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ASSOCIATION BETWEEN AMYGDALA VOLUME AND SOCIAL ANXIETY SYMPTOM
SEVERITY: A MULTI-METHOD STUDY

by

REEMA JAYAKAR

Under the Direction of Erin B. Tone, PhD

ABSTRACT

Neuroimaging research has strongly influenced a biologically-based conceptualization of social anxiety, which is the fear of evaluation from others. Functional neuroimaging research has shown consistently a robust association between atypical amygdala activation and social anxiety symptoms. However, there are disparities in the small structural imaging literature on the amygdala and social anxiety. The inconsistent findings may, in part, be a function of differences across studies in the methods used to obtain amygdala volumes. Freesurfer and manual tracings are two common segmentation techniques, and the use of one over the other involves different tradeoffs. The present study directly compared amygdala volumes generated based on Freesurfer's boundaries to those generated based on manually corrected boundaries, in neurotypical adults with varying levels of social anxiety. Also, it examined whether amygdala volume predicted social anxiety symptom severity. The Liebowitz Social Anxiety Scale – Self-Report version served as a measure of social anxiety. Participants ($N = 76$) were selected from three larger archival projects. They had social anxiety scores ranging from 0 - 108 ($M = 54.59 \pm$

33.34). The results suggest Freesurfer's boundaries consistently produced larger amygdala volumes than manually corrected boundaries. However, in neurotypical individuals with and without social anxiety, manual correction did not provide added benefit over the use of Freesurfer with regard to predicting social anxiety symptoms. The present findings strongly suggest that volumetric measurement of the amygdala is not helpful for understanding variability in social anxiety symptom severity and call into question numerous aspects of existing volumetric studies of the neural correlates of social anxiety.

INDEX WORDS: Amygdala, Volume, Social anxiety, Structural neuroimaging, Freesurfer, SAD

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REEMA JAYAKAR

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

in the College of Arts and Sciences

Georgia State University

2017

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Reema Jayakar
2017

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SEVERITY: A MULTI-METHOD STUDY

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August 2017

DEDICATION

The
dedication
of this project
is split
many ways:
to my parents,
to my grandparents,
to my Tardeo family,
to my Walnut Grove family,
and to my childhood friends,
Pooja & Kshitija,
for standing by
my side
unconditionally.

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1 INTRODUCTION

Researchers have conceptualized anxiety, which is a common human experience characterized by anticipatory distress, as biologically-based and associated with distinct patterns of neurological anomalies (Etkin & Wager, 2007; Perusini & Fanselow, 2015; Tovote, Fadok, & Lüthi, 2015). Neuroimaging research has strongly influenced the biologically-based conceptualization of anxiety-related conditions, including social anxiety, which encompasses the fear of evaluation from others (Iza et al., 2014; Miskovic & Schmidt, 2012). Evidence of neural differences between individuals with and without anxiety disorders has emerged from two types of imaging research: functional and structural. Functional and structural imaging yield distinct, but complementary types of information about the brain, with functional studies generating measures of brain activity and structural studies producing measures of brain morphology, or its form and structure. With regard to the neural correlates of social anxiety, functional neuroimaging studies have taught us the bulk of what we know. One of the more consistent findings in the social anxiety and functional neuroimaging literature is that atypical amygdala activation and social anxiety are associated (Brühl, Delsignore, et al., 2014; Etkin & Wager, 2007; Freitas-Ferrari et al., 2010). That said, surprisingly little research has examined whether the amygdala's structural characteristics relate to social anxiety.

Structural measurements are valuable complements to functional data for a number of reasons. They are presumably invariant across transient psychological states and unaffected by the task environment. They also have proven useful in efforts to delineate the brain circuitry associated with many emotional functions. The role that structural data have played in predicting clinical phenotypes of autism spectrum disorder (e.g., Jiao et al., 2010) and Alzheimer disease (e.g., Querbes et al., 2009) suggests that knowledge about brain morphology has potential to aid

more broadly in clinical diagnosis. Moreover, recent work that integrates structural findings with functional evidence has yielded useful insight into mechanisms of mental illness, such as depression (Nixon et al., 2014) and schizophrenia (Dauvermann et al., 2012). Extending such integrative approaches to the study of social anxiety could help us better understand how the condition develops and is maintained. However, before taking such integrative approaches, further attention needs to be paid to potential differences in brain structure that are associated with social anxiety, and inconsistencies in the small structural imaging and social anxiety literature must be resolved. These inconsistencies likely reflect a number of factors, including highly varied approaches to the analysis of structural imaging data across studies and inadequately powered study designs.

The current study was designed to take an initial step toward addressing inconsistencies in the existing literature and clarifying current knowledge about the structural neural correlates of social anxiety. I proposed to do so by examining associations between self-reported social anxiety and estimates of amygdala volume, derived using two different structural imaging methods (automated and manual), in a sample of adults obtained by pooling participants from multiple studies. These individuals endorsed varying levels of social anxiety, ranging from none to clinically significant, and participated in neuroimaging studies at one of three sites. In the following sections, to lay groundwork for the proposed study, I provide a brief overview of what social anxiety is, review current knowledge about its neural correlates, and briefly describe the amygdala. I then discuss functional neuroimaging findings in individuals with social anxiety, with a particular focus on the amygdala and evidence that this structure shows atypical patterns of activation and decreased functional coupling in the context of social anxiety. Next, I delve into the relatively small body of structural neuroimaging findings, with a focus on volumetric

findings about the amygdala and the value of structural imaging research. I also provide an overview of methodological issues in structural imaging as they relate to the social anxiety literature.

1.1 Social Anxiety and its Neural Correlates

Social anxiety, or fear and avoidance of social and performance situations, is associated with distress that ranges in severity from low or moderate uneasiness to incapacitating dread (Morrison & Heimberg, 2013; Rapee & Heimberg, 1997; Rosen & Schulkin, 1998). Whereas social interactions evoke some degree of discomfort for many individuals, for a smaller number of people, they evoke fears that are powerful enough to induce active behavioral avoidance of all such encounters. The term “shyness” captures less impairing manifestations of social anxiety that appear to affect up to 40% of the general population (Morrison & Heimberg, 2013; Rapee & Heimberg, 1997). In contrast, a smaller number of people with extreme levels of social anxiety and pervasive avoidant behavior may meet diagnostic criteria for social anxiety disorder (SAD) or avoidant personality disorder (Morrison & Heimberg, 2013). The estimated lifetime and 12-month prevalence rates for SAD are 13% and 7%, respectively (Grant et al., 2005; Magee, Eaton, Wittchen, McGonagle, & Kessler, 1996; Ruscio et al., 2008), with an annual incidence rate of roughly 11% (Beesdo et al., 2007). At clinically significant levels, social anxiety can result in marked disability – comorbid mood disorders, psychosocial impairment (e.g., substance abuse and restricted socialization), poor quality of life, and career difficulties are all common among affected individuals (Beesdo et al., 2007; Grant et al., 2005; Magee et al., 1996; Rapee & Heimberg, 1997; Weiller, Bisslerbe, Boyer, Lepine, & Lecrubier, 1996; Wittchen & Fehm, 2003).

At least three lines of evidence suggest that the construct of social anxiety is most appropriately captured as a continuous variable, consistent with recommendations of the

National Institute of Mental Health (NIMH) Research Domain Criteria (RDoC); <http://www.nimh.nih.gov/research-priorities/rdoc/index.shtml>) (Hershenberg & Goldfried, 2015; Lilienfeld, 2014; Van Orden & Areán, 2015). First, factor analytic findings indicate that the latent structure of social fears is the same for people with and without SAD (Iza et al., 2014). Specifically, feared social situations cluster into three categories – public performance, close scrutiny, and social interaction – across individuals with a broad range of sociodemographic characteristics and symptom severities. Second, findings from at least one study in 355 college students indicate that the rate of comorbidities and functional impairment is significantly greater for both people with SAD (72%) and those with sub-syndromal social anxiety signs and symptoms (50%), than for controls (29%) (Filho, 2010). Third, some neurobiological structures implicated in the experience of social anxiety at clinical levels of severity have also been implicated at non-clinical levels of severity (Ayling, Aghajani, Fouche, & van der Wee, 2012; Beaton et al., 2008, 2009; Brühl, Delsignore, Komossa, & Weidt, 2014; Laeger et al., 2012; Miskovic & Schmidt, 2012).

The brain structures that show atypical activation in socially anxious individuals form an interconnected system that is thought, broadly, to support complex socio-emotional functions relevant to social anxiety. These structures include limbic regions – amygdala, cingulate gyrus, and thalamus – as well as the insula, dorsolateral prefrontal cortex, and ventromedial prefrontal cortex (Brühl, Delsignore, et al., 2014; Freitas-Ferrari et al., 2010; LeDoux, 2000). Some structures within this system, such as the amygdala, appear to be especially important. Across studies of both humans and animals, the amygdala has been consistently identified as a critical player in experiences of fear or anticipatory anxiety (Ayling et al., 2012; Brühl, Delsignore, et

al., 2014; Etkin & Wager, 2007; Freitas Ferrari, Busatto, McGuire, & Crippa, 2008; Kilts et al., 2006; Lanteaume et al., 2007; Phan, Fitzgerald, Nathan, & Tancer, 2006; Tovote et al., 2015).

1.1.1 The Amygdala

The amygdala is a subcortical gray matter structure located deep within the temporal lobes that comprises functionally distinct, structurally contiguous nuclei (Entis, Doerga, Barrett, & Dickerson, 2012; Fernando, Murray, & Milton, 2013; Whalen & Phelps, 2009). The cytoarchitectonic and connectional organization of the human amygdala is complex. It has 13 distinct nuclei and cortical areas (Amaral, 2002; Whalen & Phelps, 2009), that are often classified as follows: (1) “Deep nuclei” or “Basolateral nuclear group”, which include the lateral nucleus, basal nucleus, accessory basal nucleus, and paralaminar nucleus. The lateral nucleus forms a larger portion of the amygdala in humans compared to other species and is further divided into lateral and medial components. (2) “Superficial nuclei” or “Corticomедial nuclear group”, which include the medial nucleus, periamygdaloid cortex, anterior amygdaloid area, and nucleus of the lateral olfactory tract. (3) “Remaining nuclei” or “Central nucleus”, which include the central nucleus and intercalated nuclei. (4) “Extended amygdala”, which forms a part of the basal forebrain region traditionally known as the substantia innominata (Heimer, Harlan, Alheid, Garcia, & De Olmos, 1997). Although endocrine, autonomic and somatomotor aspects of emotional and motivational states are attributed in part to the extended amygdala, this subdivision is often overlooked by researchers due to the difficulty in accessing it. A detailed description of amygdala cytoarchitecture, connections, and chemistry for human and nonhuman primates can be found in previously published works (see Amaral, 2002; Heimer, Harlan, Alheid, Garcia, & De Olmos, 1997; Whalen & Phelps, 2009).

Connections of the amygdala with other brain regions are not as well studied and characterized in humans as in nonhuman primates (Whalen & Phelps, 2009). However, evidence from comparative studies indicates that the nonhuman primate amygdala can provide a realistic representation of the human amygdala (Whalen & Phelps, 2009). As such, the main features of the connective organization of the human amygdala are extrapolated from studies in other primate species. The amygdala has historically been considered as having connections mainly to and from the hypothalamus and brain stem, but neuroanatomical studies over the last 30 years clearly demonstrate that the amygdala has a wide-reaching network of connections with a diverse array of brain regions (Aggleton, Burton, & Passingham, 1980; Amaral, 2002; Carmichael & Price, 1995; Freese & Amaral, 2005; Whalen & Phelps, 2009). These include projections *to and from* the basal forebrain, the hippocampal formation, the thalamus, and the neocortex, as well as *to* the striatum and claustrum. In particular, the amygdala has substantial connections with many regions of the neocortex and neocortical inputs are primarily received by the lateral nucleus of the amygdala.

It comes as no surprise then that many psychological phenomena appear to be supported, in part, by the amygdala. Not only is the amygdala involved in fear learning (Gaffan, Gaffan, & Harrison, 1989; Hooker, Germine, Knight, & D'Esposito, 2006), fear memory (Fadok, Darvas, Dickerson, & Palmiter, 2010; Packard, Cahill, & McGaugh, 1994), rapid threat appraisals (Klumpp, Angstadt, Nathan, & Phan, 2010; Robinson, Charney, Overstreet, Vytal, & Grillon, 2012), and a broad range of attentional (Pessoa, Kastner, & Ungerleider, 2002) and emotional functions (Adolphs, Tranel, Damasio, & Damasio, 1994; Barrett, Bliss-Moreau, Duncan, Rauch, & Wright, 2007), but it is also implicated in encoding appetitive stimuli (Fernando et al., 2013) and in social behavior (Amaral, 2002; Machado et al., 2008). In the context of human

neuroimaging research, technical limitations have precluded the detailed study of amygdalar subdivisions and its connections. However, it is important to keep the complexity of this structure and its connections in mind when interpreting data. As new technologies (e.g., scanners with higher signal/noise ratio, more refined acquisition protocols) become readily available to researchers a more nuanced understanding of the anatomy of the amygdala in the context of neuropsychiatric disorders could emerge.

1.1.2 The Amygdala and Social Anxiety: Functional Imaging Findings

Robust evidence, summarized in both qualitative reviews and meta-analyses, indicates that individuals who report elevated social anxiety show atypical amygdala function (Brühl, Delsignore, et al., 2014; Etkin & Wager, 2007; Freitas-Ferrari et al., 2010). This evidence comes primarily from functional imaging studies, which record brain activity using changes in blood flow (functional magnetic resonance imaging) or by measuring collection of a radioactive tracer in cells based on energy consumption (positron emission tomography). As published reviews of the social anxiety functional imaging literature indicate, several findings emerge consistently (Brühl et al., 2014; Etkin & Wager, 2007; Freitas-Ferrari et al., 2010; Miskovic & Schmidt, 2012).

1.1.2.1 Atypical amygdala activation

First, fear conditioning and exposure to social threat cues consistently elicit amygdala activation for individuals with and without social anxiety. Etkin and Wager (2007) examined activity in 117 healthy individuals across 10 studies and showed that the amygdala is hyperactive during fear conditioning (effect sizes not reported). Second, amygdala hyperactivity is more pronounced in adults with SAD, compared to matched controls, when they are processing salient environmental cues – a finding that has emerged in Etkin and Wager’s (2007) meta-analysis of

eight SAD studies, in a recent meta-analysis of 36 studies conducted by Brühl, Delsignore, et al., and in a qualitative review of 70 published studies by Miskovic and Schmidt (2012). As such, pronounced amygdala activation in adults with SAD was evident across studies that used a variety of behavioral paradigms, many with stimuli that were specifically relevant to social anxiety, such as emotional faces or social transgressions. In other words, individuals with SAD recruit their amygdalae more strongly than do controls when processing social-anxiety-relevant stimuli.

Furthermore, persons with SAD show aberrant amygdala recruitment both during and in anticipation of a task. For instance, patients with SAD show increased amygdala activation compared to controls when responding to harsh or fearful faces, relative to happy or neutral faces (Blair et al., 2008; Phan et al., 2006). Similarly, individuals with SAD also show greater amygdala activity compared to controls prior to performing a public speaking task (Lorberbaum et al., 2004). Interestingly, in Etkin and Wager's (2007) meta-analysis, not only did adults with SAD show more amygdala hyperactivation than healthy subjects, but their amygdalae were also more active in comparison to those of adults with post-traumatic stress disorder (PTSD). This finding suggests that the amygdala may play a particularly important role in feelings of social threat, above and beyond its role in triggering some other anxiety-related feeling states.

An exaggerated amygdala response to social anxiety-provoking cues occurs not only in those who meet diagnostic criteria for SAD, but also in "shy" individuals, many of whom may have clinically subthreshold social anxiety. One fMRI study has demonstrated that shy adults exhibit greater amygdala activation in response to neutral faces of both strangers and familiar persons than do controls (Beaton et al., 2008). A second study has similarly shown that shy adults exhibit significantly greater right amygdala activation than socially outgoing adults in

response to faces of strangers (Beaton et al., 2009). The fact that an exaggerated amygdala response occurs in individuals with SAD, as well as for those with sub-syndromal social anxiety signs and symptoms, raises the possibility that social anxiety is associated with amygdala activation in a dose-dependent way. In other words, the greater a person's baseline level of social anxiety, the higher that person's amygdala activation in response to social threat cues. At least two studies provide evidence for this notion. Namely, in patients with SAD, a positive correlation between the extent of amygdala activation and severity of social anxiety symptoms has been reported (Phan, Fitzgerald, Nathan, & Tancer, 2006; Shah, Klumpp, Angstadt, Nathan, & Phan, 2009).

1.1.3 Hemispheric Specialization of Amygdala Function

It remains unclear whether patterns of atypical amygdala activation in the context of SAD are lateralized. Broadly, empirical data from early lesion studies and recent functional activation studies have demonstrated that emotional perception, expression, and experience all show cortical lateralization (for a review, see Demaree, Everhart, Youngstrom, & Harrison, 2005; Freitas-Ferrari et al., 2010). Theories on the hemispheric specialization in emotional processing (i.e., Right Hemisphere, Valence, Approach-withdrawal, Behavioral inhibition-activation system) have also variously suggested that the brain's two halves play different roles (Heilman & Valenstein, 2003; Katarzyna, 2003; Kucharska-Pietura, David, Dropko, & Klimkowski, 2002; Kucharska-Pietura, Phillips, Gernand, & David, 2003; Narumoto, Okada, Sadato, Fukui, & Yonekura, 2001). For example, some have argued that the right hemisphere is specialized to process all affective information regardless of valence (right hemisphere theory), whereas others have posited that the left hemisphere is specialized for processing positive affect and the right for negative affect. Because many affective phenomena are partly supported by the amygdala, it is

possible that the hemispheric specialization for affective information, suggested by the aforementioned empirical data and theories, may also extend to the amygdala.

Functional imaging studies of atypical amygdala activation have variously reported significant findings for only the left amygdala (e.g., Klumpp, Fitzgerald, Angstadt, Post, & Phan, 2014; Stein, Goldin, Sareen, Zorrilla, & Brown, 2002), only the right amygdala (e.g., Cooney, Atlas, Joormann, Eugène, & Gotlib, 2006; Evans et al., 2008), and both amygdalae (e.g., Shah, Klumpp, Angstadt, Nathan, & Phan, 2009; Yoon, Fitzgerald, Angstadt, McCarron, & Phan, 2007). This diversity of findings cannot be explained by the type of tasks completed, because all of these experiments used visual affective processing tasks during scanning, with the majority using emotional faces as stimuli and a few using emotionally evocative images from the International Affective Picture System (IAPS). However, the mixed literature may in part be a function of small samples and low numbers of stimuli or stimulus blocks included in functional tasks. The studies listed above, for example, include samples that range in size from 10 to 21, and tasks used vary notably with regard to trial numbers and structure. Until an adequate body of well-powered research with some consistency in the number of stimuli and blocks can be established, questions regarding amygdalar laterality as it relates to elevated social anxiety will remain difficult to answer.

In the meantime, we may be able to extrapolate from preliminary evidence of hemispheric specialization of the amygdala with regard to socio-emotional functions. Indeed, in a study of patients with drug-resistant partial epilepsy, direct intracerebral stimulation of the left amygdala induced pleasant (happiness) as well as unpleasant (fear, anxiety, sadness) emotions, but stimulation of the right amygdala induced only unpleasant emotions (fear, sadness) (Lanteaume et al., 2007). In another example, functional imaging case studies of patients with

amygdalar damage indicate that affective information encoding is associated with activity in the left amygdala, whereas affective information retrieval is associated with activity in the right amygdala (Markowitsch, 1998). These studies suggest that the left and right amygdala may be specialized to play different roles in the experience of fear or anticipatory anxiety.

1.1.3.1 Functional coupling

A second noteworthy finding about the amygdala and social anxiety comes from fMRI studies of functional coupling that examine brain activity during relaxed wakefulness, or the state of being awake with eyes open, but not performing a specified task, inside the MRI scanner. These fMRI studies have found that individuals with SAD, compared to controls, show decreased functional coupling between the amygdala and various other brain regions, such as the medial prefrontal cortex, posterior cingulate cortex, inferior temporal gyrus, and visual cortex (Hahn et al., 2011; Liao et al., 2010; Liao et al., 2011). In other words, there is less inter-regional communication between the amygdala and other neural structures for individuals with SAD.

Thus, the functional imaging literature robustly indicates that the amygdala is a critical player in the pathophysiology of social anxiety. Although amygdala activation during fear conditioning and exposure to social threat cues appears to be a common, if not universal, experience, the effect is more pronounced in people with mild and clinically-significant levels of social anxiety. An important next step toward developing a comprehensive understanding of the amygdala and its role in social anxiety will involve accurately documenting any anxiety-related structural characteristics of the region. Researchers have already explored this in other mood-based diagnoses, such as pediatric bipolar disorder (Blumberg et al., 2005; Chen et al., 2004; Kalmar et al., 2009).

1.1.4 The Amygdala and Social Anxiety: Structural Findings

Relative to the sizable literature that documents distinct amygdala activation patterns in response to anxiety-inducing stimuli in socially anxious individuals, surprisingly little research has examined whether and how the amygdala's structural characteristics and its connections to other regions relate to social anxiety. Volumetric measurement of the amygdala, which is the process of quantifying the amount of grey and/or white matter within a structure's boundaries, has proven helpful for understanding many anxiety-related constructs, such as PTSD, obsessive-compulsive disorder, and trust behavior (Haas, Ishak, Anderson, & Filkowski, 2015; Karl et al., 2006; Koepp & Woermann, 2005). Further, amygdala volume has been found to relate to various aspects of social behavior. Healthy adults who foster and maintain more extensive and complex social networks, for instance, tend to have larger amygdalae than do their less socially-connected peers (Bickart et al., 2012). Furthermore, when these findings are put into context with a different line of cross-species research in non-human primates (Barton & Aggleton, 2000; Bickart, Wright, Dautoff, Dickerson, & Barrett, 2011) they suggest that the pressures of increasingly complex social life may have contributed to the evolution of the primate amygdala.

Thus, independent groups of researchers have linked variations in the amygdala's structural features to aspects of anxiety, as well as to social behavior. It therefore seems plausible that examination of amygdala structure could yield useful information about the neural underpinnings of social anxiety, too. Indeed, structural studies may provide data that complement those obtained from functional studies in at least two key ways.

1.1.4.1 Structural data are state-independent

First, functional neuroimaging studies typically capture brain activity changes that correspond temporally to performance of a task. Therefore, even though a group of studies may

focus on similar cognitive or emotional processes, they can yield varying results, depending on the task performed and on each participant's mental or emotional state at the time of scanning (Talati, Pantazatos, Hirsch, & Schneier, 2015; Talati, Pantazatos, Schneier, Weissman, & Hirsch, 2013). As such, it can be difficult to discern whether an anxious individual shows an atypical pattern of amygdala response as a function of task demands, baseline distress levels, or anxiety provoked by the scanning context, which has been shown to exacerbate performance concerns and induce anxiety (Chapman, Bernier, & Rusak, 2010; Ellerbrock & May, 2014; Grey, Price, & Mathews, 2000).

Structural data, in contrast, can be obtained without concurrent measurement of the cognitive or emotional construct under study (Kanai & Rees, 2011). Such data should be stable regardless of an individual's transient emotional state and should also yield results that can then be correlated with responses to conventional psychological tasks or questionnaire-based trait measures administered outside the MRI scanner. In other words, structural neuroimaging data are state-independent and presumably unaffected by the task environment.

1.1.4.2 Structural data are a useful tool for understanding brain circuitry

Second, structural data can be an aid to clinical diagnosis, as demonstrated by their utility in predicting clinical phenotypes of autism spectrum disorder (Jiao et al., 2010) and Alzheimer disease (Querbes et al., 2009). Furthermore, not only can anatomical MRI data show differences between groups of persons with and without neurological disease, but they can also reflect more subtle inter-individual differences within a population of healthy adults (Kanai & Rees, 2011). Indeed, there is ample evidence that data about regional brain structure can serve as a predictor of performance on measures of a wide range of human behaviors and characteristics, including the size and complexity of social networks (Bickart, Hollenbec, Barrett, & Dickerson, 2012),

interpersonal trust (Haas et al., 2015), empathy (Banissy, Kanai, Walsh, & Rees, 2012), loneliness (Kanai et al., 2012), political orientation (Kanai, Feilden, Firth, & Rees, 2011), morality (Lewis, 2012), perceptual decision-making (Forstmann et al., 2010) and social cognition (Lewis & Barton, 2006). Structural neuroimaging could similarly add distinct value to the study of social anxiety by enabling us to examine whether morphology recapitulates functional activation findings.

1.1.4.3 Methodological heterogeneity in social anxiety structural imaging studies

I was able to locate a total of 18 published structural neuroimaging studies of social anxiety, most of which focus on grey matter (Brühl, Hänggi, et al., 2014; Cassimjee et al., 2010; Frick et al., 2014; Frick et al., 2013; Eva Irle, Barke, Lange, & Ruhleder, 2014; E. Irle et al., 2010; Machado-de-Sousa et al., 2014; Meng et al., 2013; Potts, Davidson, Krishnan, & Doraiswamy, 1994; Syal et al., 2012; Talati, Pantazatos, Hirsch, & Schneier, 2015; Talati, Pantazatos, Schneier, Weissman, & Hirsch, 2013) and a few that document white matter findings (Baur et al., 2013; Baur et al., 2011; Liao et al., 2011; Phan et al., 2009; Qiu et al., 2014). Overall, these studies that examine volume or other structural indices (e.g., cortical thickness) have yielded mixed evidence with regard to areas of the brain that differ significantly between persons with SAD and healthy subjects.

One likely reason for the lack of coherent findings in this literature is methodological heterogeneity. Studies vary markedly, for example, with regard to how they search for structural differences – across the whole brain (exploratory whole-brain approach) (Cassimjee et al., 2010; Frick et al., 2014; Frick et al., 2013; Irle, Barke, Lange, & Ruhleder, 2014; Liao et al., 2011; Meng et al., 2013; Talati, Pantazatos, Hirsch, & Schneier, 2015; Talati, Pantazatos, Schneier, Weissman, & Hirsch, 2013) or with a focus on specific regions that are selected *a priori* based

on theory or empirical data (hypothesis-driven region of interest approach) (Irle et al., 2014; Irle et al., 2010; Machado-de-Sousa et al., 2014; Potts, Davidson, Krishnan, & Doraiswamy, 1994). Even among those that take a hypothesis-driven region of interest (ROI) approach, there are inconsistencies in the structures targeted for investigation as likely candidates for showing structural differences associated with social anxiety. Other sources of heterogeneity further complicate interpretation of findings; these include the numerous ways in which one can characterize the morphology of a particular neural region (e.g., volume, cortical thickness, surface area, shape, and integrity of tracts travelling to and from that region); technical decisions that vary across studies, such as electing to use automated versus manual approaches to generate measurements of brain structure, and the choice to use one or another type of software.

Despite the many methodological differences across studies, research has yielded some evidence that socially anxious and non-anxious adults show differences in the morphology of a few grey and white matter regions. Areas that have been found to differ structurally between groups of socially anxious and non-anxious persons in at least two studies include the amygdala, hippocampus, orbitofrontal cortex (OFC), insula, uncinate fasciculus (white matter fibers connecting the OFC and amygdala), and superior longitudinal fasciculus (fibers connecting the parietal, occipital, and temporal lobes with frontal cortices) (Ayling et al., 2012; Irle et al., 2010; Machado-de-Sousa et al., 2014; Syal et al., 2012; Talati et al., 2013). Amygdala-specific findings are reported in four studies (Brühl, Hänggi, et al., 2014; E. Irle et al., 2010; Machado-de-Sousa et al., 2014; Syal et al., 2012), with two of those studies noting significant volumetric differences among adults with levels of social anxiety that vary from minimal to extreme.

Given that the amygdala is a consistent point of focus in functional imaging studies of social anxiety, it seems surprising that associations between social anxiety and the grey matter

volume of this region have received relatively little attention. However, this difference in emphasis between structural and functional studies appears to stem, at least in part, from researcher decisions in the context of structural research to take a strictly bottom-up approach that extracts any significant findings from analyses of the whole brain versus a top-down, ROI approach. Studies that used the former approach have yielded limited evidence of amygdala volume differences between socially anxious and non-anxious adults. In contrast, studies that used the latter approach and closely examined the amygdala in isolation, have produced evidence of such differences. In the following sections, I briefly summarize the findings from studies that have used each type of methodology.

1.1.4.4 Exploratory approaches

Voxel based morphometry (VBM), which allows for the comparison of gray and/or white matter concentration or volume between groups of interest, is the most commonly used exploratory whole-brain technique in the social anxiety literature. Findings are disparate across the social anxiety studies that have used a VBM approach (Frick et al., 2014; Frick et al., 2013; Liao et al., 2011; Syal et al., 2012; Talati et al., 2015; Talati et al., 2013) to measure cortical thickness and volume. VBM has been unsuccessful in consistently identifying any one or more brain regions as differing between socially anxious and non-anxious groups across studies: some VBM studies have reported significant differences for a variety of brain regions (e.g., orbitofrontal cortex, insula, parietal cortex, inferior temporal cortex, parahippocampal cortex, striatum, thalamus, cerebellum) and some VBM studies have reported no differences in these same regions (Frick et al., 2014; Frick et al., 2013; Irle et al., 2014; Liao et al., 2011; Syal et al., 2012; Talati et al., 2015; Talati et al., 2013). A meta-analysis of this literature, although beyond

the scope of the current study, could help identify patterns among these disparate findings and should be considered by future researchers.

When a literature is nascent, as has been the case for social anxiety and structural neuroimaging in recent years, exploratory approaches such as VBM can be helpful. This is mainly because, in VBM, comparisons are made at the level of each voxel, which is a digitally represented cube of tissue, in the brain. As such, the researcher does not need to define regional margins, such as the boundaries of the amygdala, in advance (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM>; Ashburner & Friston, 2000; Mechelli, Price, Friston, & Ashburner, 2005). As a result, approaches such as VBM provide a way to identify targets for more focused explorations because they do not depend on *a priori* information or theoretical models to drive examination of possible group differences or symptom correlates. However, as the literature starts to develop and an empirical database evolves, hypothesis-driven ROI approaches offer a next step forward.

1.1.4.5 Hypothesis-driven ROI approaches

In contrast to exploratory whole-brain approaches such as VBM, ROI-based approaches involve focusing on one or more regions defined *a priori*, based on theory or prior empirical findings. To date, four structural imaging ROI-based studies have examined variations in amygdala size as they relate to social anxiety symptoms (Brühl, Hänggi, et al., 2014; E. Irle et al., 2010; Machado-de-Sousa et al., 2014; Syal et al., 2012). Findings are mixed (see Table 1). One study yielded evidence of smaller amygdala volumes in people with SAD relative to healthy controls (Irle et al., 2010), but the difference was significant only for men (Irle et al., 2010). In contrast, a second study found the amygdala to be larger in both men and women with SAD, as well as in those with clinically subthreshold social anxiety symptoms, compared to healthy

controls (Machado-de-Sousa et al., 2014). Findings from two other studies indicated no significant differences in amygdala volume between socially anxious and non-anxious persons (Brühl, Hänggi, et al., 2014; Syal et al., 2012).

Table 1 Amygdala results for volumetric studies in social anxiety

Name	Subjects (n)	M/F	Age (mean±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	7/5 11/3	20.2 19.8 SD not given	12 patients had a diagnosis of SAD and 12 patients had sub-threshold social anxiety on the SCID. Excluded organic brain syndromes, relevant general medical condition, epilepsy, psychiatric diagnosis, history of drug abuse, pregnancy, current/past psychotropic medication/psychotherapy	Manual	1.5T	↑ L 1.85 R 1.56
Irle et al., 2010	24 patients 24 controls	12/12 12/12	32±10 31±9	Patients had a diagnosis of generalized social phobia on the SCID and an LSAS>30, as well as a primary diagnosis of social phobia on ADIS-IV (i.e., most severe social anxiety cases). Excluded neurologic disease, severe medical condition, psychotic/affective disorders, prominent risk of self-harm, current substance use diagnosis, PTSD, traumatic exposure, personality disorder, psychotherapy or pharmacologic treatment	Manual	3T	↓(for men only) L -1.16 R -0.53
Brühl et al., 2014	46 patients 46 controls	29/17 29/17	33.1±10.6 33.0±8.9	Patients had a diagnosis of SAD based on psychiatrist/psychologist interview. Excluded cognitive impairment, current/previous psychiatric/neurological diagnosis, Axis-I comorbidities (except 1 patient), head trauma, pregnancy, >10 units OH/week, cigarettes >2 packs/day, caffeine >10 cups/day	Freesurfer	3T	Not significant L .06 R .01
Syal et al., 2012	13 patients 13 controls	8/5 8/5	35.3±11.8 33.6±11.2	Patients had a primary diagnosis of generalized SAD on the SCID. Excluded comorbid psychiatric diagnosis, psychotropic medication (except 1 patient)	Freesurfer	3T	Not significant L -.79 R -1.05

Note. M/F: Number of males and females in the sample; Amygdala results: ↑ means significantly greater amygdala volume for SAD group; ↓ means significantly smaller amygdala volume for SAD group; Values reported in the amygdala results column are effect sizes (Cohen's d) that were computed based on the means and standard deviations published in those studies.

A number of factors may have contributed to these inconsistencies across ROI-based studies of the amygdala, including variation in analytic approaches and in sampling (see Table 1 for an overview of each study's findings and sampling details). Two of the studies used Freesurfer, an automated technique, for delineating the amygdala (Brühl, Hänggi, et al., 2014; Syal et al., 2012), and two used a manual technique (Irle et al., 2010; Machado-de-Sousa et al., 2014). In the manual ROI-based approach the researcher identifies a desired cerebral region by drawing the boundary of that region on a three-dimensional digital image of the brain (Freitas Ferrari et al., 2008). In order to minimize observer biases, anatomical landmarks and rules for determining the boundaries are defined *a priori*. Also, tracers must be rigorously trained to visually inspect a variety of features in the image, such as image intensity and the global position of the desired structure in the brain (Fischl et al., 2002; Grimm et al., 2015).

Although many researchers regard the manual ROI-based approach as the “gold standard”, the procedure is laborious and time-consuming, which limits the number of brain regions as well as the number of participants that can be analyzed in a single study (Freitas Ferrari et al., 2008). The advent of imaging software packages over the past decade has begun to address these limitations by making it possible to examine ROIs in an automated manner. Broadly speaking, the software automatically assigns a neuroanatomical label to each voxel in the MRI image. Label assignment occurs through probabilistic estimates based on several features of the image, including voxel intensity (brightness) and spatial data derived from a brain atlas (Fischl et al., 2002; Morey et al., 2009). Using this kind of automated approach, researchers can work quickly and efficiently with large datasets and feasibly expand their focus to examine multiple brain regions in a single study.

However, there is evidence that results for volume estimates differ, depending on the approach with which they are obtained. Researchers have reported that Freesurfer – an automated ROI-based technique – generates estimates of amygdala volume that significantly exceed estimates generated by manual tracing, within the same sample of individuals (Morey et al., 2009; Schoemaker, Buss, Head et al., 2016). Correlations between Freesurfer and manually derived volumes of the amygdala range from .45-.59 and concordance coefficients range from .46-.62 (Grimm et al., 2015; Morey et al., 2009), indicating that there are systematic differences in amygdala volumes and the agreement between these two techniques is less than ideal. Researchers have suggested that differences in delineation of anatomic boundaries could be contributing to the variation in volumes obtained across automated and manual volumetric techniques (Grimm et al., 2015; Schoemaker, Buss, Head et al., 2016). As such, the use of varied ROI-based volumetric techniques across studies may be contributing to the mixed findings about the amygdala's structure as it relates to social anxiety.

Thus, there appears to be a clear need for research within individuals with varying levels of social anxiety that directly compares estimates of amygdala size obtained with automatically delineated boundaries versus boundaries that are delineated based on anatomical landmarks that are decided *a priori*. If structural neuroimaging is to play a role in helping us to identify biomarkers, such as amygdala volume, for diagnosis and prognosis, it is critical that we pay more attention to neuroimaging methodology when interpreting existing findings and developing new research. At this stage, however, even though some evidence suggests that Freesurfer and manual tracing are likely to yield different volumetric estimates for the amygdala, no published multi-method studies have examined whether the volumes obtained through amygdala boundaries determined by Freesurfer versus boundaries determined by manually identifying anatomical

landmarks relate differentially to social anxiety symptoms. To fill this gap, the proposed study is designed to examine associations between social anxiety symptoms and amygdala volume, estimated using automated as well as manual techniques.

A second possible reason for inconsistencies in findings across studies is variability in sample characteristics. The only study to report larger amygdala volume in socially anxious persons than controls (Machado-de-Sousa et al., 2014), had a disproportionately large number of males in their control group (11 males, 3 females) relative to the other studies and also relative to their own group of socially anxious individuals. The study also had younger participants (mean age = 21 years) than did the other three studies of social anxiety and amygdala volume (mean age range = 31-35 years).

Furthermore, although the inclusion and exclusion criteria for the four studies were broadly similar, there were slight variations (for a thorough characterization of each sample see Table 1). For example, whereas one study only included people with severe, clinically-significant symptoms (E. Irle et al., 2010), another included people with severe and clinically-significant symptoms as well as those with subthreshold social anxiety (Machado-de-Sousa et al., 2014). In addition, when conducting statistical analyses for group comparisons, studies varied according to the demographic variables (gender, age, education level, etc.) that they included as covariates, with some including several of these and others including none of them. Across studies, limited, if any, theoretical or statistical rationale was provided regarding the decision to include particular covariates, and none of the four studies provided descriptive information about how demographic variables relate to volumetric estimates in their samples. Thus, it is difficult to evaluate how each of these covariates may have affected results. This issue is especially problematic, given that none of the studies reported power profiles for their analyses and that

sample sizes were particularly small for two of the four investigations (Irle et al., 2010; Syal et al., 2012).

In addition to these issues, the four neuroimaging studies of amygdala volume and social anxiety have all relied on between-group comparisons. In other words, the researchers grouped participants based on whether or not they met diagnostic criteria for SAD (and in the case of one study, a third subthreshold group was included). As a result, their analyses provide limited information about individual differences in social anxiety symptom severity. More precisely, we do not know how differences in amygdala volume may correspond to social anxiety when it is operationalized as a continuous variable. Such knowledge is valuable, given evidence that social anxiety ranges in severity from low or moderate distress to incapacitating dread (Morrison & Heimberg, 2013; Rapee & Heimberg, 1997; Rosen & Schulkin, 1998). Thus, categorizing participants into groups may lead to inaccurate exclusion or misplacement of those who do not meet full criteria for a DSM diagnosis but experience some social anxiety symptoms. For a detailed discussion of the diagnostic threshold of social anxiety see a review paper on the etiology of social phobia (Rapee & Spence, 2004). Furthermore, measuring social anxiety on a continuous scale, rather than conceptualizing it as a categorical construct is consistent with the current DSM-5 nosological system (Black & Grant, 2014; Kraemer, 2007) and the National Institute of Mental Health's RDoC initiative (Hershenberg & Goldfried, 2015; Lilienfeld, 2014; Van Orden & Areán, 2015).

1.2 Summary and Aims

Researchers have conceptualized social anxiety, which is the fear of evaluation from others, as a biologically-based condition. Individuals with and without social anxiety show functional and possibly structural neural differences. Much of what we know about these

differences has emerged from functional neuroimaging research; an association between elevated social anxiety symptoms and enhanced amygdala activation has been a robust and consistent finding (Brühl, Delsignore, et al., 2014; Etkin & Wager, 2007; Freitas-Ferrari et al., 2010). Structural imaging research focused on the amygdala has potential to yield valuable complementary findings to those from functional studies. Indeed, research into other conditions such as autism spectrum disorder (Jiao et al., 2010) and Alzheimer disease (Querbes et al., 2009) has shown the potential of brain morphology (form and structure) studies to advance the literature. Furthermore, volumetric measurement of the amygdala has proven helpful for understanding many anxiety-related constructs, such as PTSD, obsessive-compulsive disorder, and trust behavior (Haas, Ishak, Anderson, & Filkowski, 2015; Karl et al., 2006; Koepp & Woermann, 2005).

To date, only a few structural imaging studies have examined amygdala volume as it relates to social anxiety. These vary dramatically in terms of image analysis methodology, as well as sampling. Such variability makes it difficult to synthesize study findings and to interpret disparities that have emerged in this small literature. A particularly problematic variation across studies is the decision to use automated versus manual techniques, which can yield strikingly different volumes for the same structure in an individual. Estimates of associations between social anxiety and amygdala volume may thus differ, depending on the technique used to delineate the amygdala's boundaries. No published studies of social anxiety and the brain have used a multi-method approach within the same sample. As such, it remains unclear whether volume measurements yielded by varied structural boundaries impact estimates of the association between amygdala volume and social anxiety.

The current study was designed as a first step toward addressing inconsistencies in the existing literature and clarifying current knowledge about whether and how amygdala structure relates to social anxiety in a large sample of adults, obtained by pooling participants from multiple studies. These individuals endorsed varying levels of social anxiety, ranging from low to clinically-significant and participated in neuroimaging studies at one of three sites. In the current study, I examined associations between estimates of amygdala volume – derived using two distinct methods to define the structure’s boundaries – and self-reported social anxiety. Also, as we know little about how the volume of the amygdala relates to social anxiety symptom severity in anxious and non-anxious adults, I operationalized social anxiety as a continuous variable, instead of taking a group comparison approach. To that end, the aims follow.

1.2.1 Aim 1:

To compare amygdala volumes obtained through automated Freesurfer versus manually corrected boundaries in adults who report varying levels of social anxiety.

Hypothesis 1: Volumes for amygdala boundaries that were automatically defined would, on average, be larger than those that were manually defined.

1.2.2 Aim 2:

To examine whether amygdala volumes predict social anxiety symptom severity, as indexed by a psychometric measure of social anxiety. For this aim, I analyzed data for left and right amygdala volumes separately, given mixed findings regarding lateralization in prior studies of social anxiety and the amygdala.

Hypothesis 2: Amygdala volume will significantly predict social anxiety symptom severity in adults who report varying levels of social anxiety.

1.2.3 Aim 2a:

To compare the correlation between automated Freesurfer amygdala volumes and social anxiety with that of manually corrected amygdala volumes and social anxiety.

2 METHOD

2.1 Participants

2.1.1 *Aggregating data across sites*

Participants in the current study were selected from three larger archival projects that were conducted at Georgia State University/Emory University (GSU), University of Illinois Chicago (UIC), and University of Michigan (UMich). Initially, I contacted four research laboratories to gain access to legacy datasets that appeared likely to contain both structural MRI scans and LSAS-SR (Baker, Heinrichs, Kim, & Hofmann, 2002) scores for participants. Of those contacted, one group of researchers (collaborators based at UIC and UMich) responded and provided data. Functional MRI data from the UIC and UMich samples have been presented in published studies in the past. However, the structural MRI data that are the focus of the current study have never been previously analyzed or reported. MRI data were de-identified and stored on a password-protected server at the Georgia State / Georgia Tech Center for Advanced Brain Imaging. Demographic, psychiatric, and LSAS-SR data were de-identified and stored in a separate password protected digital database. 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.

All studies from which participants were drawn gathered demographic information, including participant gender, age, level of education, and race/ethnicity. These data were used to describe the samples from the three study sites. When possible, each participant's data were coded to indicate co-morbidities, such as Axis-I diagnoses (e.g., depression, generalized anxiety). However, as the data were drawn from archival studies that varied in the degree to

which they gathered psychiatric/medical data, these data were not available for some participants. All available psychiatric data from each site are summarized in Tables 3 and 4.

Furthermore, because the current sample emerged from three different legacy datasets, the data were collected using three different MRI scanners. At least one study has shown that combining data across scanner platforms (i.e., Siemens Magnetom Trio, GE MR 750 Discovery, GE Signa) introduces a volume difference bias in MRI-derived measurements (Jovicich et al., 2009). As such, I dummy-coded participant data categorically according to scanner (GSU, UIC, UMich), so that scanner type could be used as a covariate in planned analyses. Specifically, each participant was coded as either 0 (GE Signa) or 1 (Siemens, GE MR750) for one set of dummy codes, and 0 (Siemens) or 1 (GE Signa, GE MR750) for the other set. Also, I checked for scanner-specific differences for each of the volumetric indices to be used and scanner-specific differences in social anxiety symptom severity. This was important because if participants from the different research laboratories varied with regard to their level of social anxiety, then covarying for scanner type would not be a viable solution and an alternate statistical strategy would have to be identified.

2.1.2 *Inclusion criteria*

For all of the archival studies, individuals were excluded if they had a primary language other than English or if they were left-handed. Men and women of all racial and ethnic backgrounds were eligible for participation. Other criteria varied across the studies. Inclusion criteria for the project based out of GSU were absence of vision and hearing impairments that would interfere with completion of study tasks, absence of a central nervous system disorder or a history of traumatic brain injury leading to loss of consciousness, and being free from safety contraindications for MRI scans (e.g., pregnancy, metallic implants, braces/orthodontic metal).

Also, individuals were only included in the GSU parent study if they endorsed either high or low levels of social anxiety (LSAS-SR total > 63 or < 33 ; cut-offs determined based on 75th and 25th percentiles for screened sample of participants), if they were free of major medical conditions, were un-medicated or had stable (3 months) psychotropic medication regimens, were free of co-morbid Axis-I disorders, had no history of mania, schizophrenia, and other psychoses.

Inclusion criteria for the projects based out of UIC and UMich were absence of current or past major medical or neurologic illness, absence of safety contraindications for MRI scans, and being un-medicated or having stable psychoactive medication regimens (3 months). Also, individuals were only included in the UIC and UMich parent studies if they were free of prominent suicidal ideation, history of mania, schizophrenia, and other psychoses.

In addition, for the purposes of the present study, I excluded individuals from the three parent studies if they were younger than 18 or older than 39 years of age because amygdala volume shows an inverse correlation with age (for a review, see Brierley & David, 2002). Individuals were also excluded if they had failed to complete the Liebowitz Social Anxiety Scale – Self-Report version (LSAS-SR; Baker, Heinrichs, Kim, & Hofmann, 2002), a social anxiety questionnaire. Of the 354 participants' data that were available to me, 297 met inclusion criteria for my study. However, I anticipated attrition from this sample during neuroimaging preprocessing steps, given that some participants' brain images might not be of adequate quality for inclusion (e.g., due to movement or failed brain registration).

In summary, all participants were either university students or community-dwelling adults in the metro Atlanta, Chicago, or Michigan areas, spoke English as their primary language, and were right-handed. All participants provided written informed consent at the time of enrollment in the parent studies.

2.2 Procedure

2.2.1 *GSU*

Participants who were scanned at GSU had each participated in one of two studies. The participants in the first study (recruited through the Psychology department undergraduate subject pool at GSU) completed self-report questionnaires as part of an online screening process and were selected for full study participation if they expressed interest in receiving a brain scan and endorsed either high (LSAS-SR scores >75th percentile within the screened sample) or low (LSAS-SR scores <25th percentile within the screened sample) levels of social anxiety. Those selected were evaluated for MRI safety and if deemed eligible they were invited to continue in the next part of the study. The second research appointment involved a brain scan at the Brain Imaging Technology Center at Emory University (Siemens Magnetom Trio) and lasted for 60-90 minutes.

Participants in the second study (recruited through referrals from area professionals, posted fliers, public service announcements, media ads) completed a telephone screening process and self-report questionnaires that they received via mail. After completion of these steps, they were invited to a research laboratory at GSU and administered a Structured Clinical Interview for the DSM-IV Axis I Disorders (First & Gibbon, 2004) by a licensed psychologist or a supervised doctoral clinical psychology student. Those who met diagnostic criteria for social phobia were eligible to participate further and were invited to continue in the next part of the study. The second research appointment involved a brain scan at the Brain Imaging Technology Center at Emory University (Siemens Magnetom Trio) that lasted 60 minutes. Both groups of participants received monetary compensation for their time and travel at the completion of the visit.

2.2.2 UIC and UMich

For participants that were part of the larger parent projects at UIC and UMich the procedural details were consistent across sites, as they took part in research studies that spanned the two locations. After recruitment, participants completed an initial screening assessment and were administered the Structured Clinical Interview for the DSM-IV Axis I Disorders (First & Gibbon, 2004) by a licensed psychologist or a supervised doctoral clinical psychology student. They also completed questionnaires and provided information about their medical history. If deemed eligible, they were invited to a second visit that involved a brain scan at UIC (GE MR 750 Discovery) or UMich (GE Signa). Participants received financial compensation for their time and travel.

2.3 Neuroimaging parameters

All structural images of the brain used in the current study were T1-weighted. The three-dimensional digital image of each brain consisted of contiguous slices. In other words, there were no gaps between the digital images of each plane of the brain, and the slices shared a common border. As the present study used archival neuroimaging data from different geographic locations, the MRI scanners that yielded these data differ. Scanner and acquisition parameters are provided in Table 2.

Table 2 Neuroimaging parameters

	GE Signa	GE MR 750 Discovery	Siemens Magnetom Trio
<i>n</i>	42	8	26
Site	Univ. of Michigan	Univ. of Illinois Chicago	Emory Univ.
T1 sequence	SPGR	SPGR BRAVO	MPRAGE
Strength	3T	3T	3T
Head coil	Quad	8 channel	12 channel
Repetition time	9ms	9.3ms	2300ms
Echo time	1.8ms	3.8ms	3.02ms
Acquisition plane	Axial	Axial	Sagittal
Inversion time	500ms	Unknown	1100ms
Flip angle	15°	13°	8°
Matrix	256x256	256x256	256x256
Field of view	256mm	220mm	256mm
Slices	124	186	176
Slice thickness	1.2mm	1.00mm	1.00mm

2.4 Image preprocessing, Quality Control, Attrition

Image analysis steps were conducted on Linux computers in a distributed computing system. All T1 images for the 297 participants who were eligible for the present study were visually inspected to check for image quality issues. Participants had to be excluded due to poor quality T1s (e.g., poor contrast, unusual plane of acquisition, high degree of movement, radiofrequency artifacts; $n = 132$), artifacts obscuring (for an illustrative example, see Figure 1) the brain's anatomy (e.g., top of the brain is cut off, large parts of the brain are missing, unknown aberration on parts of the brain; $n = 25$), or enlarged ventricles ($n = 5$). Although I 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 (i.e., 55%) exceeded expectations. In order to examine the effects of this considerable data loss, I conducted exploratory *post-hoc* analyses in addition to the analyses specified *a priori*.

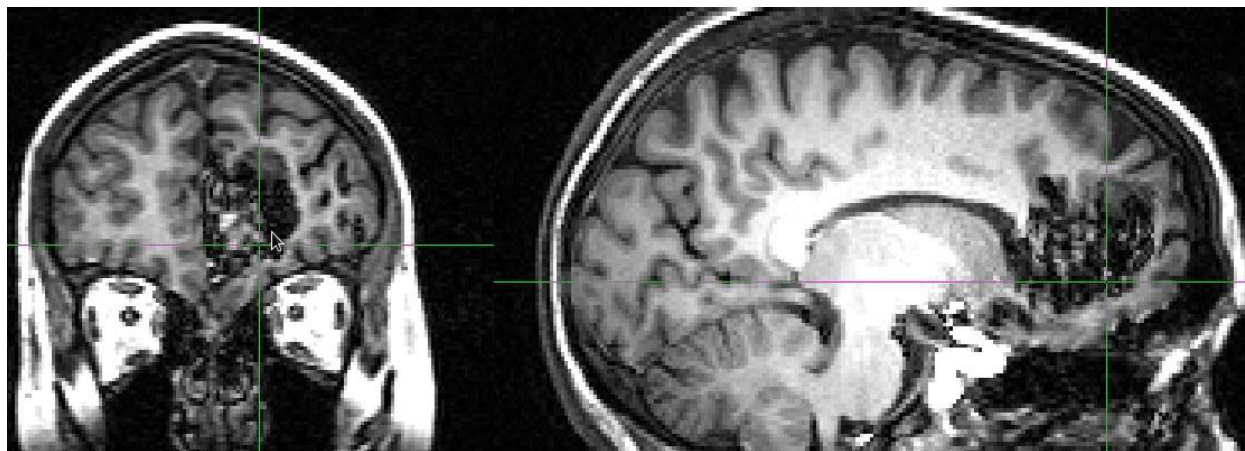


Figure 1 Frontal lobe artifact

Thus, inspection aimed at ensuring high quality brain scans that were free of defects and distortions decreased the sample to 135. There was further attrition at later stages of the image analysis pipeline due to poor anatomical landmark visibility for amygdala boundaries, leading to a final usable sample of 76 participants (refer to CONSORT diagram in Figure 2) for the purposes of Aims 1 and 2.

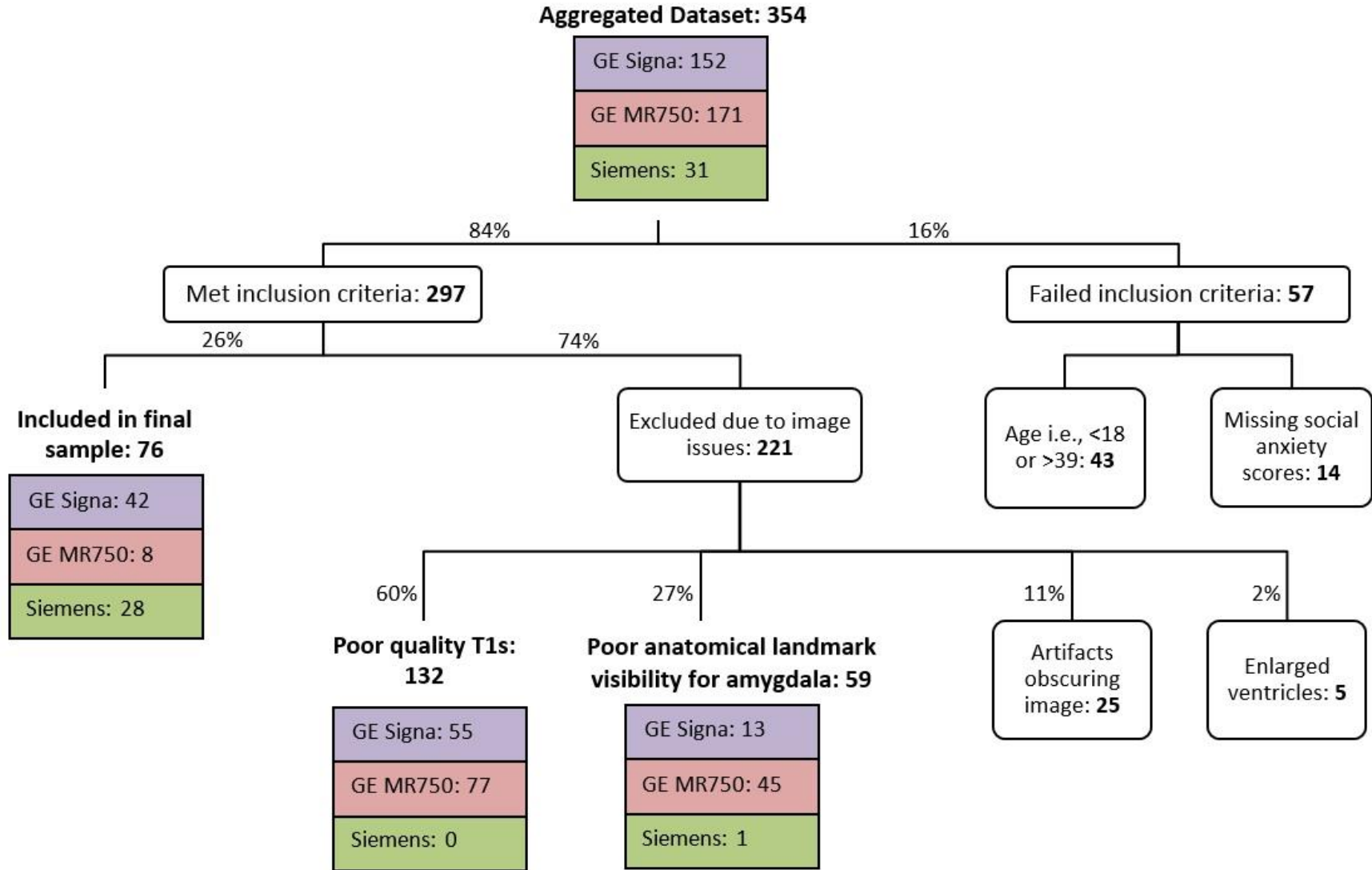


Figure 2 CONSORT flow diagram

2.5 Measures

2.5.1 Amygdala volumes

To obtain amygdala volumes I used Freesurfer's Version 5.3.0 image analysis suite (Fischl et al., 2002; <http://surfer.nmr.mgh.harvard.edu/>). Freesurfer is a set of publicly available, automated tools for segmentation (i.e., labelling units of neural matter) and visualization of three-dimensional digital images of the brain. The image analysis suite includes tools that are designed for use with subcortical brain tissue (Fischl et al., 2002). Not only does Freesurfer have an automated volume-based processing stream that generates estimates of volumes of specific structures, but it also includes tools for brain extraction and registration. I used the *reconall* tool that invokes a multi-step series of commands to do both.

First, the original image is affine registered (12 parameter transform) with MNI305 atlas space. During this step, data from different individuals' brains undergo a linear transformation so that they map onto a common coordinate system. After registration, in order to delineate biologically meaningful structures, Freesurfer assigns an initial label from its database to every voxel in the image based on voxel intensity or brightness. Then it employs a process called intensity normalization, a typical step of many image processing streams, that corrects for variation in voxel intensities by changing the range of voxel intensity values. This step helps with areas of the digital image that have poor contrast, for instance. Sometimes the brain registration or intensity normalization steps fail, leading to tissue misclassification errors (e.g., pial surface captures dura, white matter incorrectly coded as grey matter). Therefore, I visually inspected all of Freesurfer's processed images for such errors. For brains images ($n = 40$) where such errors were identified, I used the *tkmedit* tool in Freesurfer to erase offending voxels (e.g.,

dura erroneously considered as brain) or place control points to help with proper identification of missed white matter. Then I re-ran the image processing pipeline.

The aforementioned steps are followed by a final assignment of labels, which Freesurfer completes based on a complex mathematical algorithm, taking into account numerous types of subject-independent as well as subject-specific probabilities. Places where different labels get assigned to adjacent voxels demarcate the boundaries between structures. Finally, the segmented image is back-transformed to native space. Estimates of structural volume can then be generated in terms of voxels, which are the basic elements of an MRI volume (analogous to a pixel in a two-dimensional image). For further technical details of the image analysis pipeline please refer to prior publications (Fischl et al., 2002; Fischl et al., 2004).

2.5.1.1 Freesurfer volumes

Freesurfer has been used to obtain estimates of total amygdala volume in numerous published empirical studies (Brühl et al., 2014; Grimm et al., 2015; Hanson et al., 2012; Morey et al., 2009; Syal et al., 2012). Technical details of this procedure are described in prior publications (Dale, Fischl, & Sereno, 1999; Dale & Sereno, 1993; Bruce Fischl & Dale, 2000; B. Fischl, Liu, & Dale, 2001; Bruce Fischl et al., 2002; Bruce Fischl et al., 2004; Morey et al., 2009; Wenger et al., 2014). For the present study, Freesurfer volumes were those amygdalar volume estimates that were based on Freesurfer's automated and probabilistically-determined boundaries through their volume-based processing pipeline. After this pipeline had been run, I visually checked all of the amygdala boundaries for errors. In cases where $\geq 35\%$ of the coronal slices contained boundary errors, volumes were excluded (right = 6, left = 4) from analyses. This allowed me to limit Freesurfer's error variance from an anatomical standpoint by excluding the brains for which Freesurfer's drawing was deemed to be too compromised. The next stage

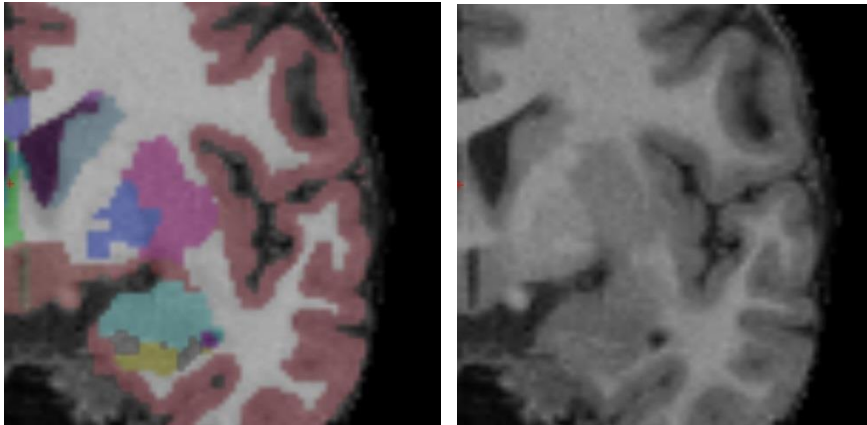
involved using the automated *asegstats* tool in Freesurfer to count the number of voxels within the amygdala's boundaries to generate its volume. The volume of each participant's left and right amygdalae were measured in mm³. Amygdala volumes generated in this manner were used in analyses as a dependent variable for Aim 1 and predictor variable for Aim 2. In the interest of clarity, I identify amygdala volume estimates that are based on Freesurfer's automated boundaries as "Freesurfer volumes."

2.5.1.2 Manually corrected volumes

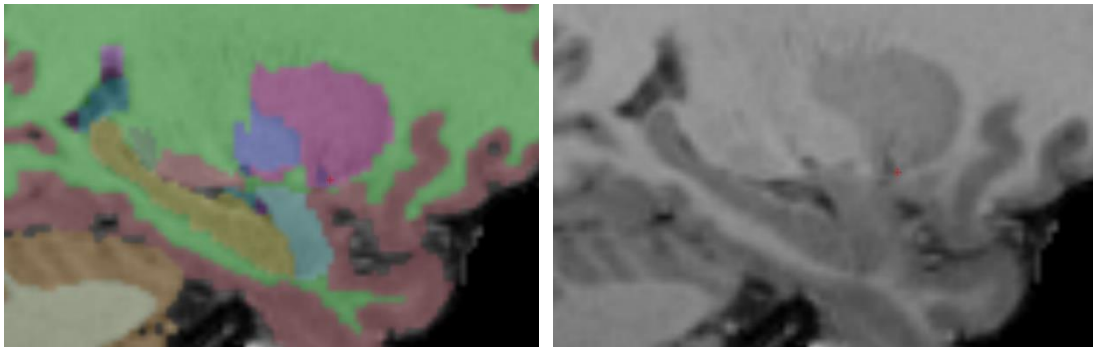
I used the Freeview tool in the Freesurfer software suite to perform manual boundary corrections of Freesurfer's automated, probabilistically-determined boundaries. Landmarks and visualization procedures described in published manual segmentation studies (Entis, Doerga, Barrett, & Dickerson, 2012; Morey, Petty, Xu et al., 2009; Pruessner, Li, Serles et al., 2000) were used to identify accurate boundaries. These publications offered good guidance for visualization as they contained numerous high quality pictures of their amygdalar segmentations. Incorrect amygdala boundaries were rectified by erasing or adding voxels. Multiple published procedures had to be combined, as not all landmarks covered within any one study were visible in the available images.

Broadly, corrections began in the coronal plane, which has been the focus of manual segmentation studies with regard to anatomical landmarks. After these were completed, smoothness of borders was checked in the axial plane and further corrections made, especially with regard to excluding white matter if it had been incorrectly captured. Next, the sagittal plane was used for corrections and checking changes made in other planes; this view was particularly useful for defining the anterior extent of the amygdala as well as delineating its inferior border from the hippocampus. For an illustrative view of segmented amygdalae see Figure 3.

Left amygdala: Coronal view



Right amygdala: Sagittal view



Left amygdala: Axial view

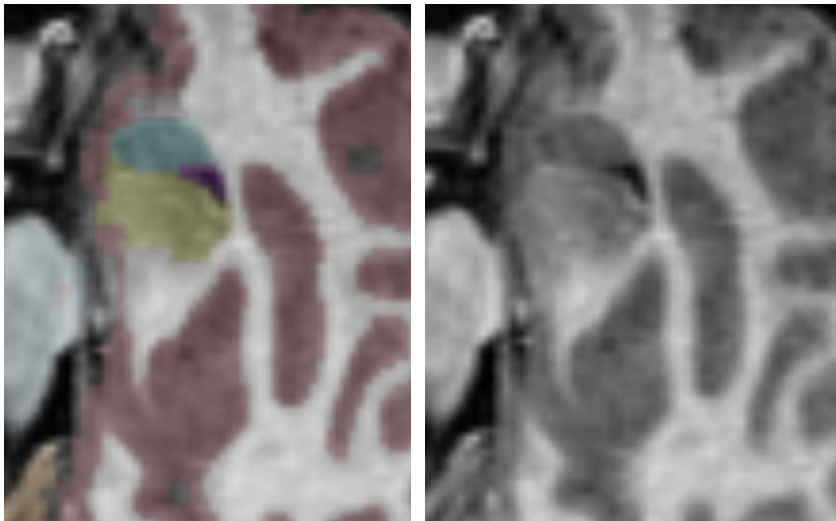


Figure 3 Amygdala segmentations

Left panel shows segmented amygdalae in light blue.

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 the mid-level slices (i.e., not too ventral and not too dorsal) in the horizontal plane 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, if it could be identified then it was excluded from the amygdala. Then slices were corrected in the sagittal plane, where the ventral boundary was identified by the presence of the hippocampus. Across all of the slices in the three planes, if white matter or blood vessels were noticed as being captured as part of the amygdala, then those borders were adjusted.

There are numerous features available in Freeview that facilitated the boundary correction process. Throughout the aforementioned steps the cursor used for corrections within the magnified view of a particular plane (e.g., coronal) was also visible in smaller side panel views of other planes (e.g., horizontal, sagittal). Also, I was able to switch back and forth between magnified views of different planes as needed to zoom in and out of the image and to toggle Freesurfer boundaries on and off.

I calculated the volume of the amygdala by using the automated *asegstats* tool in Freesurfer to count the number of voxels within the manually corrected boundaries. The volumes of each participant's left and right amygdala were each measured in mm^3 . Amygdala volumes

generated in this manner were used in analyses as a dependent variable for Aim 1 and predictor variable for Aim 2. Amygdala volume estimates that were based on manually corrected boundaries are identified as “manually corrected volumes” for clarity. Of note, from the 135 participants’ data that were available to me, only 76 were amenable to manual correction of the amygdala’s boundaries; the rest had to be excluded, as I was unable to identify many of the necessary anatomical landmarks in those images.

2.5.2 Intracranial vault volumes (ICV)

An estimate of ICV, or the overall volume in mm³ of the cranium, including white matter, grey matter, and CSF, was used to correct volumetric measurements of the amygdala for individual differences in head size. This correction was necessary, because volumes of the amygdala and other brain structures scale with overall head size (<https://surfer.nmr.mgh.harvard.edu/fswiki/BrainVolume>). In other words, people with larger heads typically also have larger amygdalae. Volumetric analysis of the intracranial vault was performed using Freesurfer (<https://surfer.nmr.mgh.harvard.edu/fswiki/BrainVolume>). Buckner et al. (2004) provides a detailed description of how Freesurfer computes an estimate of ICV.

2.5.3 Social anxiety

The Liebowitz Social Anxiety Scale – Self-Report version (LSAS-SR) (Baker et al., 2002), an individually-administered questionnaire designed to assess fear and avoidance of situations involving social interaction and performance observation by others (Heimberg et al., 1999; Safren et al., 1999), served as a measure of social anxiety. It was adapted from the LSAS (Liebowitz, 1987), a brief clinician-completed interview, that was among the first scales developed for the assessment of social anxiety, and that has since become one of the most

frequently used outcome assessment measure in research on social anxiety (Heimberg et al., 1999; Safren et al., 1999).

On the questionnaire, participants rate their level of fear/anxiety and frequency of avoidance of 24 situations in the preceding week, using a Likert-type scale. Situations involve activities in the social interaction (e.g., talking to people in authority, going to a party) and performance/observation (e.g., telephoning in public, participating in small groups) realms (Heimberg et al., 1999; Safren et al., 1999). Based on these ratings, which can range from 0 (no fear/anxiety; never avoid) to 3 (severe fear/anxiety; usually avoid), the LSAS-SR yields four subscale scores: fear of social interaction, fear of performance, avoidance of social interaction, and avoidance of performance. The subscales can be combined to provide an overall total score. The total LSAS-SR score served as the dependent variable for Aim 2 of the present study.

The LSAS produces scores that have adequate reliability, validity, and sensitivity (see Heimberg et al., 1999). Cronbach's alpha was high for all subscales, ranging from .81 to .96 for the total score and for the subscales, when data from over 300 patients enrolled in several treatment studies were pooled (Brown, Heimberg, & Juster, 1995; Heimberg et al., 1998; Juster & Heimberg, 1995; Leung & Heimberg, 1996; Schneier et al., 1998). These data suggest that the measure has excellent internal consistency. The convergent validity of the LSAS, demonstrated via significant correlations with other commonly used measures of social anxiety and avoidance (e.g., Social Avoidance and Distress Scale, Social Interaction Anxiety Scale) is also high (Beard, Rodriguez, Weisberg, Perry, & Keller, 2012; Heimberg et al., 1999; Iza et al., 2014; Safren et al., 1999). Moreover, the LSAS-SR has been shown to detect the effects of pharmacological treatments for social phobia over time (Cassimjee et al., 2010; Talati, Pantazatos, Hirsch, & Schneier, 2015), indicating that it is particularly sensitive to changes in social anxiety symptoms.

Several studies have demonstrated the utility of the self-report adaptation of the original measure (Baker, Heinrichs, Kim, & Hofmann, 2002; Cox, Ross, Swinson, & Dorenfeld, 1998; D. M et al., 2001; Oakman, Ameringen, & Mancini, 2003).

2.6 Study Design

The study design consisted of two overarching processes: (1) comparing Freesurfer volumes to manually corrected volumes, and (2) examining whether amygdala volume calculated via either method predicted social anxiety symptom severity. All analyses were conducted separately for the left and right amygdala because of mixed evidence regarding the hemispheric specialization of the amygdala, particularly in the context of social anxiety (Demaree et al., 2005; Freitas-Ferrari et al., 2010; Heilman & Valenstein, 2003; Kucharska-Pietura et al., 2003; Lanteaume et al., 2007; Markowitsch, 1999; Narumoto et al., 2001). Data analysis steps are detailed in the following sections.

2.6.1 Data entry and preparation

First, I exported volumetric data from Freesurfer to Microsoft Excel and then imported those data from Excel into an SPSS database containing demographic, social anxiety, and other variables. All statistical analyses were conducted using SPSS version 17 (Chicago: SPSS Inc.). I presented descriptive statistics for all demographic, independent, and dependent variables relevant to the study in a tabular format. Careful attention was paid to descriptive statistics and the distribution of scores on each measure before conducting statistical analyses.

2.6.2 Test of data assumptions

With regard to assumptions of parametric statistical tests, scatter plots were reviewed to visualize extreme values (>3 standard deviations from the final sample's mean) and to visualize non-linear associations between variables of interest. As the initial step for all analyses, I

inspected variable scores for normality of distribution and transformations were conducted as necessary. If violations such as skewed distributions or outliers were identified I used the appropriate data transformations to correct for them. Also, in such cases the untransformed as well as the transformed scores for the variable in question were analyzed. Other statistical assumptions (e.g., homogeneity of variance, heteroskedasticity, non-independence of residuals) were also tested as the initial step for analyses where they were needed; consistency with those assumptions and violations thereof are described in the results.

2.6.3 Aim 1

The goal of this aim was to examine whether previously published findings about differences in automated and manually traced volumes in neurotypical samples could be replicated in a sample with varying levels of social anxiety. I compared Freesurfer volumes and manually corrected volumes in three complementary ways. For all analyses, values of $p < .05$ were considered statistically significant. Although multiple statistical analyses were planned to test this aim, I had specified *a priori* directional hypotheses.

First, I conducted two separate (left, right) paired samples *t*-tests to compare Freesurfer volume and manually corrected volume. Second, I created a visual representation of the two volumetric measurement methods with Bland-Altman plots (Bland & Altman, 1999). The Bland-Altman plot is a graphical depiction of the agreement between two measures. As the present study involved amygdala segmentation by two different techniques, each participant's amygdala measurement yielded two data points for volume, ultimately yielding $2n$ data points for the entire sample. In a Bland-Altman plot, each of the n samples is shown on a graph by assigning the mean of the two measurements $(S1+S2/2)$ as the *x*-axis value and the difference between the two values $(S1-S2)$ as the *y*-axis (Grimm et al., 2015). In other words, for each pair-wise comparison

of volumes (agreement between methods), the volume differences (y-axis) are plotted against the volume means (x-axis) for each subject (Jovicich et al., 2009). As such, Bland-Altman plots show the spread of data, the mean difference, and the 95% limits of agreement (based on standard deviation of the difference). For excellent agreement between methods, the mean difference should be zero with a narrow distribution of data around zero, across the range of volume measurements.

Finally, I calculated an intra-class correlation coefficient (ICC) for data obtained using the two methods. The ICC is a descriptive statistic of how strongly measurements in the same group resemble each other (Wenger et al., 2014). A high ICC (approaching the value of 1) indicates excellent agreement between methods (Howell, 1992). Furthermore, as the MRI scanners differed across subjects who participated at different sites, I examined whether results for the aforementioned analyses followed the same pattern for each of the three scanners.

2.6.4 Aim 2

The goal of this aim was to examine whether amygdala volume calculated via either method predicted social anxiety symptom severity. To this end, first, I generated a power profile; second, I examined demographic variables as potential covariates; and finally, I statistically tested whether amygdala volume predicts social anxiety symptom severity using planned hierarchical regression.

Existing research studies (see Table 1) comparing amygdala volumes between groups of individuals with and without social anxiety report effect sizes that range from medium to large (Cohen's $d = .53$ to 1.85). These effect sizes provided a basis for the *a priori* power profile generated for the current study using *G*-power (Faul, Erdfelder, Lang, & Buchner, 2007). The profile indicated that, when employing a .05 criterion for statistical significance, I would need a

minimum sample size of 68 to detect a medium effect in a planned hierarchical regression with two predictors. The anticipated sample size ($N > 200$) of the aggregated legacy data available for the current study was much larger than the minimum required. As such, there was also room for substantial attrition, which was expected because the data emerged from multiple sources and applying stringent quality control standards to the structural imaging data could be necessary. Although the level of attrition ended up exceeding expectations, the final usable sample was 76 subjects. A sensitivity analysis showed that with this sample size the current study would have 80% power to detect a medium effect size ($f^2 = .13$) or higher. In other words, the power profile was deemed adequate to detect effects that were evidenced in past research.

Next, I evaluated whether any demographic variables (gender, age, education, and race/ethnicity) should be included as covariates in the hierarchical regression model. On the one hand, inclusion of covariates in a model reduces degrees of freedom, and thus may decrease power. On the other hand, inclusion of well-chosen covariates also increases precision of the model by reducing potential bias in the statistical estimates of population parameters (Cohen, Cohen, West, & Aiken, 2003). I set the following *a priori* criteria for inclusion of a demographic variable as a covariate: (1) it should not be highly correlated (large effect size, i.e., $r > .50$) with amygdala volume or scanner type; (2) it should be highly correlated with total LSAS-SR score; and (3) if more than one demographic variable meets criteria 1 and 2, *and* those demographic variables are significantly correlated with each other, only the variable that has the highest correlation with the LSAS-SR would be included in the model.

Finally, I statistically tested whether amygdala volume predicted social anxiety symptom severity using planned hierarchical regression. Overall a value of $p < .05$ was considered statistically significant. However, as multiple statistical analyses (left, right; Freesurfer volumes,

manually corrected volumes) were involved within the framework of this aim, with no directional hypotheses, I planned to counteract the problem of multiple comparisons. In case of significant findings, I intended to adjust for FWE rate (overall probability of committing one or more Type I errors) using the Holm-Bonferonni method (Aickin & Gensler, 1996).

Left and right amygdala volume (two separate regressions) served as the predictor variable and total LSAS-SR score served as the dependent variable. Specifically, the hierarchical regression blocks entered were as follows: Block 1 – Dummy-coded scanner type; Block 2 – Demographic variables that met inclusion criteria for covariates; Block 3 – Amygdala volume. This planned regression analysis was run separately for Freesurfer volume and manually corrected volume. Given the possibility that the association between amygdala volume and total LSAS-SR score may be nonlinear (e.g., volume could relate strongly to LSAS-SR scores when symptom severity is high, but not when the symptom severity is low), I tested for quadratic effects by adding a quadratic term to each model. Also given that the MRI scanner used differed across subjects in the sample, I examined whether results for the aforementioned analyses followed the same pattern for each of the three scanners.

2.6.5 Aim 2a

The goal of this aim was to compare the correlation between Freesurfer volumes and social anxiety with that of manually corrected volume and social anxiety. Steiger's (1980) procedure (as cited by Howell, 1992) was used. The Steiger equation tests the difference between two non-independent correlation coefficients – r value for Freesurfer volume and LSAS-SR; r value for manually corrected volume and LSAS-SR – and assumes that the correlations for both pairs of variables have been computed on the same set of subjects. The procedure takes into

account that the two correlations are not independent and incorporates a term representing the degree to which the two techniques are themselves correlated.

3 RESULTS

3.1 Sample characteristics

Descriptive statistics for participants' ($N = 76$) demographic, psychiatric (i.e., current and past co-morbidities), independent, and dependent variables of interest (i.e., left and right amygdala volumes, self-reported social anxiety) are presented in Table 3. Because this final sample represents only a subset of the original aggregated data, information regarding attrition is presented in a consort diagram (see Figure 2). Given that 74% of data from the original aggregated set had to be excluded, I conducted *post-hoc* exploratory analyses to better understand the generalizability of my findings.

Differences in imaging parameters across the three scanners, as well as similarities and differences in demographic, volumetric, and social anxiety variables of interests, across the three scanners are documented in Table 4. The total volumes of the left and right amygdalae have been adjusted for ICV, by dividing each amygdalar volumetric measure with ICV obtained from Freesurfer's automated subcortical segmentation processing stream, and multiplying by 1000. As such, all volumetric data presented and used in analyses have been corrected for differences in head size across participants. For group averages of left and right amygdala volumes with and without manual correction, see Table 3.

The LSAS-SR average total score for the final sample was 54.59 ± 33.35 (Range = 0 – 108). Sixty-seven percent of the sample had scores that were above 30 on the LSAS-SR, which is recommended as the cutoff that provides the best balance between sensitivity and specificity when using the LSAS-SR for identifying patients with social anxiety disorder (see Rytwinski, Fresco, Heimberg et al., 2009).

Table 3 Sample characteristics

Variable	Mean \pm SD
<i>N</i>	76
Gender	
Male	41%
Female	59%
Age	24.67 \pm 5.38 (Range: 18-38)
Education (n = 56)	15.70 \pm 1.80 (Range: 12-19)
Ethnicity	
White	63%
Black	17%
Hispanic	7%
Asian	8%
Other	5%
Diagnoses	
Social anxiety disorder (SAD)	40%
SAD + Other*	13%
None	24%
Unknown	24%
Left amygdala volume [^] (n = 72)	
Freesurfer boundaries	1.088 \pm .1592
Manually corrected boundaries	1.055 \pm .1569
Right amygdala volume [^] (n = 70)	
Freesurfer boundaries	1.094 \pm .1922
Manually corrected boundaries	1.060 \pm .1789
LSAS-SR Total	54.59 \pm 33.35 (Range = 0-108)
Median	65

Note. *Other included generalized anxiety disorder, major depressive disorder, specific phobia, past major depressive episode, panic disorder without agoraphobia, impulse control disorder NOS. [^]All data are mean relative volumes. Relative volumes were calculated by dividing each amygdalar volumetric measure by total ICV and multiplying by 1000.

3.2 Differences across scanners

Differences and similarities in demographic, volumetric, and social anxiety variables of interests, across the three scanners are documented in Table 4.

Table 4 Sample characteristics across scanners

Variable	GE Signa	GE Discovery	Siemens Magnetom
<i>n</i>	42	8	26
Gender			
Male	45%	63%	27%
Female	55%	38%	73%
Age	25.81 ± 4.90	24.00 ± 4.14	23.04 ± 6.14
Ethnicity [†]			
White	76%	38%	50%
Black	10%	13%	31%
Hispanic	7%	13%	4%
Asian	7%	38%	0%
Other	0%	0%	15%
Diagnoses [~]			
SAD	55%	25%	19%
SAD + Other*	7%	50%	12%
None	38%	25%	0%
Unknown	0%	0%	69%
Left amygdala volume [^]			
FreeSurfer boundaries	1.050±.1569	1.044±.1005	1.157±.1560
Manually corrected	1.012±.1541	1.016±.0809	1.131±.1513
Right amygdala volume [^]			
FreeSurfer boundaries	1.012±.1998	1.096±.1510	1.210±.1232
Manually corrected	.9773±.1836	1.070±.1302	1.174±.1096
LSAS Total	52.65±34.88	73.88±31.86	51.81±30.41

Note. [^]All data are mean relative volumes. Relative volumes were calculated by dividing each amygdalar volumetric measure by total ICV and multiplying by 1000. [†]Pearson chi-square statistically significant. *Other included generalized anxiety disorder, major depressive disorder, specific phobia, past major depressive episode, panic disorder without agoraphobia, impulse control disorder NOS.

3.2.1 Left amygdala

One-way between-groups analyses of variance (ANOVA) (two separate models), determined *a priori*, showed there were medium to large and statistically significant group

differences in Freesurfer volumes ($F(2, 69) = 4.18, p = .02, \eta^2 = .11$) and in manually corrected volumes ($F(2, 69) = 5.32, p = .01, \eta^2 = .13$) across the three scanners. *Post-hoc* comparisons using the Tukey HSD test indicated that the mean Freesurfer and manually corrected left amygdala volumes from the GE Signa were significantly smaller than those from the Siemens Magnetom. However, left amygdala volumes obtained using the GE Discovery did not differ significantly from those obtained with either the GE Signa or the Siemens Magnetom.

3.2.2 Right amygdala

Likewise, ANOVAs (two separate models), determined *a priori*, showed that there were large and statistically significant differences in Freesurfer volumes ($F(2, 67) = 10.22, p < .001, \eta^2 = .23$) and in manually corrected volumes ($F(2, 67) = 12.29, p < .001, \eta^2 = .27$) across the three scanners. *Post-hoc* comparisons using the Tukey HSD test indicated that the mean Freesurfer and manually corrected right amygdala volumes from the GE Signa were significantly smaller than those from the Siemens Magnetom. However, right amygdala volumes obtained using the GE Discovery did not differ significantly from those obtained with either the GE Signa or the Siemens Magnetom.

3.2.3 LSAS-SR

An ANOVA, determined *a priori*, showed that there were no statistically significant differences in LSAS-SR scores across participants from the three scanners, $F(2, 73) = 1.52, p = .23$.

3.3 Test of data assumptions

Volume measurements for two participants were identified as extreme values (> 3 standard deviations from the final sample mean). These values were assigned a raw score that

was one unit larger than the next highest score in the distribution (see Tabachnik & Fidell, 2001). No outliers with regard to LSAS-SR scores were identified.

The assumption of normality was met for all variables except for the distribution of LSAS-SR scores. Although not truly bimodal, the distribution had two peaks (see Figure 4). I transformed the variable using a square root transformation to attempt to correct for this violation. However, although the transformation made the distribution distinctly unimodal, it did not fully normalize it. Further, analyses yielded comparable results regardless of whether the untransformed or the transformed variable was used. Therefore, only results for the untransformed variable are presented.

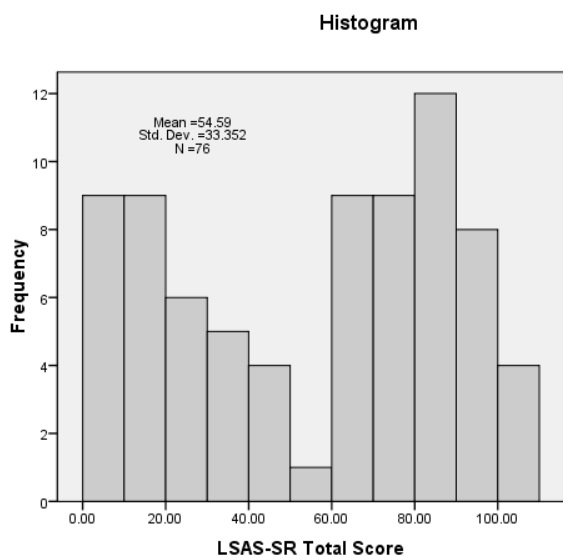


Figure 4 Distribution of LSAS-SR Scores

3.4 Aim 1: Comparing Freesurfer amygdala volumes to manually corrected amygdala volumes

First, two separate paired-samples *t*-tests (left, right), determined *a priori*, were conducted to evaluate the impact of manual boundary correction on amygdala volume

measurements. All assumptions were satisfied. There was a large and statistically significant decrease in left amygdala volume after the amygdala boundaries were manually corrected, $t(71) = 8.79, p < .001, \eta^2 = 0.52$ (see Table 3). The mean decrease in volume was .0328 (95% CI [.0253, .0402]). There was also a large and statistically significant decrease in right amygdala volume after the amygdala boundaries were manually corrected, $t(69) = 9.17, p < .001, \eta^2 = 0.55$ (see Table 3). The mean decrease in volume was .0343 (95% CI [.0268, .0417]). Statistical *post-hoc* analyses for each scanner indicated that the results for the paired-samples t-tests (left, right) followed the same pattern for each of the three scanners (Left amygdala: GE Signa $t(38) = 6.80, p < .001$; GE MR750 $t(6) = 2.66, p = .04$, Siemens $t(25) = 5.02, p < .001$; Right amygdala: GE Signa $t(36) = 6.48, p < .001$; GE MR750 $t(6) = 2.64, p = .04$, Siemens $t(25) = 5.82, p < .001$).

Next, I created visual representations of the volumetric measurements obtained with each method using Bland-Altman plots (See Figures 5 and 6). The differential line (i.e., the line that indicates the mean difference in measurements plotted on the y-axis) is near zero (mean difference between the methods was .0328 for the left and .0343 for the right), which indicates that the systematic difference stays within narrow limits. Qualitative inspection of the distribution of the data around the mean for the left as well as the right shows a modest spread across the range of volume measurements. However, an increase in variance with increasing volume of the amygdala was evident for the right hemisphere. Statistical *post-hoc* analyses for each scanner indicated that the results for the Bland-Altman plots (left, right) followed the same pattern for each of the three scanners.

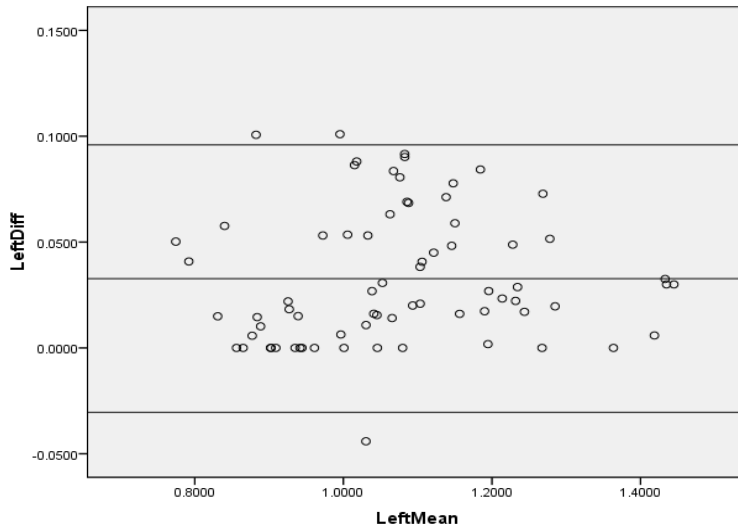


Figure 5 Bland-Altman Plot: Left Amygdala

The top and bottom lines define \pm standard deviation (i.e., upper and lower limit) and the middle line defines the mean difference. The middle line would deviate strongly from zero if there were a systematic difference between Freesurfer and manually corrected volumes.

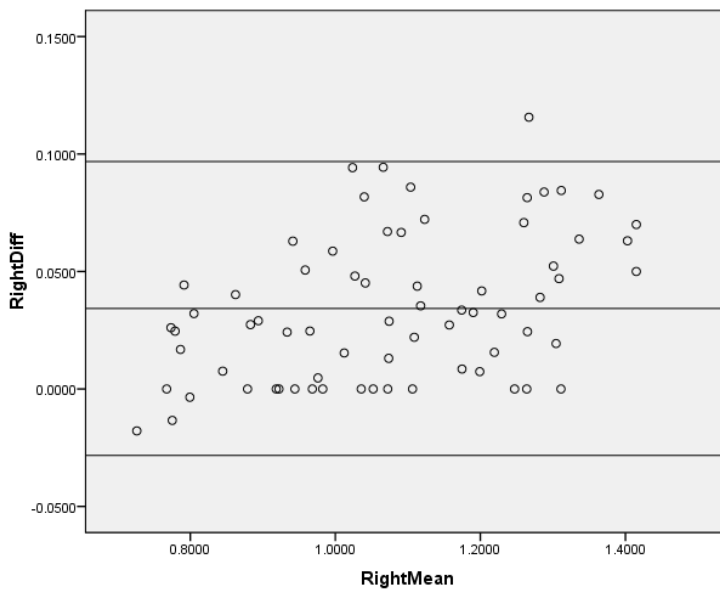


Figure 6 Bland-Altman Plot: Right Amygdala

The top and bottom lines define \pm standard deviation (i.e., upper and lower limit) and the middle line defines the mean difference. The middle line would deviate strongly from zero if there were a systematic difference between Freesurfer volumes and manually corrected volumes.

Further, I calculated ICCs for data obtained using the two methods to see how closely measurements resembled each other. For both left and right, ICCs for Freesurfer and manually corrected left amygdala volumes were high and statistically significant (left ICC: .97, $p < .001$; right ICC: .97, $p < .001$). This suggests excellent agreement between Freesurfer and manually corrected volumes. Statistical *post-hoc* analyses for each scanner indicated that the results for ICC (left, right) were comparable for each of the three scanners (Left: GE Signa ICC = .95, $p < .001$; GE MR750 ICC = .92, $p < .001$; Siemens ICC = .97, $p < .001$; Right: GE Signa ICC = .97, $p < .001$; GE MR750 ICC = .97, $p < .001$; Siemens ICC = .92, $p < .001$).

3.5 Aim 2: Examining whether amygdala volume predicts social anxiety symptoms

3.5.1 Examination of potential covariates

Pearson bivariate correlations showed that none of the demographic variables were highly correlated with social anxiety scores (r values ranging from -.12 to .08) and thus according to criteria set *a priori* for inclusion of covariates, they were not included in overall statistical models. Scanner type was dummy coded and used as a covariate at step 1 of the hierarchical regression models.

3.5.2 Amygdala Freesurfer volume and social anxiety

I used planned hierarchical multiple regressions (separate models for left and right amygdalae) to assess associations between amygdala volumes, derived based on Freesurfer boundaries, and LSAS-SR scores (see Figures 7 and 8). Preliminary checks indicated that there was no violation of the assumptions of linearity, multicollinearity, and homoscedasticity. Although LSAS-SR raw scores were not normally distributed, the residuals of the regression model showed only subtle departures from normality. Further, transformation of LSAS-SR scores with a square root function helped normalize the distribution, but all analyses yielded

comparable results regardless of whether the untransformed or the transformed variable was used. Therefore, only results for the untransformed variable are presented. Please refer to Appendix A, where Aim 2 results for the two LSAS-SR score clusters (i.e., low and high) are presented.

Dummy coded variables for scanner type were entered as covariates at Step 1. Amygdala volume was entered at Step 2. The entire model for the left amygdala was not statistically significant ($F(3, 68) = .61, p = .61$), and neither was the one for the right amygdala ($F(3, 66) = .77, p = .52$). Neither the left ($B = 7.56, SE = 26.24$) nor the right ($B = .50, SE = 24.12$) amygdala volume accounted for any additional variance in LSAS-SR scores, after controlling for scanner type (Left: $\Delta R^2 = .001, \Delta F(1, 68) = .08, p = .77$; Right: $\Delta R^2 < .001, \Delta F(1, 66) < .001, p = .98$).

Post-hoc analyses to test for quadratic effects (i.e., adding a quadratic term to Block 3) were also non-significant. Statistical *post-hoc* analyses for each scanner indicated that the results for the hierarchical multiple regressions (left, right) were non-significant for each of the three scanners. However, for the left amygdala, in contrast to the very small correlation coefficients of the GE Signa ($r = .13$) and Siemens ($r = -.08$), the correlation coefficient for the GE MR750 was negative and moderate in size ($r = -.25$).

Partial Regression Plot (Controlling for Scanner Type)

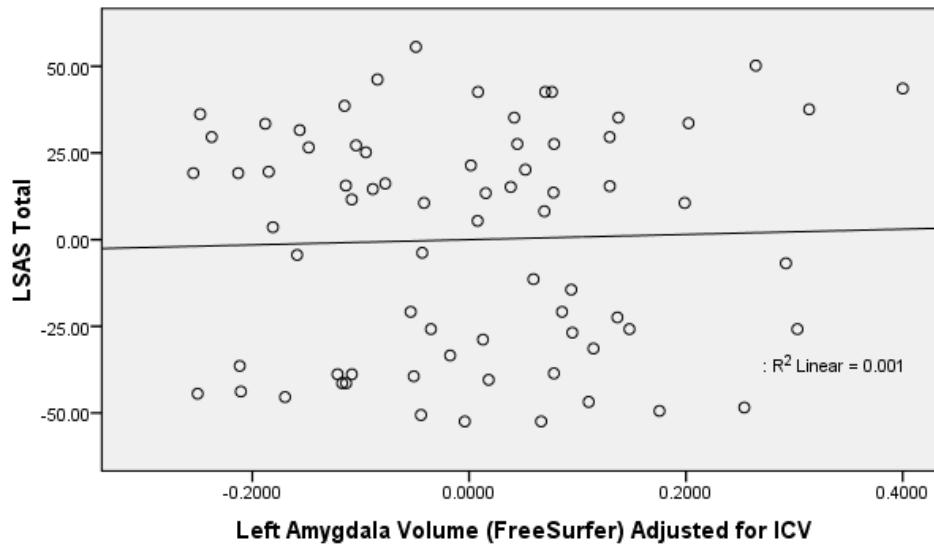


Figure 7 Partial regression plot: Left Amygdala (Freesurfer)

Partial Regression Plot (Controlling for Scanner Type)

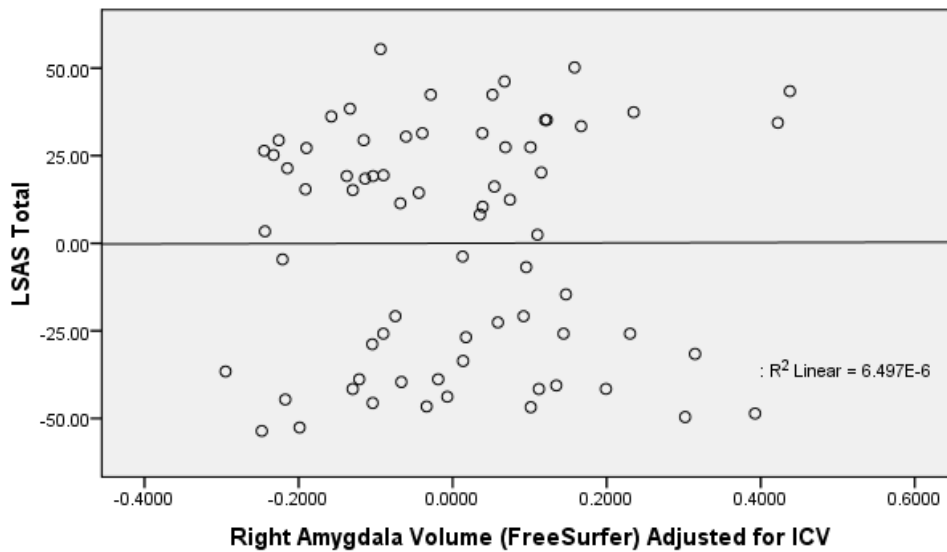


Figure 8 Partial regression plot: Right Amygdala (Freesurfer)

3.5.3 *Amygdala manually corrected volume and social anxiety*

I used planned hierarchical multiple regression (separate models for left and right amygdalae) to evaluate associations between amygdala volume, derived based on manually corrected boundaries, and LSAS-SR scores (see Figures 9 and 10). Preliminary checks indicated that there was no violation of the assumptions of linearity, multicollinearity, and homoscedasticity. With regard to the non-normal distribution of LSAS-SR raw scores the same findings and solutions reported in the aforementioned section (3.5.2) apply.

Dummy coded variables for scanner type were entered as covariates at Step 1. Amygdala volume was entered at Step 2. The entire model for the left amygdala was not statistically significant ($F(3, 68) = .73, p = .54$), and neither was the one for the right amygdala ($F(3, 66) = .77, p = .51$). Neither the left ($B = 17.71, SE = 26.95$) nor the right amygdala ($B = 4.40, SE = 26.52$) accounted for any additional variance in LSAS scores, after controlling for scanner type (Left: $\Delta R^2 = .01, \Delta F(1, 68) = .43, p = .51$; Right: $\Delta R^2 < .001, \Delta F(1, 66) = .03, p = .87$).

Post-hoc analyses to test for quadratic effects (i.e., adding a quadratic term to Block 3) were also non-significant. Statistical *post-hoc* analyses for each scanner indicated that the results for the hierarchical multiple regressions (left, right) were non-significant for each of the three scanners. However, for the left amygdala, in contrast to the small correlation coefficients obtained for the GE Signa ($r = .19$) and Siemens ($r = -.05$), the correlation coefficient for the GE MR750 was negative and moderate in size ($r = -.32$).

3.5.4 *Comparison of correlations*

William's procedure, which tests the difference between two non-independent correlation coefficients showed that the correlation between Freesurfer volume and LSAS-SR scores did not differ significantly from the correlation between manually corrected volume and social LSAS-

SR scores (Left: $z = -1.83$, Difference in Fisher's z 's = -0.04 , $SE = .02$, $p = .07$, Right: $z = -0.90$, Difference in Fisher's z 's = -0.02 , $SE = .02$, $p = .37$).

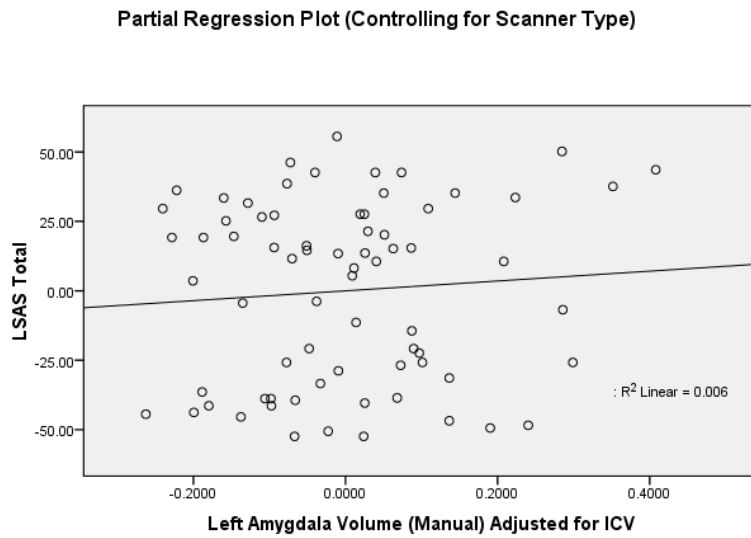


Figure 9 Partial regression plot: Left Amygdala (Manual)

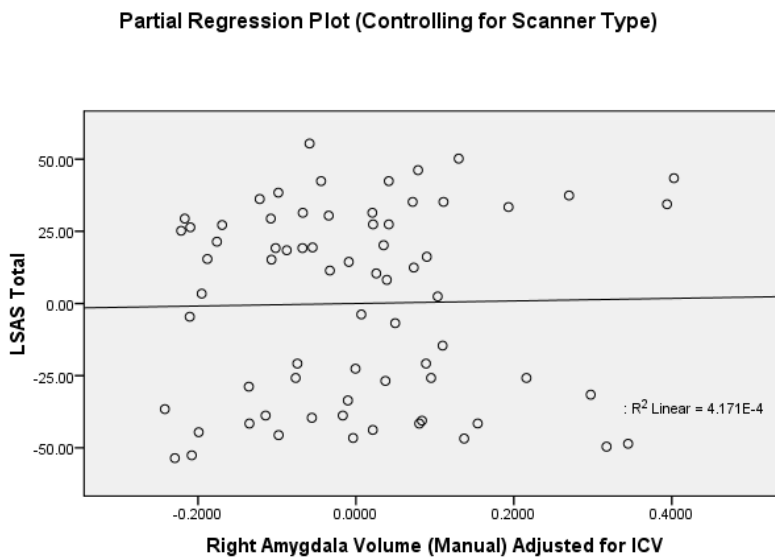


Figure 10 Partial regression plot: Right Amygdala (Manual)

3.6 Effects of attrition: Exploratory analyses

Exploratory *post-hoc* analyses focused on those participants ($n = 59$) for whom Freesurfer generated amygdala volume estimates, but whose brain images were of insufficient quality to permit manual correction of amygdala boundaries. More precisely, although most had acceptable-quality T1 images in general, the subcortical anatomical landmarks required to delineate boundaries were not readily visible.

To evaluate whether amygdala volumes for this group differed from those participants whose amygdala boundaries were amenable to manual editing ($n = 76$), I conducted a one-way between-groups analysis of covariance (ANCOVA) with subgroup (included versus excluded from study) as the independent variable and Freesurfer volumes as the dependent variable. Type of scanner served as the covariate.

Results indicated a marginally significant difference in left amygdala volumes ($F(1, 131) = 3.90, p = .05, \text{partial } \eta^2 = .03$) between the brains that were excluded ($M = 1.13, SE = .03$) and those that were included ($M = 1.06, SE = .02$). Right amygdala volumes were significantly larger ($F(2, 131) = 15.22, p < .001, \text{partial } \eta^2 = .10$) for brains that were excluded ($M = 1.25, SE = .03$) than for those that were included ($M = 1.10, SE = .02$).

Notably, when I combined Freesurfer volume data from the 76 included and 59 excluded participants to examine associations between amygdala volume and LSAS-SR scores, findings about the association between amygdala volume and social anxiety were unchanged from the aforementioned results (see section 3.5.2) obtained originally. Specifically, hierarchical multiple regressions (separate models for left and right) indicated that neither left nor right amygdala volume accounted independently for a significant amount of variance in LSAS-SR scores, after

controlling for scanner type, Left: $\Delta R^2 = .001$, $\Delta F(1, 131) = .15$, $p = .70$; Right: $\Delta R^2 < .001$, $\Delta F(1, 131) = .01$, $p = .91$.

4 DISCUSSION

The overarching purpose of the present study was to take a step toward addressing some of the disparities in the small structural imaging literature on amygdala volume and social anxiety. There are multiple factors that could have led to mixed findings, but one key difference is that previous studies have varied in their use of automated versus manual techniques.

Although researchers have shown that the different techniques used to delineate the amygdala's boundaries can yield strikingly different estimates of volume (Grimm et al., 2015; Morey et al., 2009; Schoemaker, Buss, Head et al., 2016), no published studies have examined whether differences in volume measurements impact estimates of the association between amygdala volume and social anxiety. Consequently, my first aim was to compare amygdala volumes based on fully automated Freesurfer boundaries to those that were based on manually corrected boundaries within the same sample of subjects. My second aim was to examine associations between amygdala volume – derived using these two techniques – and self-reported social anxiety.

The study yielded mixed support for my hypotheses. For Aim 1, as predicted, and in line with previous research, Freesurfer volumes were significantly larger than manually corrected volumes with regard to absolute difference in the size of the amygdala. However, the correspondence between Freesurfer volumes and manually corrected volumes was high. For Aim 2, the results did not support the hypothesis that amygdala volume would predict social anxiety symptom severity, regardless of the volumetric technique used. In the sections below, I discuss these findings, situating them in the literature and identifying future directions.

4.1 Aim 1

As hypothesized, average volume estimates based on Freesurfer's boundaries were significantly larger than those based on manually corrected boundaries and the effect size for this difference was large. Thus, the current findings replicate those of others (Morey et al., 2009; Schoemaker, Buss, Head et al., 2016), indicating that automated approaches overestimate amygdala volume. However, the concordance coefficient between volumes yielded by the two techniques was high, suggesting that despite large differences in absolute volume there was consistency between the two techniques.

The high correspondence may be explained by the qualitative observation that during manual boundary correction errors needing to be fixed occurred in all dimensions (i.e., x - y - z axis) and not just along one specific boundary. This implies that Freesurfer is susceptible to liberal criteria for the inclusion of voxels around all borders of the amygdala. Furthermore, although Hasan and Pedraza (2009) have conjectured that discrepancies in boundaries between various volumetric techniques may be due to their differing sensitivities to varied acquisition parameters, *post-hoc* analyses for each scanner in the current study indicated that the results for the paired-samples t-tests (left, right), Bland-Altman plots, and ICCs followed the same pattern for each of the three scanners.

Another finding that bears mention, although it is exploratory, has to do with the brains that had to be excluded ($n = 59$) from manual boundary determination because the images were of insufficient quality to permit identification of landmarks. The Freesurfer volumes of these amygdalae were larger than those that were included ($n = 76$) in the study. It seems likely that Freesurfer may be even more liberal in the positioning of boundaries when the image quality is lower. This also means that simply checking for anatomical landmark visibility at the subcortical

level, and excluding brains where those landmarks are not visible, may lead Freesurfer to generate more conservative volume estimates. Of note, these finer aberrations at the subcortical level can be missed when one does quick quality inspections of the overall integrity of the image, and consequently such brains may be erroneously included as part of a study's final sample. In line with this notion, one review of volumetric MRI studies of the human amygdala attempted to quantify study quality by developing an index to rate various study parameters (Brierly & David, 2002). They found that higher scores on the index corresponded with smaller amygdala volumes. To the best of my knowledge, studies rarely report the aforementioned level of methodological detail about their image preprocessing pipeline. Consequently, this precludes us from understanding whether and to what extent quality checking was applied and how it may be affecting findings. Therefore, it behooves neuroimaging researchers to be more rigorous in their reporting of methodological details.

4.2 Aim 2

The study failed to yield support for the hypothesis that amygdala volume would predict social anxiety. The present findings were consistent with results of two prior studies (Brühl et al., 2014; Syal et al., 2012), but diverged from those of two other small studies that found either larger (Machado-de-Sousa et al., 2014) or smaller (Irle et al., 2010) amygdalae in individuals with social anxiety. Notably, whether I used an automated technique to determine amygdala volume or I manually delineated amygdala boundaries based on published landmarks—a recommendation that emerged from Brühl et al. (2014)—amygdala size and social anxiety were not associated significantly. Further, the very high concordance coefficient between volumes yielded by the two techniques indicate that manual correction does not provide added benefit over the use of Freesurfer in neurotypical individuals, especially in the context of social anxiety

research. Therefore, it is likely acceptable for researchers to use Freesurfer volumes when they are conducting case control studies. However, if researchers wish to examine anatomical MRI data for more subtle inter-individual level differences, then manual correction of Freesurfer boundaries is recommended.

Overall, the outcomes of the current study suggest that inconsistencies in the existing social anxiety literature likely reflect factors other than variations in the approach used to determine amygdala volume. First, there is the notion that variability across samples in existing research studies, with regard to social anxiety symptom severity, could be influencing findings. Also worth considering are limitations of small sample sizes in past studies. Yet another problem is that researchers often make arbitrary choices about study design and other methodological variables. Second, there is the issue that the amygdala comprises functionally distinct but structurally contiguous nuclei. And finally, there is the broader question of whether and how morphological features of the brain, such as volume, map onto brain function. The merits and implications of these factors are discussed in the sections that follow.

One group of researchers (Machado-de-Sousa et al., 2014) suggested that discrepant findings in the extant literature may have stemmed, in part, from variability across study samples with regard to the severity of social anxiety symptoms that are represented. Whereas some studies included only those individuals who met criteria for clinical disorders in their socially anxious groups, others also included individuals who had clinically subthreshold social anxiety symptoms. However, all of the amygdala volume and social anxiety studies took a group-comparison approach, and none of them included measures of symptom severity for their control groups. These decisions precluded them from examining whether amygdala volumes are associated with social anxiety scores in a dose-dependent way.

Nevertheless, there are several reasons why it seems unlikely that variations in the level of social anxiety across samples have contributed to discrepant findings about amygdala volume. First, the factor structure of social fears in those with SAD and those without has been shown to be the same (Iza, Wall, Heimberg et al., 2014), which indicates that regardless of symptom severity, fears manifest in similar ways. Second, at least one group of researchers has demonstrated that individuals with subthreshold SAD display prominent psychiatric comorbidities and psychosocial impairment, despite the lesser severity of their social anxiety symptoms (Filho, Hetem, Ferrari et al., 2010). This suggests that the underlying etiology may be similar. Third, a review of the neuroscientific literature on social fearfulness implicates similar neurobiological substrates in the experience of social anxiety at clinical and non-clinical levels of severity (Ayling, Aghajani, Fouche, & van der Wee, 2012; Beaton et al., 2008, 2009; Brühl, Delsignore, Komossa, & Weidt, 2014; Laeger et al., 2012; Miskovic & Schmidt, 2012). Overall, these lines of evidence call into question the reification of diagnostic cut-offs as meaningful, and suggest that variations in severity across study samples are unlikely to be the reason for discrepant findings with regard to amygdala volume. The findings of the current study further support the idea that social fearfulness is likely expressed on a continuum of severity. In contrast to past research, and in line with the idea of a dimensional view of SAD, even though my study operationalized social anxiety as a continuous variable it failed to show an association between bilateral amygdala volume and social anxiety across a range of LSAS-SR scores. Moreover, *post-hoc* analyses (see Appendix A) showed that the association between amygdala volume and social anxiety was comparable for individuals with low and high LSAS-SR scores.

Another factor that merits particular consideration is sample size and study power to detect effects. My sample was markedly larger ($N = 76$) than the samples used in three of the

other studies (N s ranging from 26 to 48) (Irle et al., 2010; Machado-de-Sousa et al., 2014; Syal et al., 2012), two of which found evidence of significant differences of medium to large effect size. However, even though the power profile for the current study showed that it had the sensitivity to detect medium effect sizes when employing a .05 criterion for statistical significance, I did not find the effects that Irle et al. (2010) and Machado-de-Sousa et al., 2014) reported.

There are several ways in which the use of small samples in those studies may have led to findings that neither Brühl et al. (2014) nor I were able to replicate. First, as several groups of researchers have recently suggested, many MRI studies are vulnerable to an increased proportion of false positives relative to true positives because they are statistically underpowered (Button, Ioannidis, Mokrysz et al., 2013; Mar, Spreng, DeYoung, 2013; Poldrack, Baker, Durnez et al., 2017; Wicherts, Veldkamp, Augusteijn et al., 2016). As typical publishing standards reinforce the production of novel and significant results, the overall consequence is that not only does low power reduce the probability of detecting a true result when it exists (i.e., Type II error), but also it makes it more likely that any positive result is in reality false (i.e., Type I error) (Button, Ioannidis, Mokrysz et al., 2013; Wicherts, Veldkamp, Augusteijn et al., 2016). It is therefore possible that the significant results from the small samples of both Irle and colleagues' and Machado-de-Sousa and colleagues' studies reflect Type I errors.

In addition, researchers often make arbitrary choices about their study designs and approaches to collecting, analyzing, and reporting neuroimaging data that can affect the outcome of significance tests applied to the data (Evans, 2017; Wicherts, Veldkamp, Augusteijn et al., 2016). For example, in many studies researchers do not set boundaries for their analyses, they fail to specify a plan for data collection before it begins, they may choose not to report null findings, or they present data from exploratory analyses without clarifying that those findings

were determined *post-hoc* (Wicherts, Veldkamp, Augusteijn et al., 2016). Published research findings that are hard to replicate in independent samples are thought to be the result of these “researcher degrees of freedom,” combined with small sample sizes (Wicherts, Veldkamp, Augusteijn et al., 2016), – an issue that has especially plagued the human neuroimaging literature (Poldrack, Baker, Durnez et al., 2017). All of these issues underscore the importance of a priori registration of hypotheses, power profiles, and a detailed plan for data analyses as protections from incidental findings and to increase confidence in reported results. Fortunately, some journals (for a complete list, visit: <https://cos.io/rr/>) now allow for such comprehensive pre-registration and peer review of the study design and hypotheses, and guarantee publication regardless of statistical significance of results (Poldrack, Baker, Durnez et al., 2017).

4.2.1 Amygdalar sub-regions

In addition to the aforementioned methodological issues, there are other reasons why morphology may not recapitulate functional activation findings with regard to the amygdala and social anxiety. For one, the amygdala comprises functionally distinct, but structurally contiguous, nuclei (Amunts, Kedo, Kindler et al., 2005; Entis, Doerga, Barrett, & Dickerson, 2012; Fernando, Murray, & Milton, 2013; Whalen & Phelps, 2009; Yang, Fan, Chu et al., 2016). Specifically, the amygdala can be further segmented into four component subregions of interest: the basolateral complex, the centromedial nucleus, the basomedial complex, and the amygdaloid cortical region. Research studies involving animal models have proposed distinct functional roles for some of the subregions. For example, the basolateral nucleus of the amygdala, which receives and processes information from temporal lobe structures, has been implicated in defeat behaviors (e.g., submissiveness, defensiveness, avoidance of novel conspecifics) with Syrian hamsters (Clinard, Bader, Sullivan, & Cooper, 2016). One study with rats has also shown that the

basolateral complex of the amygdala is engaged in the learning and inhibition of fear responses (e.g., freezing) (Laurent & Westbrook, 2010). Together, these studies indicate that there may be a specific link between the basolateral complex and social anxiety. Manually delineating this region for analysis in humans could be a useful next step to examine if it is linked to socially anxious behaviours more closely than other subregions.

Although the current study's images lacked adequate resolution to capture amygdalar subdivisions, ultra-high resolution images would allow us to readily address such questions in the future. Such images can be acquired through magnets and head coils that yield higher signal/noise ratios. For example, Entis et al. (2012) have developed a valid and reliable protocol to delineate amygdalar subdivisions by using a prototype custom-built 32-channel head coil to acquire images. As new technologies become increasingly available, researchers will be able to examine whether findings from animal models about amygdalar subnuclei translate to humans within the context of social anxiety.

4.2.2 *What does volume mean?*

Another reason why amygdala morphology findings from my study did not parallel the findings of the functional activation literature may have to do with the complexity of how brain morphology maps onto brain function. In simpler terms, what do smaller or larger volumes of a particular brain region mean? Macroscopic grey matter volume consists of neuronal cell bodies, dendrites, axon terminals, and glial cells. Immunohistochemistry work in mice shows that alterations in neuron number and size, astrocyte number and size, as well as increased neurogenesis, are the microstructural basis of MRI-detectable volume changes (Lerch, Yiu, Martinez-Canabal, 2011). However, it is unclear if the underlying nature of changes reported in human MRI studies would be consistent with animal studies (Kanai & Rees, 2011; Thomas &

Baker, 2012). Moreover, how the amount and balance of these microstructures translate into the computational capacity of a particular brain region is also currently poorly understood (Kanai & Rees, 2011). For instance, a higher number of neurons, as well as fewer synapses (e.g., synaptic pruning during childhood/adolescent brain development), could both lead to better performance of a particular brain region (Kanai & Rees, 2011). As such, better understanding how brain morphology maps onto brain function is a rich area for future research.

4.3 Limitations and future directions

Data collection of MRI images is inherently expensive and time-consuming. Therefore, for the purpose of this study I had opted to aggregate data that were previously collected by other researchers across three different types of scanners. Although this is a strength of the current study, it also posed limitations with regard to data loss. Whereas some attrition in neuroimaging studies is inevitable due to image quality issues, the level of attrition (i.e., 55%) in the present study exceeded expectations (see Figure 2). In such cases, describing demographic and behavioral characteristics of the subjects who had to be excluded from the sample allows readers to better evaluate the generalizability of findings and is in keeping with the spirit of transparent methods reporting (for guidelines, see reviews by Poldrack, Baker, Durnez et al., 2017; Wicherts, Veldkamp, Augusteijn et al., 2016).

To that end, I examined whether participants' whose brain images were included in the final sample ($n = 76$) represented a group that was less socially anxious, or in other ways unique, in comparison to those excluded ($n = 221$) because their neuroimaging data could not be reliably analyzed. There were no significant differences in gender distribution (Pearson chi-square test of significant), age (Independent samples t-test), education (Independent samples t-test), or social anxiety scores (independent samples t-test) between the two groups (see Table 5). As such,

despite the substantial attrition, this lack of differences between included and excluded participants alleviates some concern around generalizability.

Table 5 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-SR Total	54.59±33.35 (Range=0-108)	46.78±36.07 (Range=0-121)
Median	65	55

Furthermore, as the sample for the current study emerged from legacy datasets comprising multiple research protocols, each with slightly different inclusion criteria, some information cannot be fully accounted for. Due to the variation in concurrent disorders and past comorbidities (see Table 4) the current sample is somewhat heterogeneous. While this increases the external validity of my findings, it does pose a mild threat to internal validity. Of note, some of the data for psychiatric characteristics of subjects are missing and I do not have the ability to obtain this information, as the data were de-identified prior to being shared. As recommended by Mar, Spreng, and De Young (2013), future studies may benefit from retaining contact information for participants who contributed the data, even though this may complicate the

process of establishing data sharing agreements and obtaining IRB approval. If available, contact information could then be used to collect additional psychiatric, behavioral, or cognitive data to be linked to the neuroimaging findings.

Another limitation is that of selection bias. It is possible that individuals chose to participate in the parent studies because their social anxiety problems were salient. Alternatively, it may be that participants with fewer social anxiety symptoms were better able to participate because of higher levels of willingness to interact with the research teams. Although a large proportion (67%) of participants in our aggregated sample obtained scores that fell within the clinical range on the LSAS-SR, it is possible that those whose social anxiety was very severe and impairing never agreed to participate in the research study in the first place. Therefore, all interpretations that have emerged from the current research should be considered in these contexts.

With regard to volumes for the manually corrected amygdala boundaries, the absence of a second tracer to provide a reliability check poses a limitation. Given the sheer complexity of published landmarks and rules for determining the boundaries around the amygdala, the second tracer would have to be an individual who is familiar not only with basic neuroanatomy, but also with visualizing structural features on a T1 MRI image. Moreover, rigorous training and laborious practice with the aforementioned manual correction methods would be required. Nevertheless, identifying an individual who meets these criteria and having them manually correct amygdala boundaries on a subset of randomly chosen images would strengthen the study.

Finally, it is important to acknowledge that the “extended amygdala,” which forms part of the basal forebrain region traditionally known as the substantia innominata (Heimer, Harlan, Alheid, Garcia, & De Olmos, 1997), cannot be accessed by in vivo studies of the human brain.

As endocrine, autonomic, and somatomotor aspects of emotional and motivational states are attributed, in part, to the extended amygdala it may be a relevant structure with regard to social anxiety.

4.4 Strengths and conclusion

The current study was designed in an effort to examine associations between amygdala volume and social anxiety, using volumetric estimates generated in two ways (i.e., delineating the amygdala with both automated and manual techniques), within a single sample of participants that was large enough to permit detection of meaningful effects. My findings did not support the hypothesis of an association between amygdala volume and social anxiety, regardless of the technique used delineate the amygdala's boundaries. Nevertheless, they lend strong support to the findings of Brühl et al. (2014) and call into question numerous methodological aspect of existing volumetric studies of the neural correlates of social anxiety.

This is meaningful, because the present study has numerous strengths. First, it includes subjects that represent almost the full spectrum of social anxiety scores on the LSAS-SR, making it possible to operationalize the construct of social anxiety as a continuous variable. Not only is this approach consistent with several lines of empirical evidence, but also it is consistent with recommendations of the National Institute of Mental Health (NIMH) Research Domain Criteria (RDoC; <http://www.nimh.nih.gov/research-priorities/rdoc/index.shtml>) (Hershenberg & Goldfried, 2015; Lilienfeld, 2014; Van Orden & Areán, 2015). Second, the choices made with regard to study design, data collection, and analyses, were theoretically driven, with regions of interest and behavioral outcomes that were decided *a priori*. This approach protects the internal validity of the study by reducing the threats posed by “researcher degrees of freedom” to the outcomes of statistical significance tests. Third, in line with many of the guidelines for

transparent methods reporting in neuroimaging, recommended by the Organization of Human Brain Mapping (Poldrack, Baker, Durnez et al., 2017), I have thoroughly documented neuroimaging methods and findings, which fosters reproducibility. Finally, the results are based on a large sample that allows for adequately powered analyses and increases confidence in the findings. The sample size was achieved because the study capitalizes on a large aggregated dataset. In doing so, the study highlights the value of groups of researchers compiling a common database of structural (i.e., anatomical) scans and is in line with recommendation for open platforms for data exchange (Landis, Courtney, Dieringer et al., 2016). All of these strengths lend reliability to my results and speak to the importance of applying methodological rigor in neuroimaging studies of psychosocial constructs.

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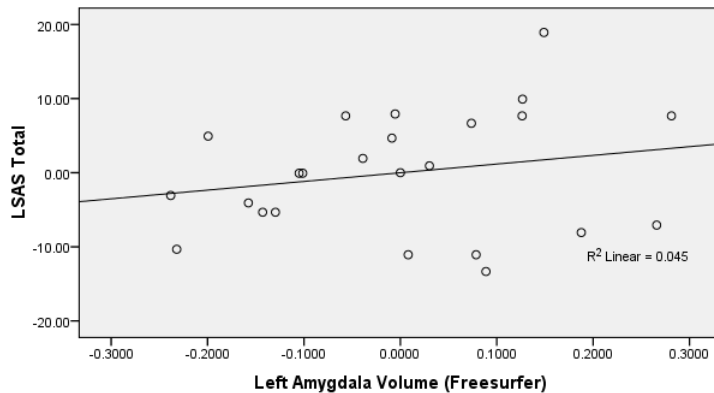
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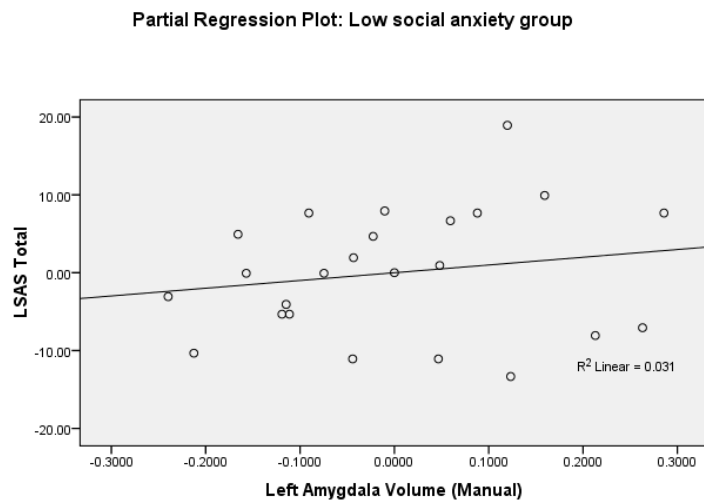
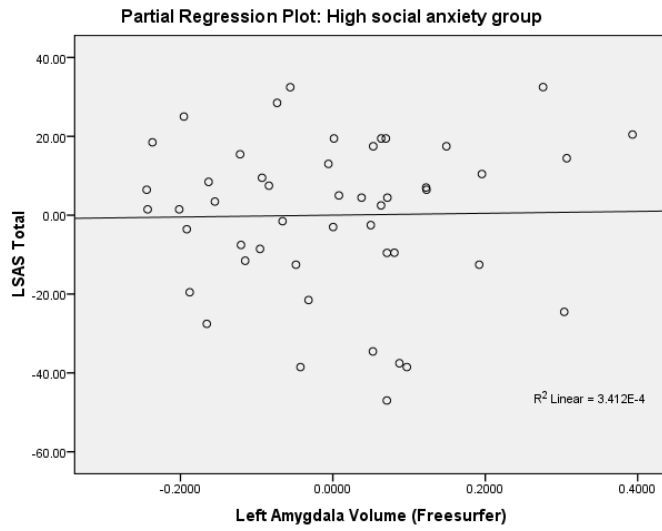
APPENDICES

Appendix A

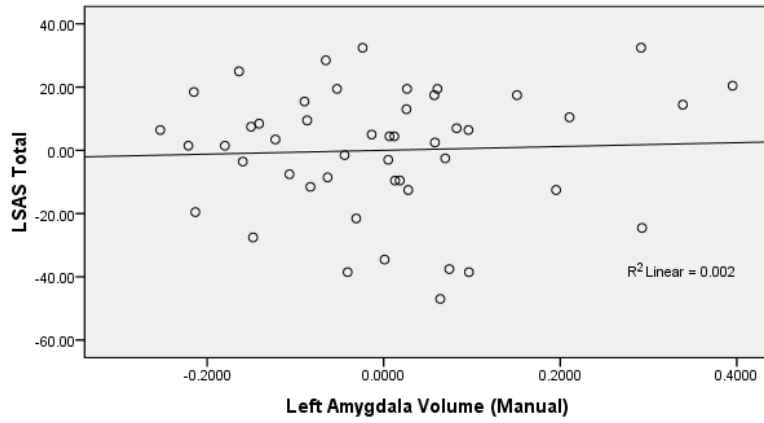
In order to better understand my sample, I examined whether the slopes and relationships between amygdala volume and social anxiety were similar for individuals with low versus high LSAS-SR score. This was important because the distribution of LSAS-SR scores had two peaks, although it was not truly bimodal. The empirically recommended (see Rytwinski, Fresco, Heimberg et al., 2009) LSAS-SR cutoff score of 30 was used to divide the sample into low versus high social anxiety subgroups. Partial regression plots for the left and right amygdala are provided below for comparison with Figures 7-10 in the manuscript.

Partial Regression Plot: Low social anxiety group

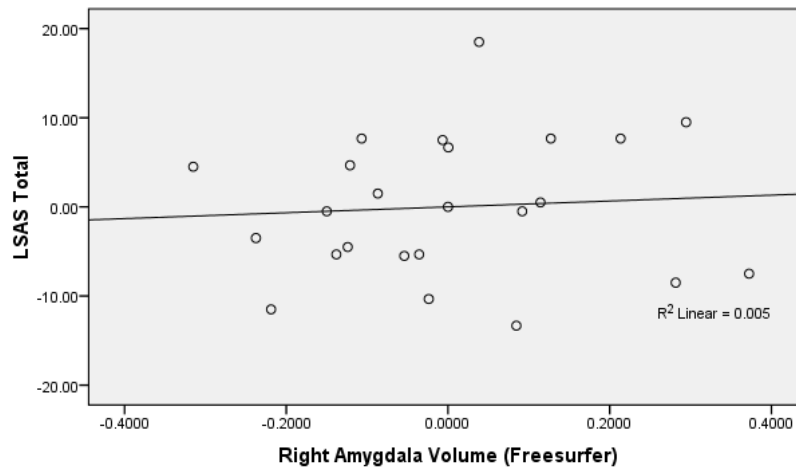




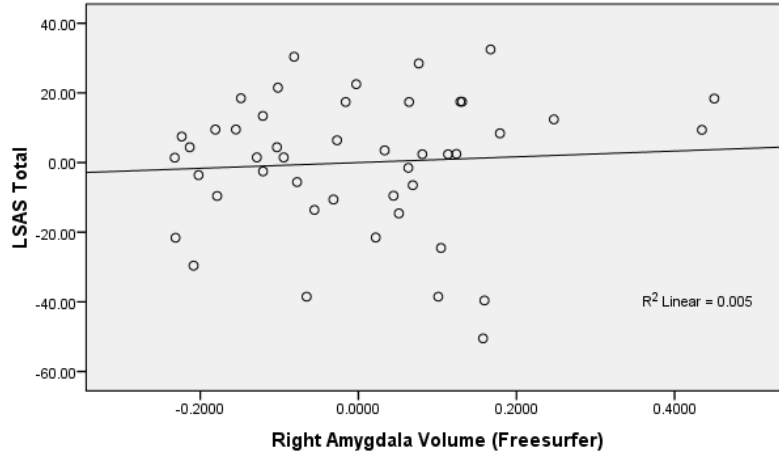
Partial Regression Plot: High social anxiety group



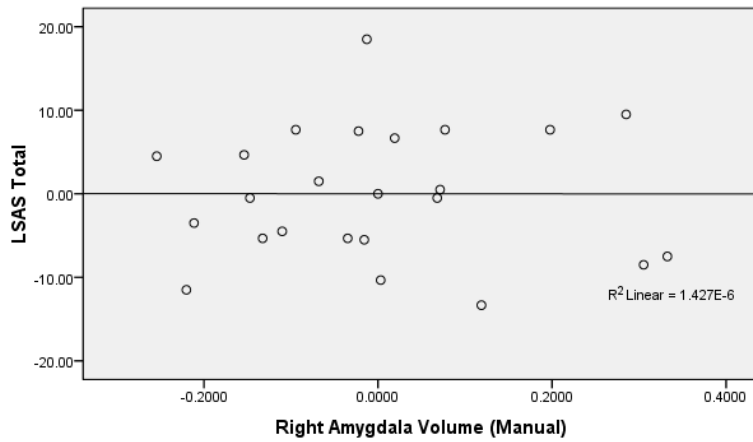
Partial Regression Plot: Low social anxiety group



Partial Regression Plot: High social anxiety group



Partial Regression Plot: Low social anxiety group



Partial Regression Plot: High social anxiety group

