

Anterolateral entorhinal cortex volume is associated with memory retention in clinically unimpaired older adults



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ABSTRACT

The entorhinal cortex is subdivided into anterolateral entorhinal cortex (alERC) and posteromedial entorhinal cortex (pmERC) subregions, which are theorized to support distinct cognitive roles. This distinction is particularly important as the alERC is one of the earliest cortical regions affected by Alzheimer's pathology and related neurodegeneration. The relative associations of alERC/pmERC with neuropsychological test performance have not been examined. We examined how alERC/pmERC volumes differentially relate to performance on 1) the Modified Rey Auditory Learning Test (ModRey), a verbal memory test designed to assess normal/preclinical populations, 2) the Montreal Cognitive Assessment (MoCA), and 3) the National Alzheimer's Coordinating Center neuropsychological battery. We also examined whether alERC/pmERC volumes correlate with Alzheimer's disease cerebrospinal fluid (CSF) biomarkers. In 65 cognitively healthy (CDR = 0) older adults, alERC, but not pmERC, volume was associated with ModRey memory retention. Only alERC volume differentiated between participants who scored above and below the MoCA cutoff score for impairment. Evaluating the MoCA subdomains revealed that alERC was particularly associated with verbal recall. On the National Alzheimer's Coordinating Center battery, both alERC and pmERC volumes were associated with Craft story recall and Benson figure copy, but only alERC volume was associated with Craft story retention and semantic fluency. Neither alERC nor pmERC volume correlated with CSF levels of amyloid or tau, and regression analyses showed that alERC volume and CSF amyloid levels were independently associated with ModRey retention performance. Taken together, these results suggest that the alERC is important for memory performance and that alERC volume differences are related to a pattern of neuropsychological test performance (i.e., impairments in episodic memory and semantic fluency) typically seen in clinical Alzheimer's disease.

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1. Introduction

The entorhinal cortex (ERC) is a critical brain region that connects the hippocampus to the cortex (Duvernoy et al., 2013; Witter et al., 2000) and supports memory processes (Coutureau and Di Scala, 2009). Functional connectivity studies suggest the ERC can be subdivided into two subfields: the anterolateral entorhinal cortex (alERC) and the posteromedial entorhinal cortex (pmERC) (Maass et al., 2015; Navarro Schröder et al., 2015; however, see also Doan et al., 2019). How the ERC subfields differentially support ERC-dependent mnemonic processes has not been clearly addressed. This question is particularly important as Alzheimer's disease (AD)-

related tau pathology appears earliest in the aERC (Braak and Braak, 1991; Khan et al., 2014), and positron emission tomography imaging studies show that tau deposition matches the pattern of neurodegeneration in AD (Ossenkoppele et al., 2016). In healthy older adults, ERC volume differences are associated with the combined presence of abnormal levels of AD cerebrospinal fluid (CSF) amyloid and tau biomarkers (Desikan et al., 2011), which in turn are related to differences in cognition (Desikan et al., 2012). Furthermore, larger aERC, but not pmERC, volumes are related to abnormal CSF tau and amyloid levels in patients with AD (Holbrook et al., 2019), as well as to better performance on the Montreal Cognitive Assessment (MoCA), a short assessment for cognitive decline (Olsen et al., 2017).

The “posterior medial, anterior temporal” (PMAT) model suggests that the aERC is involved with learning about or representing concepts and items, whereas the pmERC is involved with constructing and applying contextual models (Ranganath and Ritchey, 2012; Ritchey et al., 2015; however, see also Nilssen et al., 2019 and Wang et al., 2020 for different interpretations of the cognitive functions of the ERC subfields). Experimental studies suggest that the aERC is involved in remembering and distinguishing similar visual objects (Berron et al., 2018; Reagh and Yassa, 2014; Schultz et al., 2012), processing spatial properties of objects (Tsao et al., 2013; Wilson et al., 2013; Yeung et al., 2019b, 2017), and encoding temporal information in episodic memory (Bellmund et al., 2019; Montchal et al., 2019; Tsao et al., 2018). In contrast, direct recording experiments suggest that the pmERC is important for spatial representation/navigation (Hafting et al., 2005; Jacobs et al., 2013; Killian et al., 2012, 2015; Meister and Buffalo, 2018; Sargolini et al., 2006; Solstad et al., 2008).

ERC volume loss is associated with verbal memory performance decline in both community samples (Gicas et al., 2019; Hays et al., 2019) and patients with AD (Di Paola et al., 2007). A retrospective study of temporal lobe resection in patients with epilepsy shows that the amount of ERC removed scales with decline in verbal memory (Liu et al., 2017), and smaller ERC volume is associated with worse verbal delayed recall in mild cognitive impairment (Guzman et al., 2013). Longitudinal ERC shrinkage is associated with decline in story and word recall (Stoub et al., 2010). In addition, experimental work with tau positron emission tomography tracers shows that selective deposition of tau in the ERC/hippocampus and ERC atrophy are related to poorer episodic memory, including delayed verbal memory (Knopman et al., 2019; Maass et al., 2018).

Given the association between ERC volume and verbal memory, are aERC and pmERC volumes differentially related to aspects of verbal recall? The PMAT model would suggest that the aERC's role in item memory underlies verbal memory, and the pmERC's role in context memory also supports verbal recall. The Modified Rey Auditory Learning Test (“ModRey”, Hale et al., 2017) is a verbal memory test designed to be more sensitive to individual differences among preclinical/nonclinical populations than standard neuropsychological instruments. We previously showed that ModRey retention is specifically related to ERC cerebral blood volume (Brickman et al., 2014), which is related to subsequent conversion to AD (Khan et al., 2014), and is correlated with CSF AD biomarkers in older adults without dementia (Yeung et al., 2019a).

This study addressed four questions. First, how do aERC and pmERC volumes in cognitively healthy older adults independently relate to verbal memory? Second, building on our previous work (Olsen et al., 2017), how do aERC and pmERC volumes relate to performance on the MoCA, a multidomain screening instrument for cognitive impairment, particularly when accounting for differences in the hippocampal subfield volumes? Third, do the relationships of aERC and pmERC volumes with memory generalize to other cognitive domains, and do they match a prototypical AD cognitive profile? Fourth, how do aERC and pmERC volumes relate to AD biomarkers, and how do ERC subfield volumes and AD biomarkers interact in their effects on cognition? We hypothesized that 1) larger aERC volume is related to better ModRey verbal memory performance, 2) larger aERC volume is related to better MoCA performance, 3) larger aERC volume is related to better performance on standard clinical neuropsychological instruments that track with clinical AD progression, including episodic memory and semantic fluency, and 4) larger ERC subfield volumes are related to lower CSF tau biomarker levels.

2. Methods

2.1. Participants

Sixty-five older adults without cognitive impairment (clinical dementia rating [CDR] = 0) who were enrolled in the Columbia University Alzheimer's Disease Research Center were included in the study. Participant demographic data are shown in Table 1. Participants were screened for neurological disease, as well as

Table 1
Participant demographics

Measure	Mean	Standard deviation	Range
Age	70.5	7.1	56–93
Education (years)	16.6	2.0	12–20
Gender	32 women/33 men		
Race/ethnicity	50 Non-Hispanic white (76.9%) 11 African American (16.9%) 2 Asians/Pacific Islander (3.1%) 2 Hispanic white (3.1%)		
MoCA	26.5	2.3	20–30
Craft story immediate	22.6	6.1	7–33
Craft story delayed	19.8	6.0	4–33
Craft story retention	0.878	0.164	0.571–1.500
Digit span forward	7.0	1.3	4–9
Digit span backward	5.3	1.5	2–8
Trails A	35.0s	19.1s	15s–148s
Trails B	89.1s	51.7s	33s–300s
MINT	30.1	1.9	22–32
Semantic fluency	36.8	8.1	20–66
Verbal fluency	30.7	7.4	17–46
Benson figure copy	16.1	1.0	14–17
Benson figure delayed	11.9	2.8	4–17

Key: MINT, Multilingual Naming Task; MoCA, Montreal Cognitive Assessment.

contraindications for magnetic resonance imaging (MRI) and gadolinium injection. All participants gave informed consent to participate in this study. The Columbia University Institutional Review Board approved all procedures used in this study. We recently reported data on a subset of these participants (Yeung et al., 2019a) with different analyses.

2.2. Structural image acquisition

Structural magnetic resonance images (MRI) were acquired on a 3T GE MR750 scanner using a 32-channel head coil (56 subjects) and a 3T GE Signa Premier scanner using a 48-channel head coil (9 subjects) because of a scanner upgrade. There were no systematic differences in brain volume across the 2 scanners: total ERC volume ($M = 1516.67 \text{ mm}^3$, standard deviation (SD) = 216.57 mm^3 for the GE MR750, $M = 1522.12 \text{ mm}^3$, $SD = 126.38 \text{ mm}^3$ on the GE Signa Premier, $t(63) = -0.073$, $p = 0.942$), aERC volume ($M = 1169.29 \text{ mm}^3$, $SD = 187.89 \text{ mm}^3$ on the GE MR750, $M = 1206.89 \text{ mm}^3$, $SD = 110.19 \text{ mm}^3$ on the GE Signa Premier, $t(63) = -0.582$, $p = 0.563$), and pmERC volume ($M = 347.39 \text{ mm}^3$, $SD = 66.64 \text{ mm}^3$ on the GE MR750, $M = 315.24 \text{ mm}^3$, $SD = 57.77 \text{ mm}^3$ on the GE Signa Premier, $t(63) = 1.365$, $p = 0.177$). Participants received a T1-weighted magnetization-prepared, rapid acquisition with gradient echo image (MPRAGE) whole-brain anatomical scan (TE/TR = 2.63 ms/2000 ms, 176 sagittal slices perpendicular to the AC-PC line, flip angle = 12° , voxel size = $1 \times 1 \times 1 \text{ mm}$). The T1-weighted MPRAGE scan was used to obtain measures of brain and head size and for slice placement during the acquisition of a subsequent high-resolution T2-weighted scan in an oblique-coronal plane, perpendicular to the hippocampal long axis (TE/TR = 68 ms/3000 ms, 40 slices, flip angle = 125° , voxel size = $0.43 \times 0.43 \times 2 \text{ mm}$). For the high-resolution T2-weighted scan, the first slice was placed anterior to the first appearance of the collateral sulcus, and the last slice placed posterior to the hippocampal tail, to ensure full coverage of the entire hippocampus and medial temporal lobe (MTL) cortices.

2.3. Manual ERC subregion segmentation

Manual segmentation of the ERC subfields was performed on the T2-weighted images ($0.4 \times 0.4 \text{ mm}$ in plane), in the participants' native space, on the oblique coronal plane, perpendicular to long axis of the hippocampus. A single rater (L.-K.Y.) manually delineated the ERC and subdivided it into aERC and pmERC subregions in FSLView (FMRIB, Oxford, UK). The segmentation protocol used in this study was previously described in Olsen et al. (2017).

The ERC was defined following the protocol described by Insausti et al. (1998) based on histological study. The subdivision of the ERC into aERC and pmERC was adapted from the protocol of Maass et al. (2015), based on functional connectivity between the ERC and the perirhinal and parahippocampal cortices, respectively. The 2 protocols were combined by first defining the entire ERC based on the Insausti protocol and subsequently subdividing the ERC into aERC and pmERC subfields using the Maass protocol. Moving anterior to posterior, the ERC appears at the level of the frontal-temporal junction. The pmERC appears at the superior tip of the ERC where the hippocampal head first appears and increases in size moving posteriorly. The pmERC and aERC are equal in size approximately two-thirds of the anterior/posterior extent of the hippocampal head, as described by Maass et al. (2015). The last slice of the ERC appears just posterior to the uncus apex. At this level, the pmERC covers the entire ERC.

As in our previous work (Olsen et al., 2017), slight alterations were made to the Maass protocol to accommodate the thicker slices used in the present study. Furthermore, as the Insausti protocol

defines the lateral edge of the ERC based on the depth of the collateral sulcus (as opposed to the Maass protocol, which places the lateral edge of the ERC on the medial edge of the collateral sulcus), the ERC subfields were extended laterally based on the Insausti protocol's definition (see Fig. 1).

All ERC volumes were corrected for head size using a regression-based method, to account for differences in brain size between participants. Estimated total intracranial volume was derived from the whole-brain T1-weighted scans using FreeSurfer (v6.0) (Buckner et al., 2004). A regression slope was obtained for each ERC subfield in each hemisphere, by regressing the volume of that subfield with the total intracranial volume, and each individual participant's subfield volume was corrected using that regression slope based on the difference between their intracranial volumes compared with the mean intracranial volume. Corrected subfield volumes were subsequently summed across the 2 hemispheres, giving a single total volume for each region for each participant.

Intrater reliability was established by comparing the segmentation of 10 randomly selected scans, after a delay of several months. Reliability was assessed using the intraclass correlation coefficient, which evaluates volume reliability (Shrout and Fleiss, 1979) and the Dice metric, which also takes spatial overlap into account (Dice, 1945), computed separately for each region in each hemisphere. Dice was derived using the formula $2 \times (\text{intersecting region}) / (\text{original segmentation} + \text{repeat segmentation})$; a Dice overlap metric of 0 represents no overlap, whereas a metric of 1 represents perfect overlap. Intrater reliability results are shown in Table 2 and are similar to intrater reliability for ERC subfields in our previous work (Olsen et al., 2017) and for manual segmentation of MTL regions more generally (Wisse et al., 2012; Yushkevich et al., 2015).

2.4. Automated hippocampal subfield segmentation

Automated segmentation of the hippocampal subfields (CA1, CA2, CA3, dentate gyrus, subiculum) and the MTL cortices (BA35, BA36, parahippocampal cortex) was performed using the Automatic Segmentation of Hippocampal Subfields package (Yushkevich et al., 2015) on both the T1-weighted MPRAGE scans and the T2-weighted high-resolution scans, using the Magdeburg atlas (Berron et al., 2017, see Fig. 1). Note that subfields were partitioned in the hippocampal head and body sections, but not in the tail section, where the stratum radiatum lacunosum moleculare, a strip of white matter that separates the subfields, is not visible at the $0.4 \times 0.4 \text{ mm}$ in-plane resolution. Because it is unclear where the subfield boundaries lie in the hippocampal tail, it was excluded from the analyses in this study (see also Wisse et al., 2020 addressing concerns about segmenting hippocampal subfields where the stratum radiatum lacunosum moleculare is not visible).

2.5. ModRey

Participants were administered the ModRey (Hale et al., 2017), a test of list learning and declarative memory. Fifty-six participants were administered the ModRey on the day of MRI scanning. Of the remaining 9 participants, the median delay between scanning and testing was 9 days (average delay = 43.8 days, $SD = 48.1$ days). Seven of the 9 participants were tested within 1.5 months of scanning, and the remaining 2 were tested within 4 months of scanning. Cognitive performance did not differ on any measure tested in this study between participants who received the ModRey on the same day as scanning versus those who did not. The design of the ModRey is illustrated in Fig. 2. In brief, participants first received 3 learning trials, where they were read 20 unique, semantically and phonetically unrelated words (list A), and were asked to recall these words

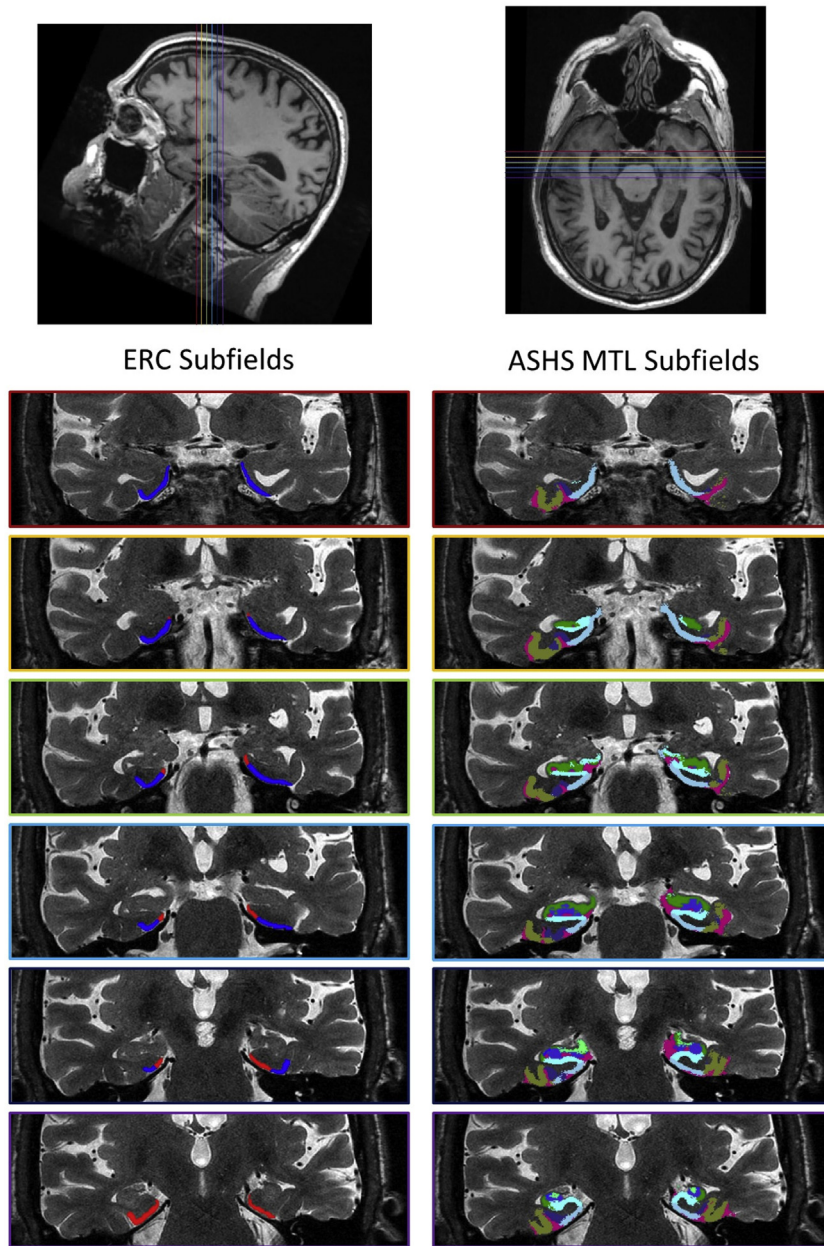


Fig. 1. Example of manual segmentation of ERC subfields (left) and ASHS automated segmentation of hippocampal subfields/MTL regions (right), shown on coronal plane. Position of color-coded coronal slices on axial and sagittal views shown at top. In the ERC subfield segmentation, aERC is indicated in blue and pmERC is indicated in red. In the ASHS hippocampal/MTL segmentation, CA1 is indicated in green, dentate gyrus in blue, CA3 in light green, subiculum in light blue, BA35 is indicated in violet, BA36 is indicated in tan. CA2 and PHC were also measured, but are not visible on these slices. ERC (navy) and empty spaces (magenta) are also shown in figure, but not used for analyses. Abbreviations: aERC, anterolateral entorhinal cortex; ERC, entorhinal cortex; pmERC, posteromedial entorhinal cortex. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

after each trial. These trials were immediately followed by a 4th learning trial, where participants were read a distractor list of 20 additional semantically and phonetically unrelated words (list B). Subsequently, participants were asked to freely recall as many words

Table 2
Intrarater reliability for ERC subfield segmentations

Subregion	Intrarater: Dice		Intrarater ICC	
	Left	Right	Left	Right
aERC	0.82	0.81	0.86	0.93
pmERC	0.75	0.77	0.84	0.93

Key: aERC, anterolateral entorhinal cortex; ERC, entorhinal cortex; ICC, intraclass correlation coefficient; pmERC, posteromedial entorhinal cortex.

as possible from list A (the “short-delay free recall”). After an approximately 40-minute delay, during which unrelated cognitive testing occurred, participants were asked to freely recall as many words as possible from both list A and list B separately (“long delay free recall”). These trials were followed by a 66-item forced-choice recognition test where participants were asked to decide if a presented word belongs to list A; distractors came from both list B and a selection of phonetically/semantically related words. Finally, source memory was assessed by asking the participant to match words on a list to either list A or list B.

The primary ModRey outcome variable was the “short-delay retention” score: the ratio of the number of items from list A correctly recalled freely at the short delay to the number of items

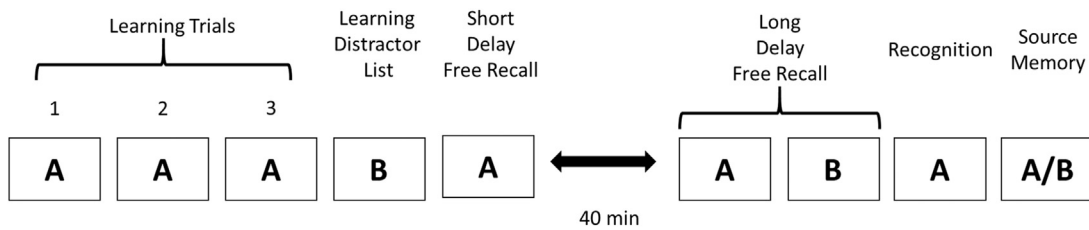


Fig. 2. Experimental design of the ModRey verbal memory task. Abbreviations: ModRey, Modified Rey Auditory Learning Test.

correctly recalled freely during the last list A learning trial. This variable was chosen because it is tightly linked to function of the ERC (Brickman et al., 2011, 2014; Fernández et al., 1999; Schon et al., 2004). Additional ModRey outcome variables analyzed in this study included total learning, short-delay free recall, long-delay free recall, recognition discrimination, and source memory.

Two psychometrically similar (Hale et al., 2017) versions of the ModRey were used, each with its own unique lists A and B. Half of the participants received one version of the ModRey, and the other half received the other. There were no differences in short-delay retention or any of the other outcome variables between the 2 versions. Accordingly, all analyses collapsed across the 2 versions.

2.6. NACC-UDS3 neuropsychological battery

All the participants received the National Alzheimer's Coordinating Center (NACC) Uniform Data Set (UDS) neuropsychological battery, version 3 (NACC-UDS3, Weintraub et al., 2018). This is a standardized set of neuropsychological tests used by Alzheimer's disease centers across the United States. The battery includes the MoCA (Nasreddine et al., 2005), Craft Story (Craft et al., 1996), Digit Span (Wechsler, 1987), Semantic and Verbal Fluency (Morris et al., 1989), Trail Making Test Parts A and B (Reitan and Wolfson, 1985), Benson Complex Figure Test (Possin et al., 2011), and the Multilingual Naming Task (Gollan et al., 2012). The NACC-UDS3 is administered to all participants in the Columbia Alzheimer's Disease Research Center cohort annually.

Because of concerns about practice effects, the baseline performance on the entire NACC-UDS3 battery was used for each participant. 42 out of the 65 participants (64.6%) received the NACC-UDS3 battery for the first time within 1 year before scanning, and additional 13 participants (20%) received the battery for the first time within 2 years prior scanning. No participant received the battery for the first time more than 3.5 years before the scan. There were no significant differences in performance between participants who were scanned within one year of taking the battery versus participants who were scanned before. The median delay between NACC-UDS3 testing and the scan was 103 days.

2.7. CSF biomarkers

CSF was obtained via lumbar puncture, performed with standard clinical research methods in aseptic fashion by a board-certified neurologist. CSF was obtained for 59 of the 65 participants in this study. CSF was always collected during a separate session from testing to avoid influencing performance on cognitive testing. Up to 15 cc of CSF was removed using a Sprotte 24G spinal needle and placed in 2 12 cc polypropylene tubes. All samples were centrifuged briefly, aliquoted using polypropylene pipettes within 30 minutes, and stored for both biomarker analysis and CSF banking at -80°C . Levels of 3 CSF biomarkers (A β 42, phosphorylated tau, total tau) were analyzed in duplicate by a bead-based multiplex method using the Innogenetics Alz-Bio3 kits on a Luminex (LS-100)

platform with 96-well plates. Coefficient of variation was generally less than 10% (samples with higher coefficient of variation were repeat analyzed).

2.8. Statistical analysis

2.8.1. ModRey performance and aERC/pmERC volumetric analyses

A multiple regression model was run to evaluate the relationship between aERC/pmERC volumes and ModRey performance. In this model, ModRey short-delay retention was the dependent variable, with aERC volume and pmERC volume as predictors and age, years of education, gender, and race/ethnicity as covariates.

These analyses were followed by additional exploratory multiple regression models that examined each of the other ModRey outcome measures (total learning, short-delay free recall, long-delay free recall, recognition discrimination, and source memory) as the dependent variable, again with aERC and pmERC volume as predictors and age, years of education, gender, and race/ethnicity as covariates.

2.8.2. MoCA performance and aERC/pmERC volumetric analyses

To replicate analyses performed in our previous work in a separate sample (Olsen et al., 2017), participants were divided into two groups based on their MoCA score (<26 , ≥ 26 ; with 26 being the MoCA threshold score). The volumes of the aERC, pmERC, as well as the other segmented hippocampal subfields and MTL cortices were compared across these two groups using t-tests. Note that since these two groups did not differ in age, $t(63) = 0.397$, $p = 0.693$, 95% confidence interval (CI) $[-3.000, 4.488]$, years of education, $t(63) = 1.169$, $p = 0.247$, 95% CI $[-0.433, 1.653]$, or gender, $\chi^2(1, N = 65) = 0.190$, $p = 0.663$, structural volumes for these t-tests were not adjusted for age, gender, or years of education.

Given that Olsen et al. (2017) found that only aERC volume differed between the MoCA groups after correction for multiple comparisons, a multiple regression model was run with all the regional volumes as predictors, age, years of education, gender, and race/ethnicity as covariates and MoCA score as the dependent variable, with the goal of testing if aERC volume, or any other MTL/hippocampal subfield volume, was related to MoCA score after accounting for other subfield volume differences.

As a posthoc analysis, performance on the MoCA was divided into subdomains (visuospatial/executive, naming, attention, language/abstraction, memory, and orientation). Pearson's correlations were used to evaluate the association of each MoCA subdomain score with aERC/pmERC volumes and age, years of education, gender, and race/ethnicity as covariates.

2.8.3. NACC UDS-ERC subfield analyses

Exploratory multiple regression models were also used to evaluate the relationship of the aERC and pmERC (as regressors), with each of the NACC-UDS3 measures (as the dependent variable): Craft immediate recall, Craft delayed recall, Craft retention, digit span, semantic fluency, Trails A & B (and the ratio of Trails B to Trails

A), Benson figure copy, Benson figure delayed recall, Multilingual Naming Task, and verbal fluency. These multiple regression models included age, years of education, gender, and race/ethnicity as covariates.

Pearson's correlations were used to test if ERC subfield volumes correlated with 3 CSF AD biomarkers ($A\beta$ -42, phospho-tau, and total tau levels), or with ratios of the CSF tau biomarkers to the $A\beta$ -42 biomarker, with age, years of education, gender, and race/ethnicity as covariates. Posthoc analyses examined the correlations between hippocampal subfield volumes and CSF AD biomarker levels. Because our previous results in this sample showed that CSF amyloid was related to ModRey short-delay retention in cognitively healthy older adults (Yeung et al., 2019a), multiple regression analysis was also used to determine if CSF amyloid and aERC volume were independent predictors of ModRey performance, with age, years of education, gender, and race/ethnicity as covariates.

3. Results

3.1. ModRey performance and aERC/pmERC volumetric analyses

The multiple regression model examining how ModRey short-delay retention performance was related to aERC and pmERC volumes showed that larger aERC volume was associated with better performance on the primary ModRey outcome measure, short-delay retention, $t(58) = 2.009$, $p = 0.049$, $\beta = 0.258$, 95% CI [0.001, 0.514], $sr = 0.255$ (Fig. 3A). However pmERC volume was not

significantly associated with ModRey performance, $t(58) = 0.301$, $p = 0.764$, $\beta = 0.043$, 95% CI [-0.243, 0.330], $sr = 0.040$.

Exploratory multiple regression analyses examining the effects of aERC and pmERC volume on other ModRey measures showed that larger aERC volume was also significantly associated with better performance on total learning, short-delay accuracy, and number of false positive errors (Table 3), whereas pmERC volume was only significantly associated with the number of intrusions.

3.2. MoCA performance and aERC/pmERC volumetric analyses

The mean MoCA score across all participants was 26.5 (SD = 2.3, range = 20–30). Independent samples t-tests showed that participants with MoCA scores of 26 or above (i.e., above the MoCA threshold score) had larger aERC volumes than those with MoCA scores below 26 (see Table 4). In contrast, the volumes of pmERC and the other measured hippocampal subfields or MTL cortical areas did not differ between the 2 groups.

Because the volume of MTL regions and hippocampal subfields is necessarily highly correlated (see Table 5), multiple regression analysis was performed to evaluate the specific associations of each regional volume (aERC, pmERC, BA35, BA36, CA1, CA2, CA3, dentate gyrus, subiculum) with MoCA score. Among all the regions evaluated, only aERC volume uniquely related to MoCA score positively, $t(50) = 3.532$, $p = 0.001$, $\beta = 0.518$, 95% CI [0.224, 0.813], $sr = 0.417$ (Fig. 3B).

As a posthoc analysis, the association between MoCA subdomain scores (visuospatial/executive, naming, attention, language,

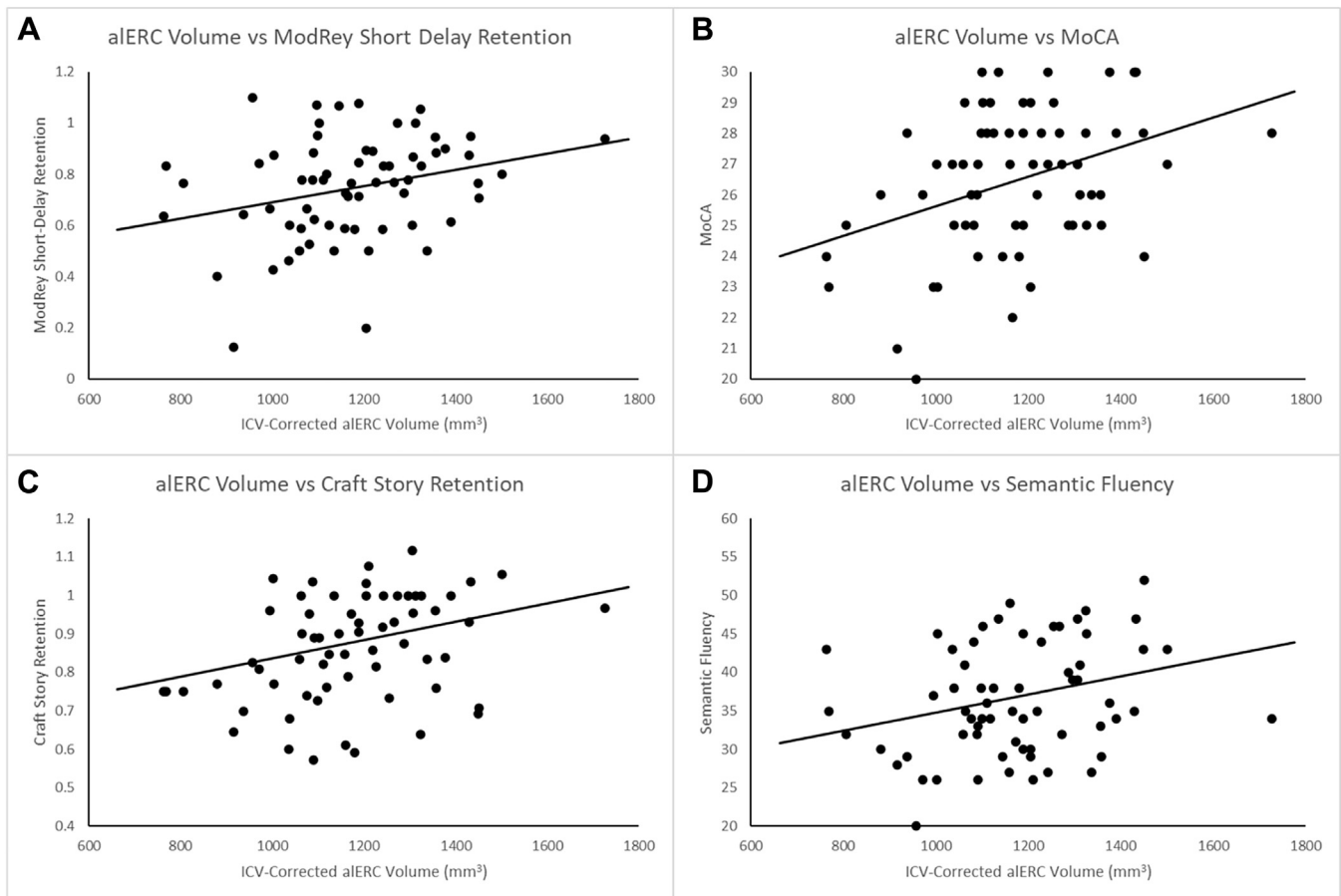


Fig. 3. Correlation of aERC volume with A) ModRey short-delay retention, B) MoCA, C) Craft story retention, and D) semantic fluency. Abbreviations: aERC, anterolateral entorhinal cortex; MoCA, Montreal Cognitive Assessment; ModRey, Modified Rey Auditory Learning Test.

Table 3
aERC/pmERC volumes as predictors in multiple regression models for additional ModRey outcome variables

Measure	aERC				pmERC			
	β	95% CI	t	p	β	95% CI	t	p
Total learning	0.276	[0.032, 0.520]	2.268	0.027	0.144	[-0.129, 0.416]	1.055	0.296
Total perseverations	0.086	[-0.157, 0.330]	0.708	0.482	-0.045	[-0.317, 0.227]	-0.329	0.743
Total intrusions	0.020	[-0.237, 0.278]	0.159	0.874	-0.288	[-0.576, -0.001]	-2.006	0.049
Short-delay accuracy	0.341	[0.099, 0.583]	2.818	0.007	0.092	[-0.179, 0.363]	0.680	0.499
Long-delay accuracy	0.195	[-0.054, 0.444]	1.567	0.123	0.119	[-0.159, 0.397]	0.856	0.396
Long-delay retention	0.046	[-0.211, 0.304]	0.360	0.720	0.085	[-0.203, 0.373]	0.590	0.557
Recognition (hits)	0.006	[-0.257, 0.269]	0.045	0.964	0.122	[-0.172, 0.416]	0.828	0.411
Recognition (false positives)	-0.259	[-0.510, -0.008]	-2.064	0.044	-0.071	[-0.352, 0.209]	-0.510	0.612
Source memory	0.159	[-0.103, 0.421]	1.215	0.229	0.122	[-0.171, 0.415]	0.835	0.407

Key: aERC, anterolateral entorhinal cortex; CI, confidence interval; ModRey, Modified Rey Auditory Learning Test; pmERC, posteromedial entorhinal cortex. Each row represents an individual multiple regression model, with the specified ModRey outcome measure as the dependent variable, and aERC volume, pmERC volume, age, years of education, gender, and race/ethnicity as predictors. $p < 0.05$ indicated in bold.

abstraction, memory, orientation) and aERC volumes was tested using Pearson's correlations. These analyses showed that only the memory subdomain score, which is a measure of delayed recall, was significantly positively associated with aERC volume, $r(59) = 0.367$, 95% CI [0.123, 0.569], $p = 0.004$. We note that aERC volume was also marginally correlated with the visuospatial/executive subdomain, $r(59) = 0.248$, 95% CI [-0.008, 0.473], $p = 0.054$, and the orientation subdomain, $r(59) = 0.241$, 95% CI [-0.016, 0.468], $p = 0.061$.

3.3. NACC UDS-ERC subfield analyses

Multiple regression analyses were run to examine the associations of aERC and pmERC volume with performance on each measure from the NACC-UDS3 battery. Table 6 lists the association of the ERC subfield predictors in these models. Notably, pmERC volume was significantly associated with performance on Craft story immediate recall and delayed recall. aERC volume was significantly associated with both Craft story delayed recall and retention (Fig. 3C). Both subfield volumes were associated with Benson figure copy performance, whereas aERC volume, but not pmERC volume, was significantly related to performance on a test of semantic fluency (Fig. 3D).

3.4. CSF biomarker analyses

Pearson's correlations were run to explore the associations between CSF biomarkers in this participant cohort. A β 42 levels were not correlated with phosphorylated tau levels, $r(59) = 0.159$, 95% CI [-0.101, 0.398], $p = 0.228$, but A β 42 levels were inversely correlated with total tau, $r(59) = 0.307$, 95% CI [0.056, 0.522], $p = 0.018$. Phosphorylated tau and total tau levels were strongly correlated, $r(59) = 0.656$, 95% CI [0.481, 0.780], $p < 0.001$.

Pearson's correlations were run to examine the relationship between ERC subfield volume and CSF biomarkers of AD. Neither aERC volume nor pmERC volume correlated with CSF levels of A β -42, phospho-tau, or total tau, or the ratios of the tau measures with A β -42 (see Table 7). Exploratory posthoc analyses using Pearson's correlations to examine the relationship between MTL region/hippocampal subfield volumes and CSF biomarkers showed that among all the segmented regions, only the dentate gyrus volume was significantly correlated with CSF A β -42.

As both aERC volume and CSF A β -42 (Yeung et al., 2019a) have been shown to relate to ModRey short-delay retention in this cohort, multiple regression analysis was performed to examine the independence of their contributions to ModRey short-delay retention. aERC volume and CSF A β -42 levels were entered as predictors for ModRey short-delay retention performance. This model showed that aERC volume, $t(52) = 2.043$, $p = 0.046$, $\beta = 0.257$, 95% CI [0.004, 0.510], $sr = 0.254$, and CSF A β -42 levels, $t(52) = 2.186$, $p = 0.033$, $\beta = 0.345$, 95% CI [0.029, 0.662], $sr = 0.271$, were independently associated with ModRey short-delay retention, accounting for age, education, gender, and race/ethnicity as covariates. Posthoc analyses revealed no significant interaction between aERC volume and CSF A β -42 (i.e., no moderation), and neither variable mediated the relationship between the other and ModRey short-delay retention.

To address concerns that the presence of biomarker-positive participants might drive the observed structure-cognition associations, the multiple regression models predicting ModRey retention were repeated, excluding participants who were biomarker positive, based on previously established CSF thresholds (A β -42 < 325 pg/mL, t-tau > 72 pg/mL, p-tau > 31 pg/mL, p-tau/A β -42 ratio < 0.1, see Yeung et al., 2019a). As shown in Table 8, excluding participants who were biomarker positive did not greatly affect the observed effect of the aERC predictor on ModRey retention.

Table 4
Average volumes of entorhinal cortex subfields, hippocampal subfields, and MTL cortices in participant groups divided by MoCA threshold score, and t-tests comparing volume differences between those groups, $df = 63$, $p < 0.05$ indicated in bold

Region	MoCA ≥ 26 (N = 43, M \pm SD mm ³)	MoCA < 26 (N = 22, M \pm SD mm ³)	95% CI of difference	t	p
pmERC	345.66 \pm 65.34	337.61 \pm 68.56	[-26.75, 42.85]	0.454	0.651
aERC	1211.14 \pm 164.56	1102.86 \pm 187.94	[17.82, 198.75]	2.254	0.028
CA1	1310.35 \pm 189.26	1271.32 \pm 220.41	[-65.83, 143.88]	0.705	0.484
CA2	60.90 \pm 23.37	59.94 \pm 21.83	[-11.03, 12.93]	0.162	0.872
DG	693.38 \pm 158.88	694.39 \pm 148.21	[-82.41, 80.40]	0.025	0.980
CA3	244.76 \pm 84.30	230.41 \pm 60.70	[-26.11, 54.81]	0.786	0.435
Subiculum	2156.78 \pm 276.51	2148.36 \pm 240.61	[-130.43, 147.27]	0.127	0.900
BA 35	1065.91 \pm 194.73	1071.42 \pm 253.12	[-118.62, 107.62]	0.089	0.929
BA 36	4410.12 \pm 742.56	4349.05 \pm 831.62	[-344.05, 466.18]	0.289	0.773
PHC	735.20 \pm 161.92	760.07 \pm 173.78	[-111.81, 62.07]	0.556	0.580

Key: aERC, anterolateral entorhinal cortex; CI, confidence interval; MoCA, Montreal Cognitive Assessment; pmERC, posteromedial entorhinal cortex; SD, standard deviation.

Table 5
Pearson's correlations between the volumes of all segmented regions

Region		aERC	pmERC	CA1	CA2	DG	CA3	Sub.	BA35	BA36	PHC
aERC	r	1	0.251	0.093	0.361	0.312	0.235	0.044	0.331	0.061	0.216
	p		0.044	0.463	0.003	0.011	<i>0.059</i>	0.730	0.007	0.627	<i>0.084</i>
pmERC	r	0.251	1	0.376	0.054	0.106	0.369	0.445	0.299	0.042	0.276
	p	0.044		0.002	0.669	0.399	0.002	0.000	0.016	0.741	0.026
CA1	r	0.093	0.376	1	0.409	0.646	0.391	0.516	0.348	0.230	0.351
	p	0.463	0.002		0.001	0.000	0.001	0.000	0.004	0.066	0.004
CA2	r	0.0361	0.054	0.409	1	0.736	0.203	0.122	0.388	0.443	0.402
	p	0.003	0.669	0.001		0.000	0.104	0.331	0.001	0.000	0.001
DG	r	0.312	0.106	0.646	0.736	1	0.173	0.215	0.368	0.321	0.401
	p	0.011	0.399	0.000	0.000		0.167	<i>0.085</i>	0.003	0.009	0.001
CA3	r	0.235	0.369	0.391	0.203	0.173	1	0.328	0.530	0.194	0.344
	p	<i>0.059</i>	0.002	0.001	0.104	0.167		0.008	0.000	0.121	0.005
Sub.	r	0.044	0.445	0.516	0.122	0.215	0.328	1	0.487	0.323	0.185
	p	0.730	0.000	0.000	0.331	<i>0.085</i>	0.008		0.000	0.009	0.140
BA35	r	0.331	0.299	0.348	0.388	0.368	0.530	0.487	1	0.591	0.411
	p	0.007	0.016	0.004	0.001	0.003	0.000	0.000		0.000	0.001
BA36	r	0.061	0.042	0.230	0.443	0.321	0.194	0.323	0.591	1	0.156
	p	0.627	0.741	<i>0.066</i>	0.000	0.009	0.121	0.009	0.000		0.216
PHC	r	0.216	0.276	0.351	0.402	0.401	0.344	0.185	0.411	0.156	1
	p	<i>0.084</i>	0.026	0.004	0.001	0.001	0.005	0.140	0.001	0.216	

Key: aERC, anterolateral entorhinal cortex; pmERC, posteromedial entorhinal cortex. df = 65 for all comparisons. $p < 0.05$ indicated in bold, $p < 0.10$ indicated in italics.

4. Discussion

In a group of cognitively unimpaired older adults, we demonstrated that larger aERC volume is related to better performance short-delay retention on the ModRey (Hale et al., 2017), a memory test designed for preclinical and cognitively healthy older adults. Among hippocampal and ERC subfields, aERC volume is also selectively positively associated with performance on the MoCA, a short assessment that tracks cognitive decline, and differs between participants who score above/below the MoCA threshold score; this observation replicates the findings of Olsen et al. (2017) in a separate participant sample. Extending this finding, we showed that the positive relationship between aERC and MoCA is largely based on the delayed recall component of the MoCA. Furthermore, we demonstrated that on the NACC-UDS3 neuropsychological battery, the volume of both aERC and pmERC is positively related to clinical measures of memory and visuospatial processing, whereas aERC volume, but not pmERC volume, is also positively related to memory retention and semantic fluency. Finally, we showed that higher aERC volume and CSF amyloid levels (i.e., healthier levels of CSF amyloid) have independent positive associations with ModRey retention.

Taken together, our results suggest that the aERC broadly supports memory processing, including recall, retention, and rejecting false positives, across multiple neuropsychological instruments, whereas the pmERC may be necessary only when contextual information is needed for memory. Interpreting our results within the PMAT framework (Ritchey et al., 2015), aERC volume is positively related to memory measures across numerous different neuropsychological instruments and to semantic fluency scores because these require memory for single items and their associations, without necessarily requiring additional contextual information. In turn, pmERC volumes are positively related to memory only on the Craft story task because the narrative structure of the stimuli makes contextual information more useful for recall. Likewise, the positive relationship of both aERC and pmERC volume to better Benson copy performance might be explained by the importance of spatial relations between the different line segments in that task. Interestingly, aERC volume was positively associated with immediate recall on both the Benson complex figure task and the ModRey, but not with delayed recall. However, on the Craft story, aERC volume was positively associated with performance on both immediate and delayed recall trials. This pattern of aERC-memory relationships

Table 6
aERC and pmERC volumes as predictors in multiple regression models for performance on NACC battery tasks (with age, gender, race/ethnicity, and years of education as covariates)

Measure	aERC				pmERC				
	β	95% CI	t	p	β	95% CI	t	p	
Craft story immediate	0.148	[-0.100, 0.396]	1.194	0.237	0.369	[0.091, 0.646]	2.662	0.010	
Craft story delayed	0.274	[0.028, 0.519]	2.230	0.030	0.296	[0.021, 0.571]	2.161	0.035	
Craft story retention	0.246	[-0.013, 0.504]	1.899	<i>0.062</i>	-0.009	[-0.292, 0.274]	-0.063	0.950	
Digit span forward	-0.009	[-0.259, 0.241]	-0.071	0.943	-0.111	[-0.389, 0.166]	-0.802	0.426	
Digit span backward	-0.058	[-0.303, 0.187]	-0.477	0.635	0.116	[-0.157, 0.390]	0.852	0.398	
Trails A	-0.178	[-0.428, 0.073]	-1.421	0.161	0.225	[-0.055, 0.504]	1.608	0.113	
Trails B	-0.065	[-0.311, 0.182]	-0.525	0.601	-0.066	[-0.342, 0.209]	-0.481	0.632	
Trails B/Trails A	0.041	[-0.211, 0.292]	0.324	0.747	-0.246	[-0.527, 0.035]	-1.756	<i>0.084</i>	
MINT	0.123	[-0.128, 0.374]	0.980	0.331	0.007	[-0.274, 0.289]	0.051	0.959	
Semantic fluency	0.277	[0.023, 0.530]	2.187	0.033	-0.062	[-0.346, 0.221]	-0.441	0.661	
Verbal fluency	0.084	[-0.173, 0.342]	0.657	0.514	0.144	[-0.143, 0.431]	1.003	0.320	
Benson figure copy	0.323	[0.086, 0.561]	2.721	0.009	0.257	[-0.009, 0.523]	1.935	<i>0.058</i>	
Benson figure delayed	0.152	[-0.109, 0.413]	1.167	0.248	-0.028	[-0.320, 0.264]	-0.193	0.847	

Key: aERC, anterolateral entorhinal cortex; CI, confidence interval; MINT, Multilingual Naming Task; pmERC, posteromedial entorhinal cortex.

Each row represents an individual multiple regression model, with the specified NACC-UDS3 outcome measure as the dependent variable, and aERC volume, pmERC volume, age, years of education, gender, and race/ethnicity as predictors. $p < 0.05$ indicated in bold, $p < 0.10$ indicated in italics.

Table 7
Pearson's correlations of CSF biomarker levels with aERC/pmERC volumes, controlling for age, years of education, gender, and race/ethnicity, $df = 53$

Region		A β 42	Phospho-tau	Total tau	Phospho-tau/A β 42	Total tau/A β 42
aLER	r	0.055	-0.082	-0.145	-0.119	-0.171
	p	0.688	0.553	0.292	0.387	0.213
	95% CI	[-0.218, 0.320]	[-0.344, 0.192]	[-0.399, 0.130]	[-0.377, 0.156]	[-0.421, 0.104]
pmER	r	0.001	-0.105	-0.099	-0.003	-0.039
	p	0.996	0.447	0.473	0.984	0.777
	95% CI	[-0.269, 0.271]	[-0.364, 0.170]	[-0.359, 0.176]	[-0.273, 0.267]	[-0.306, 0.233]
CA1	r	0.201	0.120	0.162	-0.056	-0.039
	p	0.141	0.385	0.237	0.686	0.778
	95% CI	[-0.073, 0.447]	[-0.155, 0.378]	[-0.113, 0.414]	[-0.321, 0.217]	[-0.306, 0.233]
CA2	r	0.186	0.216	0.107	-0.026	-0.098
	p	0.174	0.114	0.435	0.853	0.479
	95% CI	[-0.088, 0.434]	[-0.057, 0.459]	[-0.168, 0.366]	[-0.294, 0.246]	[-0.358, 0.176]
DG	r	0.268	0.181	0.226	-0.202	-0.167
	p	0.048	0.185	<i>0.097</i>	0.140	0.224
	95% CI	[0.000, 0.502]	[-0.093, 0.430]	[-0.047, 0.467]	[-0.447, 0.072]	[-0.418, 0.108]
CA3	r	0.055	0.128	0.167	0.211	0.185
	p	0.688	0.351	0.223	0.122	0.177
	95% CI	[-0.218, 0.320]	[-0.147, 0.384]	[-0.108, 0.418]	[-0.062, 0.455]	[-0.089, 0.433]
Sub.	r	-0.144	0.076	-0.125	0.229	0.064
	p	0.295	0.582	0.362	0.092	0.641
	95% CI	[-0.398, 0.131]	[-0.198, 0.339]	[-0.382, 0.150]	[-0.044, 0.470]	[-0.209, 0.328]
BA35	r	0.186	0.128	0.132	0.009	-0.053
	p	0.174	0.351	0.336	0.945	0.699
	95% CI	[-0.088, 0.434]	[-0.147, 0.384]	[-0.143, 0.388]	[-0.261, 0.278]	[-0.318, 0.220]
BA36	r	0.175	0.191	0.028	-0.060	-0.178
	p	0.201	0.163	0.840	0.666	0.192
	95% CI	[-0.100, 0.425]	[-0.083, 0.438]	[-0.244, 0.296]	[-0.325, 0.213]	[-0.427, 0.096]
PHC	r	-0.068	-0.005	-0.066	-0.054	-0.085
	p	0.622	0.970	0.632	0.696	0.537
	95% CI	[-0.332, 0.206]	[-0.274, 0.265]	[-0.330, 0.208]	[-0.319, 0.219]	[-0.347, 0.189]

Key: aLER, anterolateral entorhinal cortex; CI, confidence interval; CSF, cerebrospinal fluid; pmERC, posteromedial entorhinal cortex.
 $p < 0.05$ indicated in bold, $p < 0.10$ indicated in italics.

does not seem to be related to the length of the delay, as the delay for the ModRey (40 min) was greater than the Benson complex figure task (15 minutes) or the Craft story (20 minutes). Rather, our results might reflect the importance of the aLERC (as the main hippocampal input) for narrative events (Rosenbaum et al., 2008) versus single items, or the greater impact of aLERC dysfunction on more interconnected stimuli. The lack of association between aLERC volumes and ModRey long-delay recall in our study is not necessarily discrepant with previously reported association between delayed recall and functional activity in the ERC (Brickman et al.,

Table 8
The effect of the aLERC predictor in multiple regression models predicting ModRey short-delay retention in biomarker-negative only subgroups, with pmERC, age, education, gender, and race/ethnicity as additional predictors

Group	N	β	sr	t	p
All participants	65	0.258	0.249	2.009	0.049
A β -42 negative	51	0.313	0.303	2.178	0.035
P-tau negative	52	0.244	0.235	1.685	<i>0.099</i>
T-tau negative	52	0.290	0.276	2.040	0.047
P-Tau/amyloid <0.1	54	0.304	0.293	2.194	0.033
Negative for all 3 biomarkers	41	0.354	0.350	2.177	0.037

Key: aLERC, anterolateral entorhinal cortex; ModRey, Modified Rey Auditory Learning Test; pmERC, posteromedial entorhinal cortex.
 $p < 0.05$ indicated in bold, $p < 0.10$ indicated in italics.

2011). A recent study highlighted how performance on spatial navigation tasks within a reasonable range of normal performance—which have previously been shown to elicit functional activity in the hippocampus—does not relate to hippocampal volume in healthy participants (Clark et al., 2020). This model suggests that within a certain range, functional activation and cognitive performance might be linked independent of brain structure volume. Relationships between brain volume and cognitive function may emerge only when brain volume limits the amount of functional activation possible, for example, when task performance is sufficiently taxing, or when participants are undergoing volumetric decline. In the context of aging, this model would predict that it is plausible that structure-cognition relationships become more significant as atrophy progresses. This idea aligns with the prediction that arises from the PMAT model that we should expect to see pmERC volume differences have a larger effect on memory performance in more cognitively impaired populations because contextual information would become more helpful for recall as memory for individual items weakens. In addition, the aLERC results are broadly in line with the revised models (Doan et al., 2019; Nilssen et al., 2019), which argue that the aLERC is a critical convergence zone for sensory information representing the content of episodic memory. We note, however, that the associations between pmERC volume and cognition we report do not necessarily align with the allocentric spatial coding proposed by this model. This discrepancy

may arise through our functional connectivity-based definition of the ERC subfields, which could attribute some portion of the aERC as being pmERC instead, in contrast to the histological definition used in the revised model.

It is well established that ERC atrophy is commonly observed in patients with AD (Bobinski et al., 1999; Du et al., 2001; Herukka et al., 2008; Juottonen et al., 1998; Killiany et al., 2000; Pennanen et al., 2004) and correlates with clinical severity (Du et al., 2003; Fennema-Notestine et al., 2009; McDonald et al., 2009). Based on the lack of associations between aERC volume and CSF biomarkers in our sample (in contrast to the findings of Holbrook et al., 2019 in patients with AD) and the independent effects of CSF amyloid and aERC volume on ModRey retention, one interpretation is that our results represent either a range of normal variation in aERC volume relating to cognitive performance in healthy older adults, or a non-AD-related neurodegeneration effect. On the other hand, aERC volume in our data is related specifically to a canonically AD-like pattern of cognitive test performance on the NACC-UDS3 neuropsychological battery (i.e., specific deficits in episodic memory and semantic fluency), with relatively spared performance in other cognitive domains (Salmon et al., 2002, 1999). Speculatively, when our findings are viewed in context with those of Holbrook et al. (2019), it is possible that our results reflect some antecedent cause of aERC neurodegeneration that combined with the presence of amyloid or tau biomarkers may subsequently be related to development of AD. Longitudinal follow-up testing on these participants will help untangle which of these 2 interpretations is more accurate.

There are important limitations that need to be considered in interpreting these findings. It is important to note that our participant groups are all cognitively unimpaired (CDR = 0), and it is unknown whether they will go on to develop AD. Thus, any interpretation of our data in the context of AD is necessarily speculative. Because our sample is drawn from an ongoing study involving both lumbar punctures to collect CSF and gadolinium-contrast MRI, the sample size is not particularly large. The sample was mostly white and highly educated, and the findings may not generalize to a more diverse or less educated population. Furthermore, there is an underlying assumption that ERC volume loss and synaptic/neuronal loss are linearly related, which may not actually be the case. It is possible that neuronal loss might not initially cause detectable volume loss using MRI, which would suggest our data are understating the strength of the aERC-cognition association. As we examined gray matter volumes, our method does not account for changes in white matter structure (e.g., the integrity of the perforant pathway from the ERC into the hippocampus). Our study has a cross-sectional design, and we can only speak to the association of ERC subfield volume differences to cognition. Although it is possible to extrapolate that our results reflect individuals at different points along a trajectory of neurodegeneration, longitudinal data are necessary to confirm this hypothesis.

An interesting implication of the results is that the ModRey might be a particularly sensitive instrument for detecting cognitive changes in otherwise asymptomatic older adults, which may be related to AD. We previously showed in the same group of participants that ModRey short-delay retention is related to CSF levels of amyloid and tau (Yeung et al., 2019a), which are biomarkers for AD. This study shows ModRey performance in cognitively healthy older adults is related to aERC volume differences, a neurodegenerative biomarker for AD. Strikingly, we find that aERC volumes are related to a classical neuropsychological presentation of AD: relative deficits in delayed recall and semantic fluency. Combining these two results suggests that ModRey performance might reflect subtle preclinical cognitive changes that are not currently regarded as pathological, but may predict subsequent decline. If that is true,

then the ModRey may be a useful instrument for the early detection of AD and for tracking progression in very early clinical phases.

Disclosure statement

A.M.B., S.A.S., and L.-K.Y. have filed an invention report on the ModRey task with Columbia University. The Institutional Review Board of Columbia University approved all procedures used in this study. All participants gave informed consent before participating in this study.

CRediT authorship contribution statement

Lok-Kin Yeung: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Christiane Hale:** Investigation, Data curation. **Batool Rizvi:** Software, Investigation. **Kay Igwe:** Software, Investigation. **Richard P. Sloan:** Resources. **Lawrence S. Honig:** Investigation, Resources. **Scott A. Small:** Resources, Funding acquisition. **Adam M. Brickman:** Conceptualization, Resources, Writing - review & editing, Supervision, Funding acquisition.

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