Novelty-Related fMRI Responses of Precuneus and Medial Temporal Regions in Individuals at Risk for Alzheimer Disease

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Abstract

Objective: We assessed whether novelty-related fMRI activity in medial temporal lobe (MTL) regions and precuneus follows an inverted U-shape pattern across the clinical spectrum of increased Alzheimer disease (AD) risk as previously suggested. Specifically, we tested for potentially increased activity in individuals with higher AD risk due to subjective cognitive decline (SCD) or mild cognitive impairment (MCI). We further tested whether activity differences related to diagnostic groups were accounted for by CSF markers of AD or brain atrophy.

Methods: We studied 499 participants aged 60-88 years from the German Center for Neurodegenerative Diseases Longitudinal Cognitive Impairment and Dementia Study (DELCODE) who underwent task-fMRI. Participants included 163 cognitively normal (healthy control, HC) individuals, 222 SCD, 82 MCI, and 32 patients with clinical diagnosis of mild AD. CSF levels of β-amyloid 42/40 ratio and phosphorylated-tau181 were available from 232 participants. We used region-based analyses to assess novelty-related activity (novel > highly familiar scenes) in entorhinal cortex, hippocampus and precuneus, as well as whole-brain voxel-wise analyses. First, general linear models tested differences in fMRI activity between participant groups. Complementary regression models tested quadratic relationships between memory impairment and activity. Second, relationships of activity with AD CSF biomarkers and brain volume were analyzed. Analyses were controlled for age, gender, study site, and education.

Results: In the precuneus, we observed an inverted U-shape pattern of novelty-related activity across groups, with higher activity in SCD and MCI compared to HC, but not in AD patients who showed relatively lower activity than MCI. This non-linear pattern was confirmed by a quadratic relationship between memory impairment and precuneus activity. Precuneus activity was not related to AD biomarkers or brain volume. In contrast to precuneus, hippocampal activity was reduced in AD dementia compared to all other groups and related to AD biomarkers.

Conclusion: Novelty-related activity in the precuneus follows a non-linear pattern across the clinical spectrum of increased AD risk. While the underlying mechanism remains unclear, increased precuneus activity might represent an early signature of memory impairment. Our results highlight the non-linearity of activity alterations that should be considered in clinical trials using functional outcome measures or targeting hyperactivity.
Introduction

Network-level dysfunction occurs early in AD, and can be measured indirectly with fMRI. Task-based fMRI studies have yielded increased activity in MTL regions and the precuneus in older adults with MCI compared to HC\textsuperscript{1–3}. Similar or reduced activity compared to HC has been found in patients with AD dementia\textsuperscript{4}. Studies with AD biomarkers suggest that early increased activity in non-demented individuals is related to increased A\textsubscript{β} burden or MTL tau pathology\textsuperscript{5–10}, and late reduced activity accompanied by clinical impairment is linked to pronounced AD pathology and neurodegeneration. It remains unclear whether increased brain activity reflects early pathology or rather compensatory mechanisms that enable sustained memory performance\textsuperscript{7,14,15}. The activity pattern changes across the spectrum from HC, to groups with increased AD risk, such as SCD and MCI\textsuperscript{16,17}, towards patients with AD dementia have been described as an inverted U- or J-shape\textsuperscript{11–13}. Individuals with SCD - a relatively young diagnosis\textsuperscript{16} - are twice as likely to develop dementia as individuals without SCD\textsuperscript{17} and its functional characterization is crucial for clinical trials. FMRI studies in SCD indicate increased task-related parietal and frontal activity\textsuperscript{13,18,19}. However, these studies were limited by small sample sizes and lacked AD CSF biomarkers. Therefore, we examined how fMRI-task activity during novelty processing differs across the AD risk spectrum using the DELCODE cohort\textsuperscript{20}. We expected a non-linear pattern with increased activity in the MTL and precuneus in SCD and MCI explained by CSF biomarkers of AD pathology, followed by decreased activity with clinical progression and more advanced AD pathology. We further investigated the regional pattern of activity deviations and how this compares to the pattern of atrophy by means of whole-brain analyses.

Methods

Participants

The DELCODE study is a German multicentric observational study and details are provided in [20] and the eMethods. Here, we analyzed baseline data from 499 participants who completed a task fMRI. CSF samples were available for 232 participants (see Table 1), and APOE \textepsilon 4 status for 488 participants. Our study sample included 163 HC, 222 SCD, 82 MCI and 32 patients with a clinical diagnosis of AD dementia. HC was defined as having memory test performances within 1.5 SD of the age-, sex-, and education- adjusted normal performance on all subtests of the CERAD (Consortium to Establish a Registry of AD test battery). SCD was defined as the presence of subjective cognitive decline as expressed to the physician of the memory center\textsuperscript{16} and normal cognition as assessed with the CERAD.
Participants were classified as MCI when displaying an age-, sex-, and education-adjusted performance below –1.5 SD on the delayed recall trial of the CERAD word-list episodic memory tests. Finally, only participants with a clinical diagnosis of mild AD\textsuperscript{21} obtaining ≥ 18 points on the Mini Mental State Examination (MMSE) were included in DELCODE. All participants were 60 years or older, fluent speakers of German and had a relative who completed informant questionnaires. Exclusion criteria are described in the eMethods\textsuperscript{20}.

**Standard Protocols, Approvals, Registrations, and Patient Consents**

The study protocol was approved by Institutional Review Boards of all participating study centers of the DZNE\textsuperscript{20}. The process was led and coordinated by the ethical committee of the medical faculty of the University of Bonn (trial registration number 117/13). All participants provided written informed consent.

**Cognitive measures**

We assessed memory performance by a latent cognitive factor score for learning and memory, derived from a confirmatory factor analysis from the extensive DELCODE neuropsychological battery (see eMethods) as described previously\textsuperscript{22}.

**Cerebrospinal fluid (CSF) measures**

Procedures of CSF acquisition, processing, and analysis in the DELCODE cohort have been previously described\textsuperscript{20}. Here, we focused on Aβ42/Aβ40 and phospho-tau181 (p-tau) CSF measures of Aβ (A) and tau pathology (T), as well as on the ratio CSF-Aβ42/p-tau as a single continuous measure of AD pathology. For supplementary group analyses, we categorized individuals according to the AT(N) biomarker classification system\textsuperscript{23} based on cutoffs reported in\textsuperscript{20} (T+ ~ p-tau >57pg/ml; A+ ~ Aβ42/40<0.07).

**(f)MRI acquisition and fMRI task**

The T1-weighted structural image (1mm\textsuperscript{3} isotropic resolution) and fMRI data (3.5mm isotropic resolution) were acquired at 3T and sequences are reported in the eMethods\textsuperscript{20,24}. Subjects performed a modified version of an incidental encoding task lasting about 9min originally reported in\textsuperscript{25,24}. Participants were presented with 88 novel scenes (half outdoor/half indoor), and 44 repetitions of two pre-familiarized scenes (one indoor and one outdoor, presented 22 times each) using Presentation (Neurobehavioral Systems Inc.). Participants were instructed to classify each scene as “indoor” or “outdoor” by pressing a button. Each
scene presentation lasted 2500ms, with an optimized inter-trial jitter for statistical efficiency.

After a retention delay of 60min, memory was tested with a 5-point recognition-confidence rating for the former novel images and new distractor scenes, to assess successful incidental encoding. The current study focused on the novel>familiar contrast, which is independent of later memory performance, owing to the poor recognition-memory performance in the MCI/AD dementia groups. Associations between fMRI task-memory performance, hippocampal activity and Aβ x tau interactions in the non-demented groups with CSF data have been examined by other studies\textsuperscript{24,26}.

\textit{fMRI preprocessing and first-level analysis}

Preprocessing included slice-time correction, unwarping, realignment and spatial smoothing with isotropic Gaussian kernel of Full Width at Half Maximum (FWHM) 6mm in SPM12 (r7771, Wellcome Trust Centre for Human Neuroimaging, London, UK). First-level general linear models (GLM) were calculated in native space using a hemodynamic response function with a 128s high-pass filter, no global scaling. The first-level GLM included a maximum of 12 regressors of interest: five regressors for novel images ordered by subsequent confidence rating plus one regressor for the familiar image, each separately for indoor and outdoor images. Six motion regressors from the realignment process were also included. Familiar and novel stimuli (irrespective of confidence rating) were used to calculate a novelty contrast (novel>familiar).

\textit{Spatial normalization to template space}

T1-weighted images were processed using SPM and CAT-Toolbox (r1615, Structural Brain Mapping group, Jena University Hospital, Jena, Germany, http://www.neuro.uni-jena.de/cat/).

First, a correction for field inhomogeneities was applied. Thereafter, images were segmented into Grey Matter (GM), White Matter, and CSF maps that were iteratively warped to generate a study-specific template in MNI space using Geodesic Shooting approach\textsuperscript{27}. The first-level fMRI contrast images from pre-smoothed data were warped to MNI space using the obtained deformation fields and smoothed further by 2mm FWHM. The spatially normalized fMRI novelty contrast images (further referred to as ‘activity’) were used for (i) region-based analyses using \textit{a-priori} defined regions of interests (ROIs) and (ii) whole-brain voxelwise analyses as outlined below. ‘Activation’ (‘deactivation’) refers to positive (negative) contrast values (activity). GM tissue maps were warped and modulated by the Jacobian determinant to enable voxel-based comparisons of local GM volume across subjects and smoothed with 6mm
FWHM.

Region-of-interest (ROI)-based measures
Based on previous fMRI studies showing increased task-related activation in early preclinical stages of AD, we focused on 3 ROIs: entorhinal cortex, hippocampus, and precuneus. While the entorhinal cortex and hippocampus within the MTL show early tau pathology, the precuneus shows early Aβ burden. Postcentral gyrus was used as a control region as it is only affected by AD pathology in the latest stages of AD. ROIs were derived from the Desikan–Killiany atlas in Freesurfer 6.0 (http://surfer.nmr.mgh.harvard.edu/). To extract regional activity in MNI space, we used the FreeSurfer MNI aparc+aseg.mgz template and resliced ROIs to EPI space. We also derived corresponding regional volumes by segmentation of the individual T1 images with FreeSurfer, which were adjusted for total intracranial volume. Bilateral means were calculated as we had no hemisphere-specific hypotheses.

For ROI analyses, we excluded 13 subjects with extreme activity values (eMethods), leaving 486 subjects of whom 224 had CSF data (122 A-T-, 10 A+T-, 59 A+T+, 33 A-T+).

Statistical analyses Cognitive, demographic, and ROI data were analyzed using SPSS 24 (IBM, Armonk, NY). Demographic variables were compared between groups with ANOVAs, t-tests, and chi-square tests. Differences in activity were assessed in ROI-based and whole-brain analyses, as described below. For all analyses, if not otherwise stated, we included fMRI site (n=8), age, sex and years of education as covariates.

i) ROI-based analyses
We performed three complementary types of analyses to test for an inverted U-shape pattern of activity across the continuum from HC to at-risk stages for AD to AD dementia. First, we assessed differences in activity between diagnostic groups, hypothesizing a pattern of increased activity in participants with subjective or mild objective memory deficits followed by similar/decreased activity in participants with AD dementia relative to HC. To test this hypothesis, we computed a MANCOVA to predict activity in the three a priori defined ROIs by diagnostic group. Significant MANCOVAs were followed by univariate ANCOVAs for each ROI and post-hoc t-tests using Bonferroni-Holm correction for multiple comparisons (one-tailed p-values, 5 group comparisons to test U-shape pattern AD<HC<SCD/MCI). Furthermore, a univariate ANOVA was performed for a control region, the post-central gyrus, in which we did not expect activity differences between groups. Second, we performed
supplementary non-parametric Spearman rank correlations between activity in each ROI and diagnostic groups recoded by the order of expected activity increases. Third, the non-linear pattern of activity increases and decreases with increasing memory deficits was tested by quadratic models using memory performance as a continuous measure instead of diagnostic groups. The Akaike information criterion (AIC) was used to determine which model (e.g. linear or quadratic) better fit the data while also accounting for model complexity. A smaller AIC value indicates a better model.

In a next step, we tested our hypothesis that the pattern of increased activity in AD-risk stages followed by relatively decreased activity in AD dementia would be explained by AD biomarkers or measures of atrophy (in a quadratic manner). To do so, we performed three sets of analyses. First, we tested whether activity differences between diagnostic groups were accounted for by measures of pathology or atrophy by including measures of CSF Aβ42/40, p-tau, Aβ42/p-tau or ROI volume as covariates in our ANCOVAs. Second, we ran regression models (without diagnostic group as factor) to directly test for a U-shape relationship by predicting ROI activity by continuous measures of Aβ42/40, p-tau, Aβ42/p-tau or volume including linear and quadratic effects. Third, group comparisons also assessed the effect of AD pathology on activity by binary categorization of individuals according to the AT-biomarker classification scheme hypothesizing increased activity in the presence of abnormal Aβ levels followed by decreased activity when also CSF p-tau becomes abnormal (A+T+<A-T-<A+T-). We excluded the A-T+ with suspected non-AD pathologic change from the analysis as we had no hypothesis for this group. Finally, we explored in Supplementary analyses whether activity differences were related to APOE ε4 status, which has been related to increased activity in previous fMRI studies.

ii) Whole-brain voxelwise second-level analyses
Complimentary to our ROI analyses, we performed whole-brain exploratory analyses to assess the spatial pattern of activity deviations to test the same hypotheses for effects of diagnostic status as well as AD pathology using continuous measures and categorical AT staging on novelty responses. ANCOVAs with planned post-hoc independent samples t-tests were performed in SPM12. Results are FWE-corrected at cluster level with $p_{\text{cluster}}<0.05$ using a cluster-forming threshold of $p_{\text{voxel}}<0.005$ (uncorrected). For this purpose, an explicit whole-brain GM mask excluding cerebellum and basal ganglia was applied.
Similarly, voxel-based morphometry (VBM) analyses were conducted to examine the patterns of local morphological differences in terms of GM volumes in the same groups, and to explore whether the pattern of activity alterations is seen in areas of reduced GM, and whether functional alterations precede or follow reduced GM volume, which could indicate compensatory mechanisms. Total intracranial volume was included as an additional covariate. VBM results are reported at $p_{\text{cluster}} < 0.05$ using FWE cluster-level correction and a cluster-forming threshold of $p_{\text{voxel}} < 0.001$ (uncorrected).

Data Availability Statement

Data, study protocol and biomaterials can be shared with partners based on individual data- and biomaterial transfer agreements

Results

Participants and demographics

Demographics are reported in Table 1. Diagnostic groups significantly differed in age, years of education, sex, APOE ε4 status, Aβ42/40, p-tau, MMSE and memory performance factor (see Table 1 for statistics and pairwise group comparisons). Compared to HC, the SCD group was significantly older by one year, included fewer females, more APOE ε4 carriers, had higher CSF p-tau concentrations, and had worse cognition (as reported previously).22

Differences in regional activity across the clinically defined AD-risk spectrum

We conducted MANCOVAs to examine diagnostic group differences in activity in the three a priori ROIs (see Figure 1A). The effect of diagnostic group was significant (Pillai's Trace = .044, F(9, 1416) = 2.37, P = .012; Partial Eta$^2 = 0.015$, power=.921). Follow-up univariate ANCOVAs revealed a significant effect of diagnostic group on activity in the hippocampus (F(3,472) = 2.79, P = .040) and precuneus (F(3,472) = 4.31, P = .005). The group effect in the entorhinal cortex was not significant but trending (F(3,472) = 2.57, P = .054). Univariate ANCOVAs on activity in the postcentral gyrus as a control region showed no significant effect of group (F(3,472) = 2.32, p = .0745).

Post-hoc t-tests (see Table 2) showed reduced hippocampal activity in the AD dementia group relative to MCI, SCD and HC, but no difference between SCD or MCI and HC.

In the precuneus, novelty-related activity was higher in the MCI group compared to HC and compared to AD dementia. Precuneus activity was also higher in the SCD group relative to HC. Precuneus activity did not significantly differ between the AD dementia group and HC.
Thus, activity in the precuneus follows an inverted U-shape pattern with increased activity in SCD and MCI, but similar activity levels as HC in the AD dementia group, which was further confirmed by supplementary Spearman correlations between ROI activity and diagnostic group ranked by expected activity increases (Supplementary eResults 1).

Third, evidence for a non-linear pattern of precuneus activity deviations with increasing cognitive impairment was provided by quadratic models using the memory factor score as a continuous measure instead of diagnostic groups (Table 3 and Figure 1B). While lower hippocampal activity was linearly predicted by higher memory impairment, precuneus activity followed a quadratic pattern i.e. increasing followed by decreasing activity with advancing memory deficits. Model comparisons (Table 3) supported that the linear model was favorable for hippocampus (ΔAIC~2) but the quadratic model for precuneus (ΔAIC~3). We further note that there was a significant positive association between precuneus activity and memory impairment (ascending branch of inverted U) when excluding the dementia patients (r=-.126, p=.007).

Relationship between regional activity and AD biomarkers and APOE ε4 status

We next tested our hypothesis that the inverted U-pattern of precuneus activity would be accounted for by AD pathology or measures of atrophy (see eTable 1). In the subsample of individuals with CSF markers, the effect of diagnostic group on precuneus activity remained significant with similar group differences as seen in the full sample (eTable 2), whereas the group effect on hippocampal activity was only marginal. When covarying for CSF biomarkers (eTable 1) the effect of diagnostic group on precuneus activity remained significant. Activity in the different diagnostic groups separated by A- or T-biomarker status is further displayed in eFigure 1.

Subsequent regression models testing linear and quadratic (U-shape) effects of AD pathology on activity directly are summarized in Table 3. Here, we found that hippocampal activity was significantly predicted by Aβ42/Aβ40 in a quadratic rather than in a linear manner (Table 3 and eFigure 2a), whereas linear or quadratic effects of p-tau or Aβ42/p-tau were not significant (all p-values>0.055). Precuneus activity was not predicted by Aβ42/Aβ40 (Table 3), p-tau or Aβ42/p-tau, neither in models with linear nor quadratic effects (all p-values >0.5).

A MANCOVA on the effect of AT-biomarker groups (excluding A-T+) on activity revealed no significant multivariate effect of group (Pillai’s Trace = .048, F(6, 354) = 1.46, P = .190; Partial Eta2 = 0.024, power=.567). ROI-specific activity separated by AT-biomarker group is depicted in the eFigure 2b.
Similarly, we tested whether activity differences between diagnostic groups were explained by differences in regional volume. The effect of diagnostic group on hippocampal activity and precuneus remained significant when covarying for regional volume (eTable 1). Subsequent regression analyses did not reveal a significant linear or quadratic effect of ROI-specific volume on hippocampal or precuneus activity. However, we found a trend quadratic effect for the hippocampus ($F(1,473)=3.84, p=0.051$).

Partial correlations between brain activity, AD biomarkers and brain volume are further reported in Supplementary Table e-3. In summary, AD biomarkers or regional volume did not account for the inverted U-shape pattern of precuneus activity across groups. Supplementary analyses showed that the effect of diagnostic group on precuneus activity remained also significant when covarying for APOE ε4 status (eTable 2).

ii) Whole-brain analyses (fMRI & VBM)

In HC, positive activity (i.e. activation) during processing of novel versus familiar scenes was found in frontal regions, the MTL, and occipital regions bilaterally (see Figure 2A). In contrast, deactivation (novel < familiar) was evident in the lateral temporal cortex, precuneus, posterior cingulate, angular, and middle frontal gyrus (see Figure 2B), covering parts of the default mode network (DMN\(^31\)).

When assessing activity differences between diagnostic groups, significantly higher activity was found in the precuneus of the SCD and MCI groups compared to HC, confirming our ROI analyses (Figure 3A/B). Notably, higher precuneus activity represented reduced novelty-related deactivation (see Figure 2A). No significant decrease in activity was found in any diagnostic group compared to HC.

Morphometric analyses revealed reduced GM volume in the MCI group compared to HC in the hippocampus, amygdala, lateral orbital gyrus, middle frontal gyrus, angular gyrus, and precuneus (Figure 3B) but no volume differences between the SCD and HC. As depicted in Figure 3, regions of atrophy in the MCI group overlapped partly with regions of higher novelty-related activity, particularly in the precuneus.

There were no significant associations between novelty-related activity and continuous measures of p-tau or Aβ42/40 and no differences between AT-biomarker groups when applying cluster-level correction.
Discussion

The present study investigated how novelty-related fMRI activity in the MTL and the precuneus deviates with increasing clinical risk for AD in a large and well characterized cohort. In the precuneus, we observed an inverted U-shaped pattern of activity alterations with higher fMRI activity in the precuneus of SCD and MCI participants compared to HC and lower activity in AD dementia patients relative to MCI. This quadratic pattern of activity deviations with increasing memory deficits was further confirmed by regression analyses. Higher precuneus activity in our study corresponded to a reduced deactivation during processing of novel versus familiar images. The precuneus is the most interconnected node of the DMN\(^3\) and our results are in-line with previous studies reporting reduced task-related deactivation of DMN regions in at-risk stages of AD ranging from cognitively normal APOE ε4 carriers to MCI patients\(^33,34\). However, to date only few studies have examined fMRI task activity in SCD. For example, increased activity in the prefrontal cortex\(^18,19\) compared to HC was suggested to be compensatory in memory and attention tasks. A recent study\(^13\) in 28 SCD-plus individuals (SCD with smaller hippocampal volumes compared to HC and/or with APOE ε4 positivity) observed increased encoding activity in the hippocampus, precuneus, temporal and superior parietal cortex. Moreover, left superior parietal activity followed an inverted U-shape pattern with proxies of pathology (i.e., atrophy and cognition). Together with our findings in a much larger sample, this suggests that fMRI activity is increased in individuals with SCD and MCI most prominently in posterior midline brain regions, which can be measured with different fMRI paradigms. In contrast to the precuneus, hippocampal activity was not increased in individuals with SCD or MCI relative to HC, but was reduced in AD dementia patients relative to all other groups.

When considering AD biomarkers, most previous studies have linked increased task activity in HC and MCI to abnormal levels of Aβ using PET imaging\(^5–8\). More recently, with the advent of tau-specific PET tracers, a few studies in HC have suggested that increased task activity in the hippocampus\(^9,10,35\) and posterior-midline\(^9\) regions is more strongly associated with temporal lobe tau than with Aβ burden. Together, these findings are in-line with animal models in which Aβ or tau pathology has been linked to higher neural excitability\(^36,37\). However, in contrast to these previous studies we did not find a relationship between CSF AD biomarkers and increased precuneus activity, neither when considering continuous levels of CSF Aβ42/40 or p-tau, nor with categorical AT-staging. We note that only half of our sample provided CSF samples. However, despite the reduced sample size, we found similar group differences in precuneus activity as observed in the full sample, which remained significant.
when covarying for AD biomarkers or atrophy. Although further analysis in a bigger sample enriched for abnormal AD biomarkers in HC and SCD individuals would increase the power to detect such a relationship, the null findings observed here are unlikely to be explained solely by the lack of power. Hyperactivity in posterior-midline regions could be related to early MTL tau\textsuperscript{9,35} pathology that is unlikely to be detected with CSF biomarkers. According to the cascading network-model\textsuperscript{38}, high MTL tau burden might be related to a compensatory load shift to the posterior DMN (that might relate to fMRI activity and connectivity changes), which fails before Aβ plaques are measurable. It appears to initiate a connectivity cascade that continues throughout the AD spectrum. Furthermore, at early stages of the disease, increased activation in the precuneus could represent a marker of a behavioral or clinical phenotype\textsuperscript{39} that can be observed even before pathological changes become measurable. In the presence of AD dementia, we observed reduced activity in the hippocampus. Regression models further suggested that hippocampal activity followed an inverted U-shape dependency pattern on Aβ pathology, where activity slightly increased with mildly increased Aβ burden and then declined at high levels of pathologic Aβ. Recent findings from the DELCODE cohort, focusing on Aβ and tau interactions on hippocampal novelty responses in non-demented individuals, suggest that Aβ pathology is permissive for tau-related hippocampal dysfunction\textsuperscript{26}. Together, these findings highlight the presence of non-linear region-specific relationships between AD-related pathology, fMRI activity and memory impairment.

It is debated whether increased activity in at-risk stages of AD represents compensation for early AD pathology or brain atrophy, or whether aberrant activity might be directly driving protein accumulation and vice-versa. On the one hand, greater hippocampal task activation has been related to a faster cognitive decline in MCI\textsuperscript{40} and reduced cortical thickness\textsuperscript{1}. On the other hand, a study on episodic memory encoding of scenes found increased task-positive activation in A+ compared to A- HC in the hippocampus and occipital regions that was linked to more detailed memories, in accordance with compensation\textsuperscript{15}. In our study, increased precuneus activity in SCD and MCI was not linked to AD CSF biomarkers or brain volume. Moreover, higher precuneus activity was related to worse memory performance in the non-demented groups. Previous longitudinal studies have shown that worse memory in SCD and MCI at baseline is also related with increased risk for conversion to AD dementia\textsuperscript{41}. Whether compensatory or not, our results support previous studies showing hyperactivity in the precuneus as an early signature of memory impairment that could arise before AD pathology is detected in CSF biomarkers.
Our voxel-wise group comparisons of whole brain activity and GM volume further suggest that functional activity might deviate from HC even without significant structural decline or cognitive impairment, as seen in the SCD group. In MCI, a diagnosis with higher conversion risk to AD\textsuperscript{42}, the site of lower deactivation in the precuneus overlapped with regions of reduced GM volume, which additionally covered AD-typical regions of atrophy\textsuperscript{43}. Individual differences in GM volume did not account for altered precuneus activity. Taken together, our results indicate that increased precuneus activity is not associated with GM loss. Our findings are in accordance with the hypothesized sequence that neural dysfunction precedes brain structural changes. Nevertheless, we note that altered precuneus activity might already reflect early neurodegeneration or synaptic damage not detectable with standard MRI.

Future studies will need to investigate what underlies and causes the increased novelty-related fMRI activity that we observed in SCD and MCI. We assume that the increased precuneus activity represents reduced deactivation during processing of novel stimuli compared to familiar stimuli\textsuperscript{5,35}. However, this pattern could also reflect lower activation to familiar items in SCD and MCI compared to HC. The additional inclusion of a baseline condition could help to resolve this question. Furthermore, it is not clear whether increased fMRI activity represents aberrant neuronal activity or whether it also reflects altered microglia activity or vascular changes that affect the BOLD signal. Future studies, which further include measures of neuroinflammation and cerebral blood flow, will help to elucidate these questions. The additional assessment of brain metabolism via FDG PET, which shows characteristic patterns of AD neurodegeneration earlier than MRI, could give further insight into the underlying mechanisms of altered fMRI activity. While FDG data in SCD are scarce, one previous study found hypometabolism in the precuneus in SCD relative to HC\textsuperscript{44}. Several other PET studies have reported a non-linear pattern of metabolic changes across the AD continuum similar to fMRI findings, showing hypermetabolism in MCI subjects or HC with increased tau pathology\textsuperscript{45–47} at low levels of Aβ but hypometabolism when Aβ becomes abnormal. Hyperactivity could be an early sign of subtle pathology that lasts until pathology is so advanced that the BOLD signal decreases. This might be coupled with changes in network connectivity that follow a similar non-linear pattern of early hyperconnectivity, which has been also observed in the precuneus of SCD individuals\textsuperscript{48}, followed by hypoconnectivity and cortical network failure\textsuperscript{38,49} when pathology and brain atrophy progress further towards AD.
This study has strengths and limitations. A major strength is the large SCD sample with more than 200 well characterized individuals, of which about half had CSF measures of AD pathology. Moreover, the study included MCI patients with and without abnormal AD biomarkers. A limitation is its cross-sectional nature, which allows only indirect inferences about activity changes with AD progression by comparing different groups. With the availability of follow-up fMRI and cognitive data, future studies will need to test whether precuneus activity increases with clinical progression and whether increased activity might serve as an early functional predictor of progression to AD.

In conclusion, our results highlight the non-linearity of activity alterations that have to be considered when activity is used as an outcome measure, e.g. in clinical trials. While the drivers and consequences of fMRI hyperactivity in the precuneus are still to be determined, it might potentially serve as an early functional marker of pathological changes observed in subjects at increased risk for AD. Our findings further suggest that abnormally increased precuneus activity could be a potential biomarker to monitor early therapeutic interventions to reduce the risk to AD conversion, as has been proposed for hippocampal hyperactivation50. While decreasing precuneus activity might be beneficial in diagnoses with increased risk for cognitive decline, increasing its activity might be related to better cognitive performance in later stages of the disease. Moreover, as precuneus activity is apparent before brain atrophy, it might aid stratification in clinical trials for subjects at-risk for cognitive decline.
References


### Tables

**Table 1. Sample characteristic**

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<tr>
<th>Feature</th>
<th>HC</th>
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<th>MCI</th>
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<td>222</td>
<td>82</td>
<td>32</td>
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<td>70±6</td>
<td>73±5</td>
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<td>100 (45)</td>
<td>42 (51)</td>
<td>21 (66)</td>
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<td>15±3</td>
<td>14±3</td>
<td>13±3</td>
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<td>N APOE ε4+ (%)</td>
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<td>68±31</td>
<td>36±45</td>
<td>21±66</td>
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<td>48</td>
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<td>N A+ (%)</td>
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<td>26 (26)</td>
<td>22 (46)</td>
<td>20 (95)</td>
<td>p &lt; .001</td>
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<td>p-tau181 (pg/ml)</td>
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<td>97.4±46.7</td>
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</tr>
<tr>
<td>N T+ (%)</td>
<td>16 (25)</td>
<td>34 (34)</td>
<td>29 (60)</td>
<td>17 (81)</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td>MMSE</td>
<td>29.4±0.83</td>
<td>29.2±1.10</td>
<td>27.0±1.52</td>
<td>24.3±3.39</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td>Memory factor</td>
<td>0.65±0.42</td>
<td>0.41±0.58</td>
<td>-0.72±0.62</td>
<td>-1.74±0.61</td>
<td>p &lt; .001</td>
</tr>
</tbody>
</table>

Unless otherwise stated variables denote mean ± standard deviation. Subscripts denote number of missing values. Percentages are based on number of valid cases; Statistics show p-values for the effect of group in ANOVAs or chi-square tests (without additional covariates). Significant differences (at p < .05 uncorrected) for paired group comparisons are further denoted. MMSE = Mini-Mental State Examination; APOE ε4 = carriers of apolipoprotein E ε4 allele; T+ ~ p-tau > 57 pg/ml; A+ ~ Aβ42/40 < 0.09
<table>
<thead>
<tr>
<th>Group comparison</th>
<th>Mean difference</th>
<th>SE</th>
<th>P&lt;sub&gt;uncorr&lt;/sub&gt; (1-tailed)</th>
<th>P&lt;sub&gt;corr&lt;/sub&gt; (1-tailed)</th>
<th>P-value rank (lowest to highest)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hippocampal Activity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCI&gt;AD</td>
<td>1,345</td>
<td>.459</td>
<td>0.002</td>
<td>0.01*</td>
<td>1</td>
</tr>
<tr>
<td>SCD&gt;AD</td>
<td>1,156</td>
<td>.414</td>
<td>0.0025</td>
<td>0.01*</td>
<td>2</td>
</tr>
<tr>
<td>HC&gt;AD</td>
<td>1,099</td>
<td>.424</td>
<td>0.005</td>
<td>0.015*</td>
<td>3</td>
</tr>
<tr>
<td>MCI&gt;HC</td>
<td>.246</td>
<td>.302</td>
<td>0.2085</td>
<td>0.417</td>
<td>4</td>
</tr>
<tr>
<td>SCD&gt;HC</td>
<td>.056</td>
<td>.228</td>
<td>0.4025</td>
<td>0.4025</td>
<td>5</td>
</tr>
<tr>
<td><strong>Precuneus Activity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCI &gt; HC</td>
<td>1279</td>
<td>.368</td>
<td>0.001</td>
<td>0.003*</td>
<td>1</td>
</tr>
<tr>
<td>MCI &gt; AD</td>
<td>1319</td>
<td>.559</td>
<td>0.010</td>
<td>0.038*</td>
<td>2</td>
</tr>
<tr>
<td>SCD &gt; HC</td>
<td>.618</td>
<td>.278</td>
<td>0.0135</td>
<td>0.041*</td>
<td>3</td>
</tr>
<tr>
<td>SCD &gt; AD</td>
<td>.658</td>
<td>.504</td>
<td>0.096</td>
<td>0.192</td>
<td>4</td>
</tr>
<tr>
<td>HC &gt; AD</td>
<td>.040</td>
<td>.516</td>
<td>0.469</td>
<td>0.469</td>
<td>5</td>
</tr>
</tbody>
</table>

Post-hoc tests (after significant univariate ANCOVAs) in the whole cohort tested whether novelty activity differed between AD<HC<SCD/MCI (5 group comparisons). Corrected p-values denote Bonferroni-Holm correction. Significant group differences are shown in bold.
Table 3. General linear models predicting regional activity by linear and quadratic effects of memory or Aβ

<table>
<thead>
<tr>
<th>Predicted Variable</th>
<th>Model</th>
<th>Model AIC</th>
<th>Model F</th>
<th>Model p</th>
<th>Predictor</th>
<th>B</th>
<th>SE</th>
<th>T</th>
<th>p</th>
<th>Partial ( \eta^2 )</th>
<th>Obs. Power</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hippocampus Act.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Memory</td>
<td>.35</td>
<td>.13</td>
<td>2.62</td>
<td>.009**</td>
<td>.014</td>
<td>.742</td>
</tr>
<tr>
<td>Linear</td>
<td>769</td>
<td>1,808</td>
<td>.050</td>
<td></td>
<td>Memory</td>
<td>.34</td>
<td>.17</td>
<td>1.95</td>
<td>.052</td>
<td>.008</td>
<td>.495</td>
</tr>
<tr>
<td>Quadratic</td>
<td>771</td>
<td>1,655</td>
<td>.074</td>
<td></td>
<td>Memory</td>
<td>.34</td>
<td>.17</td>
<td>1.95</td>
<td>.052</td>
<td>.008</td>
<td>.495</td>
</tr>
<tr>
<td>Quadratic</td>
<td>966</td>
<td>1,556</td>
<td>.109</td>
<td></td>
<td>Memory</td>
<td>.23</td>
<td>.17</td>
<td>1.39</td>
<td>.164</td>
<td>.004</td>
<td>.285</td>
</tr>
<tr>
<td>Quadratic</td>
<td>963</td>
<td>1,827</td>
<td>.042</td>
<td></td>
<td>Memory</td>
<td>.29</td>
<td>.13</td>
<td>2.16</td>
<td>.031*</td>
<td>.010</td>
<td>.578</td>
</tr>
<tr>
<td><strong>Precuneus Act.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Aβ42/40</td>
<td>7.02</td>
<td>5.69</td>
<td>1.24</td>
<td>.218</td>
<td>.007</td>
<td>.233</td>
</tr>
<tr>
<td>Linear</td>
<td>374</td>
<td>.813</td>
<td>.627</td>
<td></td>
<td>Aβ42/40</td>
<td>2.93</td>
<td>5.92</td>
<td>.50</td>
<td>.622</td>
<td>.001</td>
<td>.078</td>
</tr>
<tr>
<td>Quadratic</td>
<td>371</td>
<td>1,176</td>
<td>.302</td>
<td></td>
<td>Aβ42/40</td>
<td>-4.28</td>
<td>6.55</td>
<td>-.65</td>
<td>.514</td>
<td>.002</td>
<td>.100</td>
</tr>
<tr>
<td>Quadratic</td>
<td>955</td>
<td>1,625</td>
<td>.086</td>
<td></td>
<td>Aβ42/40</td>
<td>5.68</td>
<td>6.90</td>
<td>-.82</td>
<td>.412</td>
<td>.003</td>
<td>.130</td>
</tr>
<tr>
<td>Quadratic</td>
<td>953</td>
<td>1,739</td>
<td>.067</td>
<td></td>
<td>Aβ42/40</td>
<td>4.28</td>
<td>6.55</td>
<td>-.65</td>
<td>.514</td>
<td>.002</td>
<td>.100</td>
</tr>
<tr>
<td>Quadratic</td>
<td>955</td>
<td>1,625</td>
<td>.086</td>
<td></td>
<td>Aβ42/40</td>
<td>-5.68</td>
<td>6.90</td>
<td>-.82</td>
<td>.412</td>
<td>.003</td>
<td>.130</td>
</tr>
</tbody>
</table>

Regression models tested whether novelty-related fMRI activity in hippocamps and precuneus follows an inverted U-shape curve across disease severity defined by memory performance (memory factor score) or Aβ42/40 ratio as marker of early AD pathology. To do so models were run first including a linear term of the predictor and second adding a quadratic term. Note that predictor variables were mean centered beforehand and then squared. Significant effects are highlighted in bold. Additional covariates of no interest in all models included age, sex, years of education and site. Only sex was a significant covariate in the linear model on Precuneus activity predicted by memory (results for covariates not shown). AIC= Akaike information criterion.
Figure legends

Figure 1. Differences in region-specific novelty activity between diagnostic groups and with increasing memory impairment

(A) Mean fMRI activity (raw betas) for the novelty contrast (novel – familiar scenes) in hippocampus and precuneus across diagnostic groups. Hippocampal activity was reduced in AD relative to all other groups. Precuneus activity followed an inverted U-shape pattern with more advanced risk stages for AD. *significant group differences surviving Bonferroni-Holm correction for the 5 group comparisons of interest (AD<HC<SCD/MCI) with p<0.05. (B) Activation deviations related to memory performance as continuous measure of clinical impairment. The memory factor score was inverted (×-1) to represent memory impairment for display purposes. Hippocampus showed a linear but precuneus a quadratic pattern of activity deviations with increasing memory impairment. HC= cognitively normal older controls, SCD=subjective cognitive decliners, MCI=mild cognitive impairment, AD= Alzheimer’s disease dementia

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Figure 2. Whole-brain voxel-wise novelty activation pattern in cognitively normal older adults
(A) Higher fMRI activity for novel than familiar scenes in cognitively normal older adults (N=163) is seen in a frontal network, the supplementary motor cortex, the MTL including the hippocampus and parahippocampal cortex, and the occipital regions bilaterally. (B) Lower fMRI activity for novel compared to familiar scenes (i.e. novelty-related deactivation) is seen in posterior midline, lateral temporal, temporo-parietal and frontal regions. Results are depicted at <0.05 (FWE, cluster-level, cluster forming threshold p=0.001).

Figure 3. Whole-brain voxel-wise pattern of increased activity and reduced GM volume in SCD and MCI.
Two-sample t-tests revealed higher novelty activity in SCD (A) and MCI (B) relative to cognitively normal older adults in the precuneus (red colors), a region usually “deactivating” for novel relative to familiar scenes (Figure 2B). FMRI results are depicted at p-voxel<0.005 (uncorrected), p-cluster<0.05 FWE corrected. Reduced gray matter volume was seen in the MCI only (B) comprising temporal lobe, frontal regions and the precuneus (blue colors). Voxel-based morphometry results are depicted p<0.05 (FWE, cluster-level, cluster forming threshold p=0.001).
Novelty-Related fMRI Responses of Precuneus and Medial Temporal Regions in Individuals at Risk for Alzheimer Disease
Ornella V. Billette, Gabriel Ziegler, Merita Aruci, et al.
Neurology published online June 3, 2022
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