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Abstract

Compared to men, women are disproportionately affected by Alzheimer's disease (AD) and have an accelerated trajectory of cognitive decline and disease progression. Neurobiological factors underlying gender differences in AD remain unclear. This study investigated brain beta-amyloid (Aβ)-related neural system differences in cognitively normal older men and women (N = 61, 41 females, 65 – 93 years old). We found that men and women showed different associations between Aβ load and hippocampal functional connectivity. During associative memory encoding, in men greater Aβ burden was accompanied by greater hippocampus-prefrontal connectivity (i.e., more synchronized activities), whereas in women hippocampal connectivity did not vary by Aβ burden. For resting-state data, the interaction of gender x Aβ on hippocampal connectivity did not survive multiple comparison in the whole-brain analyses. In the ROI-based analyses, resting-state hippocampal-prefrontal connectivity was positively correlated with Aβ load in men and was negatively correlated with Aβ load in women. The observed Aβ-related neural differences may explain the accelerated trajectory of cognitive decline and AD progression in women.

Keywords: Alzheimer's disease, gender, beta-amyloid, functional connectivity, neural compensation
**Abbreviations:**
Alzheimer’s disease – AD  
beta-Amyloid – Aβ  
functional magnetic resonance imaging – fMRI  
default mode network – DMN  
generalized psychophysiological interactions – gPPI  
mild cognitive impairment – MCI  
apolipoprotein E – ApoE  
Pittsburgh Compound-B – PiB  
echo-planar imaging – EPI  
repetition time – TR  
echo time – TE  
T1-weighted – T1w  
magnetization-prepared rapid gradient echo sequence – MPRAGE  
Montreal Neurological Institute – MNI  
averted image registration – AIR  
standardized uptake value ratio – SUVR  
Mini–Mental State Examination - MMSE
Introduction

Beta-Amyloid (Aβ) plaques are one of the key pathological hallmarks of Alzheimer’s disease (AD). The accumulation of Aβ in the brain starts very early in the cascade of AD, and there is a prolonged delay, approximately 10 to 20 years, until the onset of clinical dementia (Jack et al., 2010; Perrin et al., 2009; Sperling et al., 2011). Consistent with this, Aβ plaque pathology is frequently observed in the brains of older adults without cognitive impairment at autopsy (Bennett et al., 2006) or in vivo using positron emission tomography (PET) amyloid imaging (Aizenstein et al., 2008; Mintun et al., 2006).

Much research has focused on processes that allow elderly adults to maintain normal cognition despite Aβ plaques. Studies have demonstrated that brain activation and functional connectivity are associated with brain Aβ burden in cognitively unimpaired older adults. During episodic memory, increased brain activation in task-positive regions (Edelman et al., 2017; Elman et al., 2014; Mormino et al., 2012; Oh and Jagust, 2013; Vannini et al., 2012) and reduced deactivation in task-negative regions (e.g., default mode network -DMN) (Huijbers et al., 2014; Sperling et al., 2009; Vannini et al., 2012) were associated with elevated Aβ burden. At rest, both increased (Lim et al., 2014; Mormino et al., 2011) and decreased (Hedden et al., 2009; Huijbers et al., 2014; Mormino et al., 2011; Sheline et al., 2010) functional connectivity in the DMN network were found with increased Aβ burden. Further, most recent studies suggested nonlinear relationships between brain activation during spatial distance judgement and brain Aβ burden (Foster et al., 2018), and between resting-state functional connectivity and brain Aβ burden (Schultz et al., 2017).
Gender is an important factor associated with AD risk and progression. Compared to men, women are disproportionately affected by AD (Carolyn M Mazure, 2016) and two thirds of individuals living with AD are women (Hebert et al., 2013; Alzheimer's Association, 2017). Although men may have a greater risk of mild cognitive impairment (MCI) (Petersen et al., 2010; Roberts et al., 2012), women with mild cognitive impairment (MCI) deteriorate in cognition almost twice as fast as men with MCI (Holland et al., 2013; Lin et al., 2015; Mielke et al., 2014), suggesting a faster rate of disease progression in women versus men. In addition, genetic studies have shown that the APOE ε4 variant confers greater risk effects on women relative to men (Bretsky et al., 1999; Farrer et al., 1997; Payami et al., 1994; Poirier et al., 1993).

Neurobiological factors underlying gender differences in AD remain unclear. The current study investigates whether there are gender differences in the association between brain Aβ burden and hippocampal functional connectivity (gender x Aβ) during associative encoding and at resting state in older adults without cognitive impairment. Given gender differences in the prevalence, incidence and progression of AD, we hypothesized that cognitively normal older men and women would show different Aβ-related alterations in hippocampal functional connectivity. We also hypothesized that these gender differences in relations between Aβ load and hippocampal functional connectivity would be greater in the context of a challenging associative memory task, and would persist at resting state but with a moderate effect size.

Methods

Participants
Sixty-one cognitively normal older adults were included in this study. Participants were between the ages of 65 and 93 years (mean ± standard deviation: 75.8 ± 6.4) and 41 (67.2%) were female. Inclusion criteria included 65 years or older, fluency in English, and a minimum of 12 years of education. Exclusion criteria included: a) diagnosis of MCI or dementia, b) history of a major psychiatric or neurological condition; c) an unstable medical condition that could affect cognition; d) visual, auditory, or motor deficits sufficient to impair ability to perform the tests; e) medications affecting cognitive performance; f) MR-related contraindications: presence of any metallic implant, claustrophobia, pregnancy, or excessive weight. All participants were evaluated in multiple cognitive domains including memory, visuospatial construction, language, and attention and executive functions to ensure cognitive normality using a comprehensive neuropsychological testing battery nearly identical to that used by the University of Pittsburgh Alzheimer Disease Research Center. Detailed descriptions of the testing battery and diagnostic criteria for MCI or dementia were previously described (Edelman et al., 2017). Individuals who met the criteria for MCI or dementia were excluded from this study. See Table 1 for summarized demographic and clinical characteristics, and Table 2 for summarized neuropsychological scores of the sample by gender and amyloid load group [PiB(+) and PiB(-)] (Pittsburgh Compound-B). The Human Use Subcommittee of the Radioactive Drug Research Committees and the institutional review board of the University of Pittsburgh approved all studies. The collected data were previously reported in Edelman et al. (Edelman et al., 2017) which focused on brain activation changes in preclinical AD, and in Nebes et al. (Nebes et al., 2013) which focused on the effects of amyloid burden, white matter hyperintensities and normal aging on cognitive performance, but neither examined the effects of gender and amyloid burden on hippocampal functional connectivity.
Image Acquisition and Processing

**PiB PET imaging**

$[^{11}\text{C}]$PiB was synthesized by a simplified radiosynthetic method based on the captive solvent method (Wilson et al., 2000). Fifteen mCi of $[^{11}\text{C}]$PiB with high specific activity (~2.1 Ci/µmol at end of synthesis (EOS)) was injected intravenously over 20 seconds. A 20-min PiB PET scanning was performed (4 x 300 second frames) beginning 50 minutes after the $[^{11}\text{C}]$PiB injection. The PET scanning was conducted using a Siemens/CTI ECAT HR+ scanner (Siemens Medical Solutions, Knoxville, TN) in 3D imaging mode: 63 axial slices, slice thickness = 2.4mm, field of view (FOV) = 15.2cm, intrinsic in-plane resolution = 4.1 mm full-width at half-maximum (FWHM) at FOV center). The scanner is equipped with a neuro-insert to reduce the contribution of scattered photons. PET emission data was reconstructed using filtered back projection with corrections for attenuation, scatter, and radionuclide decay.

**MRI acquisition**
All MR scanning was performed on a 3T Siemens Trio scanner with 12-channel head coil at the University of Pittsburgh Magnetic Resonance Research Center. Whole-brain functional MR (fMRI) data were acquired axially using gradient-echo echo-planar imaging (EPI) sequence with the following parameters: repetition time (TR) = 2 s, echo time (TE) = 32 ms, flip angle = 90°, FOV = 256 x 256 mm², acquisition matrix 128 x 128, slice thickness/gap = 4/0 mm (voxel size = 2 x 2 x 4 mm³), 28 axial slices. T1-weighted structural images were acquired in the axial plane using a magnetization-prepared rapid gradient echo sequence (T1w MPRAGE) with the following parameters: TR = 2 ms, TE = 3.4 ms, flip angle = 9°, FOV = 240 x 256 mm², matrix = 240 x 256, slice thickness/gap = 1/0 mm (voxel size = 1 x 1 x 1 mm³), 160 slices and generalized autocalibrating partially parallel acquisitions (GRAPPA) acceleration factor = 2. T1w MPRAGE images were used to facilitate and improve the normalization of fMRI data into the Montreal Neurological Institute (MNI) template space. Resting state fMRI data (5 mins) were collected and participants were instructed to fixate on a central crosshair and to stay awake during image acquisition. Task fMRI data were collected while participants were performing a face-name memory associative memory task. The face-name memory encoding task is a mixed block/event-related design task. Each run of the task lasted 4 mins 36 secs, consisting of 2 experimental blocks and 2 control blocks, interspersed with 25-sec fixation. Each block lasted 48 secs, containing 8 sequential trails of face-name pairs, 5 secs each trial with 1 sec inter-trial interval. During the experimental blocks, participants were presented novel face-name pairs and were asked to subjectively decide and respond with an MR-compatible glove whether or not each name was a good fit for the face. This subjective decision was designed to strengthen the associative encoding of the face-name pairs (Sperling, Chua, et al., 2003). For the control blocks, two familiar face-name pairs, one female face-name pair and one male face-name pair, were
repeatedly presented to the participants. The participants were trained and thus familiar with these two face-name pairs in the pre-scan session. Of the 61 participants, 44 participants completed three runs of the face-name task, 11 completed two runs, and 4 completed one run.

**PET data processing**

The dynamic [11C]PiB acquisition frames are inspected for evidence of inter-frame motion. If suspected, the automated image registration (AIR) algorithm with parameters optimized for PET to PET registration is applied to the dynamic [11C]PiB images on a framewise basis to correct for inter-frame motion (Woods *et al.*, 1993). A summed PET image is then generated over the 50-70-minute post-injection interval. Structural T1w MPRAGE MR image was reoriented such that the axial image planes are parallel to the plane intersecting both the anterior and posterior cerebral commissure (AC-PC). The AC-PC aligned MPRAGE MR image was then co-registered with the summed [11C]PiB image using the AIR algorithm (PET-MR) (Woods *et al.*, 1993). The resulting PET-MR spatial transformation was applied to the summed [11C]PiB image, which was resliced into the AC-PC aligned MPRAGE image space.

A set of volumes of interests (VOIs), as previously defined (Cohen *et al.*, 2009), were separately hand-drawn on the AC-PC aligned MPRAGE MR image, which include frontal cortex (FRC; ventral and dorsal), anterior cingulate gyrus (ACG: subgenual and pregenual), anteroventral striatum (AVS), mesial temporal cortex (includes hippocampus and amygdala), precuneus/posterior cingulate cortex (PRC; ventral, middle and dorsal thirds), parietal cortex (PAR), lateral temporal cortex (LTC), occipital cortex (OCC; calcarine and pole), and
cerebellum (CER). These hand-drawn VOIs are used to sample the resliced $[^{11}C]PiB$ image, and regional radioactivity concentrations were calculated and converted into units of standardized uptake value (SUV) using the injected dose of $[^{11}C]PiB$ and the subject’s body mass. The unitless SUV outcome is normalized to non-specific uptake (CER), yielding a SUV ratio (SUVR) measure that compares favorably to fully quantitative measures of specific radiotracer retention (Lopresti et al., 2005). Regional SUVR outcomes were partial volume corrected using a previously validated method that corrects for the dilution of PET signal attributable to the limited spatial resolution of the PET scanner (Lopresti et al., 2005; Meltzer et al., 1996; 1998; 2000; Price et al., 2005). This method includes a two-component approach that corrects PET data for the dilutional effect of expanded CSF spaces accompanying normal aging and disease-related cerebral atrophy using the FSL software (University of Oxford, Oxford, UK). A global PiB retention index reflecting cerebral amyloid load is computed from the SUVR values from the six most relevant VOIs (ACG, FRC, LTC, PAR, PRC, and AVS). Participants were classified as PiB positive or negative [PiB(+) or PiB(-)] by using a sparse k-means cluster analysis (Cohen et al., 2013).

**FMRI data preprocessing**

Functional images were preprocessed in SPM12 (Wellcome Trust Centre for Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm/) implemented in Matlab 2015b (Mathworks, Natick, MA) for slice timing correction, motion correction, co-registration, image normalization, resampling at a $2 \times 2 \times 2$ mm$^3$ voxel size, and 8-mm Gaussian smoothing. The mean functional image was co-registered to individual T1w MPRAGE structural image using an affine registration (mean functional image -> T1 MPRAGE image). The T1w MPRAGE structural image was manually skull-stripped in ITK-SNAP, segmented and warped into the MNI common
template space using SPM’s unified segmentation/normalization procedure (T1 MPRAGE image->MNI template). After slice timing correction (the temporally middle slice as the reference), the functional data were realigned to the mean functional image using the two-pass rigid-body realignment procedure for motion correction, which were further resliced and normalized into the MNI common space using the functional-structural co-registration matrix (mean functional image -> T1 MPRAGE image) and the structural-MNI deformation field (T1 MPRAGE image->MNI template). Further, for resting state fMRI data, motion artifacts were removed using the wavelet despike method (Patel et al., 2014), and nuisance signals from the white matter, cerebrospinal fluid and residual motion effect were regressed out in a multiple linear regression with regressors of no interest including principal time series from the WM and CSF and six motion parameters. The residual resting state fMRI data were then temporally band-pass filtered (a second-order Butterworth bandpass filter) with the frequency range of 0.008–0.15 Hz to extract the low-frequency resting-state BOLD signal.

Functional connectivity

Both task-based and resting-state connectivity of the hippocampus were examined in this study. Two seed regions, left and right hippocampus, were created with the anatomically defined automated anatomical labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002) using the WFU PickAtlas tool. For task-based connectivity, generalized psychophysiological interaction (gPPI) analysis (Cisler et al., 2014; Friston et al., 1997; McLaren et al., 2012) was performed to estimate functional connectivity between hippocampus and voxels in the brain. Principal time series (i.e., the eigenvariate) was generated for each seed region, left and right hippocampus, using singular value decomposition (SVD, implemented in Matlab 2015b) from hippocampal
fMRI data during the face-name task. Although differences are subtle, compared to using average time series, principal component analysis (PCA) based method can identify the primary BOLD time-series (Carbonell et al., 2012; Zhou et al., 2009) and is robust to inclusion of voxels that do not follow the primary time-series of the region. This is, particularly relevant for brain regions defined anatomically, such as the whole hippocampus. Since the anatomic definition includes diverse voxels, which have different functional patterns, it is appropriate to weight the voxels accordingly using PCA. This approach generates a time-series more representative of the principal signal and minimizes the contribution of irrelevant voxels. Principal time series of the seed region (left or right hippocampus), task conditions (novel face-name pairs, familiar repeated face-name pairs), interaction variables (seed times series × task condition), as well as motion parameters were included in the design matrix. PPI connectivity maps (left or right hippocampus) during associative encoding (i.e., novel face-name pairs versus repeated familiar face-name pairs), and during novel blocks (novel face-name pairs versus fixation) and during repeated blocks (repeated familiar face-name pairs versus fixation) were computed for each participant.

Resting-state hippocampal connectivity was estimated using a seed-based correlation analysis. Similarly, using SVD principal time series of the resting state data was computed from left or right hippocampus. Resting-state connectivity between the seed region (left or right hippocampus) and a given voxel was calculated as the correlation between the seed principal time series and the voxel times series. For each participant, correlation was calculated voxel-wise within the brain, generating functional connectivity or correlation coefficient maps of left and right hippocampus respectively. The connectivity or correlation maps were converted into Z-score maps via Fisher’s r-to-z transformation.
Second-level analyses

Task-based hippocampal functional connectivity maps (left or right hippocampal gPPI maps) were entered into a statistical non-parametric mapping method (SnPM, http://warwick.ac.uk/snpm) to test the effects of gender, Aβ load and the interaction of gender x Aβ on hippocampal connectivity during associative memory encoding. SnPM uses the general linear model to construct pseudo t-statistic images, and uses permutation testing to compute non-parametric p-values, control for multiple comparisons and assess significance (Nichols and Holmes, 2002; 2004). To test if the association between connectivity and Aβ varies by gender, a linear regression model was used with main effects of gender and Aβ, as well as the interaction of gender x Aβ. In these tests, Aβ burden was treated as a continuous variable (PiB SUVR). To control for non-parametric multiple comparisons, a permutation-based method was used in SnPM with 5,000 permutations and a whole-brain intracranial volume (ICV) mask. The ICV mask has a total volume of 1885673 mm$^3$ (558718 voxels, 1.5 x 1.5 x 1.5 mm$^3$). An initial cluster-forming threshold of $p < 0.001$ was used and clusters with an extend threshold of $p < 0.05$ family-wise errors (FWE) were considered to be significant. These effects were further tested, controlling for age, education, and number of runs for the fMRI task.

Sensitivity analysis was performed to test the robustness of the results with a subset of the original sample, excluding those with extreme values of Aβ burden or functional connectivity. Subjects with extreme values of Aβ burden were identified with the Tukey Method (1.5 x interquartile range [IQR]) (Hoaglin et al., 2012) in SPSS (SPSS 24.0 version, Chicago IL USA). Regression outliers of functional connectivity and Aβ burden were identified with Cook’s distance ($D > 0.5$) in SPSS (Cook, 1977).
For post-hoc analyses, a region of interest (ROI) mask was created from prefrontal regions that showed significant gender x interactions. Task-based connectivity between the left or right hippocampus and the corresponding prefrontal ROI mask was extracted. To dissect the direction of the interaction and the magnitude of the effect size, Pearson correlation was performed in SPSS to examine the linear relationships between functional connectivity and Aβ load stratified by gender. To explore whether the observed interaction on novel – repeated blocks were driven by novel blocks or by repeated blocks, hippocampal functional connectivity during novel blocks (novel face-name pairs – fixation) and during repeated blocks (repeated familiar face-name pairs – fixation) were also extracted from above prefrontal ROI.

For resting state data, both voxelwise and ROI-based analyses were performed to evaluate the effects of gender and Aβ on hippocampal connectivity. In the voxelwise analyses, similar to task-based data, resting state hippocampal (left or right) connectivity maps were entered into SnPM to evaluate the effects of gender, Aβ load and the interaction of gender x Aβ. In the post-hoc ROI-based analyses, resting state connectivity between hippocampus and the prefrontal ROIs (from gender x Aβ interactions on task-based connectivity) was extracted and Pearson correlation was then performed to examine the associations between resting state connectivity and Aβ load stratified by gender.

ANOVA s were performed in SPSS to examine the effects of gender, PiB status [PiB(+) and PiB(-)] and the interaction of gender x PiB status on neurocognitive data. In addition to classifying Aβ levels categorically [PiB(+) and PiB(-)], Aβ load was also modeled as a continuous variable (PiB SUVR). Pearson correlation analyses were performed in SPSS to assess the linear associations between PiB SUVR and neurocognitive performances. We also tested these effects controlling for age, education and/or ApoE status.
Results

Participant Characteristics

Demographics, clinical characteristics and neurocognitive data are presented in Table 1 and Table 2. Males and females did not significantly differ on age, education, race, Global PiB SUVR, PiB(+)%, or Mini–Mental State Examination (MMSE) scores. Of 61 participants, APOE genotype results were available on 44 participants (31 women and 13 men). In this sample, more men had at least one ApoE4 allele (n = 6) than did women (n=3) (p = 0.01, Fisher’s exact test).

Neurocognitive outcomes

There were no gender differences in memory, visuospatial construction, language and attention, or executive domains (see Table 2). However, compared to men, women performed significantly better on word-list learning and Stroop color-word interference, and marginally better on word list delayed recall tests. Conversely, men performed slightly better than women on Boston Naming Test. There was no significant main effect of PiB status on neurocognitive measures of memory, visuospatial construction, or language domains. There were no gender x
PiB interactions in relation to neurocognitive outcomes. Subsequent face-name recognition accuracy was also measured and there was no significant main effect of gender, PiB status or gender x PiB interaction on subsequent face-name recognition accuracy (Table 2).

Compared to PiB(-) participants, PiB(+) participants performed significantly worse in attention and executive subtests including the trail making part B. PiB(+) participants performed better than PiB(-) participants in clock drawing. PiB status remained significantly or of a trend toward significance associated with trail making part B and clock drawing scores when controlling for age and education (trail making part B: F(1,54) = 6.56, p = 0.01, clock drawing: F(1,54) = 5.70, p = 0.02), as well as additionally ApoE status (a subset of 43 subjects had ApoE status, trail making part B: F(1,36) = 4.77, p = 0.04, clock drawing: F(1,36) = 3.38, p = 0.07).

When Aβ load was modeled as a continuous variable (PiB SUVR), controlling for gender, PiB SUVR was positively associated with trail making part B score ($r(57) = 0.30, p = 0.02, 95\%$ confidence interval (CI) [0.17 0.48]) and negatively associated with digital symbol substitution score ($r(57) = -0.29, p = 0.02, 95\%$ CI [-0.50 -0.06]) and Stroop color-word interference ($r(55) = -0.33, p = 0.01, 95\%$ CI [-0.54 -0.05]) scores. PiB SUVR showed a trend toward a significant correlation with trail making part B ($r(55) = 0.23, p = 0.09, 95\%$ CI [0.08 0.41]), digital symbol substitution score ($r(55) = -0.22, p = 0.10, 95\%$ CI [-0.43 0.01]), and Stroop color-word interference ($r(53) = -0.28, p = 0.04, 95\%$ CI [-0.50 -0.02]) when controlling for gender, age and education. PiB SUVR remained marginally significantly correlated with trail making part B ($r(37) = 0.29, p = 0.07, 95\%$ CI [0.11 0.52]) but not with digital symbol substitution score or Stroop color-word interference ($p’$s > 0.13, n = 43) when controlling for gender, age, education, and ApoE within a subset of the subjects.
Functional connectivity

Table 3 and Fig. 1A present the main and interaction effects of gender and Aβ load on hippocampal connectivity during associative encoding (corrected $p < 0.05$). There were no significant main effects of gender or Aβ load on hippocampal functional connectivity. Gender x Aβ interactions were observed for both left and right hippocampal connectivity with prefrontal regions encompassing medial prefrontal cortex, anterior cingulate, left superior frontal gyrus and left middle frontal gyrus. A gender x Aβ interaction was also observed for right hippocampal connectivity with left occipital gyrus. The gender x Aβ interaction for hippocampal connectivity persisted when controlling for age, education, and number of runs for the fMRI task.

Scatterplots in Fig. 1B visualize the gender x Aβ interactions on task-based functional connectivity between hippocampus and prefrontal regions. Specifically, hippocampal-prefrontal connectivity was positively correlated with Aβ load in men (left hippocampal connectivity: $r(18) = 0.70, p = 0.001, 95\% \text{ CI} [0.53, 0.92]$; right hippocampal connectivity: $r(18) = 0.65, p = 0.002, 95\% \text{ CI} [0.43, 0.86]$) but not in women (left hippocampal connectivity: $r(39) = 0.01, p = 0.95, 95\% \text{ CI} [-0.35, 0.27]$; right hippocampal connectivity: $r(39) = 0.06, p = 0.71, 95\% \text{ CI} [-0.48, 0.27]$). There are no significant associations between functional connectivity and subsequent recognition accuracy of the face/name pairs (left hippocampal connectivity: $r(59) = -0.06, p = 0.67$; right hippocampal connectivity: $r(59) = -0.16, p = 0.22$) or between functional connectivity and cognitive measures (uncorrected $p$'s $>0.05$). Further, the observed interaction on hippocampal connectivity were driven by both novel and repeated blocks, with a greater functional connectivity during novel blocks and a lower functional connectivity during repeated blocks in PiB+ men than in PiB+ women, as shown in Supplementary Fig. 3.
We did not observe significant main effects of gender and Aβ load on resting-state functional connectivity in the voxelwise analyses. The interaction effect of gender x Aβ on resting-state hippocampal connectivity did not survive multiple comparison either (e.g., left hippocampus: left superior frontal gyrus/medial frontal gyrus (BA 10, 32), peak \( t = 3.63 \), peak xyz: [-20 48 6], cluster size: 552 mm\(^3\), uncorrected \( p < 0.005 \)). Prefrontal ROIs from the gender x Aβ interactions on task-based connectivity (in Fig. 1A) were used to extract resting state connectivity between hippocampus in the ROI-based post-hoc analyses (Fig. 1C). We found significant interaction effects of gender x Aβ (left hippocampus: \( F(1,54) = 3.60, p = 0.063 \); right hippocampus: \( F(1,54) = 4.60, p = 0.037 \)), marginally significant main effects of gender (left hippocampus: \( F(1,54) = 4.31, p = 0.043 \); right hippocampus: \( F(1,54) = 2.91, p = 0.094 \)) and no significant main effects of Aβ (p’s > 0.52) on the extracted resting state connectivity. As shown in Fig. 1C, the extracted resting-state connectivity showed a lower magnitude positive association with Aβ load in men (moderate effect size: left hippocampal connectivity: \( r(18) = 0.32, p = 0.17, 95\% \text{ CI } [-0.25 0.77] \); right hippocampal connectivity: \( r(18) = 0.28, p = 0.23, 95\% \text{ CI } [-0.22 0.62] \)), while resting-state connectivity was negatively correlated with Aβ load in women (moderate effect size: left hippocampal connectivity: \( r(36) = -0.24, p = 0.15, 95\% \text{ CI } [-0.57 0.12] \); right hippocampal connectivity: \( r(36) = -0.32, p = 0.05, 95\% \text{ CI } [-0.61 0.01] \)).

In the sensitivity analysis, six participants were removed from the original sample based on amyloid load (n = 5, Aβ load > 2.06 based on the Tukey’s Method) and functional connectivity (n = 1, Cook’s Distance D > 0.5), yielding a subset of 55 participants (subset N = 55) (Supplementary Fig. 1). Consistent with the primary analysis, sensitivity analysis showed significant gender x Aβ interactions on both left and right hippocampal connectivity with
prefrontal regions during associative memory encoding (Supplementary Fig. 2A, corrected $p < 0.05$). In this subset, hippocampus-prefrontal connectivity was also positively correlated with Aβ load in men (left hippocampal connectivity: $r(16) = 0.72, 95\%$ CI $[0.55, 0.88]$); right hippocampal connectivity: $r(16) = 0.67, 95\%$ CI $[0.34, 0.85]$) and not in women (left hippocampal connectivity: $r(35) = -0.09, 95\%$ CI $[-0.37, 0.19]$); right hippocampal connectivity: $r(35) = -0.28, 95\%$ CI $[-0.55, 0.14]$) (Supplementary Fig. 2B). In contrast to moderate effect on resting-state connectivity in the post-hoc ROI analyses with the full resting state sample (N=58), sensitivity analysis did not find significant association between resting-state hippocampal-prefrontal connectivity and Aβ load in men or women ($p$’s > 0.4).

Discussion

This study investigated gender differences in the associations between brain Aβ deposition (i.e., global PiB retention) and hippocampal functional connectivity in cognitively intact older adults. We found men and women have different patterns in hippocampal functional connectivity with increased amyloid burden. Specifically, in men, greater Aβ burden was accompanied by greater functional connectivity between hippocampus and prefrontal regions, whereas in women hippocampal connectivity did not vary by amyloid burden. We found this pattern of associations using voxel-wise analyses of hippocampal functional connectivity during a face-name associative memory fMRI task. The effect was significant for both right and left hippocampal connectivity during associative encoding. With prefrontal regions identified from task-based analyses, we performed ROI-based analyses on resting-state fMRI data and found that hippocampal-prefrontal connectivity at rest was positively correlated with Aβ load in men and
was negatively correlated with Aβ load in women. However, whole-brain voxel-wise analyses of resting-state hippocampal connectivity did not survive multiple comparison.

Gender differences in Aβ-related compensation may relate to different trajectories of steroid hormones, estradiol in particular, with aging between men and women (Farage et al., 2012). In contrast to no significant change of estrogen with advancing age in men, there is a substantial decrease of estrogen, particularly estradiol, in midlife and older women (Farage et al., 2012). Extensive animal studies have revealed the effects of estrogen on the structural and synaptic plasticity of hippocampus (Foy et al., 2008; Hara et al., 2012; Liu et al., 2008; MCEWEN, 2002; Morrison et al., 2006; Woolley and McEwen, 1993; Woolley et al., 1990) and the prefrontal cortex (Dumitriu et al., 2010; Hao et al., 2006; Morrison et al., 2006; Rapp et al., 2003; Wang et al., 2010). Human studies have also implicated the effects of estradiol on the memory circuitry (Barth et al., 2016; Duff and Hampson, 2000; Dumas et al., 2010; Grigorova et al., 2006; Shaywitz et al., 1999). In the current study, a data-driven (principal component analysis) method was used to select the hippocampal voxels most associated with encoding during the face-name associative memory task. The particular voxels identified are located in the anterior hippocampus, primarily CA3, which matches the location previously identified with this task using high-resolution MR/fMRI (Zeineh et al., 2003). Although previous findings focused on the effects of estrogen on the synaptic plasticity of hippocampal CA1 subfield (McEwen, 2002; Woolley and McEwen, 1993; Woolley et al., 1990), new studies have extended to the dentate gyrus and CA3 subfield of the hippocampus (Briz et al., 2015; Kim et al., 2006; Zhang et al., 2013). Specifically, long-term estrogen deprivation was found to cause gender-specific hypersensitivity of the CA3 subfield to ischemic stress and to the neurotoxic effects of Aβ1–42 in ovariectomized (i.e., surgical menopause) female rats, but not in orchietomized male rats.
(Zhang et al., 2013). It is possible that the dramatic drop in estrogen levels during the menopause transition may render this circuitry particularly vulnerable to synaptic plasticity loss and Aβ neurotoxicity in post-menopausal women. Men generate estrogen from testosterone and adrenal androgens and do not experience as dramatic a loss of estrogen with aging. Thus, the estrogen-related neural plasticity loss and hypersensitivity to Aβ-induced damage may be specific for women, which may explain the reduction in fMRI markers of compensation and the faster rate of cognitive decline and AD progression. Although men show no dramatic change in estrogen, they do show a modest gradual decline in testosterone with advancing age (Snyder, 2017). Recent studies have shown the effects of testosterone on hippocampal synaptic plasticity in male rats (Atwi et al., 2016; Schulz et al., 2010), and high concentrations of serum testosterone are associated with better cognitive performance in older men (Matsumoto et al., 2002). Therefore, it is also possible that age-related decline in testosterone concentrations might contribute to cognitive decline in older men.

The effects of hippocampal hyperactivity/hyperconnectivity on cognitive performance and outcome are mixed in the literature. Some studies have shown that hippocampal hyperactivity/hyperconnectivity is beneficial. Greater hippocampal activation was observed during successful associative encoding (Miller et al., 2008) and was significantly related to a better visual memory factor score (Mormino et al., 2012). Greater hippocampal connectivity during associative encoding and during resting state was linked with better memory performance in older adults without cognitive impairment (Lim et al., 2014; Nyberg, 2016; Salami et al., 2014) and in healthy individuals (25 - 80 years of age) (Nyberg, 2016; Salami et al., 2014). In contrast, others have suggested that hippocampal hyperactivity is deleterious and is associated with quicker cognitive decline (Dickerson et al., 2004; O'Brien et al., 2010). Dickerson et al. reported
that greater clinical decline at 2.5-year follow-up was associated with greater extent of activation in right hippocampus at baseline. Using longitudinal fMRI, O’Brien et al. found that, in older adults without dementia, individuals with more rapid decline at 2-year follow-up had the highest hippocampal activation at baseline and greatest loss of hippocampal activation at 2-year follow-up. Further, treatment studies showed that normalizing hippocampal hyperactivity in MCI patients have been shown to improve task-related memory performance (Bakker et al., 2012; 2015). In our study we did not find a significance association between cognitive performance and fMRI connectivity. We suspect this may be because the neural system response (measured by fMRI) reflects a physiologic homeostatic response, which can lead to disparate effects on behavior. For example, hypertension may maintain perfusion and also lead to hypoperfusion. The hippocampal frontal hyper-connectivity observed in pre-clinical AD may serve a compensatory function and maintain performance in some individuals. It also can be seen as de-differentiation in-so-far as it may involve engagement of less specific neural resources.

Many studies have demonstrated the progression of AD from MTL/hippocampal hyperactivity/hyperconnectivity at a preclinical stage [in cognitively normal older adults with high Aβ burden (Edelman et al., 2017; Mormino et al., 2012; Oh and Jagust, 2013; Vannini et al., 2012) and in early MCI patients (Celone et al., 2006; Dickerson et al., 2005; Hämäläinen et al., 2007; Pizzi et al., 2018)] to hypoactivity/hypoconnectivity at a later stage of the disease [in late MCI and AD patients (Hämäläinen et al., 2007; Pizzi et al., 2018; Sperling, Bates, et al., 2003)] (for review see (Sperling, 2011)). Specifically, with a large sample size (N = 135), Pizzi et al. reported that compared to cognitively unimpaired older adults, nc-MCI individuals (i.e, did not convert to AD in 24 months) showed MTL/hippocampal hyperconnectivity and c-MCI
individuals (i.e., converted to AD in 24 months) showed MTL/hippocampal hypoconnectivity (Pizzi et al., 2018). These evidences collectively suggest the transition of hyper- to hypo- activity and connectivity of MTL/hippocampal happens at the late stage of MCI. On the other hand, brain activity and resting state functional connectivity have been shown to follow a nonlinear (i.e., quadratic) relationship with Aβ SUVR in cognitively unimpaired older adults, suggesting this transition may occur at the preclinical stage of AD (Foster et al., 2018; Schultz et al., 2017). Specifically, Foster and colleagues found that during a spatial distance judgement task, participants with slightly elevated Aβ showed hyperactivity while those with extreme Aβ showed hypoactivity in bilateral angular/temporal and medial prefrontal cortices (Foster et al., 2018). Schultz et al. reported a similar non-linear pattern on resting state connectivity of the default mode and salience networks (interaction effect of Aβ x Tau), with increased functional connectivity in Aβ+ participant with low neocortical Tau level and decreased functional connectivity in Aβ+ participants with high Tau level (Schultz et al., 2017). Although in our current sample men and women did not significantly differ on global PiB SUVR (p = 0.92) or PiB(+)% (p = 0.36), the distribution of global PiB SUVR seemed to be imbalanced (four women but no men showed extreme PiB SUVR values > 2.06). To address this imbalance, sensitivity analyses were performed with a subset of the sample (N=56) and a narrow range of PiB SUVR (<=2.06). Similar gender differences in hippocampal connectivity were found with this subset as with the entire sample in the primary analyses. However, with our current sample, which focuses on cognitively unimpaired older adults, we are unable to test and rule out the possibility of hypoconnectivity with extreme high Aβ load. However, using the same face-name associative encoding task, multiple studies have shown that individuals at the early phase of MCI exhibited MTL hyperactivity (Celone et al., 2006; Dickerson et al., 2005). This suggests, that at least for
this task, a sample with a greater range of cognitive impairment (further along in the AD biomarker cascade) may be necessary to demonstrate the non-linear pattern.

Several limitations of this study should be considered. Sex hormones were not measured, and we were not able to explore how functional connectivity is related to levels of estradiol, testosterone, or other sex steroids. Current medications were recorded and no participants were on estrogen at the time of the scans. In this study, we did not observe amyloid-related functional connectivity changes in women. It is possible that women showed more subtle changes in hippocampal connectivity that did not survive stringent multiple comparisons. This study has a relatively modest sample size (N = 61), which may not have sufficient power to detect these subtle changes. It is also possible that women may have amyloid-related connectivity changes in brain regions other than hippocampus. This study only evaluated hippocampal functional connectivity and thus did not capture such changes in women. A cross-sectional instead of longitudinal design was used in this analysis. The cross-sectional design evaluates A\(\beta\)-related differences through correlations, which are inherently vulnerable to inter-subject variance and cohort effects. ApoE genotype results were only available on 44 participants (31 women and 13 men) and more men had at least one ApoE4 allele (n = 6) than did women (n=3) in this study. Using a comprehensive neuropsychological testing battery, participants were evaluated in multiple cognitive domains to ensure cognitive normality. Of these tests, women performed better in the Word List Learning (WLL) test and men performed better in the Boston Naming test, which may reflect different patterns of cognitive performance in older men and women. The face-name memory encoding task used in this study is a mixed block/event-related design task. The event-related aspect of this task is not optimized for event-related analyses (not jittered and
rapid events). Further block-design in general has been shown to have a greater effect size and is less susceptibility to between subject variability in HRF, a particular concern in older populations (Huettel et al., 2001). Therefore, in this and previous (Edelman et al., 2017; Jahani et al., 2017; Sperling, Bates, et al., 2003), we and others have focused on the block-design aspect of the task. However, including event-related behavior regressors in the gPPI model could potential address interpretive issues with the task. Future studies with more participants, longitudinal designs, task behavior regressors, hormonal measures and ApoE genotype data will be important to understand gender differences in amyloid burden, hippocampal functional connectivity, and cognitive performance.

In summary, an Aβ-related increase in hippocampal-prefrontal functional connectivity occurs prior to memory decline, and may be an early marker reflecting a homeostatic response in the memory network. We found that these responses are greater in men than women. We suggest that this finding may explain gender differences in disease trajectories. Longitudinal studies are required to confirm this finding. Nonetheless, characterizing gender differences in responses for AD-related pathologies, as in the current study, can help guide the development of gender specific prevention and treatment strategies.

Acknowledgment

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Woolley CS, McEwen BS. Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat. Journal of Comparative Neurology 1993; 336: 293–306.


Table 1. Demographic variables and clinical characteristics by gender (N = 61*).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group; mean (SD)</th>
<th>Statistical test</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female, n = 41</td>
<td>Male, n = 20</td>
<td></td>
</tr>
<tr>
<td>Age, year†</td>
<td>75.5 (6.4)</td>
<td>76.5 (6.6)</td>
<td>F&lt;sub&gt;1,59&lt;/sub&gt; = 0.32 0.58</td>
</tr>
<tr>
<td>Education, year</td>
<td>14.3 (2.4)</td>
<td>15.3 (2.3)</td>
<td>F&lt;sub&gt;1,59&lt;/sub&gt; = 2.37 0.13</td>
</tr>
<tr>
<td>Race composition, no. (%)</td>
<td></td>
<td></td>
<td>χ² = 1.09 0.58</td>
</tr>
<tr>
<td>White</td>
<td>33 (80.5)</td>
<td>18 (90.0)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>7 (17.1)</td>
<td>2 (10.0)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>1 (2.4)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>APOE genotype, no. (%)‡</td>
<td></td>
<td></td>
<td>χ&lt;sup&gt;1&lt;/sup&gt; = 7.49 0.01</td>
</tr>
<tr>
<td>ε2/ε3</td>
<td>4 (9.8)</td>
<td>3 (15.0)</td>
<td></td>
</tr>
<tr>
<td>ε3/ε3</td>
<td>24 (58.5)</td>
<td>4 (20.0)</td>
<td></td>
</tr>
<tr>
<td>ε3/ε4</td>
<td>3 (7.3)</td>
<td>5 (25.0)</td>
<td></td>
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<tr>
<td>ε4/ε4</td>
<td>0 (0.0)</td>
<td>1 (5.0)</td>
<td></td>
</tr>
<tr>
<td>Global PiB SUVR</td>
<td>1.58 (0.40)</td>
<td>1.57 (0.29)</td>
<td>F&lt;sub&gt;1,59&lt;/sub&gt; = 0.01 0.92</td>
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<tr>
<td>PiB(+), no (%)</td>
<td>8 (19.5)</td>
<td>6 (30.0)</td>
<td>χ² = 0.84 0.36</td>
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<tr>
<td>MMSE score§</td>
<td>28.7 (1.4)</td>
<td>28.8 (1.4)</td>
<td>F&lt;sub&gt;1,58&lt;/sub&gt; = 0.07 0.80</td>
</tr>
</tbody>
</table>

*Unless otherwise indicated.
†Age range 65 – 93 years.
‡APOE genotyping was available on 44 out of 61 participants (31 females and 13 males).
§MMSE score (Mini–Mental State Examination) was available on 60 out 61 participants (40 females, 20 males).
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Female (n=41)</th>
<th>Male (n=20)</th>
<th>Main and Interaction Effects</th>
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<tr>
<td></td>
<td>PiB(+) (8)</td>
<td>PiB(-) (33)</td>
<td>PiB(+) (6)</td>
</tr>
<tr>
<td>Memory</td>
<td></td>
<td></td>
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<tr>
<td>WLL learning trials</td>
<td>22.0(4.3)</td>
<td>22.1(3.1)</td>
<td>19.2(3.7)</td>
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<td>WLL delayed recall</td>
<td>7.1(2.5)</td>
<td>7.7(1.8)</td>
<td>7.0(2.1)</td>
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<td>Rey figure (max=24)</td>
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<tr>
<td>Immediate recall</td>
<td>16.1(2.7)</td>
<td>16.1(3.7)</td>
<td>17.8(2.5)</td>
</tr>
<tr>
<td>Delayed recall</td>
<td>14.8(2.8)</td>
<td>16.2(3.7)</td>
<td>17.5(2.5)</td>
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<tr>
<td>Logical Memory Story A</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Immediate recall</td>
<td>15.0(4.4)</td>
<td>16.5(3.8)</td>
<td>15.2(3.7)</td>
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<tr>
<td>Delay recall</td>
<td>14.5(6.5)</td>
<td>15.1(4.3)</td>
<td>14.4(3.8)</td>
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<tr>
<td>Visuospatial construction</td>
<td></td>
<td></td>
<td></td>
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<td>Block design (max=24)</td>
<td>14.5(4.6)</td>
<td>13.6(3.6)</td>
<td>17.2(5.5)</td>
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<tr>
<td>Rey figure copy</td>
<td>19.0(2.9)</td>
<td>19.8(2.4)</td>
<td>20.1(1.5)</td>
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<td>Language</td>
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<tr>
<td>Semantic fluency (animals)</td>
<td>19.8(4.7)</td>
<td>20.1(5.8)</td>
<td>21.0(4.3)</td>
</tr>
<tr>
<td>Letter fluency (FAS)</td>
<td>48.1(14.9)</td>
<td>44.1(14.7)</td>
<td>45.2(19.2)</td>
</tr>
<tr>
<td>Boston Naming Test (max=30)</td>
<td>29.0(1.7)</td>
<td>29.5(0.9)</td>
<td>29.8(0.4)</td>
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<tr>
<td>Attention and executive</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Trail Making Part A (sec)</td>
<td>31.8(9.4)</td>
<td>29.3 (9.1)</td>
<td>27.8(10.9)</td>
</tr>
<tr>
<td>Trail Making Part B (sec)</td>
<td>113.7(61.9)</td>
<td>71.9(20.6)</td>
<td>92.6(33.3)</td>
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<tr>
<td>Digit Symbol</td>
<td>44.1(17.2)</td>
<td>55.1(10.9)</td>
<td>47.3(12.7)</td>
</tr>
<tr>
<td>Stroop color-word</td>
<td>33.0(15.5)</td>
<td>40.5(8.9)</td>
<td>30.2(5.2)</td>
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<tr>
<td>Clock drawing (max=15)</td>
<td>14.8(0.5)</td>
<td>14.2(0.8)</td>
<td>14.7(0.5)</td>
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<tr>
<td>Face/Name Recognition %</td>
<td>70.0(9.3)</td>
<td>71.0(11.2)</td>
<td>65.5(11.3)</td>
</tr>
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</table>
Table 3. Gender-by-\(\beta\) load ANOVAs of hippocampal functional connectivity during the face-name associative memory task (corrected \(p < 0.05\)).

<table>
<thead>
<tr>
<th>Gender -by-(\beta) ANOVA</th>
<th>Brain region</th>
<th>Brodmann area (BA)</th>
<th>Peak MNI coordinates (x,y,z)</th>
<th>t-score</th>
<th>Size (mm(^3))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Left hippocampal functional connectivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main effect of gender</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main effect of (\beta)</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Gender by (\beta) interaction</td>
<td>mPFC/ACC, L SFG, L MFG</td>
<td>BA 10, 32, 24</td>
<td>-4, 50, 8</td>
<td>4.21</td>
<td>3192</td>
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<td><strong>Right hippocampal functional connectivity</strong></td>
<td></td>
<td></td>
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<tr>
<td>Main effect of gender</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main effect of (\beta)</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender by (\beta) interaction</td>
<td>mPFC/ACC, L SFG, L MFC</td>
<td>BA 10</td>
<td>-22, 54, -2</td>
<td>5.34</td>
<td>1592</td>
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<tr>
<td></td>
<td>L MCG/Cuneus</td>
<td>BA 17, 18, 19, 39</td>
<td>-26, -82, 8</td>
<td>4.47</td>
<td>1352</td>
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</tbody>
</table>

Abbreviations: MNI-Montreal Neurologic Institute; L-left; mPFC-medial prefrontal cortex, ACC-anterior cingulate cortex; SFG-superior frontal gyrus, MFG-middle frontal gyrus, MCG-middle occipital gyrus.
A

B

C

L hippocampus
R hippocampus
Global PiB

Global PiB

Global PiB

Global PiB
Highlights

1. The effects of brain amyloid on functional connectivity in preclinical AD differ by sex.
2. In men greater amyloid burden was associated with greater hippocampal-prefrontal connectivity.
3. In women hippocampal-prefrontal connectivity did not vary by amyloid burden.
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