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Coenraad Jacobus Hattingh

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Neurobiological Aspects of Social Anxiety Disorder

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Neurobiological Aspects of Social Anxiety Disorder

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B.A.(Psych), Hons.B.A.(Psych)

Thesis

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ABSTRACT:

Background: There is growing evidence that social anxiety disorder (SAD) is characterized by specific changes in functional neuroanatomy. However, little work has been done to integrate activation results across functional studies and little work on alterations in neuroanatomical structure has been conducted. This thesis investigates the functional neuroanatomy of SAD using an activation likelihood-estimate meta-analysis (ALE meta-analysis), and explores the structural basis of SAD using a cortical thickness and subcortical gray matter volume analysis.

Methods: In the ALE meta-analysis, 11 studies were included for analysis. Brain activation coordinates were extracted from the articles and compiled using GingerALE software. In the cortical thickness and subcortical volumetric analysis, 13 SAD subjects and 13 demographically matched healthy controls were studied using high-resolution magnetic resonance imaging (MRI). Cortical thickness and volumes of subcortical structures were assessed using Freesurfer, and compared.

Results: In the ALE meta-analysis, consistent activation was observed in the the right head of the caudate nucleus, right claustrum, the left parahippocampal gyrus and amygdala, right globus pallidus, left cuneus, right putamen, right insula and left posterior lobe of the cerebellum. In the cortical thickness and subcortical volumetric analysis, compared to healthy controls, SAD patients had significant cortical thinning of the right amygdala, right and left medial orbitofrontal cortex, left and right insula. There were, however, no significant correlations between symptom severity measures and either cortical or subcortical abnormalities.

Conclusion: The results of the ALE meta-analysis are consistent with the animal literature on fear neurocircuitry, and with previous systematic reviews and meta-analyses of SAD. The cortical and subcortical structural abnormalities found in the cortical thickness and subcortical volumetric analyses are consistent with previous work demonstrating alterations in cognitive-affective processing in SAD. Additional work is needed to investigate the causal mechanisms involved in such functional and structural abnormalities.

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Introduction

Social anxiety disorder (SAD) is characterised by a persistent fear of one or more social or performance situations in which the person is concerned about negative evaluation or scrutiny by others (*Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR)*, 2000), for example: public speaking, writing, eating, drinking in public or initiating or maintaining conversations. People with SAD fear humiliation or embarrassment and may express anxiety through blushing or sweating. Typically, the feared social or performance situation is avoided or endured with intense anxiety and distress (*Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR)*, 2000)

SAD is an extremely prevalent disorder (Kessler, Chiu, Demler, Merikangas, & Walters, 2005), the epidemiology of which will be discussed in more detail later on. Typically, the course of the disorder is protracted, starting relatively early in life, and continuing for many decades (Coupland, 2001).

There has been significant progress in understanding the neurobiology of SAD. Brain imaging studies have focused on measuring haemodynamic changes in certain brain regions in the case of functional magnetic resonance imaging (fMRI) (Freitas-Ferrari et al., 2010), and the absorption and distribution of

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radiopharmaceutical substances throughout the brain in the case of nuclear imaging techniques such as single photon emission computerised tomography (SPECT) (Phan, T. Wager, Taylor, & Liberzon, 2002).

Neurotransmitter systems implicated in SAD include the monoaminergic neurotransmitters (Condren, Lucey, & Thakore, 2003), as well amino acid neurotransmitters (Shlik, Maron, Tru, Aluoja, & Vasar, 2004). Studies of the pathogenesis of SAD have focused on both genetic and environmental factors. Nevertheless, many important questions about the neurobiology of SAD remain unanswered.

In Chapter 1 of this thesis, I will review both what is known about the neurobiology of SAD, as well as some of the questions that remain unanswered. I will note that no recent brain imaging meta-analysis has used activation-likelihood methods and I will provide such an analysis in Chapter 2. I will also note that very few imaging studies have provided data on cortical thickness and subcortical volumetric measures in SAD, and I will provide such an analysis in Chapter 3.

Before going on to discuss the neurobiology of SAD in more detail, I will briefly note some key aspects of the disorder, which make it an important object of study. These include 1) the high prevalence of SAD, and 2) the significant morbidity associated with this condition.

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1.

Prevalence of SAD

Twelve month prevalence rates in South Africa are estimated at 1.9% (Williams et al., 2008) whereas in the United States, SAD is identified as the third most prevalent psychiatric disorder, after major depression and alcohol dependence. SAD is estimated to affect between 2.4% and 13% of the population (Kessler et al., 2005; Ruscio et al., 2008; Fehm, Pelissolo, Furmark, & Wittchen, 2005). Cross-cultural findings suggest that there may be a lower prevalence rate in non-Western countries (Gureje, Lasebikan, Kola, & Makanjuola, 2006; Mei, Xiao, He, & Fan, 2010).

Subjects with SAD demonstrate a broad range of symptom severity, and there appears to be no specific cut-off-point in terms of the number of social fears that differentiates different SAD subtypes (Stein et al, 2010). Nevertheless, subjects with more social fears are considered to have a generalized form of SAD(*Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR),* 2000), and generalized SAD is characterized by greater impairment, higher heritability, and presumably more evidence of underlying neurocircuitry dysfunction (Stein et al., 2000;Stein and Stein, 2008).

SAD has an early onset, typically beginning in the mid-teens, sometimes emerging out of a childhood history of social inhibition or shyness. Some individuals also report an onset in

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early childhood. The onset of SAD may closely follow a stressful or humiliating experience, or it may be insidious. The course of SAD is often continuous and duration is frequently lifelong, although the disorder may attenuate in severity or remit during adulthood. Severity of impairment may fluctuate with life stressors and demands (*Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR)*, 2000).

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2.

Morbidity

SAD brings with it significant distress and impairment. Indeed, patients with SAD have been shown to have significantly greater distress when compared to some other anxiety disorders (Simon et al., 2002; Merikangas et al., 2007). Thus quality of life is significantly reduced in people with SAD, even in individuals with subclinical severity(Cramer, Torgersen, & Kringlen, 2005).

Furthermore, people with SAD may experience financial difficulties due to work impairment (Davidson et al.,1993), and younger individuals with SAD may drop out of school due to impaired school performance (Merikangas et al., 2007). Weakened social support systems accompany the difficulties sufferers of SAD experience (van Ameringen, Mancini, & Farvolden, 2003).

Impairment may encompass underachievement in school due to test anxiety or avoidance of classroom participation and underachievement at work because of anxiety during, or avoidance of, speaking in groups, in public, or to authority figures and colleagues (*Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR)*, 2000).

SAD may be comorbid with other anxiety disorders, mood disorders, substance-related disorders, and bulimia nervosa and typically precedes these disorders. In clinical samples, avoidant personality disorder is frequently present in individuals with SAD (*Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR)*, 2000). Comorbidity may often be attributed to the difficulties people with SAD experience (for example, depression may follow SAD), and it may in turn contribute to distress and impairment.

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3.

Conclusion

In summary, SAD is an important psychiatric disorder, characterized by high prevalence, and significant morbidity. As a better understanding of the neurobiology of SAD could lead to more effective treatments, research in this area is key. I review such work in the next chapter.

Chapter One

The Neurobiology of Social Anxiety Disorder

Introduction

In this chapter, I will discuss the various neurobiological aspects of social anxiety disorder. Firstly, I will refer to genetic aspects that may contribute to SAD pathogenesis, and then move on to discussing various neurotransmitter systems involved in SAD. Models from animal research on the neurobiology of anxiety will be discussed to provide a background for the synopses on human neuroimaging research, discussed last.

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1.

Genetic Aspects

There have been various studies investigating the genetic predispositions associated with SAD. Several large family studies have been published – investigating whether SAD aggregates in families. First amongst them was Fyer et al., (1993) who found that approximately 16% of the relatives of individuals with SAD met the diagnostic criteria for SAD compared to 5% in controls. The greater percentage of

associated relatives with SAD versus healthy control families indicate that there may be a trend for the aggregation of genetic factors in family members with SAD.

Stein M.B. et al (1998) identified a relative risk of 9.7% in their direct-interview family study of 106 first-degree relatives of 23 individuals with SAD. This finding seems to correspond with the earlier study by Fyer et al (1993).

Twin and adoption studies provide an additional perspective on the heritability of SAD and help to distinguish between genetic and environmental contributions to its aetiology. Several early twin studies investigated the heritability of SAD in monozygotic vs dizygotic twins (Kendler et al., 1992; Skre et al., 2000), but by far the largest twin study to date was conducted by Ogliari et al., (2006) who established an approximate heritability of 60% for the 378 twins evaluated.

Gelernter, Page, M. B. Stein, & Woods, (2004)investigated the association of specific genetic variants in SAD. A variant in the SLC6A2 norepinephrine transporter protein was associated with increased risk of developing SAD amongst 163 people who were relatives of and individual with SAD. Lochner et al., (2007) genotyped serotonergic and dopaminergic candidate genes in 63 patients with SAD and matched controls, and found that the 5-HT_{2A} T102C polymorphism was associated with SAD. No single gene appears to be responsible for the

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pathogenesis of SAD, but it is apparent that several genetic variations may contribute to the development of SAD. A comprehensive review of the more extensive literature on genetic factors in SAD is however beyond the scope of this thesis. It can be hypothesized, however, that these various genetic aetiological contributors of SAD pathogenesis mainly function in the context of sensitising the individual towards developing SAD when being exposed to environmental stressors.

2.

Neurotransmitter systems

Specific neurochemical and neuropeptide systems have been suggested to play a role in the pathogenesis of SAD. One such system is the dopaminergic system. Several SPECT and PET studies have been conducted where radiopharmaceuticals that bind specifically to dopamine reuptake transporters or to selected dopamine receptors have been utilised to measure dopamine function (Tiihonen et al., 1997; Schneier et al 2000). Reduced striatal dopamine reuptake site densities were markedly lower in people with SAD(Tiihonen et al., 1997), suggesting dopaminergic dysfunction as pathogenic in SAD. Other neurotransmitter systems such as serotonin (Lanzenberger et al., 2007) and glutamate (Phan et al., 2005) have also been

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investigated using PET radioligands or magnetic resonance spectroscopy (MRS). Phan et al., (2005) found glutamate (relative to creatine) levels were significantly higher in patients than controls in the anterior cingulate. Lanzenberger et al., (2007) found significantly decreased serotonin binding in several limbic and paralimbic areas. However, findings to date are preliminary. The central focus of this thesis is not, however, on the role of neurotransmitter systems in the pathogenesis in SAD, and I will therefore not discuss these issues in more detail.

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3.

Neuroanatomy of Anxiety

In this section I will provide an overview of anxiety and its associated neuropathology. The amygdala has repeatedly been identified as being involved in fear processing in both basic neuroscience research, as well as in clinical research with anxiety disorder patients. The amygdala is a compounded nucleus, comprised of gray matter, contained within the white matter of the temporal lobe. The amygdala has been implicated in the coordination of threat responses (LeDoux et al., 2003) and integrates information from sensory features and prior experience via cortical and subcortical projections (Price et al., 2003)

In rodents, very distinct amygdalar pathways have been distinguished for fear; a brief stimulus-specific response, and anxiety; a more pervasive response prompted by conditions of unforeseen danger (Davis et al., 2006). A previous meta-analysis conducted by Phan et al (2002) is consistent with the findings in animal literature, that fearful stimuli specifically activate the amygdala in healthy human subjects.

Reciprocal connections between the amygdala and the prefrontal cortex are integral in the mechanism of fear extinction (Quirk et al., 2006). Fear extinction is the decrease in conditioned fear responses that normally occurs when a conditioned stimulus is repeatedly presented in the absence of the aversive unconditioned stimulus (Milad, Rauch, Pitman, & Quirk, 2006).

With advances in neuroimaging and more sophisticated methods of data analysis, other brain regions have emerged as important in the role they play in anxiety. The insula, a distinct area of cortex hidden underneath the frontal and temporal lobes, has been implicated as important during the processing of interoceptive cues(Craig, 2003). Altered insular activity has been reported in patients with obsessive-compulsive disorder (OCD), post-traumatic stress disorder (PTSD) (Rauch et al., 1997), specific phobia (Wright et al., 2003), and in SAD (Lorberbaum et al., 2003). Warwick et al

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(2006) found decreased regional cerebral blood flow (rCBF) measured with SPECT in bilateral insular cortices after with citalopram or moclobemide administration during an 8 week period in subjects with SAD, further implicating the insular cortex as etiological in SAD.

4.

Structural Studies

The systematic morphometric evaluation of the brain as a whole can be achieved by automated techniques of voxel by voxel analysis, or so called voxel-based morphometry (VBM). VBM allows one to compare the concentration or volume of grey and white matter between groups of interest. FreeSurfer is a brain imaging analysis package developed by the Athinoula Martinos Center for Biomedical Imaging that enables one to conduct structural and functional brain mapping of the cerebral cortex (B Fischl & Dale, 2000). It has been shown to be a reliable method of data analysis in structural MRI paradigms(Makris et al., 2005). FreeSurfer modifies the representation of the cortical surface by inflating it so that activity buried inside sulci may be visualized, cuts and flattens an entire hemisphere, and transforms a hemisphere into a simple parameterizable surface such as a sphere for the purpose of establishing a surface-based coordinate system (B

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Fischl, Sereno, & Dale, 1999). FreeSurfer tessellates the gray–white boundary, and performs an automated topology correction and surface deformation to locate the gray–white and gray– pial boundaries based on signal–intensity gradients. For each subject, FreeSurfer generates the white-matter surface for each hemisphere by tiling the superficial aspect of white matter for that hemisphere; in a similar fashion, FreeSurfer generates the pial surface. FreeSurfer calculates cortical thickness as the closest distance between the gray- and white-matter boundary and the pial mesh at each vertex on the tessellated surface (B Fischl & Dale, 2000). A cortical atlas that is based on statistics computed from manually labeled cortical regions is used; this atlas divides cerebral cortex into several structures(Desikan et al., 2006). These structures are then mapped onto a spherical space to achieve point-to-point correspondence for each subject(Bruce Fischl et al., 2004). The final segmentation of surface-based labeling is then based on both a subject-independent probabilistic atlas and on subject-specific measured values. Combining the cortical thickness map and surface-based labels the average cortical thickness for each cortical region is then computed.

In 1994, Potts et al. investigated regional brain volumes of 22 patients diagnosed with SAD. Based on the hypothesized association between SAD and dopaminergic dysfunction, this

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study used the ROI-based approach to target the following regions of interest: caudate, putamen, thalamus, and whole brain. No significant differences in the caudate, putamen, thalamus, or whole brain were found between patients and healthy controls. Milham et al., (2005) investigated the neural correlates of pediatric anxiety disorders using voxel based morphometry, and found reductions in left amygdala volumes in patients with anxiety disorders as compared to healthy controls. Van Tol et al., (2010) investigated regional brain volumes in depression and anxiety using VBM and found reduced left middle/superior temporal volume in anxiety disorders. However, this study did not specifically focus on SAD. More recently, Liao et al (2011) investigated 18 patients with SAD using VBM. The subjects with SAD demonstrated significantly decreased gray matter volumes in the right posterior inferior temporal gyrus and the right parahippocampal gyrus. Importantly, gray matter volumes in these two regions correlated negatively with the fear factor of the Liebowitz Social Anxiety Scale. Relatively few studies have utilized structural MRI paradigms to investigate the neuropathology of SAD.

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5.

Functional Magnetic Resonance Imaging Studies

Functional Magnetic Resonance Imaging (fMRI) is a specific neuroimaging sequence where hemodynamic responses are measured and then related to neural activity. The measurement of the hemodynamic response is based on changes in relative concentrations of oxyhemoglobin and deoxyhemoglobin, which is thought to reflect neural activity (Detre & Floyd, 2001). fMRI offers the advantage of very high spatial resolution. Table 1 lists all fMRI studies in SAD published to date.

Table 1
fMRI studies in SAD

Name	Year	Subjects (n)	Results
Birbaumer et al.	1998	7 SAD/ 5 HC	Increased amygdala activity to facial stimuli.
Schneider et al.	1999	12 SAD / 12 HC	Increased amygdala and hippocampus activity associated with negative odor.
Stein et al.	2002	15 SAD / 15 HC	Increased allocortical activity for contemptuous and angry faces
Loberbaum et al.	2004	8 SAD / 6 HC	Increased subcortical and limbic activity.
Straube et al.	2004	10 SAD / 10 HC	Increased insula activity for angry vs neutral faces.
Straube et al.	2005	9 SAD / 9 HC	Increased amygdala activity to angry vs happy faces.
Amir et al.	2005	11 SAD / 11 HC	Increased anterior cingulate cortex and insula activity.
Phan et al.	2006	10 SAD / 10 HC	Increased amygdala activity in harsh faces vs happy faces.
Cooney et al.	2006	10 SAD / 10 HC	Increased right amygdala activation vs neutral faces
Yoon et al.	2007	11 SAD / 11 HC	Increased bilateral amygdala activation to

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			high vs low intensity emotional faces
Sareen et al.	2007	10 SAD / 10 HC	Decreased activation in the left caudate nucleus, left inferior parietal lobe, and bilateral insula to implicit learning.
Campbell et al.	2007	14 SAD / 14 HC	Delayed amygdala response to fearful, angry and happy faces.
Evans et al.	2008	11 SAD / 11 HC	Increased right amygdala activation.
Quadflieg et al.	2008	12 SAD / 12 HC	Increased orbitofrontal activation in response to angry vs neutral voices.
Gentili et al.	2008	8 SAD / 7 HC	Increased activation of the left amygdala and insula.
Guyer et al.	2008	8 SAD / 14 HC	Increased amygdala and ventrolateral prefrontal cortex activation.
Blair et al.	2008a	17 SAD / 17 HC	Increased amygdala and medial prefrontal cortex activation to criticism.
Blair et al.	2008b	17 SAD / 17 HC	Increased amygdala and superior temporal cortex activation.
Goldin et al.	2009a	27 SAD / 27 HC	Decreased amygdala activation in cognitive reappraisal.
Goldin et al.	2009b	15 SAD / 17 HC	Increased medial orbitofrontal cortex, anterior cingulate, and bilateral parahippocampal gyrus activation.
Sripada et al.	2009	26 SAD/ 26 HC	Decreased activation in the medial prefrontal cortex.
Shah et al.	2009	11 SAD / 11 HC	Increased bilateral insula and amygdala activation.
Gentili et al.	2009	8 SAD / 7 HC	Decreased activation of the precuneus and posterior cingulate regions.
Liao et al.	2010	22 SAD / 21 HC	Decreased influence from inferior temporal gyrus to amygdala.
Liao et al.	2010	20 SAD / 20 HC	Functional connectivity was decreased in the somato-motor and visual networks.
Nakao et al.	2010	SAD 6 / HC 9	Decreased activation in the left cerebellum, left precuneus, and bilateral posterior cingulate cortex.

Most of studies in SAD to date have found increased activation in the amygdala (Blair, Geraci, et al., 2008a; Blair, Shaywitz, et al., 2008b; Campbell et al., 2007; Cooney, Atlas, Joormann, Eugène, & Gotlib,

2006; Evans et al., 2008; Gentili et al., 2008; Goldin, Manber, Hakimi, Canli, & Gross, 2009a; Phan, Fitzgerald, Nathan, & Tancer, 2006; Franklin R Schneier, Kent, Star, & Hirsch, 2030; Shah, Klumpp, Angstadt, Nathan, & Phan, 2009; M. B. Stein, Goldin, Sareen, Zorrilla, & Brown, 2002; Straube, Kolassa, Glauer, Mentzel, & Miltner, 2004; Yoon, Fitzgerald, Angstadt, McCarron, & Phan, 2007). Secondly, a few of these studies have noted the involvement of cingulate cortex (Amir et al., 2005; Gentili et al., 2009; Goldin, Manber-Ball, Werner, Heimberg, & Gross, 2009b; Lorberbaum et al., 2004), the prefrontal cortex (Blair, Geraci, et al., 2008a; Campbell et al., 2007; Gentili et al., 2008; Guyer et al., 2008; Lorberbaum et al., 2004; Sripada et al., 2009), the fusiform gyrus (Gentili et al., 2008; Lorberbaum et al., 2004; Straube et al., 2004), and the insula (Amir et al., 2005; Gentili et al., 2008; 2009; Sareen et al., 2007; Straube et al., 2004).

Regions in the parietal lobe (Gentili et al., 2008; Sareen et al., 2007), and the temporal lobe (Blair, Shaywitz, et al., 2008b; Gentili et al., 2008) have also been implicated. To a lesser extent, the caudate nucleus (Sareen et al., 2007) and the precuneus (Gentili et al., 2009) have also been earmarked.

A number of meta-analyses have attempted to summarize these functional imaging studies (Etkin & T. D. Wager, 2007; Freitas-Ferrari et al., 2010; Phan et al., 2002). Phan et al (2002) reviewed 55 PET and fMRI activation studies, and concluded that (1) The medial prefrontal cortex had a general role in emotional processing; (2) fear specifically engaged the amygdala; (3) sadness was

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associated with activity in the subcallosal cingulate; (4) emotional induction by visual stimuli activated the occipital cortex and the amygdala; (5) induction by emotional recall/imagery recruited the anterior cingulate and insula; (6) emotional tasks with cognitive demand also involved the anterior cingulate and insula (Etkin & T. D. Wager, 2007; Freitas-Ferrari et al., 2010; Phan et al., 2002).

Etkin et al (2007) conducted a quantative meta-analysis and included studies that compared negative emotional processing to baseline, neutral or positive emotional conditions, and concluded that subjects with SAD consistently showed greater activation in the amygdala and insula than matched comparison subjects. These structures are linked to negative emotional responses, and the same kind of activation was seen in healthy controls during fear conditioning.

Freitas-Ferrari et al (2010) conducted a systematic review of the literature and found (1) most of the studies demonstrated increased activity in limbic and paralimbic regions in SAD; (2) the predominance of evidence implicated hyperactivity of the amygdala; (3) reduced activity in striatal and parietal areas as well as alterations in activity of prefrontal regions.

Nevertheless, to date, there has been no Activation-Likelihood Meta-analysis (ALE) of SAD fMRI studies. An ALE meta-analysis is important because this not only describes

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compounded data, but it plots these on a neuroanatomical template and presents a likelihood of brain region activation on a much greater scale (Turkeltaub, Eden, Jones, & Zeffiro, 2002). Such a method effectively allows for the visual representations of all activation coordinates reported in analyzed studies, superimposed on a standardized neuroanatomical surface.

6.

Conclusion

In this chapter I have reviewed studies of the neurobiology of SAD. SAD may well reflect both genetic and environmental factors, which in turn act to influence neuroanatomy and neurochemistry. This thesis will focus in particular on functional and structural neuroanatomical disruptions in SAD. Chapter Two will explore the functional neuroanatomy of SAD using an ALE approach. Chapter Three will explore the structural neuroanatomy of SAD using a Freesurfer based approach.

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Chapter Two

Activation Likelihood-Estimate Meta-Analysis

1.

Introduction

There is a rapidly increasing literature of functional brain abnormalities in patients with SAD. Although studies employing functional magnetic resonance imaging (fMRI) typically observe differences in activation patterns in people with SAD relative to healthy controls, interpretation of these findings is complicated by between-study variability in the specific locations that seem affected amongst patients. As a result, data from these studies have been synthesized using meta-analytic techniques, in an attempt to identify consistent patterns of activation(Phan et al., 2002). Several meta-analyses and systematic reviews have implicated subcortical gray matter and limbic association cortices in SAD(Etkin & T. D. Wager, 2007; Freitas-Ferrari et al., 2010).

However, no Activation-Likelihood Meta-Analysis (ALE meta-analysis) has been undertaken to date. The ALE meta-analysis was designed to maximize the quantification of interstudy concordance while minimizing the subjectivity of the analytic

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technique - more so in comparison to traditional meta-analytic techniques that simply enumerate regions of activation (Laird et al., 2005; Turkeltaub et al., 2002). It can be used to generate a statistical parametric map of consistent brain activation across studies through calculating the union of the probabilities that particular voxels will be activated, based on the location of study-specific coordinates. This allows for an integrated understanding of the results from all included studies, versus the seemingly disjointed individualized presentation of the conventional narrative reviews.

The aims of the study were to conduct a search for relevant studies in the SAD neuroimaging literature and to perform an activation likelihood-estimate meta-analysis of the reported activation coordinates, which would provide an overview of functional neuroanatomy involved in the pathological expression of SAD(Etkin & T. D. Wager, 2007).

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2.

Methodology

a. Selection criteria

In order to be included in the meta-analysis, studies had to satisfy the following selection criteria: All fMRI studies that included a SAD patient group as well as a healthy control group. Patients had to have been diagnosed with SAD according to validated diagnostic criteria (DSM-IV or ICD-10). In addition, we only included studies that reported coordinates for differences in activation between these groups across the whole brain.

b. Search strategy

A search strategy was constructed to retrieve potentially eligible studies. The titles and abstracts of records contained within the PubMed, Scopius, Embase and Google Scholar electronic databases were searched using a combination of the following: fMRI, functional Magnetic Resonance, SAD, "Social Anxiety" and "Social Phobia". The database-specific search queries can be found below:

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((((("fMRI"[Title/Abstract]) OR "functional Magnetic Resonance*" [Title/Abstract]AND "SAD"[Title/Abstract]) OR "Social Anxiety*"[Title/Abstract]) OR "Social Phobia"[Title/Abstract])))). The search strategy was entered into several databases including PubMed, Scopius, Embase and Google Scholar.

c. Selection process

The study selection process consisted of three stages. The first pass selection stage requires that the titles of all of the articles obtained by the search be assessed for relevance. Obviously irrelevant articles were excluded. The second pass selection stage required that all the articles that survived the first pass, be assessed on the basis of their abstracts to which some of the selection criteria was applied.

Finally, all the articles that survived the second pass were assessed for inclusion based on the full text of the article. If they met all the selection criteria, they were included. Fig 2. demonstrates the numbers of obtained papers at each level of the selection process.

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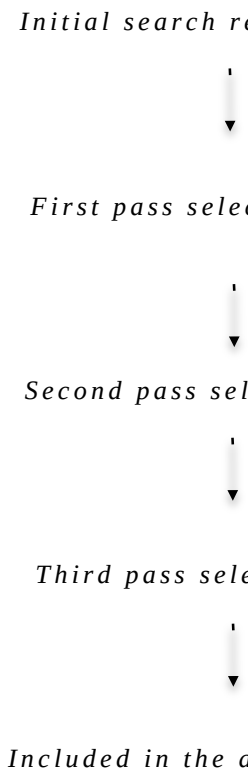
Figure 2.



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d. Data extraction

The data was extracted using a template created for this purpose. Information extracted from the articles included the title, authors, year of publication, journal name, imaging characteristics, fMRI paradigm, and activation coordinates. Main attributes are summarized in table 2.

e. ALE meta-analysis

Activation coordinates formatted in the Talairach space were extracted and analyzed in GingerALE (Eickhoff et al., 2009).

Previous versions of GingerALE would calculate the probability of each voxel in the brain being activated on the basis of all the foci reported in the studies, as if these 3D coordinates were independent of each other. This was problematic in that those studies which reported a greater number of coordinates (perhaps because they used a lower statistical threshold) would receive a greater weight with regards to their contribution to the findings of the meta-analysis.

The latest version of GingerALE, on the other hand, identifies each reported coordinate with the study that generated it, by obtaining a single modeled activation (MA) map for each study. The probability that a particular voxel is activated is then calculated as the union of the probabilities for that voxel across studies. This revised algorithm allows the weighing of the precision of the location of the foci reported by each study by its sample size (technically, by calculating a study-specific FWHM value which determines how widely-dispersed or "blurred" that activation is), on the assumption that larger studies provide more reliable estimates of activation. It is this modeling of within-study variance, which differentiates a random-effects from a fixed-effect analytical model, with the consequence that one can now generalize the findings of the

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meta-analysis to the SAD population (with a fixed-effects analysis, this was not possible). GingerALE2 also restricts voxels of interest to those areas of the brain which have a greater than 10% probability of containing grey matter, as no BOLD signal will typically be observed in white matter.

Data from studies which reported stereotactic (x, y, z) coordinates were aggregated as part of an Activation Likelihood Estimation (ALE) meta-analysis. ALE involves the generation of a statistical parametric map (SPM) of brain activity through the quantitative synthesis of whole-brain coordinate data across multiple studies. The likelihood that activation in particular voxels occurs by chance can subsequently be determined through reference to an empirically derived probabilistic map of brain activity (Laird, et al. 2005a; Turkeltaub, et al. 2002). ALE makes optimal use of the voxel-wise resolution of the study-level data, overcomes between-study heterogeneity in the positioning of activated voxels introduced through measurement error, and bypasses reliance on subjective and error-prone anatomical labeling (Laird, et al. 2005c). Moreover, ALE is fully automated and conclusions drawn from it can be statistically defended through reference to a null hypothesis distribution. Specific parameters included the coordinate space: Talairach, a more conservative mask size, Eickhoff 2009 as the ALE method, a

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0.05 false discovery rate and a cluster minimum volume of 160mm^3. Clusters corresponding to areas of deactivation in the patient versus control group were not included in the meta-analysis (Eickhoff et al., 2009).

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Authors	Year	Journal	Scan Sequence	Paradigm
Blair, K. et al	2008	Am J Psychiatry	1.5T, T2, TE = 30ms, TR = 3000ms, FA = 20, fov 240mm, Voxels = 3.75x3.75x4mm, isotropic 6mm Gaussian Kernel	fMRI assessed the neural response to facial expressions in generalised social phobia and generalized anxiety disorder
Blair, K. et al	2008	Arch Gen Psychiatry	1.5T, T2, TE = 30ms, TR = 3000ms, FA = 20, fov 240mm, Voxels = 3.75x3.75x4mm, isotropic 6mm Kernel	Examined the neural response to receipt of praise or criticism in GSP; to show if patients with GSP have an increased response to the receipt of both praise and criticism and whether self-relevance modulates this relationship
Goldin, P. et al	2009	Arch Gen Psychiatry	3T, T2, TE = 30ms, TR = 1500ms, FA = 60, fov = 22cm, 4mm isotropic gaussian spatial smoothing, high pass filtering (0.011Hz)	Investigated behavioural and neural correlates of emotional reactivity and cognitive regulation in patients and controls during processing of social and physical threat stimuli
Stein, M. et al	2002	Arch Gen Psychiatry	1.5T, T2, TE = 40ms, TR = 3000ms, FA = 90, fov = 220, 7mm thick axial slices	Examined subjects with GSP with reference to greater amygdala activation in response to harsh (angry, fearful, and contemp- tous) vs accepting (happy) facial emotional expressions compared with healthy control subjects (HCs)
Sripada, C. S. et al	2009	Neuro Report	3T, T2, TE = 25ms, TR = 2000ms, FA = 77, fov = 24cm, spatial smoothing through Gaussian 8mm full-width half-maximum kernal	Authors coupled fMRI and a “trust game” to probe mentalizing in SAD
Gentili, C. et al	2008	Brain Research Bulletin	1.5T, T2, TE = 40ms, TR = 2000ms, FA 90, fov = 24cm, Gaussian kernel 8mm half-width spatially smoothed	Investigated how face processing is altered in the distributed neural system for face perception in Social Phobia
Straube, T.	2005	Neuropsychobiology	1.5T, T2, TE = 60ms, TR = 4300ms, FA 90, fov = 192mm, Voxels 1x1x1mm size, 8mm FWHM isotropic Gaussian kernel spatially smoothed, high-pass filter	Investigated specific brain activation that is associated with the processing of threat and safety signals in social phobics
Gentili, C. et al	2009	Brain Research Bulletin	1.5T, T2, Gaussian kernel 10mm half-width spatially smoothed	Investigated potential differences in default mode network (DMN) activity between Social Phobia patients and healthy controls
Yoon, K. L. et al	2007	Neuroimaging	4T, TE = 11-78ms, TR = 2000ms, FA = 90, fov = 192mm, matrix 32x32, smoothed with 8mm3 kernel, 128-s high pass filter	Authors measured amygdala reactivity to faces varying on emotional intensity in subjects with generalized social phobia (GSP) and matched healthy controls
Shah, S. G. et al	2009	J Psychiatry Neurosci	3T, T2, TE = 25ms, TR = 2000ms, FA = 77, fov = 24cm, spatial smoothing through 8mm kernal	Examined brain response to emotionally evocative images in patients with gSAD and matched healthy controls
Evans K. C. et al	2008	Depression and Anxiety	1.5T, T2, TE = 40ms, TR = 2800ms, FA = 90, smoothed with 6mm Gaussian kernel	Investigated whether an angry ‘‘schematic face’’ would evoke exaggerated amygdalar responses in SAD patients compared with healthy control

Table 2. Summary of the characteristics of included studies.

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3.

Results

The ALE revealed several prominent areas of activation (see table 3 and figure 1). The right head of the caudate nucleus, right claustrum, the left parahippocampal gyrus and amygdala, right globus pallidus, left cuneus, right putamen, right insula and the left posterior lobe of the cerebellum were all significantly more active in the patient than the control groups across the paradigms included in the meta-analysis.

		Weighted Centre				Coordinates in Talirach Space		
Brain Region	**Volume mm3**	**x**	**y**	**z**	**Extrema value**	**x**	**y**	**z**
R.Head of the Caudate	1552	20.45	6.15	22.25	0.028053103	18	6	22
R.Claustrum					0.013352961	30	8	16
L.Parahippocampal Gyrus & Amygdala	1184	-25.64	-4.55	-15.22	0.018140156	-26	-4	-16
R.Lateral Globus Pallidus	920	20.48	-4.65	-6.18	0.023225226	20	-4	-6
L.Cuneus	256	-1.25	-82.47	8.71	0.013963579	-2	-82	8
R.Putamen	232	22.28	12.73	7.3	0.013697622	22	12	8
R.Insula	224	44.67	3.2	12.53	0.012632912	46	2	14
L.Cerebellum – posterior lobe	168	-27.68	-63.91	-16.39	0.013037634	-28	-64	-16

Table 3. Summary of main results.

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4. Conclusions

The main findings of this meta-analysis were hyperactivity in SAD in the right head of the caudate nucleus, right claustrum, the left parahippocampal gyrus and amygdala, right globus pallidus, left cuneus, right putamen, right insula and the left posterior lobe of the cerebellum. These findings are consistent with the animal literature on fear neurocircuitry(Davis, 1992; J. E. LeDoux, 1993), and with previous systematic reviews and meta-analyses (Etkin & T. D. Wager, 2007; Freitas-Ferrari et al., 2010; Phan et al., 2002) in SAD.

In particular the finding that the insula(Amir et al., 2005; Gentili et al., 2008; Straube et al., 2004) and striatum (F R Schneier et al., 2000; Tiihonen et al., 1997; Warwick, Carey, Jordaan, Dupont, & D. J. Stein, 2008) are consistently implicated across several functional brain imaging studies in SAD(Freitas-Ferrari et al., 2010), reinforces their likely involvement in the pathogenesis of anxiety disorders and SAD specifically.

The insula is likely involved with interoception: the representation of the body's internal state (Craig et al., 2002, 2009), while the striatum is involved in stimulus bound behavior (Grahn, Parkinson, & Owen, 2008). One can therefore argue that hyperexcitability of the striatum results in heightened sensitivity towards stimuli and hyperactivation of the insula equally suggests a hypersensitivity to interoceptive cues. People with

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SAD may therefore be hypersensitive to how they are perceived, because they are so acutely aware of how they perceive themselves, therefore the fear of embarrassment in social and performance situations.

Additional findings include increased activation in the parahippocampal gyrus, the cuneus and the cerebellum. The parahippocampal gyrus has been implicated in contextual fear conditioning in humans and animals (Alvarez, Biggs, Chen, Pine, & Grillon, 2008). Increased activation in this area may signify that people with SAD have an aberrant attribution of fearful perceptions to nonthreatening contextual cues. The cuneus forms part of the primary visual cortex, hyperactivation of this region may therefore be attributed to increased sensitivity to visual stimuli, and hypervigilance. Recent data suggest that distinct regions of the cerebellum have a significant role in cognition and learning (Diamond, 2000). Additional research suggests that the cerebellum may be subject to experience-dependent changes in structure (Bauer, Hanson, Pierson, Davidson, & Pollak, 2009).

The amygdala has consistently been implicated across much of the basic literature on fear as well as the neuroimaging literature in anxiety disorders. In SAD, the amygdala seems to play an important role in mediating anxiety states. In people without SAD, the amygdala appears to be involved in the emotional contextualization of events. Whereas in animals, the amygdala

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has been implicated in stress-induced abnormalities of emotional memory (LeDoux et al., 1993; Davis et al., 1992). It may be that hyperactivation of the amygdala may be involved in dysfunctional emotional contextualization of memories resulting in hyperreactivity to non-threatening stimuli.

Several limitations of this study must be emphasized. First, there are relatively few studies included in this meta-analysis. The estimation provided by the results of the analysis may not be an exact estimation of the functional neuroanatomy involved in SAD. That said, this is the largest meta-analysis of SAD to date. Second, it must be noted that the functional paradigms in each of these studies used to elicit emotional responses from the subjects, were heterogeneous. Although they were in the same broad category, the task-specific differences between them might account for heterogeneity of the ALE map of brain activation that was not directly due to the specific neuropathology of SAD. Third, we were unable to control for a range of variables such as socio-demographic characteristics like age, gender, IQ and neurogenetic variables such as differences in genotyping of integral candidate genes in the scanned subjects.

Despite these limitations, the findings suggest that there are further possible directions for future research in functional neuroimaging in SAD. It would be particularly useful if different research centers used similar event related paradigms to elicit

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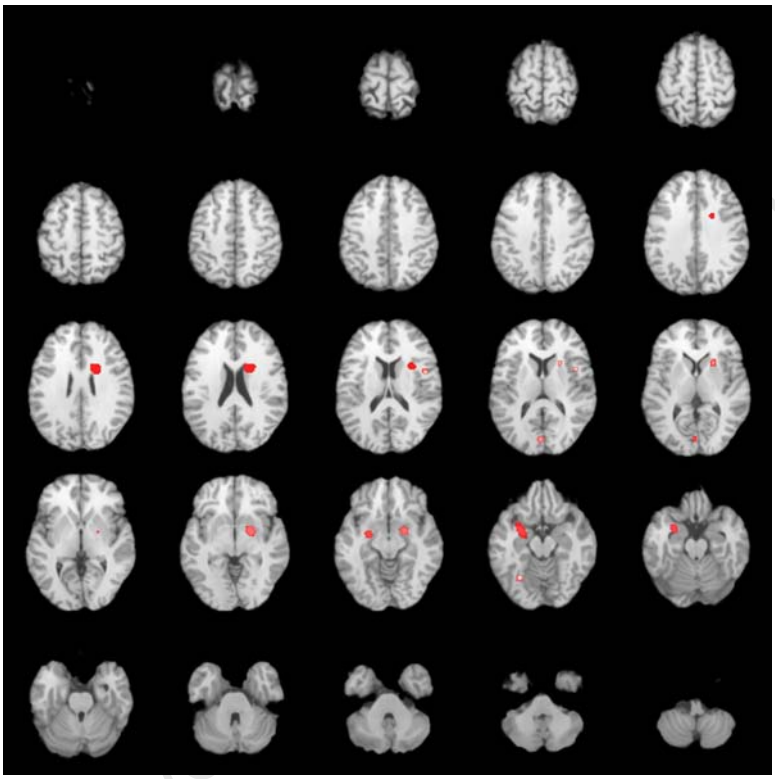
emotional responses from subjects with SAD in order to bolster interstudy concordance. This would allow for more careful analyses of socio-demographic variables.

Figure1. Summary of brain activation regions of the ALE



Chapter 3

Cortical Thickness and Subcortical Volumetric Analysis

1.

Introduction

The cerebral cortex is comprised of a thin sheet of highly folded neurons. This sheet of gray matter is constituted by 6 cytoarchitectonic layers, each as diverse as the next in terms of parallel and serial circuits within them (von Economo, C. 1929). The thickness of the cerebral cortex varies between 1 and 4.5mm with an average thickness of 2.5mm (Zilles, K. 1990). The thickness of the cerebral cortex is of great interest in psychiatric disorders. Pathological changes in the brain typically manifests as a loss of brain tissue (Takao, Abe, & Ohtomo, 2010). Cortical thinning is often region specific and the progress of the atrophy can consequently reveal a great deal about the evolution and aetiological factors of a disease process (B Fischl & Dale, 2000).

Several cortical regions have been implicated in social anxiety disorder, but no cortical thickness analysis to date has been conducted to investigate variations of cortical thickness as a possible aetiological factor in SAD. Functional MRI work has

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demonstrated that SAD is characterized by specific alterations in functional neuroanatomy; particularly increased responsiveness of amygdala to emotional stimuli, but also changes in prefrontal and other cortical areas (Freitas-Ferrari et al., 2010).

Recent studies have shown reduced resting state functional connectivity between the left amygdala and the medial orbitofrontal cortex (Hahn et al., 2011) as well as compromised integrity of the white matter tract that connects the amygdala with the medial OFC(Phan et al., 2009). These data tie in well with the increasing evidence for the role of the OFC in social information processing(Beer, John, Scabini, & Knight, 2006); a faculty that may be compromised in social anxiety disorder (Amir et al., 2005; Straube et al., 2004).

Advances in brain imaging analytic techniques have led to improved estimations of measures such as cortical thickness and subocortical volumes (B Fischl & Dale, 2000). Although functional neuroanatomical findings (those found with fMRI investigation) to date are consistent with a view that SAD is characterized by dysfunction in emotional processing (Etkin & T. D. Wager, 2007), data on structural aspects of the cortical domains that underlie these social-emotional functions are lacking. With this end in mind, we undertook high-resolution structural magnetic resonance imaging of subjects with SAD patients and matched

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controls. Based on the role of the OFC in social processing(Beer et al., 2006), longitudinal findings of a strong correlation between thinner orbitofrontal cortices and a more inhibited temperament(Schwartz et al., 2010), and the density of the reciprocal projections between the amygdala and the medial OFC(Stefanacci & Amaral, 2000), we hypothesized that the orbito-frontal cortices of SAD individuals would likely be thinner than that of control subjects.

The overwhelming majority of studies that have looked at social behavior in individuals with social anxiety have reported anomalies in face perception or face recognition, believed to be subserved, in part, by the fusiform face area (FFA)(Haxby, Hoffman, & Gobbini, 2000; Yovel & Kanwisher, 2005). Therefore, we also expected to find grey matter abnormalities in the fusiform gyrus. Finally, given the close parallels between some of the symptomology social anxiety disorder and autism spectrum disorder (ASD), including impaired social and emotional skills, we were also interested in regions that have been implicated in the social deficits in individuals with autistic disorders such as the mirror neuron system including the pars opercularis of the inferior frontal gyrus (Yamasaki et al., 2010), inferior parietal lobule and the superior temporal sulcus (Hadjikhani, Joseph, Snyder, & Tager-Flusberg, 2006).

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2.

Methodology

a. Participants

Participants with SAD were recruited from a wide range of sources (e.g. local psychiatrists and psychologists, community advocacy groups). Gender- and age-matched controls were recruited from the community and our university campus through advertisements in the media. Participants were included if they were right-handed and between 18 and 65 years of age.

Subjects with a primary diagnosis of generalized SAD (n=13) were included if they had no comorbid psychiatric disorders on the Structured Clinical Interview for DSM-IV (First et al, 1994). Healthy controls (n=13) were included if they had no history of psychiatric disorders. Neither SAD nor healthy controls were on psychotropic medication at the time of the scans, excepting for one patient who used benzodiazepines.

The institutional review board of the University of Stellenbosch approved the study protocol. Participation was voluntary and all patients and controls provided written informed consent before participation.

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b. Measures

SAD patients were administered the Liebowitz Social Anxiety Scale (LSAS) to determine symptoms severity (Liebowitz et al., 1999) and the Montgomery Äsberg Depression Rating Scale (MADRS) to assess level of depressive symptomatology(Fantino & Moore, 2009). The overall clinical severity of symptoms were assessed using the Clinical Global Impression (CGI) scale(Kadouri, Corruble, & Falissard, 2007).

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c. Structural MRI

Magnetic resonance imaging was conducted on the 3T Siemens system (MAGNETOM Allegra, Erlangen, Germany) at the Cape Universities Brain Imaging Centre. Whole brain T1-weighted 3D magnetization-prepared rapid gradient echo (MPRAGE) (Mugler and Brookeman, 1990) images were acquired using the following parameters: spatial resolution = 1.0×1.0×1.0 mm3; slices = 160; matrix = 179 × 256; TR = 2300 ms; TE = 3.93 ms; TI = 1100 ms and flip angle = 12 degrees.

d. MRI data analysis

Cortical thickness and subcortical volumes were estimated from the T1-weighted images with Freesurfer (Dale, Fischl et al.,

1999), which is a toolset for reconstructing the brain surface. Firstly cerebral white matter was segmented and the grey-white matter boundaries were estimated. Topographical defects in gray-white matter boundaries were fixed.

The gray-white matter estimate was used as the starting point for a deformable surface algorithm to search for the pial surface. The whole cortex of each individual subject was visually inspected for inaccuracies in segmentation and manual corrections were performed if it was deemed necessary. No intervention was required in this case.

Local cortical thickness was measured by calculating the differences in corresponding vertices between pial and grey-white matter surfaces. The cortex was parcellated into different regions as defined by gyral and sulcal structure. Subcortical regions were segmented into different tissue classes by applying Markov random field theory.

Thickness values for the superior temporal, medial and lateral orbitofrontal cortex, anterior cingulate and superior frontal gyrus were extracted as well as volumetric measures for the amygdala and hippocampus. These regions are implicated by previous studies as being involved with social cognition, social anxiety and autism (Etkin & T. D. Wager, 2007; Freitas-Ferrari et al., 2010; Hadjikhani et al., 2006). These values were exported to SPSS 18.0 for statistical analysis.

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e. Statistical analysis

To investigate differences in volume and cortical thickness between controls and SAD patients, an unpaired two-sample Student t-test was performed. Intracranial volume, age and gender were controlled for in the design and results were post-hoc Bonferroni corrected for multiple comparisons.

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Table 1. Demographic summary n= number of participants

	Subjects with social anxiety disorder	**Healthy controls**	**p <0.05**
n	11	13	
Age	36.6	33.6	0.261
Gender	5 female; 7 male	4 female; 9 male	0.579

Table 2. Significance values of cortical thickness value based t-tests. * Indicates statistically significant results

Region	**Hemisphere**	**p <0.05**
Temporal lobe		
Superior temporal gyrus	**L**	0,003*
	R	0,077
Superior temporal sulcus	**L**	0,143
	R	0,075
Fusiform gyrus	**L**	0,007*
	R	0,038*
Inferior temporal gyrus	**L**	0,268
	R	0,038*
Frontal lobe		
Medial orbitofrontal cortex	**L**	0,011*
	R	0,005*
Lateral orbitofrontal cortex	**L**	0,004*
	R	0,213
Anterior cingulate cortex	**L**	0,289
	R	0,074
Superior frontal gyrus	**L**	0,008*
	R	0,009*
Pars opercularis	**L**	0,091
	R	0,008*
Pars orbitalis	**L**	0,240
	R	0,157
Pars triangularis	**L**	0,083
	R	0,088
Inferior frontal gyrus	**L**	0,058
	R	0,023*
Insula	**L**	0,012*
	R	0,030*
Mid frontal gyrus	**L**	0,139

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	R	0,001*
Parietal lobe		
Inferior parietal lobule	**L**	0,518
	R	0,010*
Occipital lobe		
Inferior occipital gyrus	**L**	0,308
	R	0,333

Table 3. Volumetric measures

Region	**Hemisphere**	**p <0.05**
Amygdala	**L**	0,106
	R	0,031*
Hippocampus	**L**	0,121
	R	0,312
ICV		0.706

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3.

Results

Areas of significantly decreased cortical thickness were found in the 1) left superior temporal gyrus; 2) right amygdala; 3) right and left medial orbitofrontal cortex; 4) left lateral orbitofrontal cortex; 5) left and right superior frontal gyrus; 6) right pars opercularis; 7) right inferior frontal gyrus; 8) right inferior parietal lobule; 9) left and right fusiform gyrus; 10) right inferior temporal gyrus; 11) right middle frontal gyrus; 12) right thalamus; 13) left and right insula. There were no significant correlations between symptom severity and structural findings. Figure 1. demonstrates superficial areas of decreased cortical thickness (A, B and C)

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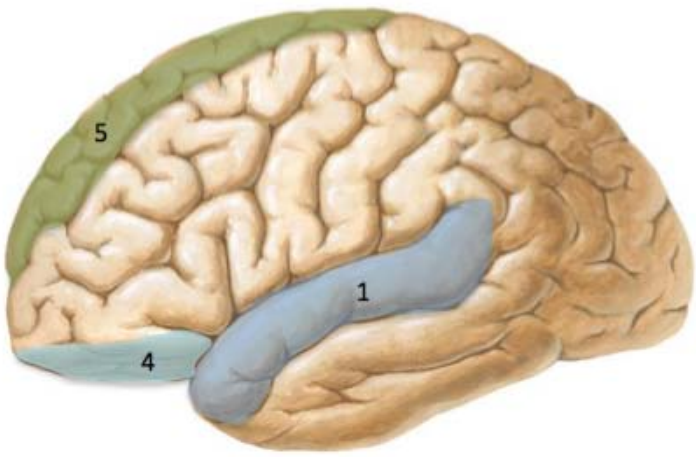
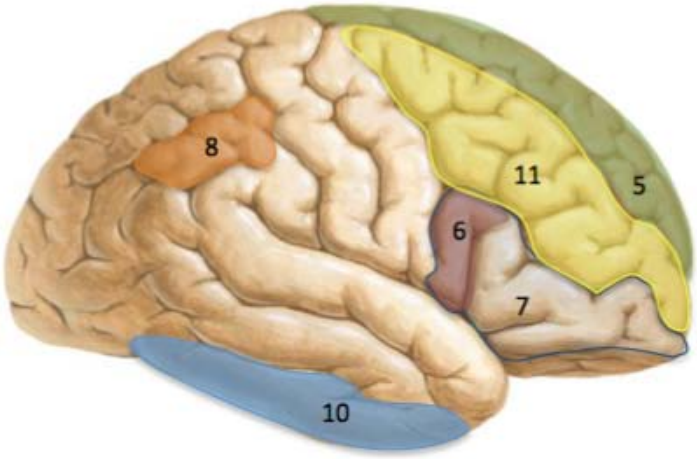
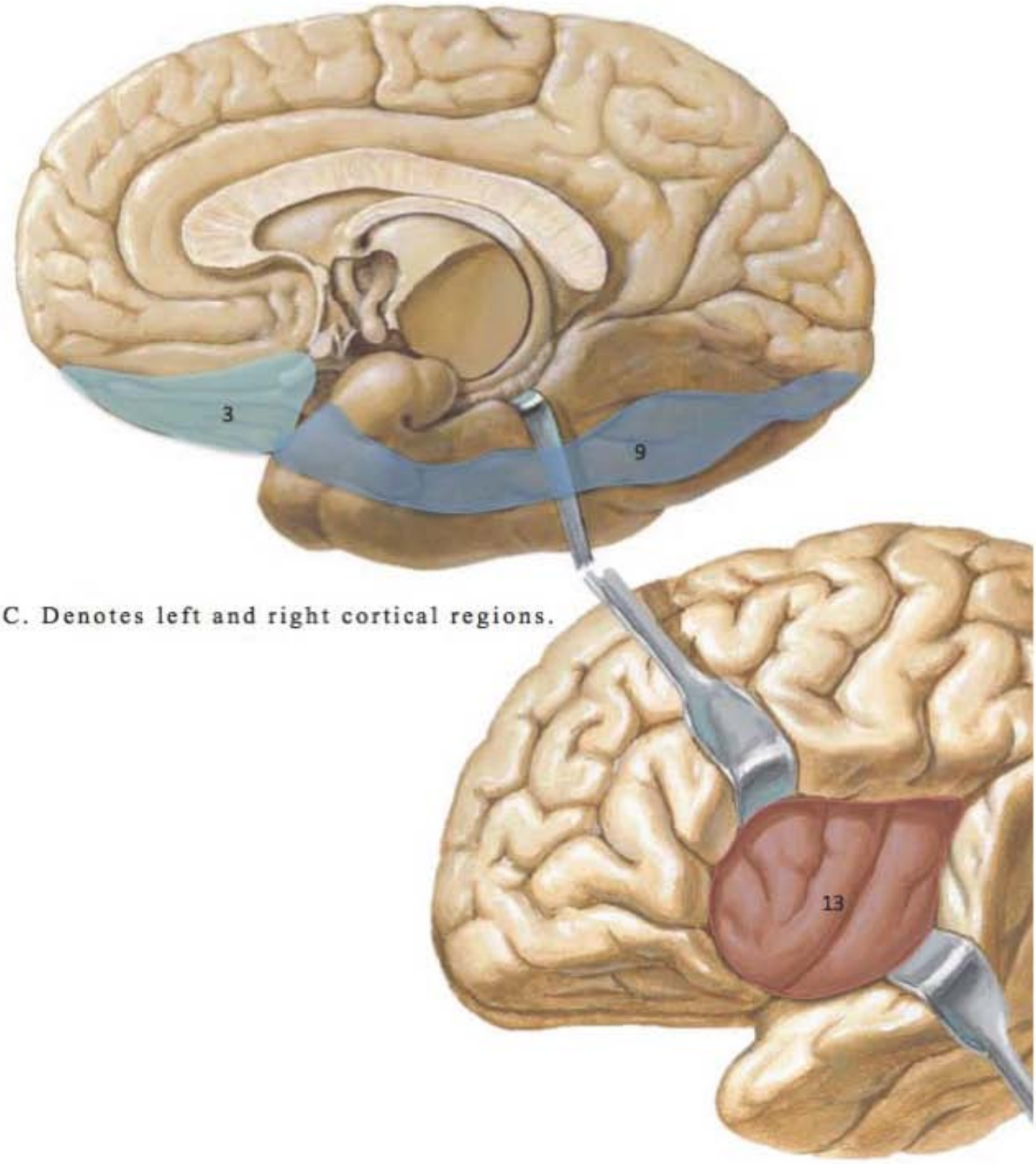


A. Left hemisphere



B. Right Hemisphere



C. Denotes left and right cortical regions.

4.

Conclusion

The main findings of this study were 1) that compared to healthy controls, SAD patients had significant cortical thinning in practically the entire lateral frontal lobe on the right. Specifically, the following regions were found to be significantly thinner: left superior temporal gyrus; right amygdala; right and left medial orbitofrontal cortex; left lateral orbitofrontal cortex; left and right superior frontal gyrus; right pars opercularis; right inferior frontal gyrus; right inferior parietal lobule; left and right fusiform gyrus; right inferior temporal gyrus; right middle frontal gyrus; right thalamus; left and right insula, and 2) that there were no significant correlations between symptom severity measures and either cortical or subcortical variations in thickness.

The structural findings here are consistent with a growing database of imaging studies that have emphasized alterations in functional neuroanatomy in SAD. While the most consistent finding from the functional imaging literature has been of amygdala hyperactivity, previous work has also emphasized alterations in prefrontal and parietal cortex(Hadjikhani et al., 2006). A small structural literature has suggested that SAD is characterized by abnormalities in cortical and subcortical volumes(Milham et al., 2005). There are, however, some

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inconsistencies in the literature; for example, we could not replicate previous findings of increased prefrontal volume in SAD (van Tol et al., 2010).

Previous work has emphasized that imaging findings in social anxiety disorder patients are consistent with a basic literature on fear conditioning in animals(J. E. LeDoux, 1993). Thus, increased amygdala activation in response to threatening emotional stimuli is taken to represent facilitation of fear responses in SAD patients. Our findings add to this body of work in two ways. First, decreased cortical thickness in several different regions suggests that a number of additional cognitive-affective processes may be impaired in SAD; these might include dysfunction in prefrontally mediated emotional regulation (Hadjikhani et al., 2006), temporal cortex mediated facial perception (Farrow et al., 2011) or others' state of mind (Baird et al., 2006).

Second, the finding of decreased amygdala volume supports the notion that neuronal atrophy may contribute to various mood and anxiety disorders, including SAD (RempelClower et al., 2007). Increased amygdalar volumes have been associated with large and complex social networks (Hadjikhani et al., 2006), it therefore stands to reason that a decrease in amygdalar volume in people with social anxiety disorder, may reflect the inverse.

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Several limitations should, however, be emphasized. In particular, the data here are correlational in nature, and so any discussion of underlying causal mechanisms is conjectural. It is possible, for example, that some cortical and subcortical changes in SAD reflect compensatory, rather than originating, changes. The fact that there were no significant associations here between symptom severity and structural findings does not strengthen a causal hypothesis. On the other hand, this finding may simply reflect a type II error, and the relatively low sample size studied here deserves emphasis. It must also be noted that various other psychosocial factors may play a confounding role in the findings such as differences in socioeconomic status, and level of education.

Despite these limitations, the data here are consistent with most previous work on SAD, and extend that work by finding reductions in cortical thickness and in amygdala volume. Further work with larger samples is needed in order to have sufficient statistical power to investigate relationships between clinical phenomenology (eg generalized vs non-generalized) and structural brain findings. It would also be useful to assess the relationship between particular genetic and environmental variables and structural measures, in order to address the causal processes at play.

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Chapter 4

Conclusion

Social anxiety disorder is one of the most prevalent (Kessler et al., 2005) disorders affecting almost 13% of the general population (Ruscio et al., 2008). People suffering from SAD may exhibit a wide range of psychosocial impairment (Simon et al., 2002) ranging from financial ruin because of not being able to function at work, to becoming reclusive (*Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR)*, 2000).

Several studies have found possible genetic contributors to the pathogenesis of SAD (Gelernter et al., 2004; Lochner et al., 2007; Ogliari et al., 2006), but no single gene factor appears to be responsible. Rather, several genetic variations may contribute to its pathogenesis. Neurotransmitter systems that may be instrumental in the expression of SAD seem to involve the dopaminergic system (Tiihonen et al., 1997), serotonergic system (Lanzenberger et al., 2007) and the glutamatergic system (Phan et al., 2005). Work on animal and human models of anxiety and its corresponding neuroanatomy (Davis, 1992; J. LeDoux, 2003) have identified some of its neural signature.

Functional neuroimaging work has attempted to further define this body of work in anxiety, and apply it to social anxiety disorder (Etkin & T. D. Wager, 2007; Freitas-Ferrari et al., 2010). Few structural studies have been conducted to investigate the

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cortical and white matter contributors to the pathogenesis of SAD.

Many questions remain about social anxiety, including what structural changes in the cerebral cortex and subcortical gray matter contribute to the pathogenesis of SAD. In this thesis, I attempted to attenuate this gap by: 1) gathering a greater perspective of the current constellation of functional neuroimaging work in SAD, by extracting activation coordinates and conducting an activation likelihood-estimate meta-analysis, and 2) to conduct an analysis on variations in cortical thickness in people with SAD versus healthy controls.

The main findings of my ALE meta-analysis were increased activation in the right head of the caudate nucleus, right claustrum, the left parahippocampal gyrus and amygdala, right globus pallidus, left cuneus, right putamen, right insula and the left posterior lobe of the cerebellum. These areas were all significantly more active in the patient than the control groups across the paradigms included in the meta-analysis. The implications of the results of the ALE meta-analysis are that these findings are consistent with the animal literature on fear neurocircuitry(Davis, 1992; J. E. LeDoux, 1993), and with previous systematic reviews and meta-analyses (Etkin & T. D. Wager, 2007; Freitas-Ferrari et al., 2010; Phan et al., 2002) in SAD.

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In particular the finding that the insula(Amir et al., 2005; Gentili et al., 2008; Straube et al., 2004) and striatum (F R Schneier et al., 2000; Tiihonen et al., 1997; Warwick et al., 2008) are consistently implicated across several functional brain imaging studies in SAD(Freitas-Ferrari et al., 2010), reinforces their likely involvement in the pathogenesis of anxiety disorders and SAD specifically.

The main results of the cortical thickness and subcortical volumetric analysis were that areas of significantly decreased cortical thickness were found in the 1) left superior temporal gyrus; 2) right amygdala; 3) right and left medial orbitofrontal cortex; 4) left lateral orbitofrontal cortex; 5) left and right superior frontal gyrus; 6) right pars opercularis; 6) right inferior frontal gyrus; 7) right inferior parietal lobule; 8) left and right fusiform gyrus; 9) right inferior temporal gyrus; 10) right middle frontal gyrus; 11) right thalamus; 12) left and right insula. There were no significant correlations between symptom severity and structural findings.

The insula is likely involved with interoception: the representation of the body's internal state (Craig, 2002, 2009), while the striatum is involved in stimulus bound behavior (Grahn et al., 2008). One can therefore argue that hyperexcitability of the striatum results in heightened sensitivity towards stimuli and hyperactivation of the insula equally suggests a hypersensitivity to interoceptive cues. People with SAD may

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therefore be hypersensitive to how they are perceived, because they are so acutely aware of how they perceive themselves, therefore the fear of embarrassment in social and performance situations.

The implications of the cortical thickness analysis are firstly, that decreased cortical thickness in several different regions suggests that a number of additional cognitive-affective processes may be impaired in SAD; these might include dysfunction in prefrontally mediated emotional regulation (Hadjikhani et al., 2006), temporal cortex mediated facial perception (Farrow et al., 2011) or others' state of mind (Baird et al., 2006). Secondly, the finding of decreased amygdala volume supports the notion that neuronal atrophy may contribute to various mood and anxiety disorders, including SAD (RempelClower et al., 2007). Increased amygdalar volumes have been associated with large and complex social networks (Hadjikhani et al., 2006), it therefore stands to reason that a decrease in amygdalar volume in people with social anxiety disorder, may reflect the inverse.

One particularly interesting finding is the hyperactivation of the left amygdala found in the ALE meta-analysis, and the decreased volume in the right amygdala in people with SAD found in the volumetric analysis. Volume of subcortical gray matter structures, as well as the thickness of the cerebral cortex

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are both indicators of neuronal integrity. Pathological changes in the brain typically manifests as a loss of brain tissue (Takao et al., 2010). Cortical thinning is often region specific and the progress of the atrophy can consequently reveal a great deal about the evolution and aetiological factors of a disease process (B Fischl & Dale, 2000). It can be hypothesized that a lack of output from the right amygdala to the right frontal lobe contributed to the loss of cortical thickness in (almost) the entire right frontal lobe as discussed earlier. It makes sense therefore to have discovered increased activity in the left amygdala only, by means of the ALE, and previous work done in functional neuroimaging in SAD. It will be useful if the white matter connectivity is assessed in the context of a functional MRI paradigm and then correlated with cortical and subcortical thickness and density measures to gain a more integrated perspective of what this finding might mean.

Several limitations must be noted however as previously discussed. Limitations applying to the ALE meta-analysis are that there are relatively few studies included in this meta-analysis. The estimation provided by the results of the analysis may not be an exact estimation of the functional neuroanatomy involved in SAD. It must also be noted that the functional paradigms in each of these studies used to elicit emotional responses from the subjects, were heterogeneous.

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Although they were in the same broad category, the task-specific differences between them might account for heterogeneity of the ALE map of brain activation that was not directly due to the specific neuropathology of SAD. Thirdly, we were unable to control for a range of variables such as socio-demographic characteristics like age, gender, IQ and neurogenetic variables such as differences in genotyping of integral candidate genes in the scanned subjects. Limitations pertaining to the cortical thickness analysis are that the data here are correlational in nature, and so any discussion of underlying causal mechanisms is conjectural. It is possible, for example, that some cortical and subcortical changes in SAD reflect compensatory, rather than originating, changes. The fact that there were no significant associations here between symptom severity and structural findings does not strengthen a causal hypothesis. On the other hand, this finding may simply reflect a type II error, and the relatively low sample size studied here deserves emphasis.

Possible future directions for research in this area: 1) It would be particularly useful if different research centers used similar event related paradigms to elicit emotional responses from subjects with SAD in order to bolster interstudy concordance. This would allow for more careful analyses of socio-demographic variables. 2) Further work with larger samples is

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needed in order to have sufficient statistical power to investigate relationships between clinical phenomenology (eg generalized vs non-generalized) and structural brain findings. Third, It would also be useful to assess the relationship between particular genetic and environmental variables and structural measures, in order to address the causal processes at play.

Since the advent of modern neuroscience and its integration with psychiatry – it became apparent, that human behavior is grounded in our neurobiology. It was always suspected as thus from the days of Descartes and the pineal gland, but never before has it become so resoundingly apparent.

We therefore require a more detailed global picture of social anxiety disorder to reach any conclusions regarding its pathogenesis. The integrity of white matter connectivity must be assessed, along with the chemical composition of key brain regions to provide more novel information on the pathogenesis of this disorder. New insights combined with existing knowledge may lead to the breakthrough that will improve the quality of life for literally hundreds of thousands of people.

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References

Alvarez, R. P., Biggs, A., Chen, G., Pine, D. S., & Grillon, C. (2008). Contextual fear conditioning in humans: cortical-hippocampal and amygdala contributions *Journal of Neuroscience*, *28*(24), 6211–6219. doi:10.1523/JNEUROSCI.1246-08.2008

Amir, N., Klumpp, H., Elias, J., Bedwell, J. S., Yanasak, N., & Miller, L. S. (2005). Increased activation of the anterior cingulate cortex during processing of disgust faces in individuals with social phobia *BPS*, *57*(9), 975–981. doi:10.1016/j.biopsych.2005.01.044

Bauer, P. M., Hanson, J. L., Pierson, R. K., Davidson, R. J., & Pollak, S. D. (2009). Cerebellar volume and cognitive functioning in children who experienced early deprivation *Biological Psychiatry*, *66*(12), 1100–1106. doi:10.1016/j.biopsych.2009.06.014

Beer, J. S., John, O. P., Scabini, D., & Knight, R. T. (2006). Orbitofrontal cortex and social behavior: integrating self-monitoring and emotion-cognition interactions *Journal of cognitive neuroscience*, *18*(6), 871–879. doi:10.1162/jocn.2006.18.6.871

Blair, K., Geraci, M., Devido, J., McCaffrey, D., Chen, G., Vythilingam, M., Ng, P., et al. (2008a). Neural response to self- and other referential praise and criticism in generalized social phobia *Archives of general psychiatry*, *65*(10), 1176–1184. doi:10.1001/archpsyc.65.10.1176

Blair, K., Shaywitz, J., Smith, B. W., Rhodes, R., Geraci, M., Jones, M., McCaffrey, D., et al. (2008b). Response to emotional expressions in generalized social phobia and generalized anxiety disorder: evidence for separate disorders *American Journal of Psychiatry*, *165*(9), 1193–1202. doi:10.1176/appi.ajp.2008.07071060

Campbell, D. W., Sareen, J., Paulus, M. P., Goldin, P. R., Stein, M. B., & Reiss, J. P. (2007). Time-varying amygdala response to emotional faces in generalized social phobia *BPS*, *62*(5), 455–463. doi:10.1016/j.biopsych.2006.09.017

Condren, R. M., Lucey, J. V., & Thakore, J. H. (2003). A preliminary study of baclofen-induced growth hormone release in generalised social phobia *Human psychopharmacology*, *18*(2), 125–130. doi:10.1002/hup.443

Cooney, R. E., Atlas, L. Y., Joormann, J., Eugène, F., & Gotlib, I. H. (2006). Amygdala activation in the processing of neutral faces in social anxiety disorder: is neutral really neutral *Psychiatry research*, *148*(1), 55–59. doi:10.1016/j.pscychresns.2006.05.003

Coupland, N. J. (2001). Social phobia: etiology, neurobiology, and treatment. *The Journal of Clinical Psychiatry*, *62 Suppl 1*, 25–35.

Craig, A. D. (2003). Interoception: the sense of the physiological condition of the body *Current opinion in neurobiology*, *13*(4), 500–505.

Cramer, V., Torgersen, S., & Kringlen, E. (2005). Quality of life and anxiety disorders: a population study *The Journal of nervous and mental disease*, *193*(3), 196–202.

Davis, M. (1992). The role of the amygdala in fear and anxiety *Annual review of neuroscience*, *15*, 353–375. doi:10.1146/annurev.ne.15.030192.002033

University of Cape Town

Desikan, R. S., Ségonne, F., Fischl, B., Quinn, B. T., Dickerson, B. C., Blacker, D., Buckner, R. L., et al. (2006). An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest *NeuroImage*, *31*(3), 968–980. doi:10.1016/j.neuroimage.2006.01.021

Detre, J. A., & Floyd, T. F. (2001). Functional MRI and its applications to the clinical neurosciences *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry*, *7*(1), 64–79.

Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR). (2000). *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR)* (4th ed. Vol. 1). Arlington, VA: American Psychiatric Association. doi:10.1176/appi.books.9780890423349

Diamond, A. (2000). Close interrelation of motor development and cognitive development and of the cerebellum and prefrontal cortex *Child development*, *71*(1), 44–56.

Eickhoff, S. B., Laird, A. R., Grefkes, C., Wang, L. E., Zilles, K., & Fox, P. T. (2009). Coordinate-based activation likelihood estimation meta-analysis of neuroimaging data: a random-effects approach based on empirical estimates of spatial uncertainty *Human brain mapping*, *30*(9), 2907–2926. doi:10.1002/hbm.20718

Etkin, A., & Wager, T. D. (2007). Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. *The American journal of psychiatry*, *164*(10), 1476–1488. doi:10.1176/appi.ajp.2007.07030504

Evans, K. C., Wright, C. I., Wedig, M. M., Gold, A. L., Pollack, M. H., & Rauch, S. L. (2008). A functional MRI study of amygdala responses to angry schematic faces in social anxiety disorder *Depression and Anxiety*, *25*(6), 496–505. doi:10.1002/da.20347

Fantino, B., & Moore, N. (2009). The self-reported Montgomery-Asberg Depression Rating Scale is a useful evaluative tool in Major Depressive Disorder *BMC psychiatry*, *9*, 26. doi:10.1186/1471-244X-9-26

Fehm, L., Pelissolo, A., Furmark, T., & Wittchen, H.-U. (2005). Size and burden of social phobia in Europe *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology*, *15*(4), 453–462. doi:10.1016/j.euroneuro.2005.04.002

Fischl, Bruce, van der Kouwe, A., Destrieux, C., Halgren, E., Ségonne, F., Salat, D. H., Busa, E., et al. (2004). Automatically parcellating the human cerebral cortex *Cerebral cortex (New York, N.Y. : 1991)*, *14*(1), 11–22.

Fischl, B, & Dale, A. M. (2000). Measuring the thickness of the human cerebral cortex from magnetic resonance images *Proceedings of the National Academy of Sciences of the United States of America*, *97*(20), 11050–11055. doi:10.1073/pnas.200033797

Fischl, B, Sereno, M. I., & Dale, A. M. (1999). Cortical surface-based analysis. II: Inflation, flattening, and a surface-based coordinate system *NeuroImage*, *9*(2), 195–207. doi:10.1006/nimg.1998.0396

Freitas-Ferrari, M. C., Hallak, J. E. C., Trzesniak, C., Filho, A. S., Machado-De-Sousa, J. P., Chagas, M. H. N., Nardi, A. E., et al. (2010). Neuroimaging in social anxiety disorder: a systematic review of the literature *Progress in neuro-*

University of Cape Town

psychopharmacology & biological psychiatry, *34*(4), 565–580. doi:10.1016/j.pnpbp.2010.02.028

Gelernter, J., Page, G. P., Stein, M. B., & Woods, S. W. (2004). Genome-wide linkage scan for loci predisposing to social phobia: evidence for a chromosome 16 risk locus *The American journal of psychiatry*, *161*(1), 59–66.

Gentili, C., Gobbini, M. I., Ricciardi, E., Vanello, N., Pietrini, P., Haxby, J. V., & Guazzelli, M. (2008). Differential modulation of neural activity throughout the distributed neural system for face perception in patients with Social Phobia and healthy subjects *Brain research bulletin*, *77*(5), 286–292. doi:10.1016/j.brainresbull.2008.08.003

Gentili, C., Ricciardi, E., Gobbini, M. I., Santarelli, M. F., Haxby, J. V., Pietrini, P., & Guazzelli, M. (2009). Beyond amygdala: Default Mode Network activity differs between patients with social phobia and healthy controls *Brain research bulletin*, *79*(6), 409–413. doi:10.1016/j.brainresbull.2009.02.002

Goldin, P. R., Manber, T., Hakimi, S., Canli, T., & Gross, J. J. (2009a). Neural bases of social anxiety disorder: emotional reactivity and cognitive regulation during social and physical threat *Archives of general psychiatry*, *66*(2), 170–180. doi:10.1001/archgenpsychiatry.2008.525

Goldin, P. R., Manber-Ball, T., Werner, K., Heimberg, R., & Gross, J. J. (2009b). Neural mechanisms of cognitive reappraisal of negative self-beliefs in social anxiety disorder *Biological Psychiatry*, *66*(12), 1091–1099. doi:10.1016/j.biopsych.2009.07.014

Grahn, J. A., Parkinson, J. A., & Owen, A. M. (2008). The cognitive functions of the caudate nucleus *Progress in neurobiology*, *86*(3), 141–155. doi:10.1016/j.pneurobio.2008.09.004

Gureje, O., Lasebikan, V. O., Kola, L., & Makanjuola, V. A. (2006). Lifetime and 12-month prevalence of mental disorders in the Nigerian Survey of Mental Health and Well-Being *The British Journal of Psychiatry*, *188*, 465–471. doi:10.1192/bjp.188.5.465

Guyer, A. E., Lau, J. Y. F., McClure-Tone, E. B., Parrish, J., Shiffrin, N. D., Reynolds, R. C., Chen, G., et al. (2008). Amygdala and ventrolateral prefrontal cortex function during anticipated peer evaluation in pediatric social anxiety *Archives of general psychiatry*, *65*(11), 1303–1312. doi:10.1001/archpsyc.65.11.1303

Hadjikhani, N., Joseph, R. M., Snyder, J., & Tager-Flusberg, H. (2006). Anatomical differences in the mirror neuron system and social cognition network in autism *Cerebral cortex (New York, N.Y. : 1991)*, *16*(9), 1276–1282. doi:10.1093/cercor/bhj069

Hahn, A., Stein, P., Windischberger, C., Weissenbacher, A., Spindelegger, C., Moser, E., Kasper, S., et al. (2011). Reduced resting-state functional connectivity between amygdala and orbitofrontal cortex in social anxiety disorder *NeuroImage*. doi:10.1016/j.neuroimage.2011.02.064

Haxby, J., Hoffman, E., & Gobbini, M. (2000). The distributed human neural system for face perception *Trends in Cognitive Sciences*, *4*(6), 223–233.

Kadouri, A., Corruble, E., & Falissard, B. (2007). The improved Clinical Global Impression Scale (iCGI): development and validation in depression *BMC psychiatry*, *7*, 7. doi:10.1186/1471-244X-7-7

University of Cape Town

Kessler, R. C., Chiu, W. T., Demler, O., Merikangas, K. R., & Walters, E. E. (2005). Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication *Archives of general psychiatry*, *62*(6), 617–627. doi:10.1001/archpsyc.62.6.617

Laird, A. R., Fox, P. M., Price, C. J., Glahn, D. C., Uecker, A. M., Lancaster, J. L., Turkeltaub, P. E., et al. (2005). ALE meta-analysis: controlling the false discovery rate and performing statistical contrasts. *Human brain mapping*, *25*(1), 155–164. doi:10.1002/hbm.20136

Lanzenberger, R. R., Mitterhauser, M., Spindelegger, C., Wadsak, W., Klein, N., Mien, L.-K., Holik, A., et al. (2007). Reduced serotonin-1A receptor binding in social anxiety disorder *BPS*, *61*(9), 1081–1089. doi:10.1016/j.biopsych.2006.05.022

LeDoux, J. (2003). The Emotional Brain, Fear, and the Amygdala. *Cellular and Molecular Neurobiology*, *23*(4/5), 727–738. doi:10.1023/A:1025048802629

LeDoux, J. E. (1993). Emotional memory systems in the brain *Behavioural brain research*, *58*(1-2), 69–79.

Liao, W., Qiu, C., Gentili, C., Walter, M., Pan, Z., Ding, J., Zhang, W., et al. (2010). Altered effective connectivity network of the amygdala in social anxiety disorder: a resting-state FMRI study *PloS one*, *5*(12), e15238. doi:10.1371/journal.pone.0015238

Lobbestael, J., Leurgans, M., & Arntz, A. (2011). Inter-rater reliability of the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID I) and Axis II Disorders (SCID II) *Clinical psychology & psychotherapy*, *18*(1), 75–79. doi:10.1002/cpp.693

Lochner, C., Hemmings, S. M. J., Seedat, S., Kinnear, C., Schoeman, R., Annerbrink, K., Olsson, M., et al. (2007). Genetics and personality traits in patients with social anxiety disorder: a case-control study in South Africa *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology*, *17*(5), 321–327. doi:10.1016/j.euroneuro.2006.06.010

Lorberbaum, J. P., Kose, S., Johnson, M. R., Arana, G. W., Sullivan, L. K., Hamner, M. B., Ballenger, J. C., et al. (2004). Neural correlates of speech anticipatory anxiety in generalized social phobia *Neuroreport*, *15*(18), 2701–2705.

Makris, N., Schlerf, J. E., Hodge, S. M., Haselgrove, C., Albaugh, M. D., Seidman, L. J., Rauch, S. L., et al. (2005). MRI-based surface-assisted parcellation of human cerebellar cortex: an anatomically specified method with estimate of reliability *NeuroImage*, *25*(4), 1146–1160. doi:10.1016/j.neuroimage.2004.12.056

Mei, L., Xiao, Z.-P., He, Y.-L., & Fan, Q. (2010). [Survey on the prevalence of anxiety disorders in internal medical outpatients at general hospital] *Zhonghua yi xue za zhi*, *90*(45), 3172–3175.

Merikangas, K. R., Ames, M., Cui, L., Stang, P. E., Ustun, T. B., Korff, von, M., & Kessler, R. C. (2007). The impact of comorbidity of mental and physical conditions on role disability in the US adult household population *Archives of general psychiatry*, *64*(10), 1180–1188. doi:10.1001/archpsyc.64.10.1180

Milad, M. R., Rauch, S. L., Pitman, R. K., & Quirk, G. J. (2006). Fear extinction in rats: implications for human brain imaging and anxiety disorders *Biological psychology*, *73*(1), 61–71. doi:10.1016/j.biopsycho.2006.01.008

University of Cape Town

Milham, M. P., Nugent, A. C., Drevets, W. C., Dickstein, D. P., Leibenluft, E., Ernst, M., Charney, D., et al. (2005). Selective reduction in amygdala volume in pediatric anxiety disorders: a voxel-based morphometry investigation *BPS*, *57*(9), 961–966. doi:10.1016/j.biopsych.2005.01.038

Ogliari, A., Citterio, A., Zanoni, A., Fagnani, C., Patriarca, V., Cirrincione, R., Stazi, M. A., et al. (2006). Genetic and environmental influences on anxiety dimensions in Italian twins evaluated with the SCARED questionnaire *Journal of anxiety disorders*, *20*(6), 760–777. doi:10.1016/j.janxdis.2005.11.004

Phan, K. L., Fitzgerald, D. A., Cortese, B. M., Seraji-Bozorgzad, N., Tancer, M. E., & Moore, G. J. (2005). Anterior cingulate neurochemistry in social anxiety disorder: 1H-MRS at 4 Tesla *Neuroreport*, *16*(2), 183–186.

Phan, K. L., Orlichenko, A., Boyd, E., Angstadt, M., Coccaro, E. F., Liberzon, I., & Arfanakis, K. (2009). Preliminary evidence of white matter abnormality in the uncinate fasciculus in generalized social anxiety disorder *Biological Psychiatry*, *66*(7), 691–694. doi:10.1016/j.biopsych.2009.02.028

Phan, K. L., Fitzgerald, D. A., Nathan, P. J., & Tancer, M. E. (2006). Association between amygdala hyperactivity to harsh faces and severity of social anxiety in generalized social phobia. *Biological Psychiatry*, *59*(5), 424–429. doi:10.1016/j.biopsych.2005.08.012

Phan, K. L., Wager, T., Taylor, S. F., & Liberzon, I. (2002). Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. *NeuroImage*, *16*(2), 331–348. doi:10.1006/nimg.2002.1087

Ruscio, A. M., Brown, T. A., Chiu, W. T., Sareen, J., Stein, M. B., & Kessler, R. C. (2008). Social fears and social phobia in the USA: results from the National Comorbidity Survey Replication *Psychological medicine*, *38*(1), 15–28. doi:10.1017/S0033291707001699

Sareen, J., Campbell, D. W., Leslie, W. D., Malisza, K. L., Stein, M. B., Paulus, M. P., Kravetsky, L. B., et al. (2007). Striatal function in generalized social phobia: a functional magnetic resonance imaging study *BPS*, *61*(3), 396–404. doi:10.1016/j.biopsych.2006.05.043

Schneier, F R, Liebowitz, M. R., Abi-Dargham, A., Zea-Ponce, Y., Lin, S. H., & Laruelle, M. (2000). Low dopamine D(2) receptor binding potential in social phobia *The American journal of psychiatry*, *157*(3), 457–459.

Schneier, Franklin R, Kent, J. M., Star, A., & Hirsch, J. (2030). Neural circuitry of submissive behavior in social anxiety disorder: A preliminary study of response to direct eye gaze. *Psychiatry Research: Neuroimaging*, *173*(3), 248–250. doi:10.1016/j.pscychresns.2008.06.004

Schwartz, C. E., Kunwar, P. S., Greve, D. N., Moran, L. R., Viner, J. C., Covino, J. M., Kagan, J., et al. (2010). Structural differences in adult orbital and ventromedial prefrontal cortex predicted by infant temperament at 4 months of age *Archives of general psychiatry*, *67*(1), 78–84. doi:10.1001/archgenpsychiatry.2009.171

Shah, S. G., Klumpp, H., Angstadt, M., Nathan, P. J., & Phan, K. L. (2009). Amygdala and insula response to emotional images in patients with generalized social anxiety disorder *Journal of psychiatry & neuroscience : JPN*, *34*(4), 296–302.

Shlik, J., Maron, E., Tru, I., Aluoja, A., & Vasar, V. (2004). Citalopram challenge in

University of Cape Town

social anxiety disorder *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*, *7*(2), 177–182. doi:10.1017/S146114570300405X

Simon, N. M., Otto, M. W., Korbly, N. B., Peters, P. M., Nicolaou, D. C., & Pollack, M. H. (2002). Quality of life in social anxiety disorder compared with panic disorder and the general population *Psychiatric services (Washington, D.C.)*, *53*(6), 714–718.

Sripada, C. S., Angstadt, M., Banks, S., Nathan, P. J., Liberzon, I., & Phan, K. L. (2009). Functional neuroimaging of mentalizing during the trust game in social anxiety disorder *Neuroreport*, *20*(11), 984–989. doi:10.1097/WNR.0b013e32832d0a67

Stefanacci, L., & Amaral, D. G. (2000). Topographic organization of cortical inputs to the lateral nucleus of the macaque monkey amygdala: a retrograde tracing study *The Journal of comparative neurology*, *421*(1), 52–79.

Stein, M. B., Goldin, P. R., Sareen, J., Zorrilla, L. T. E., & Brown, G. G. (2002). Increased amygdala activation to angry and contemptuous faces in generalized social phobia *Archives of general psychiatry*, *59*(11), 1027–1034.

Straube, T., Kolassa, I.-T., Glauer, M., Mentzel, H.-J., & Miltner, W. H. R. (2004). Effect of task conditions on brain responses to threatening faces in social phobics: an event-related functional magnetic resonance imaging study *BPS*, *56*(12), 921–930. doi:10.1016/j.biopsych.2004.09.024

Takao, H., Abe, O., & Ohtomo, K. (2010). Computational analysis of cerebral cortex *Neuroradiology*, *52*(8), 691–698. doi:10.1007/s00234-010-0715-4

Tiihonen, J., Kuikka, J., Bergström, K., Lepola, U., Koponen, H., & Leinonen, E. (1997). Dopamine reuptake site densities in patients with social phobia *The American journal of psychiatry*, *154*(2), 239–242.

Turkeltaub, P. E., Eden, G. F., Jones, K. M., & Zeffiro, T. A. (2002). Meta-Analysis of the Functional Neuroanatomy of Single-Word Reading: Method and Validation. *NeuroImage*, *16*(3), 765–780. doi:10.1006/nimg.2002.1131

van Ameringen, M., Mancini, C., & Farvolden, P. (2003). The impact of anxiety disorders on educational achievement *Journal of anxiety disorders*, *17*(5), 561–571.

van Tol, M.-J., van der Wee, N. J. A., van den Heuvel, O. A., Nielen, M. M. A., Demenescu, L. R., Aleman, A., Renken, R., et al. (2010). Regional brain volume in depression and anxiety disorders *Archives of general psychiatry*, *67*(10), 1002–1011. doi:10.1001/archgenpsychiatry.2010.121

Warwick, J. M., Carey, P. D., Jordaan, G. P., Dupont, P., & Stein, D. J. (2008). Resting brain perfusion in social anxiety disorder: a voxel-wise whole brain comparison with healthy control subjects *Progress in Neuropsychopharmacology & Biological Psychiatry*, *32*(5), 1251–1256. doi:10.1016/j.pnpbp.2008.03.017

Yamasaki, S., Yamasue, H., Abe, O., Suga, M., Yamada, H., Inoue, H., Kuwabara, H., et al. (2010). Reduced gray matter volume of pars opercularis is associated with impaired social communication in high-functioning autism spectrum disorders *Biological Psychiatry*, *68*(12), 1141–1147. doi:10.1016/j.biopsych.2010.07.012

Yoon, K. L., Fitzgerald, D. A., Angstadt, M., McCarron, R. A., & Phan, K. L. (2007).

University of Cape Town

Amygdala reactivity to emotional faces at high and low intensity in generalized social phobia: a 4-Tesla functional MRI study *Psychiatry research*, *154*(1), 93–98. doi:10.1016/j.pscychresns.2006.05.004

Yovel, G., & Kanwisher, N. (2005). The neural basis of the behavioral face-inversion effect *Current biology : CB*, *15*(24), 2256–2262. doi:10.1016/j.cub.2005.10.072