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Individual differences in trait anxiety are associated with gray matter alterations in hypothalamus: Preliminary neuroanatomical evidence

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ABSTRACT

Trait anxiety is particularly a prone phenotype for the development of anxiety disorders and depression. Studying the neural underpinnings of trait anxiety can further inform our understanding of the etiology of these disorders. To investigate the structural correlates of trait anxiety, high resolution structural images were acquired from 76 right-handed healthy participants and gray matter volumes were extracted from a priori regions of interest (ROIs) that were earlier implicated in anxiety like behaviour (i.e., hippocampus, amygdala, anterior cingulate cortex, thalamus, hypothalamus and prefrontal (dorsolateral, rostrolateral, ventrolateral) cortex. In a partial correlation analysis (with age, gender, and Beck Depression Inventory (BDI) scores as covariates of no interest), trait anxiety was found to be negatively correlated with the gray matter volume of hypothalamus bilaterally and positively correlated with the gray matter volume of left thalamus. In a hierarchical multiple regression analysis, grey matter volume of hypothalamus and left thalamus were found to be the significant predictors of trait anxiety. Our findings thus suggest that a smaller gray matter volume in the hypothalamus and an increase in the gray matter volume of left thalamus is related to a disposition to high anxiety personality trait.

1. Introduction

An anxious person is always on high alert and since worries preoccupy their working memory capacity, individuals with high anxiety trait show deficits during tasks that require an efficient attention regulation (Spielberger, 1983; Grachev and Apkarian, 2000; Eysenck et al., 2007). Further, high anxiety trait is particularly a prone phenotype for the susceptibility to the adverse effects of stress. It is also a vulnerability factor for the development of various anxiety disorders and depression (Sandi and Richter-Levin, 2009; Greening and Mitchell, 2015). Understanding the inter-relation between brain structure, function and such personality traits will help scientists develop interventions to target specific brain regions in healthy population (Hu and Dolcos, 2017). Preventive interventions can also be designed to train the brain to function better, that may help in preventing these 'at-risk' individuals from moving on to more severe anxiety or depression.

The anxiety trait within the normal population is generally assessed by measures of anxiety such as the Spielberger's State-Trait Anxiety Inventory (STAI) (Spielberger, 1983; Eysenck et al., 2007). The STAI is a self-administered assessment scale for the evaluation of severity of anxiety that consists of two scales (20 items each) – Y2, which measures the participants' disposition to anxiety (trait), and Y1, which measures how anxious the subject/participant feels at the moment (state). The modern techniques of neuroimaging offer a way to identify specific biomarkers i.e., any measurable indicator (functional brain activity or morphological change) that could be objectively measured and evaluated as an indicator of a normal biological process (Lenzenweger 2013; Donzuso et al., 2014). Many studies have explored the macro-structural biomarkers of anxiety-related personality traits (Blackmon et al. 2011; Baur et al. 2012; Kuhn et al. 2011; Spampinato et al. 2009; Fuentes et al. 2012; Cherbuin et al. 2008; Donzuso et al., 2014; Hu and Dolcos 2017; Barrós-Loscertales et al., 2006; Pujol et al., 2002). These studies highlight the presence of different degrees of relationship (positive, negative or no association) between the anxiety in a nonclinical population as assessed by various scales (STAI, Beck Anxiety Inventory (BAI), Hamilton scale for anxiety (HARS), Temperament and Character Inventory (TCI) and Behavioral Inhibition System (BIS)) and regional brain volumes studied (Table 1).

Using VBM, Yamasue et al. (2008) found that higher scores on harm avoidance (HA) were associated with smaller regional grey matter (GM) volume in the right hippocampus in both males and females. On the other hand, in females higher scores on HA were also correlated to smaller regional brain volume in the left anterior prefrontal cortex. In a VBM study a positive correlation was obtained between sensitivity to

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Table 1
Morphometric findings in anxiety related personality traits.

Reference	Subjects (n)	M/F	Age (mean ± SD)	Anxiety-related personality trait studied/ scale used	Approach	Findings	Comments
Blackmon et al. 2011	18 healthy individuals	10/8	39 ± 11	Anxiety using Beck Anxiety Inventory (BAI) and STAI	Semi-automated subcortical segmentation and an automated procedure for thickness measurements	A decreased amygdala volume was found to be associated with higher state and trait anxiety. A positive correlation between anxiety and cortical thickness in left lateral orbitofrontal cortex was also obtained.	Structural abnormalities may result in a greater vulnerability to anxiety or conversely, elevated anxiety symptoms may result in focal structural changes.
Baur et al. 2012	32 healthy subjects	17/18	24.9 ± 4.6	Trait anxiety using STAI	Volumetric segmentation of sub-cortical structures using the Free-Surfer image analysis suite	Trait anxiety was found to be positively correlated with left amygdala volume and negatively correlated with left uncinate fasciculus volume.	Uncinate Fasciculus-amygdala complex is pivotal for the control of trait anxiety.
Kuhn et al. 2011	34 healthy subjects	14/20	30.5 (age range 19 - 47 years	Trait anxiety using STAI	Automatic subcortical segmentations of Freesurfer was used to obtain a measure of cortical thickness	Trait anxiety was negatively correlated with cortical thickness in the right medial orbitofrontal cortex (mOFC) and positively correlated with the bilateral volume of nucleus accumbens	Authors suggest that this reduction in cortical thickness is a structural precondition rather than a consequence of psychiatric illnesses.
Spampinato et al. 2009	30 healthy right-handed participants	16/14	28 ± 5.6	State and trait anxiety using STAI	VBM analysis using the optimized procedure in SPM2	Inverse correlation between anxiety measures and distributed neural network, including the parahippocampal-amygdalar regions, the posterior cingulate cortex, and medial and dorsolateral prefrontal cortex	Volumetric variability of these regions may have a correlation with the development of an anxious personality trait.
Fuentes et al. 2012	114 healthy participants	114	26.76 ± 07.48	Behavioural Inhibition System (BIS) sensitivity using the BIS/Behavioural Activation System (BAS) questionnaire	VBM using the VBM8 toolbox for the SPM8 package	Negative correlation between the BIS scores and the volume of the right and medial orbitofrontal cortices and the precuneus.	Individual differences in anxiety-related personality traits may be associated with reduced brain volume in structures relating to emotional control and self-consciousness.
Cherbuin et al. 2008	430 healthy subjects	197/233	46.63 ± 1.51(males); 46.73 ± 1.37 (females)	BIS/BAS sensitivity using BIS/BAS questionnaire	Manually outlining the periphery of the ROI on the coronal T1-weighted slices using Analyze 5.0	Hippocampal volumes were positively associated with BIS sensitivity and to a lesser extent with BAS sensitivity.	Role of the hippocampus in the regulation of defensive/approach behaviours and anxiety
Donzuso et al., 2014	121 healthy subjects	54/67	38.7 ± 15.1	Anxiety using STAI and Hamilton scale for anxiety (HARS)	Automated labeling and quantification of amygdala and hippocampal volumes and cortical thickness analysis using Freesurfer and VBM using SPM8	Anatomical variability in the anterior cingulate cortex was the best predictor of the HARS scores whereas STAI related measures did not show any significant relationship with regions of limbic circuits, but their scores were predicted by gender.	HARS and STAI are neurobiologically different; HARS is more related to subclinical expression of anxiety disorders, whereas the STAI captures sub-dimensions of personality linked to anxiety.
Hu and Dolcos 2017	62 healthy young adults	27/35	23.129 ± 3.877	Trait anxiety using STAI	Freesurfer was used to extract gray matter volume form inferior frontal cortex (IFC)	Trait anxiety was found to be correlated negatively with left IFC volume	Results suggest IFC involvement in anxiety at the structural level, and may inform the development of intervention programs targeting anxiety
Barrós-Loscertales et al., 2006	63 healthy undergraduates	63/0	mean age = 22.43; range 18–34)	Sensitivity to Punishment (SP) scale of BIS measure	Voxel-by-voxel regression analysis between gray matter volume and scores on the SP scale using SPM2 (VBM)	Positive correlation between SP scores and gray matter volume in the right amygdala and the hippocampal formation.	Present results are compatible with the association between the greater volume of a structure and its hyperfunctionality.
Pujol et al., 2002	100 healthy subjects	50/50	25.5 ± 3.9	Harm avoidance using Temperament and Character Inventory (TCI)	Manual tracing of the ROI	Surface measurements of the right anterior cingulate gyrus accounted for a 24% score variance in harm avoidance.	A large right anterior cingulate is related to a temperamental disposition to fear and anticipatory worry in both genders

punishment (SP) scores (a BIS measure) and GM volume in the amygdala and the hippocampal formation (Barrós-Loscertales et al., 2006). A temperamental disposition to fear and anticipatory worry in both genders has been shown to be associated with an enlarged anterior cingulate cortex (ACC) (Pujol et al., 2002). In a recent review carried out to identify the brain regions and circuits underlying anxiety and stress disorders, prefrontal cortex, ACC and the limbic regions such as amygdala, hippocampus and thalamus were found to be either structurally or functionally altered in these disorders (Duval et al., 2015). According to the authors, structural imaging studies report overall mixed results, with reports of both increased and decreased regional volumes in anxiety patients. Further, given the role played by the HPA axis in sustaining anxiety, Terlevic et al. (2012) explored in vivo anatomy of the hypothalamus in generalized anxiety disorder (GAD) and panic disorder (PD) patients. A decreased hypothalamic volume was obtained in GAD patients but not in those with PD. The structural imaging studies on trait anxiety are rather limited. Using subcortical segmentation, Baur et al. (2012) reported a positive correlation between the left amygdale volume and trait anxiety. In the cortical thickness analysis and VBM study by Donzuso et al. (2014), STAI-related measures did not show any significant relationship with the regions of limbic circuits. A negative correlation between trait anxiety and left inferior frontal cortex volume is reported by Hu and Dolcos (2017) using Desikan atlas in Freesurfer for ROI extraction. In a VBM analysis, an inverse correlation was also obtained between trait anxiety and cortical volume in left parahippocampal gyrus and the left amygdala, rostral ACC and prefrontal cortex (bilaterally); the regions implicated in the pathogenesis of anxiety disorders (Spampinato et al. 2009). Using semi-automated subcortical segmentation, Blackmon et al. (2011) found a negative correlation between left amygdala volumes and state-trait anxiety along with a positive correlation between anxiety and cortical thickness in left lateral orbitofrontal cortex.

Although STAI and HARS are the two of the most widely used measurements to assess anxiety, HARS is mainly used in psychiatric contexts (Donzuso et al., 2014). HA as assessed by TCI is defined as a tendency to respond intensely to aversive stimuli and as an aversion to punishment, novelty, and non reward (Kim and Whalen, 2009). Similarly, BAI is best considered a measure of state rather than trait anxiety (Blackmon et al., 2011). SP measures behavioral inhibition in response to novelty or punishment cues and cognitive worry in response to failure or punishment cues (Barrós-Loscertales et al., 2006). Thus, various measures of anxiety related behavior reflect different aspects of anxiety with STAI being the most direct measure of anxiety as a personality dimension in sub-clinical population. Therefore, given a limited literature available on the morphological changes associated with trait anxiety in healthy population, methodological differences in the existing studies and the inconsistencies in the findings, we explored the relationship between individual differences in trait anxiety scores and regional gray matter volumes in the regions earlier implicated in anxiety. Hence, the hippocampus (Spampinato et al., 2009), amygdale (Blackmon et al., 2011, Spampinato et al., 2009), anterior cingulate cortex (ACC) (Donzuso et al., 2014), thalamus (Duval et al., 2015), hypothalamus (Terlevic et al., 2012) and regions of prefrontal cortex (dorsolateral prefrontal cortex (DLPFC) (Shang et al., 2014), rostrolateral prefrontal cortex (RLPFC) (Blackmon et al., 2011; Hu and Dolcos, 2017) and ventrolateral prefrontal cortex (VLPFC) (Spampinato et al., 2009)) were chosen as the ROIs (separately for both the hemispheres) in this particular study. Since the majority of the studies point towards a negative correlation between anxiety and the regional brain volumes studied, we hypothesized an inverse correlation between the GM volumes of the ROIs studied and self-reported levels of trait anxiety. Further, since the main objective of the study was to study the association between trait anxiety and GM volume, age, gender, and current depression (as assessed by the Beck Depression Inventory (BDI) (Beck et al., 1996)) were taken as covariates of no interest in all the analyses carried out (except the bivariate correlation analysis between trait anxiety and GM volumes of the ROIs) (Baur et al., 2012).

2. Materials and methods

2.1. Subjects

83 right-handed healthy participants (male – 40, female – 43, mean age – 22.66 years, SD – 2.71 years) were screened for current or past illness using the Hindi version of the diagnostic interview for genetic studies (Deshpande et al., 1998). None of the subjects chosen for the study had any clinical evidence of stroke, head injury, cardiovascular diseases, or history of alcohol or drug dependence, hypertension, neurological, psychiatric disorder or sensori-cognitive impairment, nor did they have any cortical infarctions on the T2-weighted MR images. The procedure followed in the current study was in accordance with the Declaration of Helsinki and also in accordance with the guidelines set by the ethical committee of the Institute. The study was approved by the ethics committee of the local institutional review board. Further, all subjects gave their consent to participate in the study and the study procedure was thoroughly explained to them. Participants' trait anxiety levels were assessed using STAI self-report questionnaires for adults (Spielberger, 1983). Participants also completed the BDI (Beck et al., 1996). Three subjects had a BDI score above 12 and therefore have to be eliminated from the study, resulting in a total of 80 subjects whose MRI scans were acquired.

2.2. Imaging protocol

The MRI scans were acquired using 3T whole-body MRI system (Magnetom Skyra, Siemens, Germany) with a 20-channel head and neck coil and 45 mT/ m actively-shielded gradient system. Subjects lay in the supine position with their heads supported and immobilized within the head coil using foam-pads (vendor provided), to minimize head movement and gradient noise. High-resolution MR images were obtained with a T1- weighted three-dimensional gradient echo sequence (magnetisation prepared rapid acquisition gradient echo, 160 sagittal slices, slice thickness = 1 mm, field of view = 256 mm, TR = 1900 ms, TE = 2.07 ms). In addition, a T2-weighted turbo spin echo (TE = 100 ms, flip angle = 150°, field of view = 220 mm) scan with 25 axial 4-mm slices and a 1.2-mm gap of the whole brain was acquired for diagnostic evaluation to rule out any cortical infarcts.

2.3. Data pre-processing

Data were pre-processed using statistical parametric mapping (SPM8, Wellcome Department of Cognitive Neurology, Institute of Neurology, London, UK) implemented in MATLAB R2008a, version 7.6.0 (Mathworks, Sherborn, MA, USA). All the steps were carried out as suggested by Ashburner, 2010 (www.fil.ion.ucl.ac.uk/~john/misc/VBMclass10.pdf). In brief, the anatomic images were first manually reoriented so that the anterior commissure matched the origin (0, 0, 0), and the orientation approximated Montreal Neurological Institute (MNI) space. Next, T1-weighted images were classified into GM, white matter (WM) and cerebrospinal fluid (CSF) using the 'new-segment' routine implemented in SPM8, that gives both the native space versions and Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL) imported versions of the tissues. The DARTEL imported versions of GM and WM were used to generate the flow fields (which encode the shapes) and a series of template images by running 'DARTEL (create templates)' routine. During this step, the accuracy of inter-subject alignment is increased by using parameters equal to 3 times the number of voxels. Finally, the flow fields and the final template image created in the previous step are used to generate smoothed (8 mm full width at half maximum), modulated, spatially normalised and Jacobian scaled GM and WM images resliced to 1.5 × 1.5 × 1.5

mm voxel size, in Montreal Neurological Institute (MNI) space by running the 'normalise to MNI space' option.

2.4. ROI analysis

Using the WFU Pickatlas (Maldjian et al., 2003; 2004), masks for various regions of interest (ROIs) were created. The regions previously implicated in anxiety like behavior such as, hippocampus (Spampinato et al., 2009), amygdale (Spampinato et al., 2009), anterior cingulate cortex (ACC) (Donzuso et al., 2014), thalamus (Duval et al., 2015), hypothalamus (Terlevic et al., 2012), dorsolateral prefrontal cortex (DLPFC) (Shang et al., 2014), rostrolateral prefrontal cortex (RLPFC) (Blackmon et al., 2011; Hu and Dolcos, 2017) and ventrolateral prefrontal cortex (VLPFC) (Spampinato et al., 2009) were chosen as the ROIs in this particular study. The gray matter (GM) volume was extracted from the ROIs using a MATLAB script (https://www.researchgate.net/post/How_do_I_perform_the_ROI_analysis_of_MRI_volumetric_data_ using_MarsBar). The extracted GM volumes were normalized by dividing them with total intracranial volumes (TIV) of the individual subjects. An index of the adjusted volume for each ROI was obtained by multiplying the normalized volumes with 100. For outlier identification, absolute deviation around the median (MAD) was calculated based on the method given by Leys et al. (2013). The method identified 4 outliers and therefore 76 out of 80 participants were included in the subsequent analysis. The mean and standard deviation of the normalized adjusted GM volume of the ROIs in the 76 subjects is given in supplementary Table 1.

2.5. Statistical analysis

A partial correlation analysis was carried out between the normalized adjusted gray matter values from various ROIs and the trait anxiety scores of the subjects, with age, gender, and BDI score of each subject as covariates of no interest using SPSS (version 15.0, SPSS Inc, Chicago, IL, USA) statistical software. Since there is a discrepancy in the literature regarding the directionality of the relationship between GM volumes of ROIs investigated and the anxiety like behavior, we tested for the possibility of the relationship in both directions and therefore, p values of $p \leq 0.05$ (2-tailed) were considered to be significant.

Further, confounding variables age, gender and BDI scores have been shown to be correlated with anxiety scores. As a consequence, part of the variation explained by anxiety could be confused with the variation explained by the confounding variables and vice versa. As a consequence, the mutual correlations between anxiety and the confounding variables could result in false positive or false negative findings. Therefore, bivariate correlation analysis between anxiety scores and the GM volumes of the ROIs was also carried out (without any correction for the confounding variables age, gender and BDI scores).

In order to examine if the variables of interest (gray matter volumes of ROIs showing correlation with trait anxiety as obtained using partial correlation analysis) explain a statistically significant amount of variance in dependent variable i.e. trait anxiety, after accounting for other covariates, a hierarchical multiple regression analysis was carried out. (http://www.ucdenver.edu/academics/colleges/nursing/Documents/PDF/HierarchicalRegressionHowTo.pdf). Hierarchical multiple regression analysis was conducted with the normalized adjusted gray matter volumes of all the ROIs as predictors and trait anxiety scores as predicted variable. The effect of age, gender and BDI scores was controlled for by entering them into the model first. This ensures that they will get "credit" for any shared variability that they may have with the predictors. The predictors that are actually of interest were entered in the next step. Any observed effect of the predictors can then be said to be "independent of" the effects of the variables that have already been controlled for. p values of $p \leq 0.05$ were considered to be significant.

The subjects were also grouped into two groups (median split on the basis of trait anxiety score): High anxiety group ($N = 41$, trait anxiety scores = 38–57) and low anxiety group ($N = 35$, trait anxiety scores = 21–37). In order to assess if there are statistically significant mean differences among the two groups in the gray matter volumes of the ROIs studied, a Multivariate Analysis of COVAriance (MANCOVA) using general linear model (SPSS (version 15.0, SPSS Inc, Chicago, IL, USA) statistical software) was carried out. Controlling for the effects of age, gender, sub-clinical depression (BDI scores), anxiety group and normalized adjusted GM volumes of all the ROIs were analyzed with MANCOVA. Adjustment for multiple comparisons in all the analyses was done by applying Bonferroni correction. In order to maintain 95% confidence in our set of analyses as a whole, the threshold of significant becomes $p < 0.00454$ (dividing 0.05 by 11 (total number of predictors – 8 independent variables and 3 control variables) for partial correlation analysis and MANCOVA and $p < 0.00625$ (dividing 0.05 by 8 (total number of independent variables – 8 in bivariate correlation analysis).

2.6. Whole Brain Voxel Wise Analysis

A whole brain voxel based morphometry analysis of the data to assess the relation between trait anxiety and the GM volumetric changes on a voxel by voxel basis was also carried out. The analysis details are reported in the supplementary material.

3. Results

3.1. Behavioral data

Descriptive statistics for self-report measures are as: Trait anxiety score (STAI-Y2) = 38.18 ± 8.22 (range 21 - 57); BDI = 6.92 ± 3.37 (range 0 - 12). Women were found to be more trait anxious (STAI-trait: 39.84 ± 7.74) than men (STAI-trait: 36.53 ± 8.46), without reaching a significant threshold ($p = 0.079$). Subjects with trait anxiety score between 21 and 38 were considered as low trait anxiety group and those with trait anxiety score above 38 were considered as high trait anxiety group.

3.2. Correlation between VBM measures and trait anxiety

Trait anxiety was found to be negatively correlated with the gray matter volume of right hypothalamus ($N = 76$; $r = -0.301$, $p = 0.010$; 2-tailed; Fig. 1, Fig. 2a). A negative association was also obtained between trait anxiety and the GM volume of left hypothalamus ($N = 76$; $r =$ ~~-0.211~~ -0.232, $p =$ ~~0.074~~ 0.048; 2-tailed; Fig. 1, Fig. 2b). A partially significant positive association was obtained between trait anxiety and the GM volume of left thalamus ($N = 76$; $r = 0.218$, $p = 0.064$; 2-tailed; Fig. 1). The obtained findings are however, not statistically significant when thresholded against corrected p values (Table 2).

Bivariate correlation results also showed a negative correlation between GM volume of right hypothalamus ($N = 76$; $r = -0.303$, $p = 0.008$; 2-tailed) and a positive association between GM volume of left thalamus and trait anxiety ($N = 76$; $r = 0.252$, $p = 0.028$; 2-tailed; Table 3), though not statistically significant when corrected for multiple comparisons.

The predictors in hierarchical multiple regression model were input in three steps. Age, gender and BDI scores were entered first (model 1); next the GM volumes of ROIs that showed an association with trait anxiety (left hypothalamus and left thalamus) were entered (model 2). All the remaining ROIs in the left hemisphere were entered in the model at last (model 3). All the three multiple regression models were statistically significant (Table 4) (Model 1: $F(3, 72) = 8.523$, $p = 0.000$, $R^2 = 0.262$; Model 2: $F(5, 70) = 9.972$, $p = 0.000$, $R^2 = 0.416$; Model 3: $F(11, 64) = 4.617$, $p = 0.000$, $R^2 = 0.442$). Thus, the predictive power increased from 26.2% in model 1 to 41.6% in model 2 by the addition of GM volumes of left hypothalamus and left thalamus as

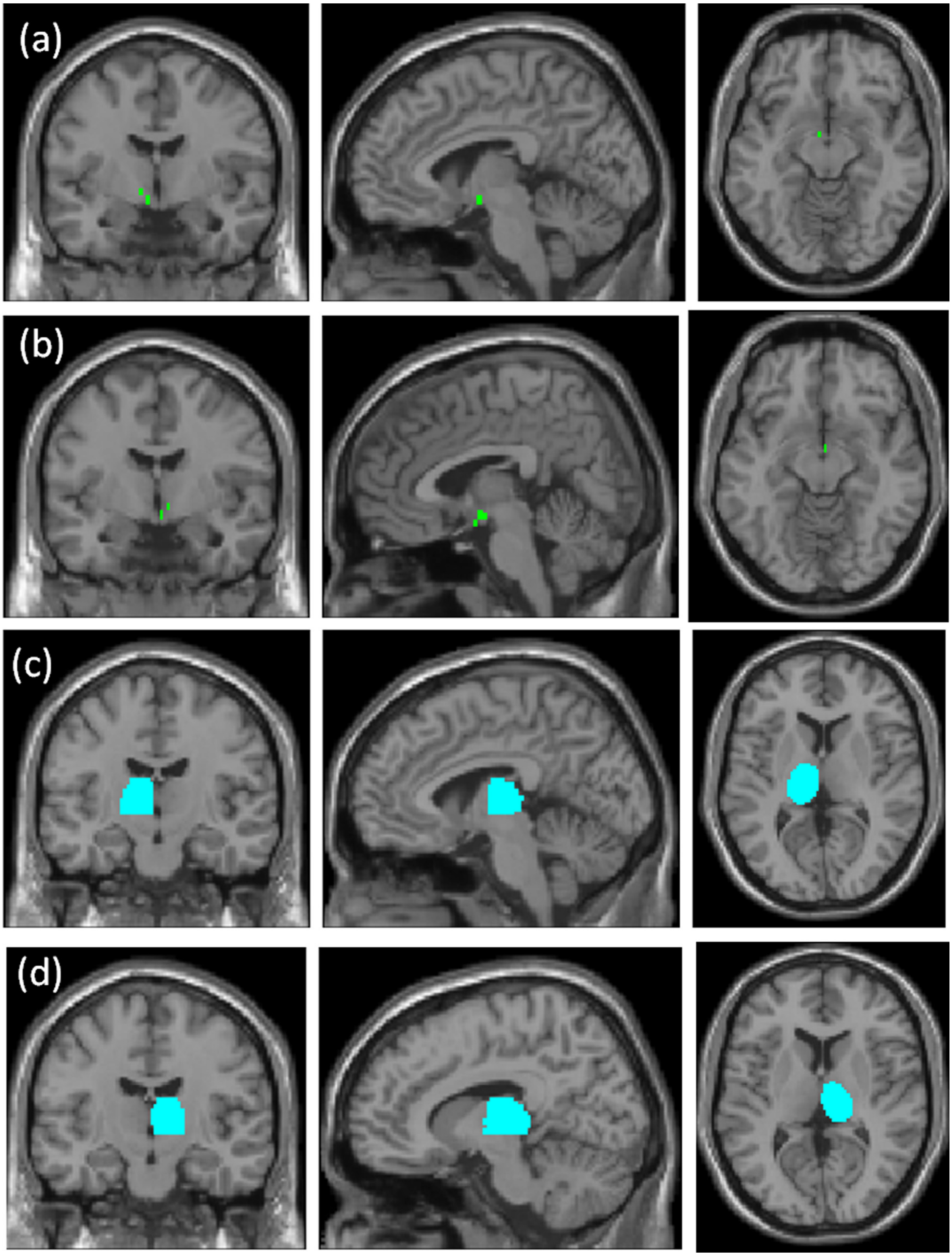

Fig. 1. A priori regions of interest (ROIs) (a) Left Hypothalamus (b) Right Hypothalamus (c) Left Thalamus (d) Right Thalamus included in the analysis.

predictors. The predictive power further increased to 44.2% on addition of other predictors in model 3. Analysis showed that trait anxiety was significantly predicted by the BDI scores ($p = 0.000$), and with marginal significance by age ($p = 0.060$) and gender ($p = 0.057$) in model 1; BDI score ($p = 0.000$), age ($p = 0.023$) and GM volume of left hypothalamus ($p = 0.000$) and left thalamus ($p = 0.000$) in model 2 and BDI score ($p = 0.000$), age ($p = 0.020$) and GM volume of left hypothalamus ($p = 0.001$) and left thalamus ($p = 0.001$) in model 3.

Similar analysis was carried out for the ROIs of right hemisphere (Table 5). All the three multiple regression models were statistically significant (Model 1: $F(3, 72) = 8.523, p = 0.000, R^2 = 0.262$; Model 2: $F(5, 70) = 8.521, p = 0.000, R^2 = 0.378$; Model 3: $F(11, 64) = 3.930, p = 0.000, R^2 = 0.403$). Thus, the predictive power increased from 26.2% in model 1 to 37.8% in model 2 by the addition of GM volumes of right hypothalamus and right thalamus as predictors. The predictive power further increased to 40.3% on addition of other predictors in model 3. Analysis showed that trait anxiety was significantly predicted by the BDI scores ($p = 0.000$), and with marginal significance by age ($p = 0.060$) and gender ($p = 0.057$) in model 1; BDI score ($p = 0.000$), GM volume of right hypothalamus ($p = 0.001$) and with marginal

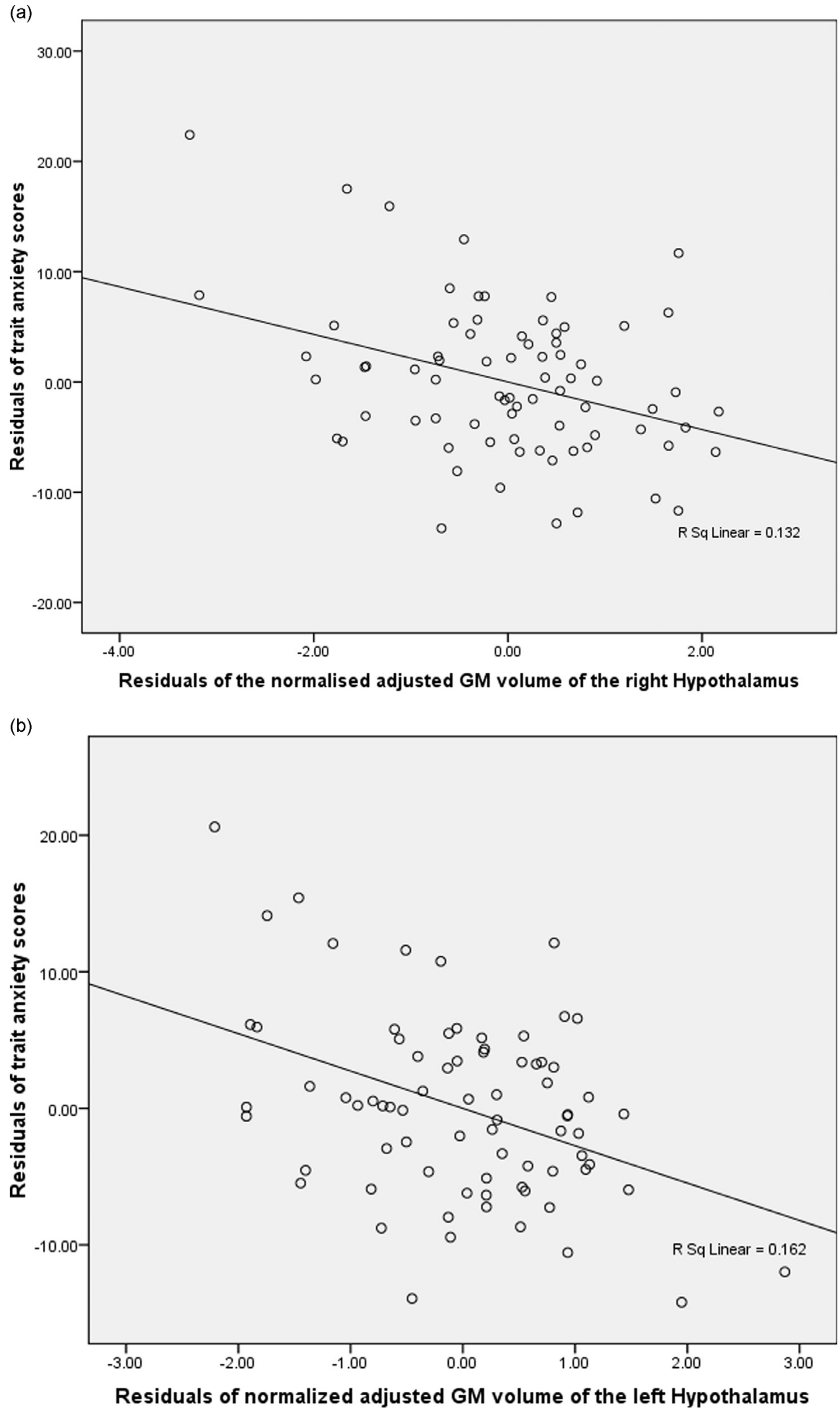

Fig. 2. Partial regression plot between normalized adjusted gray matter volume in (a) right hypothalamus and (b) left hypothalamus and trait anxiety scores.

significance by GM volume of right thalamus (p = 0.021), age (p = 0.073) and gender (p = 0.056) in model 2 and BDI score (p = 0.000), GM volume of right hypothalamus (p = 0.003) and with marginal significance by GM volume of right thalamus (p = 0.076) in model 3.

3.3. Between-group differences in VBM measures

The overall F statistic in MANCOVA showed that the group (high/low trait anxiety) had a significant effect on GM volumes of the ROIs in the left hemisphere (Wilk's Lambda = 0.775, F (8, 64) = 2.320, p = 0.030, partial η^2 = 0.225, observed power = 0.843) after

Table 2
Associations between gray matter volumes across various a-priori ROIs with trait anxiety.

ROI	Partial Correlation[a] r value	p*
Left ACC	−0.190	0.107
Right ACC	-0.080	0.501
Left Amygdala	−0.029	0.809
Right Amygdala	−0.119	0.316
Left Hippocampus	0.016	0.891
Right Hippocampus	0.070	0.558
Left Thalamus	*0.218*	*0.064*
Right Thalamus	0.160	0.176
Left Hypothalamus	**−0.232**	**0.048**
Right Hypothalamus	**−0.301**	**0.010**
Left DLPFC	0.055	0.644
Right DLPFC	−0.052	0.659
Left VLPFC	−0.055	0.646
Right VLPFC	−0.052	0.662
Left RLPFC	−0.065	0.587
Right RLPFC	−0.105	0.377

[a] with age, sex and BDI scores as covariates of no interest
* Boldface indicates $p < 0.05$ (2-tailed); marginally significant correlations at $p < 0.10$ are indicated in italics

Table 3
Bivariate correlation between gray matter volumes across various a-priori ROIs and trait anxiety.

ROI	Bivariate Correlation r value	p*
Left ACC	−0.095	0.416
Right ACC	−0.010	0.931
Left Amygdala	−0.131	0.258
Right Amygdala	−0.087	0.453
Left Hippocampus	−0.002	0.984
Right Hippocampus	0.087	0.456
Left Thalamus	**0.252**	**0.028**
Right Thalamus	0.086	0.459
Left Hypothalamus	−0.160	0.168
Right Hypothalamus	**−0.303**	**0.008**
Left DLPFC	0.143	0.217
Right DLPFC	0.040	0.729
Left VLPFC	0.006	0.962
Right VLPFC	0.066	0.570
Left RLPFC	−0.036	0.759
Right RLPFC	−0.002	0.988

* Boldface indicates $p < 0.05$ (2-tailed)

Table 4
Hierarchical regression analysis of predictors (left hemispheric ROIs) of trait anxiety.

Predictors	Standardized coefficients		
	Model 1	Model 2	Model 3
Age	0.199#	0.219*	0.243*
Gender	0.196#	0.130	0.127
BDI scores	0.475**	0.467**	0.470**
Normalized adjusted left hemispheric ROI volumes			
Hypothalamus		−0.407**	−0.419**
Thalamus		0.413**	0.406**
ACC			−0.121
Amygdala			0.105
Hippocampus			−0.029
DLPFC			0.150
VLPFC			0.083
RLPFC			−0.107

\# $p<0.1$,
* $p<0.05$,
** $p<0.01$

Table 5
Hierarchical regression analysis of predictors (right hemispheric ROIs) of trait anxiety.

Predictors	Standardized coefficients		
	Model 1	Model 2	Model 3
Age	0.199#	0.177#	0.169
Gender	0.196#	0.186#	0.161
BDI scores	0.475**	0.473**	0.473**
Normalized adjusted left hemispheric ROI volumes			
Hypothalamus		−0.331**	−0.327**
Thalamus		0.240*	0.197#
ACC			0.026
Amygdala			−0.164
Hippocampus			0.199
DLPFC			0.016
VLPFC			0.038
RLPFC			−0.046

\# $p<0.1$,
* $p<0.05$,
** $p<0.01$

controlling for covariates. In the right hemisphere, the high/low trait anxiety group had partially significant effect on the GM volumes of the ROIs (Wilk's Lambda = 0.813, F (8, 64) = 1.834, p= 0.087, partial η^2 = 0.187, observed power = 0.729).

3.4. Whole Brain Voxel Wise Analysis

In a multiple regression analysis (with age, gender and BDI as covariates of no interest), significant positive correlation was obtained between trait anxiety and GM volume in left Area PGa, (inferior parietal lobule (IPL)), PFm (IPL, bilaterally), right Area 1 (post central gyrus), right Area PFop (IPL), right Lobule VI (Verm) of cerebellum, Lobule VIIIa (Verm) of cerebellum bilaterally, left Area FG1 (cerebellum), left Area 45 (inferior frontal gyrus) and left Area 44 (Fig. 3a).

In the analysis without taking age, gender and BDI scores as covariates, a positive correlation was obtained between trait anxiety and GM volume in right Area PFop (IPL) and right Area 1 (post central gyrus) (Fig. 3b). No negative correlation was obtained between trait anxiety and GM volume in any region in both the whole brain VBM analysis carried out.

4. Discussion

In the current study, using partial correlation analysis, we report higher levels of trait anxiety in healthy young adults to be associated with smaller gray matter volumes in the hypothalamus in both left and right hemispheres (with a greater effect in the right hemisphere). The study also showed an association of an increased left thalamic volume with the trait anxiety (though with partial significance). Bivariate correlation analysis between anxiety scores and the GM volumes of the ROIs (without any correction for the confounding variables age, gender and BDI scores) also revealed a negative association between the volume of the right hypothalamus and the trait anxiety along with a positive association between the left thalamic GM volumes and trait anxiety. The multiple regression results also suggest that along with BDI scores, the hypothalamic and thalamic GM volumes in both the hemispheres are significant predictors of the trait anxiety of the individuals. The present findings of reduced hypothalamic volumes in young healthy participants with higher trait anxiety levels is in lines with the findings in an earlier study in patients with generalized anxiety disorders (GAD) (Terlevic et al., 2012). According to the authors the patients with GAD are subjected to a higher cumulative dose of cortisol that might be associated with worrying thoughts and an abnormal hypothalamic pituitary axis (HPA) axis activation. A negative correlation was also obtained between total hypothalamic volumes and levels

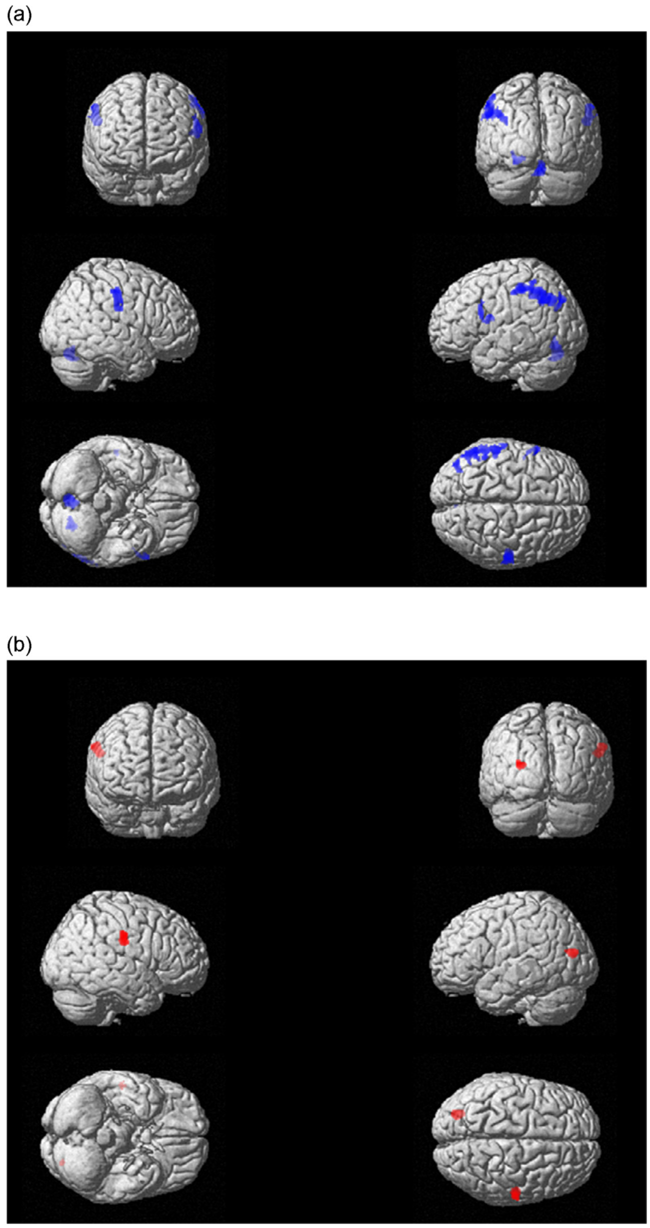

Fig. 3. 3D rendered view showing areas of positive correlation between regional gray matter volume and trait anxiety scores (uncorrected $p < 0.001$) (a) with age, gender and Beck Depression Scores as covariates of no interest and (b) without any covariates.

of anxiety as measured by Hamilton Rating Scale for Anxiety (HAMA) scores, which might indicate a causal relationship between pathological anxiety and disruption of hypothalamic morphology (Terlevic et al., 2012). The HAM-A is a clinician administered rating scale and is widely used to measure the severity of anxiety symptoms and their change over time in response to treatment. It is a 14-item scale that includes both psychological and somatic symptoms of anxiety (Hamilton, 1959). Hypothalamic corticotropin-releasing hormone (CRH) has been known to regulate neuroendocrine functions such as adrenal glucocorticoid release (Zhang et al., 2017). Authors speculated that the increased levels of circulating glucocorticoids (GCs) in the paraventricular nucleus of the hypothalamus might result in a reduction in the hypothalamic volumes through the action on glucocorticoid receptors and finally leading to neuronal atrophy, neurotoxicity and neuroendangerment.

Thalamus has also been implicated in the fear and anxiety circuitry to generate and modulate fear responses to imminent and identifiable threat (Duval et al., 2015). Our findings in thalamus are in lines with an earlier study that has shown a positive association between anxious arousal and left thalamic gray matter volumes (Castagna et al., 2017). A heightened activation of thalamic circuits might have resulted in the use dependent increase in the GM volumes of the thalamus.

In the current study, BDI scores, age and gender were also added into the model as predictor variables, since previous studies have demonstrated a relation between aging and anxiety levels (Schneider and Heuft 2012) as well as gender dependent effects on the relationship between brain anatomy and anxiety (Montag et al. 2012; Donzuso et al., 2014). The multiple regression analysis showed that trait anxiety was predicted by these three variables (age and gender with a partial significance) along with hypothalamus and thalamic volumes in both the hemispheres. Age, BDI and female gender showed a positive association with trait anxiety. However, the addition of the ROI volumes to the regression model increased the predictive power from 26.2% to 41.6% in the left hemisphere and from 26.2% to 37.8% in the right hemisphere suggesting their contribution over and above BDI, age and gender.

The whole brain voxel based study showed a positive correlation between trait anxiety and GM volume in the regions of inferior parietal lobule, post central gyrus, cerebellum and inferior frontal gyrus (Fig. 3). These observations are in line with a recent brain cortical thickness analysis study that reported both anxious arousal and apprehension to be significantly predictive of cortical thickness in various frontoparietal regions (including the dlPFC and the inferior parietal lobe) that are involved in attention and cognition control networks and emotion regulation (Castagna et al., 2017). Similarly, cerebellum has been suggested to be linked to increased arousal present in posttraumatic stress disorder (PTSD), generalized anxiety disorder (GAD), and social anxiety disorder (SAD) (Phillips et al., 2015). The obtained findings thus suggest a use dependent increase in the GM volume of obtained regions to be associated with the trait anxiety. However, the heterogeneity in the obtained results of the ROI and VBM analysis in the present study clearly illustrate the dependency of the study outcome to the opted analysis settings. Though VBM has been shown to be superior to that of ROI technique, it is recommended that ROI labeling to be used in combination with a clear hypothesis.

The earlier neuroimaging studies that investigated the morphological neural correlates of trait anxiety had methodological differences (e.g. Baur et al. (2012), included 32 healthy controls (subcortical segmentation using FreeSurfer; applied partial correlation analysis (1-tailed) between trait anxiety and amygdala/hippocampus volume); Spampinato et al. (2009), included 30 healthy controls (VBM study using SPM2 (smoothing kernel size 12 mm FWHM) with state and trait anxiety scores in multiple regression analyses); Blackmon et al. (2011), included 34 healthy controls (Semi-automated subcortical volume estimation); Kuhn et al. (2011) included 34 healthy controls (cortical thickness estimation using Freesurfer (Gaussian smoothing kernel with a FWHM of 20 mm); whole brain analysis in order to explore brain regions where cortical thickness varies with trait anxiety (controlling for age and sex)). The volumetric study by Donzuso et al. (2014) investigated a large cohort (121 healthy controls) where using VBM and cortical thickness analysis it was suggested that the relationship between anxiety and brain anatomy is critically influenced by gender, a critical variable not considered in previous studies cited above. However, in the same study STAI-related measures did not show any significant relationship with the volume of the regions of limbic circuits, but their scores were predicted by gender (Donzuso et al., 2014). The discrepancy of the findings in this study with those of Donzuso et al. (2014) might have resulted due to the methodological differences and the difference in the age group of the subjects studied. Donzuso et al. (2014) did cortical thickness analysis using FreeSurfer (circularly symmetrical Gaussian smoothing kernel across the surface with a standard deviation of 10 mm) and VBM analysis (using SPM 8 with a smoothing kernel size of 12 mm FWHM) on a-priori ROIs created using WFUPickatlas. The age of the subjects studied was mean - 38.7 years, SD- 15.1 whereas in the present study it was mean - 22.66 years, SD - 2.71. In a whole brain voxel-based morphometry study, Spampinato et al. (2009) found an inverse correlation between anxiety measures (state and trait anxiety) and cortical volume in regions of the limbic system and prefrontal cortex implicated in the pathogenesis of anxiety disorders. They also reported a positive correlation between anxiety scores and regions of the inferior frontal gyrus bilaterally. Our findings in left Area 44 and 45 (regions of the inferior frontal gyrus) (though negating the effect of covariates age, gender and BDI scores) are in lines with the findings of Spampinato et al. (2009). However, we did not find negative correlation between trait anxiety and GM volume in any region. This discrepancy warrants further multi-centric studies in different samples in future.

The earlier ROI based volumetric studies mainly focused on the relation between the volumes of amygdala and the orbitofrontal cortex with trait anxiety (Baur et al., 2012; Blackmon et al., 2011; Kuhn et al., 2011). In the study by Baur et al. (2012), amygdalar volumes were found to be positively correlated with trait anxiety in left hemisphere were as in the right hemispheres the volumes were inversely associated with trait anxiety. However in the study by Blackmon et al. (2011), left amygdala volumes predicted anxiety, with decreased amygdala volume being associated with higher anxiety on both state and trait anxiety measures. In the same study, a positive correlation between anxiety and cortical thickness was obtained in left lateral orbitofrontal cortex. A negative correlation between the cortical thickness in the right medial orbitofrontal cortex (mOFC) and trait anxiety was reported by Kuhn et al. (2011). In the same study a positive correlation was obtained between trait anxiety and the volume of nucleus accumbens bilaterally. The obtained findings in the above studies have been attributed to either a structural precondition to development of an anxious personality trait (Kuhn et al., 2011; Spampinato et al., 2009; Blackmon et al., 2011) or conversely to a consequence of elevated anxiety symptoms (Blackmon et al., 2011). To the best of our knowledge this is the first study reporting the association of both hypothalamic and thalamic volumes with trait anxiety phenotype.

The similarity between the findings obtained in the current study on healthy individuals and previous findings obtained in patients with GAD suggests that subclinical and clinical populations might share the mechanisms linking anxiety and hypothalamic brain volumes. Thus, studying the healthy population that is at risk of developing affective disorders can further inform our understanding of the etiology of these disorders (Montag et al., 2013) and may help in designing strategies to mitigate them. Nevertheless, further volumetric studies are required to delineate the neural correlates of transdiagnostic facets of anxiety: anxious arousal (worry) and anxious apprehension (somatic anxiety or arousal). While worry is primarily a cognitive response, the somatic anxiety is predominantly physiological. The two facets of anxiety are both distinct and related to each other and often collapsed into one domain namely, trait anxiety (Castagna et al., 2017; Sharp et al., 2015). Further, the use of a ROI selection method based on an averaged brain anatomical atlas as against the methodology with an optimised ROI definition to each individual brain separately as is done by FreeSurfer (https://surfer.nmr.mgh.harvard.edu/) or BrainSuite (http://brainsuite.org/) is a limitation of the present study. The results should be validated with other ROI-labeling software such as FreeSurfer or BrainSuite before they can be generalized.

Declarations of interest

None.

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Contributors

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All authors contributed to and have approved the final manuscript.

Conflict of interest

None

Supplementary materials

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