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Smaller amygdala volume and increased neuroticism predict anxiety symptoms in healthy subjects: A volumetric approach using manual tracing

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ABSTRACT

Volume reductions in the amygdala (AMY) have been found in patients with anxiety disorders, but findings are mixed in subclinical participants with high trait anxiety scores, in whom both reductions and increases in AMY volume have been identified. One potential reason for such discrepancies could be the employment of different methods to determine the AMY volume (i.e., manual tracing in psychiatric research vs. automated methods), in non-patient research. In addition to trait anxiety, smaller AMY volume has also been linked to neuroticism, a personality trait consistently linked to increased vulnerability to anxiety. However, it is not clear how AMY volume and neuroticism together may contribute to anxiety symptoms in healthy functioning. These issues were investigated in a sample of 46 healthy participants who underwent anatomical MRI scanning and completed questionnaires measuring trait anxiety and neuroticism. AMY volume was assessed using manual tracing, based on anatomical landmarks identified in each participant's anatomical image. First, smaller left AMY volume was linked to higher levels of neuroticism (p = .013) and trait anxiety (p = .024), which in turn were positively correlated with each other. Moreover, AMY volume had a significant indirect effect on trait anxiety through neuroticism (ab = − .009, 95% CI [− .019, − .002]). This effect was not bidirectional, as trait anxiety did not predict AMY volume through neuroticism. Collectively, these findings provide support for a brain-personality-symptom framework of understanding affective dysregulation, which may help inform the development of prevention and intervention paradigms targeting preservation of AMY volume and reduction of neuroticism, to protect against anxiety symptoms.

1. Introduction

Anxiety has become one of the most common mental health problems in the United States (Kessler et al., 2005), particularly among young adults (Reetz et al., 2016). Notably, although more than half of American college students (~ 92% aged 18–24, and ~ 8% aged ≥ 25) reported anxiety symptoms within a 12-month interval, only about 20% reported having been diagnosed with or treated for anxiety disorders (American College Health Association, 2017). This seems to suggest that on the one hand, college is a critical period when young adults may be particularly vulnerable to affective disturbances. On the other hand, there is also great potential for prevention/intervention programs during this period to alleviate anxiety symptoms, as well as to prevent the worsening of symptom manifestation and the development of clinical anxiety or other emotional disturbances.

The alarming anxiety statistics and the pressing need for interventions at preventative stages highlight the importance of comprehensive investigations in the vulnerable young adult population *prior* to the emergence of full-blown anxiety disorders. Multi-modal assessments of both neural correlates and personality traits enable investigations of possible associations between different aspects of functioning, and thus holds promise to reveal the mechanisms of psychopathological development (Cuthbert, 2014; Miller, 2010). Volumetric abnormalities in the amygdala (AMY) and individual differences in neuroticism have been consistently associated with anxiety (e.g., Irle et al., 2010; Meng et al., 2013; Ormel et al., 2013), but it has not been clear how these factors together may contribute to vulnerability to anxiety. Investigating this issue in non-patient populations provides the opportunity to identify

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individual difference factors associated with the range of symptom presentation that may index risks and vulnerabilities to psychopathologies (Cuthbert and Insel, 2013). In this study, we adopted a brain-personality-symptom framework, and examined how AMY volumes may be associated with neuroticism and the presence of anxiety symptoms in a group of college-age individuals with no history of psychiatric disorders.

1.1. Variation in amygdala volume linked to clinical anxiety

The AMY is an almond-shaped region located deep within each hemisphere of the medial temporal lobe (Janak and Tye, 2015). The AMY has been shown to be critical in processing emotion (Davis and Whalen, 2001; Pessoa and Adolphs, 2010; Tovote et al., 2015), motivation (Cardinal et al., 2002; Haber and Knutson, 2010), as well as emotion-related cognitions (Dolcos and Denkova, 2014; Dolcos et al., 2011; Pessoa, 2015). Furthermore, AMY dysfunction in these domains has been noted as one of the key abnormalities associated with affective disorders, such as anxiety and depression (Bruhl et al., 2014; Etkin and Wager, 2007; Patel et al., 2012; Shin and Liberzon, 2010; Zoladz and Diamond, 2013). Specifically, increased AMY activation has been consistently reported across meta-analyses examining emotional processing using various task paradigms (Bruhl et al., 2014; Etkin and Wager, 2007; Patel et al., 2012; Shin and Liberzon, 2010), and decreased AMY activation after intervention and/or treatment (Bruhl et al., 2014; Nechvatal and Lyons, 2013; Phan et al., 2013). The AMY hyperactivation has been suggested to reflect a common exaggeration of the engagement of the fear circuity shared by many anxiety disorders (Etkin and Wager, 2007).

In addition to AMY dysfunction, AMY structural abnormalities have also been identified in affective disorders. For instance, volume reduction in AMY has been extensively examined and reported in depression (Burke et al., 2011; Kronenberg et al., 2009), and seems to be modulated by gender (Hastings et al., 2004; Sheline et al., 1998, 1999), medication (Hamilton et al., 2008), and depression history (Frodl et al., 2003). Although the AMY morphometry in anxiety has received less attention, extant evidence also points to possible volume reduction in this region, across anxiety disorders (Alemany et al., 2013; Fisler et al., 2013; Hayano et al., 2009; Irle et al., 2010; Meng et al., 2013), although some evidence for enlargement has also been identified (De Bellis et al., 2000 in adolescents; Machado-de-Sousa et al., 2014). For instance, in a group of patients with panic disorders, AMY volumes were found to be bilaterally smaller, compared to healthy controls, and left AMY volumes were negatively correlated with state anxiety (Hayano et al., 2009), suggesting that the smaller AMY volume may be associated with anxiety in panic disorder. There is also evidence of smaller AMY volumes in patients with social phobia, and of more pronounced magnitude of reduction in men (Irle et al., 2010). Anxiety-related AMY volume reduction has also been replicated in patients with generalized social anxiety disorder (Meng et al., 2013). Notably, the majority of these studies investigating volumetric differences in AMY in psychiatric research measured the AMY volume by manually tracing the structure on magnetic resonance (MR) images.

Taken together, previous neuroimaging research has suggested that variations in the structure of AMY meaningfully index the variations in the clinical status of affective disorders, particularly with respect to anxiety. In contrast to increased AMY activation, volume reduction in AMY, commonly determined by manual tracing, has been consistently identified in patients with anxiety disorders compared to healthy controls. Although the exact mechanism between structural and functional abnormalities in the AMY has not been fully understood, a possible explanation may be that over-stimulation of AMY may result in neural toxicity that in turn manifests as reduced volume of the structure, similar to other structures in the MTL (Chambers et al., 1999; Shekhar et al., 2005).

1.2. Variation in amygdala volume linked to trait anxiety and neuroticism

In contrast to the more systematic findings of AMY volume reduction in patient groups with anxiety disorders, inconsistent results regarding the association between AMY volumes and trait anxiety levels have been reported in people without clinical diagnosis of anxiety (Baur et al., 2012; Blackmon et al., 2011). For example, one study (Blackmon et al., 2011) reported a negative correlation between the volume of the left AMY and trait anxiety, measured with the State-Trait Anxiety Inventory-Trait scale, whereas another study (Baur et al., 2012) identified a positive correlation between the left AMY volume and trait anxiety, measured with the same scale. Whereas these discrepancies may be related to such factors as heterogeneity in sample characteristics, it is also possible that Freesurfer estimations might not capture accurately individual differences in AMY volumes, which may explain inconsistent results across studies. Therefore, one possible cause for discrepancies between studies in the clinical vs. healthy populations may be related to their different traditions of using manual tracing vs. automated methods (most commonly Freesurfer), respectively. AMY is known to be highly variable in its shape and difficult to delineate from the surrounding subcortical regions on MR images, similar to other medial temporal lobe (MTL) structures (Moore et al., 2014), and this may pose a challenge for automated methods such as Freesurfer (Fischl, 2012). Freesurfer has demonstrated high accuracy and reliability in parcellating cortical regions, but it tends to render larger estimations of AMY volume compared to manual tracing (Morey et al., 2009). Therefore, slice-by-slice manual tracing, based on comprehensive consideration of image quality, anatomical landmarks, the relative symmetry between the hemispheres, and its relative position among other MTL structures, may be a better methodological approach that captures the subtle details of AMY morphometry, and continues to be the “gold standard” in volumetric studies (Morey et al., 2009). Nonetheless, manual tracing is also subject to biases in its own right (e.g., tracer-induced biases), and thus careful procedures need to be in place to minimize influences of such biases.

Another reason for the discrepant findings could be that AMY may be linked to trait anxiety through multiple mechanisms, which may not necessarily affect its volume directly. One such possibility is that AMY volume variation may be more closely related to other stable individual differences that reflect fundamental tendencies in emotion processing, which in turn may manifest as anxiety symptoms. Here, we propose that one such construct is neuroticism, a trait that indexes a general propensity for negative affect (Costa and McCrae, 1991). Although the construct of neuroticism is closely related to the constructs of anxiety and depression, the nature of their association remains unclear. Whereas neuroticism has been construed by some theorists as indexing a broader emotion-related propensity that encompasses anxiety and depression (Costa and McCrae, 1991, 1995), according to other authors neuroticism captures the common variance shared by anxiety and depression (Clark and Watson, 1991). Moreover, it is also possible that neuroticism, anxiety, and depression are all constructs tapping into the same underlying trait (Bishop and Forster, 2013). Empirically, there is also evidence that neuroticism can be distinguished from trait anxiety, and seems to capture unique individual differences in addition to anxiety (Brown and Rosellini, 2011). Therefore, given its reported correlation with both AMY volume and trait anxiety, neuroticism seems a likely mediating link between AMY volume and trait anxiety. Including both neuroticism and trait anxiety in a mediation model and examining their relation to AMY volume can help inform the theoretical debate regarding the relation of these two constructs.

Moreover, recent longitudinal evidence in teens has shown that neuroticism is a strong predictor of developing both anxiety and depressive disorders (Zinbarg et al., 2016). Therefore, including depression as one of the measurements in this study can potentially be informative regarding the theoretical delineations of these constructs. For instance, if neuroticism indexes the common variance shared by anxiety

and depression, it would be similar to both constructs in its relation to AMY volumes. On the other hand, if neuroticism reflects a broader emotional propensity than anxiety or depression, its pattern of relating to AMY volumes may be similar to either construct, or may even be distinguished from them.

Taken together, it remains an open question whether AMY volume in healthy participants is related to trait anxiety in the same fashion as what has been found in anxiety patients, which would be consistent with a continuum in the AMY-anxiety relation from healthy to clinical statuses. So far the literature has not supported such continuity. Here, we investigated two reasons for such discrepancy: the first reason is related to the use of different morphometric methods to assess brain structure volumes in the two traditions, and the second reason is the lack of consideration of other constructs related to negative affect. To clarify these issues, the current study used manual tracing to assess AMY volumes on high-resolution MR images, following an established protocol (Moore et al., 2014), which allows us to test whether similar methods used in healthy populations as those used in patients would produce similar outcomes to what clinical research has obtained. The current study also explored the possible mediating role of neuroticism between AMY volume and trait anxiety in a group of healthy young adults. Incorporating anxiety-related constructs such as neuroticism could not only provide novel insights into the potential mechanisms of the link between AMY and anxiety, but also have important implications on the theoretical development of these constructs.

1.3. The present study

Overall, while AMY volume has been consistently reported to be smaller in anxiety disorders, findings regarding its relation to trait anxiety have yielded mixed results in non-patient groups without anxiety diagnoses. Available evidence suggests that the discrepancy may be the *combined* result of the different morphometric methods used to measure AMY volumes in non-patient research, and the lack of consideration of other constructs related to negative affect. Therefore, to clarify these issues, the current study used manual tracing to assess AMY volumes on high-resolution MR images, following an established protocol (Moore et al., 2014), and explored the possible mediating role of neuroticism between AMY volume and trait anxiety in a group of healthy young adults. By adopting this framework and incorporating factors that reflect individual differences in amygdala volume, neuroticism and trait anxiety, the current study integrates affect-related constructs measured by multiple units of analysis, and thus has the potential to advance our understanding of vulnerability to anxiety and its mechanisms (Dolcos et al., 2016; Hu and Dolcos, 2017). Integrated understanding of anxiety vulnerability in a sample of healthy young adults will not only inform the research on anxiety as a dimensional construct (Cuthbert, 2014), but also have great impact on developing novel prevention and/or intervention programs that help alleviate anxiety symptoms in current college students. Based on the results reviewed above, the current study tested the hypothesis that AMY volume would be negatively correlated with (1) trait anxiety and (2) neuroticism, and (3) neuroticism would mediate the relation between AMY volume and trait anxiety.

2. Methods

2.1. Subjects

Analyses are based on data from 46 participants (18–33 years old, M = 23.69, SD = 3.92; 29 females), after elimination of 11 datasets during the quality assurance process (See Section 2.4, for more details.). All participants were right-handed. This sample size is comparable with or larger than similar studies in the literature (Baur et al., 2012; Blackmon et al., 2011). One subject was missing the Beck Depression Inventory (BDI), and hence analyses concerning this measure were performed on data from 45 subjects. None of the subjects had previously been diagnosed with neurological, psychiatric, or personality disorders. The subjects were recruited from the university campus and the larger metropolitan area, through various advertisement outlets (flyers, newspapers, etc.). The research protocol employed in the present study was approved for ethical treatment of human participants by the Institutional Review Board at the University of Alberta and at the University of Illinois.

2.2. Behavioral measures

2.2.1. Trait anxiety

Trait anxiety was assessed using the State-Trait Anxiety Inventory-Trait (STAI) (Spielberger et al., 1970). Subjects rated how they generally felt about 20 statements, such as *"I worry too much over something that really doesn't matter"*, using a 1–4 Likert scale (1 = not at all, 4 = very much so). Ratings of individual statements were summed to obtain a total score for each subject (ranging from 20 to 80). Higher scores are considered to reflect a possible vulnerability factor for anxiety disorders, and lower scores are potentially indexing lower vulnerability. The Cronbach's alpha for STAI in our sample was .881.

2.2.2. Neuroticism

Neuroticism was measured with the Neuroticism-Extraversion-Openness Five-Factor Inventory, Neuroticism subscale (NEO_N) (Costa and McCrae, 1991). The questionnaire consisted of 12 statements, such as "*I often feel tense and jittery*". Subjects rated how much they agreed with each statement, using a 1–5 Likert scale (1 = strongly disagree or the statement is definitely false, 3 = neutral or undecided or the statement is about equally true and false, 5 = strongly agree or the statement is definitely true). The ratings were summed to obtain a score of for each subject. Higher scores were taken to indicate higher level of neuroticism. The Cronbach's alpha for NEO_N in our sample was .854.

2.2.3. Depression

Depression was also measured and included in the analysis as a "control" construct, to examine the specificity of identified effects to anxiety symptoms. Depression symptoms were measured with the Beck Depression Inventory (BDI) (Beck et al., 1961). For each of the 21 items of the BDI, subjects were instructed to choose one of four statements that corresponded with how they felt, such as "*I do not feel sad*", "*I feel sad*", "*I am sad all the time and I can't snap out of it*", and "*I am so sad or unhappy that I can't stand it*", which were subsequently scored as 0, 1, 2, 3. The ratings were then summed to obtain a total score on BDI, with higher scores reflecting more severe depression symptoms. The Cronbach's alpha for BDI in our sample was .805.

2.3. Imaging protocol and MRI data processing

Structural scanning was conducted on a 1.5-T Siemens Sonata scanner at the University of Alberta (Canada). After the sagittal localizer, 3-D MPRAGE anatomical images were obtained using the following parameters: TR = 1600 ms; TE = 3.82 ms; FOV = 256 mm × 256 mm; 112 axial slices; voxel size of 1 × 1 × 1 mm^3. Manual tracing was performed on T1 images in native space. Manual tracing on all subjects was completed by one tracer, blind to subject information, following a previously published protocol (summarized below), initially developed by F.D. (Dolcos et al., 2004). Following manual tracing, two expert tracers (Y.H. & M.M.) visually inspected the tracing for quality assurance. The visual inspection was done blind to subject information and their scores on the personality measurements. As a result, manual tracings that did not meet the standard requirements were eliminated (N = 11). It should be noted that our results were not contingent on the elimination procedure, as analyses on the full sample yielded the same significant results. We decided, however, to report the findings based on the more conservative criteria, which were also applied to the automatic tracings, for comparison. Namely, to further examine the

contrast between manual and automated methods in estimating AMY volumes, the two expert tracers also visually inspected the AMY segmentations that Freesurfer produced in the full sample (N = 58), against the same standards that they used when inspecting manual tracing. As a result, more than half (N = 36, 62% of the total sample) of the data sets did not meet the same accuracy criteria applied to the manual tracings. ITK-SNAP (Yushkevich et al., 2006) was used for labeling, 3D construction, and volume calculation.

In order to account for the individual differences in head size, Freesurfer (version: 5.3.0) (Fischl, 2012) was used to obtain estimated total intra-cranial volumes (TIVs) for each subject. A semi-automated workflow was adopted to ensure quality control at Talairach registration, skull stripping, white matter surface and pial surface reconstruction. The outputs at each stage were manually inspected and corrected if necessary before the next stage was implemented, and all data sets passed this quality control procedure. At the end of the processing pipeline, the TIVs were extracted from the parcellation results, which would be included in the regression analyses as a covariate of no interest (see Section 2.5). The use of manual tracing for the AMY and automatic tracing for the TIV is in line with previous studies that employed manual tracing to estimate AMY volumes and automated methods, including Freesurfer, to extract TIV (Irle et al., 2010; Machado-de-Sousa et al., 2014; Wright et al., 2006). Freesurfer has been demonstrated to be both accurate and efficient in estimating TIV (Buckner et al., 2004). A previous study comparing the effects of Freesurfer vs. manually traced TIV reported that both methods were equally able to identify robust results, with Freesurfer TIVs being slightly more conservative compared to manually traced TIVs in identifying the more subtle patterns in the data (Tae et al., 2009). Although not of primary interest, Freesurfer estimations of AMY volumes were also extracted from its subcortical segmentation pipeline, and were submitted to statistical analyses to serve as a comparison to manual tracing results.

2.4. Manual tracing protocol

Manual tracing was performed by a single tracer (Z.B.) based on an established protocol (Moore et al., 2014). In short, the first slice of the AMY was identified when the limen insula initially appeared, and the AMY was traced in the coronal view with references to the axial and sagittal views as needed, from the anterior to the posterior. Anterior to the onset of the hippocampus, the AMY was traced counter-clockwise using the entorhinal sulcus, the fundus of the semianular sulcus along with its imaginary extension to the angular bundle, and the temporal stem as boundaries. After the appearance of the hippocampus, the hippocampus (along with alveus) was used as the inferior border of the AMY, an imaginary line from the fundus of the inferior circular sulcus of the insula to the optic tract served as the lateral border of the AMY, and the semilunar gyrus was used as the superomedial border. The last slice of AMY was identified when the structure appeared superior to the medial extension on the lateral ventricle, above the hippocampus, and lateral to the uncinate gyrus. Intra-rater reliability was assessed in 13 data sets based on published recommendations (Shoukri et al., 2004). The single measure intraclass correlation (ICC) was .980, for left AMY, with a 95% confidence interval [.940, .994] ($F(12,13) = 101.314$, $p < .001$), and .937, for right AMY with a 95% confidence interval [.815, .980] ($F(12,13) = 30.964$, $p < .001$) (see Fig. 1 for tracing examples and scatter plots showing the correlations between the 1st and 2nd time tracing).

2.5. Statistical analyses

IBM SPSS 24 (IBM Corp., Released 2016) was used to perform statistical analyses. To address our first two hypotheses, multiple regressions were used to regress scores of NEO_N or STAI on AMY volumes, with TIV, age and gender entered as covariates of no interest. Separate regression analyses were done for AMY in the left and right hemispheres. Bivariate outliers were examined using Mahalanobis distance with a threshold of $p < .001$, and no outliers were detected except for one subject who had a squared Mahalanobis distance of 14.66 in the bivariate distribution of NEO_N and BDI scores. This subject was removed from relevant analysis. Collinearity among regressors was monitored using variance inflation factors (VIFs) due to possible covariance between TIV and AMY volumes. In the current study, all the VIFs were less than 2, thus indicating that collinearity was not a problem in the regression analyses. When testing the hypotheses, we focused on the standardized regression coefficient (β) of the regressor of interest as an index of their association. A family-wise error rate of $\alpha = .05$ was used to control for Type I errors, and the Bonferroni method was used to account for repeated testing involving AMY volume in each hemisphere. Since anxiety was the main dependent variable of interest, additional analyses concerning depression were performed for the purpose of comparison, for which uncorrected p values are reported.

To address our third hypothesis, mediation analysis was performed using PROCESS macro for SPSS (Hayes, 2013). Since mediation models generally assume that the mediator (M) should be associated with both the predictor (X) and the outcome variable (Y), the mediation analysis was contingent on the identification of such associations in the analysis for our first hypothesis (James and Brett, 1984). Once identified, a parallel multiple mediation model was constructed, with age, gender, and TIV entered as covariates. In this model, regression coefficients, a and b, were first calculated from regressing M on X (path a), and regressing Y on both M and X (path b), respectively. The indirect effect was then estimated by the product term, ab. A bias-corrected bootstrap 95% confidence interval (CI) was generated for ab based on 10,000 bootstrap samples, along with a completely standardized effect (ab_{cs}) as the effect size measure (Hayes, 2013; Preacher and Kelley, 2011).

3. Results

Demographic characteristics of the current sample and descriptive statistics of the variables included in this study are presented in Table 1.

3.1. Amygdala volume linked to trait anxiety and neuroticism

Consistent with our first two hypotheses, the AMY volumes were found to be negatively associated with both trait anxiety and neuroticism. The left AMY volume was negatively associated with STAI scores ($\beta = -.341$, $p = .024$, n = 46; Fig. 2**A**). The right AMY volume also showed a negative trend in predicting STAI scores, although not as strong as the left AMY ($\beta = -.214$, $p = .172$, n = 46). Similar to the results concerning STAI scores, AMY volume was found to be negatively associated with NEO_N scores, stronger in the left ($\beta = -.373$, $p = .013$, n = 46) than right ($\beta = -.295$, $p = .057$, n = 46; Fig. 2**B**) hemisphere. Given these apparent differences between left and right AMY in relation to STAI and NEO_N scores, we then tested whether they were statistically significant, using Fisher's Z transform. Neither results reached the threshold for significance (Z = .639 for STAI and Z = .407 for NEO_N). As expected, NEO_N scores were positively associated with STAI scores ($\beta = .728$, $p < .001$, n = 46). Importantly, neither left ($\beta = -.204$, $p = .190$, n = 46) nor right ($\beta = -.040$, $p = .801$, n = 45) AMY volumes were significantly associated with BDI scores, despite positive associations between BDI scores and NEO_N scores ($\beta = .577$, $p < .001$, n = 44), as well as STAI scores ($\beta = .728$, $p < .001$, n = 45).

For the purpose of comparison, we also examined the relation between Freesurfer-extracted left AMY volumes and these trait level variables. The Freesurfer volumetric measures did not show significant associations with STAI scores ($\beta = -.016$, $p = .932$, n = 46), NEO_N scores ($\beta = -.219$, $p = .225$, n = 46), or BDI scores ($\beta = -.244$, $p = .181$, n = 45).

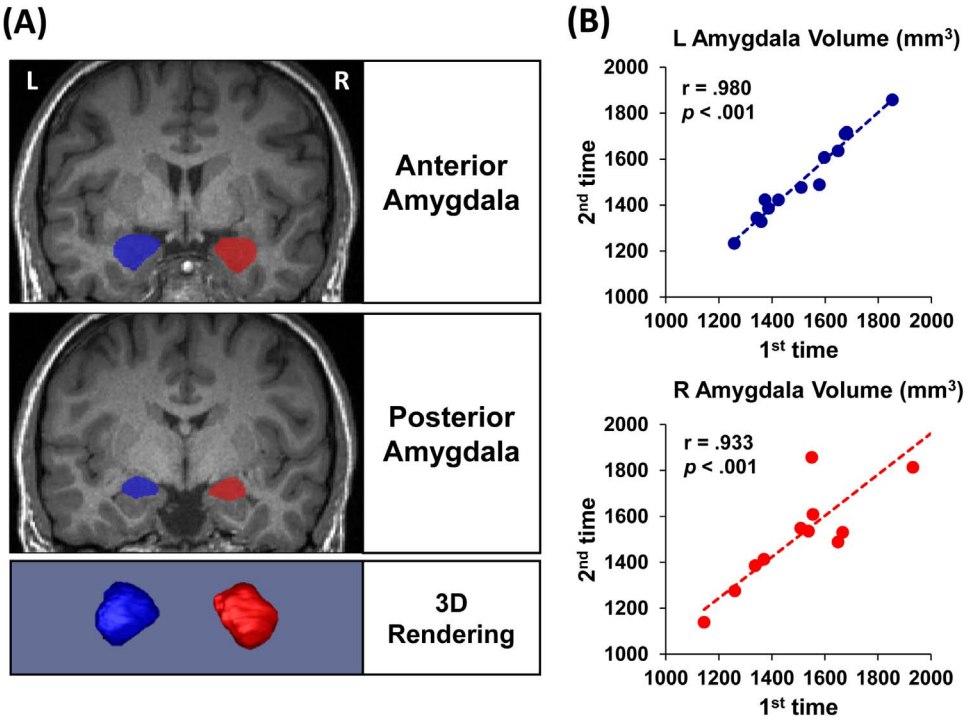

Fig. 1. Exemplar slices of manually traced amygdala and intra-tracer reliability. (A) Manual tracing of amygdala illustrated based on a representative exemplar subject, showing two slices from anterior and posterior amygdala. The 3D model was constructed in ITK-SNAP, smoothed using Gaussian blurring (SD = 1 mm). (B) Scatter plots and Pearson correlations for volumetric data from 13 subjects, randomly chosen to be traced twice for assessment of intra-rater reliability. Correlations coefficients and *p* values are presented here for easy reference. L, left; R, right.

3.2. Amygdala volume indirectly linked to anxiety-related traits

Given the above results showing common associations identified between left AMY volume, NEO_N scores and STAI scores, we then proceeded to test our second hypothesis that neuroticism would mediate the relation between AMY volume and trait anxiety. Our results confirmed this hypothesis, showing that left AMY volume had a negative indirect effect on STAI scores via NEO_N scores ($ab = -.009$, 95% CI [−.019, −.002], $ab_{cs} = -.250$; $a = -.014$, $b = .668$, $c = -.013$, [$p = .024$], $c' = -.004$, [$p = .442$]) (Fig. 3). Mediation models with AMY volume as the mediator or outcome variable were not significant.

4. Discussion

The current study found that variation in the AMY volumes in a group of healthy young adults meaningfully indexed the variation in trait anxiety-related constructs. Specifically, smaller left AMY volumes were linked to higher levels of trait anxiety and neuroticism. In exploring the mediation relationship among these variables, we found that neuroticism mediated the negative association between AMY volumes and trait anxiety, and this mediation effect was directional, as AMY volume was found to be a significant predictor of trait variables, but not vice versa.

4.1. Amygdala volume variation linked to trait anxiety and neuroticism

Our first finding that smaller AMY volume was associated with trait vulnerabilities to anxiety is consistent with our first hypothesis. AMY plays a key role in registering affective information and is critically involved in the interaction between emotion and cognition, particularly so in threat-related, negative emotions (Grupe and Nitschke, 2013). Anxiety is characterized by negative bias in attention, memory, and interpretation (Craske et al., 2009; Moran, 2016; Van Bockstaele et al., 2014), which has often been linked to AMY hyperactivation in functional neuroimaging studies (Bruhl et al., 2014; Etkin and Wager, 2007; Patel et al., 2012). Therefore, volume reductions in AMY previously reported in patients with anxiety disorders seem to be consistent with the idea that AMY may undergo volume reduction due to hyperactivation, although the exact mechanism is not clear. The current finding extends previous results by showing that in healthy young adults with high anxiety scores (but without anxiety diagnoses), AMY volume may already be negatively associated with anxiety symptoms, thus lending further support to the notion linking structure to function.

The nature of anxiety-related volumetric changes in AMY and its relation to AMY function has not been clear (Christoffel et al., 2011). One common hypothesis linking brain structure and function is the hypothesis of use-dependent neuroplasticity, which postulates a

Table 1
Demographic characteristics and descriptive statistics of behavioral and volumetric measures from manual tracing.

Demographic characteristics						
	Mean	SD	Range		Female N	Female %
Age	23.69	3.92	18–33	Gender	29	63.04%
Descriptive statistics						
Behavioral measures	Mean	SD	Range	Volumetric measures	Mean (10^3 mm^3)	SD	Range (10^3 mm^3)
STAI	37.50	8.52	[23, 57]	L AMY	1.51460	225.90	[1.11975, 2.07925]
NEO_N	29.87	8.49	[14, 49]	R AMY	1.57526	282.85	[1.03875, 2.30025]
BDI	4.00	4.11	[0, 17]	TIV	1302.92	19,2876.17	[874.93, 1902.68]

Note. N = 46, except for BDI (N = 45). STAI, State-Trait Anxiety Inventory-Trait; NEO_N, Neuroticism-Extraversion-Openness Five-Factor Inventory, Neuroticism subscale; BDI, Beck Depression Inventory; L, left R, right; AMY, amygdala; TIV, total intra-cranial volume.

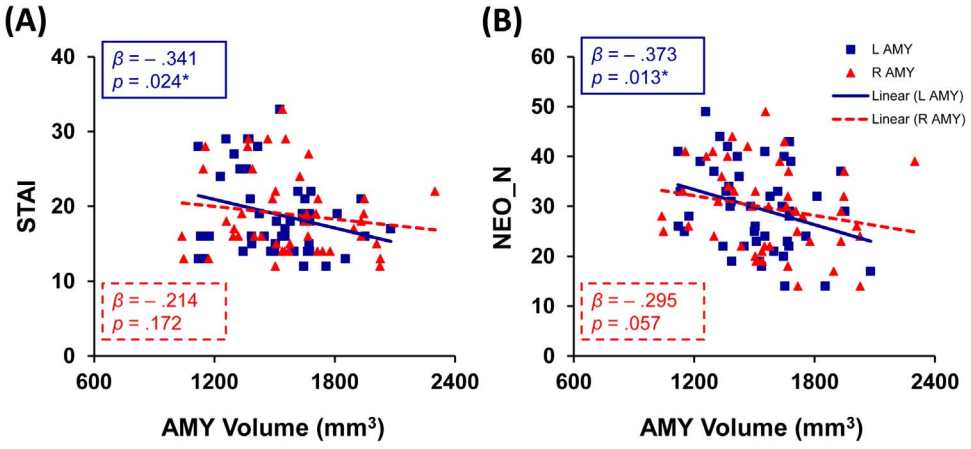

Fig. 2. Amygdala volume negatively associated with trait anxiety and neuroticism. Amygdala volume was found to negatively predict trait anxiety (A) and neuroticism (B), with significant results identified in the left (blue solid line) but not in the right (red dashed line) amygdala. Presented here are scatter plots with standardized regression coefficients and their *p* values. STAI, State-Trait Anxiety Inventory-Trait; NEO_N, Neuroticism-Extraversion-Openness Five-Factor Inventory, Neuroticism subscale; AMY, amygdala; L left; R right. *$p < .05$.

positive correlation between the structure and function of a brain region, such that the more frequent engagement of the region, the larger its gray matter volume will be (Hebb, 1949). Pertaining to individual differences related to anxiety and emotion regulation, we have previously obtained evidence consistent with this hypothesis in several regions in the prefrontal cortex (PFC) (Dolcos et al., 2016; Hu and Dolcos, 2017; Moore et al., 2016), showing that volumetric measures of brain regions associated with cognitive control and emotion regulation (i.e., inferior frontal cortex, orbitofrontal cortex, and dorsolateral prefrontal cortex) positively correlated with habitual use of an adaptive emotion regulation strategy (Moore et al., 2016), and negatively predicted anxiety symptoms (Dolcos et al., 2016; Hu and Dolcos, 2017). However, given the previous evidence of AMY hyperactivation in anxiety, the current finding of a negative association between the AMY volume and anxiety does not seem consistent with this hypothesis. This discrepancy may suggest that mechanisms other than use-dependent neuroplasticity (i.e., more use leading to volume increase) may exist in the AMY. This may include neural plasticity mechanisms induced by excessive activation, as suggested by Shekhar et al. (2005), or mechanisms that induce neural toxicity, similar to what have been found in the hippocampus (Chambers et al., 1999). It is also likely that neural plasticity may manifest through different mechanisms in the AMY, as it has been shown that in rodents stress-related neuroplasticity can manifest differently in different AMY substructures – as decreased spine density in the medial AMY, but as hypertrophy of dendritic arborization and increased spine density in the basolateral AMY (Christoffel et al., 2011). However, differences in sample characteristics have also hindered the progress of synthesizing findings from human and animal studies (Christoffel et al., 2011). In humans, there has been evidence that long-term structural neuroplasticity in AMY as a result of trauma correlated with AMY activation to negative stimuli, such that the smaller the volume, the greater the BOLD signal (Ganzel et al., 2008). Together, although there has been neuroimaging evidence supporting the link between AMY hyperactivity and reduced volume in anxiety, the exact biological mechanism awaits further clarification.

The present finding is consistent with previous psychiatric literature linking anxiety disorders with reduced AMY volume (Hayano et al., 2009; Irle et al., 2010), and stands in contrast to the mixed findings of anxiety vulnerabilities in healthy/subclinical populations (Baur et al., 2012; Blackmon et al., 2011). The fact that AMY volume is associated with trait vulnerability to anxiety in a group of healthy young adults, as well as in clinical anxiety, is in support of the dimensional conceptualization of anxiety disorders in line with the recent RDoC initiative (Cuthbert, 2014). Notably, this consistency is likely made evident by the use of manual tracing, which allows a more precise assessment of AMY volume in MR images, and has been routinely used in psychiatric research (unlike automated methods, such as Freesurfer, which has been more frequently used in studies with non-patient samples). Previously, it has been pointed out that Freesurfer tends to over-estimate the boundaries at some aspects of AMY structure (Morey et al., 2009), which may mask over statistical differences in volumetric research. Despite its importance and potential impact on the research results, the influence of different morphometric methods has not received enough attention (Moore et al., 2016), and extant reviews and meta-analyses have not included the differences in methodology as a factor of interest (Dresler et al., 2013; Hamilton et al., 2008; Koolschijn et al., 2009; Mincic, 2015). Supporting this idea, our findings are consistent with those from a previous study (Blackmon et al., 2011) that used Freesurfer as a main method and also applied necessary manual edits to Freesurfer outputs, following quality inspection, but not with results from another study (Baur et al., 2012) that used Freesurfer but did not incorporate subsequent manual edits as part of the inspection/correction procedure. It is possible that manual correction when using automated methods might have made a difference, although other possible factors (such as the

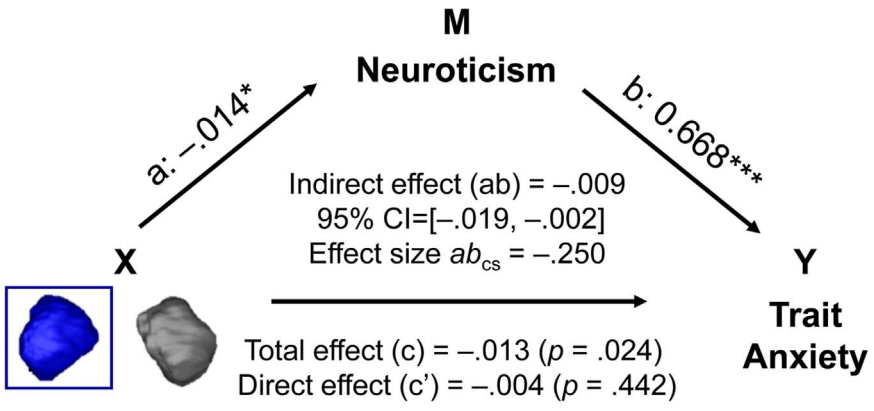

Fig. 3. Neuroticism mediated the negative association between left amygdala volume and trait anxiety. Unstandardized regression coefficients are presented here: *a* is the regression coefficient for regressing M (the mediator variable) on X (the predictor variable), *b* for regressing Y (the outcome variable) on M and X; *c* is the regression coefficient for the total effect from X to Y, and c' for the direct effect from X to Y controlling for M. The indirect effect was represented by the interaction term *ab*, and the significance of these effects was tested using bias-corrected bootstrapped 95% confidence intervals (CIs). Effect size was indexed by the completely standardized indirect effect (ab_{cs}). This mediation model shows that neuroticism fully mediated the relation between left amygdala volume and trait anxiety, indicated by a significant indirect effect and the change from a significant total effect (*c*) to insignificant direct effect (*c*) when mediator variable was introduced to the model. * $p < .05$; *** $p < .001$.

different characteristics of the control group), might also have contributed to the different results. Taken together, the current findings suggest that morphometric methods need to be considered carefully when conducting volumetric studies. This is particularly important for structures like AMY (along with other MTL structures), which are known to be challenging for automated segmentation methods, and where manual tracing, or at least inspection/manual correction of automated outputs, is recommended to ensure accuracy.

To help illustrate the differences made by the morphometric assessment, we also extracted the AMY volumes estimated by Freesurfer, and tested for their association with our targeted trait level variables. The findings of these analyses did not replicate the results obtained with manually traced AMY volumes. This is not surprising, given notable differences between the outputs resulting from the two types of analyses. Our visual inspection of Freesurfer outputs, against the same standard that we used for manual tracing, revealed that more than half of the Freesurfer outputs would need correction through manual edits. This raises an important issue regarding the need for visual inspection and manual edits of the results of automated segmentation. It has been shown that the errors in estimation are not limited to a specific location or aspect of AMY, but tend to occur consistently at the boundaries of the structure on all sides (Schoemaker et al., 2016). This suggests that these errors are not likely to be easily fixed by following simple steps in visual inspection, unless manual tracing techniques are also used to edit the segmentation on a slice by slice basis. Hence, we decided to rely on manual tracing, which requires knowledge about AMY/MTL anatomy and manual tracing skills.

Our second finding of a negative association between AMY volume and neuroticism is also consistent with previous findings in the literature (DeYoung et al., 2010). Previous results document negative associations between AMY volume and other anxiety-related constructs, such as exposure to childhood maltreatment (Edmiston et al., 2011), childhood trauma (Veer et al., 2015), and negative affect (Dennison et al., 2015; Hayano et al., 2009). The finding of a negative association of AMY volume with both neuroticism and trait anxiety may not be surprising, since neuroticism has been associated with negative bias in attention, emotion processing, and memory, all of which are manifestations of anxiety (Ormel et al., 2013). The association between AMY volume and trait-level constructs may reflect trait-related differences in basic processing of emotion and cognition (Denkova et al., 2012; Ormel et al., 2013). For instance, neuroticism has been previously linked to a tendency to rehearse negative memories and inefficient use of adaptive emotion regulation strategies (Denkova et al., 2012), which may in turn be linked to increased vulnerability to anxiety. Noteworthy, given the link between neuroticism and other forms of psychopathologies (Ormel et al., 2013), it is likely that the current finding of the negative associations of AMY volume with neuroticism and trait anxiety may not be specific only to anxiety.

4.2. Amygdala volume indirectly linked to anxiety-related traits

Our mediation analysis findings, where AMY volume predicted anxiety-related traits through neuroticism, provide support for the notion that volumetric variation of AMY may be a biological risk factor that reflects anxiety vulnerability in healthy young adults. This possibility is further supported by genetic research showing smaller AMY volumes in twins with a lifetime history of anxiety and depression, compared to healthy twins. This suggests that the link between AMY volume and anxiety may also be influenced by genetic factors (Alemany et al., 2013). The identification of mediation effects involving anxiety-related traits suggests that examining trait anxiety alone may be neglecting some aspects of variation on trait anxiety that may be meaningful and informative. Incorporating multiple self-report measurements that index correlated yet distinct constructs in vulnerability research can also help to reveal potential transdiagnostic factors and/or mechanisms.

Our results offered insights into the relation between neuroticism, trait anxiety, and depression. Although neuroticism was closely linked to both anxiety and depression, its role in relation to AMY volume was significant for trait anxiety only. These results suggest that neuroticism captured more anxiety- than depression- related variation, and thus lend support to the theoretical account postulating neuroticism as encompassing both anxiety-related and depression-related elements (Costa and McCrae, 1991), rather than as the construct capturing the intersection between anxiety and depression (Bishop and Forster, 2013; Clark and Watson, 1991). In addition, comparing the results involving neuroticism and trait anxiety to those involving depression also helped shed light on the specificity of the reported results. The lack of association between the AMY volume and depression, despite the latter's significant associations with trait anxiety and neuroticism, suggests that AMY volume is not linked to these emotion-related trait in a non-specific manner; instead the present findings distinguish neuroticism and trait anxiety from depression, in this regard.

The general dissociation between anxiety-related traits and depression symptoms in their relation to AMY volume may also reflect the differential relation between anxiety and depression and AMY-mediated processing in emotion and cognition. For instance, previous literature has shown that only anxiety has been reliably associated with an early attention bias to negative information (Crocker et al., 2013; Koster et al., 2006; Sass et al., 2010), which putatively takes place in the bottom-up pathway driven by an oversensitive AMY (e.g., Ormel et al., 2013). On the other hand, depression has been associated with a negative bias at later stages of processing, often reaching the level of consciousness and mediated by other cognitive processes (Koster et al., 2011; Teachman et al., 2012), thus less AMY-dependent.

The current study identified stronger mediation effects in the left AMY compared to the right, but statistically the left and right AMY did not differ in their strength of association with anxiety or neuroticism. This suggests that our results are not likely pointing to a laterality effect in the AMY. In the literature, laterality effects have not been consistently reported. For instance, in research with anxiety patients, AMY volume reduction has been observed both in the right hemisphere (Fisler et al., 2013; Hayano et al., 2009; Meng et al., 2013) and bilaterally (Alemany et al., 2013; Irle et al., 2010), whereas in individuals without anxiety diagnoses, anxiety-related volumetric differences in AMY has been reported in the left (Baur et al., 2012; Blackmon et al., 2011), right (Hayano et al., 2009) as well as bilaterally (Veer et al., 2015). The lack of a significant mediation effect with the right AMY volume in the current results should not be taken to indicate a laterality effect, and should be subjected to further research.

The following limitations of this study should also be noted. First, the current study used a cross-sectional design and examined the statistical predictions among the variables of interest. Although the mediation effects identified here suggest that AMY volumes might be more likely to contribute to changes in anxiety-related traits than the other way around, the exact causal influence between brain regions and personality traits needs to be demonstrated with a longitudinal design.

Second, the current study only investigated volumetric variations in AMY linked to the measures of interest, and further studies are needed to fully articulate the mechanisms of the mediations identified here. Future studies could focus on the role of other personality and biological factors implicating AMY in relation to anxiety vulnerabilities, such as linked to other cortical regions (Albaugh et al., 2013; Donzuso et al., 2014), or linked to intrinsic resting-state functional connectivity patterns (Adelstein et al., 2011). Such relations were not the goal of the present study, but could be targeted in future investigations.

Third, even though we did not administer additional measures of social desirability to rule out biases coming from its possible correlation with anxiety scores, we think that social desirability very likely did not influence our results. Previous research has shown that social desirability tendencies do not moderate the relation between implicit (Implicit Association Test) and explicit (STAI-Trait) measures of anxiety (Egloff and Schmukle, 2003). In addition, it has also been argued that

the correlation between STAI-Trait and social desirability measurements may be more of a problem for the traditional, interview-based surveys, rather than for assessments conducted in a confidential setting (Booth et al., 2016). In this study, subjects completed the STAI-Trait questionnaire independently in an enclosed cubicle, and were informed that their responses would not be traced back to their identities, and will remain strictly confidential. Therefore, we believe that social desirability did not play a significant role in the results reported in this study.

Fourth, although our results showed that there is overlap in the constructs of neuroticism and anxiety, and distinguished them from depression, they did not completely elucidate the common vs. unique elements of these constructs. Nevertheless, the current study is an important step toward the theoretical clarification of these constructs, and provides insights for future studies. For instance, future research could also investigate the role of other related constructs, such as negative affect (Dennison et al., 2015; Hayano et al., 2009), in the link between AMY and anxiety.

Another interesting direction is to explore possible links between the volume of specific AMY subregions and measures of anxiety and neuroticism. There has been some evidence suggesting that the volume of the basolateral AMY, as measured from ROIs generated by probability maps, was more closely related to trait anxiety levels during early childhood, compared to other AMY nuclei (Qin et al., 2014). Thus, future research using established protocols for manual tracing of AMY subregions (Entis et al., 2012) would be helpful in clarifying such relations in adult samples, both with and without clinical status.

5. Conclusion

Overall, the current study showed that variation in AMY volumes may reflect individual differences in anxiety-related traits in a sample of healthy young adults. Using mediation analysis, we found that variation of the left AMY volume predicted individual differences in neuroticism and trait anxiety, but trait-level differences did not predict volumetric variations. This suggests that AMY volume and neuroticism may be a biological risk factor contributing to vulnerabilities to anxiety in healthy individuals. Thus, these current findings can help inform the development of prevention and intervention paradigms, such as designing interventions that specifically target the preservation of AMY volume. Although there are no established procedures for this purpose, research on training-related neuroplasticity may be a promising direction. So far, treatment-related functional plasticity of AMY activity has been consistently observed (Nechvatal and Lyons, 2013), with AMY showing reduced activity in response to emotional stimulation after treatment, which can be linked to therapy-related symptom reduction and improvements on indices of emotional processing. In healthy participants, recent research on cognitive training has shown promising results of changes in AMY connectivity with prefrontal cortical regions after a short-term cognitive training (Cohen et al., 2016). Since these changes may be brought out by the elimination of the source of chronic stress or the improvement of cognitive skills in coping with stress, it seems reasonable to expect that the mechanisms that caused AMY volume reduction should also stop or even reverse this process, possibly leading to the preservation of AMY volume. More research is needed, however, to clarify the conditions for structural plasticity beyond changes in the functional patterns.

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Authors' Note

S.D. and F.D. conceived the study; S.D. contributed to data collection; Y.H., M.M., S.D., and F.D. contributed to the analytical approach, with feedback from K.L.P.; Y.H. and Z.B. performed the analyses; Y.H., S.D., and F.D. wrote the manuscript. All authors provided feedback to, and approved the content of, the manuscript.

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