. Like education, LEQ-total score did not show any association with FC with and without CRM as covariable. We found no significant associations between the CRM and INC of any networks when the number of education years was replaced with LEQ-t (available data of participants n=186).

ASSOCIATIONS BETWEEN CRM AND FC OF MEM-RELATED SEED REGIONS

A seed-to-voxel analysis revealed a negative association of CRM with FC of MEM-related region seed of the FPN with the right angular gyrus, a lateral DMN region (**Table 4, Fig. 1-D and Supplementary Fig. 5-B**) in the ADN group. More, CRM was associated with lower FC of the FPN seed region with the DMN (posterior cingulate cortex) in the A+T/N+ group (**Table 4, Fig. 1-D and Supplementary Fig. 5-B**).

DISCUSSION

The present study provides further evidence on the neural underpinnings of CR, i.e. neurobiological changes associated with the resting-state functional connectivity alteration estimated using residualized cognitive performance in INC of the RSNs of interest. The CRM was associated with commonly used socio-behavioral CR proxies, including years of education, and the LEQ total score, assessing mental activity levels over the lifespan. Our experiment extends previous research by including the aspect of biomarker-defined A/T/N groups, revealing neural associations of residual CRM, especially in the earlier AD stages (A+T/N- and A+T/N+). In ADN, higher CRM is associated with INC changes within and between cognitive RSNs, particularly the DMN, the FPN and the SAL. This finding supports previous findings suggesting that reserve has its most considerable impact in the transitional stage between physiological aging and advanced neurodegeneration [3,54].

The observed positive associations between INC changes and memory scores align with the literature, showing similar associations in the DMN, particularly in the posterior cingulate cortex and precuneus [50,55]. We found increased network connectivity in the DMN and FPN with higher performance in the memory domain and increased connectivity between networks of the posterior DMN regions and the FPN. We also found negative associations between memory composite scores, between-network connectivity for SAL-DMN and SAL-FPN and between the FPN and the anterior DMN. In contrast, no association was observed between memory and INC in the DAN.

We found higher INC in the DMN in subjects with higher CR when tested in the entire cohort. Here, A/T/N groups revealed different associations between INC and CRM, with a

higher anti-correlation between DMN and SAL only found in the A+T+N+ group. Higher INC in the DMN might contribute to CR when disruptions in the functional network due to AD-related neurodegenerative changes occur. Therefore, the associations between CRM and INC within the DMN may suggest inter-individual variability in network properties, pointing towards a possible neural representation of CR. More, the INC of the FPN showed a lower FC in the medial frontal region, also part of the DMN [55], suggesting a possible association between CRM and DMN-FPN anti-correlation. Also the seed-to-voxel analyses in the present study revealed associations of CRM with FC of MEM-related seed region in FPN anti-correlations between FPN and DMN in ADN and A+T/N+ subgroups in right lateral regions and medial regions of DMN, respectively. In line with these findings, a previous study suggested a CR-related higher anti-correlation between DMN and the left frontal cortex (i.e, Brodmann area 6/44), a hub region of FPN [26]. Previous studies also proved that more efficient networks are associated with higher CR, particularly involving regions in the DMN [22,23,56] and FPN [27,57].

Our findings support the major role of the FPN in CR and, more precisely, in neural compensation. In previous work, an association between CR proxies such as education and functional connectivity of FPN was found in preclinical AD, i.e. mild cognitive impairment [54]. However, another study suggested no association between the activity of FPN and increased compensation, as no temporary changes in FPN activity were observed with disease progression [27]. Besides their role in CR, FPN and FPN-DMN connectivity coupling might also play a more general protective role, with proven associations for CR and other lifestyle

factors such as sleep [58]. Similarly, the results of the present study show differences between the entire cohort and individuals in ADN, suggesting compensatory changes.

Interestingly, the association between CRM and FC remained significant for INC of the DMN after analysis accounted for education in contrast to the anti-correlation between DMN and FPN in the entire cohort. Additionally, CRM was associated with higher INC within the FPN when the general linear model was adjusted for education. However, all associations between CRM and FC were no longer significant when LEQ-t was accounted for. This finding might suggest that the CRM can predict interindividual FC differences beyond education, while LEQ-t removed the associations between CRM and FC. Therefore, we speculate that LEQ-t can more effectively estimate the residualized cognition than years of education alone. In line with this observation, we reported in our previous study that LEQ predicts FC changes within DMN, while years of education revealed no association with DMN connectivity [59]. An alternative explanation can be the potential loss of statistical power for LEQ due to missing data.

The socio-behavioral proxies revealed no significant associations with INC in any RSN, even when CRM was excluded from the statistical model. These findings might indicate a unique effect of residualized cognition, i.e. CRM, while capturing the FC alterations related to CR. These findings align with a previous report defining residual approaches as a resilience measure apart from socio-behavioral proxies [60]. However, the residualized cognitive reserve approach can estimate the inter-individual variance possibly partly due to the lifetime

experiences [32] and, therefore, might be not only unique but also more suitable. Future studies are needed to identify the relationship between both approaches to measuring CR.

Our data suggest also that FC in the pre-supplementary motor area and the anteromedial prefrontal cortex are associated with the cognitive reserve in ADN, considering the ICA results for FPN. The first region has been described in the cognitive motor control network. It is involved in complex processes such as learning and cognitive functions [61], while the latter was identified as critical for specific components of social interpretation and behavioral interactions [62].

A recent interventional study identified the effects of cognitive intervention, showing improved FPN activity and better maintenance of DMN activity in amnestic mild cognitive impairment after a vision-based speed of processing training [63]. This pilot study provides an approach to explaining the functional neural alterations associated with CR and demonstrates the practical value of the concept for developing effective intervention strategies against cognitive decline. Other non-invasive stimulation techniques, such as transcranial magnetic stimulation and focused ultrasound pulse stimulation, may have similar beneficial effects on RSNs [64,65].

A limitation of our work is the cross-sectional study design, precluding firm conclusions on causality. Moreover, an important cohort-relevant limitation is that the participants grouped as AD continuum might underrepresent participants with moderate and severe AD dementia. However, due to relatively large group sizes, our results are sufficiently powered to support the validity of the observed associations. Future studies with longitudinal

datasets are needed to examine causal relationships between functional network measures and residual CRM. We recommend a further characterization of residual CRM in biomarker-stratified cohorts in future studies. As the residual approach has been studied using different statistical approaches and modalities (26), it is less established compared to socio-behavioral proxies of CR; this shortcoming should be addressed in future studies. However, we conducted regression analyses to validate the residual approach to investigate the associations between CRM, education, and lifelong experiences.

To conclude, our results advance the understanding of the neurobiological substrates of CR by delineating mechanisms of neural implementation in functional RSNs. The detailed characterization of CRM-related network differences among individuals with AD pathology and controls will be relevant for designing future clinical trials and preventive strategies in AD.

ACKNOWLEDGEMENTS
The DZNE-Longitudinal Cognitive Impairment and Dementia Study (DELCODE) study was funded by the German Center for Neurodegenerative Diseases (Deutsches Zentrum für Neurodegenerative Erkrankungen, DZNE), reference number BN012.

DISCLOSURES
Ersin Ersoezlue, Boris-Stephan Rauchmann, Maia Tatò, Julia Utecht, Carolin Kurz, Jan Häckert, Selim Guersel, Lena Burow, Gabriele Koller, Sophia Stöcklein, Daniel Keeser, Boris Papazov, Marie Totzke, Tommaso Ballarini, Frederic Brosseron, Katharina Buerger, Peter Dechent, Laura Dobisch, Michael Ewers, Klaus Fliessbach, Wenzel Glanz, John Dylan Haynes, Michael T Heneka, Daniel Janowitz, Ingo Kilimann, Luca Kleineidam, Christoph Laske, Franziska Maier, Matthias H Munk, Oliver Peters, Alfredo Ramirez, Sandra Röske, Nina Roy, Klaus Scheffler, Anja Schneider, Björn H Schott, Annika Spottke, Eike Jakob Spruth, Stefan Teipel, Chantal Unterfeld, Michael Wagner, Xiao Wang, Jens Wiltfang, Steffen Wolfsgruber, Renat Yakupov and Emrah Düzel report no disclosures. Frank Jessen received fees for consultation from Eli Lilly, Novartis, Roche, BioGene, MSD, Piramal, Janssen and Lundbeck. Josef Priller received fees for consultation, lectures and patents from Neurimmune, Axon, Desitin and Epomedics. Robert Perneczky received speaker honoraria and fees for consultation from Janssen, Roche, Biogen, Eli Lilly, Abbott, Schwabe and Grifols.

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TABLES

Table 1. Demographic and clinical characteristics of the study cohort. Mean values are presented if not indicated otherwise.

Abbreviations: HC, healthy controls; ADN, Alzheimer's disease neuropathological-continuum; Aβ42, Amyloid-beta 42; tTau, total tau; pTau, phosphorylated tau 181; CRM, cognitive reserve marker; MEM, memory cognitive composite score; MMSE, mini-mental state examination; CDR-sb, Clinical Dementia Ratio – sum of boxes; LEQ, lifetime experiences questionnaire; SD, standard deviation; SE, standard error. *Total score of LEQ was available in n=186 (n=63 in HC, n=123 in A+) participants.

				ADN			
	HC (N=112)	**ADN (N=206)**	**P (HC vs A+)**	A+T-N- (N=106)	A+T/N+ (N=28)	A+T+N+ (N=72)	**P (overall)**
Age (SD)[a]	69 (6)	72 (6)	<0.001	70 (6) [h]	71 (6)	74 (6) [e, f]	<0.001
Sex (female, N/%) [b]	52 (46.4)	101 (49)	0.66	49 (46)	14 (50)	38 (53)	0.81
Years of formal education (SD) [a]	15 (3)	14 (3)	0.16	14 (3)	14 (3)	14 (3)	0.22
APOE Ɛ4-allele (carrier, N/%) [b]	18 (16)	113 (55)	<0.001	44 (42) [e, h]	17 (61) [e]	52 (72) [e, f]	<0.001
APOE Ɛ2-allele (carrier, N/%) [b]	20 (18)	20 (10)	0.04	12 (11)	3 (11)	5 (0.07)	0.16

MMSE (SD) [a]	29 (1)	27 (3)	<0.001	28 (2) [h]	28 (2) [h]	26 (3) [e, f, g]	<0.001
CDR-sb (SD) [a]	0 (0.1)	2 (2)	<0.001	1 (1) [e]	2 (2) [e]	3 (2) [e, f]	<0.001
Mean hippocampal volume (mm^3, SD) [c]	3149 (30)	2820 (32)	<0.001	2944 (42) [e, h]	2807 (87) [e]	2584 (49) [e, f]	<0.001
Mean cortical thickness (cm, SD) [c]	2.71 (0.1)	2.6 (0.01)	<0.001	2.64 (0.1) [e, h]	2.62 (0.3) [e]	2.53 (0.02) [e, f]	<0.001
CSF biomarkers							
Aβ42 (pg/ml, SE) [c]	898 (18)	428 (13)	<0.001	450 (12) [e]	419 (23) [e]	415 (14) [e]	<0.001
tTau (pg/ml, SE) [c]	299 (8)	524 (22)	<0.001	302 (11) [g, h]	584 (20) [e, f]	904 (33) [e, f]	<0.001
pTau (pg/ml, SE) [c]	43 (0.7)	68 (3)	<0.001	42 (1) [g, h]	68 (2) [e, f]	113 (5) [e, f]	<0.001
Global cognitive composite (Z-score, SE) [d]	0.4 (0.04)	-0.44 (0.06)	<0.001	-0.19 (0.09) [e, h]	-0.19 (0.17) [e, h]	-0.97 (0.1) [e, f, g]	<0.001
MEM (Z-score, SE) [d]	0.51 (0.04)	-0.56 (0.07)	<0.001	-0.12 (0.09) [e, h]	-0.34 (0.19) [e, h]	-1.29 (0.11) [e, f, g]	<0.001
MMSE (SE) [d]	29 (0.1)	27 (0.2)	<0.001	28 (0.2) [h]	28 (0.5) [h]	26 (0.4) [e, f, g]	<0.001
CRM (Residuals, SE) [c]	-0.007 (0.05)	0.004 (0.67)	0.83	-0.04 (0.06)	0.25 (0.14)	-0.03 (0.08)	0.15

LEQ-total [a]*† (SD)	120 (25)	115 (27)	0.38	115 (25)	115 (28)	114 (28)	0.74

[a]Kruskal-Wallis-test, [b]Chi-Square-test, [c]Analysis of Covariance tests were conducted, adjusting for age, sex and sites. Means and frequencies are shown, [d]Analysis of Covariance tests were conducted, adjusting for age, sex, years of education and site, [e]Bonferroni-p<0.05 versus HC, [f]Bonferroni-p<0.05 versus A+T-N-, [g]Bonferroni-p<0.05 versus A+T/N+, [h]Bonferroni-p<0.05 versus A+T+N+. †LEQ-total values are available for N_{HC}=63, N_{ADN}=123, N_{A+T-N-}=63, $N_{A+T/N+}$=17 and N_{A+T+N+}=46 participant.

Table 2. Associations between memory cognitive composite score and intrinsic network connectivity on whole brain level. *Network regions identified through the independent component analysis. **Atlas regions in the Harvard-Oxford Atlas.

Abbreviations: RSN, resting-state network; DMN, default mode network; FPN, frontoparietal network; SAL, salience network, DAN, dorsal attention network; FDR, false-discovery rate; tri-IFG, Inferior Frontal Gyrus, pars triangularis; PC, Cingulate Gyrus, posterior division; SFG, superior frontal gyrus: PostCG, postcentral gyrus; l, left; r, right.

RSN	Cluster (x,y,z)	Cluster size (voxels)	p-FDR	Overlapping network ROI* (x,y,z)	Main atlas region** (voxel size)
DMN	-04 -74 +40	483	<0.001	DMN, n=7675 (0,-51,34)	Precuneous (453)
	-52 +28 +14	257	0.002		l tri-IFG (176)
	+58 +34 +04	212	0.006	SAL, n=3433 (-44,12,20)	r tri-IFG (153)
	-04 -26 +32	180	0.01		PC (153)
	-04 -42 +46	122	0.03		Precuneous (98)
FPN	-04 +52 +36	127	0.042		l SFG (60)
					Precuneous (56)
SAL	-04 -46 +56	165	0.046		r PostCG
DAN	n.s.				

Table 3 Associations between CRM and intrinsic connectivity of resting-state networks on whole brain level using general linear models in the entire cohort and in the A/T/N groups. *Network regions identified through the independent component analysis. **Atlas regions in the Harvard-Oxford Atlas.

Abbreviations: CRM, cognitive reserve marker; DMN, default mode network; FPN, frontoparietal network; SAL, salience network; DAN, dorsal attention network; ROI, region of interest; FDR, false discovery rate; p-FDR, FDR-corrected p-value; SFG, Superior Frontal Gyrus; FP, Frontal Pole; PC, Cingulate Gyrus, posterior division; ICC, Intracalcarine Cortex; l, left; r, right.

	RSN	Cluster (x,y,z)	Cluster size (voxels)	p-FDR	Overlapping network ROI (x,y,z)*	Main atlas region (voxel size)**
Entire cohort	**DMN**	-04 -26 +32	170	0.02	DMN, size=7675 voxels (0,-51,34)	PC (103) Precuneus (38)
	FPN	-04 +56 +32	217	0.003	-	l SFG (100) l FP (51)
	SAL	n.s.				
	DAN	n.s.				

A+T-N-	DMN	n.s.				
	FPN	-10 +56 +14	293	<0.001	-	l FP (112)
	SAL	n.s.				
	DAN	n.s.				
A+T/N+	DMN	n.s.				
	FPN	n.s.				
	SAL	+10 -84 +04	115	0.02	-	r ICC (81)
	DAN	n.s.				
A+T+N+	DMN	n.s.				
	FPN	n.s.				
	DMN	n.s.				
	SAL	+04 -50 +28	120	0.04	DMN, size=7675 voxels (0,-51,34)	PC (60) Precuneus (48)

Table 4 Associations between CRM and seed-to-voxel functional connectivity of memory domain score related regions for each resting-state network using general linear models in the ADN and in A/T/N groups. *Network regions identified through the independent component analysis. **Atlas regions in Harvard-Oxford Atlas.

Abbreviations: CRM, cognitive reserve marker; A+, Amyloid-β positive; DMN, default mode network; FPN, frontoparietal network; SAL, salience network; DAN, dorsal attention network; AG, angular gyrus; sLOC, Lateral Occipital Cortex, superior division; pSMG, Supramarginal Gyrus, posterior division; PC, cingulate gyrus, posterior division; ROI, region of interest; FDR, false discovery rate; p-FDR, FDR-corrected p-value;

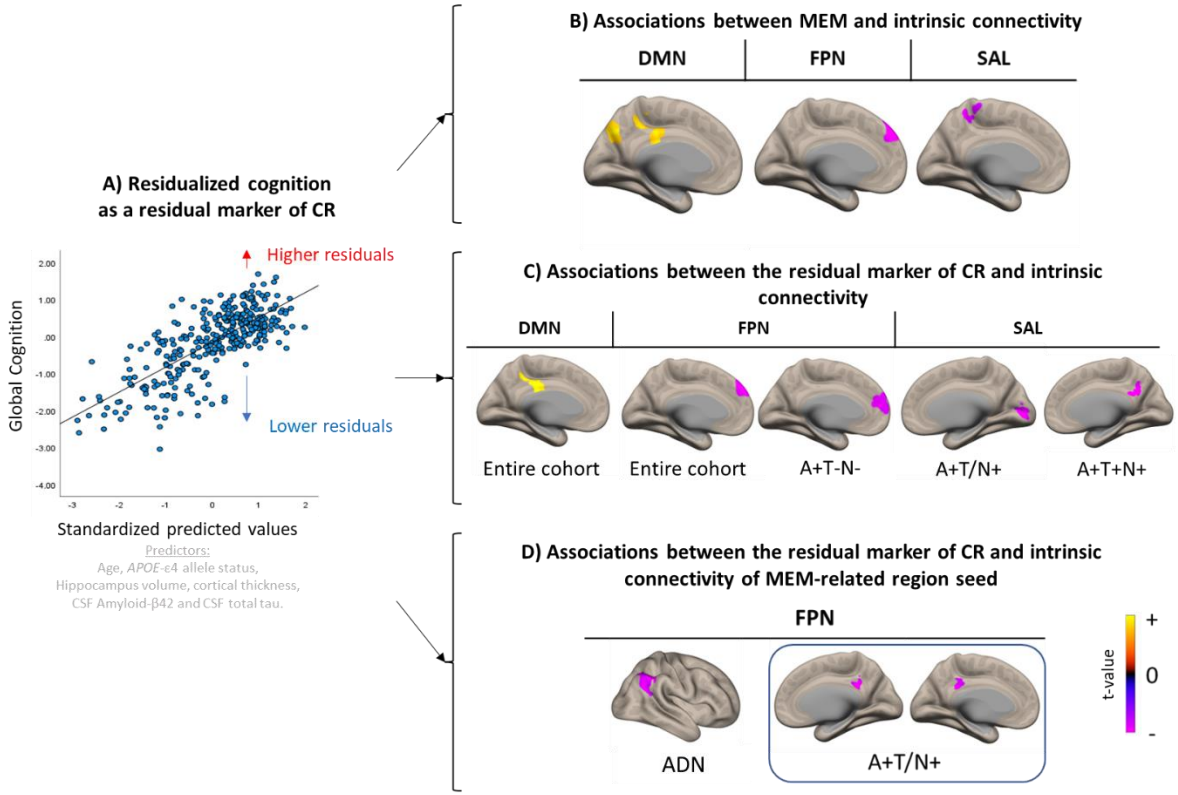
	RSN with MEM-related seed connectivity	Cluster (x,y,z)	Cluster size (voxels)	p-FDR	Overlapping network ROI (x,y,z)*	Main atlas region (voxel size)**
A+	DMN	n.s.				
	FPN	+52 -46 +50	779	<0.001	FPN, n=2321 voxels (44,-53,46)	r AG (525)

						DMN, n=1140 voxels (49,-58,30)	r sLOC (132) r pSMG (94)
	SAL	n.s.					
	DAN	n.s.					
A+T-N-	DMN	n.s.					
	FPN	n.s.					
	SAL	n.s.					
	DAN	n.s.					
A+T/N+	DMN	n.s.					
	FPN	0 -42 +32	120	0.01		DMN, n=7675 voxels (0,-51,34)	PC (111)
	SAL	n.s.					
	DAN	n.s.					
A+T+N+	DMN	n.s.					
	FPN	n.s.					

SAL	n.s.
DAN	n.s.

FIGURE LEGENDS

Figure 1. A) Estimation of cognitive reserve marker as residuals in a multilinear regression model. **B)** Associations between memory cognitive composite score and intrinsic network connectivity to derive MEM-related seed regions. **C)** Associations between CRM and intrinsic connectivity of resting-state networks in whole brain in the entire cohort and A/T/N subgroups. **D)** Associations between CRM and functional connectivity of seeds of MEM-related regions in each resting-state network in the entire cohort and A/T/N subgroups. The color bar represents t-values. Abbreviations: MEM, memory cognitive composite score; DMN, default mode network; FPN, frontoparietal network; SAL, salience network; DAN, dorsal attention network; FDR, false-discovery rate; CRM, cognitive reserve marker; ADN, Alzheimer's disease neuropathological-continuum; p-FDR, FDR-corrected p-value.


A) Residualized cognition as a residual marker of CR
Higher residuals
Lower residuals
Global Cognition
Standardized predicted values
Predictors:
Age, APOE-ε4 allele status,
Hippocampus volume, cortical thickness,
CSF Amyloid-β42 and CSF total tau.
B) Associations between MEM and intrinsic connectivity
DMN
FPN
SAL
C) Associations between the residual marker of CR and intrinsic connectivity
DMN
FPN
SAL
Entire cohort
Entire cohort
A+T-N-
A+T/N+
A+T+N+
D) Associations between the residual marker of CR and intrinsic connectivity of MEM-related region seed
FPN
ADN
A+T/N+
t-value
+
0
-

Supplementary material to

A residual marker of cognitive reserve is associated with resting-state intrinsic functional connectivity along the Alzheimer's disease continuum

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Running title: Residual marker of CR captures network FC
Keywords: Cognitive reserve; Alzheimer's disease; cognition; fMRI; resting-state functional connectivity; intrinsic network connectivity

Assignments of the single neuropsychological tests to the cognitive domain-specific composite scores

The cognitive domain-specific composite scores were derived by assigning the following neuropsychological tests from the neuropsychological assessment battery (DELCODE-NP) via the confirmatory factor analysis for each cognitive domain-specific composite score as reported by Wolfsgruber et al. [1]:

- Learning and memory: Word List 1, 2 and 3, Word List Delayed Recall, Word List Recognition, Free and Cued Selective Reminding Test (FCSRT) Free Recall, FCSRT Cue Efficiency, Wechsler Memory Scale Logical Memory 1 and 2, Figure Savings, Incidental Learning (Symbol digit modality test) and Face Name Associative Recognition Test (Computerized test).
- Executive functions: Trail Making Test A and B, Number Cancellation, Symbol Digit Modality Test and Flanker Task (Computerized test))
- Language abilities: Verbal Fluency Groceries, Verbal Fluency Animals, Boston Naming Test and FCSRT Naming Part.
- Visuospatial abilities: Clock copying, Clock drawing and Figure copying
- Working memory: Digit Span Forward, Digit Span Backward and FCSRT Subtraction

The examination of the relationship among variables in the multilinear regression model on global cognition

To provide more insight into the nature of the variables in the multilinear regression model from which the residual cognitive reserve marker was derived, we tested the simple, unique and shared effects [2] of variables of interests, namely age, total-tau and phospho-tau. We found that (1) age (adjusted-R^2=0.17), total-tau (adjusted-R^2=0.28) and phospho-tau (adjusted-R^2=0.22) had simple effects on global cognitive composite score; (2) both total-tau (R^2-change=0.19, $p<0.001$) and phospho-tau (R^2-change=0.15, $p<0.001$) revealed unique effects on global cognitive composite score, that is, the component of the effect of the respective variable on global cognition that is unrelated to age; (3) shared effect between age and total-tau was 10% and between age and phospho-tau was 7% on global cognition; and more interestingly (4) only total-tau had a unique effect on global cognitive composite score over phospho-tau (R^2-change=0.06, $p<0.001$), while phospho-tau had a negligeable part of its effect on global cognition that is unrelated to total-tau (R^2-change=0.002, $p=0.41$). The results suggest, in summary, that phospho-tau provides no additional predictive value for global cognition over total-tau.

References:

[1] Wolfsgruber S, Kleineidam L, Guski J, Polcher A, Frommann I, Roeske S, Spruth EJ, Franke C, Priller J, Kilimann I, Teipel S, Buerger K, Janowitz D, Laske C, Buchmann M, Peters O, Menne F, Fuentes Casan M, Wiltfang J, Bartels C, Düzel E, Metzger C, Glanz W, Thelen M, Spottke A, Ramirez A, Kofler B, Fließbach K, Schneider A, Heneka MT, Brosseron F, Meiberth D, Jessen F, Wagner M, DELCODE Study Group (2020) Minor neuropsychological deficits in patients with subjective cognitive decline. *Neurology* **95**, e1134–e1143.

[2] Lindenberger U, Pötter U (1998) The complex nature of unique and shared effects in hierarchical linear regression: Implications for developmental psychology. *Psychol Methods* **3**, 218–230.

Supplementary Table 1. The valid percentages of the available cases, number of cases, mean values and standard deviations of LEQ subscores and LEQ total score. One participant with an outlier LEQ-l value (z-score > 3) was excluded from the table, and analyses included LEQ-latelife scubscore.

Abbreviations: LEQ, Lifetime Experiences Questionnaire.

	Healthy controls				Alzheimer's disease neuropathological-continuum			
	Valid N %	Valid N	Mean	Standard Deviation	Valid N %	Valid N	Mean	Standard Deviation
LEQ early adulthood	79%	89	38	9	81%	166	36[a]	8
LEQ mid-life	80%	90	45	11	75%	155	42	12
LEQ latelife	58%	65	37	9	65%	134	35	10
LEQ total	56%	63	120	25	60%	123	115	27

[a]Univariate analysis of covariance $p<0.05$ in comparison to heatlhy controls.

Supplementary Table 2. Associations between CRM and intrinsic connectivity of resting-state networks using general linear models in the entire cohort and in the A+ group. *Network regions from the independent component analysis. **Atlas regions in the Harvard-Oxford Atlas.

Abbreviations: CRM, cognitive reserve marker; DMN, default mode network; FPN, frontoparietal network; SAL, salience network; ROI, region of interest; FDR, false discovery rate; p-FDR, FDR-corrected p-value; FP, Frontal Pole; PC, Cingulate Gyrus, posterior division; r AG, Angular Gyrus Right; r sLOC, Lateral Occipital Cortex, superior division Right; r SPL, Superior Parietal Lobule Right; r pSMG, Supramarginal Gyrus, posterior division Right; l, left; r, right; n.s., not significant.

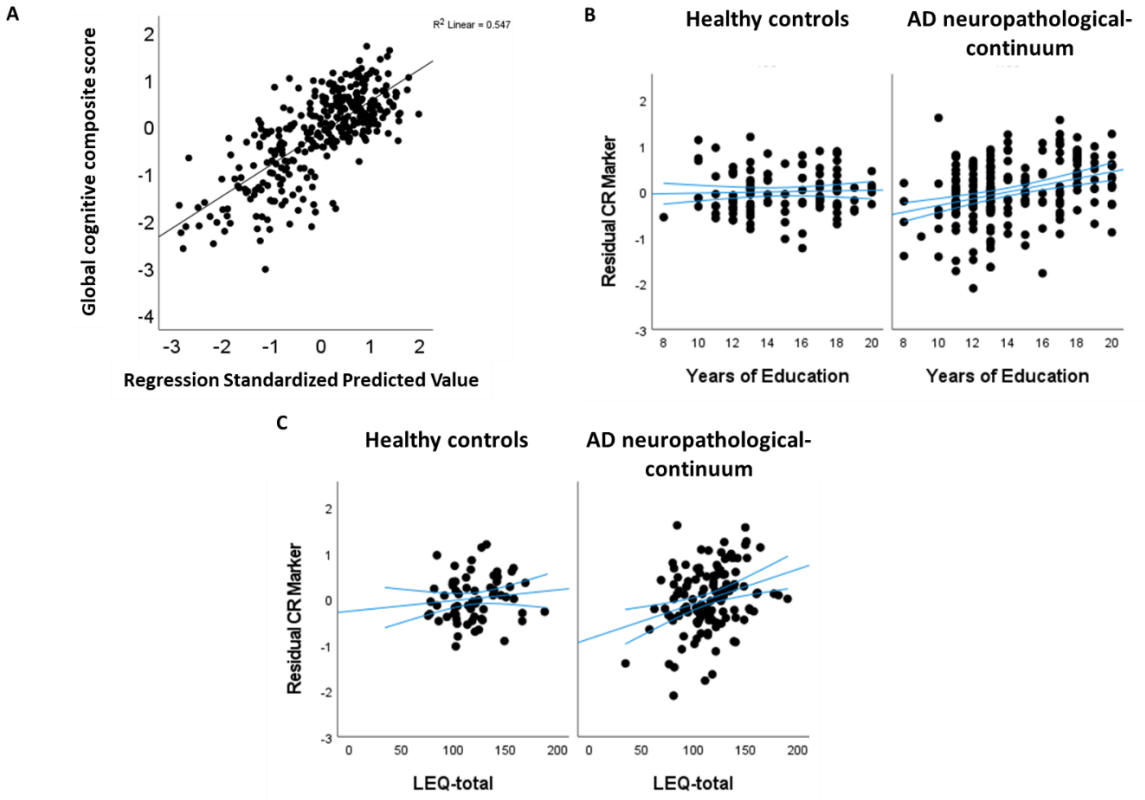
	RSN	Cluster (x,y,z)	Cluster size (voxels)	p-FDR	Overlapping network ROI (x,y,z)*	Main atlas region (voxel size)**
Entire cohort	**DMN**	-04 -42 +46	156	0.03	DMN, size=7675 voxels (0,-51,34)	PC (89) Precuneus (46)
	FPN	+42 -54 +54	163	0.02	FPN, n=2321 voxels (44,-53,46)	r AG (81) r sLOC (32) r SPL (22) r pSMG (20)

	SAL	n.s.
	DAN	n.s.
A+	DMN	n.s.
	FPN	n.s.
	SAL	n.s.
	DAN	n.s.

Supplementary figure legends

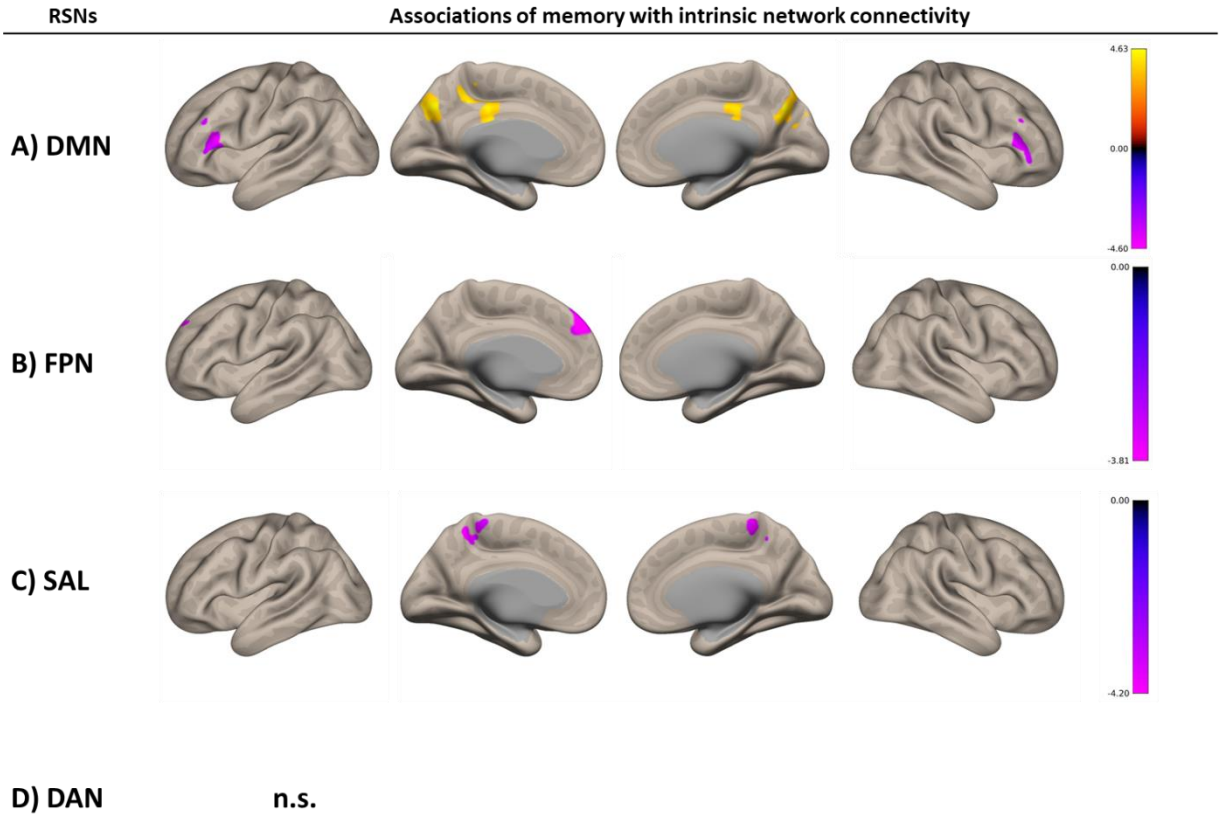
Supplementary Figure 1. **A)** Plot graphic that shows the global cognitive composite score on the vertical axis and the predicted global cognitive performance as predicted by the included (dependent) variables on the horizontal axis in the entire cohort. Multilinear regressions of years of education **(B)** and lifetime experiences questionnaire **(C)** on the residual cognitive reserve marker in the healthy controls and AD neuropathological-continuum groups.

Abbreviations: CR, cognitive reserve, AD, Alzheimer's disease; LEQ-total, total lifetime experiences questionnaire score.



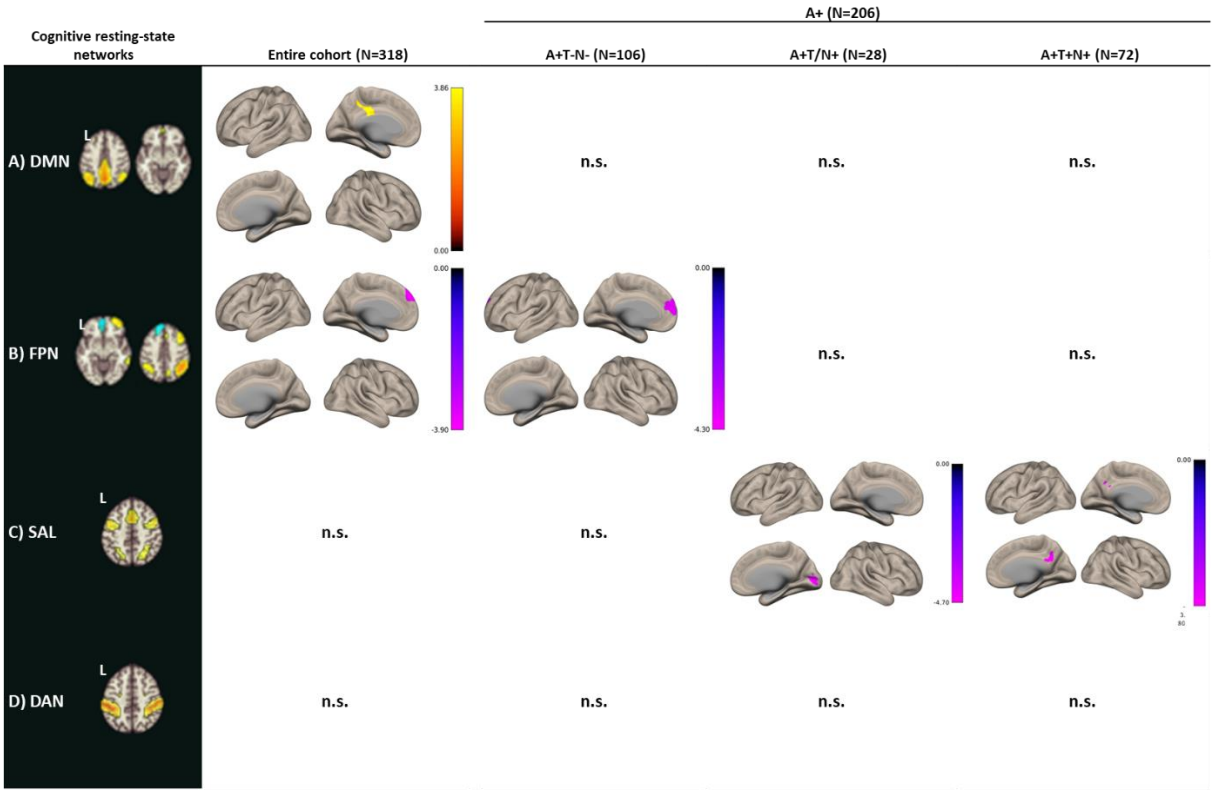
Supplementary Figure 2. Associations between memory cognitive composite score and intrinsic network connectivity. The color bar represents T-values.

Abbreviations: DMN, default mode network; FPN, frontoparietal network; SAL, salience network; DAN, dorsal attention network; FDR, false-discovery rate; n.s., not significant.



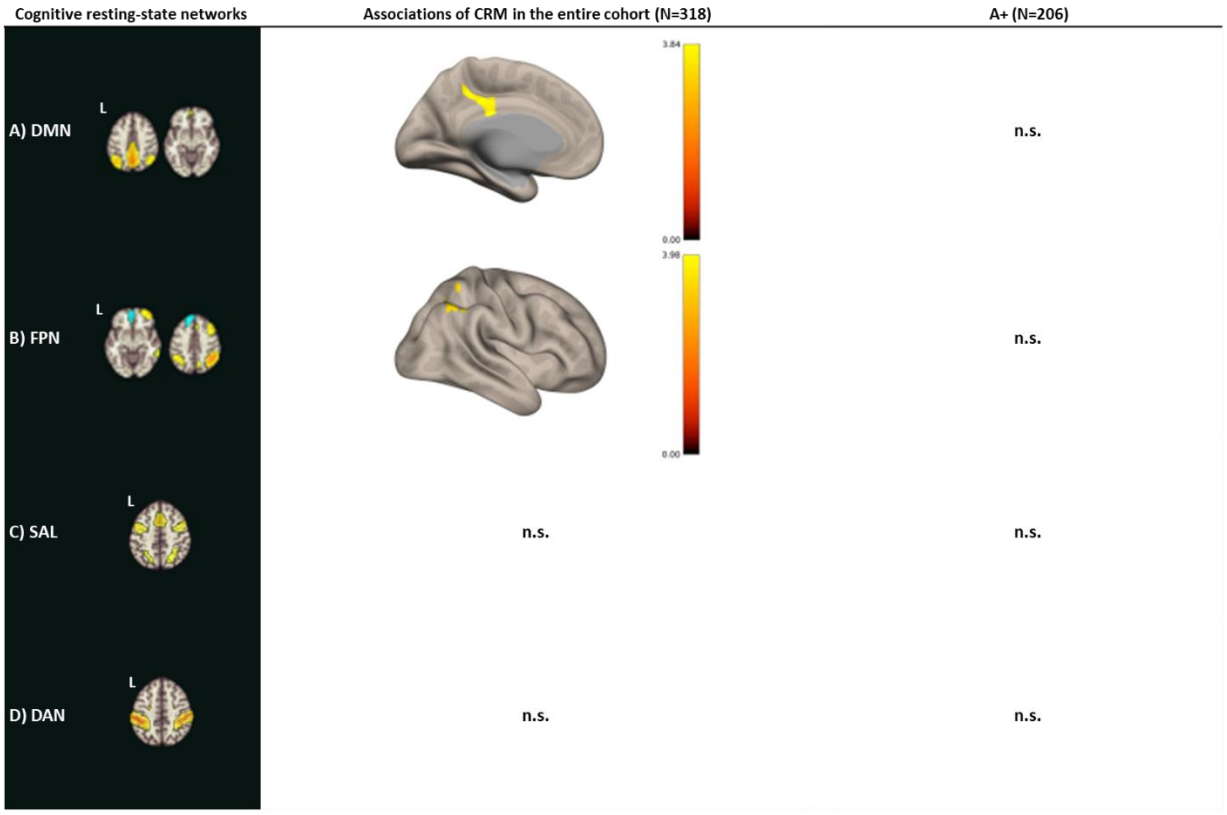
Supplementary Figure 3. Associations between CRM and intrinsic connectivity of cognitive resting-state networks in the entire cohort and A/T/N subgroups.

Abbreviations: CRM, cognitive reserve marker; A+, Alzheimer's disease; DMN, default mode network; FPN, frontoparietal network; SAL, salience network; DAN, dorsal attention network; FDR, false discovery rate; p-FDR, FDR-corrected p-value; n.s., not significant; L, left.



Supplementary Figure 4. Associations between CRM and intrinsic connectivity of cognitive resting-state networks in the whole brain in the entire cohort and A+ group. The color bars represent T-values.

Abbreviations: CRM, cognitive reserve marker; A+, Alzheimer's disease; DMN, default mode network; FPN, frontoparietal network; SAL, salience network; DAN, dorsal attention network; n3.s., not significant; L, left.



Supplementary Figure 5. Associations between CRM and functional connectivity of seeds of MEM-related regions in each cognitive resting-state network in the entire cohort and A/T/N subgroups. The color bar represents T-values.

Abbreviations: CRM, cognitive reserve marker; A+, Amyloid-β positive; DMN, default mode network; FPN, frontoparietal network; SAL, salience network; DAN, dorsal attention network; RSN, resting-state network; n.s., not significant.