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Iron deposition and distribution across the hippocampus is associated with pattern separation and pattern completion in older adults at risk for Alzheimer's disease

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Abstract)

Elevated iron deposition in the brain has been observed in older adult humans and persons with Alzheimer's disease (AD), and has been associated with lower cognitive performance. We investigated the impact of iron deposition, and its topographical distribution across hippocampal subfields and segments (anterior, posterior) measured along its longitudinal axis, on episodic memory in a sample of cognitively unimpaired older adults at elevated familial risk for AD (N = 172, 120 females, 52 males; mean age = 68.8 $\pm$ 5.4y). MRI-based quantitative susceptibility maps were acquired to derive estimates of hippocampal iron deposition. The Mnemonic Similarity Task was used to measure pattern separation and pattern completion, two hippocampally-mediated episodic memory processes. Greater hippocampal iron load was associated with lower pattern separation and higher pattern completion scores, both indicators of poorer episodic memory. Examination of iron levels within hippocampal subfields across its long axis revealed topographic specificity. Among the subfields and segments investigated here, iron deposition in the posterior hippocampal CA1 was most robustly and negatively associated with lower fidelity memory representations. This association remained after controlling for hippocampal volume and was observed in the context of normal performance on standard neuropsychological memory measures. These findings reveal that the impact of iron load on episodic memory performance is not uniform across the hippocampus. Both iron deposition levels as well as its spatial distribution, must be taken into account when examining the relationship between hippocampal iron and episodic memory in older adults at elevated risk for AD.

Significance statement

The objective of this study was to map hippocampal iron deposition and its topographical distribution in cognitively unimpaired older adults at risk for AD, and its relationships to hippocampal-mediated episodic memory processes, i.e., pattern separation and pattern completion. Results revealed that elevated hippocampal iron, particularly within the posterior CA1 subfield, was strongly associated with lower pattern separation and higher pattern completion, both markers of poorer episodic memory. This is the first evidence that the spatial distribution of iron deposition in the human hippocampus has specific impacts on memory performance, and may be a more precise early neuropathological marker of insipient memory dysfunction in older adults at elevated risk for AD, but who remain clinically asymptomatic.

Keywords: Quantitative susceptibility mapping; iron; hippocampus; hippocampal subfields; pattern separation; pattern completion; Alzheimer's disease

Introduction

Iron accumulation in the brain is associated with neuropathological changes and neurodegenerative conditions including Alzheimer's disease (AD) (Zecca, 2004; Damulina, 2020; Ayton, 2020; Moon, 2016), potentially promoting β-amyloid toxicity and tau-protein dysfunction through oxidative tissue damage (Ayton, 2015; Ayton, 2021; Cogswell, 2021; Lane, 2018). Elevated iron levels have been observed in the human hippocampus both in normal aging (Bartzokis, 2007; Rodrigue, 2013; Daugherty, 2015) as well as AD (Acosta-Cabronero, 2013; Kim, 2017), suggesting that hippocampal iron may exacerbate the impact of AD-related neuropathology, even before the emergence of a clinical syndrome (Ayton et al., 2017; Chen et al., 2021). As such, hippocampal iron deposition may be an early pathological marker of insipient memory dysfunction in presymptomatic AD.

Quantitative Susceptibility Mapping (QSM) is an MRI approach for estimating brain iron levels *in vivo* (Lin, Chao, & Wu, 2015). This method, along with earlier relaxometry-based approaches (Bartzokis et al., 2011; Rodrigue, Daugherty, Haacke, & Raz, 2013; Venkatesh, Daugherty, & Bennett, 2021), has shown that elevated human hippocampal iron globally is associated with poorer episodic memory in aging, mild cognitive impairment and AD (Ayton, 2017; Chen, 2021). However, the impact of iron distribution across functional subfields and segments of the hippocampus (Poppenk, 2013) has yet to be investigated.

Here we collected QSM in a sample of asymptomatic older adults at elevated familial risk for AD (Breitner, Poirier, Etienne, & Leoutsakos, 2016; Tremblay-Mercier et al., 2021). We selected the Mnemonic Similarity Task (MST, Kirwan, 2007; Stark, 2013) as a sensitive and specific assay of pattern separation and completion; two processes central to episodic memory (Hunsaker & Kesner, 2013). Pattern separation involves the transformation of overlapping information into orthogonal, non-overlapping, representations, necessary for high fidelity mnemonic encoding (Bakker, 2008; Bakker, 2012; Lacy, 2010; Yassa, 2010; Yassa, 2011). Pattern completion involves the 'filling in' of missing features from partial or degraded mnemonic representations, resulting in lower fidelity memory retrieval (Bakker, 2008; Lacy, 2010; Rolls, 2013, for a review). Both memory processes are altered in normal aging, MCI, and AD (Ally, Hussey, Ko, & Molitor, 2013; Bakker, Albert, Krauss, Speck, & Gallagher, 2015; Bakker et al., 2012; Brock Kirwan et al., 2012; Stark, Yassa, Lacy, & Stark, 2013; Yassa et al., 2010).

Pattern separation and completion processes have been mapped to specific hippocampal subfields (Stark, 2013), enabling us to test hypotheses regarding the impact of iron distribution across the hippocampus. The dentate gyrus (DG) is implicated in pattern separation (Bakker, 2008; Bakker, 2012; Lacy, 2010; Yassa, 2010; Yassa, 2011), while CA3 has been related to pattern completion (Bakker, 2008; Lacy, 2010; Rolls, 2013, for a review). The CA1 subfield has been associated with both pattern separation and completion (Hanert, Pedersen, & Bartsch, 2019). Although the CA4/subiculum has been less directly implicated in pattern separation and completion (Stevenson et al., 2020), it does show significant iron deposition (Spence et al., 2022). Topographical associations with pattern separation and completion have also been reported along segments of the hippocampal long-axis (Poppenk et al., 2013). Evidence from both animal models and human studies suggest that the anterior segment is associated with retention of coarse-grained (global) information, increasing demands on pattern completion at retrieval. In contrast, the posterior segment has been linked to the encoding of local or fine-grained representational detail, necessary for pattern separation (Boggan & Huang, 2011; Ekstrom, Copara, Isham, Wang, & Yonelinas, 2011; Kjelstrup et al., 2008; McTighe, Mar, Romberg, Bussey, & Saksida, 2009; Morgan, Macevoy, Aguirre, & Epstein, 2011; Stensola et al., 2012). Here we use QSM and MST to relate hippocampal iron and memory performance in older adults to test the prediction that measuring iron deposition *and distribution* across the hippocampus, is necessary to better understand its impact as a pathological marker of emergent memory dysfunction.

Methods and materials

Study Participants

Participants were recruited from the PREVENT-AD (PRe-symptomatic EValuation of Experimental or Novel Treatments for AD) dataset, collected at the Douglas Mental Health University Institute in Montreal (Breitner et al., 2016; Tremblay-Mercier et al., 2021). For inclusion in PREVENT-AD, participants had to meet the following eligibility criteria: (1) Parental or ≥2 siblings with AD history with diagnosis; (2) age 60 or older (55 to 59 if parental/sibling onset was within 15 years of participant's age); (3) cognitively intact with no diagnosable cognitive disorder. Neuroimaging data for the current study was collected on a 3 Tesla Siemens Prisma. All subjects also underwent a battery of neuropsychological tests. Inclusion criteria involved the completion of a QSM MRI scan, anatomical scan and MST neuropsychological testing. Four participants were excluded due to MST outlier performance using an interquartile range outlier calculation (see Hoaglin, 2003). Additionally,

after visually inspecting the images, one additional participant was excluded due to a failure in hippocampal segmentation caused by registration issues. The final sample of eligible participants included 172 cognitively unimpaired older adults, including 120 females and 52 males (See Table 1).

Cognitive Assessments

All participants underwent a comprehensive battery of neuropsychological tests on the same day as their MRI scan. See Table 1.

Experimental memory measure. For our primary analyses we selected the Mnemonic Similarity Task (MST) as it has shown high selectivity in mapping the structure and function of hippocampal subfields to specific memory processes (Yassar and Stark, 2011; Stark, Kirwan, & Stark, 2019). Typical MST administration involves two phases (Stark, 2013). During the incidental encoding phase participants are asked to make judgments about pictures of common, everyday objects ("Is this item commonly found indoors or outdoors?"). Phase two involves a surprise recognition test. Here participants are asked to view a series of pictures and judge whether they are "old" (previously seen targets), "similar" (lure items that are perceptually similar to targets, but were never seen), or "new" (foil items that were perceptually distinct from the targets and never seen). The task also includes a manipulation of lure similarity, wherein lure items may be binned by their degree of similarity to target items, from high to low. Successfully discriminating targets from lures on the MST requires non-overlapping, distinct representations of encoded items, a hallmark of 'high fidelity' memory that is dependent upon pattern separation. In contrast, high false recognition rates for lures as targets is evidence for lower fidelity memory representations. These type of MST errors are hypothesized to result from over reliance on pattern completion processes, leading to imprecise memory retrieval, with error rates positively tracking the degree of lure similarity.

Here we report bias-corrected measures for behavioral pattern separation (BPS) and behavioral pattern completion (BPC). We adjusted potential response biases previously observed on the MST in older adults as they tend to response "similar" or "old" significantly more. (Budson, 2006, Ally, 2012, Yeung, 2013, and see Ally et al., 2013, for review and detailed formulas for each measure). Bias-corrected BPS scores were calculated in two ways. First, to account for any bias the participant may have in using the "similar" response overall, scores were calculated as the difference between the probabilities of giving a 'similar' response to lure versus foil items [similarity-bias corrected BPS score: pattern separation rate minus similar bias rate] (BPS-S; Stark et al., 2013). Second, to account

for a general bias towards labelling items as 'old', we subtracted the rate of 'old' versus 'similar' responses to lure items [old-bias corrected BPS score: pattern separation rate minus pattern completion rate] (BPS-O; Holden, Toner, Pirogovsky, Kirwan, & Gilbert, 2013; Toner et al., 2009). Bias-corrected BPC scores were derived by subtracting the rate of 'old' responses to foil versus lure items [bias-corrected BPC score: pattern completion rate minus false alarm rate].

Finally, we calculated a measure to estimate the impact of lure familiarity on memory retrieval (Wilson, Gallagher, Eichenbaum, & Tanila, 2006; Yassa, Mattfeld, et al., 2011). This approach has been used to evaluate the fidelity of memory representations by contrasting the relative influence of pattern separation versus pattern completion during retrieval judgments (Stark et al., 2013; see Yassa, Lacy, et al., 2011, for a review). The measure is an inverse ratio of old versus other responses, calculated across lure similarity bins. An area under the curve estimate was calculated to index the fidelity of memory representations. Higher area under the curve values suggest greater influence of pattern separation versus pattern completion processes at retrieval. We refer to this measure as a memory Fidelity Index (FI) throughout the paper.

Standard clinical memory measures. While not the focus of the current report, we also conducted exploratory analyses to investigate the impact of hippocampal iron on standard neuropsychological measures of episodic memory collected as part of the PREVENT-AD neurocognitive battery (Tremblay-Mercier et al., 2021). These standard measures are among the most common clinical assays of hippocampally-mediated memory functioning (Wicking, 2014). From the Rey-Auditory Verbal Learning Test (RAVLT; Schmidt, 1996; Moradi et al., 2017) we used immediate recall (sum of trials 1-5), delayed recall, and percentage forgetting (trial 5 score, minus delayed recall score divided by the score of trial 5). From the Repeatable Battery for Assessment of Neuropsychological Status (RBANS, Randolph, 1998) we used the immediate and delayed recall index scores. Three participants failed to complete the RAVLT due to fatigue and were excluded from all analyses involving this test.

Table 1

A) Demographics	
N	172
Ages(years)	68.8 (5.4)
Sex	
Female	120
Male	52
Education(years)	15.4 (3.3)
APOE genoptype	
APOE4 positive	69
APOE4 negative	103
B) Memory measures	
Episodic memory (items recalled)	
RAVLT delay	8.64 (3.79)
RAVTL immediate	48.10 (8.94)
RAVTL percentage forgetting	0.3 (0.27)
RBANS delay	104.03 (10.03)
RBANS immediate	106.58 (12.89)
MST performance	
Pattern Separation	
Similarity- bias corrected BPS score	0.14 (0.15)
Old-bias corrected BPS score	− 0.38 (0.27)

Pattern Completion	
Bias-corrected BPC score	0.56 (0.13)
Memory Fidelity	
FI-AUC	2.53 (0.69)

Table 1. Panel A: Demographic information for study participants [Mean (SD)]. Panel B: Memory scores. RAVLT = Rey Auditory Verbal Learning Test, RBANS = Repeatable Battery for the Assessment of Neuropsychological Status, BPS = behavioral pattern separation, BPC = behavioral pattern completion, FI = Fidelity Index, AUC = Area Under the Curve across seven conditions [Mean (SD)].

Neuroimaging protocols

MRI scans were conducted on a Siemens Prisma 3T MRI scanner (Siemens Medical Solutions, Erlangen, Germany) with a 32-channel head coil at the Cerebral Imaging Centre of the Douglas Mental Health University Institute. A 3D spoiled gradient recalled echo sequence (TE=7.29ms; TR=20ms; FOV=230 mm^2; Flip angle=15^0; Voxel resolution=0.8×0.8×1.0 mm^3, 6/8 partial Fourier; GRAPPA factor =2; 144 slices; 5.13min acquisition time) was acquired for QSM and a multi-echo field map (TE=4.80ms/9.90ms/15.00ms; TR=20ms; FOV=230 mm^2;Voxel resolution=3.6×3.6×4.0 mm^3, 6/8 partial Fourier; GRAPPA factor =2; 52 slices; 0:38 min). In addition, T1-weighted (T1w) structural images were acquired with a high-resolution magnetization-prepared rapid gradient echo (MP-RAGE) (TR=2300.0ms; TE=2.96ms; FOV=256mm^2; Flip angle=9º; Voxel resolution=1.0 x 1.0 x 1.0 mm, 192 slices; 5:30 min acquisition time), and T2-weighted images (T2w) (TR=2500.0ms; TE=198ms; FOV=206 mm^2; Voxel size=0.6 x 0.6 x 0.6 mm; turbo factor=143; 7:35 min acquisition time) (Bussy et al., 2021).

Image processing

QSM data processing. QSM reconstruction involved several core preprocessing steps, summarized in Figure 1. First, we combined the phase images from the 32 channel receiver to calculate offset maps using phase-offset estimation from multi-echo method adapted from (Sun et al., 2020) (https://github.com/sunhongfu/QSM/tree/master/coil_combination) on low resolution field maps. After registration, the offset maps were subtracted from the high-resolution spoiled gradient echo the phase images to obtain offset-corrected phase images without singularities. The corrected phase image and magnitude image were then used for QSM reconstruction. The QSM maps were reconstructed using a total-generalized-variation based method (http://www.neuroimaging.at/pages/qsm.php) (Langkammer et al., 2015). This method incorporates the three individual steps involved in common QSM pipelines: phase unwrapping, background field removal, and dipole inversion in a single iteration, which greatly reduces noise and is especially suitable for low SNR data.

Segmentation and quantification of susceptibility. Hippocampal segmentations were performed using the Automatic Segmentation of Hippocampal Subfields (ASHS) package. Two separate ASHS pipelines were used to derive hippocampal subfield and segment ROIs. To identify subfields, a combined T1w and T2w pipeline for hippocampal subfield segmentation was implemented (Yushkevich et al., 2015). To identify segments, a T1w pipeline for segmenting the long axis of the

hippocampus into anterior and posterior regions. Here, anterior hippocampus (aHPC) corresponds to the head, while posterior hippocampus (pHPC) corresponds to the hippocampal body and tail (Xie L., 2016). The anterior and posterior segment ROIs was further used to separate the subfields into anterior and posterior portions. To improve delineation of tissue boundaries in the T1w images, the GRE magnitude images were first linearly coregistered with each individual's T1w, and the T2w images were coregistered with T1w. Finally, the transformation matrices were combined and applied to the QSM map. This was accomplished using FSL image processing tools (Smith et al., 2004) v.6.0.4). All ROIs were visually inspected to ensure quality of anatomical delineation, alignment across neuroimages, and non-overlap of independent ROIs. As we do not have laterality hypotheses, we averaged the left and right ROIs, resulting in combined bilateral measures for the full hippocampus as well as each subfield and segment (see Figure 1). This resulted in nine ROIs: whole hippocampus plus the anterior and posterior CA1, CA3, DG, and subiculum (SUB), selected based on evidence of involvement in pattern separation or completion and/or significant iron deposition (Baker et al., 2016; Brock Kirwan et al., 2012; Hanert et al., 2019; Poppenk, Evensmoen, Moscovitch, & Nadel, 2013; Wilson, Gallagher, Eichenbaum, & Tanila, 2006; Spence McNeil, & Waiter, 2022; Stevenson, Reagh, Chun, Murray, & Yassa, 2020). Median susceptibility values, reflecting iron deposition estimates, were adjusted for subject-wise variability in scan acquisitions by standardizing all hippocampal measures by the median susceptibility value of the corpus callosum. This region is commonly selected as a reference region due to high signal reliability (Bilgic, Pfefferbaum, Rohlfing, Sullivan, & Adalsteinsson, 2012; Meineke et al., 2018)). As such, QSM provides a measure of relative rather than absolute susceptibility (Cheng, Neelavalli, & Haacke, 2009).

Regional volumetry. Anatomical images were skull stripped using FSL '*bet2*' (Smith, 2002). Volumetric segmentation (e.g., corpus callosum and intracranial volume) was completed using the FreeSurfer software suite (Dale, Fischl, & Sereno, 1999) v.7.3.2).

Statistical analysis

Hierarchical regression analyses were conducted to examine associations between iron levels and MST scores. Level one incorporated demographic covariates (age, education, and sex) and APOE $\varepsilon 4$ status (APOE4+/-). Next, intracranial and hippocampal volumes were added to the model to test, and control for, associations between brain volume and MST performance. Finally, whole hippocampal iron deposition values were added to examine whether iron remained a significant contributor to MST performance over and above the contributions of demographics and brain volume. Separate hierarchical regressions were conducted for the four MST scores in four models: Pattern separation (BPS similarity-bias corrected, BPS old-bias corrected); Pattern completion (bias corrected); Fidelity Index (FI-area under the curve). All four models are reported to demonstrate the robustness of the observed effects, regardless of any specific MST score. For this reason, p-value corrections were not applied. To test predictions regarding topographical specificity, we next examined associations between iron deposition and MST independently in six hippocampal subregions (CA1, CA3, DG, subiculum, aHPC, pHPC) using the hierarchical regression approach outlined above. Finally, we combined our hippocampal segmentation techniques to identify posterior and anterior segments of each subfield, resulting in eight independent ROIs (e.g. anterior CA1, posterior CA1, anterior CA3 etc.). For these analyses we performed a series of stepwise regressions for the four MST measures. This approach enabled us to test the specific contributions of iron deposition within each segmented subfield to MST performance. For these analyses, we first residualized each MST explanatory variable for the effects of the control variables (age, education, sex, intracranial volume, whole and segmented hippocampal volumes, and APOE genotype). These residuals were then used as predictors in subsequent stepwise regression models. To evaluate the significance of each model we selected values for probability-of-*F*-to-enter of $\leqslant .05$ (and probability-of-*F*-to-remove of $\geqslant .10$) as entry criteria for adding subsequent variables. '*F*-change' values were calculated to measure the amount of extra variance explained from the previous model. To determine the relative contribution of each model predictor to MST performance, we implemented constrained dominance analysis (DA, Azen & Budescu, 2003), which is based on the constrained relative importance analytical approach (LeBreton, Tonidandel, & Krasikova, 2013). The eight explanatory variables (iron values from each ROI) were residualized for the control variables (e.g., age, education, sex, intracranial volume, whole hippocampal volume, specific hippocampal segment volume (e.g. posterior CA1 volume for CA1 iron) and APOE genotype). Next, dominance analysis was used to determine the incremental contribution of each explanatory variable in predicting MST performance, measured as an increase in R^2 associated with adding each predictor to the subset of the remaining

predictors. Finally, a Percentage Relative Importance (PRI) value was derived for each predictor by calculating the percentage of dominance value:

$$[\text{PRI}=\frac{average\ contribution\ (each\ predictor)}{the\ sum\ of\ average\ contribution\ (all\ predictors)}].$$

Finally, to investigate associations between hippocampal iron and standard neuropsychological measures of episodic memory (RAVLT and RBANS), we used partial correlation analyses, consistent with previous reports (Ayton, 2017; Chen, 2021). All partial correlation models included sex, age, years of education, intracranial volume, hippocampal (whole and segmented) volumes and APOE genotype as covariates. All statistical analyses were performed using IBM SPSS 28.0 (Armonk, NY, USA) and R (version 4.2.1).

Results

Estimates of QSM values for iron deposition, volume and density for the whole hippocampus, hippocampal subfields and segments and subregions, are reported in Table 2. Correlations among iron levels across the whole hippocampus, segments, and subfields are reported in Figure 2.

Table 2

ROIs	Susceptibility(ppm)	Volume(ml)	Iron density (ppm/ml)
Hippocampus	0.035 (0.009)	3781.3 (425.5)	9.26×10^{-6}
CA1	0.035 (0.010)	1215.9 (172.7)	2.88×10^{-5}
CA3	0.009 (0.019)	68.7 (35.1)	1.31×10^{-4}
DG	0.027 (0.011)	730.0 (103.4)	3.70×10^{-5}
SUB	0.059 (0.013)	425.7 (58.4)	1.39×10^{-4}
aHPC	0.042 (0.012)	1725.3 (279.3)	2.43×10^{-5}
pHPC	0.030 (0.010)	1645.4 (175.7)	1.82×10^{-5}
Anterior CA1	0.044 (0.013)	543.7 (112.2)	8.09×10^{-5}
Posterior CA1	0.031 (0.010)	567.1 (77.7)	5.47×10^{-5}
Anterior CA3	0.011 (0.020)	58.3 (15.7)	1.89×10^{-4}
Posterior CA3	0.016 (0.023)	3.0 (1.9)	5.33×10^{-3}
Anterior DG	0.034 (0.014)	367.8 (63.3)	9.24×10^{-5}
Posterior DG	0.022 (0.011)	337.6 (52.5)	6.52×10^{-5}
Anterior SUB	0.065 (0.018)	186.5 (33.5)	3.49×10^{-4}
Posterior SUB	0.051 (0.013)	176.3 (26.8)	2.89×10^{-4}

Table 2. Susceptibility (iron load), volumes and iron density (susceptibility/volume) for each ROI [Median (SD)].

330 Hippocampal iron and MST performance

331

332 *Whole hippocampus.* Results of the hierarchical regression analyses for MST performance and
333 QSM-derived estimates of iron deposition across the whole hippocampus were consistent with
334 predictions and are reported in Table 3. After controlling for demographic factors as well as hippocampal
335 volume, estimates of hippocampal iron were significantly and negatively associated with similarity-bias
336 and old-bias corrected measures of pattern separation (BPS-S, β=−.18, p=.02; BPS-O, β=−.20, p=.01).
337 In contrast, hippocampal iron was positively associated with bias-corrected pattern completion score;
338 however, the statistical reliability of this association fell below standard, non-directional significance-
339 testing thresholds (β=.14, p=.08). Whole brain hippocampal iron was also negatively associated with the
340 memory fidelity index (β=−.18, p=.03).

Table 3

	Pattern Separation		Pattern Completion	Memory Fidelity Index
	Similarity-bias corrected BPS score	Old-bias corrected BPS score	Bias-corrected BPC score	FI-AUC
	β (CI) (p value)	β (CI) (p value)	β (CI) (p value)	β (CI) (p value)
Level1: Demographic variables				
Age	–.29 (–.00, .00) (.002)	–.27 (–.00, .00) (.004)	.08 (.00, .00) (.39)	–.14 (–.00, .00) (.14)
Education	.07 (–.00, .01) (.34)	.07 (–.01, .02) (.39)	–.01 (–.01, .01) (.88)	.03 (–.03, .04) (.68)
Sex	–.19 (–.12, .00) (.04)	–.09 (–.16, .06) (.35)	.09 (–.03, .08) (.36)	–.07 (–.39, .19) (.49)
APOE4	–.11 (–.08, .01) (.15)	.02 (–.07, .10) (.77)	–.04 (–.05, .03) (.59)	.01 (–.20, .24) (.86)
R^2	0.122	0.069	0.01	0.019
F	5.827	3.071	0.413	0.827
Level 2: Control variables				
TIV	.11 (.00, .00) (.29)	.12 (.00, .00) (.27)	–.14 (.00, .00) (.22)	.12 (.00, .00) (.30)
HC vol	–.07 (.00, .00) (.51)	–.10 (.00, .00) (.36)	.08 (.00, .00) (.50)	–.08 (.00, .00) (.48)
R^2	0.126	0.073	0.017	0.023

ΔF	0.319	0.392	0.563	0.321
ΔR^2	0.004	0.004	0.007	0.004
Level 3: Variable of interest				
HC iron	–.18 (–5.35, –.55) (.02)	–.20 (–10.06, –1.2) (.01)	.14 (–.23, 4.06) (.08)	–.18 (–24.65, –1.48) (.03)
R^2	0.156	0.107	0.035	0.052
ΔF	5.867	6.298	3.097	4.961
ΔR^2	0.03	0.034	0.018	0.029

Table 3. Results of Hierarchical Regression Analyses for pattern separation and completion and iron level in the whole hippocampus. All standardized regression coefficients (β) are from the level 3 in the analysis (see Text). Data in parentheses are 95% CIs. *P* values less than .05 indicate statistical significance. TIV = Total intracranial volume, HC vol=hippocampus volume, HC = hippocampus.

Hippocampal subfields. Next, we examined associations between MST measures and iron deposition in hippocampal subfields following the hierarchical regression approach described above. Iron levels in CA1 were significantly and negatively related to similarity-bias and old-bias corrected pattern separation scores, controlling for demographic factors, APOE genotype, and structural volume (BPS-S, β=−.20, p =.008; BPS-O, β=−.21, p=.007). In contrast, CA1 iron approached significance in being positively related to the bias-corrected pattern completion scores (bias-corrected BPC, β=.16, p =.06). We also observed a significant and negative association between iron load in CA1 and the memory fidelity index (β=−.19, p =.02). No significant associations were observed for the CA3 and DG subfields. Associations between iron in the SUB and these MST measures followed a similar trend as CA1. SUB iron significantly and negatively correlated with pattern separation scores (BPS-S, β=−.20, p =.008; BPS-O, β=−.22, p=.005), and was negatively associated with memory FI (β=.19, p=.02).

Anterior and posterior hippocampal segments. We next examined associations between MST measures and iron deposition in anterior and posterior hippocampal segments. No significant associations were observed for the anterior hippocampus. As predicted, posterior hippocampal iron levels were negatively and significantly associated with similarity-bias and old-bias corrected pattern separation scores (BPS-S, β = − .17, p =.03; BPS-O, β = − .16, p=.04). Posterior hippocampal iron levels trended towards a negative association with memory FI, (β=−.14, p=.07).

Anterior and posterior hippocampal subfields. To examine our topographical specificity hypotheses further, we conducted stepwise regression analyses to test associations between the anterior and posterior CA1, CA3, DG and SUB subfields and MST measures (see Table 4). All analyses were conducted on residualized susceptibility values after controlling for demographic and structural volume measures. When added to the stepwise regression model, iron deposition in posterior CA1 was the only significant predictor of similarity-bias corrected (BPS-S) scores (R^2=.037, F_{change} (1,170) =6.484, p=.012)). Iron deposition levels in posterior subfields CA1 predicted old-bias corrected pattern separation (BPS-O) scores (R^2=.038, F_{change} (1,170) =6.801, p=.01). Additionally, posterior CA1 iron predicted the memory fidelity index (R^2=.028, F_{change} (1,170) =4.820, p=.029) There were no significant predictors of the bias corrected BPC scores (all probabilities of $F_{\text{change}} >$ p=.1) (see Table 4 for a summary of all stepwise regression model results).

Finally, constrained dominance analyses revealed that iron deposition in posterior CA1 was the most dominant predictor of performance across all MST measures. Relative contributions of all predictors for each MST score are reported in Figure 3.

Table 4

Independent variables	**Models**	**Predictors**	**Standardized β**	**SE**	**R^2**	***P* value**
Similarity-bias corrected BPS score	1	Posterior CA1	–0.192	0.011	0.037	0.012
Old-bias corrected BPS score	1	Posterior CA1	–0.196	0.02	0.038	0.01
FI-AUC	1	Posterior CA1	–0.166	0.052	0.028	0.029

Table 4. Results of stepwise regression analyses for pattern separation, completion and iron level in the anterior and posterior hippocampal subfields. Residual explanatory variables denoted for posterior CA1. The model for bias-corrected BPC score was non-significant. *P* values less than .05 indicate statistical significance. FI = Memory fidelity index (area under the curve).

Hippocampal iron and standard neuropsychological memory measures

In a series of exploratory analyses, we examined the relationship between performance on standard neuropsychological measures of memory (see Table 1) and hippocampal iron deposition using partial correlation analyses, controlling for demographic variables, APOE4 status and hippocampal volumes. No significant associations emerged for our five memory measures and whole hippocampal iron. Interestingly, at the level of hippocampal segments and subfields, several associations did pass standard statistical significance thresholds ($p < .05$). Consistent with our MST findings, iron deposition in the CA1 subfield, and its posterior aspect specifically, was associated with poorer memory performance. A similar pattern was also observed for posterior DG and the posterior segment of the hippocampus. Notably, iron deposition in the CA3 and anterior CA3 subfield was specifically related to greater percent forgetting on the RAVLT, a measure known to be sensitive to the emergence of clinical AD (Moradi et al., 2017).

Table 5

ROIs	**RAVLT** *pr*(*p*)			**RBANS** *pr*(*p*)	
	Delay recall	Immediate recall	Percentage forgetting	Delay recall	Immediate recall
Hippocampus	–.11 (.15)	–.06 (.46)	.11 (.15)	–.11 (.15)	–.14 (.07)
CA1	–.11 (.17)	–.07 (.36)	.11 (.18)	–.18 (.02*)	–.18 (.03*)
CA3	–.15 (.05)	–.00 (.99)	.16 (.04*)	–.01 (.89)	–.01 (.87)
DG	–.08 (.29)	–.04 (.60)	.09 (.26)	–.06 (.42)	–.11 (.15)
SUB	–.07 (.36)	–.05 (.49)	.11 (.15)	–.02 (.82)	–.06 (.34)
aHPC	–.12 (.13)	–.03 (.67)	.13 (.11)	–.05 (.52)	–.03 (.74)
pHPC	–.08 (.34)	–.06 (.42)	.07 (.38)	–.14 (.08)	–.20 (.01*)
Anterior CA1	–.12 (.13)	–.04 (.65)	.13 (.09)	–.16 (.04*)	–.09 (.27)
Posterior CA1	–.07 (.35)	–.07 (.39)	.06 (.45)	–.16 (.04*)	–.19 (.02*)
Anterior CA3	–.15 (.05)	–.01 (.94)	.16 (.04*)	–.02 (.78)	–.03 (.71)
Posterior CA3	–.04 (.63)	–.02 (.77)	.03 (.68)	–.02 (.83)	–.12 (.11)
Anterior DG	–.11 (.19)	–.05 (.53)	.12 (.12)	–.01 (.88)	–.01 (.88)

Posterior DG	–.08 (.33)	–.07 (.41)	.07 (.41)	–.15 (.06)	–.21 (.01*)
Anterior SUB	–.06 (.45)	.03 (.68)	.07 (.38)	–.01 (.92)	–.01 (.90)
Posterior SUB	–.06 (.44)	–.01 (.91)	.10 (.20)	–.07 (.36)	–.11 (.15)

Table 5. Results from partial association between iron load and cognitive scores of standard clinical measures of episodic memory. Values for *pr* and *P* are given for each cognitive measure. * indicates $p < .05$, uncorrected. No associations are significant with Bonferroni correction.

Discussion (1444/1500)

We used QSM to investigate the impact of iron deposition and its topographical distribution across the hippocampus on memory functioning in a large cohort of older adults who are elevated familial risk for AD, but remain asymptomatic. We derived measures of iron deposition across the whole hippocampus, within CA1, CA3, DG, and the subiculum as well as within their anterior and posterior hippocampal segments. We used these as predictors of pattern separation and completion, assessed using the Mnemonic Similarity Test. Consistent with predictions, we observed robust negative correlations between hippocampal iron and pattern separation, positive correlations with pattern completion, and an overall negative correlation between iron deposition and the fidelity of memory representations, reflected as greater reliance on pattern completion over pattern separation processes. These associations were independent of hippocampal volume and showed topographical specificity, with iron deposition in the posterior aspect of CA1 displaying the most consistent negative associations with memory fidelity.

Our findings extend two earlier reports using T2* relaxometry in typically aging older adults (Bartzokis et al., 2011; Rodrigue et al., 2013). Bartzokis and colleagues (2011) observed a negative association between global hippocampal T2* relaxation time, a related marker of iron concentration, and performance on standard memory measures. Notably, hippocampal volumes were not modelled in their analyses, leaving open the question of whether iron concentration was an independent predictor of memory performance. Rodrigue and colleagues (2013) explicitly modelled associations between global hippocampal volume, iron concentration, and an index of standard memory measures. They reported that higher hippocampal iron was associated with smaller hippocampal volumes, which together accounted for worse memory ability with age. A negative relationship between hippocampal iron deposition and delayed memory performance on standard memory measures has also been observed when directly accounting for hippocampal volume (Venkatesh et al., 2021). While these reports used relaxometry approaches, QSM methods have also been used to investigate associations between hippocampal iron deposition and memory functioning in normal aging, mild cognitive impairment and AD (Ayton et al.,

2017; Chen et al., 2021). Their findings are largely convergent with the relaxometry studies, implicating iron deposition across the hippocampus in memory decline into older age. We are not aware of any previous studies explicitly examining the topographical specificity of these effects.

In our analyses examining hippocampal subfields and segments, the largest unique effects were observed for iron deposition in CA1, which was associated with lower pattern separation, higher pattern completion and lower overall memory fidelity scores. While CA1 has been implicated in pattern completion (Duncan, Ketz, Inati, & Davachi, 2012; Kumaran & Maguire, 2007), there is emerging evidence for its role in pattern separation (Hanert et al., 2019). However, a leading theoretical account argues for a dual role of the CA1 subfield in pattern separation and completion as a downstream, 'read-out' layer from upstream DG/CA3 outputs (Guzowski, Knierim, & Moser, 2004); Knierim et al., 2016; Yassa and Stark, 2011). While speculative, functional disruption related to iron deposition in this 'read-out' layer would result in poorer pattern separation at encoding and subsequently greater reliance on pattern completion at retrieval as we observed here. Given that CA1, and its posterior aspect specifically, is the largest of the subfields investigated in the current report, it is perhaps unsurprising it would be the region most susceptible to the impact of neuropathological changes, including iron deposition. Consistent with this idea, CA1 is considered vulnerable to multiple physiological changes in aging and AD including vascular atrophy, resulting in disruptions in endothelial function and iron homeostasis (Buch, Chen, Jella, Ge, & Haacke, 2022), inflammatory impacts on iron-containing microglia (Zeineh et al., 2015) as well as increased iron-related gliosis (Venkatesh et al., 2021) all of which serve to promote iron-related oxidative damage to CA1.

In contrast to CA1, the DG and CA3 subfields have been specifically implicated in pattern separation and pattern completion processes respectively (Marr & Brindley, 1971; McClelland & Goddard, 1996;Rolls, 2013; Rolls & Kesner, 2006; Treves & Rolls, 1994; Yassa and Stark, 2011 for a review). We did not observe predicted associations with CA3 iron and MST (but note our exploratory results for RAVLT percent forgetting in Table 5). This may be an artifact of the comparatively small volume of this subfield, resulting in lower sensitivity of QSM measures to detect reliable associations. Unexpectedly, we did observe an association between subiculum iron and MST performance. While this region has been more reliably implicated in source

memory, as the main output of the hippocampus iron related toxicity is likely to impact many hippocampally-dependent memory processes (Stevenson et al., 2020) More recently, novel QSM separation methods have been identified to reliably separate susceptibility sources in QSM, distinguishing the unique contribution of paramagnetic and diamagnetic susceptibility (Dimov et al., 2022; Shin et al., 2021). Application of these methods will be an important future research direction to enhance the specificity of iron estimates from QSM imaging, as necessary to further interrogate the associations reported here.

To our knowledge, no previous studies have examined hippocampal iron across the long axis of the hippocampus, and impacts on memory function. Our preliminary predictions, hypothesizing greater impact of anterior hippocampal iron on pattern completion and posterior hippocampal iron on pattern separation, were only partially supported. We did not observe any significant associations with pattern completion scores in the anterior segment of the hippocampus. However, consistent with predictions we observed robust (and negative) associations between iron in posterior hippocampus and pattern separation. These topographical associations across the hippocampal long axis likely track with the relative subfield volumes in anterior and posterior segments. In adult humans, CA1-3 subfield volumes are relatively larger in the anterior hippocampus, consistent with its hypothesized role in pattern completion. In contrast, DG volumes are greater in the posterior aspect (Malykhin, Lebel, Coupland, Wilman, & Carter, 2010), which is more strongly implicated in pattern separation. Despite these volume differences in anterior and posterior subfields, posterior CA1 iron remained the strongest independent contributor to pattern separation performance, as well as overall memory fidelity. This finding was further confirmed through the dominance analysis wherein iron with the posterior CA1 subfield was the strongest contributor to MST performance in our participants.

While not the focus of the current study, we failed to observe associations between whole hippocampal iron and performance on standardized memory measures. This was unexpected given that previous studies have demonstrated such associations using both T2* relaxometry and QSM measures of iron in older adult cohorts. However, only one of these earlier studies (Rodrigue et al., 2013) included structural volumes directly in their statistical models. The authors used structural equation modeling to assess the impact of hippocampal iron in the context of volume differences, perhaps accounting for the differences with our findings. We also note

that their memory index was more heavily weighted towards associative memory, which is known to be more strongly related to hippocampal function than the item memory tasks used here. This may account for their observed associations between hippocampal iron concentration and performance on associative memory measures. Interestingly, we did observe associations between performance on standard memory measures and iron deposition at the level of hippocampal subfields and segments. Here again iron deposition in CA1 showed the most reliable and negative associations with memory, however these exploratory findings will need to replicated and confirmed in future research.

Here we provide novel evidence that hippocampal iron is a pathological marker associated with poorer memory function in older adults who are elevated risk for AD but who remain cognitively unimpaired. These results provide the first demonstration in humans that it is not only iron deposition, but its topographic distribution across hippocampal subfields and segments that determine the pattern of memory dysfunction. These findings open a new avenue and provide strong evidence pointing to the importance of iron deposition as both a mechanism and marker of cognitive dysfunction in later life. Iron deposition in CA1 may be a particularly robust marker of memory dysfunction and an important target for new studies testing the topographic specificity hypothesis. The use of iron chelation therapy, employing agents like deferoxamine, holds potential for alleviating iron levels in specific brain regions of individuals with AD. This approach aims to mitigate or even treat AD, underscoring the significance of iron-targeted therapeutic strategies (Liu et al., 2018). Focusing on addressing iron deposition in particular brain areas, including CA1, may has potential for therapeutic interventions. Finally, this work offers a roadmap for future investigations, highlighting the importance of precision brain and behavioral mapping to reveal the often-subtle associations between pathological markers and behavioral performance in cognitively normal older adults. This may be particularly critical for revealing early neuropathological markers in older adults who are at elevated risk for brain disease, yet are clinically asymptomatic.

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Figure Captions

Figure 1 Caption. Schematics for QSM data processing. Step 1: Phase-offset estimation from multi-echo (POEM) applied to correct the phase image. Step 2: Total generalized variation (TGV) method was applied to complete QSM reconstruction. Finally, hippocampal segmentation was performed using Automatic Segmentation of Hippocampal Subfields (ASHS). Far right panel: ASHS outputs overlayed on QSM maps. Panel A: anterior (orange) and posterior (pink) hippocampus; panel B (anterior subfield): anterior CA1(yellow), anterior CA3 (red), anterior DG (green), anterior SUB (purple); panel C (posterior subfield): posterior CA1 (yellow), posterior CA3 (red) posterior DG (green) and posterior SUB (purple).

Figure 2 Caption. Heatmap of product-moment correlations between iron measures of the whole hippocampus, anterior and posterior hippocampal segments, hippocampal subfields, and hippocampal subfields split by segment. Each square represents the coefficient value. All p's <.05, uncorrected. Regions are averaged across the left and right hemispheres.

Figure 3 Caption. Constrained dominance analyses. PRI calculated the percentage relative contribution of the independent subregions of anterior and posterior hippocampal subfields to MST cognitive performance. For each cognitive score, the percentage relative contribution of six predictors consisting of residual explanatory variables anterior CA1, posterior CA1, anterior CA3, posterior CA3, anterior DG, posterior DG and anterior SUB and posterior SUB are shown. Iron deposition in posterior CA1 consistently contributes more to explained variance in MST performance relative to other subregions. Note, we first residualized each explanatory variable for the effects of the control variables (age, education, sex, intracranial volume, hippocampal and segmented volume, APOE genotype). FI = Memory fidelity index (area under the curve).