Appendix/Supplement (Billette et al.)

e-Tables

Table e-1. Univariate ANOVAs predicting ROI activity by clinical groups and additional covariates

Factor/Covariate	ROI	Sum of Squares	df	Mean square	F	Sig.	Partial Eta2	Decentered Parameter	power
Model 0a	**N=486**								
Diagnosis Group	Entorhinal Ctx.	31,154	3	10,385	2,567	,054	,016	7,700	,632
	Hippocampus	39,889	3	13,296	2,786	**,040**	,017	8,358	,671
	Precuneus	90,929	3	30,310	4,313	**,005**	,027	12,939	,866
Model 0b	**N=224 (CSF)**								
Diagnosis Group	Entorhinal Ctx.	40,545	3	13,515	3,443	**,018**	,047	10,329	,767
	Hippocampus	38,042	3	12,681	2,566	,056	,035	7,697	,626
	Precuneus	93,468	3	31,156	4,933	**,002**	,066	14,799	,908
Model 1	**N=476**								
Diagnosis Group	Entorhinal Ctx.	32,340	3	10,780	2,626	,050	,017	7,879	,643
	Hippocampus	35,463	3	11,821	2,443	,064	,016	7,328	,608
	Precuneus	72,680	3	24,227	3,542	**,015**	,023	10,626	,785
APOE4 status	Entorhinal Ctx.	1,929	1	1,929	,470	,493	,001	,470	,105
	Hippocampus	9,189	1	9,189	1,899	,169	,004	1,899	,280
	Precuneus	,533	1	,533	,078	,780	,000	,078	,059
Model 2a	**N=224**								
Diagnosis Group	Entorhinal Ctx.	39,618	3	13,206	3,365	**,020**	,046	10,096	,757
	Hippocampus	31,100	3	10,367	2,089	,103	,029	6,267	,529
	Precuneus	94,051	3	31,350	4,953	**,002**	,066	14,858	,909
CSF Aß42/40	Entorhinal Ctx.	4,183	1	4,183	1,066	,303	,005	1,066	,177
	Hippocampus	,740	1	,740	,149	,700	,001	,149	,067
	Precuneus	3,435	1	3,435	,543	,462	,003	,543	,114
Model 2b	**N=224**								
Diagnosis Group	Entorhinal Ctx.	38,856	3	12,952	3,291	**,022**	,045	9,874	,746
	Hippocampus	31,433	3	10,478	2,112	,100	,029	6,335	,534
	Precuneus	93,393	3	31,131	4,913	**,003**	,066	14,738	,906
CSF p-tau	Entorhinal Ctx.	1,846	1	1,846	,469	,494	,002	,469	,105
	Hippocampus	,805	1	,805	,162	,688	,001	,162	,069
	Precuneus	1,973	1	1,973	,311	,577	,001	,311	,086
Model 2c	**N=224**								
Diagnosis Group	Entorhinal Ctx.	39,414	3	13,138	3,359	**,020**	,046	10,078	,756
	Hippocampus	25,836	3	8,612	1,745	,159	,024	5,235	,452
	Precuneus	93,388	3	31,129	4,906	**,003**	,066	14,719	,906
CSF Aß42/p-tau	Entorhinal Ctx.	6,954	1	6,954	1,778	,184	,008	1,778	,264
	Hippocampus	6,312	1	6,312	1,279	,259	,006	1,279	,203
	Precuneus	,315	1	,315	,050	,824	,000	,050	,056
Model 3	**N=486**								
Diagnosis Group	Entorhinal Ctx.	31,271	3	10,424	2,571	,054	,016	7,713	,632
	Hippocampus	36,485	3	12,162	2,543	,056	,016	7,628	,627
	Precuneus	90,855	3	30,285	4,300	**,005**	,027	12,901	,865
Regional Volume	Entorhinal Ctx.	,199	1	,199	,049	,825	,000	,049	,056
	Hippocampus	,033	1	,033	,007	,934	,000	,007	,051
	Precuneus	,003	1	,003	,000	,983	,000	,000	,050

Univariate follow-up ANOVAs predicting activity in each of the three a priori ROIs by clinical group (CN, SCD, MCI, AD dementia). Covariates in all models were age, sex, and site of the MRI scan (results not shown in Table). Model 0 is the original model in all subjects (Model 0a) and the subsample with CSF biomarkers (Model 0b). Model 1, 2a-c and 3 were additionally adjusted for APOE4 genotype, AD pathology or ROI-specific gray matter volume (adjusted by intracranial volume). Significant effects are highlighted in bold. The effect of clinical group on precuneus activity was significant when controlling for APOE4 genotype, CSF markers of AD pathology or precuneus volume.

Table e-2. Group comparisons for precuneus activity differences in CSF sample

Group comparison	Mean difference	SE	P_{uncorr} (1-tailed)	P_{corr} (1-tailed)	P-value rank (lowest to highest)
MCI>HC	1.61	,503	0.001*	0.005*	1
MCI>AD	2.17	,679	0.001*	0.004*	2
SCD>AD	1.42	,617	0.011*	0.033*	3
SCD>CON	.863	,415	0.020*	0.039*	4
AD<HC	-.562	,645	0.193	0.193	5

Post-hoc ttests in the subcohort of individuals with CSF data N=224 tested whether AD<HC<SCD/MCI (5 group comparisons). Group differences were mainly similar to the found pattern in the whole sample, with the only difference that activity in SCD was also higher than in the AD dementia group. Corrected p-values denote Bonferroni-Holm correction.

Table e-3. Partial correlations of ROI novelty activity with cognition, CSF markers and volume

		Prec. Act.	Hipp. Act.	Entorhinal Act.	Postcentral Act.
Memory	R	-,060	**,116**	,040	-,067
	P (2-tailed)	,191	**,011**	,381	,143
	df	479	**479**	479	479
MMSE	R	-,007	**,097**	,049	-,031
	P (2-tailed)	,876	**,033**	,282	,491
	df	480	**480**	480	480
Aß42/40	R	-,003	,104	**,144**	,028
	P (2-tailed)	,962	,124	**,033**	,679
	df	218	218	**218**	218
Aß42/p-tau	R	,018	**,153**	**,161**	,029
	P (2-tailed)	,790	**,023**	**,017**	,664
	df	218	**218**	**218**	218
p-tau181	R	,010	-,102	-,112	-,041
	P (2-tailed)	,883	,132	,098	,544
	df	218	218	218	218
t-tau	R	-,048	**-,162**	**-,155**	-,051
	P (2-tailed)	,483	**,016**	**,022**	,451
	df	218	**218**	**218**	218
Precuneus Volume	R	-,002	-,004	**-,100**	,012
	P (2-tailed)	,963	,938	**,027**	,796
	df	480	480	**480**	480
Hipp. Volume	R	-,052	,031	-,001	-,051
	P (2-tailed)	,257	,497	,975	,260
	df	480	480	480	480
Entorhinal Volume	R	-,035	,031	,007	-,013
	P (2-tailed)	,450	,495	,871	,784
	df	480	480	480	480

Partial Pearson correlations were run in the whole cohort (after excluding outliers with extreme values in activation, N=486), covarying for age, sex, education and site ID. Volumes are adjusted for intracranial volume. Significant correlations at $p<0.05$ uncorrected are highlighted in bold.

e-Figures

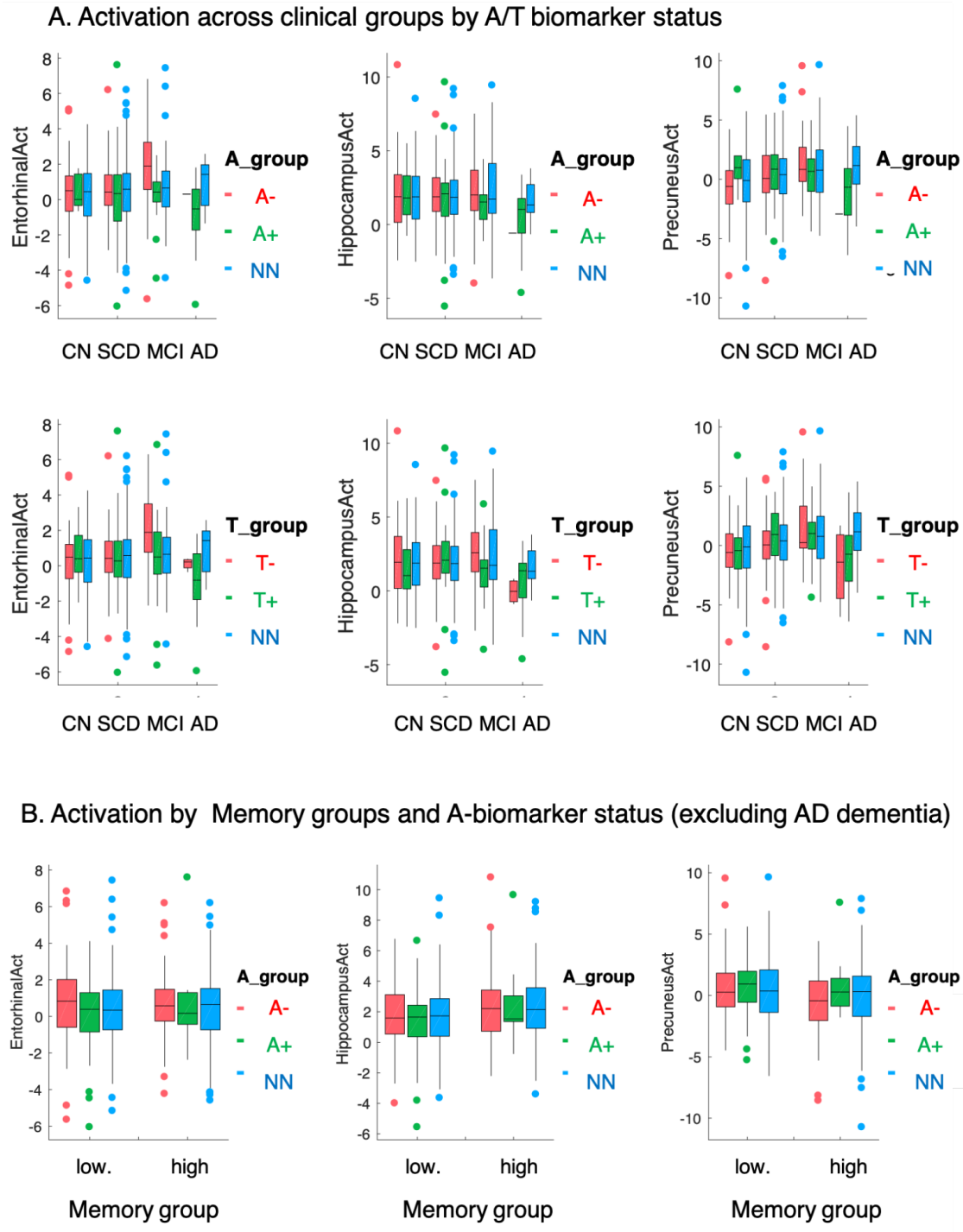


Figure e-1. Regional activation in clinical groups by A- and T-biomarker status and by high vs. low memory groups. **(A)** Activation by A+/A- and by T+/T- (**B)** Activation in predementia (HC/SCD/MCI) cases divided in low vs high memory groups by median split (Median = 0.387, including all subjects with memory performance) by A-biomarker group. NN: subject without CSF samples. N(Memory High)= 233, N(Memory low)= 234; N(CSF)=211: N(Mem low/A-)=71, N(Mem low/A+)=38, N(Mem high/A-)=83, N(Mem high/A+)=11,

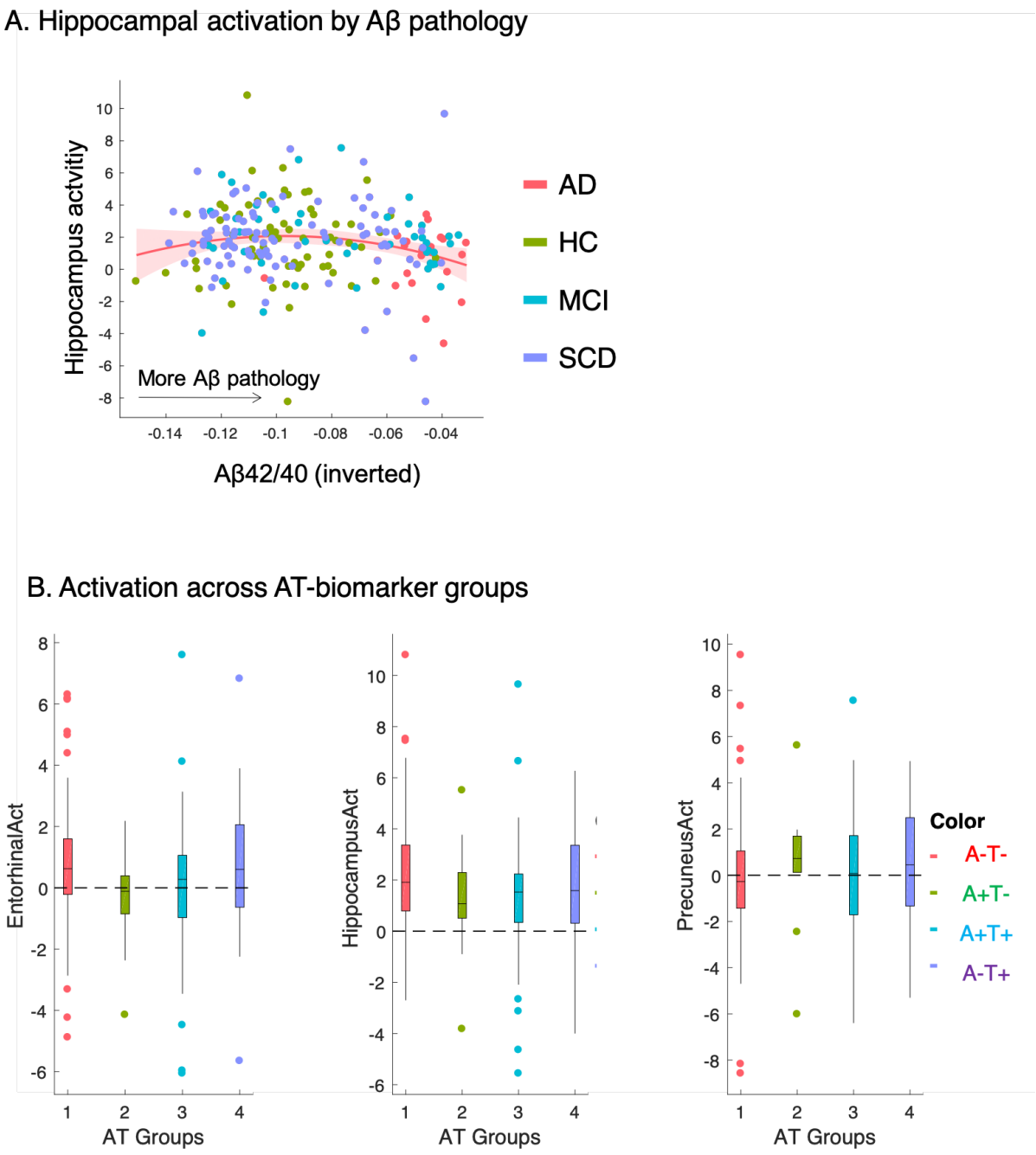


Figure e-2. Region-specific activity across the biomarker defined AD spectrum
A. Activation in hippocampus follows a nonlinear (quadratic) relationship with Aβ42/40 levels. The Aβ42/40 levels were inverted (*-1) to represent increased AD burden for display purposes.
B. Mean fMRI novelty-related activity in entorhinal cortex, hippocampus and precuneus across AT-biomarker defined groups based on CSF Aβ42/40 and p-tau CSF concentrations. N(CSF)=224: N(A-T-)=122, N(A+T-)=10, N(A+T+)=59, N(A-T+)=33

e-Methods

Delcode study participants and sample selection

DELCODE is an observational longitudinal memory clinic based multicenter study in Germany, focusing on SCD in the context of AD, carried out by ten university-based memory clinics collaborating with local sites of the DZNE. Details about the sample, data acquisition, handling, and quality control have been previously in Jessen et al., 2018[1]. As decribed in [1], the study includes individuals with MCI and mild AD as well as control subjects without subjective or objective cognitive impairment. In addition, a subgroup of first-degree relatives of patients with AD dementia were enrolled as an exploratory group at-risk for development of AD. All patient groups (SCD, MCI, AD) are referrals, including self-referrals, to the participating memory centers and were assessed clinically at the respective memory centers before entering DELCODE. The assessments include medical history, psychiatric and neurological examination, neuropsychological testing (as described below), blood sampling, and routine MRI scanning. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) neuropsychological test battery was applied at all memory centers to assess cognitive function. German age, sex, and education-adjusted norms of the CERAD neuropsychological battery are available online (www.memoryclinic.ch).

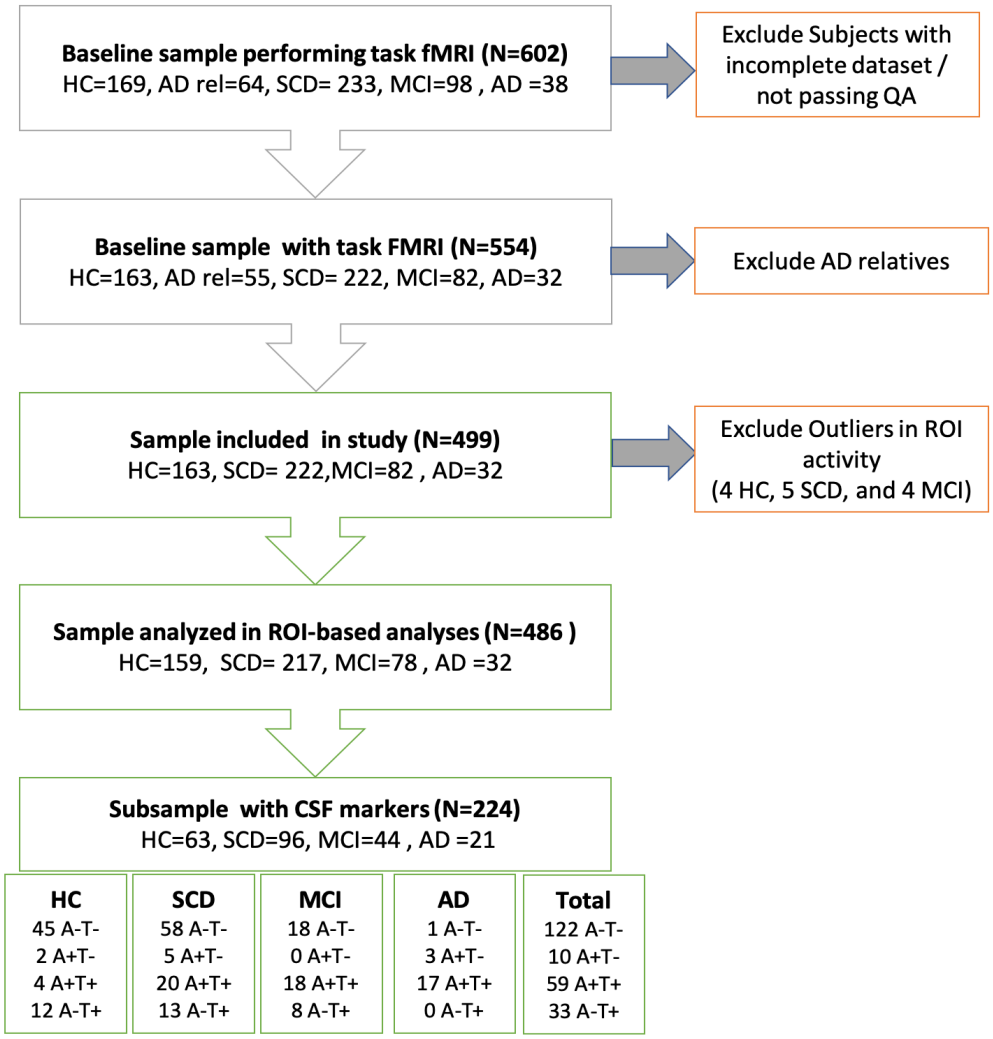
All subjects assigned to the SCD group had to report a subjectively perceived cognitive performance decline within at least the last 6 months and at most the last 5 years in a Personal Interview. In addition, they had to have average performance (<1.5 SD) in all subcategories of the CERAD-Plus administered for screening. Subjects were assigned to the MCI group if their performance on the CERAD was worse than average (>1.5 SD) on the "recall word list" subtest, they reported decreased cognitive performance, and at the same time they did not meet dementia criteria. By selecting a memory-related subtest, primarily amnestic MCI patients were included in the study. Patients with mild AD dementia[2] and >=18 points on the Mini Mental State Examination (MMSE) were included in DELCODE. SCD and amnestic MCI groups fulfill the current research criteria for SCD [3,4] or MCI[5], respectively.

The control group and the group of first-degree relatives of AD patients were recruited by identical local newspaper advertisements. In the advertisement text, individuals were explicitly sought who felt healthy and without relevant cognitive problems. All individuals who responded to the advertisement were screened by telephone with regard to SCD. The report of very subtle cognitive decline, which did not cause any concerns and was considered normal for age by the individual, was not an exclusion criterion for the control group. For the first-degree relatives of AD, the advertisement did not exclude those with concerns of cognitive decline. AD in the relative (parent or sibling) had to be documented by medical records. Both the control group and the group of first-degree relatives had to achieve unimpaired cognitive performance according to the same definition as the SCD group.

Additional inclusion criteria for all groups were age ≥ 60 years, fluent German language skills, capacity to provide informed consent, and presence of a study partner. The following medical conditions were considered exclusion criteria: current major depressive episode, major psychiatric disorders either at baseline or in the past (e.g., psychotic disorder, bipolar disorder, substance abuse), neurodegenerative disorder other than AD, vascular dementia, history of stroke with residual clinical symptoms, history of malignant disease, severe or unstable medical condition, and clinically significant abnormalities in vitamin B12. Prohibited drugs included chronic use of psychoactive compounds with sedative or anticholinergic effects, use of anti-dementia agents in SCD, amnestic MCI, and control subjects and in healthy siblings, and investigational drugs for treatment of dementia or cognitive impairment 1 month prior to entry and for the duration of the study.

As shown in the flowchart below, we included all subjects with complete fMRI task data and related logfiles, structural T1-MRI and cognitive data, excluding subjects with QA status “unusable”. We next excluded the AD relatives, which is an exploratory at-risk group, as we had no hypothesis for this group (they could be expected to show similar activity as the HC group or might show mildly increased activity). Finally, for our ROI analyses, we excluded subjects that showed extreme activity values in at least one of the 4 ROIs using SPSS 24 (IBM, Armonk, NY) based on the interquartile range (IQR) ($x > 75\%$ percentile + 3 IQR; $x < 25\%$ percentile – 3 IQR). This led to the

exclusion of 13 subjects (4 HC, 5 SCD, and 4 MCI), leaving 486 subjects for ROI-based analyses of whom 224 had CSF data (122 A-T-, 10 A+T-, 59 A+T+, 33 A-T+).



Neuropsychological testing

The test battery included the Mini Mental State Examination (MMSE), ADAScog 13, the Free and Cued Selective Reminding Test, Wechsler Memory Scale revised version (WMS-R) Logical Memory Story A, WMS-R Digit Span, semantic fluency task, the oral form of the Symbol–Digit–Modalities Test (including subsequent free recall of symbols and symbol–digit pairings), Trail Making Test A and B, Clock Drawing, and Clock Copying. In addition to these established tests, two newly developed computerized tests were implemented: the Face Name Associative Recognition Test, and a Flanker task to assess executive control of attention. The cognitive testing was performed by a trained neuropsychologist at all sites.

As described in detail in [6] the memory factor score used in the current study was originally derived by a confirmatory factor analysis (CFA) applied to the DELCODE-neuropsychological testing data at baseline. The CFA derived five cognitive domain scores: Learning & memory, language ability, executive functions and mental processing speed , working memory and visuo-spatial abilities (see Figure e-1 in ref. 6)

MRI and fMRI data acquisition

MRI data were acquired with Siemens scanners (3 TIM Trio systems, 4 Verio systems, one Skyra and one Prisma system) at 10 different scanning sites. The current analysis was performed using T1-weighted (3D GRAPPA PAT 2, 1mm3 isotropic, 256x256px, 192 slices, sagittal, ~5min, TR 2500ms, TE 4.33ms, TI 110ms, FA 7°) and a task-fMRI protocol (2D EPI, GRAPPA PAT 2, 3.5mm3 isotropic, 64x64px, 47 slices, oblique axial/AC-PC aligned, ~9 min, TR 2580ms, TE 30ms, FA 80°, 206 volumes). For more details see [1,7]. SOPs, quality assurance and assessment were provided and supervised by the DZNE imaging network[1].

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e-Results

Spearman rank correlation between clinical group and activity

Spearman rank correlation between groups and novelty-related activity in each ROI were calculated and p-values were corrected for the number of analyses (alpha=0.05, 4 ROIs; corrected p-threshold$_{1\text{-tailed}} < 0.0125$). Spearman rank correlations between the clinical group, ranked by expected activity increases (1=AD, 2=HC, 3=SCD/MCI). and activity in the different ROIs revealed a significant positive association in precuneus (rho =.130; $p_{1\text{-tailed}}$= 0.002, p_{corr}=0.008) surviving correction for multiple comparisons. There was a similar but weaker association for hippocampus (rho =.098; $p_{1\text{-tailed}}$= 0.016, p_{corr}=0.064) not surviving correction for multiple comparisons. Correlations for ERC (rho =.066; $p_{1\text{-tailed}}$= 0.074, p_{corr}=0.296) and postcentral gyrus (rho =.065; $p_{1\text{-tailed}}$= 0.078, p_{corr}= 0.312) were not significant.