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## Differential Effect of Age on Posterior and Anterior Hippocampal Functional Connectivity

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### Abstract

Aging is associated with declines in cognitive performance and multiple changes in the brain, including reduced default mode functional connectivity (FC). However, conflicting results have been reported regarding age differences in FC between hippocampal and default mode regions. This discrepancy may stem from the variation in selection of hippocampal regions. We therefore examined the effect of age on resting state FC of anterior and posterior hippocampal regions in an adult life-span sample. Advanced age was associated with lower FC between the posterior hippocampus and three regions: the posterior cingulate cortex, medial prefrontal cortex, and lateral parietal cortex. In addition, age-related reductions of FC between the left and right posterior hippocampus, and bilaterally along the posterior to anterior hippocampal axis were noted. Age differences in medial prefrontal and inter-hemispheric FC significantly differed between anterior and posterior hippocampus. Older age was associated with lower performance in all cognitive domains, but we observed no associations between FC and cognitive performance after controlling for age. We observed a significant effect of gender and a linear effect of *COMT* val158met polymorphism on hippocampal FC. Females showed higher FC of anterior and posterior hippocampus and medial prefrontal cortex than males, and the dose of val allele was associated with lower posterior hippocampus – posterior cingulate FC, independent of age. Vascular and metabolic factors showed no significant effects on FC. These results suggest differential age-related reduction in the posterior hippocampal FC compared to the anterior hippocampus, and an age-independent effect of gender and *COMT* on hippocampal FC.

### Keywords

Functional Connectivity; Aging; Hippocampus; *COMT* val158met; Gender; Vascular Risk

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## 1. Introduction

Advanced age is associated with low performance on multiple cognitive tasks (Salthouse, 2010), and magnetic resonance imaging (MRI) has been used to explore the neural correlates of this cognitive decline (for example: (Grady, 2012; Gunning-Dixon and Raz, 2000; Raz et al., 1998). Numerous brain characteristics, such as regional brain volumes, structural and diffusion properties of the white matter and task-related activation, exhibit significant age differences and have been linked to cognitive performance, see (Fjell et al., 2014; Kennedy and Raz, 2015) for reviews. Since the introduction of MRI measures of resting brain activity (resting state MRI or rsMRI; (Biswal et al., 1995; Raichle et al., 2001), various measures of functional connectivity within and among brain networks have been used to assess age-related differences on a brain network level.

The core assumption behind applying functional connectivity measures to the study of cognitive aging is that even subtle disruption of physical connections and relatively minor damage to the brain's regional integrity may affect the flow of information across the brain, which can manifest their effect in age-related differences in functional connectivity (Ferreira and Busatto, 2013). Measures of resting state functional connectivity reflect coherence between temporal fluctuations in the blood oxygen level dependent (BOLD) signal across brain regions organized into distinct networks (Damoiseaux et al., 2006; van den Heuvel et al., 2008; Zuo et al., 2010).

In the extant studies of age-related differences in resting state functional connectivity, the most consistent finding is the association between advanced age and lower functional connectivity within the default mode network (Andrews-Hanna et al., 2007; Damoiseaux et al., 2008; Ferreira and Busatto, 2013). Default mode network activity is commonly linked to a variety of cognitive processes such as episodic memory, self-referential processing, and mind wandering (Buckner et al., 2008; Raichle et al., 2001), with a positive relationship reported between strength of default mode connectivity and performance on tasks of memory and executive function (Andrews-Hanna et al., 2007; Damoiseaux et al., 2008; Wang et al., 2010).

Even though both independent component analysis (ICA) and seed-based analyses of the default mode network have consistently revealed age-related differences in functional connectivity, there is no consensus regarding the role of the hippocampus within the default mode network. Consequently, age-related differences in hippocampal functional connectivity remain unclear. Some studies examining age effects on default mode connectivity did not include the hippocampus (Bluhm et al., 2008; Damoiseaux et al., 2008), while others varied considerably in the exact demarcation of the region in question. For example, some (Andrews-Hanna et al., 2007) found lower connectivity in older adults in a posteriorly hippocampal region of interest (ROI), whereas others found either no significant connectivity differences (Koch et al., 2010) or increased functional connectivity (Salami et al., 2014) with older age in more anteriorly located ROIs. These discrepancies in reported findings may, at least partly, be explained by differences in hippocampal ROI selection, since hippocampal connectivity patterns are known to vary across hippocampal regions (Kahn et al., 2008; Zarei et al., 2013). The posterior hippocampus is part of the functional

pathway that, via the cingulum bundle, connects to parahippocampal, retrosplenial, posterior cingulate, precuneus, lateral parietal and medial prefrontal cortices - all areas of the default mode network. In contrast, the anterior hippocampus belongs to the functional pathway that via the uncinate fasciculus is connected with the amygdala, perirhinal/entorhinal cortices, and the anterior and lateral temporal lobes (Kahn et al., 2008; Poppenk and Moscovitch, 2011; Ranganath and Ritchey, 2012).

The main goal of this study is to address these discrepancies by determining the effect of age on posterior versus anterior hippocampal functional connectivity to default mode brain regions, and the relationship with cognitive performance, in an adult life-span sample. Based on the extant research outlined above, we hypothesized lower functional connectivity of the posterior hippocampus with older age, and either no age-related differences (Koch et al., 2010) or an increase in connectivity with age (Salami et al., 2014) for the anterior hippocampus.

As characteristics of participants vary across studies, another potential source of discrepancy among studies could be unaccounted variability in the samples with regards to gender, vascular risk burden and genetic risk factors, all of which can affect brain structure and function (Ferreira and Busatto, 2013; Jagust, 2013; Raz and Rodrigue, 2006). Thus, an additional, exploratory aim of this study was to determine the effect of several age-related and age-independent risk factors on hippocampal functional connectivity and cognitive performance. Among the former are risk factors such as vascular and metabolic risk (Friedman et al., 2014; Zhou et al., 2010) whereas the latter include gender and genetic variants that are associated with risk for age-related cognitive and physiological impairment, such as Alzheimer's disease, diabetes, inflammation and probable dopamine availability in the synapse (for examples see (Barnett et al., 2008; Damoiseaux et al., 2012).

## 2. Methods

### 2.1. Participants

Adult volunteers were recruited from the Metro Detroit, Michigan area through advertisements in local newspapers and flyers for an ongoing longitudinal study of cognitive and neural correlates of aging. Structural and functional MRI data were available on 167 adults (60 men, 107 women) age 18–83 years (mean age  $49.1 \pm 18.0$  years). A subset of this sample, 91 adults aged 18–78 years (mean age  $42.2 \pm 17.6$  years; 33 men, 58 women), completed cognitive testing. The Wayne State University Institutional Review Board approved the study and signed informed consent was obtained from all participants. Participants spoke English as their first language and were right-hand dominant (score over 75% on Edinburgh Inventory, (Oldfield, 1971)). They were screened with a health questionnaire for neurological, psychiatric, cardiovascular, and endocrine diseases, diabetes, cancer, and a history of loss of consciousness for more than 5 minutes. In addition, participants were screened for dementia (Mini-Mental State Exam, MMSE  $\geq 26$ ; (Folstein et al., 1975) and depression (Center for Epidemiological Study Depression questionnaire, CES-D  $\geq 16$ ; (Radloff, 1977)). There was no relationship between age and MMSE ( $r=-0.053$ ,  $p=0.498$ ), but older participants had more years of formal education ( $r=0.173$ ,  $p=0.025$ ).

## 2.2. Data acquisition

**2.2.1. MRI data**—Imaging was performed at the MRI research facility at Wayne State University on a 3-Tesla Siemens Verio (Siemens Medical AG, Erlangen, Germany) full-body magnet with a 12-channel head coil. The scan session included resting state functional MRI and anatomical MRI. For the resting state functional scan, 200 volumes of 43 axial slices were acquired sequentially using a T2\*-weighted echo planar sequence with the following parameters: repetition time (TR) = 2500 ms, echo time (TE) = 30 ms, flip angle = 90°, pixel bandwidth = 2298 Hz/pixel, GRAPPA acceleration factor PE = 2, field-of-view = 210 mm, matrix size = 64 x 64, voxel size = 3.3 × 3.3 × 3.3 mm. Participants were instructed to lie still with their eyes open. For the anatomical scan a 3D T1-weighted magnetization-prepared rapid gradient-echo (MPRAGE) sequence was acquired with the following parameters: TR = 1680 ms, TE = 3.51 ms, inversion time (TI) = 900 ms, flip angle = 9.0°, pixel bandwidth = 180 Hz/pixel, GRAPPA acceleration factor PE = 2; field-of-view = 256 mm, matrix size = 384 x 384, voxel size 0.67 mm × 0.67 mm × 1.34 mm.

**2.2.2. Cognitive data**—Cognitive performance was assessed using a comprehensive battery of cognitive tests, including: letter comparison, pattern comparison, Woodcock-Johnson-R Memory for Names (immediate and delayed); Stroop, Wisconsin Card Sorting Test (WCST), size judgment span, listening span, spatial recall, and Cattell Culture Fair Test (see (Raz et al., 2009), for a detailed description of the cognitive battery).

**2.2.3. Vascular and metabolic risk data**—We collected common indicators of vascular and metabolic risk: blood pressure, frequency of exercise, smoking, and waist-to-hip ratio. In addition, a phlebotomist collected blood samples from all participants following a 12-hr overnight fast. The Detroit Medical Center hospital laboratory analyzed these samples to determine levels of cholesterol, glucose and triglycerides.

**2.2.4. Genetic data**—DNA extraction and genotyping were performed on material obtained from buccal cell cultures that were collected in mouthwash samples. DNA was isolated with Genra Autopure LS under the standard buccal cell protocol. The Wayne State University Applied Genomics Technology Center performed DNA isolations and genotyping assays using an Applied Biosystems 7900. DNA sequencing reactions were carried out using the 0.5X protocol for ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). The sequencing extension products were purified utilizing Sephadex, and analyzed on an ABI PRISM 3700 DNA analyzer with a 50-cm capillary array. Genetic variants associated with increased risk for Alzheimer's disease, diabetes, synaptic dopamine degradation and pro-inflammatory response were determined, including: *APOE*, *CLU*, *TOMM157580*, *TOMM157581*, *TOMM207*, *TOMM5900*, *GCK*, *G6PC2*, *G6PC2G231A*, *TCF7L2*, *IL-1βC-511T*, *IL-6 C-174G* and *TNFα G308A*, and *COMT* val158met.

## 2.3. Data analysis

**2.3.1. MRI data**—Image preprocessing was carried out using tools from FMRIB's Software Library (FSL, version 4.1; (Smith et al., 2004)). For the resting state data the following pre-statistics processing was applied: motion correction (Jenkinson et al., 2002);

removal of non-brain structures (Smith, 2002); spatial smoothing using a Gaussian kernel of 6 mm full width at half maximum; and mean-based intensity normalization of all volumes by the same factor (i.e. 4D grand-mean scaling in order to ensure comparability between data sets at the group level). After pre-processing the functional scan was first aligned to the individual's high resolution T1-weighted image, which was subsequently registered to the MNI152 standard space (average T1 brain image constructed from 152 normal subjects at Montreal Neurological Institute) using affine linear registration (Jenkinson et al., 2002). ICA-based Automatic Removal Of Motion Artifacts (ICA-AROMA; (Pruim et al., 2015) was applied to these normalized images. ICA-AROMA is a data-driven method of identifying and removing motion-related independent components from functional MRI data. As a last step of preprocessing we applied high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with  $\sigma=75.0s$ ) and removed the global signal contribution from the data. The anatomical data underwent the following preprocessing: removal of non-brain structures (Smith, 2002), followed by segmentation of the high-resolution images (T1-weighted MPRAGE) into grey matter, white matter, cerebrospinal fluid and background, and lastly calculation of partial volume maps (Zhang et al., 2001). All individual grey matter partial volume maps were transformed into MNI152 standard space using affine linear registration. By averaging every participant's standard space grey matter image a sample-specific mean grey matter image was created. In addition, individual partial volume estimates were used to calculate grey matter volume for each of the ROIs used in the functional connectivity analyses. The association between grey matter volume of specific ROIs and their functional connectivity was examined using Pearson correlation (significance determined at  $\alpha=0.003$  after applying a Bonferroni correction for multiple comparisons).

ROI-based correlations were used to assess functional connectivity between the anterior and posterior hippocampal regions and default mode regions. Eight spherical ROIs were created using the coordinates specified in (Andrews-Hanna et al., 2007), which were transformed from Talairach to Montreal Neurological Institute (MNI) space using the tal2icbm transform (Lancaster et al., 2007). These ROIs were located in the bilateral posterior hippocampus (L:  $xyz = -23 -26 -15$ ; R:  $xyz = 25 -26 -15$ ), posterior cingulate/retrosplenial cortex ( $xyz = 0 -50 29$ ), medial prefrontal cortex ( $xyz = 2 44 11$ ), bilateral lateral parietal (L:  $xyz = -46 -68 30$ ; R:  $xyz = 57 -65 29$ ), and bilateral parahippocampal gyrus (L:  $xyz = -25 -41 -12$ ; R:  $xyz = 28 -41 -12$ ). Two more anteriorly located hippocampal ROIs were created using the coordinates reported by Salami and colleagues (2014) showing an age related increase in functional connectivity (L:  $xyz = -18 -14 -18$ ; R:  $xyz = 18 -14 -18$ ). A diameter of 4 mm was used for hippocampal and parahippocampal ROIs, and 8 mm for the others. The sample-specific mean grey matter image was used to constrain the ROIs to grey matter only. The time-series of bilateral ROIs were averaged to calculate posterior and anterior hippocampal functional connectivity with the default mode ROIs. Mean time-series of unilateral ROIs were used to calculate within hippocampal functional connectivity. Pearson correlations among ROIs served as indices of functional connectivity, and their Fisher's  $r$ -to- $z$  transformed values were used as dependent variables in linear regression models to assess age effects while accounting for gender. Within-subjects analysis of variance models were used to assess differences in posterior versus anterior hippocampal functional connectivity to default mode regions and the interaction with age. Significance was determined after

correcting for multiple comparisons using the Benjamini-Hochberg procedure with a false discovery rate of  $q < 0.05$ .

**2.3.2. Cognitive data**—Cognitive tests scores were available for 91 of the 167 participants. For the Stroop color task, we computed interference scores by residualizing the reading time under the incompatible condition on the time under the neutral condition. Numbers of perseverative responses and perseverative errors that served as indicators of performance on WCST were log-transformed to correct skewness. Listening span scores were log-transformed for the same reason. Stroop interference scores and the numbers of perseverative responses and perseverative errors in WCST were reversely coded, so that for all the variables, higher cognitive scores indicated better performance. All cognitive scores were standardized to avoid scaling problems. Two subjects had missing cognitive data: one missed WCST scores, and the other did not have letter comparison, pattern comparison, spatial recall, Cattell Culture Fair Tests, size judgment span and listening span scores. To determine the main cognitive constructs for analyses, we performed a confirmatory factor analysis (CFA), while taking into account missing observations via the full-information maximum likelihood (FIML) method. Missing cases were handled under the MAR (missing at random) assumption, which allows missingness to be related to the observed covariates and observed outcomes. The CFA model consisting of three latent factors: processing speed, with scores of letter comparison and pattern comparison as indicators; memory, with scores of Woodcock-Johnson-R Memory for Names (WJR memory, immediate and delayed) as indicators; and executive functions, with the following indicators: Stroop, Wisconsin Card Sorting Test, size judgment span, listening span, spatial recall, and Cattell Culture Fair Test (form 3B, tests 1, 2, 3, 4). The component loadings on common factors are as follows: letter comparison 1.000 and pattern comparison 1.026 onto processing speed; immediate WJR memory 1.000 and delayed WJR memory 0.836 onto memory; for executive functions, the loadings are Stroop tests 1.000, 1.036, size judgment span 0.830, listening span 1.226, WCST perseverative responses 1.077, WCST perseverative errors 1.078, spatial recall 1.010, and 1.245, 0.914, 0.531, 1.072 respectively for the four Cattell subtests. The goodness-of-fit indices are: CFI (Comparative Fit Index) = 0.973, RMSEA (Root mean square error of approximation) = 0.059, SRMR (standardized root mean residual) = 0.049, indicating that the CFA is a valid model. The analyses were conducted using *Mplus* 6.0 (Muthén and Muthén, 2010), and composite factor scores were calculated for each latent factor. To examine the effect of age on cognitive performance, Pearson correlations between factor scores, which contained information about an individual's placement on the factor, and age was calculated. In addition, Pearson correlations were calculated to assess the relationship between cognitive performance and functional connectivity. Significance for both correlation analyses was determined at  $\alpha = 0.002$  after correcting for multiple comparisons using a Bonferroni correction.

**2.3.3. Vascular and metabolic risk data**—Composite scores for vascular and metabolic risk were created, based on commonly known clinical risk factors, to measure potential effects of participant's health on age-related functional connectivity differences. The vascular risk composite score was calculated as  $2 \times \text{diagnosed high blood pressure} + \text{smoking} - \text{exercise} + ((\text{systolic} - 120)/10) + ((\text{diastolic} - 80)/10)$ ; where diagnosed high

blood pressure, smoking and exercise are binary (1 versus 0); and systolic and diastolic are actual blood pressure readings referenced to the hypertension cut-offs. Metabolic risk composite score was calculated as the sum of waist-hip-ratio, triglycerides, systolic blood pressure, glucose and high-density lipoprotein; where the actual values are standardized and high-density lipoprotein is reversely coded so that for all the variables, higher scores indicated increased risk. Individual composite scores were calculated and added as independent variables along with the genetic risk variables described below, age and gender in linear regression models to assess their association with functional connectivity and cognitive performance. Significance was determined after correcting for multiple comparisons using the Benjamini-Hochberg procedure with a false discovery rate of  $q < 0.05$ .

**2.3.4. Genetic data**—Genetic variants associated with increased risk for Alzheimer’s disease, diabetes, dopamine availability and pro-inflammatory response were assessed and cumulative risk scores were computed. Such composite scores are frequently computed to evaluate risks conveyed by ensembles of risk alleles rather than single genes (Potkin et al., 2016). Cumulative risk score for Alzheimer’s disease was calculated as the sum of the risk alleles on the following single nucleotide polymorphisms (SNPs): apolipoprotein E (*APOE*)  $\epsilon 4$  variant, clusterine (*CLU*), and four *TOMM40* SNPs (*TOMM157580*, *TOMM157581*, *TOMM207*, *TOMM5900*). Cumulative diabetes risk was calculated as the sum of the risk alleles on: *GCK*, *G6PC2*, *G6PC2G231A* and *TCF7L2*; and cumulative pro-inflammatory response propensity was represented as the sum of the risk alleles on: *IL-1 $\beta$ C-511T*, *IL-6 C-174G* and *TNF $\alpha$  G308A*. Probable synaptic dopamine availability (high to low) was evaluated as the dose of the val allele in Catechol-O-methyltransferase (*COMT*) val158met SNP. Individual risk scores were added as independent variables along with vascular and metabolic risk, age and gender in linear regression models to assess their association with functional connectivity and cognitive performance. Significance was determined after correcting for multiple comparisons using the Benjamini-Hochberg procedure with a false discovery rate of  $q < 0.05$ .

### 3. Results

#### 3.1. Age differences in functional connectivity

Advanced age was associated with lower functional connectivity of bilateral posterior hippocampus – posterior cingulate/retrosplenial cortex ( $F_{(2,164)}=4.149$ ,  $p=0.017$ , adjusted  $R^2=0.037$ , age  $\beta=-0.198$ ,  $p=0.010$ , gender not significant); bilateral posterior hippocampus – medial prefrontal ( $F_{(2,164)}=8.280$ ,  $p<0.001$ , adjusted  $R^2=0.081$ , age  $\beta=-0.198$ ,  $p=0.009$ , gender  $\beta=0.239$ ,  $p=0.002$ ); and bilateral posterior hippocampus – bilateral lateral parietal ( $F_{(2,164)}=4.571$ ,  $p=0.012$ , adjusted  $R^2=0.041$ , age  $\beta=-0.199$ ,  $p=0.010$ , gender not significant), see figure 1 (plotted in red). Connectivity between the other hippocampal – default mode ROI pairs evidenced no significant association with age. Direct comparison of bilateral anterior versus posterior hippocampal connectivity within participants showed significantly higher connectivity ( $F_{(1,165)}=10.823$ ,  $p=0.001$ ) for posterior hippocampus – medial prefrontal cortex and a differential effect of age on these pairs ( $F_{(1,165)}=5.249$ ,  $p=0.021$ ), such that connectivity of the posterior hippocampus showed stronger association with age than connectivity of the anterior hippocampus. Connectivity of anterior versus

posterior hippocampus to posterior cingulate/retrosplenial and lateral parietal cortices did not show any significant differences (figure 1). Note, that even though only functional connectivity of posterior hippocampal – default mode ROI pairs showed a significant reduction with older age, this association was only significantly different between anterior versus posterior hippocampus for connectivity with the medial prefrontal cortex. The association of age with connectivity of anterior hippocampus to posterior cingulate/retrosplenial and lateral parietal cortices therefore was not significant at the corrected 0.05 threshold.

To assess further differences within the hippocampus, we examined functional connectivity between unilateral posterior and anterior hippocampal ROIs. The results showed reduced inter-hemispheric functional connectivity between the left and right posterior hippocampus with advanced age ( $F_{(2,164)}=9.834$ ,  $p<0.001$ , adjusted  $R^2=0.096$ , age  $\beta=-0.298$ ,  $p<0.001$ , gender  $\beta=0.149$ ,  $p=0.045$ ). Connectivity between the left and right anterior hippocampus was unrelated to age. Direct comparison of hippocampal inter-hemispheric connectivity showed no significant difference in connectivity strength between anterior and posterior hippocampus, however, it did show a differential effect of age ( $F_{(1,165)}=5.668$ ,  $p=0.018$ ), see figure 2. Within-hemispheric functional connectivity along the posterior to anterior axis decreased with age for both left hippocampus ( $F_{(2,164)}=7.050$ ,  $p=0.001$ , adjusted  $R^2=0.068$ , age  $\beta=-0.245$ ,  $p=0.001$ , gender  $\beta=0.150$ ,  $p=0.047$ ), and right hippocampus ( $F_{(2,164)}=4.103$ ,  $p=0.018$ , adjusted  $R^2=0.036$ , age  $\beta=-0.194$ ,  $p=0.012$ , gender not significant), see figure 2. Direct comparison of this within-hemispheric connectivity revealed significantly higher connectivity strength within the left hippocampus compared to the right hippocampus ( $F_{(1,165)}=8.941$ ,  $p=0.005$ ), but no different effect of age, see figure 2. All significant results reported here were significant after correction for multiple comparisons using a false discovery rate of  $q=0.05$ . None of the correlations between ROI grey matter volume and functional connectivity of corresponding regions reached significance; therefore grey matter volume was not included as covariate in any of the functional connectivity analyses.

### 3.2. Age differences in cognitive performance and their relationship to functional connectivity

Advanced age was associated with lower performance in all cognitive domains tested: *processing speed* ( $r=-0.610$ ,  $p<0.001$ ), *memory* ( $r=-0.548$ ,  $p<0.001$ ), and *executive function* ( $r=-0.665$ ,  $p<0.001$ ). Functional connectivity between anterior hippocampus and lateral parietal cortex was positively correlated to performance on executive function tasks ( $r=0.328$ ,  $p=0.001$ ), meaning that better performance was associated with higher functional connectivity. After controlling for age and gender, this association between functional connectivity strength and cognitive performance no longer remained significant.

### 3.3. Gender differences in functional connectivity

We observed a relationship between gender and functional connectivity of posterior hippocampus – medial prefrontal cortex ( $F_{(2,164)}=8.280$ ,  $p<0.001$ , adjusted  $R^2=0.081$ , gender  $\beta=0.239$ ,  $p=0.002$ , age  $\beta=-0.198$ ,  $p=0.009$ ) and anterior hippocampus – medial prefrontal cortex ( $F_{(2,164)}=3.911$ ,  $p=0.022$ , adjusted  $R^2=0.034$ , gender  $\beta=0.213$ ,  $p=0.006$ , age not significant). A subsequent one-way ANOVA revealed significantly increased functional



connectivity in females compared to males for posterior hippocampus – medial prefrontal cortex ( $F_{(1,165)}=9.165$ ,  $p=0.003$ ; females  $M=0.134$ ,  $SD=0.148$ ; males  $M=0.061$ ,  $SD=0.155$ ) and anterior hippocampus – medial prefrontal cortex ( $F_{(1,165)}=7.764$ ,  $p=0.006$ , females  $M=0.099$ ,  $SD=0.166$ ; males  $M=0.024$ ,  $SD=0.165$ ).

### 3.4. Effect of age-related and age-independent risk factors

The examination into the effect of vascular, metabolic and genetic risk on functional connectivity and cognitive function, showed a significant effect of *COMT* val158met on functional connectivity between the posterior hippocampus and the posterior cingulate/retrosplenial cortex independent of age ( $F_{(8,109)}=2.449$ ,  $p=0.018$ , adjusted  $R^2=0.090$ , *COMT*  $\beta=-0.346$ ,  $p<0.001$ , other variables not significant). A subsequent one-way ANOVA confirmed the observed effect ( $F_{(2,129)}=7.735$ ,  $p=0.001$ ) and Tukey post-hoc tests revealed that val homozygotes ( $M=0.125$ ,  $SD=0.168$ ) showed significantly lower functional connectivity between the posterior hippocampus and the posterior cingulate/retrosplenial cortex than val heterozygotes ( $M=0.215$ ,  $SD=0.144$ ,  $p=0.013$ ) and met homozygotes ( $M=0.274$ ,  $SD=0.161$ ,  $p=0.001$ ), see figure 3. Our sample contained 38 val homozygotes, 68 val heterozygotes, and 26 met homozygotes, this distribution is consistent with the Hardy – Weinberg principle ( $\chi^2=0.199$ ,  $p=0.655$ ). No differences were found in age, gender, and years of education across allelic groups, and no association was observed between *COMT* val158met and cognitive performance. None of the other risk factors examined emerged as significant correlates of functional connectivity strength, or cognitive performance.

## 4. Discussion

The main finding of this study is a differential effect of age on functional connectivity of posterior versus anterior hippocampus in healthy adults. Our results show that advanced age is associated with lower functional connectivity between left and right posterior hippocampus and posterior hippocampus to medial prefrontal cortex, and that this is significantly different from connectivity of the anterior hippocampus, which is not affected by age. Advanced age also is linked to lower within-hemispheric, i.e. posterior – anterior, functional connectivity for both left and right hippocampus. Furthermore, we show that advanced age is associated with lower posterior hippocampus – posterior cingulate/retrosplenial, and posterior hippocampus – lateral parietal functional connectivity. However, the magnitude of these age effects was not significantly different between the posterior and anterior hippocampus. The cortical pathway that includes the posterior hippocampus is known to connect with the parahippocampal gyrus and retrosplenial cortex and, via the cingulum bundle, regions of the default mode network, such as the posterior cingulate, precuneus, parietal and medial prefrontal cortex (Ranganath and Ritchey, 2012; Ward et al., 2013). Our findings suggest a partial age-related functional disconnection of the posterior hippocampus from contra- and ipsilateral hippocampal regions and other regions within this pathway. Additional evidence of partial age-related disconnection comes from existing reports on white matter connectivity estimated from diffusion tensor imaging. These studies indicate lower fractional anisotropy in the cingulum bundle with older age (Catheline et al., 2010; Damoiseaux et al., 2009; Yoon et al., 2008). It is plausible, therefore, that disruption of white matter organization of the cingulum underlies the observed decline in functional

connectivity. Further research directly comparing structural and functional connectivity measures of this pathway is needed, however, to substantiate this claim. A pattern of reduced hippocampal connectivity has also been observed in patients with mild cognitive impairment and Alzheimer's disease (Delbeuck et al., 2003; Stoub et al., 2006; Villain et al., 2008). These findings may indicate either an accelerated form of the age-related reduction of hippocampal connectivity, similar to observed in the current study or a distinct process affecting other pathways or separate sub-regions of the cingulum bundle (Catheline et al., 2010; Damoiseaux et al., 2009; Zhang et al., 2007).

In accord with one report (Koch et al., 2010), we found no age differences in functional connectivity of the anterior hippocampus to default mode regions. Nor did we note age differences in inter-hemispheric functional connectivity, as was observed for the posterior hippocampus. As mentioned in the introduction, the anterior and posterior parts of the hippocampus likely belong to distinct functional and vascular systems, and functional differences between the two may play out in various aspects of memory, semantic, social and emotional processing (Kahn et al., 2008; Ranganath and Ritchey, 2012). Nevertheless, for most default mode regions examined here, we found no difference between their functional connectivity to anterior versus posterior hippocampus. Therefore, the role of these separate pathways in the observed differences between anterior and posterior hippocampal functional connectivity needs to be viewed with caution. Although for this study we focused on the default mode network, it must be noted that age-related functional connectivity differences in other resting state networks and in interactions among networks have been also reported (Chan et al., 2014; Ferreira and Busatto, 2013; Geerligs et al., 2015). Our results do not preclude age-related functional connectivity differences across other brain networks, but we did not find them in the anterior hippocampal ROIs, contrary to what was reported elsewhere (Salami et al., 2014). This discrepancy may stem from sample characteristics, such as the age of participants. Although as in our study, the participants included in the study by Salami and colleagues (2014) nominally covered almost the entire adult lifespan, a larger proportion of their participants were older adults, as indicated by the average age of 61.5 years versus 49.1 years in our sample. Perhaps functional connectivity differences across networks other than the default mode become more apparent at an older age. Further research is needed to address this hypothesis.

Including grey matter volume estimates as covariates in functional connectivity studies has become a common practice, in spite of lack of a clear rationale for the relationship between functional connectivity and grey matter volume. In our sample, the correlations between ROI hippocampal volume and functional connectivity of corresponding pairs revealed no significant associations. Further examination of grey matter volume and functional connectivity is needed to more thoroughly assess the need for including grey matter volume in functional connectivity analyses.

Our results replicated the reported negative associations between older age and lower performance on multiple cognitive tests; in addition, we observed a relationship between default mode functional connectivity and performance on tasks of processing speed. However, no links between functional connectivity strength and cognitive performance were found once age was taken into account. This may reflect a well-known problem of cross-

sectional mediation, see (Hofer and Sliwinski, 2001; Lindenberger et al., 2011; Lindenberger and Pötter, 1998; Maxwell and Cole, 2007) for discussions. The commonality between age, brain indices and cognitive performance measures is too strong to allow a meaningful representation of developmental dynamics (Raz and Lindenberger, 2011; Salthouse, 2011). Previous cross-sectional studies that did find a relationship between functional connectivity and cognitive function independent of age, worked with more a restricted age range and found these effects predominantly in the older adults (Andrews-Hanna et al., 2007; Damoiseaux et al., 2008). Future studies using longitudinal designs are needed to investigate the relationship between functional connectivity and cognitive function independent of age.

Our examination of the relationship between gender, vascular, metabolic and genetic risk factors and hippocampal functional connectivity yielded two findings: First, a gender difference in hippocampal – medial prefrontal functional connectivity; second, an association between dopamine availability as assessed by *COMT* val158met and posterior hippocampal – posterior cingulate/retrosplenial functional connectivity. Our findings of gender differences in functional connectivity are in line with previous reports (Gong et al., 2011; Tomasi and Volkow, 2012), and more specifically, with a report of increased functional connectivity of the medial prefrontal cortex in females (Biswal et al., 2010). Because gender is associated with functional connectivity it is important to take it into account in data analysis. Here we included gender in the models examining age effects, and our reported results are significant after controlling for gender.

*COMT* is an enzyme that catabolizes dopamine in synaptic clefts, mainly of the D1 receptor locations that predominate in the prefrontal cortex. The val allele of the *COMT* val158met polymorphisms is associated with greater activity of *COMT* at the synapse and therefore very likely with lesser dopamine availability (Chen et al., 2004; Lotta et al., 1995). The probable increase in dopamine availability associated with the met allele has been linked to better performance on several executive tasks (Bruder et al., 2005; Caldú et al., 2007; de Frias et al., 2005; Raz et al., 2009), although these results have not been replicated consistently, see (Barnett et al., 2008) for a review. The majority of existing neuroimaging literature indicates that the val allele is associated with lower neuronal efficiency, resulting in higher prefrontal and decreased medial temporal lobe activation during working memory tasks (de Frias et al., 2009; Dennis et al., 2010), and lower functional connectivity of hippocampal and other default mode regions (Dennis et al., 2010; Liu et al., 2010). Across a younger part of the life-span, from neonates to middle-aged adults, homozygosity for the val (high activity) allele of *COMT* val158met polymorphism has been linked to reduced task-related hippocampal activation (Bertolino et al., 2006; Krach et al., 2010), and coupling between the hippocampus and other cortical regions (Bertolino et al., 2006; Dennis et al., 2010). In some samples of young adults, the intermediate levels of *COMT* activity are associated with greater connectivity of the prefrontal cortex (Dang et al., 2013), and in some instances, the effect of that genetic variant was observed only in conjunction with moderating factors, such as cumulative exposure to stress (Rabl et al., 2014). Our results showing a significant linear relationship between the dose of a met allele and functional connectivity are consistent with these patterns. Although *COMT* val158met variant is considered of particular relevance to the prefrontal dopaminergic system (Dickinson and

Elvevåg, 2009), we found no effect of *COMT* on the connections involving the medial prefrontal cortex, which showed a relationship in the expected direction that did not reach significance. Notably, this is the first study to examine the effect of *COMT* val158met on functional connectivity in a sample including older adults and found its influence independent of age.

In addition to its role in cortical dopaminergic D1 synapses, dopamine – via the same receptors – acts as a vasodilator (Jose et al., 2003; Zeng et al., 2004), and the low-activity val allele has been associated with increased vascular risk (Hagen et al., 2007). Thus, in the context of an aging vascular system, probable low availability of dopamine in val homozygotes may impair vasodilatory response, and produce hypoperfusion. The impact of age differences in vasodilatory activity on functional connectivity indices – inferred from the resting state BOLD effect – merit further investigation.

None of the other risk factors (vascular, metabolic, or genetic) significantly affected functional connectivity, nor were they associated with cognitive function. Besides acknowledging the abovementioned problem of variance partitioning in a cross-sectional framework, we can only speculate that the performed health screening could have significantly mitigated the effects of these risk factors. In a sample devoid of most of the age-related disease, the effects of risk factors are quite subtle and elusive. Although the sample size of this study was reasonably large, uncovering such effects may require greater statistical power. Furthermore, it is worth noting that while we examined multiple potential risk factors there may be other factors, such as the presence of amyloid- $\beta$  or tau, affecting functional connectivity. Further research is needed to examine these additional potential risk factors.

Respiration and cardiac pulsation are known to affect the BOLD signal (Dagli et al., 1999; Shmueli et al., 2007). Monitoring respiration and cardiac pulse during fMRI scan acquisition, and subsequently filtering them out using a priori models (Birn et al., 2006; Glover et al., 2000), can reduce these effects. A limitation of the current study is that no such techniques were applied as respiratory and cardiac data were not collected during scan acquisition. Nevertheless, thorough noise reduction was performed by applying ICA-AROMA, which in addition to motion artifacts also removes other structured noise from the data (e.g. cardiac pulsation artifacts) (Pruim et al., 2015), and global signal removal, which removes global noise, including respiration and pulsation artifacts (Birn et al., 2006).

## 5. Conclusions

In this report we provide evidence for an age-related partial functional disconnection of the posterior hippocampus to other hippocampal and default mode network regions. This reduction in functional connectivity seems to specifically affect the posterior hippocampus, as functional connectivity of the anterior hippocampus is unrelated to age. In addition, we show that, independent of age, females have higher hippocampal – medial prefrontal functional connectivity than males, and *COMT* val158met polymorphism is associated with lower posterior hippocampal – posterior cingulate/retrosplenial functional connectivity.

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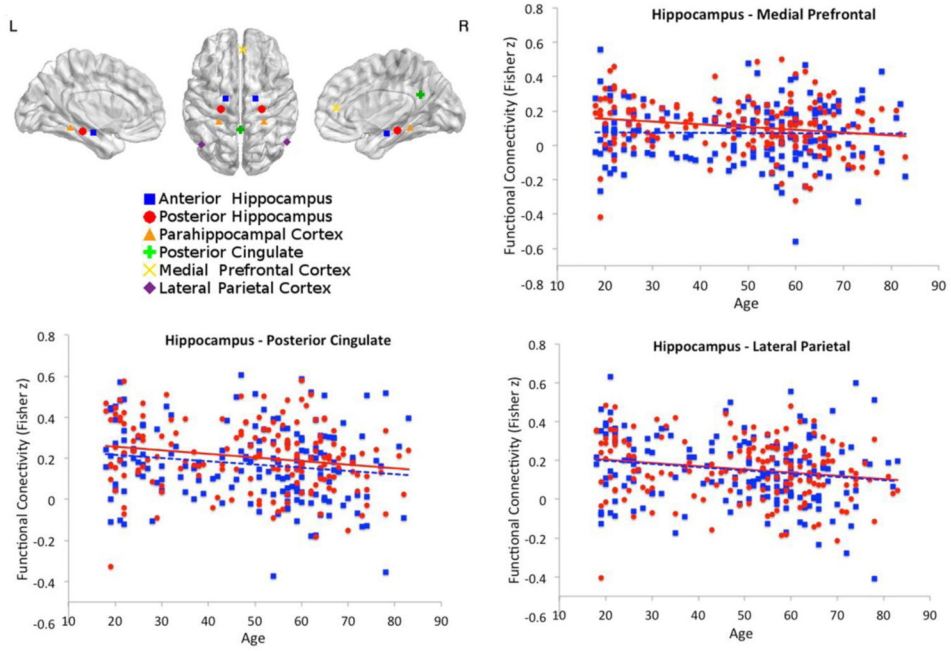
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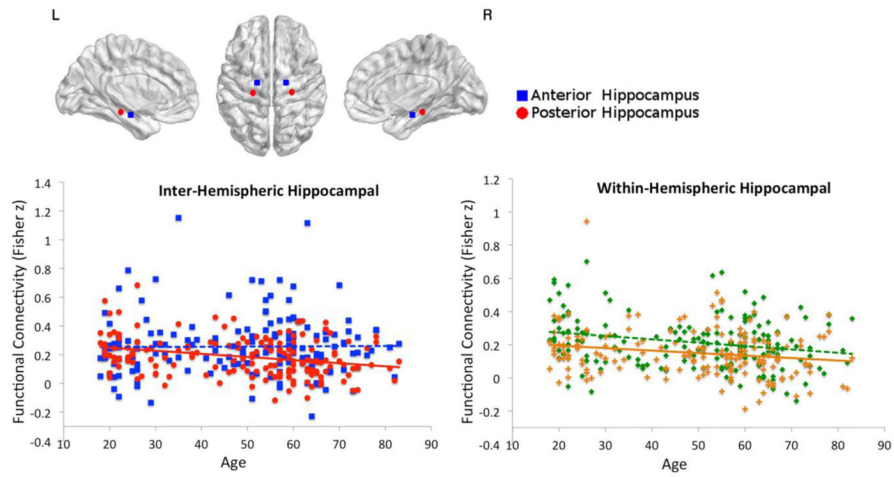
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### Highlights

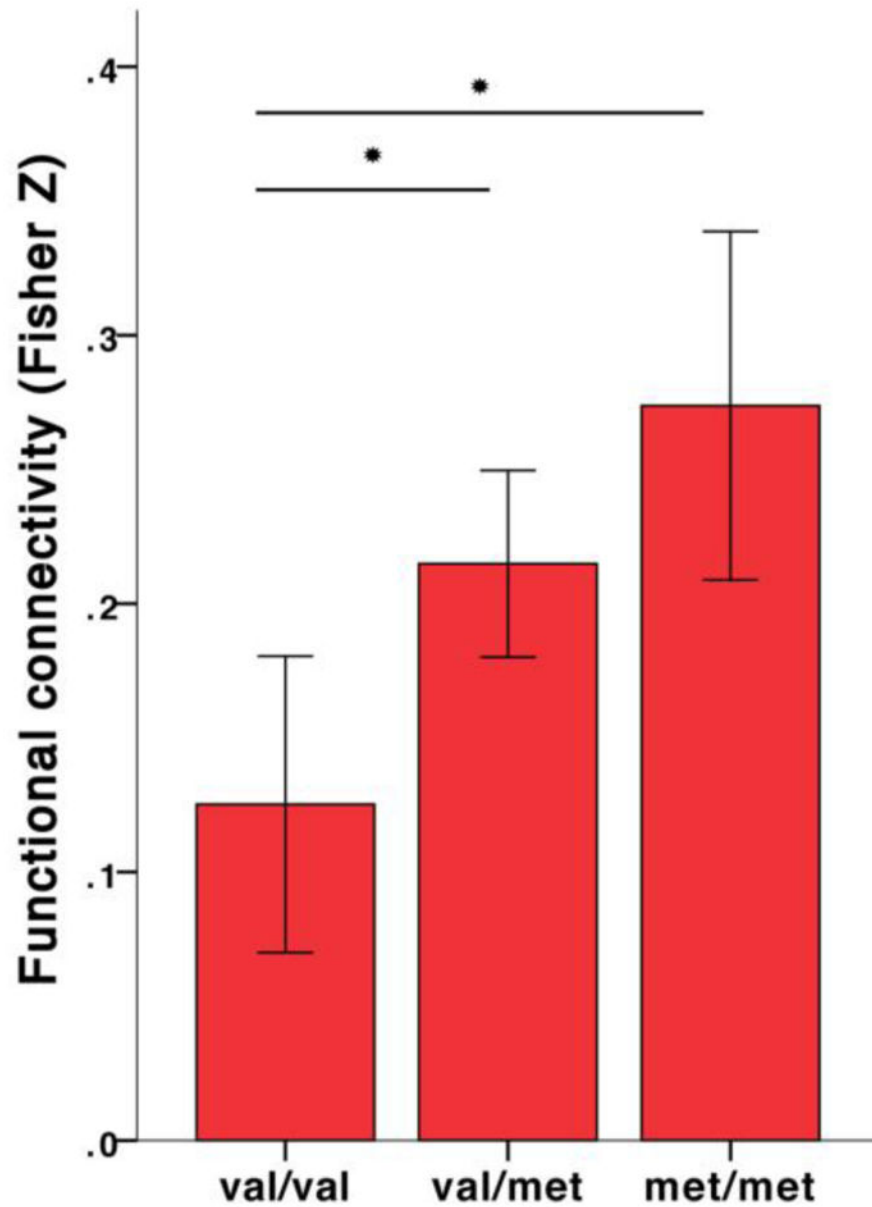
- Advanced age is related to lower posterior hippocampal functional connectivity
- Higher hippocampal – medial prefrontal connectivity in females vs. males
- *COMT* val alleles are linked to lower posterior hippocampal functional connectivity
- No association between functional connectivity and vascular or metabolic risk



**Figure 1.** Effect of age on hippocampal – default mode functional connectivity. Age-related decreases in functional connectivity between bilateral posterior hippocampus and three other default mode network regions – posterior cingulate/retrosplenial, medial prefrontal and bilateral lateral parietal are shown in red. Functional connectivity of the anterior hippocampus to the same regions is shown in blue. The effect of age on functional connectivity with the medial prefrontal cortex is significantly different between posterior and anterior hippocampus.



**Figure 2.** Effect of age on hippocampal functional connectivity. Age-related decreases in inter-hemispheric functional connectivity for left – right posterior hippocampus are shown in red in the left scatterplot. The anterior hippocampus, which does not show an age effect, is shown in blue. The effect of age on inter-hemispheric functional connectivity is significantly different between posterior and anterior hippocampus. Age-related decreases in within-hemispheric functional connectivity, along the posterior to anterior axis of the hippocampus, are shown in the right scatterplot for both the left (in green) and right (in orange) hemispheres.



**Figure 3.** Age-independent linear effect of *COMT* val158met genotype on functional connectivity strength between posterior hippocampus and posterior cingulate/retrosplenial cortex. The bar graphs show the significant differences between genotypes as revealed by post-hoc comparisons. \*Significant at  $p < 0.05$