DOES LOCUS COERULEUS STRUCTURE RELATE TO ASSOCIATIVE MEMORY RELATED-ACTIVITY IN OLDER ADULTS?

By

Riley Barrios

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University of California, Riverside

APPROVED

Dr. Ilana Bennett

Department of Psychology

Dr. Begoña Echeverria, Howard H Hays, Jr. Chair

University Honors

ABSTRACT

Older adults have shown well-documented declines in memory for relationships between items, especially as the number of items that must be bound together increases. Less is known about the neural mechanisms that support this type of associative memory, particularly the hippocampus and its neuromodulatory input from the Locus Coeruleus (LC). In the current study, cognitively healthy older adults completed the QuadMax associative memory task during fMRI scanning. They studied word pairs, triplets, and quadruplets and were subsequently tested on their ability to remember repeated, recombined, and novel word sets. Diffusion-weighted MRI measures were also collected to assess LC microstructural integrity. Results revealed that associative memory, recognition, and hit rates declined significantly as associative load increased from pairs to triplets to quadruplets. These effects were no longer significant when age and sex were included as covariates. At the neural level, associative memory encoding led to greater hippocampal BOLD activity for pairs relative to quadruplets in the left hippocampus, with a similar trend in the right hippocampus. Increased hippocampal activity for the pairs > quadruplets contrast was significantly associated with worse associative memory and recognition performance for quadruplets. Finally, LC mean diffusivity was significantly associated with poorer recognition performance for pairs, but not to hippocampal associative memory–related activity. These findings suggest that age-related variability in hippocampal engagement, but not LC integrity, contributes to individual differences in associative memory performance in older adults. Our study highlights the importance of examining both neural activity and neuromodulatory structure when studying associative memory in aging.

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Throughout this experience, Dr. Bennett created a supportive and challenging environment that pushed me to grow as a researcher and as a person. She consistently took the time to ensure I truly understood the work I was doing, while also encouraging independence and confidence. Beyond her mentorship, she entrusted me with leadership responsibilities and modeled what it means to support others with patience and care. I am incredibly grateful for the impact she has had on my academic journey.

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TABLE OF CONTENTS

INTRODUCTION

A key component of our memory in daily life is that it is inherently associative (Mayes, Montaldi, & Migo, 2007; Tulving, 1983). When we remember a past event (e.g., meeting a friend for coffee), not only do we retrieve isolated items (e.g., a mug or café table), but we also retrieve the relationships between items (e.g., who sat where or who drank what), their features (e.g., the size or color of the mug), and the environment in which they occurred (e.g., the café background noises). Relative to memory for isolated items, memory for associative relationships between items has shown a pronounced age-related decline in accuracy (Franco et al., 2022). Functional magnetic resonance imaging (fMRI) has shown that the hippocampus is active during associative memory performance (Chen & Chang, 2016), indicating that it has a central role in constructing associative memory traces and that associative memory-related activity in the hippocampus varies with age. The hippocampal memory network is thought to be shaped by neuromodulatory input from the locus coeruleus (LC), which is the brain's primary source of noradrenaline, including to the hippocampus (Khakpour-Taleghani et al., 2021). However, no studies have examined whether LC microstructure relates to hippocampal associative memoryrelated activity in older adults. This is the focus of the current study as it will help us to better understand how hippocampal and LC systems that support associative memory.

Decades of work have shown that older adults exhibit disproportionate impairments in associative memory relative to the memory of items alone (Naveh-Benjamin et al., 2004). For example, in typical paired-associative tasks, participants study pairs of items (e.g., word-word pairs) and are later tested on their ability to distinguish intact pairs from recombined pairs containing previously studied items presented in new combinations (Gold et al., 2006). In these tasks, associative memory is measured by how well participants can recognize intact pairs or sets

and reject recombined sets made up of previously studied items, whereas item memory is measured by recognition of individual items regardless of how they were paired. In these pairedassociative memory tasks, older adults reliably show lower accuracy for remembering which pairs of items were presented together (Naveh-Benjamin et al., 2004), despite a generally preserved recognition of individual items supporting the idea that aging selectively disrupts associative binding rather than just memory. These findings suggest specific age-related impairments in forming and retrieving bound representations, but they have been limited to associations between pairs of items.

Our own group used the QuadMax task to extend this literature by parametrically increasing associative load (Franco et al., 2022). Participants studied sets of two, three, or four unrelated words and were later tested on their ability to distinguish intact word sets from recombined sets containing the same words in new combinations and from entirely novel sets. This design isolates associative memory demands by holding item familiarity constant while systematically increasing the number of inter-item relationships that must be encoded and retrieved. Prior work using the QuadMax task showed that older adults' associative memory became progressively worse as set size increased from pairs to triplets to quadruplets (Franco et al., 2022). Associative memory (determined as hit rate minus recombined false alarms), recognition performance (hit rate minus novel false alarms), and hit rates (correct "old" responses to intact sets) each declined significantly with increasing set size, while recombined (containing studied words rearranged into new set) and novel false alarms (containing entirely new words not previously studied) did not differ across set sizes. Consistent with frameworks that highlight age-related vulnerability in binding processes, these findings indicate that increasing associative load places greater demands on the memory processes that support linking multiple elements into a single episodic representation and that they are impaired in aging.

Previous neuroimaging studies using paired-associative paradigms have consistently shown strong hippocampal engagement during associative encoding, with greater activity for conditions that require binding relations between items in comparison to conditions that emphasize single-item processing (Davachi, 2006). These findings support the idea that the hippocampus plays a central role in linking multiple elements of an experience into a unified representation of memory. Together, this prior work motivates the current study which aims to test whether hippocampal activity increases or changes at higher-order associative loads. The locus coeruleus (LC), being the brain's primary source of noradrenergic input to the hippocampus, plays an important role in modulating hippocampal memory function. Noradrenergic input from the LC enhances synaptic plasticity and supports memory encoding, consolidation, and retrieval processes that are critical for episodic memory (Uematsu et al., 2015). The LC's microstructure has been shown to measurably decline with age (Bennett et al., 2024) and be linked to episodic memory performance in older adults (Fukabori et al., 2020), albeit not to associative memory performance in human. This suggests that LC structural variability may contribute to individual differences in hippocampal recruitment and ultimately to associative memory success. Studies have shown that activity during associative memory tasks is found in the hippocampus, but no studies have looked at whether this activity is modulated by LC structure.

The current study aims to bridge this gap by testing whether LC microstructural integrity is related to hippocampal activity during associative memory encoding in older adults. The older adults completed the QuadMax associative memory task during fMRI scanning along with diffusion-weighted imaging to assess LC microstructure. We predicted that older adults would show worse associative memory performance as associative load increased as well as measurable hippocampal engagement during the study phase. We also predicted that individual differences in

LC integrity and the level of hippocampal activity observed during associative memory processing would be related, highlighting the LC's role in modulating hippocampal function during demanding memory tasks.

Methods

Participants

Forty-four older adults (>65 years old) were recruited from surrounding communities of the University of California, Riverside through print and online advertisements, word-of-mouth, and fliers posted within the community. Prior to study enrollment, potential participants were screened over the phone for: (1) normal global cognition (>17 score on the Montreal Cognitive Assessment [MoCA] adapted for phone screening; [Pendlebury et. al, 2013]), (2) hearing or vision loss, (3) history of neurological conditions or psychiatric disorders that could influence performance (e.g. depression, schizophrenia), (4) recent medications that could influence image quality (e.g. mood stabilizers, benzodiazepines, psychotropics), (5) major health conditions (e.g. diabetes, uncontrolled hypertension), (6) ensuring MRI scanning could be conducted safely (e.g. pregnancy, claustrophobia, having metal inside of the body), and (7) comfortability pushing keyboard buttons. After enrollment, participants were excluded from final analyses due to issues with data collection (n = 4; e.g., not recording all responses, running the same condition twice, coughing fit during fMRI scans) or if they did not respond to more than 30% of the trials (n = 1). The final sample consisted of 39 older adults. Group means for age, gender, and education levels are given in Table 1. All participants gave informed consent and received monetary compensation for participation.

Table 1. Demographic information by decade

Age Group	n	Mean Age ± SD	Gender (M/F)	Mean Education ± SD
60-69	24	65.0 ± 2.9	7/17	15.0 ± 2.4
70-79	12	72.8 ± 2.6	4/8	14.9 ± 2.6
80-89	3	84.3 ± 2.5	0/3	13.3 ± 1.5

Note. Education is reported in total years. n = sample size, SD = standard deviation

General Procedure

After informed consent was provided, participants completed two MRI sessions as part of two separate studies at the Center for Advanced Neuroimaging at the University of California, Riverside. Scan sessions were conducted on a 3.0 Tesla MRI system (Siemens Magnetom Trio) using a standard 32-channel head coil. In one session, structural MRI sequences were used to assess locus coeruleus structure. In the other, participants completed two runs of our novel associative memory task, The QuadMax task, during functional MRI sequences.

Associative Memory Task

Stimuli

High-frequency English nouns composed of 3-7 letters were acquired from the English Lexicon Project hosted by the University of Washington in St. Louis (Balota et al., 2007). For each task version (e.g., version C, versions D), 270 of these words were randomly selected and sorted into semantically unrelated sets of two (pairs), three (triplets), and four (quadruplets) words with 30 sets per set sizes.

Task Procedure

Participants completed two versions of the task in the MRI scanner, with the study and test phases of the first version (Study C, Test C) followed by the study and test phases of the second version (Study D, Test D). During the study phase, a total of 60 word sets (20 per set size)

were displayed one at a time on the computer screen. For each set, participants were instructed to indicate, using a button box, "yes" (pointer finger response) if they could "imagine the words doing something together" or indicate "no" (middle finger response) if they were not able to create a mental image of the words together. Word sets were visible for 6,000 (pairs), 7,000 (triplets), or 8,000 (quadruplets) ms and separated by a 500 ms inter-stimulus-interval (ISI). There were 14 fixation trials consisting of a black fixation cross on a white background (1,000 or 1,500 ms) that was displayed randomly throughout the study phase.

During the subsequent test phase, a total of 90 word sets (30 per set size) were displayed one at a time on the computer screen. 30 sets were identical to those seen during the study phase (repeated or intact; 10 per set size), 30 sets were recombinations of studied sets with vertical position maintained (recombined or rearranged; 10 per set size), and 30 sets were comprised of entirely new words not previously seen at study (novel; 10 per set size). Recombined sets were created by recombining all words within a given set from the 10 studied sets per set size. For example, recombined triplets were comprised of one word randomly selected from three of the five previously studied sets that contained three words (See Figure 1) and presented in any location within the recombined sets. All participants viewed the same recombined sets. Participants were instructed to indicated whether they had ("old"; pointer finger response) or had not ("new"; middle finger response) seen the exact set of words presented together at the study phase. Word sets were visible for 5,000 ms and separated by a 500 ms ISI, with a 20 rest screen as outlined above.

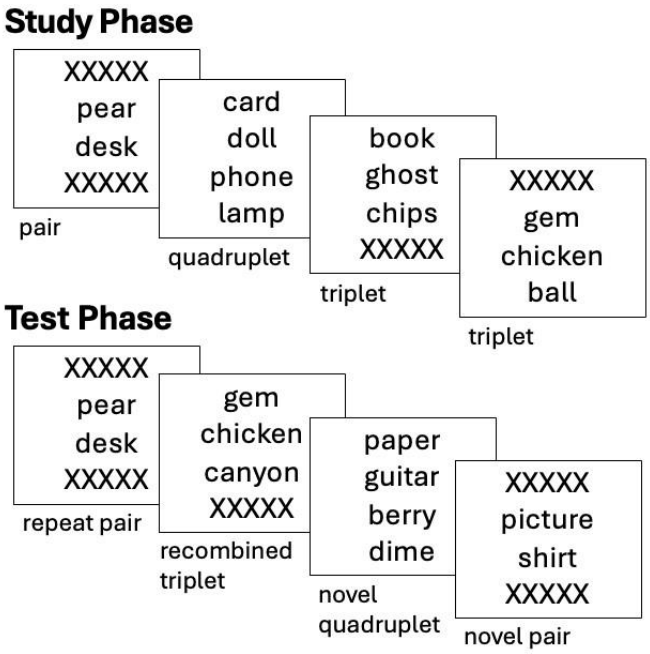

Figure 2: Example trials from the study phase (top) and test phase (bottom) of the QuadMax task show the set size (pairs, triplets, quadruplets) and trial type (repeated, recombined, novel) conditions.

Memory metrics

For each participant and each set size, the proportion of correct "old" responses to repeated (hit rate), incorrect "old" responses to recombined (recombined false alarm [FA] rate), and incorrect "old" responses to novel (novel FA rate) sets were calculated. An associative memory measure was also calculated as hit rate minus recombined FA rate. A set recognition measure was calculated as hit rate minus novel FA rate. For recognition and associative memory, average scores across set sizes and task versions were calculated.

Structural Imaging Data

Acquisition. Structural imaging data were acquired on a 3 T MRI scanner (Prisma, Siemens Healthineers, Malvern, PA) using a 32-channel receive only coil at the Center for Advanced Neuroimaging at the University of California, Riverside.

A T1-weighted MP-RAGE image was acquired with the following parameters: echo time (TE)/repetition time (TR)/inversion time = 3.02/2600/800 ms, GRAPPA acceleration factor = 2, flip angle = 8°, and voxel size = 0.8 mm^3.

Magnetization transfer-prepared gradient echo (MT-GRE) images were acquired with the following parameters: TE/TR = 3.21/385 ms, flip angle = 40°, field of view (FOV) = 220 × 186 mm^2, matrix size = 512 × 432, slice thickness = 3 mm, magnetization transfer preparation pulse (flip angle = 370°, 1.5 kHz off-resonance, duration = 10 ms), and 4 averages. Slices were positioned perpendicular to the dorsal edge of the brainstem at the midline along the fourth ventricle.

Diffusion-weighted single-shot spin-echo, echo planar images were acquired with the following parameters: TE/TR = 75/4100 ms, FOV = 202 × 170 mm^2, matrix size = 176 × 148, voxel size = 1.15 × 1.15 × 2.5 mm^3, and 32 slices with no gap. Slices were aligned parallel to the hippocampus. Monopolar diffusion-encoding gradients were applied in 30 directions with gradient strengths of b = 500 s/mm^2 and 2000s/mm2. Two sets of b = 0 images were acquired with opposite polarities of phase-encoding direction.

Locus coeruleus region of interest. A standardized atlas was used to define bilateral locus coeruleus (Langley et al., 2020) and a rectangular midline pontine reference region. For each participant, these regions were aligned from T1-weighted Montreal Neurological Institute (MNI) space to their MT-GRE (using the averaged MT-GRE image) and diffusion-weighted (using the average b = 0 image) spaces using concatenated rigid body transformations with a boundary-based registration cost function and their T1-weighted image as an intermediate step using the linear (FLIRT) and nonlinear (FNIRT) image registration tools in the FMRIB Software Library (FSL) (Jenkinson et al., 2002).

MTC metrics. For each participant, raw MT-GRE acquisitions were corrected for motion by registering them to the first image using a FLIRT rigid-body transformation and then averaged. A magnetization transfer contrast (MTC) image was calculated relative to the mean intensity of the pontine reference region as in prior work (Bennett et al., 2024). Maximum MTC metric was calculated by finding the peak MTC value within the MT-GRE-aligned locus coeruleus regions of interest. Individual values were excluded from the MTC analyses if they exceeded four standard deviations from the sample mean (2 older).

Diffusion metrics. For each participant, raw diffusion-weighted data were corrected for motion, susceptibility distortions, and eddy currents using FSL's TOPUP and EDDY (Andersson, Skare, & Ashburner, 2003; Andersson & Sotiropoulos, 2016). Mean diffusivity (MD) was estimate using the $b = 0$ and 500 data with FSL's DTIFIT. Mean MD in the LC was calculated by averaging values within the diffusion-aligned region of interest.

Functional Imaging Data

Acquisition. Separate echo-planar imaging (EPI) pulse sequences were acquired during performance of the study and test phases of each version (C, D) of the QuadMax task with the following parameters: TR/TE = 1750/32 ms, field of view = 221 × 190.4 mm, flip angle = 77 degrees, spatial resolution = 1.7 mm^3, 72 slices with no gap, AP phase encoding direction, GRAPPA acceleration factor = 2, and multiband factor = 3. To correct for susceptibility distortions in each participant's functional data, two sets of spin echo EPI images with phaseencoding directions of opposite polarity were acquired using parameters identical to the EPI sequence in the functional runs, except TR/TE = 7700/58 ms. Only the study phase data will be reported here.

Preprocessing. Using FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl), raw fMRI data were corrected for susceptibility-induced distortions (TOPUP; Andersson et al., 2003)

and then preprocessed using FEAT (FMRI Expert Analysis Tool), which included motion correction, brain extraction, spatial smoothing (5 mm FWHM Gaussian kernel), and high-pass filtering (100 s). Preprocessed data were registered to the participant's T1-weighted image using FLIRT (Jenkinson et al., 2002; Jenkinson and Smith, 2001), and then to a standard Montreal Neurological Institute (MNI) 152 template that was resampled to 1.7 mm^3 resolution using a combination of FLIRT with BBR and FNIRT (Andersson et al., 2007).

Lower-level analysis. Separate lower-level analyses were conducted for each participant and each task version (C, D) from the study phase, using FILM with local autocorrelation correction (Woolrich 2001). Analyses modeled each set size (pairs, triplets, quadruplets) and these three explanatory variables were convolved with a gamma-variate hemodynamic response function (standard deviation = 3 s, mean lag = 6 s). Two contrasts captured associative memoryrelated activity by contrasting pairs and quadruplets (pairs > quadruplets, quadruplets > pairs).

Higher-level analyses. A mid-level analysis was conducted to capture variance across task versions within each participant using fixed effects. One explanatory variable modeled activity from the lower-level analyses for each participant and one contrast captured average activity for each participant across task versions.

Higher-level analyses were conducted across participants on outputs of the mid-level analyses using FSL's Local Analysis of Mixed Effects (FLAME) stage 1. One explanatory variable modeled activity from the mid-level analyses and one contrast captured within-group mean effects by average activity across participants. We applied the default nonparametric statistical threshold using clusters determined by $Z > 3.1$ and a (corrected) cluster significance threshold of $P = 0.05$ (Worsley 2001). FSL's Featquery was used to extract parameter estimates for each contrast for each participant from the left and right hippocampus.

Results

Associative Memory Performance

Separate repeated-measures ANOVAs were conducted for each outcome metric (associative memory, recognition, hits, recombined FA, novel FA) using Set Size (pairs, triplets, quadruplets) as the within-subjects variable. Results are shown in Figure 2.

For associative memory, there was a significant main effect of Set Size, F (2, 78) = 25.2, $p < .001$. Post hoc pairwise comparisons revealed that associative memory was worse (lower) for each increase in set size from pairs (0.51 ± 0.29) to triplets (0.33 ± 0.17) to quadruplets (0.23 ± 0.24), ps < 0.022.

For recognition, there was a significant main effect of Set Size, F (2, 78) = 37.1, $p <$ 0.001. Post hoc pairwise comparisons revealed that recognition was worse for each increase in set size from pairs (0.82 ± 0.17) to triplets (0.70 ± 0.17) to quadruplets (0.60 ± 0.19), ps < 0.001.

For hits, there was a significant main effect of Set Size, F (2, 78) = 38.0, $p < 0.001$. Post hoc pairwise comparisons revealed that hits were lower for each increase in set size from pairs (0.90 ± 0.14) to triplets (0.76 ± 0.17) to quadruplets (0.66 ± 0.20), ps < 0.001.

For recombined FA, there was no significant main effect of Set Size, $p = 0.108$, with comparable recombined FAs for pairs (0.38 ± 0.25), triplets (0.44 ± 0.18), and quadruplets (0.43 ± 0.22).

For novel FA, there was again no significant main effect of Set Size, $p = 0.856$, with comparable novel FAs for pairs (0.071 ± 0.10), triplets (0.066 ± 0.09), and quadruplets (0.065 ± 0.10).

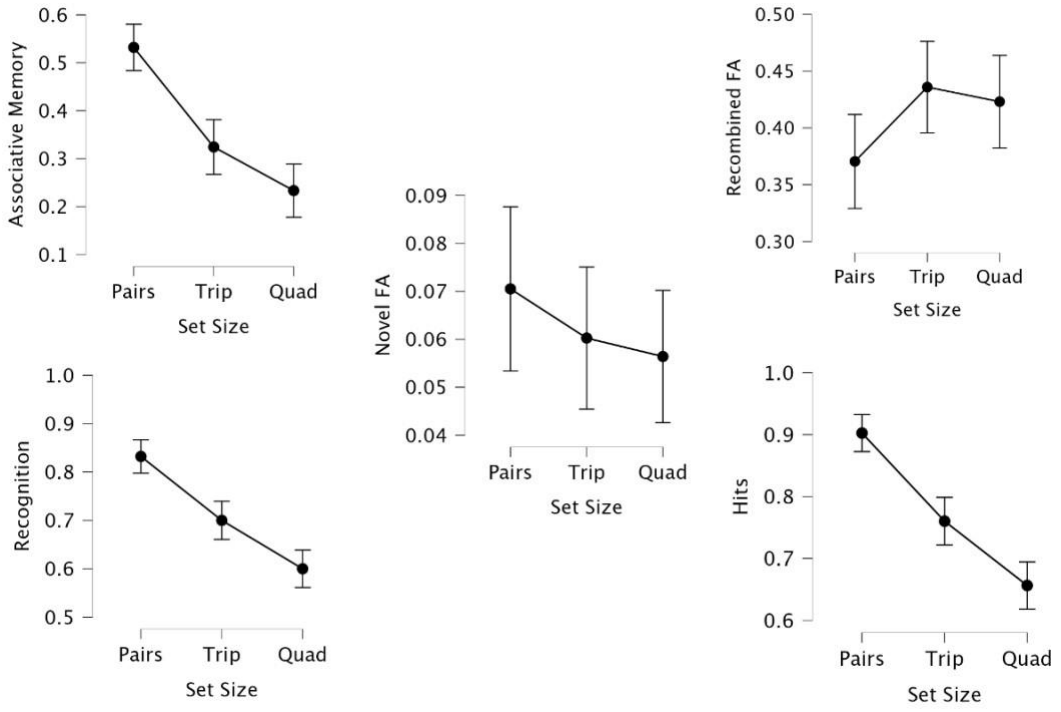

Figure 3: Increasing set size from pairs to triplets to quadruplets led to lower associative memory, recognition, and hit rates, while recombined and novel false alarms showed no significant differences between set sizes.

Effects of age and sex. The aforementioned repeated-measures ANOVAs were conducted after controlling for age and sex. The main effects of Set Size were no longer significant for any outcome metrics, *p*s > 0.10. However, age did have a significant effect on associative memory, F (1, 36) = 5.2, p = 0.029, recognition, F (1, 36) = 4.3, p = 0.044, and novel FA, F (1,36) = 4.8, p = 0.035, performance.

Associative Memory-Related Activity

Whole-brain maps showing regions with significant BOLD activation are shown in Figure 4. One sample t-tests determined whether there was significant activity in the left and right hippocampus during the study phase of our associative memory. Results revealed significant activity for the pairs > quadruplets contrast in the left hippocampus (13.0 □ 3.6), t(38) = 3.55, p = 0.001. Results revealed no significant activity for the quadruplets > pairs contrast in

the left hippocampus (2.2 □ 4.2), $p = 0.608$. Results revealed a non-significant trend for activity for the pairs > quadruplets (9.8 □ 5.1), $p = 0.059$, and quadruplets > pairs (6.8 □ 3.7), $p = 0.073$, contrasts in the right hippocampus. Although the right hippocampus only showed a trend-level effect, the results show a pattern that suggests associative memory related activity was higher for pairs compared to quadruplets in both hemispheres.

Effects of age and sex. Note that there were no significant correlations between age and task-related activity in either hippocampal hemisphere, ps > 0.36. There were also no significant sex group differences in task-related activity in the hippocampus, ps > 0.17.

Associative Memory Performance Correlates with Associative Memory-Related Activity

Partial correlations examined relationships between associative memory performance (associative memory, recognition) for each set size (pairs, triplets, quadruplet) and associative memory related-activity in the left and right hippocampus, controlling for age and sex. For associative memory, results revealed that greater activity for the pairs > quadruplets contrast in the left, $r = -0.38$, $p = 0.021$, and right, $r = -0.34$, $p = 0.040$, hippocampus was significantly related to worse associative memory for quadruplets. For recognition, results revealed that greater activity for the pairs > quadruplets contrast in the left, $r = –0.43$, $p = 0.008$, and right, $r = –0.34$, $p = 0.037$, hippocampus was significantly related to worse recognition for quadruplets.

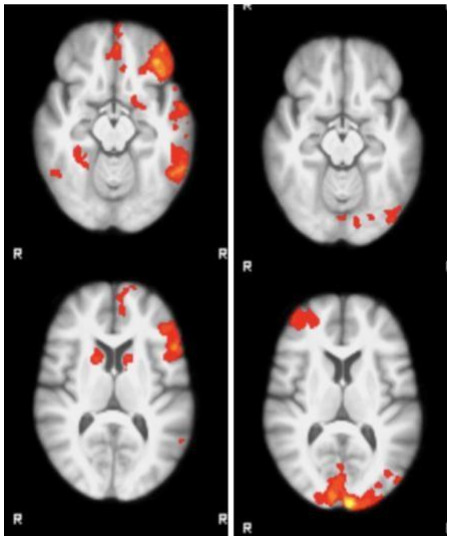

Figure 4: Whole-brain maps showing regions with greater BOLD activation for pairs compared to quadruplets (left) and quadruplets compared to pairs (right) during the study phase.

Locus Coeruleus Structure Correlates with Memory Performance

Partial correlations then examined relationships between memory performance (associative memory, recognition) for each set size (pairs, triplets, quadruplet) and locus coeruleus metrics (MD and Maximum CNR), controlling for age and sex. Results revealed that higher LC MD was significantly associated with worse recognition for pairs, $r = -0.48$, $p = 0.029$. No other relationships achieved significance, ps > 0.16.

Locus Coeruleus Structure Does Not Correlate with Associative Memory-Related Activity

Partial correlations examined relationships between locus coeruleus structural metrics (MD, Maximum CNR) and task-related hippocampal activity (pairs > quadruplets, quadruplets > pairs) in the left and right hippocampus, controlling for age and sex. Results revealed no significant relationships, ps > 0.14.

Discussion

The current study examined whether locus coeruleus (LC) microstructural integrity relates to associative memory performance and hippocampal associative memory–related activity

in older adults. Thirty-nine cognitively healthy older adults completed the QuadMax associative memory task during fMRI scanning, along with diffusion-weighted and magnetization transfer imaging to assess LC microstructure. At the behavioral level, associative memory, recognition, and hit rates declined significantly as associative load increased from pairs to triplets to quadruplets, but only when not controlling for age or sex. At the level of hippocampal BOLD activity, associative memory encoding elicited significantly greater hippocampal BOLD activity for pairs relative to quadruplets in the left hippocampus, with a similar trend observed in the right hippocampus. Greater hippocampal activity for the pairs > quadruplets contrast was significantly associated with worse associative memory and recognition performance for quadruplets. Finally, LC structure was selectively related to behavior such that higher LC mean diffusivity was significantly associated with worse recognition performance for pairs, while LC structural measures were not significantly related to hippocampal associative memory–related activity.

When not controlling for age or sex, associative memory, recognition, and hit rates were significantly worse for quadruplets than triplets and triplets than pairs. This does replicate our prior work in which we found declines in associative memory and related measures as set size increased in older adults (Franco et al. 2022). Interestingly, when controlling for age and sex, these effects were no longer significant, suggesting that age- and sex-related differences were the primary driving force for performance rather than associative load. Recombined and novel false alarm rates did not differ with set sizes, regardless of whether age and sex were controlled. This finding was unexpected given our prior work and may reflect methodological differences between the studies that reduced sensitivity to associative load effects on false alarm rates, including the use of two task versions with a larger total number of study sets as well as a substantially smaller sample size. This may have increased variability and limited the power to detect load-related differences in false alarms. Nonetheless, these behavioral findings suggest

that age and sex are key factors that play a significant role in associative memory performance in older adults.

fMRI data revealed significantly greater hippocampal BOLD activity for pairs relative to quadruplets during the study phase in our associative memory task in the left hippocampus, with a non-significant trend in the right hippocampus. This finding is consistent with prior literature that has shown that the hippocampus is critical for binding multiple items into a cohesive memory representation (Davachi, 2006). However, our finding that hippocampal activity during encoding was higher for pairs than triplets or quadruplets (i.e., higher associative loads) is not consistent with prior work, which has instead found that hippocampal activity during encoding was higher for conditions in which two items needed to be associated relative to conditions with one or zero items to be associated (Addis et al., 2006). One interpretation of the direction of our set size effects on hippocampal BOLD activity is that the hippocampus may be recruited when two items must be bound together, whereas binding four elements may exceed the capacity of hippocampal binding mechanisms in older adults, resulting in lower activity (see Reuter-Lorenz and Cappell, 2008).

Individual differences in hippocampal activity were related to memory performance at higher loads. Greater hippocampal activity for the pairs > quadruplets contrast revealed worse associative memory and recognition performance for quadruplets. This negative correlation suggests that heightened hippocampal engagement under lower associative loads may reflect inefficient processing rather than more effective memory formation. As associative load increases, the hippocampus may reach a functional limit in its ability to bind multiple elements into a cohesive representation, similar to what has been previously reported in frontal cortex during high working memory load (Reuter-Lorenz and Cappell, 2008), resulting in reduced engagement to quadruplet during the study phase, impairing encoding of the word sets and

ultimately leading to worse memory performance, especially for quadruplets. Together, these findings may suggest that reduced hippocampal engagement at higher associative loads may contribute to poorer associative memory performance in aging adults.

Finally, LC microstructure was selectively related to memory performance, but not to associative memory-related hippocampal activation. Specifically, higher LC mean diffusivity was significantly associated with worse recognition performance for pairs. This finding extends prior work that has similarly shown LC's microstructure relates to episodic memory performance in older adults (Fukabori et al., 2020) and is consistent with the notion that noradrenergic input from the LC enhances synaptic plasticity and processes that are critical for episodic memory (e.g., encoding, consolidation, and retrieval) (Uematsu et al., 2015). However, because LC microstructural measures were not significantly related to hippocampal associative memory–related activity during the study phase, the contribution of LC microstructure to individual differences in recognition accuracy may be independent of mechanisms captured by task-related hippocampal BOLD activation. Thus, our findings did not support the notion that activity in the hippocampal memory network is shaped by neuromodulatory input from the LC,

Several limitations should be considered when interpreting the present findings. Although the QuadMax task was designed to isolate associative memory demands by holding item familiarity constant and systematically increasing the number of inter-item relationships, larger set sizes may also place greater demands on working memory and strategic organization during study, particularly in older adults who must maintain and integrate multiple words simultaneously into a coherent representation. Thus, some of the observed associative load effects in performance and the BOLD response may reflect not only greater demands on binding processes, but also age-related differences in the ability to actively maintain multiple elements in working memory during study. Although hippocampal activity was measured during task

performance, fMRI cannot determine whether LC-related influences on memory occur through direct modulation of hippocampal associative binding or through broader network-level effects involving other regions that support memory. Future studies using higher-resolution LC imaging, functional connectivity approaches, and larger samples will be important for clarifying these mechanisms. Despite these limitations, the present study extends prior work using the QuadMax task by demonstrating that associative load impairs associative memory and recognition memory performance in older adults including at higher set sizes, that greater activity in the hippocampus during study of pairs relative to quadruplets was associated with worse performance (associative memory and recognition for quadruplets), whereas LC microstructural variability was selectively related to recognition performance. Together, these findings suggest that age-related difficulty remembering how elements of an event belong together may separately reflect both hippocampal engagement as well as integrity of the neuromodulatory LC system that supports memory. Because daily remembering depends on linking multiple elements of an experience into a coherent episode, these results return to the broader idea that successful memory for daily events relies on both hippocampal and LC systems that become increasingly vulnerable with aging.

REFERENCES

Addis, D. R., & McAndrews, M. P. (2006). Prefrontal and hippocampal contributions to the generation and binding of semantic associations during successful encoding. Neuroimage, 33(4), 1194-1206.

Andersson, J. L. R., Skare, S. & Ashburner, J. (2003). How to correct susceptibility distortions in spin-echo echo-planar images: Application to diffusion tensor imaging. Neuroimage 20, 870–888.

Andersson, J. L. R., Jenkinson, M., & Smith, S. (2007). Non-linear registration, aka spatial normalisation (FMRIB Technical Report TR07JA2). FMRIB Centre, University of Oxford.

Andersson, J. L. R. & Sotiropoulos, S. N. (2016). An integrated approach to correction for offresonance effects and subject movement in diffusion MR imaging. Neuroimage 125, 1063–1078.

Balota, D. A., Yap, M. J., Hutchison, K. A., Cortese, M. J., Kessler, B., Loftis, B., ... & Treiman, R. (2007). The English lexicon project. Behavior research methods, 39(3), 445-459.

Bennett, I. J., Langley, J., Sun, A., Solis, K., Seitz, A. R., & Hu, X. P. (2024). Locus coeruleus contrast and diffusivity metrics differentially relate to age and memory performance. Scientific Reports, 14(1), 15372.

Chen, P.-C., & Chang, Y.-L. (2016). Associative memory and underlying brain correlates in older adults with mild cognitive impairment. Neuropsychologia, 85, 216–225.

Corinna Y. Franco, Alexander Alcaraz-Torres & Ilana J. Bennett (2022): The QuadMax Task: Parametrically Manipulating Associative Memory Load across the Adult Lifespan, Experimental Aging Research.

Davachi L. Item, context and relational episodic encoding in humans. Curr Opin Neurobiol. 2006

Dec;16(6):693-700. Epub 2006 Nov 9. PMID: 17097284.

Fukabori, R., Iguchi, Y., Kato, S., Takahashi, K., Eifuku, S., Tsuji, S., Hazama, A., Uchigashima, M., Watanabe, M., Mizuma, H., Cui, Y., Onoe, H., Hikishima, K., Yasoshima, Y., Osanai, M., Inagaki, R., Fukunaga, K., Nishijo, T., Momiyama, T., Benton, R., & Kobayashi, K. (2020). Enhanced retrieval of taste associative memory by chemogenetic activation of locus coeruleus norepinephrine neurons. Journal of Neuroscience, 40(43), 8367–8385.

Gold, J. J., Hopkins, R. O., & Squire, L. R. (2006). Single-item memory, associative memory, and the human hippocampus. Learning & Memory, 13(5), 644–649.

Jenkinson, M., Bannister, P., Brady, M. & Smith, S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage 17, 825–841 (2002).

Jenkinson, M., & Smith, S. (2001). A global optimisation method for robust affine registration of brain images. Medical Image Analysis, 5(2), 143–156.

Khakpour-Taleghani, B., Akhoondian, M., & Jafari, A. (2021). The nucleus locus coeruleus modulatory effect on memory formation: A literature review. Journal of Current Biomedical Reports, 2(4), 142-149.

Langley, J., Hussain, S., Flores, J. J., Bennett, I. J. & Hu, X. Characterization of age-related microstructural changes in locus coeruleus and substantia nigra pars compacta. Neurobiol. Aging 87, 89–97 (2020).

Mayes, A., Montaldi, D., & Migo, E. (2007). Associative memory and the medial temporal lobes. Trends in cognitive sciences, 11(3), 126-135.

Naveh-Benjamin, M., Guez, J., & Shulman, S. (2004). Older adults' associative deficit in episodic memory: Assessing the role of decline in attentional resources. Psychonomic Bulletin & Review, 11(6), 1067–1073.

Pendlebury, S. T., Welch, S. J., Cuthbertson, F. C., Mariz, J., Mehta, Z., & Rothwell, P. M. (2013). Telephone assessment of cognition after transient ischemic attack and stroke: modified telephone interview of cognitive status and telephone Montreal Cognitive Assessment versus face-to-face Montreal Cognitive Assessment and neuropsychological battery. Stroke, 44(1), 227-229.

Reuter-Lorenz, P. A., & Cappell, K. A. (2008). Neurocognitive aging and the compensation hypothesis. Current directions in psychological science, 17(3), 177-182.

Tulving, E. (1983). Ecphoric processes in episodic memory. Philosophical Transactions of the Royal Society of London. B, Biological Sciences, 302(1110), 361-371.

Uematsu, A., Tan, B. Z., & Johansen, J. P. (2015). Projection specificity in heterogeneous locus coeruleus cell populations: implications for learning and memory. Learning & memory, 22(9), 444-451.

Woolrich, M. W., Ripley, B. D., Brady, M., & Smith, S. M. (2001). Temporal autocorrelation in univariate linear modeling of fMRI data. NeuroImage, 14(6), 1370–1386.

Worsley, K. J. (2001). Statistical analysis of activation images. In P. Jezzard, P. M. Matthews, & S. M. Smith (Eds.), Functional MRI: An introduction to methods (pp. Chapter 14). Oxford University Press.

APPENDIX

Table 1. Demographic information by decade

Age Group	n	Mean Age ± SD	Gender (M/F)	Mean Education ± SD
60-69	24	65.0 ± 2.9	7/17	15.0 ± 2.4
70-79	12	72.8 ± 2.6	4/8	14.9 ± 2.6
80-89	3	84.3 ± 2.5	0/3	13.3 ± 1.5

Note. Education is reported in total years. n = sample size, SD = standard deviation

Table 1. Demographic information by decade

This table outlines participant age, gender distribution, and years of education across three age groups (60–69, 70–79, 80–89).

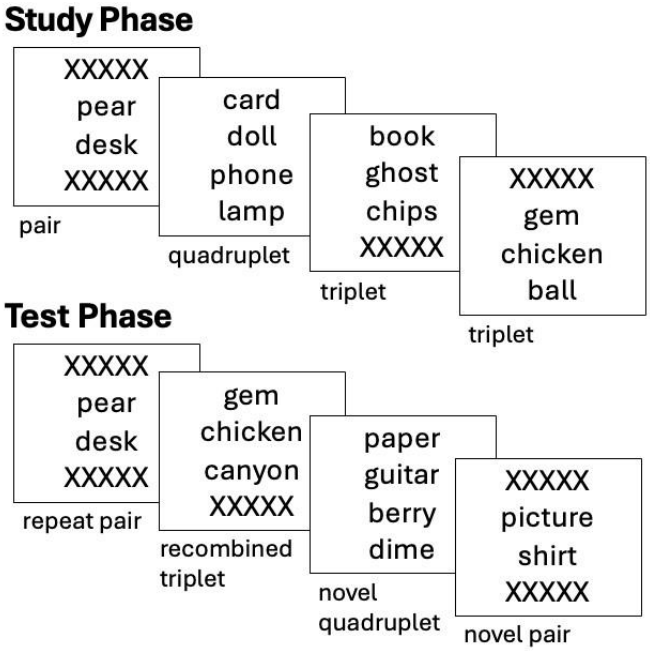

Figure 2: Example trials from the study phase (top) and test phase (bottom) of the QuadMax task show the set size (pairs, triplets, quadruplets) and trial type (repeated, recombined, novel) conditions.

Figure 2. Example trials of the study and test phase from the novel QuadMax task. This figure outlines study and test phase trials, including different set sizes and trial types (repeated, recombined, novel) used to assess associative memory.

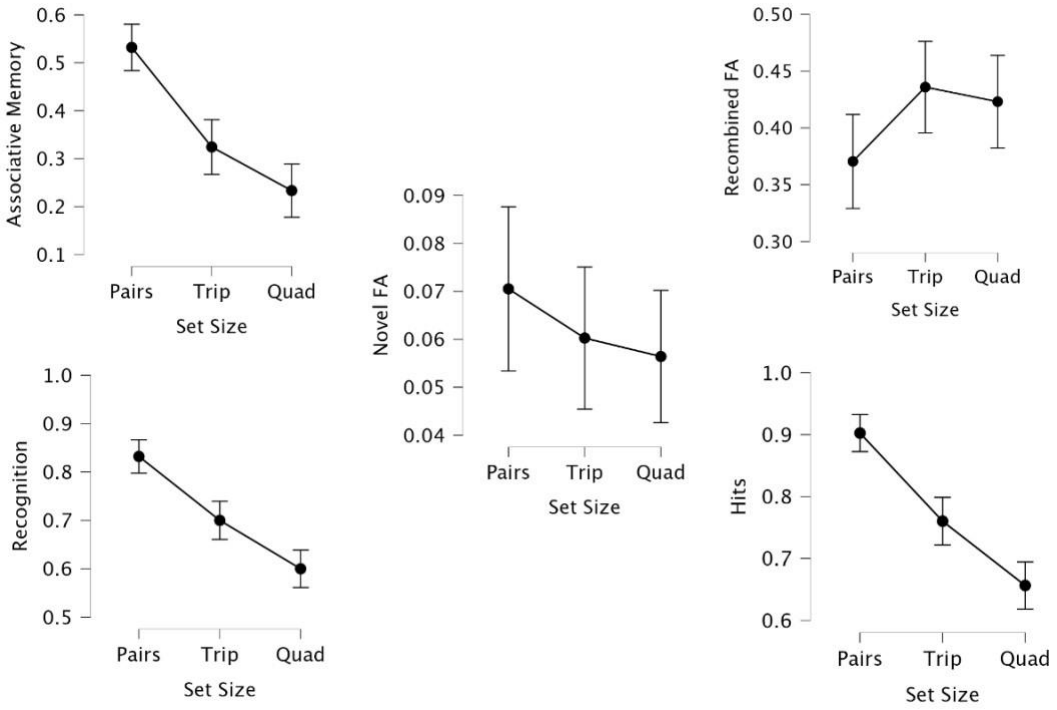

Figure 3: Increasing set size from pairs to triplets to quadruplets led to lower associative memory, recognition, and hit rates, while recombined and novel false alarms showed no significant differences between set sizes.

Figure 3. Increasing set size from pairs to triplets to quadruplets

This figure shows that as set size increases, associative memory, recognition, and hit rates decrease, while false alarm rates remain relatively stable.

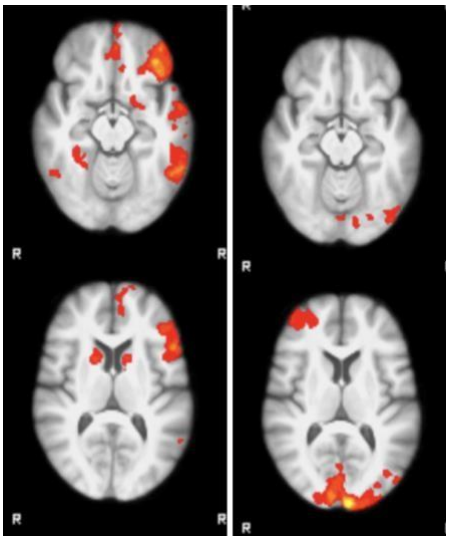

Figure 4: Whole-brain maps showing regions with greater BOLD activation for pairs compared to quadruplets (left) and quadruplets compared to pairs (right) during the study phase.

Figure 4. Whole-brain activation differences by set size

This figure shows brain regions with greater BOLD activation for pairs vs. quadruplets (left) and quadruplets vs. pairs (right) during the study phase.