Published in final edited form as:

Psychiatry Res Neuroimaging. 2020 January 30; 295: 111006. doi:10.1016/j.pscychresns.2019.111006.

Amygdala volume and social anxiety symptom severity: Does segmentation technique matter?

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Abstract

The amygdala factors prominently in neurobiological models of social anxiety (SA), yet amygdala volume findings regarding SA have been inconsistent and largely focused on case-control characterization. One source of discrepant findings could be variability in volumetric techniques. Therefore, we compared amygdala volumes derived via an automated technique (Freesurfer) against a manually corrected approach, also involving Freesurfer. Additionally, we tested whether the relationship between volume and SA symptom severity would differ across volumetric techniques. We pooled participants ($n = 76$) from archival studies. SA severity was assessed with the Liebowitz Social Anxiety Scale; scores ranged from non-clinical to clinical levels. Freesurfer produced significantly larger amygdalar volumes for participants with poor image quality. Even after excluding such participants, paired sample t -tests showed Freesurfer's boundaries produced significantly larger amygdalar volumes than manually corrected ones, bilaterally. Yet, intra-class correlation coefficients between the two methods were high, which suggests that Freesurfer's over-

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Reema Jayakar: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Visualization, Writing - original draft, Writing - review & editing. **Erin B. Tone:** Conceptualization, Data curation, Funding acquisition, Resources, Supervision, Writing - original draft, Writing - review & editing. **Bruce Crosson:** Conceptualization, Supervision, Writing - review & editing. **Jessica A. Turner:** Conceptualization, Supervision, Writing - review & editing. **Page L. Anderson:** Conceptualization, Data curation, Funding acquisition, Supervision, Writing - review & editing. **K. Luan Phan:** Funding acquisition, Resources, Writing - review & editing. **Heide Klumpp:** Funding acquisition, Data curation, Project administration, Resources, Writing - review & editing.

Informed consent

For this type of study formal consent is not required.

Ethical approval

The current study was approved for designation as Not Human Subjects Research (PI: E. Tone, IRB#: HI6586) by the Georgia State University Institutional Review board, because the data contained no identifying linkages to participants in the parent studies.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

estimation of amygdala volume was systemic. Regardless of segmentation technique, volumes were not associated with SA symptom severity. Potentially, amygdala sub-regions may yield clearer patterns regarding SA symptoms. Further, our study underscores the importance of image quality for segmentation of the amygdala, and image quality may be particularly valuable when examining anatomical data for subtle inter-individual differences.

Keywords

Structural neuroimaging; Freesurfer; Manual segmentation; Automated segmentation; Social Anxiety Disorder (SAD); Brain structure; Volumetrics

1. Introduction

There is robust evidence from functional neuroimaging research that Social Anxiety Disorder (SAD), which is characterized by the fear of negative evaluation from others and by avoidance of social situations (American Psychiatric Association, 2013; Iza et al., 2014; Miskovic and Schmidt, 2012), is associated with an amplified ‘fear circuit.’ This amplification is evidenced in case control studies that report exaggerated activation in subcortical regions underlying threat processing (e.g., amygdala, hippocampus, insula), atypical activation in cortical regions, or aberrant connectivity in pathways that modulate subcortical activation of individuals with SAD (Ayling et al., 2012; Brühl et al., 2014; Etkin and Wager, 2007; Freitas-Ferrari et al., 2010; Irle et al., 2010; Machado-de-Sousa et al., 2014; Syal et al., 2012; Talati et al., 2013; Whalen and Phelps, 2009). Among the most consistent findings, however, is exaggerated amygdala activation (for reviews, see Brühl et al., 2014; Etkin and Wager, 2007; Freitas-Ferrari et al., 2010; Whalen and Phelps, 2009). Amplified amygdala responses to social anxiety-provoking cues also occur in individuals who do not meet diagnostic criteria for SAD, but instead have subthreshold social anxiety (Beaton et al., 2008; Beaton et al., 2009) – a pattern that indicates that social anxiety may be better conceptualized as a dimensional construct. As neural function reflects brain structure to some degree (Greicius et al., 2008; Honey et al., 2010), one would expect social anxiety to be associated with aberrant amygdala volume. Yet, relative to the robust functional neuroimaging literature on SAD and amygdalar activation, previous studies on brain anatomy differences in SAD have reported inconsistent findings. The current study was designed to take an initial step toward clarifying structural findings (i.e., volume) as they related to the amygdala and social anxiety.

Studies of amygdala size, as it pertains to social anxiety, that report mixed findings are complicated by methodological differences. For example, whether they take a whole-brain approach versus an *a priori* region of interest (ROI) approach seems to be relevant. The bulk of studies that have yielded non-significant findings with regard to amygdalar volumes (e.g., Cassimjee et al., 2010; Cheng et al., 2015; Frick et al., 2014, 2013; Irle et al., 2014; Liao et al., 2011; Talati et al., 2015, 2013) have taken a whole-brain approach (typically voxel-based morphometry, VBM) to compare structural differences between socially anxious and non-anxious adults. A large voxel-based meta-analysis on SAD also failed to find differences in grey matter volume between patients and healthy participants (Wang et al., 2018), although

methodological limitations may have contributed to the lack of amygdala effects (cf. Bas-Hoogendam, 2019). To our knowledge, there is only one study that took a whole-brain approach and yielded statistically significant findings (Meng et al., 2013). In this study, SAD patients (with no comorbid diagnosis) had significantly lower grey matter density in the right amygdala, compared to healthy controls. Within the studies that have taken an ROI-based approach too, there are some that report no social anxiety related differences in amygdala volume (Bas-Hoogendam et al., 2017, 2018; Kawaguchi et al., 2016). However, further interpretation of findings from these studies is complicated by their unique samples (e.g., multi-generational families with a genetic predisposition for SAD in Bas-Hoogendam et al., 2018), composite ROI approach (e.g., amygdala-hippocampus ROI consisted of voxels bilaterally in the amygdala, hippocampus, and the anterior and posterior parahippocampal gyri, in Bas-Hoogendam et al., 2017), and the *post hoc* nature of findings (e.g., Kawaguchi et al., 2016).

There are, however, four case-control studies that took an ROI-based approach focused on the amygdala in individuals with and without SAD (see Table 1). Three of the studies reported large effect sizes when comparing amygdala volumes in individuals with SAD to healthy controls (Irle et al., 2010; Machado-de-Sousa et al., 2014; Syal et al., 2012). Of these, two reported smaller (Irle et al., 2010; Syal et al., 2012) and one reported larger (Machado-de-Sousa et al., 2014) amygdala volumes, bilaterally, in persons with SAD. In contrast, one case-control study found no differences in amygdala volumes between individuals with SAD and healthy controls (Brühl et al., 2014).

Reasons for disparate findings across the four case-control studies could include variability in factors such as participant demographic and social anxiety characteristics, as well as analytic approaches used to determine amygdala volumes. In two of the studies, researchers manually traced the amygdala (Irle et al., 2010; Machado-de-Sousa et al., 2014), whereas two others employed Freesurfer's automated protocol (Brühl et al., 2014; Syal et al., 2012). Agreement between manual tracing and Freesurfer is less than ideal according to a few studies (Grimm et al., 2015; Morey et al., 2009; Schoemaker et al., 2016), with two studies (Morey et al., 2009; Schoemaker et al., 2016) showing that automated Freesurfer produces significantly larger estimates of amygdala volume. Correlations and concordance coefficients between Freesurfer and manually derived amygdala volumes range from 0.45–0.59 in Grimm et al. (2015) and 0.46–0.62 in Morey et al. (2009). However, although many researchers regard ROI-based manual tracing as a “gold standard,” it is also prohibitively laborious and time-consuming (Freitas-Ferrari et al., 2008). One strategy to balance the labor intensiveness of manual tracing with automatic approaches is manual editing of an automated approach.

In addition to these issues, the four ROI-based case-control studies focused on individuals diagnosed with SAD, which represents an extreme manifestation of social anxiety symptoms. Although members in their comparison group did not cross the diagnostic threshold, some may have had sub-clinical social anxiety symptoms. Research shows that social fears in those with and without SAD have the same factor structure (Iza et al., 2014), and neurobiological substrates implicated in social anxiety are similar across clinical and non-clinical samples (Ayling et al., 2012; Beaton et al., 2008, 2009; Brühl et al., 2014;

Laeger et al., 2012; Miskovic and Schmidt, 2012). Studies of social anxiety related constructs among healthy women without clinical diagnoses (Günther et al., 2018) and among college students (Haas et al., 2015) have also reported higher right amygdala volume to be significantly associated with social anxiety and with trust behavior, respectively. Therefore, there may be value to measuring social anxiety on a continuous scale, rather than conceptualizing it as a categorical construct, in the context of amygdala volume research.

Given that automated and manual volumetric techniques have yielded differing amygdala size estimates (Grimm et al., 2015; Morey et al., 2009; Schoemaker et al., 2016), our overarching goal was to understand implications of these differences for studies that use a volumetric approach to predict psychosocial constructs. In order to do this, it was essential that we first try to replicate existing findings, by determining if different volumetric techniques would lead to systematic differences in estimates of amygdalar volumes for our sample. Confirming that a difference in volumetric techniques exists would then allow us to examine whether such a difference would have an impact on the association between amygdala volume and social anxiety symptoms. As such, our first aim was to compare amygdala volumes obtained using an automated approach, Freesurfer, against those obtained after manual editing of Freesurfer boundaries, in a sample of individuals with varying level of social anxiety symptoms. We hypothesized that on average, estimates of amygdala volume based on Freesurfer boundaries would be larger than volumes derived using manually corrected boundaries. Additionally, in order to evaluate how strongly measurements between techniques resembled each other, we calculated an intra-class correlation coefficient and expected consistency between approaches to be in the fair to moderate range. Our second aim was to examine associations between social anxiety symptom severity and amygdala volume in automated Freesurfer derived and manually corrected volumes, respectively. In summary, we aimed to determine whether a systematic difference between the two volumetric techniques, if detected, would have an impact on the association between amygdala volume and social anxiety symptoms.

2. Methods

2.1. Participants

We analyzed legacy data from Georgia State University (GSU), the University of Illinois Chicago (UIC), and the University of Michigan (UMich). Functional magnetic resonance imaging (fMRI) data from UIC and UMich samples have been published before (Appendix A), and their structural MRI data were part of another multi-site collaboration (see Bas-Hoogendam et al., 2017).

Participants were university students or community-dwelling adults, spoke English as their primary language, were right-handed, had no safety contraindications for MRI, were free of major medical conditions, and either were not taking psychotropic medications or had stable regimens (3 months). Given evidence of normative developmental changes in amygdala volume in youths (Giedd et al., 1996; Wierenga et al., 2014) and older adults (Brierley et al., 2002; Goodro et al., 2012), we excluded individuals younger than 18 ($n = 38$) or older than 39 ($n = 11$). We also excluded individuals with missing social anxiety (SA) data. Of the 354 participants across all legacy datasets, 297 met our inclusion criteria (see Fig. 1).

2.2. Procedure

Procedural details were largely consistent across all sites, with participants providing written informed consent and completing MRI safety screening. All participants received monetary compensation at the completion of each study visit.

2.3. Neuroimaging parameters and software

T1-weighted images were acquired from three different MRI scanners: (a) GE Signa; (b) GE MR 750 Discovery; (c) Siemens Magnetom. For acquisition parameters, see Appendix B. All image analysis steps for de-identified data were completed at the Georgia State/Georgia Tech Center for Advanced Brain Imaging (CABI). We used Freesurfer Version 5.3.0 (Fischl et al., 2002; <http://surfer.nmr.mgh.harvard.edu>) on a Linux platform (Red Hat Enterprise) in a distributed computing system.

2.4. Quality control and attrition

We visually inspected all T1 images ($n = 297$) to ensure that the final dataset of eligible participants consisted only of high-quality images. Upon inspection, 162 images were excluded from the study due to poor quality T1s ($n = 132$; e.g., low contrast, movement), artifacts obscuring anatomy ($n = 25$; e.g., top of the brain is cut off, anomaly on parts of the brain; e.g., Fig. 2), and enlarged ventricles ($n = 5$). After these exclusions, data from 135 participants remained. There was further attrition ($n = 59$) at later stages of the image analysis pipeline, due to poor visibility of subcortical anatomical landmarks, which precluded manual correction of amygdala boundaries. These steps yielded a final sample of 76 participants. Please refer to Table 2 for sample characteristics and Fig. 1 for a visual depiction of the various types of attrition.

Although we had anticipated having to disqualify many T1 images from the initial aggregated dataset, in order to ensure that the final dataset consisted only of high-quality images, the level of attrition exceeded expectations. Therefore, we conducted exploratory *post-hoc* analyses in addition to the analyses specified *a priori*.

2.5. Neuroimaging measures

2.5.1. Amygdala volumes—We used Freesurfer's *reconall* command to invoke a series of steps for brain extraction, registration, and to obtain amygdala volumes. Technical details for Freesurfer are described elsewhere (Dale et al., 1999; Dale and Sereno, 1993; Fischl and Dale, 2000; Fischl et al., 2001; Fischl et al., 2002; Fischl et al., 2004; Morey et al., 2009).

In brief, as a part of the *reconall* command, the original T1 image is affine registered (12-parameter transform) with MNI305 atlas space. During affine registration, data from different individuals' brains undergo a linear transformation to map them on to a common coordinate system; the geometry of each amygdala is maintained, whereas the shape and size are forced to conform to the MNI template. The transformation to MNI space (i.e., registration) is necessary in order for Freesurfer to employ intensity normalization and do the initial labeling of structures. Once these steps were completed, the first author visually inspected all images for tissue misclassification errors (e.g., dura erroneously considered brain), used the *tkmedit* tool to make corrections ($n = 40$), and then re-ran the processing

pipeline. These steps are followed by Freesurfer's automated subcortical segmentation stream, in which Freesurfer completes delineation of the amygdala based on a complex mathematical algorithm. Finally, the segmented image is back transformed into native space, after which estimates of volume are generated in terms of voxels. In other words, volumetric analysis of the images occurs in native space. Once these steps are completed, we used the *asegstats* tool in Freesurfer to output volumes in mm³.

After Freesurfer back transformed the segmented images, the first author visually checked all amygdalar segmentations for gross errors in placement of boundaries (i.e., where Freesurfer had clearly misclassified neighboring regions as amygdalar tissue, or vice versa). This allowed us to limit Freesurfer's error variance from an anatomical standpoint, by excluding such amygdalae (right = 6, left = 4) from further volumetric analyses.

For manually corrected volumes, we identified boundaries based on published landmarks and visualization procedures (Entis et al., 2012; Morey et al., 2009; Pruessner et al., 2000). The *FreeView* program was used to make manual corrections to Freesurfer boundaries. For an illustration of segmented amygdalae after manual editing of Freesurfer boundaries, see Fig. 3. Broadly, corrections (i.e., erasing or adding voxels) began in the coronal plane, after which, we checked smoothness of borders in the axial plane and made further corrections to exclude white matter. Next, we used the sagittal plane to define the anterior extent of the amygdala and to delineate its inferior border from the hippocampus. Starting with anterior slices in the coronal plane, the dorsal boundary was based on the appearance of the optic tract. Moving toward the posterior slices, the medial boundary was defined as the narrowest point along the isthmus of the hippocampal-amygdalar transition area; the dorsolateral boundary was identified by the presence of the putamen or a small layer of white matter; the ventral border was indicated by the alvear white matter on the superior surface of the head of the hippocampus if visible, or based on the inferior horn of the lateral ventricle. After completing corrections in the coronal plane, the amygdala was visualized from the horizontal plane (i.e., axial view). For mid-level slices (i.e., not too ventral, not too dorsal), the posterior boundary was delineated from the hippocampus by the inferior horn of the lateral ventricle; for ventral slices the entorhinal cortex blends into the amygdala medially and it was excluded if it could be identified. Then, the sagittal plane was used to identify the ventral boundary by presence of the hippocampus. After manual corrections, we re-calculated left and right volumes using the *asegstats* tool.

In the current manuscript, we identify the amygdalar volumes that are based on Freesurfer's automated boundaries as "Freesurfer volumes," and the volumes that are based on manual corrections as "manually corrected volumes." The first author, who was blind to participants' SA scores and diagnoses, completed all image processing steps.

2.5.2. Intracranial vault volumes (ICV)—ICV size (overall volume: including white matter, grey matter, cerebrospinal fluid) estimates were derived using Freesurfer (see Buckner et al., 2004, for a detailed description). The volumes of the left and right amygdala were adjusted for ICV, to correct for individual differences in head size. To do this, we divided each amygdala's volume by ICV volume, and then multiplied by 1000. As such, all volumetric data presented in the manuscript and its tables are mean relative volumes.

2.6. Social anxiety

We operationalized SA as a continuous variable, in line with current dimensional models (Black and Grant, 2014; Kraemer, 2007; Hershenberg and Goldfried, 2015; Lilienfeld, 2014; Van Orden and Areán, 2015). The 24-item Liebowitz Social Anxiety Scale (LSAS; Liebowitz, 1987), self-report version (Baker et al., 2002) or clinician-administered version, served as a measure of SA symptom severity. Higher scores reflect greater severity. The clinician-administered and self-report LSAS versions have similar psychometric properties (Fresco et al., 2001). We used the total LSAS score, which combines fear and avoidance of social and performance situations. Exploratory findings pertaining to individual subscales are presented in Appendix C.

2.7. Statistical design

All analyses were conducted in the Statistical Package for Social Sciences (SPSS) version 17 (Chicago: SPSS Inc.). To achieve the first aim of comparing automated Freesurfer volumes to manually corrected volumes, we conducted paired samples *t*-tests. In addition, we created a visual representation of the two sets of volumes with Bland-Altman plots (Bland and Altman, 1999). We plotted the mean of the two measurements on the *x*-axis and the difference on the *y*-axis. Finally, in order to evaluate how strongly measurements between techniques resemble each other, we calculated an intra-class correlation coefficient (ICC). We set *p* of <0.05 as our significance threshold. We also examined whether results for planned comparisons followed the same pattern for each MRI scanner.

For the second aim, we used hierarchical multiple regression to determine whether amygdala volume, calculated via either method, predicted SA symptom severity. As MRI scanners differed across subjects, we dummy-coded for site to account for scanner-specific differences. In addition, to increase precision of our statistical model we set three *a priori* criteria for selection of demographic variables as covariates. Criteria were based on guidelines for well-chosen covariates to reduce potential bias (see chapter 4, in Cohen et al., 2003). First, the demographic variable being considered as a covariate should not be highly correlated ($r > 0.50$) with amygdala volume or scanner type. Second, it should be highly correlated with total LSAS score. Third, if more than one demographic variable met these criteria *and* those demographic variables were significantly correlated with each other, only the variable that has the highest correlation with SA symptom severity would be included in the model.

The hierarchical regression blocks were entered as follows – Dependent variable: symptom severity; Block 1: Dummy-coded scanner type; Block 2: Demographic variables meeting inclusion criteria; Block 3: Amygdala volume. Regression analyses were conducted separately for Freesurfer volumes and manually corrected volumes. Given the possibility that the association between amygdala volume and SA symptom severity could be nonlinear, we also tested for quadratic effects. Furthermore, we examined *post-hoc* whether results followed the same pattern for each of the three scanners.

We conducted all analyses separately for the left and right amygdala, as lateralization in the context of SAD is unclear. Functional imaging studies have reported significant findings for

only the left (Klumpp et al., 2014; Stein et al., 2002), only the right (Cooney et al., 2006; Evans et al., 2008), and both (Shah et al., 2009; Yoon et al., 2007).

2.7.1. Comparison of correlations—In order to compare the correlation of Freesurfer volumes and SA symptom severity with the correlation of manually corrected volumes and SA symptom severity, we used Steiger's (1980) procedure (as cited by Howell, 1992). The Steiger equation tests the difference between two non-independent correlation coefficients. In this case, we compared the r value for Freesurfer volume and LSAS scores to the r value for manually corrected volume and LSAS scores.

3. Results

3.1. Test of data assumptions

Freesurfer and manually corrected volume measurements for two participants were identified as extreme values (> 3 standard deviations from sample mean), bilaterally. Each of these was assigned a raw score that was one unit larger than the next highest score in the distribution (see Tabachnik and Fidell, 2007). No LSAS scores were outliers. All variables, except LSAS scores, met the assumption of normality based on the Shapiro-Wilk test and visual inspection of histograms. Although not truly bimodal, the LSAS score distribution had two peaks (see Figure 4). A square root transformation made the distribution unimodal but did not fully normalize it. Analyses yielded comparable results for untransformed and transformed LSAS scores, so only results for the untransformed variable are presented. See Table 2 for demographic/psychiatric data.

3.2. Comparison of amygdala volumes as calculated by Freesurfer and manual correction

Results of paired-samples t -tests indicated that after boundaries were manually corrected, left and right amygdala volumes were significantly smaller relative to those created using Freesurfer's automated procedure; effect sizes were large (see Tables 2 and 3, for descriptive statistics and t -test results). *Post-hoc* analyses indicated similar results for each of the three scanners.

Bland-Altman plots (Fig. 5) show that the systematic difference (i.e., mean difference in measurements) stayed within narrow limits. Qualitative inspection of data distribution around the mean showed a modest spread across the range of volume measurements. *Post-hoc* inspection indicated that results followed the same pattern in Bland-Altman plots for each of the three scanners.

For left and right amygdalae, there was a high correlation between Freesurfer-derived and manually corrected volumes (left ICC: 0.97, $p < .001$; right ICC: 0.97, $p < .001$). This pattern indicates that Freesurfer's over-estimation of amygdalar volume was systematic. *Post-hoc* analyses for each scanner indicated that results were nearly identical across scanners.

3.3. Is amygdala volume associated with social anxiety symptom severity?

3.3.1. Examination of potential covariates—Pearson bivariate correlations showed that no demographic variables were highly correlated with SA (r values ranged from -0.12 to 0.08) and thus none were included in overall statistical models.

3.3.2. Amygdala volumes and social anxiety symptom severity—In both hierarchical regression models for predicting SA symptom severity, we entered dummy-coded variables for scanner type at Step 1 and amygdala volumes at Step 2. One model was based on Freesurfer volumes. The omnibus model for predicting SA was not statistically significant for the left amygdala ($F(3, 68) = 0.61, p = .61$) or for the right amygdala ($F(3, 66) = .77, p = .52$). Neither left ($B = 7.56, SE = 26.24$) nor right ($B = .50, SE = 24.12$) amygdala volume independently accounted for variance in LSAS scores, after controlling for scanner type (Left: $R^2 = .001, F(1, 68) = .08, p = .77$; Right: $R^2 < .001, F(1, 66) < .001, p = .98$). Another hierarchical regression model was based on manually corrected volumes. Again, the omnibus model was nonsignificant for the left amygdala ($F(3, 68) = .73, p = .54$) or for the right amygdala ($F(3, 66) = .77, p = .51$). Neither left ($B = 17.71, SE = 26.95$) nor right ($B = 4.40, SE = 26.52$) amygdala volume independently accounted for variance in LSAS scores, after controlling for scanner type (Left: $R^2 = .01, F(1, 68) = .43, p = .51$; Right: $R^2 < .001, F(1, 66) = .03, p = .87$). For partial regression plots of each hierarchical regression model, see Figs. 6 and 7.

Post-hoc analyses for both Freesurfer and manually corrected volumes, in which we added a quadratic term to Block 3 of the hierarchical regressions, yielded non-significant results.

Post-hoc analyses also showed that hierarchical multiple regression results were non-significant, regardless of whether data were examined collectively or separately by scanner (all p 's $> .05$). In addition, we generated partial regression plots to examine amygdala volume and SA slopes separately for individuals with low and high LSAS scores (cutoff = 30; see Rytwinski et al., 2009), and found no association between amygdala volume and SA for either sub-group (i.e., low and high LSAS scores).

3.3.3. Comparison of correlations—Steiger's procedure showed that correlations, between Freesurfer volume and LSAS scores, did not differ significantly from those between manually corrected volume and LSAS scores (Left: $z = -1.83$, Difference in Fisher's z 's = $-0.04, SE = 0.02, p = .07$; Right: $z = -0.90$, Difference in Fisher's z 's = $-0.02, SE = 0.02, p = .37$).

3.4. Attrition analyses

First, we conducted exploratory analyses to determine whether there were differences between participants who were included ($n = 76$) versus excluded from the study ($n = 221$). There were no significant differences between the two groups (Table 4) in SA symptom severity (independent samples t -test), gender distribution (Pearson χ^2 test), age (independent samples t -test), or education (independent samples t -test).

Next, we conducted exploratory analyses focused on those participants for whom Freesurfer segmented the amygdala ($n = 59$), but whose brain images were of insufficient quality to

permit manual correction of amygdalar boundaries (see Fig. 1, Box A). More precisely, although the T1 brain images of these 59 individuals had generally acceptable quality (e.g., no artifacts, minimal to no motion related distortion, etc.), the subcortical anatomical landmarks required to delineate boundaries were not readily visible, and thus they had to be excluded from our study's planned analyses. It is for these excluded participants ($n = 59$) and the those that were amenable to manual correction ($n = 76$), that we compared the Freesurfer generated amygdalar volume estimates *post-hoc*. One-way between-groups analysis of covariance (ANCOVA), with scanner type as a covariate, indicated a marginally significant difference in left amygdala volumes between excluded ($M = 1.13$, $SE = .03$) and included ($M = 1.06$, $SE = .02$) brains ($F(1, 131) = 3.90$, $p = .05$, partial $\eta^2 = .03$). Right amygdala volumes were significantly larger for excluded ($M = 1.25$, $SE = .03$) compared to included ($M = 1.10$, $SE = .02$) brains ($F(2, 131) = 15.22$, $p < .001$, partial $\eta^2 = .10$). These results suggest frank differences in Freesurfer's volume estimates for the excluded versus included participants.

4. Discussion

The purpose of the current study was to compare amygdala volumes derived with an automated method, Freesurfer, with those derived using a manually corrected approach, given evidence that different volumetric techniques yield strikingly different volume estimates (Grimm et al., 2015; Morey et al., 2009; Schoemaker et al., 2016; Wenger et al., 2014). Additionally, we examined whether estimates of associations between amygdala volume and social anxiety differed, depending on the technique used to delineate the amygdala's boundaries. As predicted, and in line with previous research, Freesurfer amygdala volumes were significantly larger than manually corrected volumes. However, inconsistent with prior studies that compared automated segmentation approaches with manual tracing (Grimm et al., 2015; Morey et al., 2009), we found high correlations between volumes derived using the two methods. Results indicate a possible systemic relationship between automated and semi-automated approaches.

Results also did not support the hypothesis that amygdala volume, estimated based on Freesurfer's boundaries or manually corrected boundaries, would predict social anxiety symptom severity. This is consistent with research by Brühl et al. (2014) and Syal et al. (2012) that also used an *a priori* ROI-based approach and found no differences in amygdalar volume between people with and without SAD. The current findings extend upon previous work, and show that the association between amygdala volume and social anxiety symptom severity is not significant, regardless of the volumetric technique used, even when social anxiety is conceptualized as a continuous variable, in a sample including individuals with and without SAD. A similar picture has emerged within the large functional imaging literature, where the associations between atypical amygdala activation and SAD in case-control studies are robust (Brühl et al., 2014; Etkin and Wager, 2007; Freitas-Ferrari et al., 2010; Whalen and Phelps, 2009), but the associations between amygdala activation and *severity* of social anxiety symptoms are unclear. Whereas some studies show a positive correlation (Ball et al., 2012; Phan et al., 2006; Shah et al., 2009), others show no correlation (Klump et al., 2010; Ziv et al., 2013), and yet others do not report on links between

amygdala response and social anxiety symptom severity (e.g., Stein et al., 2002; Yoon et al., 2007).

Although amygdala volume was uncorrelated with social anxiety symptoms in the current study, it is possible that research examining the amygdala in a more finely grained way may yield more clarity. The amygdala comprises several functionally distinct but structurally contiguous nuclei (Amunts et al., 2005; Entis et al., 2012; Fernando et al., 2013; Whalen and Phelps, 2009; Yang et al., 2016): the basolateral complex, the centromedial nucleus, the basomedial complex, and the amygdaloid cortical region. Animal models have implicated the basolateral nucleus in defeat behaviors (Clinard et al., 2015), as well as learning and inhibition of fear responses (Laurent and Westbrook, 2010). Such findings have led researchers to speculate that the basolateral complex may play a prominent role in human social anxiety (Truitt et al., 2007). Others have postulated that atypical development of the amygdala's centromedial nucleus may contribute to social anxiety (Fox and Kalin, 2014). Moreover, the extended amygdala, which may mediate endocrine, autonomic, and somatomotor aspects of emotional and motivational states may also be relevant to social anxiety (Heimer et al., 1997). Thus, manually delineating human amygdalar subregions, rather than the whole amygdala, could be a useful next step in social anxiety research. Images in the present study lacked adequate resolution to capture amygdalar subdivisions. In future research, however, ultra-high resolution images could allow researchers to readily address such questions. Such images can be acquired through magnets with higher field strength or with specially designed head coils (e.g., Entis et al., 2012).

We speculate that the discrepancy in findings between the present study's high correlations for volumes derived using the two methods, relative to low correlations reported in past studies (Grimm et al., 2015; Morey et al., 2009), could be due to various reasons. These may include subtle differences across studies in anatomical definitions for amygdalar boundaries; different versions of Freesurfer; sample characteristics (i.e., Grimm et al. and Morey et al. did not focus on individuals with social anxiety); as well as the current study's semi-manual technique (as opposed to drawing on an unsegmented image), which may have biased at least some aspect of the segmentation.

The current study contributes to a growing body of evidence on structural brain imaging research in individuals with social anxiety, especially with regard to our understanding of tools frequently used for volumetric segmentation. On the one hand, the results of paired-samples *t*-tests showed that manually corrected volumes were significantly smaller than Freesurfer's automated volumes. This indicates that if the goal of the research is to determine the structural basis of inter-individual differences (as opposed to group-level differences) in human socioemotional constructs, then a more accurate estimation of volume through manual editing of boundaries is advisable. On the other hand, the intra-class correlation coefficients between Freesurfer-derived and manually corrected volumes were high. This suggests that if the goal is to average data for groups of participants (i.e., patients vs. controls), then fully automated generation of volumes (as that offered by Freesurfer) may be acceptable, provided there is high visibility of subcortical structures in the T1 scans.

The importance of image quality for accurate segmentation of the amygdala, even when the segmentation is fully automated, is evidenced in our exploratory attrition analyses. Although we had anticipated being able to use imaging data from 135 participants, we had to exclude 59 brain images due to inadequate visibility of subcortical anatomical landmarks that are required for manual boundary correction (see Fig. 1, Box A). These analyses showed that Freesurfer produced significantly larger volumes for amygdalae in the 59 brain images that had to be excluded, relative to the brains amenable to manual editing ($n = 76$). Taken together, these findings indicate that Freesurfer is likely to position boundaries more liberally when image quality is low. Indeed, a review of volumetric MRI studies has shown that higher scores on a “study quality index” corresponded with smaller amygdala volumes (Brierley et al., 2002).

Exploratory findings suggest that implementation of rigorous, empirically-based, manual visualization and correction procedures that are specific to subcortical structures is valuable. Otherwise researchers risk erroneously including images in their studies that meet all pre-processing criteria, but are of poor quality with regard to subcortical structures, which consequently leads to inflated volume estimates when using Freesurfer. In addition, we observed during manual correction that Freesurfer made errors in all dimensions (i.e., x - y - z axis) of the 76 included images, which is consistent with findings from at least one other study (Morey et al., 2009).

The finding that Freesurfer is likely to position boundaries more liberally in images where subcortical anatomical landmarks are not readily visible to the human eye has implications. For one, in the future, researchers using Freesurfer for volumetric segmentation of the amygdala may want to exclude brains in which subcortical landmarks cannot be reliably identified visually. Also, our findings illustrate why it is advisable that brain images processed using Freesurfer's automated segmentation protocol are checked manually; in fact, this is a step that Freesurfer recommends. In addition, inclusion of detailed descriptions of image processing pipeline steps in published manuscripts could help researchers consider how quality-checking may have affected structural measurements. This has also been suggested in best practices for neuroimaging methods reporting (see Nichols et al., 2017). And finally, there may be benefit to using Freesurfer-recommended morphometry protocols (see, https://surfer.nmr.mgh.harvard.edu/fswiki/FreeSurferWiki?action=AttachFile&do=get&target=FreeSurfer_Suggested_Morphometry_Protocols.pdf) for image acquisition, as they are purported to deliver higher contrast between grey matter, white matter, and CSF, and consequently may lead to more accurate segmentations.

Our study also highlights both the utility and challenges of combining MRI data sets. Published neuroimaging findings are particularly hard to replicate in independent samples, and studies are often plagued with lack of adequate statistical power. This occurs, in part, because of small sample sizes (Wicherts et al., 2016), as acquisition of MRI images is expensive and time consuming. Small samples can also render imaging studies vulnerable to false positive results (see Button et al., 2013; Mar et al., 2013; Poldrack et al., 2017). By pooling datasets, groups of researchers can circumvent such sample size problems. Further, aggregation of structural neuroimaging data aligns with recommendations for open platforms for data exchange (see Groenewold et al., 2018; Landis et al., 2016). We were able

to amass a large sample only because we aggregated data from multiple research groups that scanned at different sites.

We faced, however, some obstacles commonly encountered when pooling datasets. For example, we had to discard much of the data from the original aggregated dataset (see Fig. 1). Although *post hoc* exploratory analyses revealed no significant differences between included and excluded participants on demographic or social anxiety measures (see Table 4), it remains possible that they may have differed on other unmeasured variables. We also had to manage variability across legacy studies, in the demographic, psychosocial, and diagnostic information that they included. As such, neuroimaging researchers may consider obtaining consent from participants to be re-contacted and retaining contact information for longer time periods. Although this step may complicate the process of establishing data sharing agreements and obtaining IRB approval (see Mar et al., 2013), it may be of value given the potential advantages that aggregated datasets offer (e.g., statistical power).

In summary, our findings suggest that social anxiety researchers should carefully consider the volumetric segmentation technique they wish to use for their study in context of their research question and study design (i.e., group comparison/case-control studies versus those examining more subtle inter-individual level differences). Our findings also underscore the importance of image quality for precise segmentation of the amygdala and show that implementation of rigorous and empirically-based quality-control procedures that are specific to subcortical structures is valuable.

Acknowledgements

This article is based, in part, on the first author's Doctoral dissertation, completed at GSU (April 2017); findings were presented to the International Neuropsychological Society (February 2018). We are grateful to the individuals who participated in this study and generously contributed their time and effort. Also, we would like to acknowledge Avery Henning for his assistance with the project, staff at the GSU/GA Tech Joint Center for Advanced Brain Imaging (CABI), and the Tone Laboratory research team. This research was supported in part by a graduate student fellowship from the Health Resources and Services Administration (R. Jayakar) and a Georgia State University (GSU) Brains & Behavior Seed Grant (E. B. Tone). It was further supported by a Senior Research Career Scientist Award from the Rehabilitation Research and Development Service of the Department of Veterans Affairs (B. Crosson, grant # B9270L) and National Institutes of Health grants (K. L. Phan, grant #R01MH101497 and #K23MH076198; H. Klumpp, grant #K23MH093679).

Funding sources

The funding sources mentioned in the acknowledgement had no role whatsoever in study design, data collection, analyses, or any other aspect of this project.

Appendix

Appendix A Published fMRI papers

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- Klumpp, H., Fitzgerald, D. A., Angstadt, M., Post, D., & Phan, K. L. (2014). Neural response during attentional control and emotion processing predicts improvement after cognitive behavioral therapy in generalized social anxiety disorder. *Psychological Medicine*, 44(14), 3109–3121. doi:[10.1017/S0033291714000567](https://doi.org/10.1017/S0033291714000567)
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- Wheaton, M. G., Fitzgerald, D. A., Phan, K. L., & Klumpp, H. (2014). Perceptual load modulates anterior cingulate cortex response to threat distractors in generalized social anxiety disorder. *Biological Psychology*, 10, 113–17. doi:10.1016/j.biopsycho.2014.06.004

Appendix B Acquisition parameters

	GE Signa	GE MR 750 Discovery	Siemens Magnetom TrioTrim
<i>n</i>	42	8	26
T1 sequence	SPGR	SPGR BRAVO	MPRAGE
Strength	3T	3T	3T
Head coil	Quad	8 channel	12 channel
Repetition time	9 ms	9.3 ms	2300 ms
Echo time	1.8 ms	3.8 ms	3.02 ms
Acquisition plane	Axial	Axial	Sagittal
Inversion time	500ms	Unknown	1100 ms
Flip angle	15°	13 °	8 °
Matrix	256 × 256	256 × 256	256 × 256
Field of view	256 mm	220 mm	256 mm
Slices	124	186	176
Slice thickness	1.2 mm	1.00 mm	1.00 mm

Appendix C Bivariate correlations among the LSAS subscales and amygdala volume (*n* = 33)

	Left Amygdala Freesurfer	Manual	Right Amygdala Freesurfer	Manual
Fear subscale	−0.16	−0.14	−0.12	−0.13
Fear of social interaction	−0.14	−0.13	−0.11	−0.12
Fear of performance	−0.17	−0.15	−0.10	−0.11
Avoidance subscale	−0.17	−0.15	−0.14	−0.15
Avoidance of social interaction	−0.17	−0.16	−0.16	−0.17
Avoidance of performance	−0.16	−0.14	−0.09	−0.10

* Note: the sample size differs from the total sample of 76 participants because we did not have access to item-level data for some participants. None of the correlations were statistically significant.

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Fig. 1.
Flow diagram for participant inclusion.

Fig. 2.
Frontal lobe anomaly.

Fig. 3.**Amygdala segmentations**

The right panel shows images prior to segmentation and the left panel shows the amygdala after manual editing of Freesurfer boundaries. For the left panel: Light blue = amygdala; Pink = putamen; Gold = hippocampus; Purple = lateral ventricle; White = left cerebral white matter; Green = Right cerebral white matter. The grey areas along the edges of the light blue amygdala (in the left panel) are voxels that were manually erased because they had been labelled incorrectly by Freesurfer as belonging to the amygdala. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article. For readers viewing the black-and-white version, the solid black arrow points to the amygdala.)

Fig. 4.
Distribution of LSAS Scores.

Fig. 5.

Bland-Altman Plots:

The top and bottom lines represent 95% limits of agreement and the middle line defines the mean difference. Minimal deviation of the middle line from zero indicates minimal systematic difference between Freesurfer and manually corrected volumes.

Fig. 6.
Partial regression plots (controlling for scanner type): Freesurfer.

Fig. 7.
Partial regression plots (controlling for scanner type): Manually corrected.

Table 1

Hypothesis-driven ROI based case-control volumetric studies in social anxiety.

Name	Subjects (n)	Age (mean $\pm$ SD)	Characterization of the sample	ROI Approach	Scanner Field Strength	Amygdala Results (Direction/Effect size)
Machado-de-Sousa et al., 2014	24 patients 14 controls	20.2 19.8 SD not given	12 participants were diagnosed with SAD; 12 participants had sub-threshold social anxiety on the SCID, 4th ed. (Brazilian version).	Manual	1.5T	$\uparrow$ L 1.85 R 1.56
Irle et al., 2010	24 patients 24 controls	32 $\pm$ 10 31 $\pm$ 9	All participants were diagnosed with generalized social phobia on the SCID, 4th ed. and ADIS-IV and had an LSAS score $>$ 30.	Manual	3T	$\downarrow$ (for men only) L -1.16 R -0.53
Brühl et al., 2014	46 patients 46 controls	33.1 $\pm$ 10.6 33.0 $\pm$ 8.9	Participants were diagnosed with SAD based on psychiatrist/psychologist interview.	Freesurfer	3T	Not significant L 0.06 R 0.01
Syal et al., 2012	13 patients 13 controls	35.3 $\pm$ 11.8 33.6 $\pm$ 11.2	Participants were diagnosed with generalized SAD on the SCID, 4th ed.	Freesurfer	3T	Not significant L -0.79 R -1.05

Note, $\uparrow$ means significantly greater amygdala volume for SAD group; $\downarrow$ means significantly smaller amygdala volume for SAD group; Values reported in the amygdala results column are Cohen's d that were computed based on the published means and standard deviations.

Table 2

Sample characteristics.

Variable	Overall sample	GE Signa (a)	GE Discovery (b)	Siemens Magnetom (c)	One-way between-groups ANOVA (a, b, c)
<i>N</i>	76	42	8	26	
Gender					
Male	41%	45%	63%	27%	
Female	59%	55%	38%	73%	
Age (Mean $\pm$ SD)	24.67 $\pm$ 5.38 (Range: 18–38)	25.81 $\pm$ 4.90	24.00 $\pm$ 4.14	23.04 $\pm$ 6.14	
Education (<i>n</i> = 56)	15.70 $\pm$ 1.80 (Range: 12–19)	-	-	-	
Ethnicity ¹					
White	63%	76%	38%	50%	
Black	17%	10%	13%	31%	
Hispanic	7%	7%	13%	4%	
Asian	8%	7%	38%	0%	
Other	5%	0%	0%	15%	
Diagnoses ¹					
Social Anxiety Disorder (SAD)	40%	55%	25%	19%	
SAD + Other ²	13%	7%	50%	12%	
None	24%	38%	25%	0%	
Unknown	24%	0%	0%	69%	
Left amygdala volume ^{3,4}					
FreeSurfer boundaries	1.088 $\pm$.1592	1.050 $\pm$.1569	1.044 $\pm$.1005	1.157 $\pm$.1560	$F(2, 69) = 4.18, p = .02, \eta^2 = .11$
Manually corrected boundaries	1.055 $\pm$.1569	1.012 $\pm$.1541	1.016 $\pm$.0809	1.131 $\pm$.1513	$F(2, 69) = 5.32, p = .01, \eta^2 = .13$
Right amygdala volume ^{3,4}					
FreeSurfer boundaries	1.094 $\pm$.1922	1.012 $\pm$.1998	1.096 $\pm$.1510	1.210 $\pm$.1232	$F(2, 67) = 10.22, p < .001, \eta^2 = .23$
Manually corrected boundaries	1.060 $\pm$.1789	.9773 $\pm$.1836	1.070 $\pm$.1302	1.174 $\pm$.1096	$F(2, 67) = 12.29, p < .001, \eta^2 = .27$
LSAS Total ⁵	54.59 $\pm$ 33.35 (Range = 0–108)	52.65 $\pm$ 34.88	73.88 $\pm$ 31.86	51.81 $\pm$ 30.41	$F(2, 73) = 1.52, p = .23$
Median	65	-	-	-	

¹ Pearson χ^2 statistically significant.

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² Other included generalized anxiety disorder, major depressive disorder, specific phobia, past major depressive episode, panic disorder without agoraphobia, impulse control disorder NOS.

³ Statistically significant group differences across the three scanners.

⁴ Total volumes of the left and right amygdalae have been adjusted for ICV, by dividing each amygdalar volumetric measure with ICV, and multiplying by 1000. As such, all volumetric data presented and used in analyses are mean relative volumes and have been corrected for differences in head size across participants.

⁵ 26 participants (c) completed the self-report version and 50 participants (a + b) completed the clinician-administered version.

Table 3

Comparison of amygdala volumes between automated and manually corrected methods.

	Mean decrease	95%CI	<i>t</i>	<i>dF</i>	<i>p</i>	η^2
Left	.0328	.0253, 0.0402	8.79	71	<0.001	.52
Right	.0343	.0268, 0.0417	9.17	69	<0.001	.55

Note. Total volumes of the left and right amygdalae had been adjusted for ICV, by dividing each amygdalar volumetric measure with ICV, and multiplying by 1000. As such, all volumetric data presented and used in analyses are mean relative volumes and have been corrected for differences in head size across participants.

Table 4

Comparison of included and excluded participants.

Variable	Included in final	Excluded from final
<i>n</i>	76	221
Gender		
Male	41%	34%
Female	59%	65%
Age	24.67 ± 5.38	23.78 ± 5.76
Education	15.70 ± 1.80	15.43 ± 2.04
Missing	20	39
Ethnicity		
White	63%	52%
Black	17%	11%
Hispanic	7%	9%
Asian	8%	19%
Other	5%	9%
Diagnoses		
Social anxiety disorder (SAD)	40%	25%
SAD + Other	13%	27%
None	24%	48%
Unknown	24%	1%
LSAS Total	54.59 ± 33.35	46.78 ± 36.07
	(Range = 0–108)	(Range = 0–121)
Median	65	55