Research article

Age and sex related variations of MRI parameters of hippocampus and amygdala in healthy humans: A cross sectional study using a small cohort

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ABSTRACT

Introduction and Aim: Aging alters limbic structure, sex and hemispheric variations with anterior-posterior dominances of which are still obscure in relation to cognition in humans.

Methodology: The present study included MRI coronal images for measurements of volumes [hippocampal volume (HV), amygdala volume (AV)], shapes [hippocampal angle (HA), medial distance ratio (MDR), parahippocampal angle (PHA)] and their hemispheric asymmetry indices (AI) in male and female healthy individuals of young and aged groups. The best-fitted formulae of 'volume index' (VI) and 'shape index' (SI) were evaluated for better interpretation.

Results: In young, males showed larger bi-hemispheric volumes and AV/HV-ratios, greater HA and MDR values with distinct hemispheric dominances and greater VI with different SI values, compared to females. Aged subjects showed lesser VI and bi-hemispheric volumes compared to young counterparts where the decrement of volumes were more in aged males resulting in same volumes and AV/HV-ratios in both aged sexes. Only aged females exhibited greater HA and MDR values with altered SI compared to young counterparts retaining the sex-specific such differences with their altered AIs. The parametric changes showed significant correlations in respective cases. PHA remained unaltered in all cases.

Conclusion: Sex-specific volume differences in young and volume reduction in age might associate with distinct alterations of hippocampal shapes viz. shifting of the (a) head (anterior portion) vertically compared to its horizontal position and (b) body (posterior portion) laterally toward temporal cortex, in both hemispheres. The recent findings claim further studies to unveil the cognitive variations corroborated with such limbic structural/shape alterations among sexes and ages.

Keywords: Human brain MRI; aging; gender; hippocampus; amygdala.

INTRODUCTION

MRI-database study indicates that the hippocampal malrotation with asymmetric appearance, predominantly in the left hemisphere, is characterized by the alterations of the anatomical position (vertical and medial) of the hippocampus and morphological changes in extra-hippocampal regions i.e., sulci of the limbic lobe in healthy subjects (1). The differential pattern of development of sub-regions (anterior vs posterior regions) of hippocampus are reported in healthy population (2). However, the variations of the structure and shape of hippocampus along with amygdale and PHG among sexes in healthy population are not clear.

Human aging causes (a) hippocampal atrophy with bilateral gray matter volume loss and alterations of the cellular (dendritic architecture and neuronal bodies) structures, (b) loss of microstructural integrity of the fornix bundle and (c) small numbers of a neurofibrillary tangle in the parahippocampal region (3, 4, 5). Aging exhibits differential rates of volume reductions in amygdala and sub-fields of hippocampus viz. cornu ammonis (CA) and dentate gyrus (DG) (6, 7). The right hemisphere shows the atrophy of CA-1 region (8) whereas CA-2, CA-3 and DG are more resistant to change in normal aging (9). Ageing associates with the (a) decline in memory performances with differential pattern of volume loss of anterior and posterior hippocampus with decrease in volume of their ratio (10), (b) same rate of atrophies in subiculum and global gray matter (9), (c) reduction in volumes of CA-3 and DG comprising posterior hippocampus (11),

posterior parahippocampus (12) and superior-posterior lateral side of the amygdala (5).

Therefore, literature indicates that the morphological/structural alterations including (a) anterior-posterior variations and (b) hemispheric distinctions of hippocampus, amygdala, and parahippocampal region with (c) their sex differences during normal aging are yet to be revealed. The present study includes evaluation of coronal MR-images to measure single-slice parameters with 1D (MDR as posterior part) and 2D (HA as anterior part and PHA as tail portion) measurements of hippocampus along with conventional three-dimensional volumetric (hippocampal and amygdala) approaches among male and female healthy subjects of young adult and aged groups. In addition, the measurements have been combined to develop the best-fitted mathematical formulas of 'volume index' (VI) and 'shape index' (SI) to find a global scenario of the structural orientations of limbic structures (hippocampus, amygdala, and parahippocampal regions) in the brain of healthy young and aged human.

MATERIALS AND METHODS

University post-graduate students (age 19-23 years; 11 males and 10 females) and senior professors (age 55-65 years; 8 males and 8 females) were selected and allowed to participate voluntarily with their consents in the present study. The inclusion criteria of subjects considered as (a) body mass index within the normal range, (b) no history of alcohol or drug abuse, (c) no history of neurological/psychiatric diseases, (d) normal standard T1- and T2-weighted MRI as assessed by a group of clinicians and (e) right-handed people. The study protocols were pre-approved by the Institutional Human Ethics Committee at IPGME&R, SSKM Hospital under State Govt. of West Bengal, Kolkata, India, and the Department of Physiology, University of Calcutta, Kolkata, India.

Mini-mental state examination (MMSE)

The subject underwent MMSE for screening before MRI study. MMSE (score ≥24 as normal cognitive status) covers neuropsychological assessment including eleven cognitive domains such as orientation, learning, attention or calculation, naming, repetition, recall, comprehension, writing, and construction and is composed of 19 individual tests.

Structural MRI study

The oblique coronal T1-weighted (3D-SPGR) images of hippocampus and amygdala were obtained using a 3-Tesla MRI scanner (Signa3THDxt, GE-healthcare) with following image acquisition protocols under supervision of an experienced radiologist and other neuro-imaging staffs: (TR/TE/TI/NEX: 9.6ms/4.2ms/470ms/1), flip-angle 15º, matrix-size 256×256, DFOV 20cm, and slice thickness 2.4mm with 1.2mm overlap. The oblique coronal sections of the hippocampus were taken perpendicular to the long axes of the temporal lobes. Transversal images of the hippocampus were taken parallel to the AC-PC line. The resultant 3D data sets were reconstructed by multi-planar reformations and the hippocampus volume was measured by proprietary software provided by the MRI vendor. The MR-images were interpreted for volumetric and linear assessments independently by both trained radiologists and non-radiologists.

Measurements of HV and AV

The anatomical boundaries of hippocampi were segmented and the volume was calculated using proprietary volume calculator software available in the workstation. “The hippocampus was traced superiorly around the choroidal fissure, curving laterally along the medial border of the temporal horn, and medially along with the gray matter of the hippocampus, up to its junction with the PHG including subiculum. It was traced posteriorly to include the entire tail up to the slice where the full length of the crus of the fornix is seen” (13). For AV measurement, “the segmentation of amygdala in coronal slices started from the level of uncinate gyrus, continued over the temporal horn of the lateral ventricle, and the amygdala was separated from hippocampus at its narrowest point. More anteriorly, the gray-white boundary was used to separate amygdala from substantia innominata, inferior putamen and claustrum” (13).

Measurements of HA, MDR and PHA

The HA of both hemispheres was measured on the most rostral slice and delineated as “the angle between the horizontal line orthogonal to the falx cerebri and the uncal sulcus line between the deepest point of the uncal sulcus and the point nearest to the side of the ambient cistern in the uncal gyrus facing the uncal sulcus” (13). The MDR was measured as “the distance between the midline and the fimbria, normalized by dividing it into the distance from the midline to the peripheral margin of the temporal lobe”, in the same or separate coronal sections with clear anatomical demarcations, in both hemispheres (13). The PHA was measured as the angle between descending and ascending portions of the PHG in both hemispheres individually in the same or separate coronal (occipital most) sections with clear anatomical demarcation. The MRI slice having fornix (presence of columns of fornix bellow corpus callosum) was used as the anatomical boundary of the posterior part of PHG for PHA measurements (13).

Measurement of hemispheric asymmetry index(AI)

The AI was measured for both linear and non-linear MRI parameters following the standardized formula reported previously (13): “[2 X (Right hemispheric measure – Left hemispheric measure) / (Right

hemispheric measure + Left hemispheric measure)]". The positive and negative signs of AI represent right (R) and left (L) hemispheric dominance respectively.

Formulation of indices of volume and shape

The average value of HV and AV of each individual was calculated to consider it the 'volume function' [(HV+AV)/2]. The mean of 'volume function' was calculated using all data comprising four groups viz. Young male (YM), Young female (YF), Aged male (AM), and Aged female (AF). Each value of the 'volume function' was expressed as a dimensionless 'Volume Index' (VI) using the equation viz. individual value of the 'volume function' divided by the mean of 'volume function' (equation-1). The angular measurements were made dimensionless taking the ratio of HA/PHA for each individual. The product of two ratios, that is, angular ratio (HA/PHA) and distance ratio (MDR) was calculated to take into account as 'shape function' [(HA/PHA)*MDR]. The calculation of the mean of 'shape function' followed by an evaluation of 'Shape Index' (SI) was performed using the procedure (equation-2) similar to the evaluation of VI. The formula connecting 'volume function' [(HV+AV)/2] and 'shape function' [(HA/PHA)*MDR] were considered after repeated trials of different mathematical criteria to create a best-fitting regression model of VI and SI.

VI = [(HV+AV)/2]/mean [(HV+AV)/2] - (equation-1)

SI = [(HA/PHA)*MDR] /mean [(HA/PHA)*MDR] -- (equation-2)

Both linear and polynomial regression of VI and SI were applied to find the best-fitted formula to achieve a minimum error term (residual error). The least-square principle was applied to minimize the error term. The correlation coefficients for both linear and non-linear fittings were evaluated for the better statistical interpretation of regression of VI and SI for four groups (YM, YF, AM, AF).

Statistical analysis

The all data sets were subject to analysis of (a) QQ-plot and Shapiro–Wilk normality test, (b) Bartlett's test (p<0.05 as significance level) for homogeneity of variance, (c) two-way ANOVA followed by post-hoc (pair-wise) analysis (Tukey-HSD test) using the significance level at p<0.05, (d) 'effect size' by evaluating 'eta-squared' (η^2) value for ANOVA to measure the contribution of any factor (age or gender) or interaction among factors to the observed dependent variable and (e) correlation (Pearson's product-moment statistic) for paired parameters. The effects (η^2 values) The results (Table-1) of two-way ANOVA indicated that the main effects of age, the main effects of gender and the interaction effects of gender:age were statistically significant for HV, AV, HA, MDR, AI of HA, AI of MDR. Furthermore, the main effects of gender and age for AV/HV-ratio and AI of PHA were considered as large (>0.14), medium (0.06-0.13), and small (0.01-0.05). The correlation coefficient values (r values) represented 'very strong' (0.8-1.0), 'strong' (0.6-0.79), and 'moderate' (0.4-0.59) correlation. 'Markov Chain Monte Carlo' (MCMC) method, a Bayesian approach, was implemented to estimate the data-dependent (prior probability distribution) posterior probability distribution of parameters considering 95% confidence interval using a robust algorithm 'Delayed Rejection and Adaptive Metropolis' (DRAM) with the optimal acceptance rate based on the multivariate normality of the marginal posterior distributions (14). The modMCMC function (15) in R-package FME (Flexible Modelling Environment) was used for the MCMC analysis with considering the first and third quantile values as lower and upper cut-off values of each parameter respectively. All statistical evaluations were performed in 'R language and environment' and data were plotted in GraphPad Prism.

RESULTS

Status of mental health

All male and female participants of both age groups were cognitively healthy with MMSE score ranged from 27.4 to 30.0 in the present study. Subjects were selected with inclusion criteria and underwent an MRI scan of the brain for further analysing the brain's structural alterations during healthy aging.

Status of brain MRI parameters

The inter-observer (radiologist vs. non-radiologist) variability (%) was non-significant (p>0.05, Student's unpaired t-test) for right and left hemispheric measurements of the data of healthy subjects (2.63±0.51 and 3.21±0.29 in men and 4.89±0.64 and 3.52±1.04 in women) by different observers for respective parameters. The inter-observer variability of the present study found to be consistent with our previous report (13).

In the present study, the QQ-plot and Shapiro–Wilk normality test showed normal distribution (data not shown) of the data sets of brain MRI parameters of four groups (both genders of young and aged). The Bartlett test was performed to verify the assumption that variances were equal across genders or two age groups. The test statistics was larger than the critical value for each combination of dataset and therefore, rejected the null hypotheses (p<0.05) and confirmed that the variance was different from the other at each group (data not shown).

respectively and the interaction effects of gender:age for the both parameters were statistically significant. However, the main effects of age and gender were non-significant for the AV/HV-ratio and AI of PHA respectively. The main effects of age, the main effects of gender and the interaction effects of gender:age were

non-significant for the PHA and AIs of HV, AV and AV/HV-ratio respectively. The results (Fig. 1 and Fig. 2) of post-hoc (pair-wise) analysis of the present study are described below categorically.

Table 1: The statistical evaluation of the status of MRI parameters measured among men and women of both young and aged groups

Parameter (Dependent Variable)	Independent variables		
	Main effects		Interaction effects
	Age	Gender	Gender:Age
HV	$F(1,66) = 111.3$, $p<0.001$, $\eta^2 = 0.43$	$F(1,66) = 20.14$, $p<0.001$, $\eta^2 = 0.22$	$F(1,70) = 6.13$, $p<0.001$, $\eta^2 = 0.12$
AV	$F(1,66) = 88.04$, $p<0.001$, $\eta^2 = 0.29$	$F(1,66) = 32.9$, $p<0.001$, $\eta^2 = 0.31$	$F(1,70) = 14.81$, $p<0.001$, $\eta^2 = 0.15$
AV/HV-ratio	$F(1,66) = 0.4$, $p = 0.5$, $\eta^2 = 0.003$	$F(1,66) = 17.04$, $p<0.001$, $\eta^2 = 0.4$	$F(1,66) = 3.92$, $p<0.01$, $\eta^2 = 0.08$
HA	$F(1,66) = 136.23$, $p<0.001$, $\eta^2 = 0.45$	$F(1,66) = 5.14$, $p<0.05$, $\eta^2 = 0.04$	$F(1,70) = 32.2$, $p<0.001$, $\eta^2 = 0.32$
MDR	$F(1,66) = 11.66$, $p<0.001$, $\eta^2 = 0.08$	$F(1,70) = 18.4$, $p<0.001$, $\eta^2 = 0.35$	$F(1,70) = 8.26$, $p<0.001$, $\eta^2 = 0.16$
PHA	$F(1,66) = 0.87$, $p = 0.09$, $\eta^2 = 0.03$	$F(1,66) = 0.77$, $p = 0.5$, $\eta^2 = 0.02$	$F(1,70) = 0.32$, $p = 0.8$, $\eta^2 = 0.01$
AI of HV	$F(1,34) = 1.76$, $p = 0.19$, $\eta^2 = 0.005$	$F(1,34) = 0.09$, $p = 0.77$, $\eta^2 = 0.002$	$F(1,34) = 0.08$, $p = 0.78$, $\eta^2 = 0.002$
AI of AV	$F(1,34) = 0.31$, $p = 0.58$, $\eta^2 = 0.009$	$F(1,34) = 0.23$, $p = 0.63$, $\eta^2 = 0.008$	$F(1,34) = 0.27$, $p = 0.6$, $\eta^2 = 0.007$
AI of AV/HV-ratio	$F(1,34) = 1.6$, $p = 0.08$, $\eta^2 = 0.01$	$F(1,34) = 2.09$, $p = 0.08$, $\eta^2 = 0.05$	$F(1,34) = 3.08$, $p = 0.06$, $\eta^2 = 0.02$
AI of HA	$F(1,34) = 5.75$, $p<0.01$, $\eta^2 = 0.07$	$F(1,34) = 59.86$, $p<0.001$, $\eta^2 = 0.61$	$F(1,34) = 3.26$, $p<0.05$, $\eta^2 = 0.04$
AI of MDR	$F(1,34) = 60.45$, $p<0.001$, $\eta^2 = 0.37$	$F(1,34) = 63.11$, $p<0.001$, $\eta^2 = 0.36$	$F(1,34) = 6.62$, $p<0.01$, $\eta^2 = 0.05$
AI of PHA	$F(1,34) = 13.38$, $p<0.001$, $\eta^2 = 0.22$	$F(1,34) = 1.09$, $p = 0.26$, $\eta^2 = 0.01$	$F(1,34) = 15.12$, $p<0.001$, $\eta^2 = 0.24$
VI	$F(1,66) = 146$, $p<0.001$, $\eta^2 = 0.43$	$F(1,66) = 90.49$, $p<0.001$, $\eta^2 = 0.25$	$F(1,70) = 30.07$, $p<0.001$, $\eta^2 = 0.09$
SI	$F(1,66) = 72.49$, $p<0.001$, $\eta^2 = 0.32$	$F(1,66) = 8.11$, $p<0.01$, $\eta^2 = 0.04$	$F(1,70) = 70.82$, $p<0.001$, $\eta^2 = 0.32$

Two-way ANOVA was applied to evaluate effects of two factors (gender and age) and their interaction in measured (dependent) variables. The two-way ANOVA results are reported as F-statistic (F-values), its associated degrees of freedom (df) and p-value. The numbers inside the parentheses account for the first df (between-groups df) and second df (within-group df or "residual" df) in F-statistics respectively. The p-values denote the "significance levels". The shaded areas represent the non-significant effects. The eta-square (η^2) values ("effect size") quantify the degree of association between effects (main effects or interaction effects) and dependent variables for the sample.

Pairwise analysis in healthy young subjects

Young males showed greater values of HV, AV, AV/HV-ratio, HA, and MDR in both hemispheres compared to respective values of females (Figs. 1b-1f). In young males, only HV exhibited significantly greater values in the right hemispheres compared to the left hemispheres (Fig. 1b). The hemispheric values remained same for other cases. The AI values represented greater right-hemispheric dominancy in HA of young females and greater left hemispheric dominancy in MDR of young males compared to their respective counterparts (Fig. 2b).

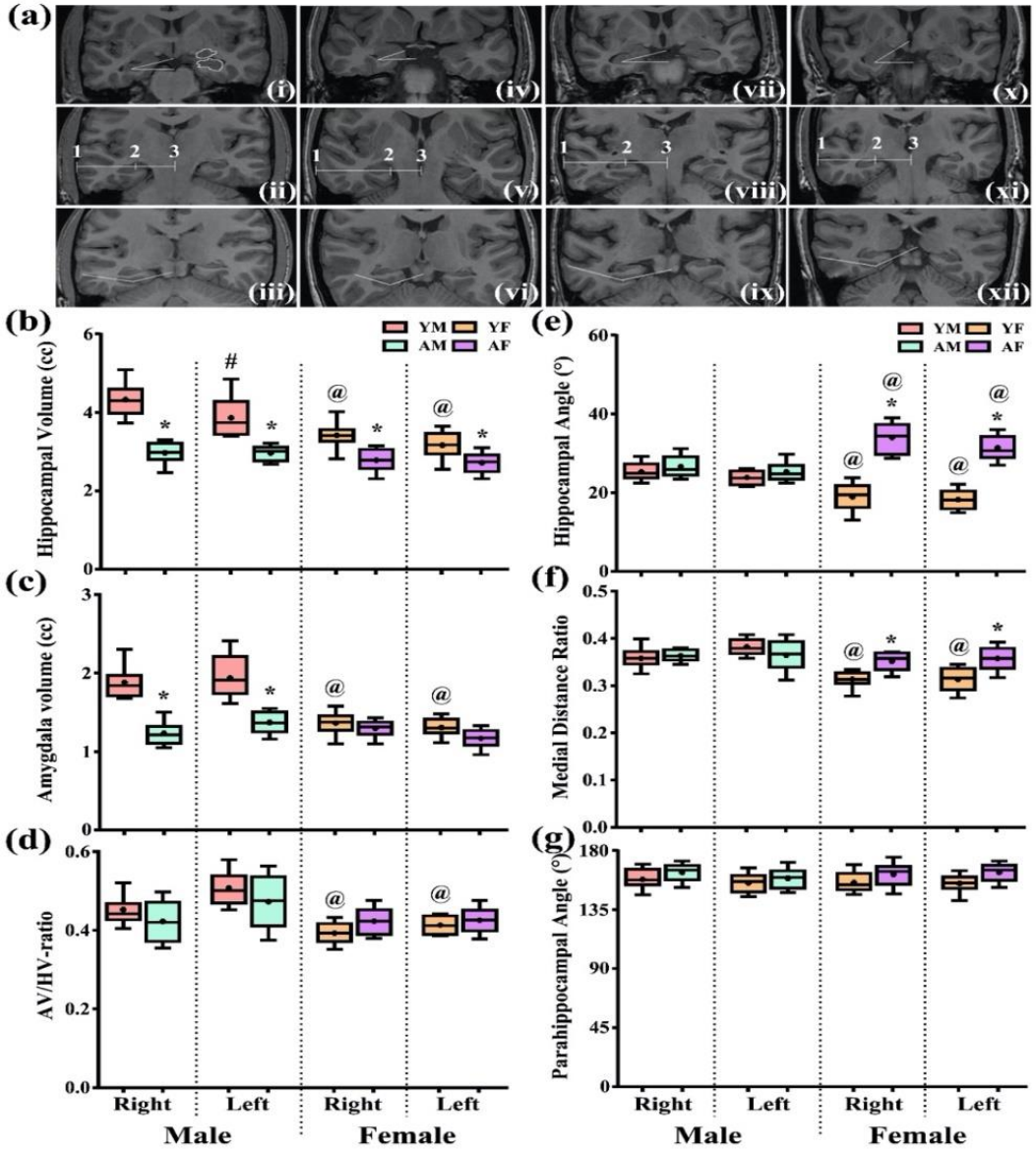

Fig. 1. Aging effect on morphometric measurements of MR-images of hippocampus and amygdala of human subjects. The figure represents (a) Coronal brain MRI (T1-weighted) illustrations of hippocampus and amygdala with anatomical markings of hippocampal measures of young male (i–iii), young female (iv-vi), aged male (vii-ix), and aged female (x–xii) healthy subjects having bilateral hemispheric changes in parameters. The anatomical markings for angular [HA (i, iv, vii, x) and PHA (iii, vi, ix, xii) and linear [MDR (ii, v, viii, xi)] measurements are drawn in right hemispheres (left-hand sides) of the images. The medial positioning (MDR) of the hippocampus is determined by the distance (2-3) between the midline (marked as '3') and the fimbria (marked as '2') normalized by the distance (1-3) from the midline (marked as '3') to the outer edge of the temporal lobe of the neocortex (marked as '1').The figure represents (b) hippocampal volume (HV), (c) amygdala volume (AV), (d) AV/HV-ratio, (e) hippocampal angle (HA) (f) medial distance ratio (MDR), and (g) parahippocampal angle (PHA) in both genders of healthy young and aged human subjects. Each boxplot represents the bottom line, top line, and middle line as the 25th percentile, 75th percentile, and 50th percentile (median) distribution of scores respectively. The lower and upper whiskers denote 5% and 95% CI values of each group respectively. The mean value of each group is represented by plus sign (+) in each box. Hash-tag (#) sign represents a significant difference in hemispheric (Right vs. Left) status of parametric values of either in young or aged individuals. Asterisks (*) indicates a significant difference in parametric values of the aged group compared to young of both genders (either in males or females). The symbol "@" represents a significant difference in parametric values among genders (Males vs. Females) either in young or aged individuals.

Pairwise analysis in healthy aged subjects

Aged females showed higher values of HA in both hemispheres (Fig. 1e) and greater right-hemispheric dominancy in HA (Fig. 2b) compared to aged males. The other parameters remained unaltered in values among both sexes.

Pairwise analysis of young vs. aged subjects

Aged males exhibited significant less HV and AV, in both hemispheres, compared to the young counterparts (Fig. 1b and Fig. 1c), whereby the changes in HV and AV were strongly and positively correlated with each other (Fig. 3a and 3b). The reduction in HV, increase in HA, and rise in MDR in both hemispheres appeared in aged females compared to younger counterparts (Figs. 1b, 1e, and 1g). The decrease in HV was strongly correlated with increases in HA and MDR, whereby increases in HA and MDR were also strongly associated in aged females in both hemispheres (Fig. 3c and Fig. 3d). The greater right-hemispheric dominancy was found in PHA of aged male subjects whereas greater left hemispheric dominancy appeared in MDR of young male subjects compared to their respective counterparts (Fig. 2b). In females, the left hemispheric dominancy was observed in MDR of young subjects whereas the right hemispheric dominancy was observed in aged subjects of the same parameter (Fig. 2b).

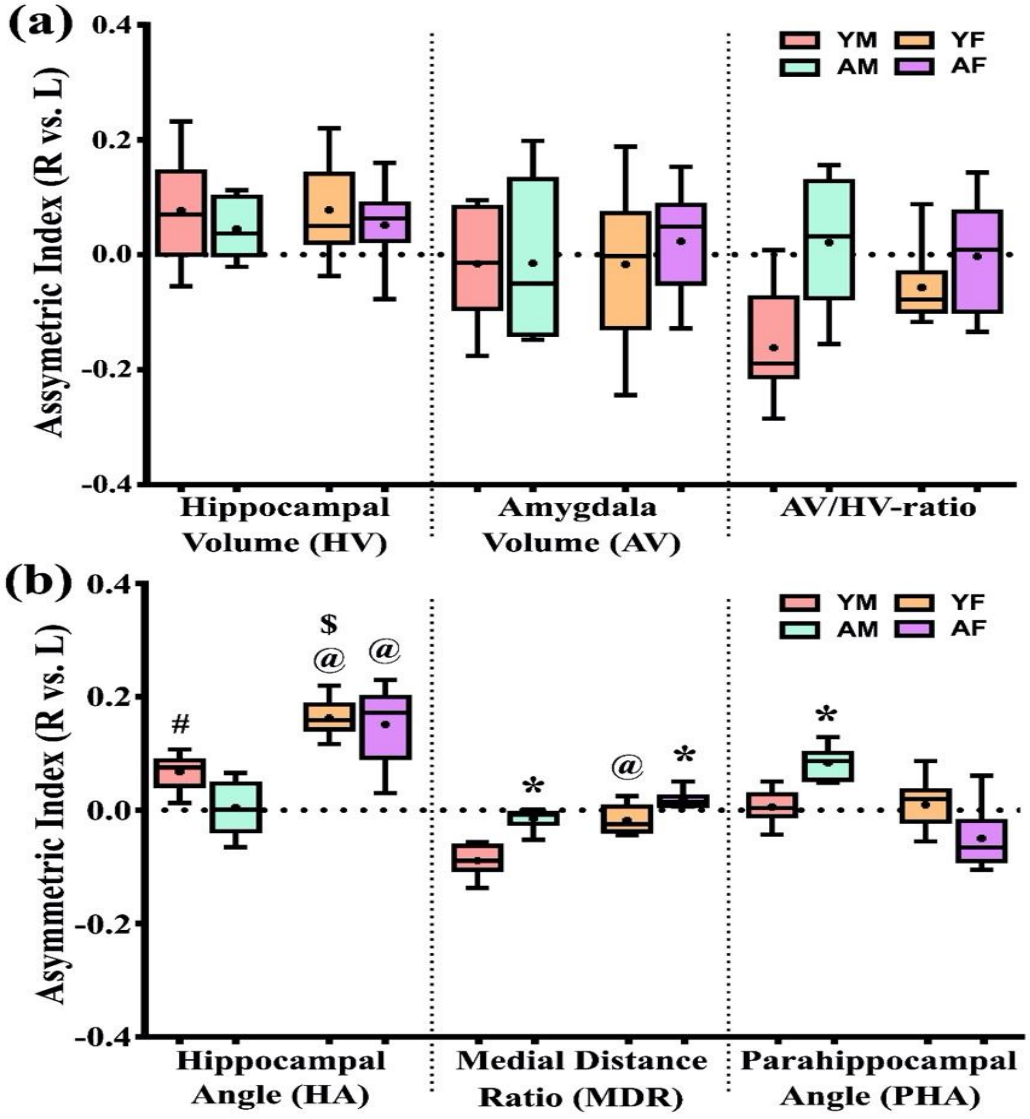

Fig. 2. The status of hemispheric asymmetry of hippocampus and amygdala in young and aged human subjects. The Figure represents the asymmetric index (AI) of (a) hippocampal volume (HV), amygdala volume (AV), AV/HV-ratio, and (b) hippocampal angle (HA), para hippocampal angle (PHA), medial distance ratio (MDR). Each boxplot represent the bottom line, top line, and middle line as the 25th percentile, 75th percentile, and 50th percentile (median) distribution of scores respectively. The lower and upper whiskers denote 5% and 95% CI values of each group respectively. The mean value of each group is represented by plus sign (+) in each box. Asterisks (*) indicate a significant difference in parametric values of the aged group compared to young of both genders (either in males or females). The symbol "@" represents a significant difference in parametric values among genders (Males vs. Females) either in young or aged individuals. The hash-tag (#) represents a significant difference in HA compared to the MDR of the young male group. The dollar ($) significant difference in HA compared to MDR and PHA of the young female group.

Relation between VI and SI of limbic structure

Four distinct groups viz. YM, YF, AM and AF had been depicted for a precise statistical interpretation (Fig. 4a). The data sets of VI and SI parameters of four groups were tested by QQ-plot and Shapiro–Wilk normality test and, Bartlett test to verify the assumption that variances were equal across genders or two age groups (data not shown).

The results (Table-1) of two-way ANOVA indicated that the main effects of age, the main effects of gender and the interaction effects of gender:age were statistically significant for the VI and SI parameters. The post-hoc (pair-wise) analysis showed significant ($p<0.001$) levels of (a) gender differences of VI in young age and SI in both ages and, (b) ageing effects with reduction of VI in both genders and alterations of SI in females only (Fig. 4a). The significant negative linear correlation (r= -0.6) in the inter-age (young vs. aged) variations of VI and SI was found in females (Fig 4b) whereas no such correlation was found in males (data not shown). Also, a significant negative linear correlation (r= -0.67) in intra-age variations of VI and SI was found in aged males (Fig. 4b). Alternatively, there were no correlations in the intra-age variations of VI and SI (data not shown) in other cases (YM, YF, AF). Hence, the results signified that females showed a specific correlation between the age-related changes in the volume and shape of limbic structure in the present study with a small cohort.

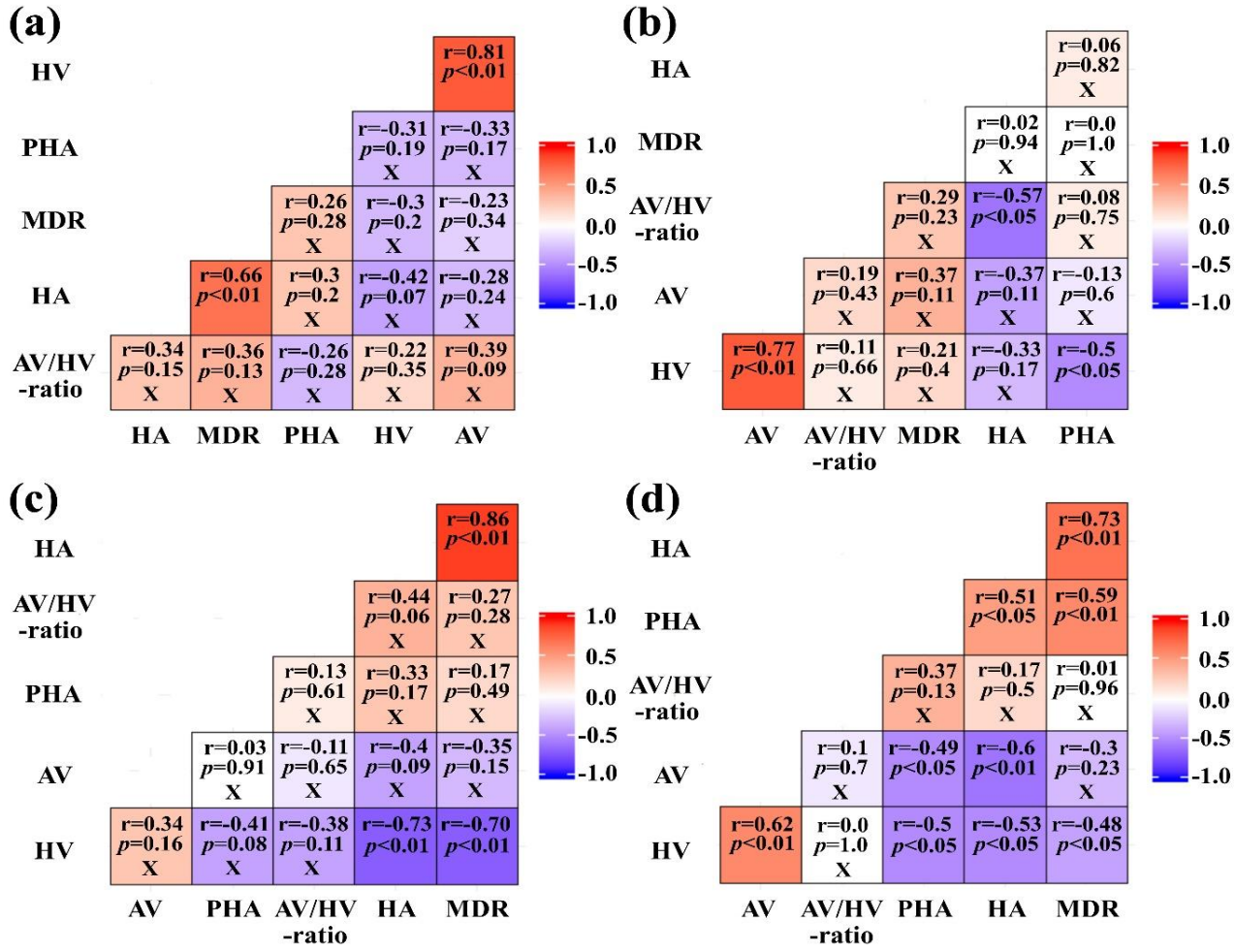

Fig. 3. Correlation (Pearson's product-moment statistic) heat map for paired MRI parameters measured during experimental conditions of young vs. aged found in right and left hemisphere of male and female individuals. The Figure represents (a) heat map matrix of the male right hemisphere, (b) heat map matrix of the male left hemisphere, (c) heat map matrix of the female right hemisphere, and (d) heat map matrix of the female left hemisphere. The values represent Pearson's correlation coefficient (r) and significance level (*p*) between pair-wise MRI parameters. A value of $p < 0.05$ was considered significant. (+) sign in "r" represents positive correlation i.e., similar kind of changes (either increase or decrease) found in both parameters, whereas (-) sign represents negative correlation i.e., one parameter increase vs. other parameter decrease or vice-versa. The symbol "X" designates without any significant correlation between MRI parameters among young and aged individuals in the respective hemisphere.

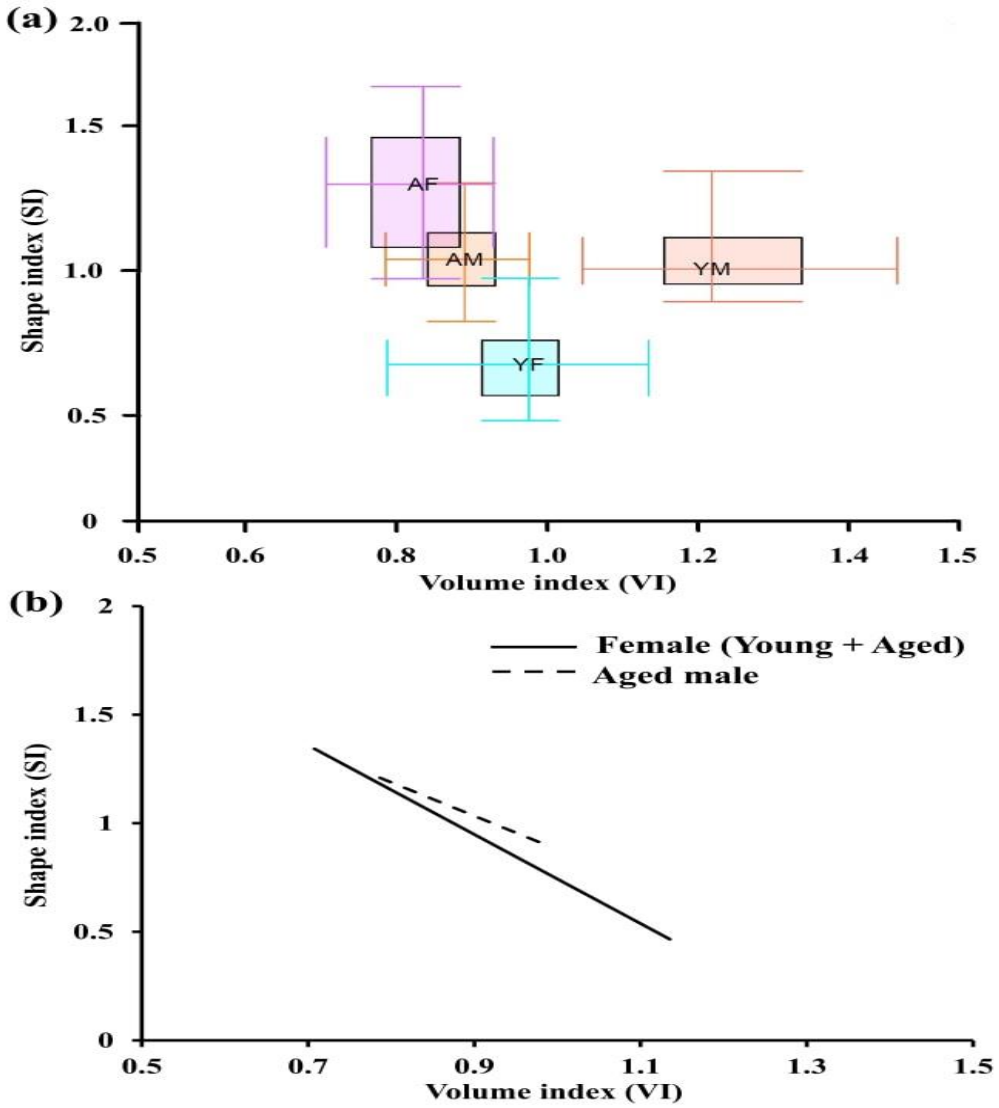

Fig. 4. Aging effects on volume index (VI) and shape index (SI) of limbic structure representing the relation between VI and SI in four groups viz. YM (Young male), YF (Young female), AM (Aged male), and AF (Aged female) in our study. All data including the right and left hemispheres of these four groups have been used to calculate VI and SI. The details of the mathematical calculation are mentioned in "Materials and Methods" (a): The graphical representation of the values of VI vs. SI depicts the four distinct groups (YM, YF, AM, and AF). Each boxplot represent the bottom line, top line, and middle line as the 25th percentile, 75th percentile, and 50th percentile (median) distribution of scores respectively. The lower and upper whiskers denote 5% and 95% CI values of each group respectively. The mean value of each group is represented by plus sign (+) in each box. The results of two-way ANOVA indicate significant ($p<0.001$) differences of VI (YM:YF, YM:AM, YF:AF, YM:AF) and SI (YM:YF, AM:AF, YF:AF, YM:AF, YF:AM) among groups. (b): Regression analysis exhibits the significant negative linear correlation ($r= -0.60$) in the inter-age (young vs. aged) variations of VI and SI in females whereas no such correlation has been found in males (data not shown). A significant negative linear correlation ($r= -0.67$) in intra-age variations of VI and SI is found in aged males. There were no correlations in the intra-age variations of VI and SI in other cases (data not shown). The results signified the presence of correlation between the age-related changes in volume vs. shape of the limbic structure in females.

DISCUSSION

The present MRI (coronal images) study reports, for the first time with our best knowledge, the gender differences of the structural status of hippocampus and amygdala with their hemispheric variations (asymmetry index) in healthy young and aged individuals using 3D volumetric (HV, AV), 2D angular (HA, PHA) and 1D linear (MDR) measurements in human. The AV/HV-ratio was measured to minimize the structure-specific approach and established the more global aspect of the structural-functional relationship between hippocampus and amygdala. The measurement of HA, MDR, and PHA using single-slice images represented the hippocampal shape. Notably, a novel approach was introduced measuring new normalized scaled scores (VI and SI)

mathematically by combining values of volumes (HA and AV) and shapes (HA, MDR and PHA), to find any correlation between volume vs. shape of limbic structures among genders and ages for better interpretation.

A robust statistical MCMC algorithm with 95% confidence intervals was used to reduce the bias-ness of the small sample size in the present study. This Bayesian approach provided a more intuitive and interpretive model of posterior probability distribution with large data sets of a parameter based on a given prior distribution of experimental data. The MCMC simulation also generated probability intervals for exact estimation from the posterior distribution. Here, the quartile values (first and third quartiles) estimated during MCMC analysis of the respective parametric value in healthy young subjects were used to delineate the age-related changes (Fig. 1b-1g).

Our results demonstrated the considerable magnitudes of significant main effects of the independent variables viz. aging (7% to 45% level of effects) and gender (4% to 61% level of effects) on the total variability, in HV, AV, AV/HV-ratio, HA, MDR, VI, SI and AIs of PHA, HA, MDR (Table-1). The main effects implied the significant influences of (a) aging condition irrespective of gender and (b) sexual dimorphism regardless ageing condition on structural MRI parameters. Moreover, the independent variables viz. age and gender, showed significant interaction effects with magnitudes of 4% to 32% levels on the aforesaid dependent variables which inferred that gender does matter for changes in structural MRI parameters, although differently in respect of aging. However, the main effects of independent variables and interaction effects of dependent variables [PHA, AIs of HV, AV, AV/HV-ratio] found non-significant in the present study (Table-1). The pair-wise comparisons of mean differences of parameters followed by correlation regression analysis executed the most desirable interacting factors which might have the significant effects of age with gender variations. Finally, the present study showed an age-dependent significant linear correlation among VI and SI of limbic structures in females without indicating such correlation in males.

Gender variations of MRI parameters in young adults

The greater HV and AV in young males in both hemispheres compared to that of young females found in the present study (Fig. 1b and Fig. 1c) are consistent with other reports compelling greater hippocampal (16, 17) and amygdala (18) volumes in men. The present study exhibited greater AV/HV-ratio in both hemispheres in young males compared to that in female counterparts (Fig. 1d). The higher value of AV/HV-ratio associates with negative bias-ness, cognitive vulnerability and high risk of depression. The AV/HV-ratio shows positive correlation with psychosis-like behaviour in early adulthood and associates with epigenetic alterations like DNA methylation in brain tissues during their prenatal life under stressful pregnancy of their mother (19). In the present study, both young males and females had normal cognitive status without having any history of depression. Therefore, the gender differences in AV/HV-ratio found in the present study claims further studies in this regard.

Developmentally, the volume reduction in the hippocampus is greater at the anterior region in males and at posterior region in females (2) which may cause the gender variation of shape of the hippocampus in a healthy adult population. The anatomical and morphological characteristics of the hippocampus in MR-images designate that (a) HA represents CA-1 morphology comprising the shape of the anterior hippocampal portion alteration of which causes structural changes as well as positional shifting for the horizontal axis of the hippocampal head (b) MDR corresponds to the medial positioning of the hippocampus and structural information at the posterior portion of the hippocampus in the horizontal axis and (c) PHA comprises the structural information of posterior part of PHG that corresponds to the tail portion of the hippocampus and represents the vertical positioning of the hippocampus (13, 20). In the present study, the significant greater values of HA and MDR in both hemispheres found in males compared to females in young (Fig. 1e).

The results signified that young males might have (a) altered internal morphology at the CA-1 subfield resulting in more vertical shifting (compared to the horizontal position) of the hippocampal head (anterior portion) leading to greater HA values and (b) different medial position of the hippocampal body (posterior portion) with more lateral shift towards temporal cortex along the horizontal axis leading to greater MDR values, in both hemispheres compared to young women (Fig. 1f). The anterior hippocampus associates with encoding memory, verbal memory, a scene recalling with novelty and decision making (20). The posterior hippocampus associates with spatial memory and visual scene perception and therefore it is supposed to have direct anatomical links with parahippocampal and retrosplenial cortices (20). Therefore, the gender differences between HA and MDR in the present study might be corroborated with cognitive variations among sexes in young adults. However, both genders showed the same PHA values indicating the similar vertical position of the hippocampus with respect to morphology (ascending and descending structure) of PHG, in both hemispheres among healthy individuals (Fig. 1f). The present finding of hippocampal volume with right-hemsipheric > left-hemispheric asymmetry appeared to be consistent with previous reports (21). In addition, the values of AI in the present study indicated the sex differences of hemispheric dominance in

hippocampal shape like (a) right-hemispheric dominancy of HA in both genders with greater extent in females and (b) left hemispheric dominancy of MDR in males where females showed a bi-hemispheric pattern (Fig. 2b). Other parameters did not show hemispheric dominances in both sexes and sex differences in AI. The sex related differences in AI of hippocampal shapes might have functional associations. Interestingly, the new indices VI and SI (Fig. 4a) indicated that men had significant different size (volume) and shape (combined angular and linear measures) of the limbic structures irrespective to hemispheric status, compare to women in the healthy young adult population. Therefore, our results signifies that the differences in the structures (size and shape) of hippocampus and amygdala in both sexes relates with the gender variations of cognitive function in a healthy young adult population.

Age-related changes in the brain MRI parameters of human subjects

Aged males showed the same magnitude (around 50%) of reduction in HV and AV (Figs. 1b and 1c) whereby these two changes exhibited a significantly positive correlation in both hemispheres (Figs. 3a and 3b). Consequently, AV/HV-ratio remains unaltered in aged males compared to younger counterparts (Fig. 1d). Several reports indicate that males have greater whole brain atrophy including hippocampus and ventricular enlargement (22) during aging. In the present study, aged females exhibited (a) reduction in HV to a lesser extent than aged males (Fig. 1b), (b) alterations in hippocampal shape by increasing in values of HA and MDR in both hemispheres without having such changes in aged males (Figs. 1e and 1f) and (c) significant and negative correlation between HV vs. HA and HV vs. MDR whereby the increase in HA positively correlated with an increase in MDR (Figs. 3c and 3d) compare to younger counterparts. In aged females, the AV did not altered (Fig. 1c) where the reduction in HV was around 18% and AV/HV-ratio remains unaltered (Fig. 1d). The altered AV/HV-ratio associates with cognitive variability (19). In the present study, aged men and women had normal cognitive status concomitant with unaltered levels of AV/HV-ratio compared to young counterparts.

The structural characteristics (13, 20) indicate that increase in HA values in the present study might delineate the structural changes in the subiculum and the CA-1 region of the anterior hippocampus in aged females. Consistently, a few reports indicate significant atrophy in the CA-1 sub-region of hippocampus during normal aging (23, 24). However, other reports claim the appearance of the CA1 atrophy in pathological conditions including MCI (mild cognitive impairment), Alzheimer's Diseases (AD) (9), and hypertension (11). Aging associates with the atrophy to the greater extent in the posterior part of the hippocampus (9). Noteworthy, in the MDR measurement, the outer edge of the temporal lobe of the neocortex remained equidistant from the midline in aged females compared to younger counterparts (data not shown). Therefore, the shifting of fimbria laterally towards the outer edge of the temporal lobe (lateral torsion) might cause the increase in MDR in aged females. Hence, the alterations of hippocampal shape (HA and MDR) in aged females might have changes in microstructure of the hippocampus in anterior and posterior regions.

The values of PHA remained unaltered in both genders (Fig. 1g) indicating unchanged shape of posterior PHG and thus vertical position of the hippocampus in the aged population. The PHG atrophy is reported in MCI (25). Nonetheless, the unaltered PHA values governed the normal cognitive status of both genders of older subjects in the present study.

The AI referred that aging was associated with alterations of hemispheric dominance in hippocampal shape but not in volumes (Fig. 2). Aging (a) showed the right hemispheric dominance of HA with greater extent in females, similar to young groups, (b) altered left-hemispheric dominances in MDR differentially in both genders where aged males remained left hemispheric dominant like young groups and aged females became right hemispheric dominant, and (c) made PHA right hemispheric dominance in aged males (Fig. 2b). It is reported that aging with cognitive decline associates with a decline in PHG volume with the greater extent in the right hemisphere whereas atrophy in the anterior medial temporal lobe is more strongly related to pathological aging (12). However, in the present study, AI of MDR and PHA signified that ageing might cause differential structural changes associated with lateral shifting of the hippocampal body at posterior portion adjacent to posterior PHG among sexes which might have functional importance.

Finally, the new indices VI and SI demarcated the gender-dependent alterations of volume and shape in limbic structure (combining hippocampus, amygdala, and parahippocampalgyrus) (Fig. 4a). Aging was associated with (a) a reduction in the VI in both genders to a greater extent in males and (b) a significant alteration in the SI in aged females. Aging exhibited a significant linear correlation among changes in VI and SI in limbic structure in females without showing such correlation in males (Fig. 4b). The age-related gender differences in structural (volume and shape) alterations of hippocampus and amygdala with their hemispheric dominances might have an association with cognitive variability during aging in the healthy individuals.

Abbreviations

Hippocampal Volume- HV; Amygdala Volume- AV; Hippocampal Angle- HA; Medial Distance Ratio-MDR; Alzheimer's Diseases- AD; Cornus Ammonis-CA; Dentate Gyrus- DG; Mild Cognitive Impairment-

MCI; Mini-Mental State Examination- MMSE; Parahippocampal Angle- PHA; Asymmetry Index- AI; Parahippocampal Gyrus- PHG;

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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