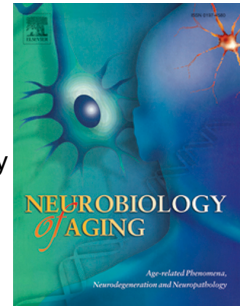# Accepted Manuscript

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Please cite this article as: Wu, M., Thurston, R.C., Tudorascu, D.L., Karim, H.T., Mathis, C.A., Lopresti, B.J., Kamboh, M.I., Cohen, A.D., Snitz, B.E., Klunk, W.E., Aizenstein, H., Amyloid Deposition Is Associated with Different Patterns of Hippocampal Connectivity in Men versus Women, *Neurobiology of Aging* (2019), doi: https://doi.org/10.1016/j.neurobiolaging.2018.11.020.

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Amyloid Deposition Is Associated with Different Patterns of Hippocampal Connectivity in Men versus Women

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Running title: Gender, amyloid and functional connectivity

Abstract

Compared to men, women are disproportionally 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
automated 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 disproportionally 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

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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.

Insert Table 1 about here

Insert Table 2 about here

Image Acquisition and Processing

PiB PET imaging

[^{11}C]PiB was synthesized by a simplified radiosynthetic method based on the captive solvent method (Wilson *et al.*, 2000). Fifteen mCi of [^{11}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}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 = 256×256 mm^2, acquisition matrix 128×128, slice thickness/gap = 4/0 mm (voxel size = 2 x 2 x 4 mm^3), 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 = 240×256 mm^2, matrix = 240 x 256, slice thickness/gap = 1/0 mm (voxel size = $1 \times 1 \times 1$ mm^3), 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

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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 [^{11}C]PiB image using the AIR algorithm (PET-MR) (Woods *et al.*, 1993). The resulting PET-MR spatial transformation was applied to the summed [^{11}C]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

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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

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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 Pick-Atlas 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).

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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.

ANOVAs 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.

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Insert Fig. 1 about here

Insert Table 3 about here

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

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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 (Table2).

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.

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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% CI [0.53 0.92]); right hippocampal connectivity: $r(18) = 0.65$, $p = 0.002$, 95% CI [0.43 0.86]) but not in women (left hippocampal connectivity: $r(39) = 0.01$, $p = 0.95$, 95% CI [-0.35 0.27]); right hippocampal connectivity: $r(39) = 0.06$, $p = 0.71$, 95% 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% CI [-0.25 0.77]; right hippocampal connectivity: r(18) = 0.28, p = 0.23, 95% 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% CI [-0.57 0.12]; right hippocampal connectivity: r(36) = -0.32, p = 0.05. 95% 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

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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

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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 orchiectomized 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/hyerconnectivity 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

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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

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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 Aβ-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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Fig. 1. There are significant gender differences in the associations between brain amyloid (Aβ) load and hippocampal functional connectivity. Brain Aβ load was measured as a global PiB retention index computed from the SUVR values from the six most relevant brain regions. **A**. Gender x Aβ interactions on left and right hippocampal functional connectivity during associative encoding (left panel: left hippocampal connectivity, right panel: right hippocampus connectivity). Hippocampal functional connectivity during associative encoding and during resting state were voxelwise extracted from prefrontal regions identified in A. The extracted functional connectivity (beta weights) were voxelwise plotted against Aβ load in B and C (small dots: voxelwise beta estimate, bold dots: median beta estimate across the prefrontal ROI). **B**. Scatterplots of Aβ load and hippocampus-prefrontal connectivity during associative encoding (blue: men, red: women). **C**. Scatterplots of Aβ load and hippocampus-prefrontal connectivity during resting state (blue: men, red: women).

References

Aizenstein HJ, Nebes RD, Saxton JA, Price JC, Mathis CA, Tsopelas ND, et al. Frequent Amyloid Deposition Without Significant Cognitive Impairment Among the Elderly. Archives of Neurology 2008; 65: 1509.

Atwi S, McMahon D, Scharfman H, MacLusky NJ. Androgen Modulation of Hippocampal Structure and Function. The Neuroscientist 2016; 22: 46–60.

Bakker A, Albert MS, Krauss G, Speck CL, Gallagher M. Response of the medial temporal lobe network in amnestic mild cognitive impairment to therapeutic intervention assessed by fMRI and memory task performance. NeuroImage: Clinical 2015; 7: 688–698.

Bakker A, Krauss GL, Albert MS, Speck CL, Jones LR, Stark CE, et al. Reduction of Hippocampal Hyperactivity Improves Cognition in Amnestic Mild Cognitive Impairment. Neuron 2012; 74: 467–474.

Barth C, Steele CJ, Mueller K, Rekkas VP, Arélin K, Pampel A, et al. In-vivo Dynamics of the Human Hippocampus across the Menstrual Cycle. Scientific Reports 2016; 6: 584.

Bennett DA, Schneider JA, Arvanitakis Z, Kelly JF, Aggarwal NT, Shah RC, et al. Neuropathology of older persons without cognitive impairment from two community-based studies. Neurology 2006; 66: 1837–1844.

Bretsky PM, Buckwalter JG, Seeman TE, Miller CA, Poirier J, Schellenberg GD, et al. Evidence for an Interaction between Apolipoprotein E Genotype, Gender, and Alzheimer Disease. Alzheimer Disease & Associated Disorders 1999; 13: 216.

Briz V, Liu Y, Zhu G, Bi X, Baudry M. A novel form of synaptic plasticity in field CA3 of hippocampus requires GPER1 activation and BDNF release. The Journal of Cell Biology 2015; 210: 1225–1237.

Carbonell F, Bellec P, Shmuel A. Global and System-Specific Resting-State fMRI Fluctuations Are Uncorrelated: Principal Component Analysis Reveals Anti-Correlated Networks. https://home.liebertpub.com/brain 2012; 1: 496–510.

Carolyn M Mazure JS. Sex differences in Alzheimer's disease and other dementias. The Lancet Neurology 2016; 15: 451.

Celone KA, Calhoun VD, Dickerson BC, Atri A, Chua EF, Miller SL, et al. Alterations in Memory Networks in Mild Cognitive Impairment and Alzheimer's Disease: An Independent Component Analysis. J. Neurosci. 2006; 26: 10222–10231.

Cisler JM, Bush K, Steele JS. A comparison of statistical methods for detecting context-modulated functional connectivity in fMRI. NeuroImage 2014; 84: 1042–1052.

Cohen AD, Mowrey W, Weissfeld LA, Aizenstein HJ, McDade E, Mountz JM, et al. Classification of amyloid-positivity in controls: comparison of visual read and quantitative approaches. NeuroImage 2013; 71: 207–215.

Cohen AD, Price JC, Weissfeld LA, James J, Rosario BL, Bi W, et al. Basal Cerebral Metabolism May Modulate the Cognitive Effects of A beta in Mild Cognitive Impairment: An Example of Brain Reserve. J. Neurosci. 2009; 29: 14770–14778.

Cook RD. Detection of Influential Observation in Linear Regression. Technometrics 1977; 19: 15–18.

Dickerson BC, Salat DH, Bates JF, Atiya M, Killiany RJ, Greve DN, et al. Medial temporal lobe function and structure in mild cognitive impairment. Annals of Neurology 2004; 56: 27–35.

Dickerson BC, Salat DH, Greve DN, Chua EF, Rand-Giovannetti E, Rentz DM, et al. Increased hippocampal activation in mild cognitive impairment compared to normal aging and AD. Neurology 2005; 65: 404–411.

Duff SJ, Hampson E. A Beneficial Effect of Estrogen on Working Memory in Postmenopausal Women Taking Hormone Replacement Therapy. Hormones and Behavior 2000; 38: 262–276.

Dumas JA, Kutz AM, Naylor MR, Johnson JV, Newhouse PA. Increased memory load-related frontal activation after estradiol treatment in postmenopausal women. Hormones and Behavior 2010; 58: 929–935.

Dumitriu D, Hao J, Hara Y, Kaufmann J, Janssen WGM, Lou W, et al. Selective Changes in Thin Spine Density and Morphology in Monkey Prefrontal Cortex Correlate with Aging-Related Cognitive Impairment. J. Neurosci. 2010; 30: 7507–7515.

Edelman K, Tudorascu D, Agudelo C, Snitz B, Karim H, Cohen A, et al. Amyloid-Beta Deposition is Associated with Increased Medial Temporal Lobe Activation during Memory Encoding in the Cognitively Normal Elderly. The American Journal of Geriatric Psychiatry 2017; 25: 551–560.

Elman JA, Oh H, Madison CM, Baker SL, Vogel JW, Marks SM, et al. Neural compensation in older people with brain amyloid-β deposition. Nature Neuroscience 2014; 17: 1316–1318.

Farage M, Miller KW, Zouboulis CC. Gender differences in skin aging and the changing profile of the sex hormones with age. Journal of Steroids … 2012

Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, et al. Effects of Age, Sex, and Ethnicity on the Association Between Apolipoprotein E Genotype and Alzheimer Disease: A Meta-analysis. JAMA 1997; 278: 1349–1356.

Foster CM, Kennedy KM, Horn MM, Hoagey DA, Rodrigue KM. Both hyper- and hypo-activation to cognitive challenge are associated with increased beta-amyloid deposition in healthy aging: A nonlinear effect. NeuroImage 2018; 166: 285–292.

Foy MR, Baudry M, Diaz Brinton R, Thompson RF. Estrogen and Hippocampal Plasticity in Rodent Models. Journal of Alzheimer's Disease 2008; 15: 589–603.

Friston KJ, Buechel C, Fink GR, Morris J, Rolls E, Dolan RJ. Psychophysiological and Modulatory Interactions in Neuroimaging. NeuroImage 1997; 6: 218–229.

Grigorova M, SHERWIN B, Tulandi T. Effects of treatment with leuprolide acetate depot on working memory and executive functions in young premenopausal women. Psychoneuroendocrinology 2006; 31: 935–947.

Hao J, Rapp PR, Leffler AE, Leffler SR, Janssen WGM, Lou W, et al. Estrogen Alters Spine Number and Morphology in Prefrontal Cortex of Aged Female Rhesus Monkeys. J. Neurosci. 2006; 26: 2571–2578.

Hara Y, Park CS, Janssen WGM, Roberts MT, Morrison JH, Rapp PR. Synaptic correlates of memory and menopause in the hippocampal dentate gyrus in rhesus monkeys. Neurobiology of Aging 2012; 33: 421.e17–421.e28.

Hämäläinen A, Pihlajamaki M, Tanila H, Hänninen T, Niskanen E, Tervo S, et al. Increased fMRI responses during encoding in mild cognitive impairment. Neurobiology of Aging 2007; 28: 1889–1903.

Hebert LE, Weuve J, Scherr PA, Evans DA. Alzheimer disease in the United States (2010–2050) estimated using the 2010 census. Neurology 2013; 80: 1778–1783.

Hedden T, van Dijk KRA, Becker JA, Mehta A, Sperling RA, Johnson KA, et al. Disruption of Functional Connectivity in Clinically Normal Older Adults Harboring Amyloid Burden. J. Neurosci. 2009; 29: 12686–12694.

Hoaglin DC, Iglewicz B, Tukey JW. Performance of Some Resistant Rules for Outlier Labeling. Journal of the American Statistical Association 2012; 81: 991–999.

Holland D, Desikan RS, Dale AM, McEvoy LK. Higher Rates of Decline for Women and Apolipoprotein E ε4 Carriers. American Journal of Neuroradiology 2013; 34: 2287–2293.

Huettel SA, Singerman JD, Neuroimage GM, 2001. The effects of aging upon the hemodynamic response measured by functional MRI. biac.duke.edu

Huijbers W, Mormino EC, Wigman SE, Ward AM, Vannini P, McLaren DG, et al. Amyloid Deposition Is Linked to Aberrant Entorhinal Activity among Cognitively Normal Older Adults. J. Neurosci. 2014; 34: 5200–5210.

Jack CR Jr, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. The Lancet Neurology 2010; 9: 119–128.

Jahani S, Fantana AL, Harper D, Ellison JM, Boas DA, Forester BP, et al. fNIRS can robustly measure brain activity during memory encoding and retrieval in healthy subjects. Scientific Reports 2017; 7: 9533.

Kim MT, Soussou W, Gholmieh G, Ahuja A, Tanguay A, Berger TW, et al. 17β-Estradiol potentiates field excitatory postsynaptic potentials within each subfield of the hippocampus with greatest potentiation of the associational/commissural afferents of CA3. Neuroscience 2006; 141: 391–406.

Kristina Schulz VK. Hippocampal Testosterone Relates to Reference Memory Performance and Synaptic Plasticity in Male Rats. Frontiers in Behavioral Neuroscience 2010; 4

Lim HK, Nebes R, Snitz B, Cohen A, Mathis C, Price J, et al. Regional amyloid burden and intrinsic connectivity networks in cognitively normal elderly subjects. Brain 2014; 137: 3327–3338.

Lin KA, Choudhury KR, Rathakrishnan BG, Marks DM, Petrella JR, Doraiswamy PM. Marked gender differences in progression of mild cognitive impairment over 8 years. Alzheimer's & Dementia: Translational Research & Clinical Interventions 2015; 1: 103–110.

Liu F, Day M, Muñiz LC, Bitran D, Arias R, Revilla-Sanchez R, et al. Activation of estrogen receptor-|[beta]| regulates hippocampal synaptic plasticity and improves memory. Nature Neuroscience 2008; 11: 334–343.

Lopresti BJ, Klunk WE, Mathis CA, Hoge JA, Ziolko SK, Lu XL, et al. Simplified quantification of Pittsburgh compound B amyloid imaging PET studies: A comparative analysis. J. Nucl. Med. 2005; 46: 1959–1972.

Matsumoto AM. Andropause: clinical implications of the decline in serum testosterone levels with aging in men. J Gerontol A Biol Sci Med Sci 2002

MCEWEN B. Sex, stress and the hippocampus: allostasis, allostatic load and the aging process. Neurobiology of Aging 2002; 23: 921–939.

McLaren DG, Ries ML, Xu G, Johnson SC. A generalized form of context-dependent psychophysiological interactions (gPPI): A comparison to standard approaches. NeuroImage 2012; 61: 1277–1286.

Meltzer CC, Cantwell MN, Greer PJ, Ben-Eliezer D, Smith G, Frank G, et al. Does cerebral blood flow decline in healthy aging? A PET study with partial-volume correction. J. Nucl. Med. 2000; 41: 1842–1848.

Meltzer CC, Smith G, Price JC, Reynolds CF, Mathis CA, Greer P, et al. Reduced binding of [18F]altanserin to serotonin type 2A receptors in aging: persistence of effect after partial volume correction. Brain Res. 1998; 813: 167–171.

Meltzer CC, Zubieta JK, Links JM, Brakeman P, Stumpf MJ, Frost JJ. MR-based correction of brain PET measurements for heterogeneous gray matter radioactivity distribution. Journal of Cerebral Blood Flow & Metabolism 1996; 16: 650–658.

Mielke M, Vemuri P, Rocca W. Clinical epidemiology of Alzheimer’s disease: assessing sex and gender differences. Clinical Epidemiology 2014: 37.

Miller SL, Celone K, DePeau K, Diamond E, Dickerson BC, Rentz D, et al. Age-related memory impairment associated with loss of parietal deactivation but preserved hippocampal activation. PNAS 2008; 105: 2181–2186.

Mintun MA, LaRossa GN, Sheline YI, Dence CS, Lee SY, Mach RH, et al. [11C]PIB in a nondemented population Potential antecedent marker of Alzheimer disease. Neurology 2006; 67: 446–452.

Mormino EC, Brandel MG, Madison CM, Marks S, Baker SL, Jagust WJ. Aβ Deposition in Aging Is Associated with Increases in Brain Activation during Successful Memory Encoding. Cereb Cortex 2012; 22: 1813–1823.

Mormino EC, Smiljic A, Hayenga AO, H Onami S, Greicius MD, Rabinovici GD, et al. Relationships between Beta-Amyloid and Functional Connectivity in Different Components of the Default Mode Network in Aging. Cereb Cortex 2011; 21: 2399–2407.

Morrison JH, Brinton RD, Schmidt PJ, Gore AC. Estrogen, Menopause, and the Aging Brain: How Basic Neuroscience Can Inform Hormone Therapy in Women. J. Neurosci. 2006; 26: 10332–10348.

Nebes RD, Snitz BE, Cohen AD, Aizenstein HJ, Saxton JA, Halligan EM, et al. Cognitive aging in persons with minimal amyloid-β and white matter hyperintensities. Neuropsychologia 2013; 51: 2202–2209.

Nichols TE, Holmes AP. Nonparametric permutation tests for functional neuroimaging: A primer with examples. Human Brain Mapping 2002; 15: 1–25.

Nichols TE, Holmes AP. Nonparametric permutation tests for functional neuroimaging. Human brain function 2004

Nyberg L. Functional brain imaging of episodic memory decline in ageing. Journal of Internal Medicine 2016; 281: 65–74.

O'brien JL, O'keefe KM, LaViolette PS, DeLuca AN, Blacker D, Dickerson BC, et al. Longitudinal fMRI in elderly reveals loss of hippocampal activation with clinical decline. Neurology 2010; 74: 1969–1976.

Oh H, Jagust WJ. Frontotemporal Network Connectivity during Memory Encoding Is Increased with Aging and Disrupted by Beta-Amyloid. J. Neurosci. 2013; 33: 18425–18437.

Patel AX, Kundu P, Rubinov M, Jones PS, V rtes PE, Ersche KD, et al. A wavelet method for modeling and despiking motion artifacts from resting-state fMRI time series. NeuroImage 2014; 95: 287–304.

Payami H, Montee KR, Kaye JA, Bird TD, Yu C-E, Wijsman EM, et al. Alzheimer's Disease, Apolipoprotein E4, and Gender. JAMA 1994; 271: 1316–1317.

Perrin RJ, Fagan AM, Holtzman DM. Multimodal techniques for diagnosis and prognosis of Alzheimer's disease. Nature 2009; 461: 916–922.

Petersen RC, Roberts RO, Knopman DS, Geda YE, Cha RH, Pankratz VS, et al. Prevalence of mild cognitive impairment is higher in men The Mayo Clinic Study of Aging. Neurology 2010; 75: 889–897.

Pizzi SD, Punzi M, Sensi SL. Functional signature of conversion in Mild Cognitive Impairment patients. bioRxiv 2018: 290783.

Poirier J, Bertrand P, Kogan S, Gauthier S, Davignon J, Bouthillier D. Apolipoprotein E polymorphism and Alzheimer's disease. The Lancet 1993; 342: 697–699.

Price JC, Klunk WE, Lopresti BJ, Lu X, Hoge JA, Ziolko SK, et al. Kinetic Modeling of Amyloid Binding in Humans using PET Imaging and Pittsburgh Compound-B. Journal of Cerebral Blood Flow & Metabolism 2005; 25: 1528–1547.

Rapp PR, Morrison JH, Roberts JA. Cyclic Estrogen Replacement Improves Cognitive Function in Aged Ovariectomized Rhesus Monkeys. J. Neurosci. 2003; 23: 5708–5714.

Roberts RO, Geda YE, Knopman DS, Cha RH, Pankratz VS, Boeve BF, et al. The incidence of MCI differs by subtype and is higher in men The Mayo Clinic Study of Aging. Neurology 2012; 78: WNL.0b013e3182452862–351.

Salami A, Pudas S, Nyberg L. Elevated hippocampal resting-state connectivity underlies deficient neurocognitive function in aging. PNAS 2014; 111: 17654–17659.

Schultz AP, Chhatwal JP, Hedden T, Mormino EC, Hanseeuw BJ, Sepulcre J, et al. Phases of Hyperconnectivity and Hypoconnectivity in the Default Mode and Salience Networks Track with Amyloid and Tau in Clinically Normal Individuals. J. Neurosci. 2017; 37: 4323–4331.

Shaywitz SE, Shaywitz BA, Pugh KR, Fulbright RK, Skudlarski P, Mencl WE, et al. Effect of Estrogen on Brain Activation Patterns in Postmenopausal Women During Working Memory Tasks. JAMA 1999; 281: 1197–1202.

Sheline YI, Raichle ME, Snyder AZ, Morris JC, Head D, Wang S, et al. Amyloid Plaques Disrupt Resting State Default Mode Network Connectivity in Cognitively Normal Elderly. Biological Psychiatry 2010; 67: 584–587.

Snyder PJ. **Overview of testosterone deficiency in older men**

[Internet]. httpswww.uptodate.comcontentsoverview-of-testosterone-deficiency-in-older-menH[cited 2017 Sep 10] Available from: https://www.uptodate.com/contents/overview-of-testosterone-deficiency-in-older-men#H28

Sperling R, Chua E, Cocchiarella A, Rand-Giovannetti E, Poldrack R, Schacter DL, et al. Putting names to faces:. NeuroImage 2003; 20: 1400–1410.

Sperling R. The potential of functional MRI as a biomarker in early Alzheimer's disease. Neurobiology of Aging 2011; 32: S37–S43.

Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, et al. Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's & Dementia 2011; 7: 280–292.

Sperling RA, Bates JF, Chua EF, Cocchiarella AJ, Rentz DM, Rosen BR, et al. fMRI studies of associative encoding in young and elderly controls and mild Alzheimer's disease. Journal of Neurology, Neurosurgery & Psychiatry 2003; 74: 44–50.

Sperling RA, LaViolette PS, O'Keefe K, O'Brien J, Rentz DM, Pihlajamaki M, et al. Amyloid Deposition Is Associated with Impaired Default Network Function in Older Persons without Dementia. Neuron 2009; 63: 178–188.

Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, et al. Automated Anatomical Labeling of Activations in SPM Using a Macroscopic Anatomical Parcellation of the MNI MRI Single-Subject Brain. NeuroImage 2002; 15: 273–289.

Vannini P, Hedden T, Becker JA, Sullivan C, Putcha D, Rentz D, et al. Age and amyloid-related alterations in default network habituation to stimulus repetition. Neurobiology of Aging 2012; 33: 1237–1252.

Wang ACJ, Hara Y, Janssen WGM, Rapp PR, Morrison JH. Synaptic Estrogen Receptor-α Levels in Prefrontal Cortex in Female Rhesus Monkeys and Their Correlation with Cognitive Performance. J. Neurosci. 2010; 30: 12770–12776.

Wilson AA, Garcia A, Jin L, Houle S. Radiotracer synthesis from [^{11}C]-iodomethane: A remarkably simple captive solvent method. Nuclear Medicine and Biology 2000; 27: 529–532.

Woods RP, Mazziotta JC, Cherry SR. MRI-PET registration with automated algorithm. J Comput Assist Tomogr 1993; 17: 536–546.

Woolley CS, Gould E, Frankfurt M, McEwen BS. Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. J. Neurosci. 1990; 10: 4035–4039.

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.

Zeineh MM, Engel SA, Thompson PM, Bookheimer SY. Dynamics of the Hippocampus During Encoding and Retrieval of Face-Name Pairs. Science 2003; 299: 577–580.

Zhang Q-G, Wang R-M, Scott E, Han D, Dong Y, Tu J-Y, et al. Hypersensitivity of the hippocampal CA3 region to stress-induced neurodegeneration and amyloidogenesis in a rat model of surgical menopause. Brain 2013; 136: 1432–1445.

Zhou Z, Ding M, Chen Y, Wright P, Lu Z, Liu Y. Detecting directional influence in fMRI connectivity analysis using PCA based Granger causality. Brain Res. 2009; 1289: 22–29.

Alzheimer's Association. Alzheimer's disease facts and figures. Alzheimer's & Dementia. 2017; 13: 325-373.

Table

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

| Characteristic	Group; mean (SD)		Statistical test	p value
	Female, n = 41	Male, n = 20		
Age, year[†]	75.5 (6.4)	76.5 (6.6)	$F_{1,59} = 0.32$	0.58
Education, year	14.3 (2.4)	15.3 (2.3)	$F_{1,59} = 2.37$	0.13
Race composition, no. (%)			$\chi^2 = 1.09$	0.58
White	33 (80.5)	18 (90.0)		
Black	7 (17.1)	2 (10.0)		
Asian	1 (2.4)	0(0.0)		
APOE genotype, no. (%)[‡]			$\chi^1 = 7.49$	0.01
ε2/ε3	4 (9.8)	3 (15.0)		
ε3/ε3	24 (58.5)	4 (20.0)		
ε3/ε4	3 (7.3)	5 (25.0)		
ε4/ε4	0 (0.0)	1 (5.0)		
Global PiB SUVR	1.58 (0.40)	1.57 (0.29)	$F_{1,59} = 0.01$	0.92
PiB(+), no (%)	8 (19.5)	6 (30.0)	$\chi^1 = 0.84$	0.36
MMSE score[§]	28.7 (1.4)	28.8 (1.4)	$F_{1,58} = 0.07$	0.80

[*]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).

Characteristics Mean(SD)*	Female (n=41)		Male (n=20)		Main and Interaction Effects		
	PiB(+) (8)	PiB(-) (33)	PiB(+) (6)	PiB(-) (14)	Gender	PiB	Gender x PiB
Memory							
WLL learning trials	22.0(4.3)	22.1(3.1)	19.2(3.7)	19.6(3.2)	**$F_{1,55}$=5.70, _p_=0.02**	$F_{1,55}$=0.05, _p_=0.82	$F_{1,55}$=0.03, _p_=0.86
WLL delayed recall	7.1(2.5)	7.7(1.8)	7.0(2.1)	6.4(2.5)	$F_{1,55}$=1.17, _p_=0.28	$F_{1,55}$=0.001, _p_=0.97	$F_{1,55}$= 0.81, _p_=0.37
Rey figure (max=24)							
Immediate recall	16.1(2.7)	16.1(3.7)	17.8(2.5)	16.6(2.4)	$F_{1,56}$=1.41, _p_=0.24	$F_{1,56}$=0.35, _p_=0.56	$F_{1,55}$=0.41, _p_=0.53
Delayed recall	14.8(2.8)	16.2(3.7)	17.5(2.5)	16.3(2.1)	$F_{1,56}$=2.07, _p_=0.16	$F_{1,56}$=0.02, _p_=0.89	$F_{1,56}$=1.52, _p_=0.22
Logical Memory Story A							
immediate recall	15.0(4.4)	16.5(3.8)	15.2(3.7)	15.2(2.7)	$F_{1,55}$=0.19, _p_=0.66	$F_{1,55}$=0.38, _p_=0.54	$F_{1,55}$=0.37, _p_=0.55
delay recall	14.5(6.5)	15.1(4.3)	14.4(3.8)	15.2(2.6)	$F_{1,55}$<0.001, _p_=0.99	$F_{1,55}$=0.24, _p_=0.62	$F_{1,55}$=0.01, _p_=0.93
Visuospatial construction							
Block design (max=24)	14.5(4.6)	13.6(3.6)	17.2(5.5)	13.9(3.6)	$F_{1,56}$=1.49, _p_=0.23	$F_{1,56}$=2.8, _p_=0.10	$F_{1,56}$=0.85, _p_=0.36
Rey figure copy	19.0(2.9)	19.8(2.4)	20.1(1.5)	19.3(1.4)	$F_{1,56}$=0.10, _p_=0.75	$F_{1,56}$=0.003, _p_=0.96	$F_{1,56}$=1.06, _p_=0.31
Language							
Semantic fluency(animals)	19.8(4.7)	20.1(5.8)	21.0(4.3)	21.4(6.4)	$F_{1,56}$=0.49, _p_=0.49	$F_{1,56}$=0.04, _p_=0.85	$F_{1,56}$<0.001, _p_=0.99
Letter fluency (FAS)	48.1(14.9)	44.1(14.7)	45.2(19.2)	39.0.1(9.4)	$F_{1,56}$=0.82, _p_=0.37	$F_{1,56}$=1.32, _p_=0.26	$F_{1,56}$=0.06, _p_=0.81
Boston Naming Test (max=30)	29.0(1.7)	29.5(0.9)	29.8(0.4)	29.7(0.6)	**$F_{1,56}$=4.45, _p_=0.04**	$F_{1,56}$=0.05, _p_=0.83	$F_{1,56}$=0.36, _p_=0.55
Attention and executive							
Trail Making Part A(sec)	31.8(9.4)	29.3 (9.1)	27.8(10.9)	32.7(14.5)	$F_{1,56}$=0.01, _p_=0.94	$F_{1,56}$=0.12, _p_=0.73	$F_{1,56}$=1.18, _p_=0.28
Trail Making Part B(sec)	113.7(61.9	71.9(20.6	92.6(3.3)	77.5(25.4	$F_{1,56}$=0.60, _p_=0.44	**$F_{1,56}$=8.11, _p_=0.006**	$F_{1,56}$=1.80, _p_=0.19
Digit Symbol	44.1(17.2	55.1(10.9)	47.3(12.7	48.4(9.3)	$F_{1,56}$=0.22, _p_=0.65	$F_{1,56}$=2.66, _p_=0.11	$F_{1,56}$=1.78, _p_=0.19
Stroop color-word	33.0(15.5	40.5(8.9)	30.2(5.2)	32.8(8.3)	$F_{1,54}$=2.72, _p_=0.11	$F_{1,54}$=2.50, _p_=0.12	$F_{1,54}$=0.59, _p_=0.45
Clock drawing (max=15)	14.8(0.5)	14.2(0.8)	14.7(0.5)	14.2(0.9)	$F_{1,56}$=0.01, _p_=0.91	**$F_{1,56}$=4.29, _p_=0.04**	$F_{1,56}$=0.05, _p_=0.82
Face/Name Recognition %	70.0(9.3)	71.0(11.2)	65.5(11.3)	61.0(12.4)	$F_{1,57}$=2.19, _p_=0.14	$F_{1,57}$=0.004, _p_=0.95	$F_{1,57}$=1.84, _p_=0.18

Table 3. Gender-by-Aβ load ANOVAs of hippocampal functional connectivity during the face-name associative memory task (corrected $p < 0.05$).

Gender -by-Aβ ANOVA	Brain region	Brodmann area (BA)	Peak MNI coordinates (x,y,z)	t-score	Size (mm³)
Left hippocampal functional connectivity					
Main effect of gender	None				
Main effect of Aβ	None				
Gender by Aβ interaction	mPFC/ACC, L SFG, L MFG	BA 10, 32, 24	-4, 50, 8	4.21	3192
Right hippocampal functional connectivity					
Main effect of gender	None				
Main effect of Aβ	None				
Gender by Aβ interaction	mPFC/ACC, L SFG, L MFC	BA 10	-22, 54, -2	5.34	1592
	L MCG/Cuneus	BA 17, 18, 19, 39	-26, -82, 8	4.47	1352

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.
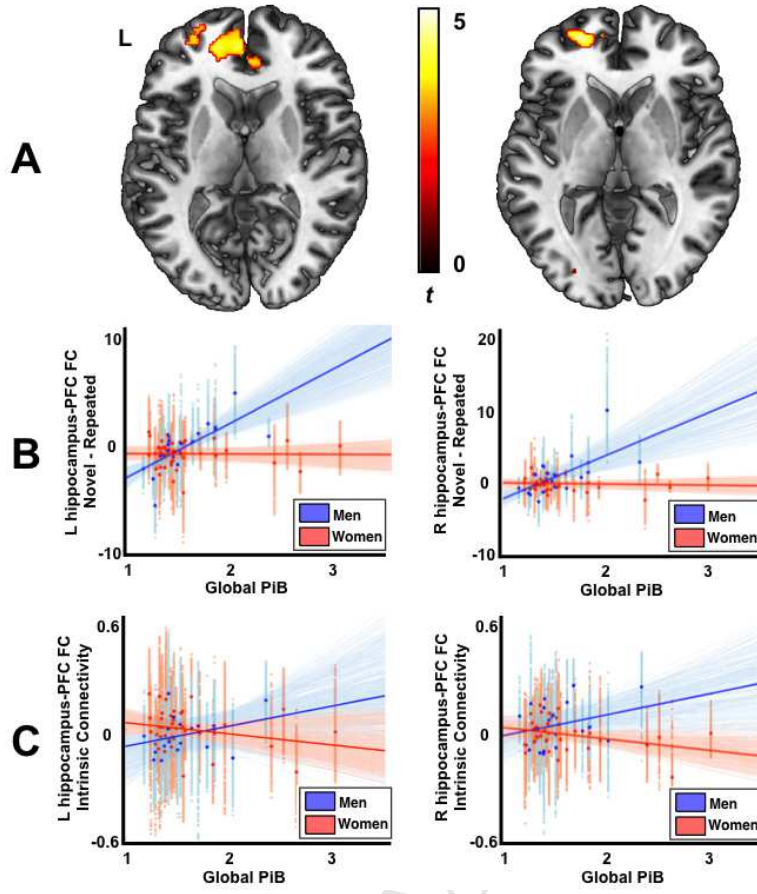
Highlights

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.