**Title: The interactive influence of chronological age and menopause status on the functional neural correlates of spatial context memory in middle-aged females**

Abbreviated Title: Menopause status and age affect memory and brain function

Arielle Crestol[1]*, Sricharana Rajagopal[2], Rikki Lissaman[2,7], Annalise LaPlume[2,8], Stamatoula Pasvanis[2], Rosanna Olsen[4], Gillian Einstein[4,5], Emily Jacobs[6], and M. Natasha Rajah[2,3,7,8]*

*Both authors contributed equally to the final manuscript

[1] Integrated Program in Neuroscience, McGill University, Montréal, QC, Canada, H3A 1A1
[2] Douglas Mental Health University Institute, Verdun, QC, Canada, H4H 1R3
[3] Department of Psychology, McGill University, Montréal, QC, Canada, H3A 1G1
[4] Rotman Research Institute, Baycrest Centre & University of Toronto, Toronto, ON, Canada, M6A 2E1
[5] Department of Psychology, University of Toronto, Toronto, ON, Canada, M5S 3G3
[6] Psychological and Brain Sciences, U. C. Santa Barbara, California, U.S.A., 93106-9660
[7] Department of Psychiatry, McGill University, Montréal, QC, Canada, H3A 1A1
[8] Department of Psychology, Toronto Metropolitan University, Toronto, ON, Canada,

Corresponding Author:
M. Natasha Rajah, Ph.D.
2114 CIC Pavilion
Douglas Hospital Research Centre, McGill University
Montreal, Quebec, H4H 1R3

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**Abstract**

Reductions in the ability to encode and retrieve past experiences in rich spatial contextual detail (episodic memory) are apparent by midlife – a time when most females experience spontaneous (natural) menopause. Yet, little is known about how menopause status interacts with chronological aging to influence the functional brain networks associated with episodic memory. Female-specific investigations of memory-related brain function at midlife are thus warranted to advance our knowledge of how endocrine and chronological aging affect cognitive brain aging in middle-aged females. We conducted an event-related task fMRI study of episodic memory for spatial context and used data driven multivariate fMRI analysis to investigate how chronological age, and retrieval accuracy correlated with encoding and retrieval activity in middle-aged premenopausal and postmenopausal females. Compared to premenopausal females, postmenopausal females exhibited lower spatial context memory retrieval accuracy. The fMRI results indicated there was a significant correlation between retrieval activity in lateral prefrontal cortex (PFC) and midline cortical networks and memory performance in premenopausal, but not postmenopausal, females. This retrieval effect was not significantly related to chronological age in either group. In addition, there were menopause group differences in the correlation between inferior parietal cortex activity at encoding and retrieval, memory performance, and age. Importantly, the correlation between age and activity in these posterior cortical regions was in opposite direction between groups – highlighting an interaction between endocrine and chronological aging.

**Significance Statement (109 Words)**

This study advances our understanding of how menopause status and chronological age interact to affect memory-related brain function in middle-aged females. The results highlight how endocrine aging may introduce non-linearities in the effect of chronological age on memory and brain function. Using a data driven multivariate fMRI analytic method we found: 1) a positive correlation between encoding activity in the recollection network and lateral prefrontal cortex and memory performance in both menopause groups, 2) menopause status, but not age, was associated with altered brain-behavior correlations in these same regions at retrieval, and 3) group differences in the correlation between age and inferior parietal function, which correlated with memory performance.

## Introduction

Chronological aging is associated with declines in the ability to encode and consciously recollect past events in rich contextual detail (episodic memory, (Tulving, 1972)). Age-related episodic memory decline is associated with altered brain activity in medial temporal, prefrontal and parietal cortices (Ankudowich et al., 2016; Spaniol & Grady, 2012). It has been assumed that age-related decline in episodic memory, and related neural systems, follows a similar linear trajectory across the adult lifespan in females and males (Craik & Rose, 2011; Spencer & Raz, 1995). However, recent fMRI studies suggest there are sex differences in the effect of age on memory-related brain function (Subramaniapillai et al., 2022; Subramaniapillai et al., 2019). Also, in females, the neuroendocrine changes associated with the natural menopause at midlife, i.e., depletion of centrally circulating 17B-estradiol (Rettberg et al., 2014), may interact with chronological aging processes, and differentially influence memory-related brain function at pre-, compared to post-, menopause (Mosconi et al., 2017). Thus, introducing non-linear patterns of cognitive brain aging in females at midlife.

Discerning whether and how menopause status and chronological aging individually and/or interactively contribute to episodic memory decline, and related differences in brain function is important for informing current models of cognitive brain aging. Such information also has clinical relevance since many females experience memory changes at menopause (Greendale et al., 2011; Maki, 2015), and more females than males are diagnosed Alzheimer's Disease (AD) – a fatal neurodegenerative disease that affects episodic memory and its related brain systems early in the disease process (Backman et al., 1999; Mielke et al., 2018). Thus, knowing whether menopause status interacts with chronological age, and possibly serves as an inflection point at which age-related declines in memory and brain function become more pronounced, is important for

determining the efficacy of using endocrine-related interventions (e.g., hormonal replacement therapy) at menopause transition to support memory and brain function at this stage in the adult lifespan (Henderson et al., 2016) .

To our knowledge, only a limited number of studies have evaluated menopause effects on memory-related brain activity (Jacobs et al., 2016; Rentz et al., 2017; Taylor et al., 2019). For example, Jacobs and colleagues (2016) examined the effect of menopause status on brain activity during verbal encoding. Postmenopausal status was associated with less activity in the hippocampus compared to pre- and perimenopausal status. However, age was matched across groups in this study, which assumes there are no cohort effects on the outcomes of interest i.e., cognition and brain health. However, early menopause has been associated with increased risk of age-related cognitive impairment and dementia (Shimizu et al., 2019; Song et al., 2020). Thus, it is unclear if the reported menopause effects are generalizable under conditions in which age and menopause status are correlated. Moreover, in this study there was no effect of menopause status on verbal episodic memory. This may be due to the age-matching done, or because episodic memory decline at midlife may only be apparent on tasks of associative context memory, e.g., object-location spatial context memory tasks (Cansino et al., 2012; Kwon et al., 2016).

Therefore, the goal of the current event-related task fMRI study was to explore how menopause status *and* chronological aging affects spatial context memory and related brain function in healthy middle-aged pre- and postmenopausal females. To this aim, a data-driven, between group, brain-behavior partial least squares (PLS) correlation analysis (McIntosh et al., 2004) was used to test for similarities, differences, and interactions in how age and memory performance correlated with brain activity in premenopausal and postmenopausal females.

Importantly, the data reduction methods used in PLS address issues of multicollinearity amongst predictors/independent variables in a model, i.e. age and menopause status (McIntosh et al., 1996), making it an ideal method for addressing our goal. We predicted that older age and post-menopause status would both contribute to decreases in spatial context memory, and that age-related differences in memory-related brain activity in temporal, prefrontal and parietal activity would be present in postmenopausal, but no premenopausal, females.

## Materials and Methods

### Participants

Ninety-six middle-aged (MA) females between the ages of 39 to 65 years participated in this study. Participants were categorized as being premenopausal (Pre-Meno females) or postmenopausal (Post-Meno females) based on the Stages of Reproductive Aging Workshop + 10 (STRAW +10) criteria (Harlow et al., 2012). Based on these criteria there were 33 Pre-Meno females participants (Age range: 39.55 – 53.30 yrs, $M_{age}$ = 44.41, $SD_{age}$ = 3.12, $M_{EDU}$ = 16.44, Ethnicity: 81.8% White, 9.1% Latin American, 3.0% Black, 3.0% Chinese, 3.0% South Asian) and 63 Post-Meno females participants (Age range: 46.70 to 65.14 yrs, $M_{age}$ = 57.05, $SD_{age}$ = 3.93, $M_{EDU}$ = 15.39, Ethnicity: 87.3% White, 3.2% Black, 3.2% Indigenous, 1.6% Chinese, 1.6% South Asian, 3.2% Middle Eastern and North African). This study was approved by the research ethics board (REB) of the Montreal West Island Integrated University Health and Social Services Centre – subcommittee for mental health and neuroscience, and all participants signed informed consent forms.

### Behavioral Methods

Enrollment

Participants were recruited via advertisements posted on websites and around the Montreal community. Participants first signed an online consent form and then proceeded to fill out an online questionnaire that collected demographics, medical history, reproductive history, and education information. This information was used to screen participants for inclusion/exclusion in the study. Individuals who met the inclusion/exclusion criteria listed below were invited to Behavioral Session 1 at the Cerebral Imaging Center (CIC) at the Douglas Mental Health University Institute (DMHUI).

Inclusion criteria: a minimum of high school education, willingness to provide a blood sample, and in general good health. Participants were excluded if they reported any of the following in their medical history: current use of hormonal birth control, current use of hormone replacement therapy, bilateral oophorectomy, untreated cataract/glaucoma/age-related maculopathy, uncontrolled hypertension, untreated high cholesterol, diabetes, history of estrogen-related cancers, chemotherapy, neurological diseases or history of serious head injury, history of major psychiatric disorders, claustrophobia, history of a substance use disorder, or currently smoking >40 cigarettes per day. Participants were also excluded if they did not meet the requirements for magnetic resonance imaging (MRI) safety (e.g., implanted pacemaker). Females who were perimenopausal or whose menopausal status was indeterminate (due to hysterectomy) were excluded from the study.

Behavioral Session

After obtaining consent, participants filled out the MRI safety questionnaire. Next, they completed a battery of standardized psychiatric and neuropsychological assessments which included: the Mini-International Neuropsychiatric Interview (M.I.N.I; (Sheehan et al., 1998)), exclusion criteria was indications of undiagnosed psychiatric illness; the Beck Depression Inventory II (BDI-II; (Beck et al., 1997)), inclusion cut-off ≤ 19, the Beck Anxiety Inventory (BAI; (Beck et al., 1988), inclusion cut-off ≤ 21; Folstein Mini Mental State Examination, inclusion cut-off ≥ 27 (MMSE, (Folstein et al., 1975)). Participants then donated blood samples for hormonal assays to confirm menopausal status and performed a practice version of the spatial context memory task in a mock MRI scanner to familiarize them with the task and ensure they felt comfortable enough to partake in the real MRI scan. Participants who met the neuropsychological inclusion criteria stated above

and who were able to perform the practice fMRI task were invited to a second visit to undergo MRI scans at the CIC, DMHUI.

Task fMRI Session

Upon arrival at the CIC, participants first took a pregnancy test. Participants who were not pregnant were then scanned while performing the spatial context memory task. Participants were asked to lie in a supine position in a 3T Siemens Prisma-Fit scanner, wearing a standard 32-channel head coil. High-resolution T1-weighted anatomical images were collected using a 3D Magnetization-Prepared Rapid Gradient-Echo (MP-RAGE) sequence (repetition time [TR] = 2300 ms, echo time [TE] = 2.36 ms, flip angle = 9°, 192 1 mm thick transverse slices, 1 mm × 1 mm × 1 mm voxels, field-of-view [FOV] = 256 $mm^2$, acquisition time = 5:03 min) to use in the registration and normalization of the fMRI data. While performing the spatial context memory task, functional BOLD images were acquired using single shot T2*-weighted gradient echo-planar imaging (EPI) pulse sequence (TR = 2000 ms, TE = 30 ms, FOV = 256 $mm^2$, matrix size = 64 × 64, in-plane resolution = 4 mm × 4 mm). A mixed rapid event-related design was used for the task fMRI portion of the experiment (Dale, 1999). Participants were scanned during encoding and retrieval phases of easy and hard spatial context memory tasks. Task difficulty was manipulated by increasing encoding load (details below). A participant could perform up to four Easy and four Hard task runs (maximum eight runs total). Easy runs included 2 easy spatial context memory tasks (total run duration: 9:42 min), whereas hard runs only consisted of 1 experimental block (total run duration: 7:14 min). Run order was counterbalanced across all participants. Total length of the task fMRI session was 1 hr 7 min and 44 sec approximately.

The tasks were presented through E-Prime software (Psychology Software Tools, PA), back projected into the scanner bore, and made visible to participants via a mirror mounted within the head coil. Participants were provided with two 4-button inline fibre optic response boxes to respond throughout the task. During each run, response options and the corresponding buttons for each task were displayed at the bottom of the screen for clarity (see Figure 1 for a visual summary of the task). Response accuracy and reaction time (RT, msec) was collected for all responses by E-Prime software.

Encoding Phase. Participants were presented with instructions for the first six seconds of each encoding phase. Participants were informed that they would see a series of either six or twelve black and white photographs of age-variant (toddler – older adults) faces. The stimulus set has been described in detail in prior publications (Rajah et al., 2008). Conditions in which six faces were presented at encoding were considered the 'easy', and those in which 12 faces were presented at encoding were considered 'hard'. Thus, the experiment included a task difficulty manipulation, which was based on the encoding load. This allowed us to assess within group and between group differences in performance as a function of increased task demands. There were a total of 48 faces presented during the hard easy condition, and 48 faces presented during the hard encoding condition.

Participants were instructed to memorize the spatial location of each face and rate each face as either pleasant or neutral by pressing the corresponding button on the provided response box. The pleasantness rating was added to ensure participants deeply encoded the stimuli (Bernstein et al., 2002). Participants were then sequentially presented face stimuli, one at a time (two sec/stimulus) in one of four quadrants on the computer screen (see Figure 1). There were 50% girl/women and 50% boy/men faces presented and the race and ethnicity of the faces presented

were 83% White, 8.5% Black, 8.5% Other. The inter-trial interval (ITI) was varied (2.5 – 7.5 seconds; mean ITI = 4.17 seconds) to add jitter to fMRI data collection (Dale & Buckner, 1997). Subsequent to the encoding phase, participants rated their confidence on how well they performed the task on a four-point scale from not well (1) to very good (4) during a 60 second break.

Retrieval Phase. Participants were presented with instructions for fourteen seconds. They were informed that they would be shown a series of faces, both new and previously seen, and would have to respond to each face by pressing one of six buttons corresponding to the following retrieval responses: (1) N – The face is NEW; (2) F – The face is FAMILIAR but you don't remember its location; (3) TL – You remember the face and it was previously on the TOP LEFT; (4) BL – You remember the face and it was previously on the BOTTOM LEFT; (5) TR – You remember the face and it was previously on the TOP RIGHT; (6) BR – You remember the face and it was previously on the BOTTOM RIGHT. Participants were instructed not to guess and only respond (3) to (6) if they recollected the face and its location. The participants were then presented with either 12 (Easy condition) or 24 (Hard condition) faces per task. Each face was presented for four seconds at a time at the center of the screen with a variable ITI (2.5 – 7.5 s, mean ITI = 4.17 s). At the end of the retrieval phase participants were given 60 seconds to rate how well they thought they performed on a four-point scale from not well (1) to very good (4). There was a total of 96 faces (48 presented at encoding, 48 new) presented during easy and hard conditions, respectively.

**Endocrine Assessments**

Estradiol-17β (E2), Follicle stimulating hormone (FSH), Luteinizing Hormone (LH) and Progesterone (P) levels were assessed to corroborate menopausal staging using STRAW+10 if categorization was unclear based on participant responses alone. Endocrine measurements were

assessed on plasma, derived from heparin blood collection tubes. Blood was drawn on non-fasting individuals by a certified research nurse at the DMHUI during session 2 (MRI visit). Specimens were analyzed at the McGill University Health Centre (Glen site) Clinical Laboratories in Montreal. Endocrine Chemiluminescent immunoassays (CLIA) were performed on an Access Immunoassay System (Beckman Coulter), using the company's reagents.

**Data analysis**

***Behavioral Analyses***

Mean accuracy and reaction time (RT, msec) were calculated for each participant for the following retrieval response types within easy and hard tasks:

1) Correct Spatial Context Retrieval (CS) easy and hard: total number of *correct associative retrieval trials* (i.e., 3, 4, 5, 6 button presses made for previously seen faces, see Figure 1) divided by the total number of previously seen faces presented at retrieval.
2) Incorrect Spatial Context Retrieval or a Source Misattribution (SM) easy and hard: total trials in which the incorrect location was misattributed to previously seen face, but the face was correctly recognized as previously seen.
3) Correct Recognition (Recog) easy and hard: total number of *correct retrieval* trials for recognition without memory for location (i.e. 2 responses for previously seen faces) divided by the total number of previously seen faces presented at retrieval.
4) Correct Rejections (CR) easy and hard: total number of *correct* retrieval responses (i.e. 1 responses to new faces) divided by the total number of new faces presented at retrieval
5) Misses easy and hard: total number of *incorrect* retrieval responses (i.e. 1 responses for previously seen faces) divided by the total number of previously seen faces presented at retrieval

6) False alarms (FA) easy and hard: total number of *incorrect* recognition responses (i.e. 2 button presses for new faces) divided by the total number of new faces presented at retrieval
7) Spatial False alarms ($FA_{spatial}$) easy and hard: total number of *incorrect* spatial location responses (i.e. 3,4, 5,6 button presses for new faces) divided by the total number of new faces presented at retrieval
8) No Response easy and hard: total number of stimuli seen for which no response was collected for old faces.

In addition, we calculated adjusted measures of correct spatial retrieval (pCS) and recognition memory (pRecog) (Snodgrass & Corwin, 1988) to account for response bias as follows:

[1] $pCS = Z_{(CS)} - Z_{(Spatial\ Errors)}$

[2] $pRecog = Z_{(Recog)} - Z_{(FA)}$

Spatial Error was calculated as Z-score of the mean $FA_{spatial}$ + SM responses for easy and hard trials to account for individual's bias towards erroneously endorsing a spatial location at retrieval for old and new items.

SPSS (Version 29, IBM Corporation) was used to conduct between-group (Pre-Meno females|Post-Meno females) × 2 Retrieval Response Types (pCS|pRecog) × Task Difficulty (Easy|Hard) repeated measures analysis of variance (RM-ANOVA) for accuracy. Post-hoc between group paired t-tests and univariate general linear models (GLMs) were conducted as needed to interpret significant effects. To examine group differences in RT (msec), we conducted RM-ANOVAs 2 Groups (Pre-Meno females|Post-Meno females) × 7 Retrieval Response Types (CS, Recog, SM, CR, Misses, FA, $FA_{spatial}$) × Task Difficulty (Easy|Hard). Analyses were conducted with and without Age (years) as a covariate of interest given the age difference in Pre- and Post-Meno females females. In addition, we conducted simple between group one-way

ANOVAs for each of the accuracy and RT response types and significance was assessed at $p<0.05$ corrected for 16 response types across both levels of difficulty for accuracy ($p<0.003125$ uncorrected) and corrected for 14 response types across both levels of difficulty for RT ($p<0.00357$).

To test the hypothesis that menopFor mean accuracy and RT on CS easy and hard tasks, we conducted further analyses to examine the relative effects of chronological age and menopause. Specifically, we ran PLS regressions in R (version 4.1.3) using the *pls* package (Liland et al., 2023). We selected this method as it is an optimal tool for analyzing data in which the predictors/independent variables are highly collinear (Abdi, 2010; Wold et al., 2001), as is the case for chronological age and menopause status in our sample (for a relevant example, see Snytte et al., 2020). We ran four models, one for mean accuracy and one for mean RT at each level of task difficult (easy, hard). In each model, we used leave-one-out cross-validation, scaled (i.e., standardized) the independent variables, and used jackknife testing. To determine the optimal number of LVs for each model, we adopted an approach based on permutation testing (van der Voet, 1994). This method is implemented backwards, removing LVs from the model that do not result in a significant deterioration in overall performance ($\alpha = .05$; $n$ permutations = 1000). Jackknife testing was used to determine the statistical significance of regression coefficients from any resulting LVs. All presented correlations of independent variables with each LV are standardized (scaled and centered).

***fMRI Analyses***

Image Preprocessing

The DICOM files were first converted to NIFTI format and organized using the Brain Imaging Data Structure (BIDS; (Gorgolewski et al., 2016)). Volumes collected during the first 10

seconds of scanning were removed to ensure that magnetization had stabilized. Preprocessing was then conducted using fMRIprep version 20.2 (Esteban et al., 2019). FMRIprep is a robust preprocessing workflow in Python version 3.0 that implements tools from various software packages including Advanced Normalization Tools version 2.3.3 (ANTs), FMRIB Software Library version 5.0.9 (FSL), and FreeSurfer 6.0.1. The following preprocessing description was generated by fMRIprep (Esteban et al., 2019):

For each of the 8 BOLD runs found per participant (across all tasks and sessions), the following preprocessing was performed. First, a reference volume and its skull-stripped version were generated using a custom methodology of fMRIPrep. A B0-nonuniformity map (or fieldmap) was estimated based on a phase-difference map calculated with a dual-echo GRE (gradient-recalled echo) sequence, processed with a custom workflow of SDCFlows inspired by the epidewarp.fsl script and further improvements in HCP Pipelines (Glasser et al., 2013). The fieldmap was then co-registered to the target EPI (echo-planar imaging) reference run and converted to a displacements field map (amenable to registration tools such as ANTs) with FSL's fugue and other SDCflows tools. Based on the estimated susceptibility distortion, a corrected EPI (echo-planar imaging) reference was calculated for a more accurate co-registration with the anatomical reference. The BOLD reference was then co-registered to the T1w reference using bbregister (FreeSurfer) which implements boundary-based registration (Greve & Fischl, 2009). Co-registration was configured with six degrees of freedom. Head-motion parameters with respect to the BOLD reference (transformation matrices, and six corresponding rotation and translation parameters) are estimated before any spatiotemporal filtering using mcflirt (FSL 5.0.9,(Jenkinson et al., 2002)). The BOLD time-series were resampled onto their original, native space by applying a single, composite transform to correct for head-motion and susceptibility distortions. These

resampled BOLD time-series will be referred to as preprocessed BOLD in original space, or just preprocessed BOLD. The BOLD time-series were resampled into standard space, generating a preprocessed BOLD run in MNI152NLin2009cAsym space. First, a reference volume and its skull-stripped version were generated using a custom methodology of fMRIPrep. Several confounding time-series were calculated based on the preprocessed BOLD: framewise displacement (FD), DVARS and three region-wise global signals. FD was computed using two formulations following Power (absolute sum of relative motions, (Power et al., 2014)) and Jenkinson (relative root mean square displacement between affines, Jenkinson et al. (2002)). FD and DVARS are calculated for each functional run, both using their implementations in Nipype (following the definitions by Power et al. 2014). Three global signals are extracted within the CSF, the WM, and the whole-brain masks. Additionally, a set of physiological regressors were extracted to allow for component-based noise correction (CompCor, (Behzadi et al., 2007)).

Principal components are estimated after high-pass filtering the preprocessed BOLD time-series (using a discrete cosine filter with 128s cut-off) for the two CompCor variants: temporal (tCompCor) and anatomical (aCompCor). tCompCor components are then calculated from the top 2% variable voxels within the brain mask. For aCompCor, three probabilistic masks (CSF, WM and combined CSF+WM) are generated in anatomical space. The implementation differs from that of (Behzadi et al., 2007) in that instead of eroding the masks by 2 pixels on BOLD space, the aCompCor masks are subtracted from a mask of pixels that likely contain a volume fraction of GM. This mask is obtained by dilating a GM mask extracted from the FreeSurfer's aseg segmentation, and it ensures components are not extracted from voxels containing a minimal fraction of GM. Finally, these masks are resampled into BOLD space and binarized by thresholding at 0.99 (as in the original implementation). Components are also calculated separately

within the WM and CSF masks. For each CompCor decomposition, the k components with the largest singular values are retained, such that the retained components' time series are sufficient to explain 50 percent of variance across the nuisance mask (CSF, WM, combined, or temporal). The remaining components are dropped from consideration. The head-motion estimates calculated in the correction step were also placed within the corresponding confounds file. The confound time series derived from head motion estimates and global signals were expanded with the inclusion of temporal derivatives and quadratic terms for each (Satterthwaite et al., 2013). Frames that exceeded a threshold of 0.5 mm FD or 1.5 standardised DVARS were annotated as motion outliers. All resamplings can be performed with a single interpolation step by composing all the pertinent transformations (i.e. head-motion transform matrices, susceptibility distortion correction when available, and co-registrations to anatomical and output spaces). Gridded (volumetric) resamplings were performed using antsApplyTransforms (ANTs), configured with Lanczos interpolation to minimize the smoothing effects of other kernels. Non-gridded (surface) resamplings were performed using mri_vol2surf (FreeSurfer).

In addition to fMriprep, further preprocessing steps were carried out using custom code in Python 3.0 and Nilearn libraries (Abraham et al., 2014). If a participant had more than 15% of all their scans showing motion greater than 1 mm FD, they were removed from the analysis. For participants with motion <15% across all scans, the normalized scans from fMRIprep were scrubbed for motion artefacts as follows: a)when less than 15% of scans across all runs were above the 1mm cut-off, and only one or two consecutive volumes exceeded this cut-off, the volume was replaced by the average of the previous and the subsequent volume in time (e.g., a scan T_i that had an FD > 1mm was replaced by the mean/avg of scans T_(i-1) and T_(i+1)), b) when less than 15% of scans across all runs exceeded 1mm FD and there were 3 or more consecutive volumes

that exceeded 1mm FD, then onsets including those volumes were excluded from the PLS analysis. The scans were then smoothed using a Gaussian filter (FWHM=6mm). Finally, confounds such as white matter, CSF and 6 motion parameters were regressed out from all the runs. The scans from each run were visually analyzed by two independent raters across different stages of preprocessing in FSL or Statistical Parametric Mapping software version 12 (SPM12).

***fMRI Analysis***

A multivariate between-group behavior partial least squares (B-PLS, (McIntosh & Lobaugh, 2004)) analysis was conducted via open source PLSGUI software (https:// www.rotman-baycrest.on.ca/index.php?section=345) in MATLAB (R2022a; Mathworks, Inc., Natick, MA). Partial least squares analysis was chosen because it is a powerful method that can be used to objectively assess spatiotemporal functional patterns in neuroimaging datasets. Moreover, B-PLS directly assesses the correlation between brain activity and different demographic and/or behavioral measures ((McIntosh et al., 2004). When conducting PLS the fMRI and behavioral data are stored in two separate matrices. The fMRI data matrix was organized with rows representing brain activity for each event-type of experimental interest nested within participants, and participants nested within Pre-Meno females and Post-Meno females groups. In the current analysis the rows included activity for the following four event types of interest, nested within participant, nested within group: all encoding events during easy conditions (EncEasy), all encoding events during hard conditions (EncHard), all retrieval events during easy conditions (RetEasy), all retrieval events during hard conditions (RetHard). The columns of the fMRI data matrix contain mean-centred BOLD activity of all brain voxels at event onset and the subsequent 7 scans/lags, wherein each lag is equivalent to the T2*-weighted gradient echo-planar imaging

(EPI) sequence's TR or 2000 msec; thus capturing 16 sec of whole-brain activity following every event onset.

The behavioral data were stored in a separate matrix. The rows of the behaviour matrix are stacked in the same order as the fMRI data matrix The columns of the behavioral matrix included vectors of participants' behavioral data for: (1) standardized age (2) standardized mean spatial context accuracy and (3) standardized mean recognition accuracy. The behavior matrix was cross-correlated with the fMRI data matrix, and underwent singular value decomposition (SVD). SVD yields a series of orthogonal latent variables (LVs) that is equal to the number of groups-by-event-type/condition (2 groups × 4 event-type/conditions = 8 LVs). Each LV is composed of a singular value, a singular image, and a correlation profile. The singular value represents the proportion of covariance accounted for by the LV. The singular image includes positive and/or negative saliences which establish the weighted contributions of each voxel at a given time lag, producing a spatiotemporal pattern of whole-brain activity. Time lags are the number of TRs after the stimulus of interest is presented. The correlation profile portrayed the association between the pattern of brain activity from the singular image with the behavioral measures.

Permutation testing was conducted on the LVs to establish significance ($p < 0.05$; McIntosh et al., 2004). One thousand permutation tests were conducted within each participant and across conditions. Bootstrapping was used to measure the stability and 95% confidence intervals of activations identified in the singular image. Five hundred bootstraps were conducted and a bootstrap ratio for each voxel was computed by dividing the voxel salience by its estimated standard error. Voxels with a bootstrapping ratio of ± 3.28 ($p < 0.001$) within a cluster of at least 10 significant voxels were retained.

Temporal brain scores were calculated to obtain time lags with the strongest correlation profile for significant LVs from each PLS analysis. Temporal brain scores reveal the strength of the pattern of brain activity for each participant at each time lag. We retained only peak coordinates from time lags 3 to 5, which exhibited maximal effects related to the PLS effect. Identified peaks were converted to Talairach space using the icbm2tal transform (Lancaster et al., 2007), implemented in GingerAle 2.3(Eickhoff et al., 2009). Relevant Brodmann areas were established using the Talairach and Tournoux atlas (Talairach & Tournoux, 1988) and confirmed in FSL. Peak coordinates from the cerebellum and brainstem were not included as the fMRI acquisition in these regions was incomplete.

Post-hoc analyses

For significant PLS LVs, in which chronological age was significantly correlated with brain activity within specific condition(s) and/or menopause group(s), we used SPSS (Version 29, IBM Corporation) to conduct relevant linear regressions to determine the degree to which chronological age predicted the mean brain scores between lags 3 to 5 for the given task fMRI condition(s) and menopause group(s) in which a significant age-related correlation was observed in the LV i.e., EncEasy, EncHard, RetEasy, and RetHard. This helped determine the degree to which chronological age contributed to observed effects. In addition, post-hoc one-way ANOVAs were conducted to determine if there were significant group differences in significant LV brain scores based on female's history of antidepressant, HBC, and HRT use; and between females with compared to without PCOS. Significance was assessed at $p < 0.05$ corrected for 4 comparisons for the 4 conditions represented by each significant LV ($p < 0.0124$ uncorrected).

**Data and code availability**

Code (MATLAB, R) used for the present study is made publicly available on our Lab GitHub page (https://github.com/RajahLab/BHAMM_TaskfMRI_scripts). Readers seeking access to data should email the Principal Investigator of the BHAMM Study, Professor Maria Natasha Rajah (maria.rajah@mcgill.ca), for further information.

## Results

### Behavior Results

A total of thirteen participants were excluded post-data-collection: 1 participant due to incidental finding, 1 participant due to scanner technical issues, 8 participants for being behavioral outliers and/or task non-compliant, 2 participants due to excessive movement (> 15% of all scans with FD > 1mm) during MRI scanning and 1 participant was identified as a PLS brain scores-behaviour correlation outlier. A behavioral outlier was defined as an individual that had a Cook's D value which was 3 stand deviations from the group mean on 3 or more response types. A non-compliant responder was defined as someone who performed poorer than chance on the correct rejections and/or had zero spatial context retrieval responses.

Demographic and fMRI behavioral data separated by reproductive stage for the final sample (N = 83) are reported in Table 1. The age range for the 31 Pre-Meno females was 39.55 to 53.30 years, with an education range of 12 – 20 years. The age range for the 52 Post-Meno females was 46.70 to 65.14 years, with an education range of 11 to 20 years. Chi-square tests were conducted to determine if there were group differences in the proportion of antidepressant medication use, use of hormonal birth control (HBC, i.e., oral contraceptive, patch, intrauterine devices), use of hormone replacement therapies (HRT), or diagnosis of polycystic ovary syndrome (PCOS). There was a significant group difference in HRT use (p= 0.023), with Post-Meno females having 17.3% (N = 9) and Pre-Meno females having 0% of females reporting HRT use. One-way

ANOVAs were conducted on demographic variables (age, education, handedness, body mass index [BMI], BDI-II scores, BAI score), blood-based measures of sex hormone levels (E2, FSH, LH, P), and task fMRI behavioral data. There was a significant group difference in age, and hormone levels. Post-Meno females were significantly older than Pre-Meno females. Post-Meno females had significantly higher FSH, LH levels, and lower E2 and P, levels compared to Pre-Meno females; consistent with their menopause staging. Detailed analysis of the task fMRI analysis data is presented below.

Task fMRI Accuracy Results

The one-way ANOVAs for each of the accuracy responses indicated there was a group difference in CS easy and hard accuracy levels between group ($p<0.05$ corrected, $p<0.0035$ uncorrected). Table 1 presents the means and SE for all response types. The between-group RM-ANOVA for accuracy identified significant main effect of Difficulty ($F(1,80) = 11.23$, $p = 0.001$, $\eta^2_p = 0.123$). In addition, there was a significant Difficulty*Retrieval Response Type interaction ($F(1,80) = 10.24$, $p <0.001$, $\eta^2_p = 0.161$), and a significant Retrieval Response Type *Menopause Group interaction ($F(1, 80) = 10.06$, $p = 0.002$, $\eta^2_p = 0.112$). To determine what contributed to the significant Difficulty*Retrieval Response Type effect, we conducted post-hoc paired t-tests which indicated there was a significant effect of Difficulty on pCS ($t(81) = 6.78$, $p < 0.001$, two-tailed), but not pRecog ($t<1$, $p = 0.534$). The between group one-way ANOVAs (Table 1) for response types, indicate that significant Retrieval Response Types x Menopause Group interaction, was due to there being a significant group difference in pCS between Pre-Meno females and Post-Meno females groups, but not between pRecog. Pre-Meno females had significantly more correct spatial context retrieval responses, compared to Post-Meno females, in both easy and hard task conditions,

after adjusting for response biases. There was no significant main effect of Menopause Group overall (F(1,80) = 3.44 p = 0.067, $\eta^2_p$ = 0.04).

When the same analysis was conducted with Age (years) as a covariate, there was a significant main effect of Retrieval Response Type (F (1,79) = 8.14, p = 0.006, $\eta^2_p$ = 0.09) and Retrieval Response Type x Age interaction (F(1,79) = 8.99 p =0.004, $\eta^2_p$ = 0.102), and no other significant effects. Post-hoc univariate GLMs with Menopause Group defined as a fixed effect and Age defined as a covariate indicated the significant Retrieval Response Type x Age interaction effect was due to Age, but not Menopause Group (F<1), being a significant predictor of pCS hard (F(1, 79) = 5.12, p = 0.026, $\eta^2_p$ = 0.061) and Age, but not Menopause Group (F<1), showing a trend for pCS easy (F(1, 79) = 3.43, p = 0.068, $\eta^2_p$ = 0.041), and no significant Age or Menopause Group effects for pRecog easy or hard.

To clarify the relative contributions of Menopause Group and Age (years), we conducted two PLS regression analyses, one for CS easy accuracy and one for CS hard accuracy. For CS easy accuracy, the analysis identified one statistically significant LV ($p < .05$), which captured 93.73% of the variance in the predictors and 22.99% of the variance in accuracy. Both Menopause Group ($\beta = -0.046$, $p < .001$) and Age (years) ($\beta = -0.057$, $p < .001$) were negatively correlated with this LV. For CS hard accuracy, the analysis again identified one statistically significant LV ($p < .05$), which captured 93.69% of the variance in the predictors and 19.88% of the variance in accuracy. Both Menopause Group ($\beta = -0.034$, $p < .001$) and Age (years) ($\beta = -0.045$, $p < .001$) were negatively correlated with this LV.

Task fMRI RT Results

The between group one-way ANOVAs indicate there were no significant group differences in RT after correcting for multiple comparisons. Due to some high performing participants not

having any Misses, FAs, or too few correct Recog responses, we ran two separate between-group RM-ANOVAs for RT: one examining CS and CR RT, and another examining SM, Misses, FA, $FA_{spatial}$, and Recog RT. The first RM-ANOVA examining CS and CR identified significant main effects of Retrieval Response Type ($F(1,81) = 147.02$, $p < 0.001$, $\eta^2_p = 0.65$), Difficulty ($F(1,81) = 24.12$, $p < 0.001$, $\eta^2_p = 0.23$), and a marginally significant Menopause Group effect ($F(1,81) = 3.81$, $p = 0.054$, $\eta^2_p = 0.05$). In addition, there was a significant Retrieval Response Type x Menopause Group interaction ($F(1, 81) = 4.69$, $p = 0.033$, $\eta^2_p = 0.06$) and a significant Retrieval Response Type x Difficulty interaction ($F(1,81) = 4.57$, $p = 0.036$, $\eta^2_p = 0.05$). To clarify the Retrieval Response Type–x Difficulty interaction effect we ran post-hoc paired t-tests and observed a significant Difficulty effect for both CS ($t(82) = -4.67$, $p <0.001$) and CR ($t(82) = -3.07$, $p = 0.001$), but with CS RT exhibiting a stronger effect. To clarify the Retrieval Response Type x Menopause Group interaction effect we conducted post-hoc univariate GLMs with Menopause Group as a fixed effect. Post-Meno females females were significantly slower than Pre-Meno females females on CS easy ($F(1,81) = 7.51$, $p = 0.008$) and CS hard ($F(1,81) = 5.96$, $p = 0.017$) but there was no significant group difference in RT on CR Response Types ($F < 1$). The RM-ANOVA for CS and CR with Menopause Group as a between group factor, with Age (yrs) as a covariate identified a significant three-way interaction between Retrieval Response Type x Difficulty x Age ($F(1,80) = 5.06$, $p = 0.027$, $\eta^2_p = 0.06$), and a significant main effect of Age ($F(1,80) = 8.68$, $p = 0.004$, $\eta^2_p = 0.10$). Post-hoc univariate GLMs with Menopause Group defined as a fixed effect and Age defined as a covariate indicated the significant three-way interaction was due to older females being significantly slower on CS easy ($F(1,80) = 6.09$, $p = 0.016$), CS hard ($F(1,80) = 4.49$, $p = 0.037$), and CR hard ($F(1,80) = 7.07$, $p = 0.009$), but not being significantly slower on CR easy trials ($p > 0.05$).

The RM-ANOVA for SM, Misses, FA, $FA_{spatial}$ and Recog RT identified significant main effects of Retrieval Response Type ($F(4, 144) = 9.24$, $p < 0.001$, $\eta^2_p = 0.204$) and Difficulty ($F(1,36) = 6.17$, $p = 0.018$, $\eta^2_p = 0.146$). There was no significant Menopause group effect, and no significant interactions. Post-hoc paired samples t-tests indicated the significant Difficulty main effect was due to participants RTs being significantly different during easy, compared to hard, conditions for Recog ($t(80) = 3.22$, $p = 0.001$) only. There was no significant effect of difficulty on Misses RT ($p > 0.05$). Post-hoc two-tailed paired t-tests were conducted to compare mean RT, averaged between easy and hard levels, between Response Types to interpret the significant main effect of Retrieval Response Type. Results indicated this main effect was due to participants Recog RTs being significantly slower on compared to Miss trials ($t(72) = 4.81$, $p <0.001$), and faster compared to SM ($t(73) = -2.95$, $p = 0.004$) and FA trials ($t(59) = -5.12$, $p <0.001$). In addition, Miss RTs were also significantly faster than SM ($t(51) = -7.04$, $p <0.001$), FA ($t(51) = -6.06$, $p <0.001$), and $FA_{spatial}$ ($t(47) = -3.82$, $p < 0.001$) RTs; and FA RTs was significant slower than $FA_{spatial}$ RTs ($t(46) = 2.08$, $p = 0.043$). The RM-ANOVA for SM, Misses, FA, $FA_{spatial}$ and Recog with Menopause Group as a between group factor, with Age (yrs) identified no significant effects.

Mirroring the accuracy analysis, we additionally conducted two PLS regression analyses, one for CS easy mean RT and one for CS hard mean RT. Interestingly, for both CS easy and hard, no statistically significant LVs were identified ($p > .05$).

**fMRI results**

The B-PLS analysis was conducted on the N = 83 sample and identified two significant LVs. Post-hoc one-way ANOVAs indicated none of the effects reported differed based on female's history of antidepressant, HBC, and HRT use, nor based on female's history of PCOS.

Latent variable 1 (LV1, $p = 0.001$) accounted for 16.35% of cross-block covariance and primarily reflected group similarities and differences in performance-related activity at encoding and retrieval. Figure 2 displays the correlation profile and singular image for LV1, and Table 2 displays local maxima from lags 2–5. LV1 identified negative salience brain regions in lateral frontal, ventral occipito-temporal cortices, and midline cortical areas including cingulate, medial prefrontal, posterior cingulate, and precuneus. In both Pre-Meno and Post-Meno females greater encoding activity in these negative salience regions was correlated with higher subsequent spatial context memory accuracy, and lower recognition accuracy, though in Pre-Meno females this effect was only for the EncHard condition. Also, only in Post-Meno females activity in these same regions during the EncEasy condition correlated with age. Post-hoc within group linear regression confirmed that age was a significant predictor of EncEasy brain scores for Post-Meno females ($F(1,50) = 6.636$, $p = 0.013$, R2adjusted = 0.10, Standardized b = 0.342), but not Pre-Meno females ($F < 1$). Older Post-Meno females exhibited less activity in these regions during EncEasy and this was linked to lower subsequent spatial context accuracy and higher subsequent recognition for the easy condition. LV 1 also indicated that at retrieval, in Pre-Meno females more activity in these same regions correlated with higher spatial context memory accuracy and lower recognition accuracy across easy and hard conditions in only Pre-Meno females. In addition, in Pre-Meno females age was correlated with activity in these brain region during the RetHard condition. However, a post-hoc within group linear regression indicated that chronological age did not significantly predict RetHard brain scores in Pre-Meno females ($F(1,29) = 2.341$, $p = 0.137$) or Post-Meno females ($F(1,50) = 1.618$, $p = 0.209$). Also, Post-Meno females did not show significant correlations between retrieval activity in negative salience brain regions and

performance. Instead, increased activity in these brain regions during the RetEasy condition in Post-Meno females was associated with higher easy recognition accuracy.

LV2 ($p = 0.011$) accounted for 10.57% of cross-block covariance (see Figure 3, Table 3) and reflected group differences in age- and accuracy-related brain activity. This LV identified negative salience brain regions that included: bilateral inferior parietal sulcus, cuneus, and precuneus. The correlation profile (Figure 3A) indicates that in Pre-Meno females more activity in these brain regions across encoding and retrieval conditions was related to lower spatial context accuracy and higher recognition accuracy, with the exception of the EncHard condition. In addition, in Pre-Meno females this effect was correlated with age during EncEasy, this was confirmed by post-hoc linear regression of chronological age on brain scores in Pre-Meno females ($F(1,29) = 6.064$, $p = 0.020$, R2adjusted = 0.144, Standardized b = -0.416). Thus, older Pre-Meno females exhibited greater activity in negative salience brain regions during EncEasy, which was also correlated with higher subsequent recognition accuracy and lower subsequent spatial context retrieval accuracy during in Pre-Meno females. In contrast, in Post-Meno females more activity in these same regions across encoding and retrieval conditions was associated with higher spatial context accuracy and lower recognition accuracy, with the exception of the RetEasy condition. In addition, in Post-Meno females this effects was correlated with age across all conditions. Post-hoc linear regressions of age on brain scores confirmed the significant chronological age effect for Post-Meno females across all conditions (EncEasy $F(1, 50) = 26.42$, $p < 0.001$, R2adjusted = 0.333, Standardized b = 0.588; EncHard $F(1,50) = 21.639$, $p < 0.001$, R2adjusted = 0.288, Standardized b = 0.550; RetEasy $F(1,50) = 5.408$, $p = 0.024$, R2adjusted = 0.08, Standardized b = 0.312; RetHard $F(1,50) = 5.707$, $p = 0.021$, R2adjusted = 0.085, Standardized b = 0.320). Thus, older Post-Meno females exhibited lower activity in negative salience brain regions across all conditions, which

was also correlated with higher recognition accuracy and lower spatial context retrieval accuracy. The linear regressions also suggest that the age correlations were stronger at encoding compared to retrieval in Post-Meno females.

## Discussion

The goal of the current study was to investigate how menopause status and chronological age affected episodic memory for face-location association (spatial context memory) and its functional neural correlates in a sample of Pre-Meno females and Post-Meno females middle-aged females. In past studies, chronological age was controlled for by matching the age of Pre- and Post-Meno females to isolate menopause effects on memory-related brain function from chronological age effects (Jacobs et al., 2016; Rentz et al., 2017). However, it is arguable that age matching Pre- and Post-Meno females results in non-representative sampling that may bias results, e.g., surgical menopause at an early age is associated with higher dementia risk (Georgakis et al., 2019; Gervais et al., 2020), and older age of menopause has been associated with lower brain volume (Ambikairajah et al., 2020). Other clinical neuroimaging studies examining structural, functional, and metabolic effects of menopause on the brain, have accounted for chronological age differences in Pre- and Post-Meno females by covarying out the effect of age and enrolling age-matched males with the assumption that chronological age effects would be similar in both sexes (Mosconi et al., 2021). However, it is likely that chronological and endocrine aging interact (Ambikairajah et al., 2020; Gervais et al., 2022). Therefore, here, we broadly sampled Pre- and Post-Meno females across a large midlife window (39.5 – 65. 14 years) so that there was a chronological age difference in the two groups and used multivariate data driven B-PLS analysis to understand how chronological and endocrine aging interactively and/or independently contribute to brain health and memory function in middle-aged females.

Our behavioral analysis indicated that increasing task demands had comparable effects in middle-aged Pre- and Post-Meno females. This suggests that both groups were similarly affected by increases in cognitive effort and processing resources (Meulenbroek et al., 2010). However, Post-Meno females exhibited lower accuracy and slower RT on easy and hard spatial context memory accuracy compared to Pre-Meno females. Post-Meno females also had lower pCS compared to Pre-Meno females, indicating even after controlling for potential differences in response bias, Post-Meno females had lower spatial context retrieval accuracy. Interestingly, when chronological age was included as a covariate in the behavioral analyses, it accounted for the aforementioned effects, with comparable effect sizes, and menopause status was no longer significant. This suggests that spatial context memory decline in middle-aged females was associated with chronological age, consistent with prior cognitive aging research (Ankudowich et al., 2016; Cansino, 2009). However, it remains possible that menopause status and chronological age interactively affected the underlying functional neural correlates of episodic memory at midlife (Becker & Koob, 2016; Mosconi et al., 2017). To address this possibility, we conducted a between group task fMRI brain-behavior PLS analysis in which we examined how brain activity during episodic encoding and retrieval correlated with standardized age, spatial context accuracy, and item accuracy in Pre-Meno females compared to Post-Meno females females.

The PLS analysis identified two significant LVs. The first LV (LV 1) identified group similarities and differences in the correlations between brain activity and memory performance, with a minimal influence of chronological age on observed affects. In contrast, the second LV (LV 2) only identified group differences in correlations which were also correlated with chronological age, particularly in Post-Meno females. Importantly, post-hoc analyses indicated that the

significant B-PLS LV effects were not significantly different between females' with, compared to without, a history of antidepressant, HBC, and HRT use, nor having a history of PCOS.

Regarding group similarities, LV 1 indicated that in both groups increased encoding activity in medial PFC, cingulate, precuneus, lateral PFC, and ventral occipitotemporal cortices, was positively correlated with subsequent memory for spatial context. The medial PFC, cingulate and precuneus regions are part of the default mode network (DMN (Greicius et al., 2003; Power et al., 2010)) – brain regions that exhibit more activity during task-free states, and are also more active during successful episodic encoding and contextual recollection (Ankudowich et al., 2017; de Chastelaine et al., 2016; Kim, 2010;Rugg & Vilberg, 2013). In addition, increased lateral PFC and ventral occipitotemporal activity has been consistently observed in episodic context memory studies (Mitchell & Johnson, 2009; Rajah et al., 2008; Rajah et al., 2010) and was also reported by Jacobs et al. (2016) and Rentz et al. (2017), who found that individual differences in lateral PFC activity during episodic memory encoding directly correlated with subsequent memory in Pre- and Post-Meno females and in males (Jacobs et al., 2016). Interestingly, chronological age was not generally correlated with the observed brain-behavior associations, with one exception: there was a significant correlation between age and easy encoding activity in Post-Meno females. Older Post-Meno females exhibited less in these brain regions during only the easy encoding condition, which was also correlated with poorer subsequent spatial context memory. However, since there was no significant age effect in Post-Meno females during the hard encoding condition nor the retrieval conditions, this suggests LV 1 effects in Post-Meno females were not reflective of general chronological age affect. Instead, the significant chronological age effect in Post-Meno females during the easy encoding condition may reflect age differences in the neurocognitive strategy employed. This interpretation aligns with past cognitive aging research indicating there

are shifts in self-initiated strategy implementation in older adulthood (Craik & Rose, 2012; Segen et al., 2022).

To our knowledge this is the first task fMRI study to compare memory-related brain activity during the retrieval of item and associative spatial context memory in middle-aged Pre- and Post-Meno females. In Pre-Meno females, individual differences in retrieval activity in medial PFC, cingulate, precuneus, lateral PFC, and ventral occipitotemporal cortices (LV 1) during both easy and hard tasks correlated with higher spatial context memory accuracy. Moreover, in Pre-Meno females this pattern was not correlated with age. Post-Meno females did not show this pattern of brain-behavior correlation at retrieval. This suggests that spatial context memory reductions observed in Post-Meno females may partially be due to a failure to re-instate DMN, lateral PFC, and occipitotemporal cortices activity observed during encoding, at retrieval (Morcom, 2014; Wing et al., 2015). Importantly, this retrieval effect in Post-Meno females was not correlated with age; and suggests a role for endocrine aging on cortical reinstatement during memory paradigms.

LV 2 from the brain-behavior PLS analysis identified group differences in the correlation between brain activity in bilateral inferior parietal cortex, precuneus, and dorsal occipital cortex with age and memory performance. In Pre-Meno, females that exhibited increased activity in these brain regions at encoding and retrieval exhibited lower spatial context retrieval and higher item retrieval. Moreover, older Pre-Meno females exhibited more activity in these regions and had lower spatial context memory during easy conditions. The opposite performance and age correlations were observed in Post-Meno females. Post-Meno females that exhibited greater activation of bilateral dorsal visual regions and inferior parietal cortex had higher spatial context memory accuracy, lower recognition memory accuracy, and were younger. Thus, LV2 identified

group differences in the direction of correlations between brain activity and age and performance, consistent with the idea that endocrine aging may introduce non-linearities in the effect of chronological age on memory-related brain function. These group differences in brain activity may reflect the use of different neurocognitive strategies to support spatial context memory in Post- compared to Pre-Meno females (Ankudowich et al., 2017; Dulas et al., 2011).

Interestingly, in the previous study by Jacobs et al. (2016) menopause status and endocrine function were not associated with differences in parietal cortex. Indeed, only males in this study exhibit memory-related differences in posterior parietal (BA 7) function. Here, we observe menopause effects in both posterior and inferior parietal function, with the former being evident at encoding and the latter being evident at both encoding and retrieval. It is possible that including males in prior studies obscured the ability to detect group differences between Pre-Meno and Post-Meno females in prior work (Jacobs et al., 2016; Jacobs et al., 2017). If so, the current study highlights the importance of conducting female only studies of cognitive neuroscience and aging to discern the effects of chronological and endocrine aging on memory-related brain function.

In conclusion, the current results indicate that endocrine age and chronological age interact and affect the functional neural correlates of episodic memory in middle-aged females. Menopause status directly affected the direction of age- and performance-related correlations with brain activity in inferior parietal, caudate, and dorsal occipital cortices across encoding and retrieval. Moreover, we found that memory-related reductions in Post-Meno females status was associated with reduced activity in DMN, PFC and ventral visual at retrieval, which was not related to chronological age. Future studies should examine how individual differences in the severity of menopause-related symptoms at peri- and early post-menopause (e.g., sleep disruptions, altered vascular function, and inflammation) affect memory and brain function.

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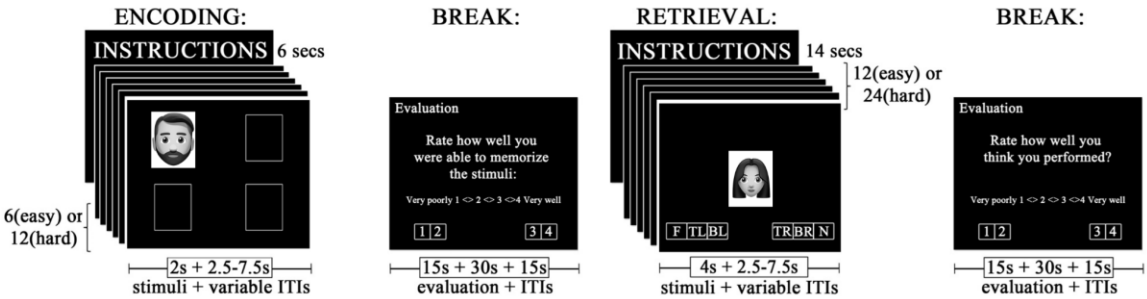
Figures

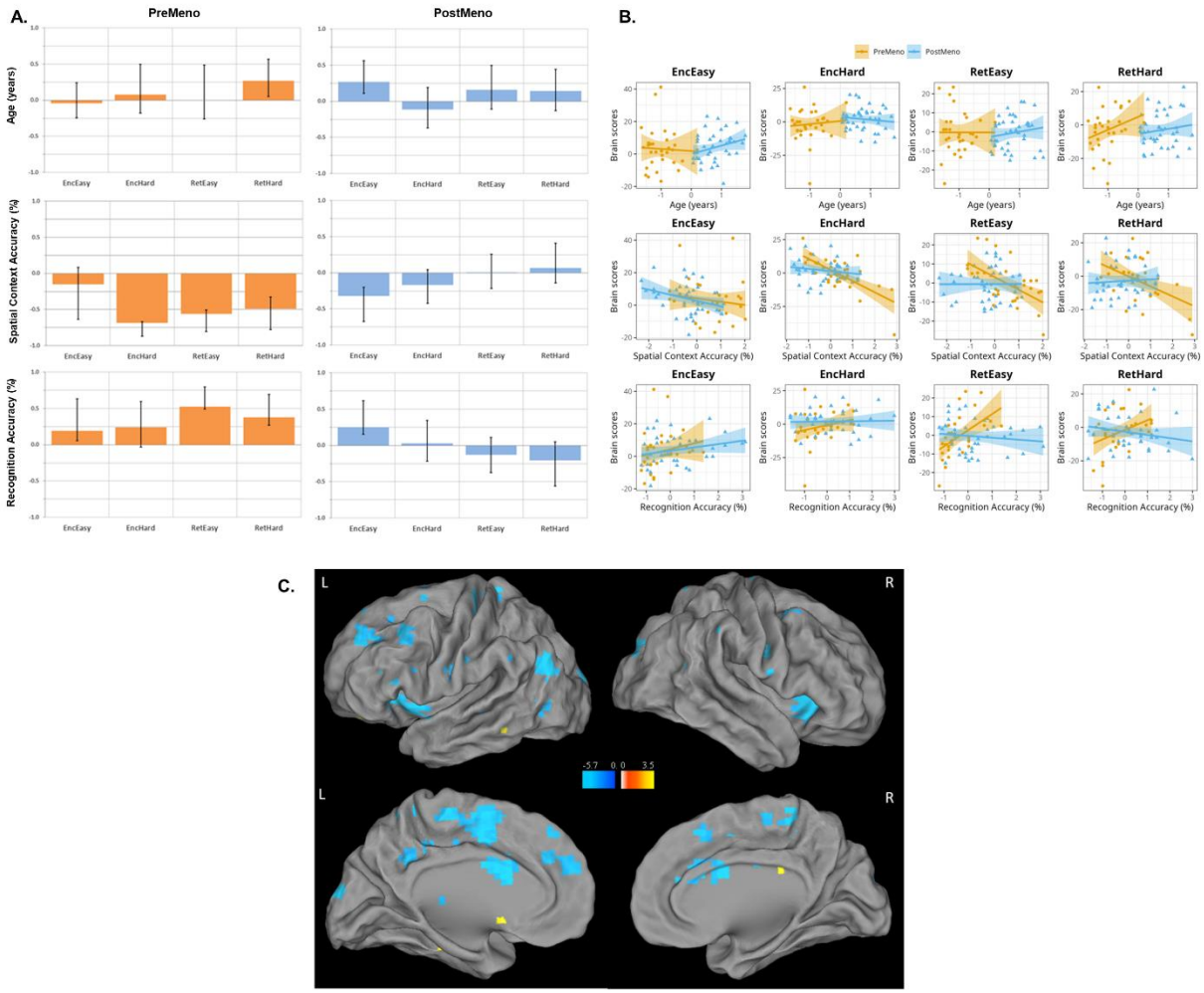
1. Figure 1: Task fMRI Paradigm Design

   Research participants were asked to encode face-location associations and make a pleasant/neutral decision for face stimuli presented in one of four quadrants. There was a 1 minute interval between encoding and retrieval. During retrieval participants were presented with an equal number of previously encoded ("old") and novel face stimuli in the centre of the screen and asked to make a 6-alterantive forced choice decision. Details about the paradigm are presented in the Materials & Methods section.



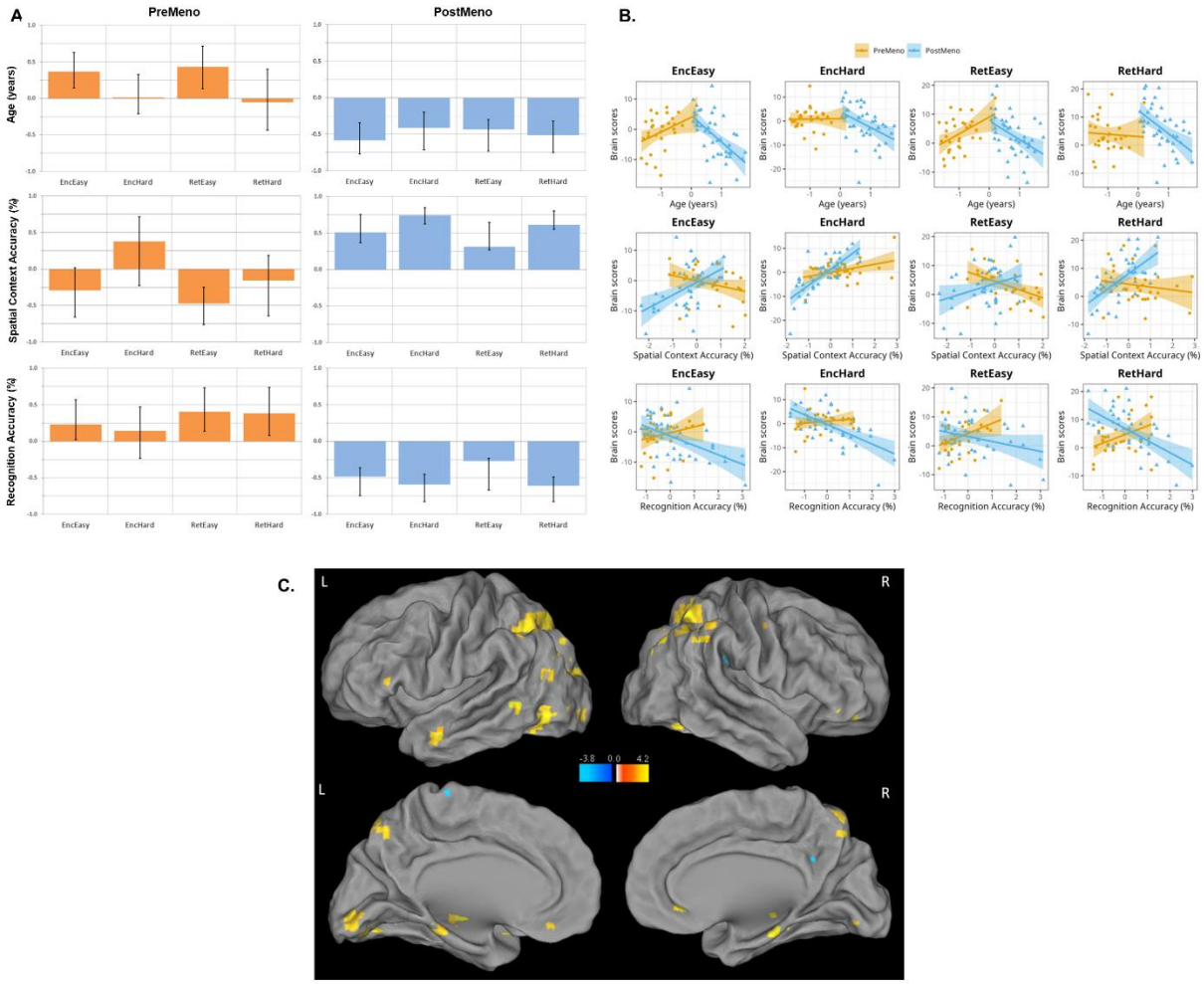

2. Figure 2: Brain-behavior PLS Results – LV 1. A) The brain-behaviour correlation profiles for LV1 depicting how chronological age, spatial context retrieval accuracy, and at item retrieval accuracy correlated with activity in regions identified inf the singular image. The bars represent the correlation values between age, spatial context retrieval accuracy, and recognition accuracy and the brain activity pattern identified by the symmetrically paired singular image. The whiskers on the bars reflect the 95% confidence intervals (CI) based on the bootstrap results. Bars in which the whiskers cross zero indicate that correlation effect did not significantly contribute to the results; B) The singular image for LV1, threshold bootstrap ratio of ± 3.28, $p < 0.001$. Only negative salience brain regions were identified and are coloured in blue. The figure highlights regions identified between TR/Lags 3 – 5 based on the

temporal brain scores. The scale colour codes effects according to the strength of BSR. Interpretation of this LV is presented in the Results section. The singular image is presented on template images of the lateral and medial surfaces of the left and right hemispheres of the brain using Caret software v5.65 (http://brainvis.wustl.edu/john/caret5).




3. Figure 3: Brain-behavior PLS Results – LV 2. A) The brain-behaviour correlation profiles for LV 2 depicting how chronological age, spatial context retrieval accuracy, and at item retrieval accuracy correlated with activity in regions identified inf the singular image; B) The singular image for LV2, threshold bootstrap ratio of ± 3.28, $p < 0.001$. Only negative salience brain regions were identified and are coloured in blue. The figure highlights regions identified

between TR/Lags 3 – 5 based on the temporal brain scores. The scale colour codes effects according to the strength of BSR. The singular image is presented on template images of the lateral and medial surfaces of the left and right hemispheres of the brain using Caret software v5.65 (http://brainvis.wustl.edu/john/caret5). Interpretation of this LV is presented in the Results section.



Tables

Table 1: Demographic Information and fMRI Behavioural Measures by Reproductive Group. Handedness assessed using Edinburgh Handedness Inventory. Antidepressant use based on self-report. BMI = body mass index; BDI-II = Beck Depression Inventory-II; BAI = Beck Anxiety Inventory; CVLT = California Verbal Learning Test; LFR = Long-form Free Recall; LCR = Long-form Cued Recall; RG = Recognition; HBC = Hormone-Based Contraceptive; HRT = Hormone Replacement Therapy (% on each medication); PCOS (%) = Polycystic ovarian syndrome (% with PCOS diagnosis). Due to detection thresholds of the ELISA used, E2 was measurable in 12 out of 48 Post-Meno females who donated blood for hormonal measures, all other hormone measures in Post-Meno females are the average from 48. For Pre-Meno females all measures are based on 28 of 31 who donated blood for hormonal analysis. CS = Correct Spatial Context Retrieval; CR = Correct Rejection; ACC = Accuracy; RT = Reaction Time. Accuracy values are shown as proportion correct per task type with standard error (SE). Reaction time values are shown in milliseconds (msec) per task type. SPSS (Version 29, IBM Corporation) was used to conduct Chi-square tests for demographics, and one-way between-group ANOVAs for hormones, and fMRI Behavioral measures to test for group differences. For the fMRI Behavioral significance was assessed at $p<0.05$ corrected for 20 multiple comparisons ($p<0.0025$ uncorrected) for accuracy. For RT, measures are only possible for 7 of the response types and corrections were for 14 response types, $p<0.0035$ uncorrected or $p<0.05$ corrected.

Table 1: Demographic Information and fMRI Behavioural Measures by Reproductive Group

| | Premenopausal | Postmenopausal |
|---|---|---|
| Sample size (*n*) | 31 | 41 |
| Age (years)* | 44.28 (0.57) | 57.56 (0.56) |
| Education (years) | 16.34 (0.40) | 15.21 (0.40) |

| | | |
|---|---|---|
| BMI (kg/m$^2$) | 25.14 (1.00) | 25.45 (0.63) |
| BDI-II | 4.10 (0.71) | 6.10 (0.87) |
| BAI | 4.52 (0.82) | 4.07 (0.74) |
| Handedness (% right) | 90.32 (2.56) | 87.07 (4.80) |
| Antidepressant (%) | 22.58 | 19.51 |
| HBC (%)* | 12.90 | 0.00 |
| PCOS (#) | 3 | 2 |
| E2 (pmol/L)* | 355.61 (42.44) | 35.23 (3.10) |
| FSH (IU/L)* | 9.19 (1.68) | 85.69 (4.45) |
| LH (IU/L)* | 6.60 (1.05) | 34.22 (1.95) |
| P (nmol/L)* | 17.52 (3.49) | 1.54 (0.15) |
| % CS easy Accuracy* | 63.91 (3.61) | 44.78 (2.86) |
| % Recog easy Accuracy* | 13.17 (1.87) | 22.68 (2.71) |
| % CS hard Accuracy* | 43.08 (3.19) | 28.42 (2.25) |
| % Recog hard Accuracy* | 25.34 (2.50) | 33.30 (2.90) |
| CS easy RT (msec)* | 2429.81 (82.32) | 2757.01 (84.94) |
| Recog easy RT (msec) | 3011.28 (113.74) | 3047.63 (125.06) |
| CS hard RT (msec)* | 2595.57 (76.04) | 2936.40 (86.12) |
| Recog hard RT (msec) | 2889.18 (105.49) | 2762.19 (99.14) |

Note: Significant p-value ($p < 0.05$) for between-group analysis using two-sided Chi-square or T-tests marked with '*'.

Table 2: Local maxima of negative and positive saliences identified in LV 1. Temporal lag represents the time after event onset when a cluster of voxels showed an effect of interest. The temporal brain score identified lags 3 – 5 as maximally representing the LV effects identified by the PLS. The bootstrap ratio (BSR) threshold was set to $\pm > 3.28$ ($p < 0.001$) and identified dominant and stable activation clusters. The spatial extent refers to the total number of voxels included in the voxel cluster (threshold = 10). The stereotaxic coordinates (measured in

millimeters), gyral location and Brodmann areas (BAs) were determined by referring to Talaraich and Tournoux (1998). HEM = Cerebral hemisphere in which the activation of interest occurred.

Table 2: Local maxima of negative and positive saliences identified in LV 1

| Temporal lag | BSR | Cluster size | Talairach Coordinates | | | Gyral location | BA |
|---|---|---|---|---|---|---|---|
| | | | x | y | z | | |
| **Negative Saliences** | | | | | | | |
| *Left Hemisphere* | | | | | | | |
| 4 | -6.03 | 39 | -34 | 30 | 25 | Middle Frontal Gyrus | 9 |
| 5 | -5.90 | 86 | -23 | 19 | 27 | Medial Frontal, Cingulate Gyrus | 9, 32 |
| 3 | -5.68 | 44 | -42 | -72 | 29 | Middle Temporal Gyrus | 39 |
| 5 | -5.31 | 130 | -31 | 24 | 6 | Insula, Inferior Frontal Gyrus | 13, 47 |
| 3 | -5.23 | 76 | -23 | 44 | 37 | Superior Frontal Gyrus | 9 |
| 4 | -4.93 | 32 | -5 | 11 | 23 | Anterior Cingulate | 33 |
| 5 | -4.91 | 126 | -9 | -6 | 47 | Cingulate Gyrus | 24 |
| 4, 5 | -4.68 | 17 | -9 | -89 | 10 | Cuneus | 17, 18 |
| 3 | -4.66 | 10 | -49 | 21 | 2 | Inferior Frontal Gyrus | 47 |
| 3 | -4.56 | 22 | -39 | 17 | 45 | Middle Frontal Gyrus | 8 |
| 5 | -4.54 | 11 | -24 | -26 | 59 | Precentral Gyrus | 4 |
| 4 | -4.53 | 12 | -24 | 7 | 30 | Precentral Gyrus | 6 |
| 3 | -4.11 | 12 | -57 | -11 | 27 | Precentral Gyrus | 4 |
| 3 | -4.08 | 20 | -9 | -50 | 32 | Precuneus, Cingulate Gyrus | 31 |
| 5 | -3.90 | 10 | -16 | -42 | 36 | Cingulate Gyrus | 31 |
| *Right Hemisphere* | | | | | | | |
| 5 | -6.02 | 103 | 29 | 17 | 6 | Claustrum | |
| 5 | -5.40 | 19 | 28 | 7 | 31 | Middle Frontal Gyrus | 9 |
| 4 | -4.97 | 70 | 28 | 15 | 24 | Medial Frontal, Cingulate Gyrus | 9, 32 |
| 5 | -4.59 | 48 | 10 | 22 | 32 | Cingulate Gyrus | 32 |
| 3 | -4.58 | 41 | 39 | -12 | 33 | Precentral Gyrus | 6 |
| 3 | -4.04 | 19 | 13 | -82 | 15 | Cuneus | 17, 18 |
| 5 | -4.00 | 14 | 6 | 16 | 53 | Superior Frontal Gyrus | 8 |
| 4 | -3.90 | 13 | 17 | -10 | 50 | Medial Frontal Gyrus | 6 |

Table 3: Local maxima of negative and positive saliences identified in LV 2. Temporal lag represents the time after event onset when a cluster of voxels showed an effect of interest. The temporal brain score identified lags 3 – 5 as maximally representing the LV effects identified by the PLS. The bootstrap ratio (BSR) threshold was set to ± > 3.28 ($p < 0.001$) and identified dominant and stable activation clusters. The spatial extent refers to the total number of voxels included in the voxel cluster (threshold = 10). The stereotaxic coordinates (measured in millimeters), gyral location and Brodmann areas (BAs) were determined by referring to Talaraich and Tournoux (1998). HEM = Cerebral hemisphere in which the activation of interest occurred.

Table 3: Local maxima of negative and positive saliences identified in LV 2

| Temporal lag | BSR | Cluster size | Talairach Coordinates | | | Gyral location | BA |
|---|---|---|---|---|---|---|---|
| | | | x | y | z | | |
| **Positive Saliences** | | | | | | | |
| *Left Hemisphere* | | | | | | | |
| 5 | 4.97 | 101 | -39 | -59 | 49 | Superior/Inferior Parietal Lobule | 7, 40 |
| 3 | 4.89 | 37 | -31 | -80 | -4 | Inferior Occipital Gyrus | 18 |
| 4 | 4.80 | 55 | -23 | -20 | -5 | Parahippocampal Gyrus | 28 |
| 5 | 4.39 | 79 | -12 | -87 | -11 | Lingual Gyrus | 18 |
| 5 | 4.37 | 27 | -42 | -61 | -6 | Middle Temporal Gyrus, Fusiform Gyrus | 37 |
| 3 | 4.15 | 30 | -24 | -82 | 14 | Cuneus, Middle Occipital Gyrus | 17, 19 |
| 5 | 4.17 | 51 | -35 | -71 | 19 | Middle Temporal Gyrus | 39 |
| 3 | 4.09 | 25 | -31 | -76 | 29 | Superior Occipital Gyrus | 19 |
| 4 | 4.09 | 10 | -34 | 16 | 20 | Insula | 13 |
| *Right Hemisphere* | | | | | | | |
| 3 | 4.84 | 62 | 10 | -22 | -16 | Culmen, Parahippocampal Gyrus | 35 |
| 5 | 4.53 | 175 | 46 | -44 | 48 | Inferior Parietal Lobule | 40 |
| **Negative Saliences** | | | | | | | |
| *Right Hemisphere* | | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 3 | -4.41 | 45 | 28 | -23 | 24 | Insula | 13 |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