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The Role of Amygdala Subregions in the Neurobiology of Social Anxiety Disorder

Simone Mizzi

A thesis submitted in total fulfilment of the requirements for the degree of

Doctor of Philosophy

And partial fulfilment of the requirements for the degree of

Master of Psychology (Clinical)

School of Behavioural and Health Sciences

Faculty of Health Science

Australian Catholic University

28th March 2022

Declaration of Authorship and Sources

This thesis contains no material that has been extracted in whole or in part from a thesis that I have submitted towards the award of any other degree or diploma in any other tertiary institution. No other person's work has been used without due acknowledgment in the main text of the thesis. All research procedures reported in the thesis received the approval of the relevant Ethics/Safety Committees (where required).

Signed: 

Date: 28/03/2022

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Abstract

Social anxiety is characterised by fear and/or avoidance of social situations in which an individual may be scrutinised by others. Social anxiety is thought to exist as a spectrum, with individuals on the high-end experiencing frequent and severe anxiety in the context of social situations. When severe social anxiety is accompanied by distress and functional impairment, a diagnosis of social anxiety disorder (SAD) can be made. SAD is a prevalent and debilitating disorder that can be unremitting and pervasive in the absence of intervention. Current psychotherapeutic and pharmacotherapeutic treatments for SAD demonstrate limited efficacy in remitting symptoms. Therefore, it is important to achieve a better understanding of the neurobiological mechanisms implicated in this disorder and identify potential neural treatment targets to develop more efficacious treatments.

This thesis aimed to further investigate the neurobiological mechanisms implicated in SAD (vs. controls) and the associations between neural functioning and social anxiety as a dimensional symptom, with a focus on the amygdala and four of its subregions (the amygdalostriatal, basolateral, centromedial, and superficial subregions). This was due to previous findings in the neuroimaging literature in SAD having consistently implicated the amygdala, albeit with mixed findings of both increased and decreased functioning in those with SAD compared to controls. In the literature to date, however, most studies had examined the amygdala as a singular homogenous region due to methodological limitations in being able to examine the functionally and structurally distinct subnuclei that make up this region. By examining the amygdala subregions through the use of multiband functional magnetic resonance imaging (fMRI), this thesis additionally sought to determine whether the mixed findings in the literature to date may be a result of amygdala subregion-specific activity and connectivity patterns.

This was achieved through three research studies. Firstly, Study 1 involved a comprehensive systematic review that summarised the literature on resting-state neuroimaging in SAD with a focus on fMRI studies and findings specific to the amygdala and its subregions (Chapter 3). This was followed by two empirical studies which investigated the role of the amygdala and its subregions during resting-state (Study 2) and emotion processing (Study 3) fMRI paradigms (Chapters 5 and 6, respectively).

Findings from the systematic review (Study 1) highlighted the mixed findings in the resting-state neuroimaging literature in SAD to date, along with methodological limitations relating to neuroimaging acquisition and analysis. The empirical studies sought to address these limitations and demonstrated differing amygdala subregion activity and connectivity patterns at rest and during emotion processing. In the resting-state fMRI study (Study 2), there were no statistically significant differences in functional connectivity of the amygdala and its subregions in those with SAD compared to controls. However, social anxiety severity was found to be positively associated with connectivity between the superficial subregion and the supramarginal gyrus. The superficial subregion, along with the basolateral and centromedial subregions, were also implicated in the task-based emotion processing fMRI study (Study 3). In response to happy, angry, and fearful faces, those with SAD (vs. controls) had hyperactivation of the superficial subregion, hypoconnectivity between the superficial subregion and the precuneus, and hyperconnectivity between the basolateral subregion and broader brain regions (i.e., the pre/postcentral gyrus and the supramarginal gyrus). Additionally, social anxiety severity was positively associated with superficial and centromedial activation.

Overall, the findings from this thesis provide novel information to the current understanding of the neurobiology of SAD by demonstrating amygdala subregion-specific alterations. This has important implications for research, theory, and clinical practice that are

detailed in the thesis discussion (Chapter 7). Briefly, in terms of research, findings from the thesis provide support for the continuing investigation of SAD using both dimensional and categorical approaches. This was evident by the findings from the two empirical papers which demonstrated positive associations between subregional activity and connectivity patterns and social anxiety severity. With regards to theory, differences in neural patterns that were observed at rest (Study 2) and during emotion processing (Study 3) provide support for distinct neurobiological models to be constructed based on whether those with SAD are in the absence or presence of social stimuli. This is in contrast to the most recently proposed neurobiological model of SAD which was informed by a combination of resting-state and task-based fMRI data. Finally, with regards to clinical practice, the findings from this thesis provide preliminary evidence of the superficial, basolateral, and centromedial subregions of the amygdala as being potential treatment targets that can be used to inform the development of more efficacious treatments for SAD.

Research Outputs

Published Peer-Reviewed Paper as Chapter of the Thesis

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List of Commonly Used Abbreviations

ABM	Attention bias modification
ACC	Anterior cingulate cortex
ACT	Acceptance and commitment therapy
ALE	Activation likelihood estimation
ALFF	Amplitude of low-frequency fluctuations
ANOVA	Analysis of variance
CBD	Cannabidiol
CBT	Cognitive behavioural therapy
CBM	Cognitive bias modification
DSM	Diagnostic and statistical manual of mental disorders
DTI	Diffusion tensor imaging
EFMT	Emotional face matching task
EPI	Echo planar imaging
FA	Functional anisotropy
FDR	False discovery rate
fMRI	Functional magnetic resonance imaging
GCA	Granger causality analysis
gPPI	Generalised psychophysiological interactions
IAM	Integrated aetiological and maintenance model
ICA	Independent component analysis
ICD	International Classification of Disease
LSAS	Liebowitz Social Anxiety Scale
MBCT	Mindfulness-based cognitive therapy
MBSR	Mindfulness-based stress reduction

MDD	Major depressive disorder
MINI	Mini-international neuropsychiatric interview
MRI	Magnetic resonance imaging
MVPA	Multi-voxel pattern analysis
NART	National adult reading test
NMA	Network meta-analysis
PET	Positron emission tomography
PFC	Prefrontal cortex
PRISMA	Preferred reporting items for systematic reviews and meta-analyses
RDoC	Research domain criteria
ReHo	Regional homogeneity analysis
ROI	Region of interest
SAD	Social anxiety disorder
SDM	seed-based D mapping
SET	Social-evaluative threat
SIAS	Social interaction anxiety scale
SMA	Supplementary motor area
SPECT	Single-photon emission computed tomography
SPM	Statistical parametric mapping
SPSS	Statistical package for the social sciences
SSRIs	Selective serotonin reuptake inhibitors
VBM	Voxel-based morphometry

CHAPTER 1: THESIS OVERVIEW

1.1 Thesis Rationale

The need for humans to be socially connected to others has been evident over centuries and is thought to be an evolutionary-driven innate tendency (Öhman & Dimberg, 1984). Social anxiety can be considered on a spectrum. For those at the low end, connecting with others is a relatively easy task to which they engage in with limited conscious cognitive effort. However, for others in the middle-range of the spectrum, socialising can be accompanied by fear, anxiety, and/or avoidance of social situations. Individuals at the severe end of the spectrum may meet the current diagnostic criteria for social anxiety disorder (SAD) due to experiencing high levels of social anxiety which is often coupled with significant distress and functional impairment.

In the most recent National Survey of Mental Health and Wellbeing, SAD was found to be the second most prevalent anxiety disorder (following post-traumatic stress disorder) in Australian adults aged 16 to 85 years, with 8.4% of people meeting the criteria for SAD during their lifetime (Australian Bureau of Statistics, 2008). Females and adults aged 25 to 64 were more likely to have a diagnosis of SAD compared to males and younger/older adults respectively (McEvoy et al., 2011). Compared to other anxiety disorders, this survey also demonstrated that SAD had the earliest onset with a median age of 13 years (McEvoy et al., 2011). Following onset, the progression of SAD is often unremitting and pervasive, and it can be regarded as a lifelong illness in the absence of treatment (Keller, 2003). The impact of having a diagnosis of SAD is significant, causing moderate levels of psychological distress and severely interfering across different life domains (including home life, employment, study, close relationships, and social life; Slade et al., 2009).

Given its prevalence in the community and the significant interference and distress SAD can have in the lives of individuals, there has been much focus on psychotherapeutic and pharmacological interventions to treat SAD. Whilst pharmacological and psychological interventions have demonstrated some efficacy in alleviating symptoms of SAD (see Chapter

1), the response rates to such interventions remain to be relatively poor, with only approximately 30% of people with SAD experiencing remission of symptoms within a year of treatment involving psychotherapy, pharmacotherapy, or a combination of both (American Psychiatric Association, 2013). Underlying individual variability, such as at the level of neurobiological mechanisms, is thought to contribute to the limited efficacy of current treatment options (i.e., why some people with SAD respond well to treatment and others do not).

A plethora of studies investigating the neurobiology of this disorder already exists, with the amygdala commonly implicated across studies (see Chapter 2). Consequentially, the amygdala has also been explored as a treatment target for people with SAD with studies investigating the effects of treatment on the neural activity and connectivity of this region. However, discrepancies across the literature continue to be observed with regards to the role of the amygdala in SAD and it is thought that these discrepancies may be occurring due to the amygdala being investigated as a single homogenous region (Klumpp & Fitzgerald, 2018). In primates, the amygdaloid complex is conventionally divided into thirteen subnuclei which can histologically be delineated ex vivo (Amaral, 1992). Given the relatively small size of the amygdala and these subnuclei, it has been more difficult studying these in humans. In humans (the focus of this thesis), these subnuclei are grouped into larger subregions with the most commonly studied being the amygdalostriatal, basolateral, centromedial, and superficial subregions. Although limitations with neuroimaging modalities have precluded many earlier studies to fully examine these subregions, current advances in neuroimaging have allowed for increased reliability in investigating these subregions in humans using neuroimaging techniques such as multiband functional magnetic resonance imaging (fMRI).

It is known that the subregions of the amygdala are functionally and structurally distinct (Bzdok et al., 2013). Therefore, by investigating these subregions, more convergent findings

regarding the role of the amygdala in SAD may be uncovered. This will lead to a clearer understanding of the neurobiology of SAD to allow for better assessment of current treatment options and the identification of neurobiological targets to guide the development of novel treatments.

1.2 Thesis Aims

The overall objective of this thesis was to better understand the neurobiological mechanisms implicated in people with SAD. This was addressed across three research investigations. The first study was a systematic review of resting-state neuroimaging studies in SAD that aimed to summarise the current knowledge of brain dysfunctions in the absence of overt stimuli (Study 1). The review focused on the findings from seed-based fMRI studies as it was the most common analysis and imaging technique used. The second and third studies of this thesis used multiband fMRI to empirically investigate the role of the amygdala and its subregions at rest (Study 2) and during an emotion processing task (Study 3). Group differences (SAD vs. controls) of localised activity and seed-to-voxel connectivity were examined as primary outcomes. Both empirical studies also investigated associations between brain function and social anxiety severity as secondary outcomes. The findings from this thesis will serve as a basis for future research to improve diagnostic accuracy through the potential identification of novel neural markers that may improve treatment approaches and outcomes for people with SAD.

1.3 Thesis Structure

This thesis comprises of manuscripts that have been published or were prepared with publication in mind. It is presented as a thesis by publication in line with the Australian Catholic University Guidelines on the Preparation and Presentation of a Higher Degree Research Thesis for Examination. This thesis comprises of seven chapters. The current chapter (Chapter 1) provides a general overview of the thesis including a brief overview of relevant background

information, the rationale, and aims. Chapter 2 provides a critical review of the broader SAD literature including existing theoretical models of SAD, treatment options, and neuroimaging findings involving the amygdala and its subregions. Chapter 3 (Study 1) presents the published systematic review of resting-state neuroimaging in SAD. Chapter 4 includes the methodology for the empirical research papers with greater detail to what is included in the publication-focused manuscripts. Chapter 5 (Study 2) presents the submitted manuscript and empirical study investigating resting-state fMRI in SAD. Chapter 6 (Study 3) presents the prepared manuscript and empirical study examining emotion processing in SAD using task-based fMRI. Finally, Chapter 7 provides a summary and broader discussion of the findings presented throughout the thesis that includes an exploration of the limitations and the implications of the findings for theories of SAD, clinical practice, and further research.

CHAPTER 2: SOCIAL ANXIETY DISORDER

2.1 Chapter Guide

This thesis aims to better understand the neurobiological mechanisms implicated in SAD, especially that of the amygdala subregions, to potentially improve treatment targets (and thereby treatment efficacy) relating to this disorder. For this reason, the current chapter begins with a comprehensive overview of SAD to provide a clear background relating to the current diagnostic criteria, classification (categorical vs. dimensional), prevalence, onset, and impairments associated with this disorder. Current biopsychosocial models of SAD are also then outlined, followed by an overview of current psychotherapeutic and pharmacotherapeutic treatments for this disorder and potential candidates for treatment. Given the focus on the neurobiology of SAD in this thesis, the neuroimaging literature and related limitations are then reviewed in more detail that includes a specific focus on findings involving the amygdala and its subregions.

2.2 General Overview

2.2.1 Diagnostic Criteria

The Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) and the International Classification of Disease, tenth edition (ICD-10) are the most common references for diagnoses of mental health (American Psychiatric Association, 2013; World Health Organization, 1992). In the DSM-5, social anxiety disorder (SAD) is categorised as one of many anxiety disorders with 10 criteria that must be fulfilled for a diagnosis to be made (see Figure 2.1). At the core of this disorder are feelings of extreme fear and/or anxiety about one or more social situations whereby a person has the potential to be scrutinised by others (e.g., when having casual conversations with a friend or with someone unfamiliar, public speaking, or eating in a food court). The disorder is also characterised by a fear of negative evaluation of one's actions and/or behaviour by others. Given this fear and anxiety, social situations are either avoided or endured with intense fear or anxiety that lingers.

Whilst it is expected that most people will experience a level of anxiety or fear during social situations, the diagnostic criteria distinguish SAD by stating that social situations must almost always provoke fear and anxiety and that this fear or anxiety is out of proportion to the actual threat present in the social situation. The remaining criteria address the fear, anxiety, or avoidance that has been characterised by this disorder. Fear, anxiety, or avoidance of social situations must be present for at least six months or more and must cause clinically significant distress or impairment in social, occupational, or other areas of functioning considered important.

Before a diagnosis of SAD can be made, the DSM-5 criteria further specify that the fear, anxiety, or avoidance should not be attributable to other factors such as the physiological effects of a substance, other medical conditions, or other mental disorders. A ‘performance only’ specifier exists wherein the fear, anxiety, or avoidance occurs only in situations where one is required to speak or perform in public and not in non-performance social situations.

Figure 2.1

The DSM-5 Diagnostic Criteria for SAD

Diagnostic Criteria for Social Anxiety Disorder - 300.23 (F40.10)

a. Marked fear or anxiety about one or more social situations in which the individual is exposed to possible scrutiny by others. Examples include social interactions (e.g., having a conversation, meeting unfamiliar people), being observed (e.g., eating or drinking), and performing in front of others (e.g., giving a speech).

   Note: *In children, the anxiety must occur in peer settings and not just during interactions with adults.*

b. The individual fears that he or she will act in a way or show anxiety symptoms that will be negatively evaluated (i.e., will be humiliating or embarrassing; will lead to rejection or offend others).

c. The social situations almost always provoke fear or anxiety.

   Note: *In children, the fear or anxiety may be expressed by crying, tantrums, freezing, clinging, shrinking, or failing to speak in social situations.*

d. The social situations are avoided or endured with intense fear or anxiety.

e. The fear or anxiety is out of proportion to the actual threat posed by the social situation and to the sociocultural context.

f. The fear, anxiety, or avoidance is persistent, typically lasting for 6 months or more.

g. The fear, anxiety, or avoidance causes clinically significant distress or impairment in social, occupational, or other important areas of functioning.

h. The fear, anxiety, or avoidance is not attributable to the physiological effects of a substance (e.g., a drug of abuse, a medication) or another medical condition.

i. The fear, anxiety, or avoidance is not better explained by the symptoms of another mental disorder, such as panic disorder, body dysmorphic disorder, or autism spectrum disorder.

j. If another medical condition (e.g., Parkinson's disease, obesity, disfigurement from burns or injury) is present, the fear, anxiety, or avoidance is clearly unrelated or is excessive.

Note. Reprinted with permission from the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, (Copyright 2013). American Psychiatric Association.

2.2.2 Classification of SAD

There has been ongoing discussion regarding the most appropriate classification of mental disorders, which includes SAD, with categorical vs. dimensional approaches being considered in current diagnostic systems such as the DSM. Traditionally, diagnoses have been

determined categorically, with the earliest DSM-III to the current DSM-5 classifying individuals merely by the presence or absence of a disorder (American Psychiatric Association, 1980, 1994, 2013). However, alterations to the classification of SAD across the DSMs have included a change to the name, with SAD originally being termed 'social phobia' in the DSM-III. SAD was introduced as an alternative name in the DSM-IV but became the primary label in the DSM-5. The change from 'social phobia' to SAD was thought to place more emphasis on the pervasiveness and impairment that occurs in SAD (Heimberg et al., 2014). Additionally, people were more likely to recommend that someone sought treatment if they had a diagnosis of SAD as opposed to a diagnosis of social phobia (Bruce et al., 2012).

Relating to SAD, the diagnostic criteria have undergone several changes with the evolvement of the DSM. The original DSM-III defined social phobia as "a persistent, irrational fear of, and compelling desire to avoid, a situation in which the individual exposed to possible scrutiny by others, and fears that he or she may act in a way that will be humiliating or embarrassing" (American Psychiatric Association, 1980; p. 228). This definition allowed for the diagnosis of people who had a discrete and specific concern related to one situation, thereby excluding those with more widespread interpersonal fears. Later DSMs changed the criterion to include those who are anxious or fearful of most social situations. Additionally, the latest DSM-5 broadened the criterion for SAD to include the fear of negative evaluation or rejection (as opposed to just fear of humiliation or embarrassment; American Psychiatric Association, 2013). This broader focus was thought to capture a larger group of people who meet the criterion for a diagnosis of SAD who may additionally benefit from treatment (Heimberg et al., 2014).

Despite these changes, the DSM continues to primarily use a categorical approach to psychiatric diagnosis. Limitations of the categorical approach have been identified, including high rates of comorbidity across disorders, the frequent use of 'not-otherwise-specified'

diagnoses, and the need for intermediate categories to define less severe disorders (e.g., schizoaffective disorder as an intermediate for schizophrenia; American Psychiatric Association, 2013). Additionally, research using a categorical framework for mental disorders has had limited success in demonstrating unique pathophysiological and psychological mechanisms for each disorder and a lack of treatment specificity across disorders (Gillan & Daw, 2016; Krystal & State, 2014; Schaeuffele et al., 2021).

Given these limitations, a shift towards using a dimensional approach to supplement the current categorical classification of mental disorders is being increasingly argued for. This would allow for variation within each person to be considered (e.g., differential severity of individual symptoms both within and outside of a disorder's diagnostic criteria as measured by intensity, duration, or the number of symptoms) rather than relying on a simple 'yes-or-no' approach. The severity measures included in the DSM-5 allow for the assessment and tracking of disorder-specific symptom severity that can be used as a clinical tool for those who have a diagnosis and for those who fall short of meeting the full criteria of a diagnosis (American Psychiatric Association, 2013).

In the DSM-5, 11 indicators were noted as potential validators to inform new groupings/conceptualisations of disorders (i.e., family traits, neural substrates, biomarkers, abnormalities in emotional or cognitive processing, temperamental antecedents, course of illness, symptom severity, high comorbidity, genetic and environmental risk factors, and shared treatment response; American Psychiatric Association, 2013). This is aligned with the National Institute of Mental Health's Research Domain Criteria (RDoC) project which aims to develop a classification system for mental disorders based upon dimensions of observable behaviour and (neuro)biological constructs (Insel, 2014). It is expected that this dimensional approach will improve understanding of disease mechanisms underlying psychiatric disorders to enhance treatment outcomes.

2.2.3 Prevalence and Onset

The most recent World Health Organisation World Mental Health Survey Initiative examined the global prevalence rates of SAD across high (e.g., Australia, New Zealand, France), upper-middle (e.g., Brazil, Mexico, South Africa), and low/lower-middle-income countries (e.g., Colombia, Peru, Ukraine) and by region (e.g., the Americas, Western Pacific, and Africa). They found that the prevalence was positively correlated with income bracket (i.e., greater prevalence in high-income countries). Regionally, the prevalence was greatest in the Americas and Western Pacific region and lowest in Africa and Eastern Mediterranean. Globally, the estimated life-time prevalence of SAD was 4% (Stein et al., 2017) and in Australia, the life-time prevalence for SAD was 8.4% (Crome et al., 2015). The age of onset of this disorder appears to be consistent globally, with the age of greatest risk of onset ranging from mid-late adolescence to the early forties (Stein et al., 2017).

2.2.4 Impairment

SAD is associated with functional impairment across multiple domains, including educational, occupational, and social dysfunction (Kessler, 2003; Wittchen et al., 2000). In terms of education, there are many opportunities in which social anxiety symptoms are exacerbated, including having to give an oral presentation, answering questions in class, and changing friendship groups. Students with SAD may stop socialising with their classmates and friends, stop attending certain classes, or refuse to attend school altogether, which could lead to impediments in their social, cognitive, or academic development (Blöte et al., 2015). Compared to controls, they are significantly more likely to have failed a grade or to have left high school prior to graduation (Stein & Kean, 2000). In the context of occupational functioning, a diagnosis of SAD is associated with decreased workplace functioning, an increased likelihood of unemployment compared to other anxiety disorders (Moitra et al., 2011), and with triple the number of disability days compared to someone without a diagnosis

(Fehm et al., 2008). SAD can also interfere with gaining employment. Compared to those without a diagnosis of SAD, people with a diagnosis are significantly less likely to have interview skills, relevant training, work experience, and social employment skills ($p \leq 0.012$) (Himle et al., 2014).

For people with SAD, impairment is most significant with regards to interpersonal relationships and social situations (compared to functioning in employment and home life; Stein et al., 2017). Those with SAD (compared to those without this diagnosis) are significantly more likely to be lonely (Eres et al., 2021), report being more dissatisfied with their family life, friends, and leisure activities (Stein & Kean, 2000), report greater difficulty in engaging and sustaining their relationships with others (Rodebaugh, 2009), and report having significantly more interpersonal problems (Tonge et al., 2020).

Findings from a meta-analysis demonstrated that a diagnosis of SAD is a significant predictor of suicidal ideation (odds ratio = 1.38, $p < 0.01$) and suicidal attempts (odds ratio = 1.67, $p < 0.01$; Bentley et al., 2016). A diagnosis of SAD has also been shown to be economically burdensome, with results from a meta-analysis demonstrating significantly increased health care costs on an individual level compared to healthy controls (ratio of means = 1.60, $p = 0.005$; Konnopka & König, 2020). Therefore, it is evident that SAD is a debilitating psychiatric disorder that is associated with impairments across multiple domains, suicidality, and a significant cost burden.

2.2.5 Comorbidities

Comorbidities with other anxiety disorders (most commonly specific phobia, followed by agoraphobia, panic disorder, and generalised anxiety disorder) are likely for those that have lifetime or 12-month SAD (Stein et al., 2017). Major depressive disorder (MDD) is also found to be comorbid with SAD, with comorbidity rates ranging from 35-70% (Koyuncu et al., 2019). It is unclear whether SAD precedes or follows the development of MDD. The impairments in

social functioning that occur in SAD and the subsequent difficulties with forming and maintaining interpersonal relationships may lead to the development of depression (Beesdo et al., 2007). However, it is also possible that the lack of social engagement and social isolation that is associated with MDD may lead to the development of anxiety in social situations once they begin to re-engage with others (Jacobson & Newman, 2017).

SAD has also been shown to be comorbid with, and likely to precede 80% of the time, substance abuse and dependence (Chartier et al., 2003; Morris et al., 2005). Substances are often used by those with SAD to self-medicate to aid with the physiological and psychological symptoms of anxiety that arise in the context of feared social situations. As people feel they can cope better, the substance use behaviour is positively reinforced and can easily become a dependence (Morris et al., 2005).

It has also been shown that the following mental disorders can also co-occur with SAD: first-episode psychosis (comorbidity rate of 25%; Michail & Birchwood, 2009), eating disorders (Kerr-Gaffney et al., 2018), bipolar disorder (comorbidity rates ranging from 3.5-21%), obsessive-compulsive and related disorders (comorbidity rates ranging from 2-19%), and alcohol use disorder (comorbidity rates ranging up to 50%; Koyuncu et al., 2019). In Australia specifically, almost 70% of those who had SAD in the past 12 months also experienced another mental disorder in their lifetime, and comorbid disorders most frequently reported in this cohort were MDD (36.5%), generalised anxiety disorder (25.1%), and post-traumatic stress disorder (22.4%). There are significant issues that arise from the high prevalence of comorbidities with SAD, including increased severity of impairment (e.g., more time out of work) and increased emotional distress (Teesson et al., 2009), which supports the need for further understanding of the neurobiology of this disorder to aid treatment to reduce impairment.

2.3 Biopsychosocial Models of Social Anxiety Disorder

Our understanding of SAD has evolved, with novel research looking at biological, psychological, and social factors continually being integrated into revised or novel models that attempt to conceptualise the disorder in its entirety. This section will begin by covering the aetiological and maintenance factors implicated in SAD and will conclude with an overview of Wong and Rapee's (2016) most recent proposed Integrated Aetiological and Maintenance (IAM) model of SAD which draws on concepts from both the literature and previous models.

2.3.1 Aetiological Factors

Several models have attempted to define the biological, psychological, and social factors that are implicated in the aetiology of SAD (Higa-McMillan & Ebesutani, 2011; Hofmann & Barlow, 2002; Kearney, 2005; Kimbrel, 2008; Rapee & Spence, 2004; Spence & Rapee, 2016). As is evident from Table 2.1, the two most widely proposed risk factors are the importance of inherited tendencies (i.e., genetic predispositions and temperament) and life events.

In terms of inherited tendencies, a meta-analysis found that the amount of variance explained by genetic factors in SAD ranges from 0.13 – 0.60, with children of parents who have SAD being more likely to have the same disorder compared to children of parents without a diagnosis of SAD (Scaini et al., 2014). Higher estimates of heritability based on inherited tendencies were found when the outcome was social anxiety severity (58%) compared to a clinical diagnosis of SAD (27%) which provides evidence for social anxiety existing as a trait, personality-like construct (Scaini et al., 2014). Temperament is thought to be influenced by genetic factors, and behavioural inhibition (i.e., heightened sensitivity and/or avoidance of unfamiliar stimuli, situations, and people) is a dimension of temperament that has been strongly implicated in the development of SAD (Spence & Rapee, 2016). The role of parenting is tied to temperament as it has been implicated in maintaining or enhancing a behaviourally inhibited

temperament. For example, parents who use supportive strategies and encourage their children to engage with others socially and encounter novel situations are more likely to assist children in being confident in social situations compared to parents who are overly controlling, intrusive, or critical about their behavioural inhibition (Ollendick & Benoit, 2012).

Non-shared environmental factors (e.g., experiences at school, participation in extra-curricular activities, and peer relationships), more so than shared environmental factors (e.g., the family environment), have also been found to contribute to the development of SAD (Scaini et al., 2014). People with SAD are thought to have poorer social skills as a child/adolescent, which may lead to rejection from their peers and may lead them to expect negative outcomes from future social interactions (Miers et al., 2011). The role of culture has only recently been considered an important factor in the development of SAD. Specifically, culture is thought to contribute to whether socially anxious tendencies are considered an impairment (which is necessary to obtain a diagnosis of SAD), with behaviours such as withdrawal and social avoidance being more accepted in certain cultures than others (Spence & Rapee, 2016).

Table 2.1

Summary of Aetiological Models of SAD

Aetiological concepts in IAM model	Hofmann and Barlow (2002)	Rapee and Spence (2004)	Kearney (2005)	Kimbrel (2008)	Higa-McMillan and Ebesutani (2011)	Spence and Rapee (2016)
Inherited tendencies	✓	✓	✓	✓	✓	✓
Parent Behaviour	×	✓	✓	✓	✓	✓
Peer experiences	×	✓	✓	✓	✓	✓
Life events	✓	✓	✓	✓	✓	✓
Culture	×	✓	×	×	×	✓
Performance deficits due to lack of age-	×	✓	✓	✓	×	✓

appropriate social skills/ knowledge									

Note. IAM = integrated aetiological and maintenance model; ✓= has been included in the listed models; × = concept has not been included in the listed models.

2.3.2 Maintenance Factors

Prior to the IAM model, several maintenance models were prominent in the literature (Clark & Wells, 1995; Heimberg et al., 2010; Hofmann, 2007; Moscovitch, 2009; Rapee & Heimberg, 1997). It is proposed that those with SAD engage in maladaptive behavioural and cognitive processes before, during, and after social-evaluative events. Across all models, the importance of several behavioural and cognitive factors is recognised (see Table 2.2).

Behavioural factors thought to be implicated in the maintenance of SAD include avoidance, escape, and safety behaviours (Wong & Rapee, 2016). Those with SAD may steer clear of social situations that they fear or leave the situation if their social anxiety increases at the time. Alternatively, they may engage in subtle safety behaviours such as minimally participating in a conversation or standing back from others in an attempt to minimise the level of anxiety they are experiencing (Piccirillo et al., 2016). However, these behavioural factors are thought to maintain the disorder by undermining one's opportunity to learn that they are able to cope with and handle social situations despite the anxiety they are experiencing (Piccirillo et al., 2016).

Cognitive factors that are thought to maintain the disorder include self-focused attention, increased attention towards threat in the environment, and negative social-evaluation cognitions (Heimberg et al., 2010). These refer to the often negatively distorted internal mental representations that an individual with SAD forms based on how they believe they are being perceived by others, on the monitoring of their own behaviours and autonomic symptoms, and on their perception of threat in the environment. For example, when a person with SAD is telling an anecdote to a group of people, they may focus on their internal dialogue in which

they may believe that others find them boring. They may have come to this conclusion based on observing a listener glance away from them as they were vigilantly scanning the environment and may also imagine that they are visibly sweating profusely. This may lead to increased social anxiety despite it potentially being an inaccurate reflection of others' perceptions and of their experience in listening to the anecdote.

Additional cognitive factors include increased anticipatory and post-event processing which involves ruminating about the behaviours and actions in a social situation they individuals with SAD are about to partake in or had participated in (Wong, 2016). This processing is thought to help them prepare to engage with others before entering the social situation or to help them potentially better understand their behaviour and/or to detect potentially embarrassing moments that had occurred in the social situation they had previously engaged in to avoid a repetition in future scenarios. It is thought that as someone with SAD continues to engage in post-event processing, a more inaccurate view of the social situation is developed based on the individual's distorted interpretations which may contribute to increased anticipatory anxiety about upcoming social situations, thus maintaining the disorder (Heimberg et al., 2010).

Table 2.2

Summary of Maintenance Models of SAD

Maintenance concepts in the IAM model	Clark and Wells (1995)	Rapee and Heimberg (1997)	Hofmann (2007)	Moscovitch (2009)	Heimberg et al. (2010)
Performance deficits due to lack of age-appropriate social skills/knowledge	×	✓	✓	×	×
Performance deficits due to anxiety	✓	✓	×	×	✓
Negative social-evaluation cognitions	✓	✓	✓	✓	✓
Self-focused attention	✓	✓	✓	✓	✓
Attention towards threats in the environment	×	✓	×	×	✓

Anticipatory processing	✓	✓	×	×	✓
Post-event processing	✓	✓	✓	×	✓
Avoidance	✓	✓	✓	✓	✓
Escape	✓	✓	✓	✓	✓
Safety behaviours	✓	✓	✓	✓	✓
Cognitive avoidance	×	×	×	×	✓

Note. IAM = integrated aetiological and maintenance model.; ✓= has been included in the listed models; × = concept has not been included in the listed models.

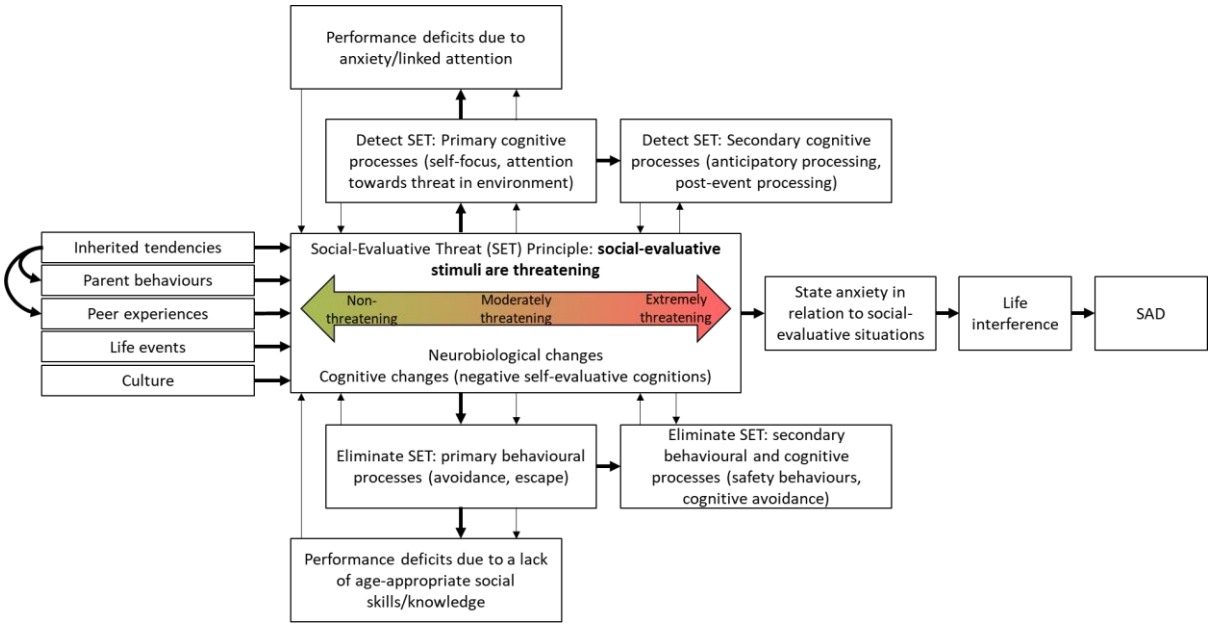
2.3.3 Integrated Model

Whilst previous models focused on either aetiology or maintenance on SAD as standalone concepts (Clark & Wells, 1995; Heimberg, 2009; Higa-McMillan & Ebesutani, 2011; Hofmann & Barlow, 2002; Hofmann, 2007; Kearney, 2005; Kimbrel, 2008; Moscovitch, 2009; Rapee & Heimberg, 1997; Rapee & Spence, 2004; Spence & Rapee, 2016), the Wong and Rapee (2016) IAM model of SAD (see Figure 2.2) proposes how risk factors for SAD increased the likelihood of disorder onset and the emergence of maintaining factors. Wong and Rapee (2016) developed the concept of the social-evaluative threat (SET) principle, which is a continuum ranging from low to high threat values on which the population exists. Aetiological factors (including temperament and parent behaviours) are thought to determine the level of one's threat value and increases in threat value are hypothesised to lead to the development of primary cognitive and behavioural processes to detect and eliminate social-evaluative threat. Primary cognitive processes involve increased self-focus and increased attention towards the social environment, whilst primary behavioural processes include escape or avoidance of social situations. These primary cognitive and behavioural processes are thought to maintain the threat value, increase performance deficit, and elicit the development of secondary cognitive and behavioural processes that further detect and eliminate social-evaluative threat. Secondary cognitive processes include anticipatory and post-event processing and cognitive avoidance, whilst secondary behavioural processes include safety behaviours or subtle avoidance. These secondary cognitive and behavioural processes are also thought to maintain the threat value

and contribute to performance deficits. It is thought that individuals on the upper end of the SET principle who experience social situations as highly threatening will experience more frequent and severe anxiety in such situations. Thus, they are more likely to experience interference with their functioning and are more likely to meet the criteria for SAD.

Figure 2.2

The Integrated Aetiological and Maintenance Model for SAD



Note. Figure adapted from original, see Wong and Rapee (2016). Bold arrows indicate aetiological pathways, other arrows indicate maintenance pathways.

2.4 Treatments for SAD

It has been recommended that the first-line treatment for SAD is either pharmacotherapy, cognitive behavioural therapy (CBT), or a combination of the two (Andrews et al., 2018; National Institute for Health and Care Excellence [NICE], 2013). CBT has been considered to be a more efficacious treatment than pharmacotherapy (antidepressants; Hofmann & Bögels, 2006), however, a recent meta-analysis reported little to no difference in the efficacy of treatment when comparing psychotherapy and pharmacotherapy in those with

SAD (Cuijpers et al., 2013). This section will review the treatments for SAD with brief summaries of both psychotherapeutic and psychopharmaceutical approaches, as well as potential candidates for treatment that have been more recently investigated.

2.4.1 Psychotherapy

2.4.1.1 Cognitive Behavioural Therapy

CBT has been widely investigated as a treatment of SAD that focuses on addressing the cognitive and behavioural factors that maintain this disorder. It is commonly used in clinical practice, with evidence from meta-analyses demonstrating its efficacy in reducing symptoms of SAD (see Table 2.3). In research, a manualised approach that is commonly used is the guidebook "Managing Social Anxiety: A Cognitive-Behavioral Therapy Approach" (Hope, 2006). In a clinical setting, these guidelines form the basis of CBT but can be adopted more flexibly. This CBT manual consists of 16 sessions that can be delivered over a duration of 16-20 weeks. It involves homework components that clients are expected to complete between sessions. The following sections outline the critical components of CBT.

2.4.1.1.1 Psychoeducation. One of the first steps involved in CBT is the provision of psychoeducation about the aetiology, maintenance factors, and the rationale for treatment for SAD. Additionally, a fear and avoidance hierarchy would be created which includes a list of situations in which the client experiences social anxiety.

2.4.1.1.2 Cognitive restructuring. This involves identifying and challenging the automatic thoughts that are contributing to the maintenance of this disorder. These thoughts are often related to negative self-evaluations, concerns that others will attach a negative label, worrying about performance inadequacy, concerns that something negative would happen for no particular reason, and concern about experiencing anxiety symptoms (either visible to others or not) or negative emotions. Upon identification of an automatic thought, the client is also asked to identify the emotions and behaviours that arise with the thought. The 'challenging'

component of the process involves moving from accepting automatic thoughts as facts to questioning them and considering the possibility of alternative ways of thinking about things. Any cognitive distortions (also known as 'thinking errors') that the client engages in are identified at this stage and 'disputing questions' can be used to challenge the automatic thoughts further. The final step in this process is to generate an alternate thought (also known as a 'rational response') to challenge the automatic thought.

2.4.1.1.3 Exposure. During the exposure component of CBT, the client and clinician pick some automatic thoughts that have a behavioural element to target with exposure. At this stage, situations identified as part of the fear and avoidance hierarchy that was created during the initial stages of treatment can be drawn upon. A behavioural goal is mutually agreed upon, and the therapist would assist in supporting the client to attempt the goal. The exposure would typically last 5 to 10 minutes. Afterwards, debriefing typically occurs whereby the client and the clinician reflect on their perspectives of the experience.

2.4.1.1.4 Barriers. Despite being effective, clinical trials demonstrate that 40-50% of people with SAD show minimal or no improvement after engaging in CBT (Hofmann & Bögels, 2006) and drop-out rates for people with SAD are high, with 18.3% of people dropping out during CBT treatment (Swift & Greenberg, 2014)s. Due to these limitations, other psychotherapeutic approaches have been introduced as potential alternative treatments for SAD and are described below.

2.4.1.2 Third-Wave CBT

The "third wave" of CBT describes a change in approach whereby the focus of the intervention is on one's relationship to their thoughts and emotions, rather than on the content of their thoughts as is the focus in classical CBT (Hayes, 2004). Approaches in this category of interventions that have been investigated in the context of treating those with SAD are described below. The efficacy of each treatment can be found in Table 2.3.

2.4.1.2.1 Acceptance and Commitment Therapy. Acceptance and commitment therapy (ACT) incorporates mindfulness and acceptance interventions and is based on the premise that any psychopathology is a result of 'fusing' to distressing thoughts and feelings and the subsequent struggle that people typically experience in attempting to control or eliminate these often unpleasant experiences (which is referred to as 'experiential avoidance'; Hayes et al., 1999). The goal of treatment is to learn how to experience fully and without defence to allow one to achieve their value-based goals. Mindfulness plays a role in helping someone be able to experience fully, being defined as the non-judgemental, moment-to-moment awareness of one's present experience (Kabat-Zinn, 2015).

Specifically, in the context of SAD, four major concepts of ACT are explored in treatment. The first phase involves the identification of values and goals to motivate the individual to persist with treatment despite how difficult they may find it. The second phase involves the concept of 'creative hopelessness' to help those with SAD understand that their past behaviours (e.g., avoidance, escape, and safety behaviours) are futile in reducing their social anxiety. Following this, the second phase introduces the concept of 'acceptance/willingness' to experience anxiety in the context of feared social situations to be able to attain their value-based goals (rather than trying to reduce their anxiety as is the goal of exposure in CBT). The third phase involves the concept of 'cognitive defusion' which involves exercises (e.g., mindfulness) that aim to help those with SAD separate from their internal experiences. An example of a defusion technique involves adding the phrase 'I'm having the thought that…' as a prefix to automatic negative thoughts that occur for those with SAD to give them a sense of separation from their internal cognitive processes (Dalrymple & Herbert, 2007).

2.4.1.2.2 Mindfulness-Based Cognitive Therapy and Mindfulness-Based Stress Reduction). Both mindfulness-based cognitive therapy (MBCT) and mindfulness-based stress

reduction (MBSR) have a significant focus on training in mindfulness. They are both typically provided over the course of 8 weekly session, with MBSR including an additional one-day meditation retreat (Kabat-Zinn & Hanh, 2009; Segal et al., 2002). MBCT and MBSR are thought to help those with SAD by training individuals to gain attentional control and increase tolerance of unpleasant emotions that are experienced during social situations, with the main components of treatment being mindfulness meditation techniques such as body scans, mindful yoga exercises, and sitting meditation (Sipe & Eisendrath, 2012). MBCT differs from MBSR through its incorporation of components derived from CBT (e.g., increase awareness of negative automatic thoughts to decrease engagement in them; Segal et al., 2002).

2.4.1.3 Cognitive Bias Modification and Attention Bias Modification

Cognitive bias modification (CBM) aims to reduce negative cognitive biases that are thought to occur when individuals with SAD process emotionally salient stimuli. Attention bias modification (ABM) is an intervention that focuses on attempting to reduce social anxiety symptoms by retraining attention through the reduction of attentional bias to threat that is thought to underlie the maintenance and aetiology of this disorder (MacLeod & Mathews, 2012). Both therapies typically involve participation in a modified version of the visual dot-probe task whereby a threatening and non-threatening stimulus is displayed in two distinct locations on a screen for a brief duration. This is followed immediately by a target stimulus (i.e., a dot) that appears in the location occupied previously by either the threatening or non-threatening stimulus to which the individual must respond to (MacLeod et al., 2002). ABM differs to CBM in that the target stimulus most often appears in the place of the non-threatening stimulus (80-100% of the time; MacLeod & Mathews, 2012). Over time, individuals are SAD are trained to respond faster to the dot that replaces the non-threatening stimulus which indicates that they are no longer biased to attending threatening stimuli (Heeren et al., 2015).

Table 2.3

Key findings of Systematic Reviews and Meta-Analyses Examining Psychological Treatments of SAD

Author	Findings
Heeren et al. (2015)	ABM had a small significant effect on self-reported SAD symptoms (n = 11; g = 0.36)
Norton et al. (2015)	MBSR: significant within-group decrease in social anxiety symptoms (n = 3; d = 0.41 – 1.54), depression (n = 3; d = 0.27 – 0.67), functional impairment (n = 2; d = 0.41 – 1.39); significant within-group increase in quality of life (n = 2; d = 0.44 – 0.54) MBCT: significant within-group decrease in social anxiety symptoms (n = 2; d = 0.32 – 0.85) ACT: significant within-group decrease in social anxiety symptoms (n = 2; d = 0.57 – 1.24)
Cuijpers et al. (2016)	CBT reduced self-reported social anxiety symptoms (SIAS: n = 7; g = 0.80)
Liu et al. (2017)	CBM had a small significant effect on SAD symptoms (n = 25; g = 0.26).

Note. ABM = attention bias modification; ACT = acceptance and commitment therapy; CBT = cognitive behavioural therapy; CBM = cognitive bias modification; d = Cohen's d; g = Hedges' g; MBCT = mindfulness-based cognitive therapy; MBSR = mindfulness-based stress reduction; n = number of studies; SAD = social anxiety disorder; SIAS = social interaction anxiety scale.

2.4.2 Pharmacotherapy

Pharmacotherapy involves targeting either a single or combination of neurotransmitter systems including the serotonergic, dopaminergic, and noradrenergic systems. There is a plethora of evidence demonstrating the involvement of these systems across a range of anxiety and depressive disorders including SAD (Furmark, 2009; Marcin & Nemeroff, 2003; Neumeister et al., 2005; Schneier et al., 2009; Stein et al., 2002). The serotonergic and noradrenergic systems have been of particular focus due to their extensive projections through cortical and subcortical structures of the brain (Ressler & Nemeroff, 2000).

Pharmacotherapy has been used as a treatment for SAD, administered alone or in conjunction with psychotherapeutic treatment. There have been several meta-analyses published on this topic in the last 10 years. For a summary of the methods and main findings of each meta-analysis, see Table 2.4. Most meta-analyses employed standard pairwise meta-

analytical techniques from which conclusions can be made about interventions that are compared directly with one another but do not allow for an understanding of comparisons of each medication class compared to one another. This limitation was addressed by network meta-analysis (NMA) techniques that allow for comparisons of all interventions with one another, even if they have not been compared head-to-head in individual studies (Mavridis et al., 2015). Williams et al. (2020) has completed the most recent NMA, and the results from this review will be discussed below. It provides an update from the previous NMA completed by Mayo-Wilson et al. (2014), and improves on it by ranking interventions and using a standardised system to assess the quality of the evidence to assist in their conclusion of best treatment options.

Table 2.4

Overview of Meta-Analyses of Pharmacotherapy for SAD

Author (year)	Approach/Methodology	Findings	
		Number of studies (number of participants)	Results
Mayo-Wilson et al. (2014)	Studies were included if they had a sample of adults with SAD that were given oral drugs.	51 (5,042)	All pharmacological interventions (aside from noradrenergic and specific serotonergic antidepressants) had greater effects on outcomes compared to waitlist (class effect SMD ranging from 0.81 – 1.01). MAOIs had the largest effect (1.01), followed by benzodiazepines (0.96), and SSRIs/SNRIs (0.91).
Curtiss et al. (2017)	Studies were included if they had a sample of adults with SAD, more than two doses of medication, a pill placebo control condition, and a measure of treatment outcome.	52 (12,153)	Pharmacotherapy contributed to greater symptom remission than pill placebo (Hedges' g = 0.41; 95% CI = 0.36-0.46; $p < 0.001$). Treatment efficacy was not significantly moderated by class of pharmacotherapy ($p > 0.05$).
Williams et al. (2017)	Studies were included if they had a sample of	66^ (11,597)	The following classes of drugs were significantly more effective in

	adults with SAD, they were administered a medication to treat SAD and that this was compared to an active or non-active placebo		treating SAD compared to placebo: anticonvulsants/GABAs (RR = 1.60), benzodiazepines (RR = 4.03), MAOIs (RR = 2.36), RIMAs (RR = 1.83),and SSRIs (RR = 1.65).
Williams et al. (2020)	Studies were included if they had a randomised, double-blind, controlled design that compared any pharmacological intervention with placebo or another intervention in the treatment of SAD.	67* (12,122)	Results outlined in Table 2.5

Note. ^ = 63 studies included in the meta-analysis; * = 21 studies including in the meta-analysis; CI = confidence interval; GABA = gamma aminobutyric acid; MAOIs = monamine oxidase inhibitors; RIMAs = reversible inhibitors of monoamine oxidase-A; RR = relative risk; SAD = social anxiety disorder; SMD = standard mean difference; SNRIs = serotonin and norepinephrine reuptake inhibitors; SSRIs = selective serotonin reuptake inhibitors.

In the most recent systematic meta-analysis, results were combined and compared from over 65 randomised controlled trials that compared the effects of pharmacotherapeutic treatments of SAD in adults (see Table 2.5) Williams et al. (2020) concluded that there was only a small difference between the drugs and placebo in treating SAD and reducing symptoms. Paroxetine (an SSRI) was found to be most effective in reducing social anxiety symptoms compared to placebo (based on the highest quality of evidence compared to other medication classes), however, was also associated with increased dropouts due to adverse side effects (odds ratio = 2.56). Based on the rankings completed, olanzapine (an anti-psychotic) was found to perform better than other medications in reducing social anxiety symptoms however this was based on the findings from one study and thus should be interpreted with caution.

Table 2.5

Outline of Pharmacotherapy Treatments for SAD

Drug class	Neuro-transmitter(s) implicated	Mechanism of Action	Drug Name	Efficacy of treatment^	Drop-out rates*
Anticonvulsants/ GABAs	GABA	Work as calcium modulator to inhibit excitatory neurotransmitter release	Pregabalin Gabapentin Levetiracetam	n.r. -11.50 -3.82	1.55 n.r. 0.98
Anti-psychotics	Serotonin Dopamine	Inhibit serotonin and dopamine reuptake by blocking the receptors	Olanzapine	-37.80	1.12
Benzodiazepines	GABA	Enhance affinity to GABA type A receptors	Clonazepam Bromazepam	-23.60 -31.60	1.03 0.48
Beta-blockers	Adrenaline	Block beta1 receptors in vascular smooth muscle and the heart to inhibit actions of catecholamines thereby inhibiting sympathetic stimulation	Atenolol	n.r.	2.76
Irreversible MAOIs	Dopamine Noradrenaline Serotonin	Unclear; may inhibit MAO (enzyme in the brain) which is thought to inactivate neurotransmitters thus increasing dopaminergic, noradrenergic, and serotonergic concentrations in neuronal synapses; MAOIs bind to and inactivate MAO for the life of the molecule	Phenelzine	-8.65	1.71
RIMAs	Dopamine Noradrenaline Serotonin	Similar to MAOIs but recovery of enzyme activity is relatively faster as these drugs have low affinity for the enzyme	Brofaromine Moclobemide	-8.10 -8.51	0.95 0.90
SNRIs	Serotonin Norepinephrine	Inhibits reuptake of catecholamines; weakly inhibit the reuptake of dopamine	Venlafaxine Mirtazapine	30.47 -14.53	0.83 2.07

SSRIs	Serotonin	Inhibit serotonin reuptake to increase the bioavailability of serotonin which binds to and activates various receptors; minimal effect on norepinephrine and dopamine reuptake	Paroxetine	-15.89	0.94
			Fluvoxamine[#]	-2.12	1.51
			Sertraline	-17.45	0.87
			Fluoxetine	n.r.	0.59
			Citalopram	n.r.	n.r.
			Escitalopram	-8.05	0.99

Note. ^ = effect size reported as mean difference scores if there was a significant change in symptoms of social anxiety in the treatment group compared to placebo; * = effect size reported as odds ratio for drop-out rates for any reason; # = the only medication that had significantly greater dropout rates and was one of the most harmful interventions; GABA = gamma aminobutyric acid; MAO = monoamine oxidase; MAOIs = monoamine oxidase inhibitors; n.r. = not reported in meta-analysis; RIMAs = reversible inhibitors of monoamine oxidase-A; SNRIs = serotonin and norepinephrine reuptake inhibitors; SSRIs = selective serotonin reuptake inhibitors.

2.4.3 Potential candidates for treatment

The limited efficacy and the potential adverse side effects of the aforementioned pharmacological treatments warrant that further pharmacological tools be investigated as potential alternative treatments for SAD. Several of these are described in the following section.

2.4.3.1 Cannabidiol

Cannabidiol (CBD) is derived from the cannabis plant, and unlike tetrahydrocannabinol, is devoid of producing euphoria and cognitive/perceptual alterations. It has been demonstrated to have potential in treating symptoms of psychosis, mood and anxiety disorders, neurodegenerative disorders, and neurodevelopmental disorders (Bonaccorso et al., 2019; Fernández-Ruiz et al., 2013; Kwan Cheung et al., 2021). In terms of mechanistic action, CBD is thought to mainly target the endocannabinoid system including cannabinoid receptor 1 which is densely expressed in neural regions associated with emotion and anxiety (Premoli et al., 2019). Evidence suggests that CBD acts as a non-competitive antagonist/negative allosteric modulator of the cannabinoid receptor 1 receptor and as an activator for the serotonin 1A receptor (Mechoulam et al., 2007). In terms of therapeutic potential, CBD is thought to have minimal adverse side effects (most commonly reported are diarrhea, somnolence, nausea,

fatigue, decreased appetite, and headaches) compared to SSRIs and SNRIs across people with differing psychiatric diagnoses (Mandolini et al., 2018).

Two clinical trials have investigated CBD as a treatment for SAD (Bergamaschi et al., 2011; Crippa et al., 2011). CBD (compared to placebo) was found to significantly reduce subjective anxiety, discomfort, and cognitive impairment during a simulation public speaking test in 24 treatment-naïve SAD participants (Bergamaschi et al., 2011. In 10 treatment-naïve participants with SAD, CBD (compared to placebo) significantly decreased their subject anxiety associated with undergoing a single-photon emission computed tomography (SPECT) scan {Crippa, 2011 #6798). These preliminary findings show that CBD may have an anxiolytic effect for people with SAD, but the small sample sizes and single-dose designs used warrant further research to determine whether this treatment could be effective for people with a diagnosis of SAD.

2.4.3.2 Oxytocin

Oxytocin, a neuropeptide that is commonly referred to as a prosocial drug, has been investigated as a potential treatment for SAD. It has been shown to reduce anxiety and depression in animal models (Ring et al., 2006; Wang et al., 2018). This effect is primarily due to oxytocin modulating the hypothalamic-pituitary-adrenal axis and the opioidergic and dopaminergic systems in limbic brain structures. In humans, oxytocin is shown to have an inhibitory effect on the adrenocorticotropic hormone and cortisol levels and alterations in endogenous oxytocin levels have been observed in post-traumatic stress disorder, mood disorders, and eating disorders (Frijling et al., 2015; Plessow et al., 2018; Scantamburlo et al., 2007; Schneider et al., 2021).

Seven randomised-controlled studies have investigated the effects of intranasal oxytocin administration in people with SAD (Dodhia et al., 2014; Fang et al., 2014; Fang et al., 2017; Gorka et al., 2015; Guastella et al., 2009; Labuschagne et al., 2010, 2012). Oxytocin

(vs. placebo) was found to significantly attenuate the heightened activity in the amygdala (in response to fearful faces) and the medial prefrontal cortex (PFC)/anterior cingulate cortex (ACC; in response to angry faces) observed in SAD (vs. controls; Labuschagne et al., 2010, 2012). Additionally, hypoconnectivity of the amygdala with the rostral ACC and medial PFC in SAD (vs. controls) during a resting-state fMRI study was enhanced after the administration of a single dose of oxytocin (vs. placebo; Dodhia et al., 2014). Further studies are required with larger sample sizes and repeated administrations of oxytocin to determine whether it may be an alternative efficacious treatment option for SAD.

2.5 Neurobiology of SAD

Many modalities have been used to investigate the neurobiology of SAD, including studies that have used electroencephalogram, positron emission tomography (PET), and SPECT. The most common modality to delineate the neurobiological underpinnings of SAD has been MRI, which was also used in this thesis. For this reason, the following section will provide a broad overview of the MRI findings in SAD to date, starting with an overview of structural MRI studies. Subsequently, resting-state fMRI studies will be briefly discussed with reference to the systematic review on resting-state fMRI in SAD (Study 1) detailed in Chapter 3. It is noted that Study 2 (Chapter 5) of this thesis draws on this literature. Then, task-based fMRI studies are reviewed with a focus on emotion processing tasks as Study 3 (Chapter 6) builds on this topic. This will be followed by a review of the treatment-focused fMRI studies in SAD that reviews the effects of pharmacological agents on brain functioning. Lastly, drawing from the aforementioned literature, this section will conclude with a summary of the amygdala and amygdala subregion findings in SAD to date given the primary focus of the amygdala and its subregions in the empirical studies (Study 2 and Study 3). Overall limitations of the neuroimaging literature thus far will be discussed to conclude this section.

2.5.1 Structural and Microstructural MRI studies in SAD

Structural MRI studies examine the volume and density of neural grey matter. Several analysis techniques can be used, however, the most common is voxel-based morphometry (VBM) in which the brain is segmented in white matter, grey matter, and cerebrospinal fluid, and an estimate is made of the volume/density at each voxel in the brain (Lerch et al., 2017). Microstructural MRI, in contrast, examines the distribution of water molecules to identify the intrinsic features of structures, with diffusion tensor imaging (DTI) being most commonly (Basser & Pierpaoli, 2011).

Wang et al. (2021) completed a meta-analysis on structural MRI differences in those with SAD compared to controls that included 12 studies using anisotropic effect-size seed-based D mapping (SDM) analysis. This method entails the combination of statistical parametric maps and peak coordinates to create maps of whole-brain effect size and variance, which are then used to perform voxel-wise random-effects analysis. This differs from traditional meta-analyses techniques that are based on peak coordinates alone, with the addition of statistical parametric maps thought to increase the sensitivity of analyses (Radua et al., 2012). Relative to controls, those with SAD were observed to have larger grey matter volume in the right superior frontal gyrus (SDM Z = 1.498), the left precuneus (SDM Z = 1.274), right angular gyrus (SDM Z = 1.148), and the right supplementary motor area (SDM Z = 1.225), and also smaller grey matter volumes in the left thalamus (SDM Z = -1.234) and left lenticular nucleus (SDM Z = -1.314); Wang et al., 2021).

However, anisotropic effect-size SDM has been criticised for having low statistical power in the presence of multiple effects and relying on the assumption that all voxels are independent of one another (Albajes-Eizagirre & Radua, 2018). To address the limitations of this method, Sheng et al. (2021) repeated the above meta-analysis using a novel coordinate-based meta-analysis method, SDM with the permutation of subject images, to validate the results. This method imputes subject images to allow for a subject-based permutation test. This

determines whether activation of a voxel is different from zero, rather than comparing whether activation of a voxel is greater than other voxels (Albajes-Eizagirre et al., 2019). Sheng et al. (2021) found no statistically significant grey matter volume alterations in those with SAD related to controls, discordant to the findings obtained in the original meta-analysis (Wang et al., 2021). The discrepancies from these two meta-analyses demonstrate the need for further research investigating alterations in the structural integrity of neural regions in those with SAD compared to controls.

In terms of microstructural MRI research in SAD, DTI has mostly been used to examine the microstructure of white matter tracts in the brain. The uncinate fasciculus is a white matter tract that connects the prefrontal cortex with the anterior medial temporal lobe (Leng et al., 2016). It is most implicated in SAD, with studies showing decreased functional anisotropy (FA) in those with SAD compared to controls (Baur et al., 2013; Baur et al., 2011; Jenkins et al., 2016; Phan et al., 2009; Qiu et al., 2014) and an association between FA of the uncinate fasciculus and social anxiety severity (Baur et al., 2013; Qiu et al., 2014). The superior longitudinal fasciculus is a major tract that connects prefrontal areas with the parietal cortices (Nakajima et al., 2020). It has also been implicated in SAD, with four studies showing decreased FA in SAD compared to controls (Baur et al., 2011; Jenkins et al., 2016; Qiu et al., 2014; Tükel et al., 2017) and an association between FA in this region and social anxiety severity (Qiu et al., 2014; Tükel et al., 2017).

2.5.2 Resting-State Functional MRI Studies in SAD

Compared to task-based paradigms, resting-state fMRI aims to examine brain functioning in the absence of overt stimuli. Several reviews have been published on this topic, including two that used a narrative approach (Kim & Yoon, 2018; Peterson et al., 2014) and one systematic review (Brühl et al., 2014). Peterson et al. (2014) examined resting-state connectivity across numerous anxiety disorders (including SAD, post-traumatic stress disorder,

and generalised anxiety disorder). Eight studies that specifically examined SAD were included, with mixed results of increased, decreased, or no difference in connectivity between regions in the default mode network and the salience network in those with SAD (vs. controls). Kim and Yoon (2018) extended this narrative review and reported on 10 resting-state studies involving those with SAD. The amygdala was notably a region of interest, with six studies using it as a region of interest (ROI) in their analysis. Four studies reported decreased connectivity and two studies reported increased connectivity between the amygdala and frontal/parietal regions (including the posterior cingulate cortex, dorsomedial PFC, precuneus, pregenual and dorsal ACC, and the medial orbitofrontal cortex) in those with SAD compared to controls.

Whilst narrative reviews are useful in reviewing previous literature, systematic reviews require a comprehensive and systematic search of the literature with strict inclusion and exclusion criteria for studies to avoid selection bias, and thus provide a more thorough overview and allow for more accurate summaries of the findings across studies (Pae, 2015). Brühl et al. (2014) systematically examined the neuroimaging literature in SAD and included 11 studies that specifically examined resting-state fMRI. However, in reporting the findings of the systematic review, they combined resting-state and task-based fMRI findings, making it difficult to distinguish the neural activity that was occurring at rest.

Since 2015, there has been an increase in resting-state neuroimaging in SAD. Given the limitations of the aforementioned systematic review, an updated systematic review that summarised and reported the results across only resting-state fMRI studies was completed as part of this thesis. Overall, mixed findings were observed across studies. However, differences in the activity of frontal regions and aberrant amygdala connectivity with temporal, parietal, and frontal regions in those with SAD compared to controls were most commonly reported. For a full summary of resting-state neuroimaging in SAD, see Chapter 3 (Study 1) for the review paper.

2.5.3 Task-based functional MRI findings in SAD

This section will briefly review findings from the most common task-based fMRI paradigms used in studies investigating SAD thus far. However, the main focus will be on studies that used emotion processing tasks in SAD cohorts given the research project presented in Chapter 6 (Study 3).

2.5.3.1 Cognitive Reappraisal

Cognitive reappraisal is a strategy used to assist with regulating emotions (Dryman & Heimberg, 2018). It involves altering one's interpretation or appraisal of an emotional-generating event. For example, the automatic thought 'that person is laughing at me' could be reframed as 'maybe that person is laughing at someone else and not me.' The ability to engage in cognitive reappraisal is thought to be impaired in SAD. Instead, individuals with SAD tend to believe their automatic thoughts to be true (Dryman & Heimberg, 2018). Wang et al. (2018) published a meta-analysis of the neurological underpinnings of cognitive reappraisal in anxiety disorders, including SAD. Of the five studies include, sample sizes of the SAD group ranged from 17 - 27 with an average of 20 participants. Wang et al. (2018) found that those with SAD had significantly decreased activity in the following clusters when using cognitive reappraisal strategies compared to controls: bilateral dorsal medial PFC, bilateral dorsal ACC, bilateral supplementary motor area, left amygdala, and the right superior parietal gyrus. No studies on this topic have been published since this meta-analysis. Of the five studies included in this meta-analysis, Gaebler et al. (2014) was ranked as being of the highest quality based on the assessment of participants, methods, and reporting of results and conclusion. Interestingly, they found no differences in neural activation in those with SAD compared to controls when reappraising negatively valenced images, and in those with SAD who were engaged in cognitive reappraisal to downregulate their emotional experience compared to those with SAD who were asked to intensify their emotional experience (Gaebler et al., 2014). Given these

findings are incongruent to the overall meta-analysis findings despite being of the highest quality, further research investigating the neurobiology underlying the mechanisms of cognitive reappraisal in SAD using larger sample sizes is warranted.

2.5.3.2 Social Stimuli

In a recent meta-analysis, Yu et al. (2021) examined differences in neural activation when participants were instructed to observe stimuli involving specific situations thought to elicit social anxiety. Situations that were used included depictions of someone giving a speech, in a job interview, in a discussion with others, and being the centre of attention (Heitmann et al., 2017; Heitmann et al., 2016). Other situations included asking someone for directions, making a complaint about something, and going to a restaurant (Boehme et al., 2014; Nakao et al., 2011). Pujol et al. (2013) had a different approach in examining neural responses to social situations. Participants were video recorded as they verbally recounted a story that they had just heard. When viewing these videos in the scanner, they were also advised that a psychologist would be evaluating their performance. Across 8 studies and 153 participants with SAD, these authors found significantly lower activation in the right supramarginal gyrus (extending to the angular gyrus) compared to controls (maximum activation likelihood estimated (ALE) value = 0.013, $p = 0.0000046$). Of the studies included, Heitmann et al. (2017) was reported as having the highest quality rating and reported increased activity in the insula, precuneus, and frontal regions in those with SAD (vs. controls) when viewing disorder-related versus neutral scenes. Further research is warranted to clarify the neurological underpinnings of how people with SAD process social situations given the limited number of studies and the small sample sizes used (ranging from 6 – 30 with an average of 19 in the SAD group). The differences in paradigms used to investigate neural responses to social situations may have contributed to the minimal findings of the pooled studies, and should also be considered in future research.

2.5.3.3 Emotion Processing

The neurobiology of emotion processing in people with SAD has been investigated using numerous paradigms. Many studies have examined how participants respond to emotional faces (e.g., sad, happy, fearful, angry) compared to neutral faces (Goldin et al., 2009; Klumpp et al., 2010; Phan et al., 2006), instructing them to respond in various manners such as pressing a button in the scanner to identify the emotion (Yoon et al., 2007) or asking them to downplay their emotional reaction using cognitive strategies (Goldin et al., 2009). Others have examined neural responses to negatively valenced statements (e.g., 'You're an idiot' and 'I am ugly') compared to positively valenced statements (e.g., 'You are loved' and 'I am a genius') from both a first- and second-person viewpoint (Blair, Geraci, et al., 2008; Blair et al., 2011).

One of the earliest reviews on this topic was Hattingh et al. (2013) meta-analysis of seven studies (across 91 participants with SAD), in which they identified neural differences in emotion recognition in those with SAD compared to controls using an ALE approach. All studies that were included used a whole-brain fMRI approach, with two examining responses to emotional statements and five examining responses to emotional faces. Findings from this meta-analysis demonstrated that, regardless of stimuli, those with SAD had greater activation in the following regions compared to controls: right anterior cingulate (extrema value = 0.0139), distal tip of right post-central gyrus (extrema value = 0.0145), left medial aspect of the inferior temporal lobe (extrema value = 0.0139), left medial temporal lobe (extrema value = 0.0177), bilateral amygdala (extrema value = 0.0239 (left) and 0.0381(right)), and the right globus pallidus (extrema value = 0.0230). Differences in amygdala activity were identified as being the most disparate between groups.

Hattingh et al. (2013) recognised the limitation in combining findings from studies that used both faces and statements as part of the fMRI task, and a subsequent ALE meta-analysis

of 23 studies (including 449 participants with SAD) focused specifically on neural responses to face processing (Gentili et al., 2016). Both published and unpublished fMRI data that used whole-brain analysis techniques were included. Whilst the majority of the 23 studies included in this review examined neural responses to emotional faces, one study included neutral faces and examined neural differences depending on whether the gaze of the stimuli was direct or averted (Schneier et al., 2011). Compared to controls, those with SAD had significantly increased activation to emotional (vs neutral faces) in frontal regions (i.e., right inferior frontal gyrus, bilateral medial frontal gyrus, left superior frontal gyrus, left subgenual ACC), temporal regions (i.e., bilateral superior temporal sulcus, bilateral middle temporal gyrus), right lingual gyrus, the left amygdala, and the right amygdala/globus pallidus ($p < 0.01$, false discovery rate (FDR) corrected). Significantly decreased activation in the right lingual gyrus and the left precuneus ($p < 0.01$, FDR corrected) was also observed. Effect sizes were not reported in this meta-analysis, and the heterogeneity of contrasts was acknowledged as a limitation (e.g., some contrasts, such as fearful vs. neutral, were more common than other contrasts, such as happy vs. neutral) as it is unclear whether different neural activation patterns would occur if contrast was controlled for.

As part of Yu et al. (2021) ALE meta-analysis investigating the cognitive neural mechanisms underpinning SAD in whole-brain MRI studies, a sub-group analysis was conducted on 15 studies (with 237 SAD participants) that used emotional faces as task stimuli. In those with SAD compared to controls, significantly decreased activation of the left cerebellar region (extending to the fusiform gyrus; maximum ALE value = 0.015, $p = 0.00000034$) was observed. This finding was not identified in either of the previous meta-analyses published on this topic. Although it was published more recently than Gentili et al. (2016) meta-analysis, it included fewer studies due to the lack of inclusion of unpublished data. The discrepancies in

findings between meta-analyses suggest a potential effect of publication bias and affirms the importance of allowing open access to data.

2.5.4 Treatment Effects on the Brain in SAD

The effects of current pharmacotherapeutic and psychotherapeutic treatments on brain functioning in adults with SAD has been summarised in a meta-analysis conducted on studies published between 2000 to 2015 which identified 12 studies that examined changes pre- to post-treatment in brain fMRI activity using a whole-brain approach (Li et al., 2016). Decreased activity was detected in the left inferior parietal gyrus, right postcentral gyrus, and the right precuneus post-treatment (compared to pre-treatment; Li et al., 2016).

Additional findings of the treatment effects on the neurobiology of SAD can be observed in studies that were not included in the aforementioned meta-analysis. The most commonly investigated treatments were CBT and SSRIs, with findings of reduced amygdala activation in response to threat stimuli and reduced functional connectivity of the amygdala – anterior cingulate cortex post-treatment compared to pre-treatment (Månsson et al., 2013; Yuan, Zhu, et al., 2016). The anxiolytic effects of SSRIs are thought to be a result of their mechanistic action of reducing serotonin synthesis in the amygdala, with Frick et al. (2016) demonstrating an attenuated rate of serotonin synthesis in the amygdala, insular cortex, and the ventromedial PFC (accompanied with reduced regional cerebral blood flow and an improvement in symptoms) after a 6-week treatment program with the SSRI citalopram.

Other psychotherapeutic treatments have been investigated including MBSR and ACT. Decreased amygdala activation and increased connectivity between the amygdala and the visual cortex/parietal regions/primary motor cortex during emotion regulation tasks were observed after those with SAD completed 12 weeks of CBT or ACT compared to pre-treatment (Young et al., 2017). Compared to waitlist, those with SAD who participated in treatment (MBSR or CBT) had greater increases pre-post treatment in the dorsomedial PFC and the

dorsal ACC (Goldin et al., 2021). Additionally, novel treatments such as oxytocin have been investigated, with the acute intranasal administration of oxytocin (vs. placebo) shown to attenuate the hyperactive amygdala response to emotional faces (Labuschagne et al., 2010), as well as lowering the heightened connectivity between the amygdala and medial PFC at rest in those with SAD compared to controls (Dodhia et al., 2014).

2.5.5 The Role of the Amygdala and its Subregions in SAD

2.5.5.1 The Whole Amygdala

Given the primary focus on amygdala subregions in the two empirical studies of this thesis (Study 2 and Study 3), this section will describe findings of the amygdala and its subregions in SAD in more detail for context. As can be seen from the aforementioned MRI literature in SAD, the amygdala as a single homogenous ROI has been frequently implicated albeit with inconsistencies in the pattern of findings. In brief, fMRI studies have demonstrated both increased and decreased activity of the bilateral amygdala in those with SAD (vs. controls) at rest and in response to varying stimuli (e.g., social situations, fearful faces, angry faces; Brühl et al., 2014; Gentili et al., 2016; Hattingh et al., 2013; Mizzi et al., 2021). Moreover, both hyper- and hypoconnectivity between the amygdala and various regions of the brain (especially frontal, parietal, and temporal regions) have also been observed in those with SAD (vs. controls) during both resting-state and task-based paradigms (involving the presentation of social stimuli; Brühl et al., 2014; Mizzi et al., 2021).

Despite the inconsistencies in the direction of amygdala dysfunction in the literature to date, it is clear that the amygdala does play a role in the neuropathology of SAD. This is expected given the broader function of the amygdala as a region of the brain that is commonly implicated in emotion processing, assigning valence to environmental stimuli, and in social functioning (LeDoux, 2007). Lesions in this region have been linked to impairment in recognising emotions in facial expressions, in fear conditioning, and in a wide range of social

deficits (Adolphs, 2010; Adolphs et al., 1994; Anderson & Phelps, 2001). However, findings from lesion studies of monkeys who had undergone an amygdalectomy demonstrate that they are still able to engage in social behaviours and respond to social cues. This suggests that the amygdala is not necessary for all aspects of social behaviour, and instead, is thought to fine-tune and add nuance/context to social functioning (Gothard, 2020) which is often impaired in those with SAD.

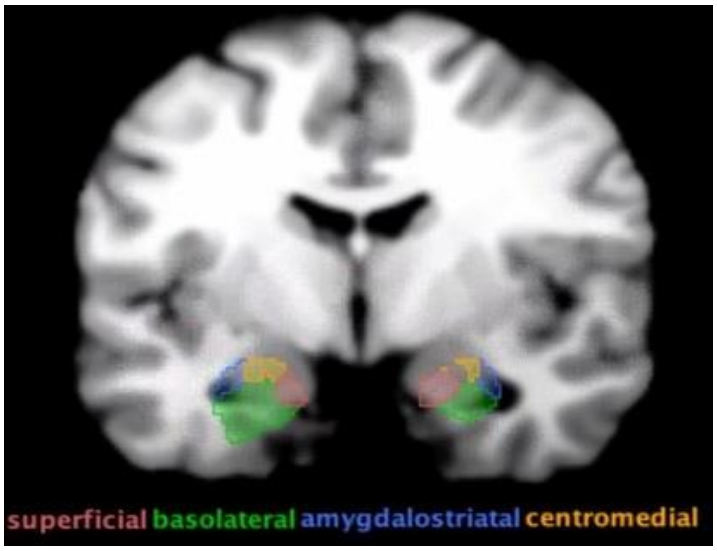
2.5.5.2 The Amygdala Subregions

The amygdala is known to be comprised of functionally and structurally distinct subnuclei which in humans, are most commonly grouped into the superficial, centromedial, basolateral, and amygdalostriatal subregions (see Figure 2.3; Amunts et al., 2005; LeDoux, 2007). Although there is limited evidence of the role of these subregions in SAD to date, all subregions (except the amygdalostriatal) have been associated with emotion processing (in particular, fear processing) and in viewing faces in humans (Bzdok et al., 2013). However, despite knowledge of subregional differences, the literature to date is limited, largely due to limitations in imaging acquisition techniques resulting in inadequate spatial resolution to delineate the amygdala subnuclei in humans (Hrybouski et al., 2016; Yuan, Zhu, et al., 2016). Studies have typically averaged neural responses across voxels of the whole amygdala, which may lead to false negatives as signals from different subregions may cancel each other out (Ball et al., 2007). Other studies have restricted their interpretation to a single peak value/cluster within the amygdala without identifying which subregion this peak value/cluster exists within, thus potentially leading to discrepancies across studies of amygdala activation and connectivity patterns. However, the development of more advanced neuroimaging acquisition techniques has allowed for a closer investigation of these subregions such as through the use of multiband MRI that is utilised in the empirical studies of this thesis (Studies 2 and 3). Preliminary

information about the anatomy and functionality of these subregions (as derived from both animal and human studies) are described below.

Figure 2.3

Four Subregions of the Amygdala



2.5.5.2.1 Superficial Subregion. The superficial amygdala has axonal connections with the olfactory cortex and is functionally connected to the anterior insula, inferior frontal gyrus, and the nucleus accumbens (Amunts et al., 2005; Roy et al., 2009). It is thought to be highly tuned to social information and is implicated in the processing of both static and dynamic visual presentations of facial emotional expressions (Goossens et al., 2009; Hurlemann et al., 2008).

2.5.5.2.2 Centromedial Subregion. The centromedial subregion of the amygdala is considered to primarily generate and mediate behavioural and autonomic responses (Bzdok et al., 2013). It is believed to receive pre-processed information from the basolateral nuclei and has axonal connections with the brain stem, hypothalamus, and basal forebrain (Amunts et al., 2005). The centromedial has been found to coactivate with regions such as the primary motor cortex, the supplementary motor area, and the basal ganglia (Bzdok et al., 2013). In response to faces, it is thought that this affective-motor pathway may play a role in mediating the emotional facial movements of respondents (Schilbach et al., 2008).

2.5.5.2.3 Basolateral Subregion. The basolateral subregion of the amygdala is considered to primarily receive inputs from the external environment and is a site of integration for sensory information. It has axonal connections with sensory areas (e.g., the visual and auditory cortex; Bzdok et al., 2013). The basolateral amygdala is thought to play a role in auditory fear conditioning and in self-focused reflection given its coactivation with the ventromedial PFC, medial prefrontal regions, and the precuneus (Bzdok et al., 2013; Sun et al., 2020).

2.5.5.2.4 Amygdalostriatal Subregion. The amygdalostriatal subregion has been the least investigated of the four subregions. Findings from animal studies demonstrate that this subregion is interconnected with the basolateral and centromedial seeds and receives inputs from secondary auditory, visual and somatosensory cortices. It is thought to participate in the regulation of fear expression (Leitermann et al., 2016; Shammah-Lagnado et al., 1999; Wang et al., 2002).

2.5.5.3 Studies investigating Amygdala Subregions in SAD

The amygdalar subregions have been implicated across psychiatric disorders including MDD, post-traumatic stress disorder, obsessive-compulsive disorder, and generalised anxiety disorder (Cao et al., 2022; Etkin et al., 2009; Gao et al., 2021; Qiu et al., 2018; Tang et al., 2019; Yuan et al., 2019). For the scope of this thesis, however, the following section will detail findings of amygdala subregions in SAD. In the existing fMRI literature in SAD, only three studies investigated amygdala subregions (Anteraper et al., 2014; Heitmann et al., 2016; Yoon et al., 2016). In addition, one study using PET investigated amygdala subregions (Faria et al., 2012). In brief, findings from these studies primarily demonstrated hyperconnectivity of the basolateral, superficial, and centromedial subregions with brain regions in those with SAD compared to controls (see Table 2.6 for a full summary of results).

Table 2.6

Studies Investigating Amygdala Subregions in SAD

Study	Participants	Method	Amygdala Seeds	Findings
Faria et al. (2012)	36 SAD with SSRI (20 responders*), 27 SAD with placebo (11 responders*)	PET scan while doing a speech in front of an audience	Amygdala	Responders < non-responders: basomedial/basolateral and ventrolateral amygdala subregions[#]
Anteraper et al. (2014)	17 SAD 17 CON	Resting-state fMRI	Centromedial	SAD > CON: centromedial – SMA, ITG, secondary visual cortex, angular gyrus, and precuneus/cingulate gyrus
Yoon et al. (2016)	20 SAD 30 CON	Resting-state fMRI	Basolateral Centromedial Superficial	SAD > CON: basolateral - dorsomedial PFC, ACC, superior and inferior frontal gyri, and the right cerebellum SAD > CON: centromedial amygdala - left TPJ SAD > CON: superficial amygdala - left dorsolateral PFC, superior and inferior frontal gyri and the cerebellum CON > SAD: right superficial amygdala - left dorsomedial thalamus
Heitmann et al. (2016)	30 SAD 30 CON	Task-based fMRI: viewing disorder-related and neutral stimuli	Basolateral Centromedial Superficial	SAD > CON: basolateral amygdala – globus pallidus SAD > CON: corticomedial activation (combination of superficial and centromedial regions)

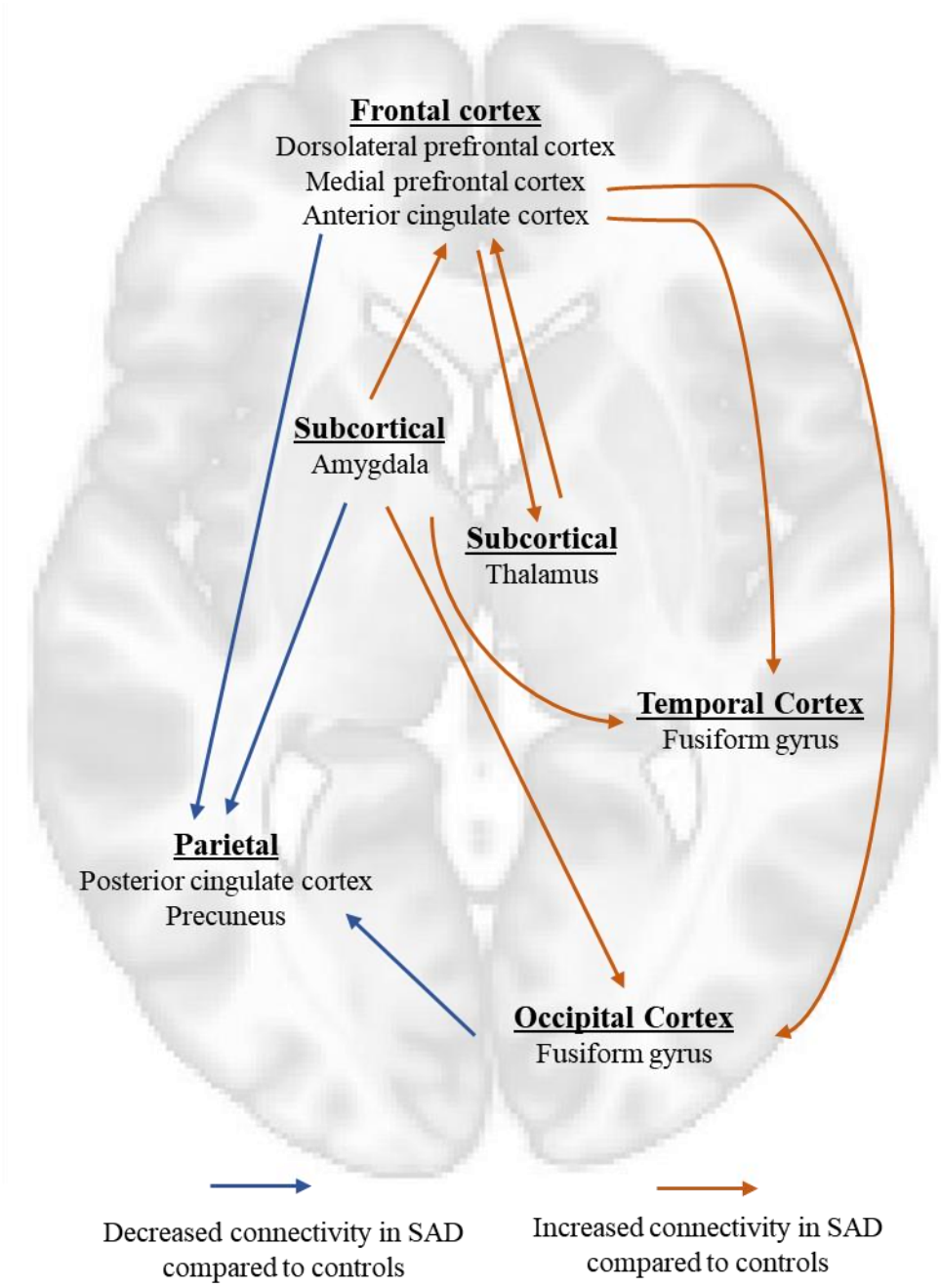
Note. ACC = anterior cingulate cortex; CON = controls; fMRI = functional magnetic resonance imaging; ITG = inferior temporal gyrus; PET = positron emission tomography; PFC = prefrontal cortex; SAD = social anxiety disorder; SMA = supplementary motor area; SSRI = selective serotonin reuptake inhibitor; TPJ = temporoparietal junction * = classified as responders if they scored 1 or 2 (very much or much improved) posttest on the Clinical Global Impression improvement item; # = subregions identified as statistical peaks in the whole amygdala seed.

2.5.6 A Neurobiological Model of SAD

Despite numerous theoretical biopsychosocial models existing for SAD (see Section 2.3), only one neurobiological model of SAD has been proposed to date (Brühl et al., 2014). This extends the previous model by Etkin and Wager (2007) that identified neural regions implicated in a 'fear circuit' across people with SAD, specific phobia, and post-traumatic stress disorder compared to controls. Brühl et al. (2014) incorporated findings from both task-based and resting-state fMRI studies to demonstrate patterns of increased localised activity in parietal and medial occipital brain regions, a decoupling of these regions with limbic areas and the ventral attention and cingulo-opercular networks, and increased connectivity between the amygdala and prefrontal/orbitofrontal regions (see Figure 2.4). This model has been discussed in greater detail in the systematic review (Chapter 3) and in the general discussion (Chapter 7).

Figure 2.4

The Neurobiological Model of SAD



*Note. F*igure adapted from original, see Brühl et al. (2014). Each region is theorised to have increased activity in those with SAD compared to controls, while the arrows represent increased or decreased connectivity in those with SAD compared to controls.

2.5.7 Limitations of MRI studies in SAD

Findings from the neuroimaging literature discussed in this chapter suggest that there are abnormalities in neural activity and connectivity in both task-based paradigms and at rest in those with SAD (vs. controls). However, the literature to date is mixed and there remains only preliminary evidence of structural and microstructural differences and changes in neural activity and connectivity in the context of other tasks (e.g., social stimuli, cognitive reappraisal), and further research is required in these areas. Furthermore, despite there being an increase in studies examining resting-state neuroimaging and emotion processing in fMRI in those with SAD, the body of literature presented to date has significant limitations, especially in their methods, that are likely to be contributing to the conflicting findings that are reported in reviews on these topics. These limitations are discussed below, including details of how this thesis and research project aimed to address some of these issues.

2.5.7.1 Low Powered Studies

In the most recent resting-state and task-based reviews published, it was noted that an average sample size of 23 and 18 SAD participants were included respectively (Mizzi et al., 2021; Yu et al., 2021). Results from small sample sizes are known to be prone to error, including increased susceptibility to false positives and false negatives (Blackford, 2017; Eklund et al., 2016). Small sample sizes (ranging from approximately 20-30 participants) also impact the ability to detect reliable and reproducible brain-behaviour relationships (Grady et al., 2021). Increasing sample sizes serves to reduce both within-subject and between-subject variance (Turner et al., 2018). This project addressed this limitation by including a larger sample size of clinical participants than the average in the literature to date, with an equal number of males and females (i.e., n = 42 SAD participants; see Chapters 5 and 6).

2.5.7.2 Heterogenous Acquisition Parameters

Differences in the scanning methods across neuroimaging studies in SAD have been shown to impact on the observed results. The repetition time used (ranging from 1000-6000 milliseconds) can influence how activated and nonactivated brain tissue is discriminated, with shorter repetition times (less than 1500 milliseconds) providing better discrimination than longer repetition times (more than 4000 milliseconds; Constable & Spencer, 2001). Additionally, the length of the scan time has been shown to affect test-retest reliability with increased scan time associated with increased reliability (Noble et al., 2017). For resting-state data, a scan time of between 5 and 13 minutes has been suggested to obtain good intra- and intersession reliability (Birn et al., 2013). In this thesis, a repetition time of 1020 milliseconds and resting-state scan length of 8 minutes 38 seconds was used to enhance the reliability of the findings.

2.5.7.3 Heterogenous Pre-Processing Pipelines

The literature is increasingly moving towards using streamlined data processing frameworks, resulting in methodological consistency between studies. One such framework is *fMRI*prep (Esteban et al., 2019), which allows for standardisation in image registration, smoothing and filtering of fMRI data, and consistency in the management of common artifacts including respiration and head motion. In the current thesis, we used *fMRIprep* to pre-process the MRI data for functional studies; see Chapters 5 and 6.

2.5.7.4 Confounding Variables

There is evidence that differences in state anxiety (often measured by the State-Trait Anxiety Inventory) and respiration may influence the fMRI findings as they can change cerebral blood flow (Giardino et al., 2007). Other potential confounding variables include whether participants are taking medication and/or have psychiatric comorbidities. Furthermore, the severity of the disorder (most often measured by the Liebowitz Social Anxiety Scale) may be confounding the results. Most studies have included a case-control group comparison,

however, a dimensional approach (normal, subclinical, abnormal) would allow for the identification of changes in neural activity that is dependent on symptom severity (Parkes et al., 2020). In the current thesis, we will be examining the neural bases of social anxiety as both a discrete categorical disorder and a spectrum of severity to contribute to the discussion of how social anxiety is classified; see Chapters 5 and 6.

2.5.7.5 Echo-Planar Imaging

Most of the existing literature examining brain functioning using fMRI in SAD has used gradient-recalled echo-planar imaging (EPI). More recently, a new technique known as multiband EPI was developed and was used for data acquisition in Study 2 and Study 3. Multiband EPI sequencing involves the simultaneous acquisition of multiple slices to increase the amount of data acquired without needing to increase the duration of time in the scanner, thus minimising motion artifacts and increasing the reliability of findings (Feinberg & Setsompop, 2013). Compared to gradient-recalled EPI, multiband EPI allows for shorter scan times without reducing the signal-to-noise ratio and improvements in the spatial and temporal resolution of fMRI data. This has resulted in improved statistical sensitivity in both task-based and resting-state fMRI studies and has allowed for increased reliability when investigating smaller brain regions such as the subregions of the amygdala (Bhandari et al., 2020).

CHAPTER 3: RESTING-STATE NEUROIMAGING IN SOCIAL ANXIETY DISORDER: A SYSTEMATIC REVIEW (STUDY 1)

3.1 Chapter Guide

The following chapter comprises a comprehensive systematic review of 35 studies investigating resting-state neuroimaging in SAD. The studies included in this review used varying neuroimaging techniques such as positron emission tomography, single-photon emission computed tomography and functional magnetic resonance imaging (fMRI). As the latter imaging technique was used by most studies (n = 31), the review primarily focused on integrating fMRI findings. Overall, this review demonstrated discrepancies across the studies in terms of regions implicated in SAD. Despite this, frontal regions were observed to be most consistently reported across studies that examined group differences (SAD vs. controls) in brain activity and connectivity and in studies that examined associations between brain functioning and social anxiety severity. However, the nature of the aberrant activity of implicated regions (including frontal regions) was mixed (i.e., findings of both hyper- and hypoactivity/connectivity). This review has been published in *Molecular Psychiatry* and has been included in this chapter without any alterations. Given the manuscript word limit required for publication, relevant information regarding methods and results are presented in the Supplementary Materials which are included following the manuscript.

Citation:

Mizzi, S., Pedersen, M., Lorenzetti, V., Heinrichs, M., & Labuschagne, I. (2021). Resting-state neuroimaging in social anxiety disorder: a systematic review. *Molecular Psychiatry*. https://doi.org/10.1038/s41380-021-01154-6

3.2 Abstract

There has been a growing interest in resting-state brain alterations in people with social anxiety disorder. However, the evidence has been mixed and contested and further understanding of the neurobiology of this disorder may aid in informing methods to increase diagnostic accuracy and treatment targets. With this systematic review, we aimed to synthesize the findings of the neuroimaging literature on resting-state functional activity and connectivity in social anxiety disorder, and to summarize associations between brain and social anxiety symptoms to further characterize the neurobiology of the disorder. We systematically searched seven databases for empirical research studies. 35 studies met the inclusion criteria, with a total of 1 611 participants (795 people with social anxiety disorder and 816 controls). Studies involving resting-state seed-based functional connectivity analyses were the most common. Individuals with social anxiety disorder (vs. controls) displayed both higher and lower connectivity between frontal-amygdala and frontal-parietal regions. Frontal regions were the most consistently implicated across other analysis methods, and most associated with social anxiety symptoms. Small sample sizes and variation in the types of analyses used across studies may have contributed to the inconsistencies in the findings of this review. This review provides novel insights into established neurobiological models of social anxiety disorder and provides an update on what is known about the neurobiology of this disorder in the absence of any overt tasks (i.e., resting-state). The knowledge gained from this body of research enabled us to also provide recommendations for a more standardized imaging pre-processing approach to examine resting-state brain activity and connectivity that could help advance knowledge in this field. We believe this is warranted to take the next step towards clinical translation in social anxiety disorder that may lead to better treatment outcomes by informing the identification of neurobiological targets for treatment.

3.3 Introduction

Social anxiety disorder (SAD) is characterized by fear, anxiety, and/or avoidance of social situations (American Psychiatric Association, 2013). Data from the World Mental Health Surveys suggest that SAD exists globally, with a lifetime prevalence rate of 5% in high-income regions (e.g., Australia, the USA and the UK; Stein et al., 2017). Living with SAD can be debilitating, with the diagnosis being associated with significant impairments in multiple domains of functioning, most substantially impacting relationships and social experiences. Despite its widespread prevalence in the community and the distressing nature of SAD, the diagnostic accuracy and treatment response rates remain poor in individuals with this disorder (Chapdelaine et al., 2018; Vermani et al., 2011). Advances in neuroimaging techniques have only begun to provide important insights into the neurobiology of SAD. The most recent models of SAD (Brühl et al., 2014; Etkin & Wager, 2007) propose that individuals with SAD (compared to controls) are characterized by dysfunctional fronto-limbic (fear) circuitry, with hyperactivity in limbic areas (i.e., amygdala, hippocampus, parahippocampus) and hypoactivity in cognitive control areas (i.e., anterior cingulate cortex, ventral medial prefrontal cortex (PFC), dorsolateral PFC). Brühl et al. (2014) added the role of medial parietal and occipital regions that are increasingly activated in those with SAD. The aforementioned reviews, however, primarily focused on interpreting the activation of different brain regions in response to overt tasks.

A recent trend in the clinical neuroimaging community is to study resting-state functional activity/connectivity paradigms. Compared to task-dependent methods, resting-state paradigms are not susceptible to the potential confounding effects of task performance and may be particularly useful in aiding our understanding of the intrinsic brain mechanisms implicated in the clinical presentation of SAD. Resting-state brain connectivity as a potential biomarker of psychiatric symptoms has been found useful in other disorders, including autism spectrum

disorder (Hull et al., 2017) and major depression (Wang et al., 2012). In SAD, there has been an increase in resting-state brain imaging studies in recent times. To our knowledge, Brühl et al. (2014) was the most recent review that systematically examined resting-state studies (n = 11). However, the results were reported in combination with task-based functional connectivity findings, making it challenging to interpret a pattern of results specifically attributed to resting-state brain imaging studies. Other narrative reviews examining resting-state neuroimaging in SAD have been published since (Kim & Yoon, 2018; Peterson et al., 2014) and show discrepancies within the findings of resting-state studies. Peterson et al. (2014) examined resting-state connectivity across anxiety disorders and found that whilst there were overlap in the brain networks underlying the full range of anxiety disorders, there were variations in connectivity between limbic and cortical regions unique to each anxiety disorder, thereby warranting a thorough review of resting-state brain activity/connectivity of SAD on its own.

Due to the growing number of studies investigating resting-state neuroimaging in SAD, the current review aimed to use a systematic approach to obtain a critical appraisal and comprehensive understanding of the brain regions and networks implicated in SAD (compared to controls) at rest. A secondary aim of this review involved summarizing any known brain-behaviour associations (i.e., links between brain function and symptoms) associated with SAD. Although the majority of the literature involves functional magnetic resonance imaging (fMRI) studies, we included a broad range of imaging modalities (including fMRI, single-photon emission computed tomography (SPECT), and positron emission tomography (PET)) and various acquisition and analysis approaches relating to both activation and connectivity studies to be as comprehensive as possible. With this review, we hope to further our understanding of the neurobiology of this disorder that could aid in informing methods to increase diagnostic accuracy and treatment targets.

3.4 Method

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines, which outlines a set of items to improve reporting of systematic reviews (Moher et al., 2009). Full details regarding the search strategy, eligibility criteria, data extraction process, data synthesis, and quality assessment can be found in the Supplement. Seven databases were searched on the 29th November 2020 and studies were included if they were published in English and measured brain function/activation at rest in a sample of participants that had a diagnosis of SAD and in a control group. Data extracted included demographic information and details regarding the method of neuroimaging acquisition and analysis and the results. All included studies were quality checked using the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies published by the National Heart, Lung and Blood Institute to evaluate the internal validity of each included study (National Institutes of Health, 2014). The primary results of interest for the qualitative synthesis of findings were group differences in resting-state neural activity and connectivity.

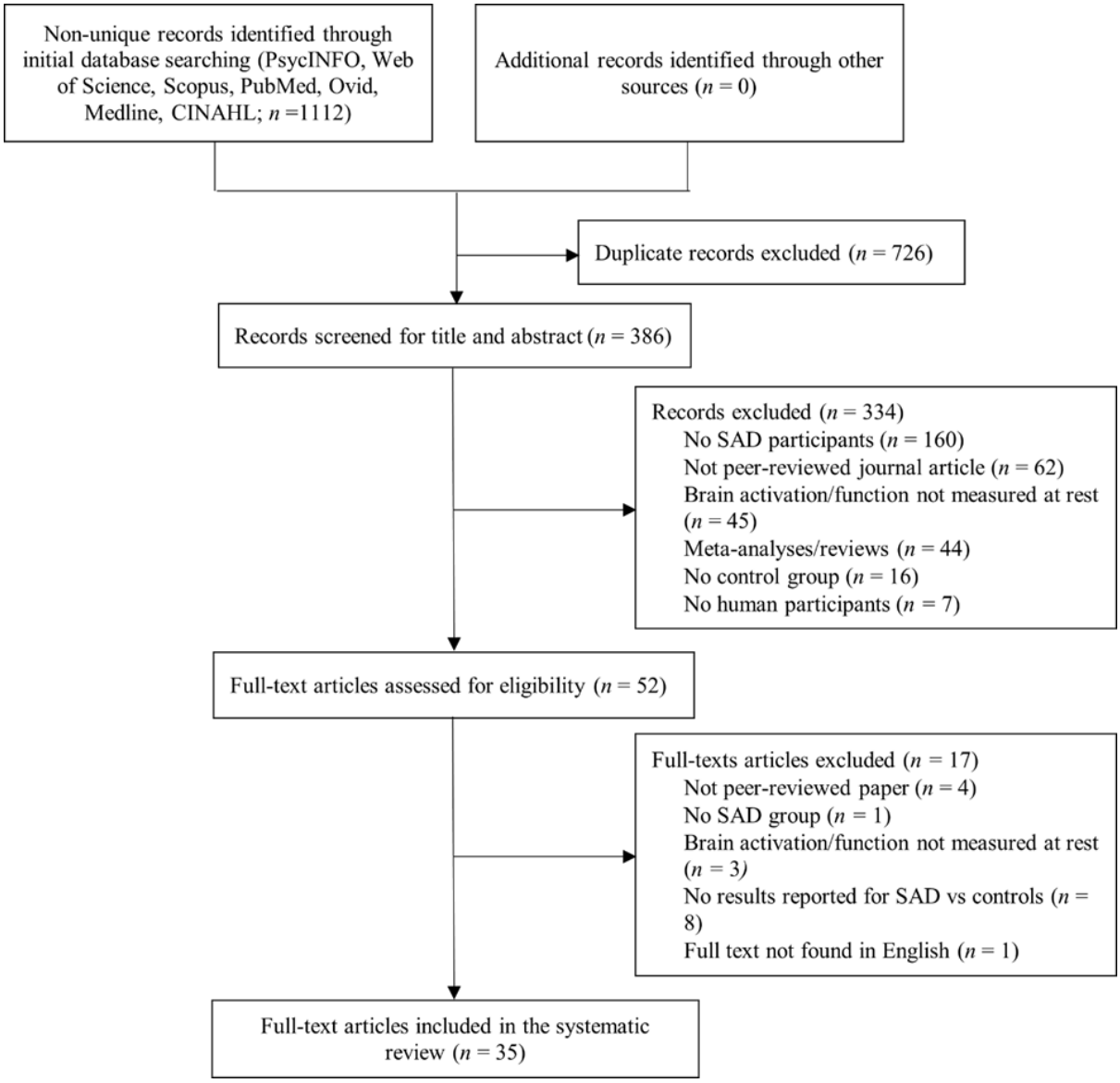
3.5 Results

3.5.1 Study Selection

The initial search identified 1 112 possible studies. After the screening of titles, abstracts, and full-text articles and the removal of duplicates and other non-suitable studies, a final 35 studies were included in the systematic review (see Figure 3.1). The primary reasons for the exclusion of studies were that no participants diagnosed with SAD were involved and the data was not published in a peer-reviewed journal.

Figure 3.1

Preferred Reporting Items for Systematic Reviews and Meta-Analyses Flowchart for Systematic Search and Identification of Studies Meeting Inclusion Criteria for the Systematic Review



Note. Studies were included based on the following criteria: i) the full text was published in English, ii) human participants were involved, iii) brain function/activation was measured at rest, and iv) a sample of participants with a diagnosis of SAD was compared to a control group. Reasons for exclusion are detailed in the flowchart. We were unable to complete a meta-analysis (as planned and reported in the PROSPERO pre-registration) variety in seeds and analysis methods used across studies, and the absence of coordinates of commonly reported seeds (i.e., the amygdala).

3.5.2 Sample Population

Information regarding demographic details (including age and sex), handedness, recruitment, diagnosis, and severity of participants are included in Table 3.1. Details regarding overlaps in samples across studies, methods of diagnosis, and medication use can be found in the Supplement**.** For the 35 studies included, all were published between 2008 to 2020 and involved a cross-sectional design. A total of 1 611 participants were included, consisting of 795 people with SAD and 816 controls. For individuals with SAD, the mean age was 26.26 (± 5.48) years, and aside from one study that used a paediatric sample (results are reported separately in the supplementary (Dorfman et al., 2016), the remaining 34 studies used adult cohorts. The average sample size for SAD participants was 23, with a range of 7 to 53 participants.

Table 3.1

Demographic Information of Participants

Author	Year	Sample n (female)		Age Years (SD)		Country	SAD				Handed-ness
		SAD	CON	SAD	CON		Diagnosis, type	Severity Measure : Mean (SD)	Comorbid disorders	Medication	
Anteraper et al. (2014)	2014	17 (9)	17 (9)	24.7 (6.3)	25 (7.5)	USA	DSM-IV, generalized	LSAS total: 77.9 (14.1)	n = 4: depression; n = 4: anxiety disorder	None at time of study	34 R
Choi et al. (2016)	2016	21 (9)[1]	20 (8)	24.1 (2.8)	24.1 (1.8)	Republic of Korea	DSM-IV-TR	LSAS total: 71 (20) SIAS: 43.6 (10.8)	None	None	41 R
Cui et al. (2017)	2017	21 (6)	20 (6)	22.05 (3.94)	21.65 (3.60)	China	DSM-IV	LSAS total: 53.9 (11.211) LSAS fear: 27.95 (6.021) LSAS avoidance: 25.95 (6.7560 STAI-T: 48.14 (6.858)	None	None	41 R
Ding et al. (2011)	2011	17 (4)	19 (5)	23.47 (4.17)	21.89 (3.77)	China	DSM-IV	LSAS total: 52.60 (11.66) LSAS fear: M=27.41 (6.25) LSAS avoidance: 25.18 (7.06) STAI-T: 48.41 (7.53)	n.r.	None	36 R
Dodhia et al. (2014)	2014	18 (0)	17 (0)[1]	29.9 (10.2)	29.4 (9.0)	USA	DSM-IV, generalized	LSAS total: 84.67 (17.5) LSAS performance: 38.11 (11) LSAS social situations: 43.56 (8.6) STAI-T: 50.44 SD= 11.5)	n.r.	None	35 R
Dorfman et al. (2016)	2016	8 (n.r.)[1]	36 (21)	13.2 (2.7)	13 (2.7)	USA	DSM-IV	----------	n.r.	None	44 R
Doruyter et al. (2016)	2016	23 (6)	15 (5)	32.8 (n.r.)	37 (n.r.)	RSA	DSM-IV	LSAS total: 102.55 (20.8)	n.r.	n = 1: alprazolam 0.25mg per day	38 R
Doruyter et al. (2018)	2018	15 (9)	15 (9)	30.5 (n.r.)	30.6 (n.r.)	RSA	DSM-5	LSAS total: 91.1 (25.4)	n.r.	None	30 R
Ergul et al. (2019)	2019	16 (5)	21 (8)	29.56 (6.53)	27.24 (6.39)	Turkey	DSM-IV, generalized	LSAS total: 72.00 (15.17)	n = 2: specific phobias	None for last 6 weeks; n = 8: SSRIs for short periods of time	37 R
Evans et al. (2009)	2009	15 (6)	10 (4)	33.3 (12)	31 (7.7)	USA	DSM-IV, generalized	LSAS total: 785 (15.9)	n = 1: current MDD and dysthymia; n = 2: current GAD; n = 3: past MDD; n = 1: past dysthymia; n = 3: past substance or alcohol use disorder	None	25 R

Geiger et al. (2016)	2016	18 (11)	12 (8)[1]	29.56 (8.97)	28.47 (8.15)	RSA	DSM-IV-TR	LSAS total: 88.6 (24.82)	n.r.	None	30 R
Hahn et al. (2011)	2011	7 (0)	11 (11)[1]	28.6 (4.3)	27.72 (7.2)	Austria	DSM-IV	STAI-T: 41.6 (11.5)	n.r.	None within 3 months of inclusion	n.r.
Jung et al. (2018)	2018	36 (19)	42 (23)	25.4 (3.1)	24.7 (3.1)	Republic of Korea	DSM-5	LSAS: 78.3 (26.2) SIAS: 54 (14.8)	n = 4: depressive disorders	n = 9: serotonergic antidepressants; n = 2: benzodiazepines and beta-blocker as-needed medication (but did not take on day of scanning)	72 R 6 AMB
Liao et al. (2010)	2010a	20 (6)	19 (5)	22.90 (2.99)	21.89 (3.77)	China	DSM-IV	LSAS total: 53.90 (11.50) LSAS fear: 28.00 (6.17) LSAS avoidance: 25.90 (6.93) STAI-T: 48.25 (7.02)	None	None	39 R
Liao et al. (2010)	2010b	22 (6)	21 (6)	22.55 (4.04)	21.71 (3.64)	China	DSM-IV	LSAS total: 51.5 (9.72) LSAS fear: 26.55 (4.82) LSAS avoidance: 24.95 (6.940) STAI-T: 46.77 (7.86)	None	None	43 R
Liao et al. (2011)	2011	18 (6)	18 (5)	22.67 (3.77)	21.89 (3.69)	China	DSM-IV, generalized	LSAS total: 54.39 (11.96) LSAS fear: 28.50 (6.20) LSAS avoidance: 25.89 (7.32) STAI-T: 48.33 (32.67)	n.r.	None	36 R
Liu et al. (2015)	2015b	20 (6)	20 (6)	22.90 (3.99)	21.75 (3.73)	China	DSM-IV	LSAS total: 53.90 (11.50) LSAS fear: 28.00 (6.17) LSAS avoidance: 25.90 (6.93) STAI-T: 48.25 (7.02)	n.r.	None	40 R
Liu et al. (2015)	2015a	20 (6)	20 (6)	22.90 (3.99)	21.75 (3.73)	China	DSM-IV	LSAS total: 53.90 (11.50) LSAS fear: 28.00 (6.17) LSAS avoidance: 25.90 (6.93) STAI-T: 48.25 (7.02)	n.r.	None at time of the study	40 R
Manning et al. (2015)	2015	53 (17)	33 (14)	29.9 (n.r.)	29.4 (n.r.)	USA	DSM-IV, generalized	LSAS total = 81.85 (ranging from 60-121)	n = 18: MDD; n = 16: comorbid anxiety disorders (12 with GAD, 1 with PTSD, 7 with specific phobia, 3 with panic disorder)	Off concurrent psychotropic meds for at least 2 weeks prior to scanning session	73 R; 13 L
Pannekoek et al. (2013)	2013	12 (7)	12 (7)	34 (8.8)	34 (7.2)	Netherlands	DSM-IV	--------	None	None	24 R
Prater et al. (2013)	2013	20 (11)	17 (10)	25.95 (5.39)	25.71 (7.15)	USA	DSM-IV, generalized	LSAS total: 79.35 (15.41) STAI-T: 46.45 (11.88)	n = 3: specific phobia, one of which also had GAD; n = 1 had	n = 2: SSRIs with no change of mediation or dosage for at least 8 weeks prior to the scan	37 R

									OCD; n = 1 had panic disorder; no patients had a major depressive episode within a 6-month prior to scanning		
Qiu et al. (2015)	2011	20 (6)	19 (5)	22.90 (2.99)	21.89 (3.77)	China	DSM-IV	LSAS total: 53.90 (11.50) LSAS fear: 28.00 (6.17) LSAS avoidance: 25.90 (6.93) STAI-T: 48.25 (7.02)	None	None	39 R
Qiu et al. (2011)	2015	20 (6)	19 (5)	22.90 (2.99)	21.79 (3.31)	China	DSM-IV	LSAS total: 53.90 (11.50) LSAS fear: 28.00 (6.17) LSAS avoidance: 25.90 (6.93) STAI-T: 48.25 (7.02)	None	None	39 R
Rabany et al. (2017)											
	2017	8 (5)	19 (6)	33 (3.53)	40.47 (3.58)	USA	DSM-5	CGI: 4.50 (0.267)	n.r.	n = 3:benzodiazepines; n = 3: antidepressants; Stabilized pharmacotherapy (type and dose) for 3 months before study entry with exception of benzodiazepines which were stabilized for at least 2 weeks	27 R
Warwick et al. (2008)	2008	28 (7)	19 (5)	34 (10)	37 (8)	RSA	DSM-IV, generalized	LSAS total: 99.6 (21.8)	n.r.	n = 1: alprazolam 0.25 mg/day	47 R
Yang et al. (2019)	2019	33 (13)	32 (13)	25 (6)	25 (4)	China	DSM-IV	LSAS total: 61 (22) LSAS fear: 30 (11) LSAS avoidance: 30 (11)	n.r.	None	65 R
Yoon et al. (2016)	2016	20 (10)	20 (10)	23.6 (2)	23.6 (2.3)	Republic of Korea	DSM-IV-TR, generalized	LSAS total: 84.8 (17.1)	None currently n = 3: past major depression which ended over 1 year ago	None	40 R
Yuan et al. (2018)	2018a	15 (5)	19 (6)	27.07 (8.11)	26.26 (4.90)	China	DSM-IV, generalized	LSAS total: 78.87 (27) LSAS fear: 38.13 (13.21) LSAS avoidance: 40.73 (15.22)	None	n = 4: stable dosage of SSRI for at least 4 weeks, but discontinued psychotropic medication due to poor response at	34 R

									least 2 weeks prior to baseline MRI	
Yuan et al. (2017)	2017	46 (18)	64 (29)	24.79 (6.07)	23.78 (3.33)	China	DSM-IV	LSAS total: 64.79 (22.37) LSAS fear: 32.04 (11.00) LSAS avoidance: 32.32 (12.32)	n = 2: depression	n = 14: taking psychotropic medications at study enrolment BUT were medication free at the time of scan	110 R
Yuan, Ren, et al. (2016)	2016	15 (5)	19 (6)	27.07 (8.11)	26.26 (4.90)	China	DSM-IV, generalized	LSAS total: 78.87 (27) LSAS fear: 38.13 (13.21 LSAS avoidance: 40.73 (15.22)	None	n = 4: stable dosage of a SSRI for at least 4 weeks but had to discontinue psychotropic medication due to poor response at least 2 weeks prior to the baseline MRI scan	34 R
Yuan et al. (2018)	2018b	43 (16)	43 (17)	29 (7.6)	30.14 (8.59)	China	DSM-IV	LSAS total: 69.23 (28.20) LSAS fear: 34.88 (14.20) LSAS avoidance: 34.33 (15.37)	n = 10: depression	Yes (but not stated how many and what medications)	86 R
Yun et al. (2017)	2017	28 (9)	27 (10)	23.5 (2.5)	24.2 (1.9)	Republic of Korea	DSM-IV-TR	LSAS total: 73.7 (12.5) SIAS: 45.2 (11.8)	n.r.	None at time of study participation	55 R
Zhang et al. (2015)	2015a	20 (6)	19 (5)	22.09 (3.99)	21.89 (3.77)	China	DSM-IV	LSAS total: 53.90 (11.50) LSAS fear: 28.00 (6.17) LSAS avoidance: 25.90 (6.93) STAI-T: 48.25 (7.02)	n.r.	None	39 R
Zhang et al. (2015)	2015b	40 (14)	40 (14)	25.95 (6.48)	24.80 (3.35)	China	DSM-IV	LSAS total: 65.42 (21.23) LSAS fear: 32.45 (10.39) LSAS avoidance: 32.47 (11.91)	None	n = 12: anti-anxiety medication but underwent at least 2-week washing-out prior to the MR examination	80 R
Zhu et al. (2017)	2017	42 (16)	42 (16)	27.33 (7.16)	29.83 (8.75)	China	DSM-IV	LSAS total: 67.40 (26.84) LSAS fear: 34.12 (13.00) LSAS avoidance: 35.29 (15.05)	n = 2: major depression; n = 1: GAD	n = 4: stable dosage of a SSRI for at least 4 weeks but had to discontinue psychotropic medication due to poor response at least 2 weeks prior to the baseline MRI scan	84 R

Note: [1] = demographics reported for larger sample size; AMBI = ambidextrous; CGI = clinical global impression; CON = controls; DSM-IV = diagnostic and statistical manual of mental disorders, fourth edition; DSM-IV-TR = diagnostic and statistical manual of mental disorders, fourth edition, text revision; DSM-5 = diagnostic and statistical manual of mental disorders, fifth edition; GAD = generalized anxiety disorder; L = left-handed; LSAS = Liebowitz Social Anxiety Scale; MDD = major depressive disorder; n = number of participants; n.r. = not reported; OCD = obsessive compulsive disorder; PTSD = post-traumatic stress disorder; R = right-handed; RSA = Republic of South Africa; SAD = social anxiety disorder; SD = standard deviation; SIAS = social interaction anxiety scale; SSRI = selective serotonin reuptake inhibitor; STAI-T = state -trait anxiety inventory, trait scale; USA = United States of America.

3.5.3 Neuroimaging Methods and Analyses

Of the 35 studies included, there was a range of neuroimaging methods and analyses included. Thirty-one studies used fMRI whilst four studies used alternative imaging modalities such as SPECT and PET (Doruyter et al., 2018; Doruyter et al., 2016; Evans et al., 2009; Warwick et al., 2008). Due to difficulty integrating SPECT and PET with findings from fMRI, results from these four studies will be reported in Supplementary Table 3.1. For this reason, the information presented from here on will only refer to the fMRI studies (n = 31). The results from the 31 fMRI studies that used multiple neuroimaging analysis methods on their data set were considered independent of one another (Cui et al., 2017; Geiger et al., 2016; Liao et al., 2010; Yuan et al., 2018; Zhu et al., 2017). Information regarding the scan parameters used and the pre-processing/first-level analyses can be found in Supplementary Tables 3.2 and 3.3 respectively. Of the 31 fMRI studies, the most frequently used method of analysis was seed-based/ROI-to-ROI functional connectivity analysis (n = 18), followed by the amplitude of low-frequency fluctuations (ALFF; n = 4). Other analysis methods included graph theory (n = 3), multi-voxel pattern analysis (n = 3), whole-brain analysis (a data-driven exploratory approach that seeks to identify significant correlations or activity patterns in different voxels of the brain at the size usually between 2 and 3 mm^3; n = 2), independent component analysis (ICA; n = 2), regional homogeneity analysis (ReHo; n = 2), Granger causality analysis (GCA; n = 1), and functional connectivity density analysis (FCDA; n = 1). We summarize the findings from these studies next, however, findings involving ICA, ReHo, GCA, and FCDA can be found in the Supplementary Table 3.4.

3.5.4 Seed-based (and ROI) functional connectivity studies (n = 18)

ROI/seed-based functional connectivity analysis finds regions of the brain that are correlated with activity in a certain seed-region or the brain region of interest (ROI), with the coupling of activation (usually the Pearson's correlation between brain regions, over the length

of an fMRI scan) assumed to reflect involvement in the same underlying functional process and therefore can be interpreted as being functionally connected or correlated. Seeds are usually derived a priori, based on a hypothesis, prior results, or from statistically significant regions from other modalities such as ALFF or ReHo calculations. Four studies used seed-to-seed (ROI-to-ROI) analyses (Cui et al., 2017; Rabany et al., 2017; Yang et al., 2019; Zhu et al., 2017), whilst the remaining 14 studies conducted seed-to-whole brain analyses. The most commonly reported seed was the amygdala (n = 11). Four studies (Choi et al., 2016; Cui et al., 2017; Ergul et al., 2019; Manning et al., 2015) reported findings of clusters of regions across brain areas and were therefore reported separately in Supplementary Table 3.5. Table 2 details a complete overview of the pairings found by seed-based analyses. Overall, there were a total of 200 pairings (i.e., significant connectivity between a seed and a cluster/ROI) across 15 of these studies. Of the 200 pairings, the most commonly reported connectivity was between frontal-amygdala regions (9 studies; 23 pairings), followed by frontal-parietal regions (7 studies; 22 pairings) and temporal-amygdala regions (6 studies; 10 pairings); see Supplementary Table 3.6. Of the 200 pairings, 186 pairings were positively correlated, 4 pairings from two studies were reported as negative correlations, 6 pairings across two studies reported as positively correlated in those with SAD and negatively correlated in controls, and 4 pairings across two studies were reported as negatively correlated in those with SAD and positively correlated in controls. Of the 200 pairings, exactly half were found to be higher in SAD compared to controls whereas the other half showed the opposite contrast (lower in SAD compared to controls).

3.5.4.1 Positive Connectivity

The majority of findings demonstrated positive connectivity between regions, with 186 pairings; see Table 3.2 for a full list of pairings and Figure 3.2 for a visual representation of the most consistent findings. Compared to controls, those with SAD had higher connectivity

between the following regions: frontal-amygdala (5 studies; 15 pairings), temporal-amygdala (4 studies; 6 pairings), and frontal-parietal (3 studies; 4 pairings). Compared to controls, those with SAD had lower connectivity between the following regions: frontal-amygdala (4 studies; 7 pairings), frontal-parietal (4 studies; 18 pairings), and temporal-temporal (2 studies; 11 pairings), and frontal-temporal (2 studies; 13 pairings).

Table 3.2

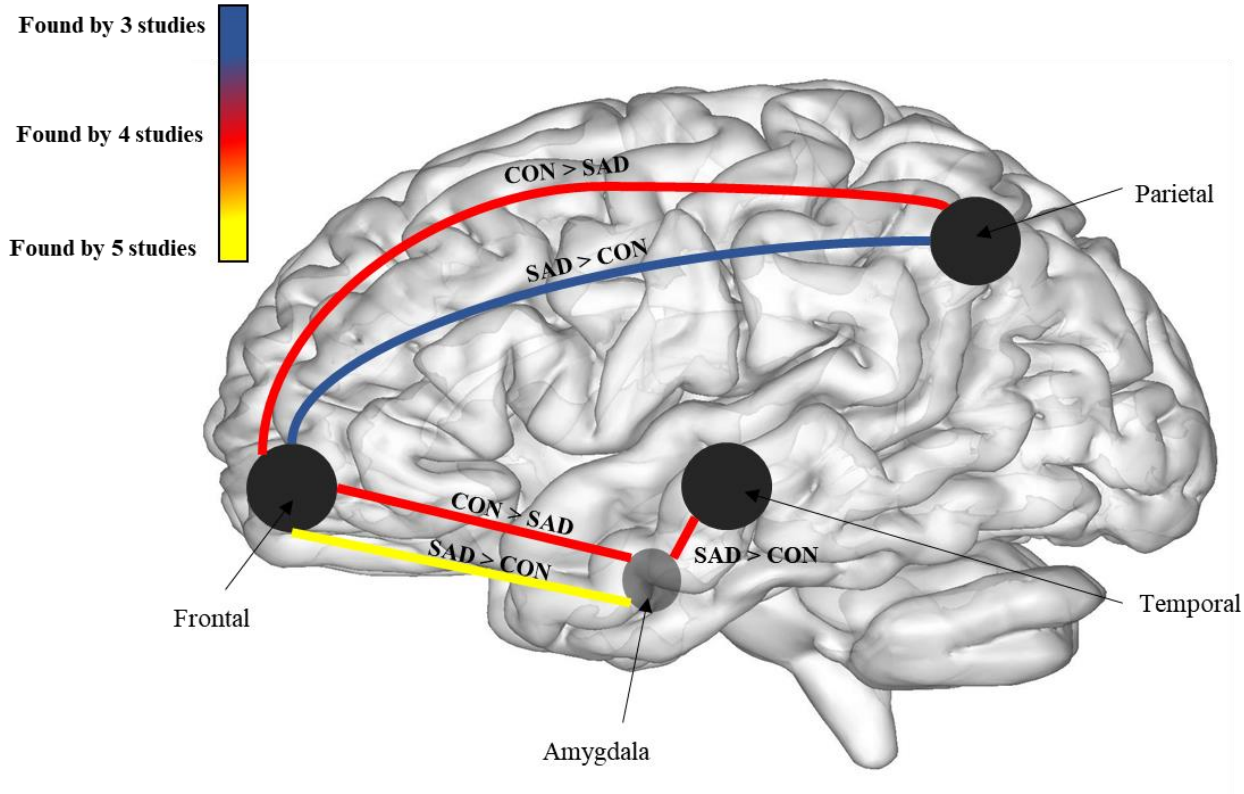
Summary of Connectivity Findings between Regions from Seed-Based fMRI Analyses – Specifically Positive Connectivity Pairings

Brain Region 1	Brain Region 2	Total pairings	Total studies	Pairings	Studies	Pairings	Studies	Pairings	Studies
				Pos Conn.	Pos Conn.	Pos Conn. SAD > CON	Pos Conn. SAD > CON	Pos Conn. CON > SAD	Pos Conn. CON > SAD
Frontal	Temporal	24	3	24	3	11	1	13	2
Frontal	Amygdala	23	9	22	9	15	5	7	4
Frontal	Parietal	22	7	22	7	4	3	18	4
Temporal	Temporal	13	4	13	4	2	2	11	2
Temporal	Amygdala	10	6	7	5	6	4	1	1
Occipital	Temporal	9	2	8	1	0	0	8	1
Frontal	Occipital	8	3	8	3	4	2	4	1
Frontal	Subcortical	8	2	7	2	6	1	1	1
Thalamus	Temporal	8	2	8	2	8	2	0	0
Parietal	Amygdala	7	5	3	2	2	1	1	1
Parietal	Temporal	7	3	7	3	1	1	6	2
Insula	Frontal	7	2	7	2	6	1	1	1
Frontal	Frontal	6	2	6	2	3	1	3	1
Parietal	Subcortical	6	2	6	2	5	1	1	1
Frontal	Cerebellar	4	1	4	1	0	0	4	1
Amygdala	Cerebellar	4	2	3	1	3	1	0	0
Thalamus	Parietal	4	1	4	1	4	1	0	0
Amygdala	Occipital	3	3	2	2	1	1	1	1
Temporal	Subcortical	3	1	3	1	3	1	0	0
Amygdala	Subcortical	3	2	2	1	0	0	2	1
Thalamus	Cerebellar	2	1	2	1	0	0	2	1
Parietal	Cerebellar	2	1	2	1	0	0	2	1
Insula	Temporal	2	1	2	1	2	1	0	0
Parietal	Parietal	1	1	1	1	0	0	1	1
Subcortical	Cerebellar	1	1	1	1	1	1	0	0
Thalamus	Frontal	1	1	1	1	1	1	0	0
Thalamus	Insula	1	1	1	1	1	1	0	0
Thalamus	Occipital	1	1	1	1	1	1	0	0
Thalamus	Amygdala	1	1	1	1	0	0	1	1
Subcortical	Occipital	1	1	1	1	1	1	0	0
Insula	Insula	1	1	1	1	1	1	0	0
Insula	Subcortical	1	1	1	1	0	0	1	1
Insula	Occipital	1	1	1	1	1	1	0	0
Insula	Parietal	1	1	1	1	0	0	1	1
Amygdala	Insula	1	1	0	0	0	0	0	0

Note. CON = controls; Conn.=connectivity; SAD = social anxiety disorder.

Figure 3.2

Group Differences from Seed-Based Connectivity Studies (n = 14)



Note. This figure shows common brain region pairings as supported by positive connectivity findings from three or more fMRI studies.

3.5.4.2 Negative Connectivity

Only two studies reported higher negative connectivity in SAD compared to controls between the following regions: amygdala-lateral occipital cortex, amygdala-middle temporal gyrus, amygdala-supramarginal gyrus, and the posterior inferior temporal gyrus-inferior occipital gyrus. Both of these studies used global signal regression, a processing step that is known to induce a substantial amount of anti-correlations. No studies reported lower connectivity in those with SAD compared to controls (Murphy & Fox, 2017). No studies reported lower connectivity in those with SAD compared to controls.

3.5.4.3 Mixed Connectivity

Two studies reported that those with SAD (compared to controls) had higher connectivity between the amygdala - precuneus, amygdala - posterior cingulate cortex, and amygdala - left superior temporal gyrus, and lower connectivity between the amygdala - cerebellum, amygdala - anterior insula, and amygdala - supramarginal gyrus. In the aforementioned pairings, positive correlations between brain regions were reported for the SAD group and negative correlations between brain regions were reported for the control group. One study reported that those with SAD (compared to controls) had higher connectivity between the amygdala - lentiform nucleus, and lower connectivity between the amygdala - supplementary motor area, and the amygdala - right middle temporal gyrus. In all these pairings, negative correlations between brain regions were reported for the SAD group and positive correlations between brain regions were reported for the control group.

3.5.5 Amplitude of Low-Frequency Fluctuations (ALFF) Studies (n = 4)

The ALFF method quantifies the power of the BOLD signal is within a low-frequency range of activation (typically in the range of 0.01 to 0.1 Hz) that is thought to be an indirect representative of neuronal activity (e.g., cortical activity or basal ganglia activity) while containing minimal artefacts (Yang et al., 2007). Four studies used ALFF as an analysis method (Qiu et al., 2015; Yuan et al., 2018; Yuan et al., 2018; Zhang et al., 2015); see Supplementary Table 3.7. Most consistently, three of these studies (Qiu et al., 2015; Yuan et al., 2018; Zhang et al., 2015) found that individuals with SAD (vs. controls) had lower ALFF across 17 frontal regions (such as inferior, middle and superior frontal gyri, median cingulate gyrus, Rolandic operculum, precentral gyrus, prefrontal, and supplementary motor areas).

3.5.6 Graph Theory Studies (n = 3)

Graph theory is the mathematical field of network science. Graph theory quantifies the topological configuration and complexity of brain network function by delineating the local

and global organization of brain networks (Bullmore & Sporns, 2009). Three resting-state brain imaging studies utilized graph theory in their methodology (Yang et al., 2007; Yun et al., 2017; Zhu et al., 2017); see Supplementary Table 3.9. Temporal (middle and inferior temporal gyrus, superior-middle temporal cortices, hippocampus), frontal (inferior frontal cortices, middle frontal gyrus) and parietal regions (angular gyri, posterior cingulate gyrus, supramarginal gyrus) were most implicated in the findings.

3.5.7 Multi-Voxel Pattern Analysis (MVPA; n = 3)

Multi-voxel pattern analysis (MVPA) is a machine learning approach (usually based on support vector machines) that can be used to predict categories from various patterns of activation across brain voxels. Three studies used MVPA as part of their neuroimaging analysis to determine whether resting-state data distinguished between groups with and without a diagnosis of SAD (Liu et al., 2015; Zhang et al., 2015; Zhu et al., 2017); see Supplementary Table 3.10. Frontal regions were most implicated in these studies in being able to distinguish between groups.

3.5.8 Associations between Brain and Dimensional Measures

Of 35 studies included in the review, 17 studies reported associations between resting-state brain activity/connectivity and behavioural measures; see Supplementary Table 3.11. The most common behavioural outcome studied involved social anxiety symptoms across 13 studies, which all used the Liebowitz Social Anxiety Scale (LSAS; n = 13), and others additionally including the Social Phobia Scale (n = 1) or the Brief Fear of Negative Evaluation (n = 1). Of those studies assessing resting-state brain connectivity (n = 7), frontal-occipital pairings were consistently positively associated with symptoms of social anxiety (11 pairings across 1 study). Other pairings associated with symptoms of social anxiety included: amygdala-frontal (3 studies; 3 pairings positively associated, 1 pairing negatively associated), amygdala-temporal (2 studies; 3 pairings negatively associated), and frontal-temporal (1 study; 1 pairing

positively associated, 1 pairing negatively associated). Other pairing combinations were found by individual studies; see Supplementary Table 3.11. Other studies measured general anxiety (Hamilton Anxiety Scale, n = 2; Spielberg State-Trait Anxiety Scale, n = 1), depression (Hamilton Depression Scale, n = 2; Beck Depression Inventory, n = 2), and illness duration (n = 2), and these findings are reported in the Supplement.

3.5.9 Risk of Bias

Results from the quality assessment showed consistency in the quality of studies included in this review; see Supplementary Table 3.12. All 35 studies stated the research question clearly, with a clearly defined study population. No studies were pre-registered, and no studies provided a sample size justification or power description for the sample used. The quality check highlighted inconsistencies across studies in whether confounding variables were adjusted for statistically when examining resting-state neuroimaging between groups (SAD vs. controls). Only 14 of the 35 studies controlled for potential confounding variables in their statistical analyses, controlling for variables such as gender, age, mean framewise displacement, medication status, and education level (Cui et al., 2017; Dorfman et al., 2016; Hahn et al., 2011; Jung et al., 2018; Liao et al., 2010; Feng Liu et al., 2015; Pannekoek et al., 2013; Rabany et al., 2017; Yang et al., 2019; Yuan et al., 2018; Yuan et al., 2017).

3.6 Discussion

This systematic review aimed to obtain a comprehensive understanding of the brain regions and networks implicated in people with SAD compared to controls, focusing on resting-state multimodal neuroimaging techniques and analysis methods. It included the examination of associations between brain and dimensional measures in people with SAD. Of the 35 studies, the most common analysis approach involved seed-based analysis. Frontal regions were most implicated across studies and analysis methods and in the relationships between brain and dimensional measures. Even when excluding findings from studies that had

smaller sample sizes that were uncorrected (Geiger et al., 2016; Hahn et al., 2011; Pannekoek et al., 2013; Rabany et al., 2017), similar findings remained. From seed-based studies, the SAD group had both higher and lower positive connectivity between the amygdala and frontal regions and between the amygdala and parietal regions, and lower positive connectivity between the amygdala and temporal regions. Findings from ALFF predominantly demonstrated lower ALFF across 17 frontal regions in those with SAD compared to controls. Likewise, across other non-seed analysis methods (e.g., graph theory, MVPA), frontal regions (i.e., superior and middle frontal gyrus) were most reported throughout. The superior frontal gyrus was most commonly implicated across all fMRI studies, being reported 63 times. It was also most consistently found to be associated with social anxiety symptoms, with results showing negative and positive correlations. The middle frontal gyrus, inferior frontal gyrus, and anterior cingulate cortex were also frequently reported across studies. Other frontal regions implicated, albeit to a lesser extent, were the dorsolateral PFC, the dorsomedial PFC, the precentral gyrus, Rolandic operculum, rectal gyrus, supplementary motor area, and the orbitofrontal gyrus.

3.6.1 Theoretical Implications

Review findings partially deviated from the two key models of SAD (Brühl et al., 2014; Etkin & Wager, 2007). These models posited that those with SAD had hyperactivation of the fear circuitry (consisting of the amygdala, insula, PFC, and anterior cingulate cortex) compared to controls. Brühl et al.'s (2014) model additionally pointed to higher activation in the cuneus, precuneus, and the posterior cingulate cortex, which were less functionally connected to other neural regions (including the fusiform gyrus, amygdala, dorsolateral/medial PFC, and the anterior cingulate cortex) in those with SAD compared to controls. Due to the lack of studies examining neural activation in resting-state neuroimaging in SAD, we cannot comment on whether findings regarding activation differed. However, the connectivity findings from this

review only partially supported Brühl et al. (2014) model of connectivity between regions; in most instances, our review demonstrated that connectivity pairings are less clear cut with findings of both hyper- and hypoconnectivity; see Supplementary Table 3.13. Furthermore, findings of higher connectivity between the amygdala and temporal regions (as demonstrated in 6 pairings across 4 studies of this review) were unfounded by Brühl's model.

These discrepancies may be a result of examining only resting-state neuroimaging data in the current review as opposed to the predominantly task-based neuroimaging data that informed these neurobiological models of SAD (e.g., Brühl et al. (2014)). Potentially, people with SAD have certain connectivity patterns between brain regions when encountering socially- and disorder-relevant information (e.g., emotional faces) or when they are anticipating events that would typically induce social anxiety (such as public speaking or social interactions). However, in the absence of such stimuli (resting-state), there may be no requirement for the same connectivity patterns to arise. Therefore, the Brühl et al. (2014) model of SAD may be more suitable as a neurobiological model of stimuli response, rather than as an accurate model of the normal underlying neuropathology (at rest) of the disorder. Next, we provide a discussion of the main findings, reporting on the neural pairings identified by most studies in this review.

3.6.2 Amygdala – Frontal Connectivity

The most common finding was alterations in positive connectivity between the amygdala and frontal areas, with 15 pairings across 5 studies reporting higher connectivity and 7 pairings across 4 studies reporting lower connectivity in SAD compared to controls. This suggests that alterations in this pathway are a core feature of SAD, however, we note that this effect may also be partially due to the amygdala being the most commonly used seed.

The inconsistency in findings of higher and lower connectivity between these regions in this review may be due to recent evidence in both controls and in varying clinical samples

demonstrating the importance of examining subregions of the amygdala (including the centromedial, basolateral, amygdalostriatal, and superficial complex) and their connectivity patterns rather than examining the amygdala as a whole. Evidence of disturbances in the fear circuitry in only specific subregions of the amygdala has been demonstrated in clinical groups (e.g., autism spectrum disorder, post-traumatic stress disorder, major depressive disorder) and in controls (Kleinhans et al., 2016; Roy et al., 2009; Tang et al., 2019; Yuan et al., 2019). For example, hyperconnectivity between the amygdala and the PFC was only found when looking at the centromedial complex (rather than basolateral or superficial complex) in people with autism spectrum disorder (vs. controls; Kleinhans et al., 2016). In this review, one of the studies contributing to evidence of hyperconnectivity between the amygdala and PFC used amygdala subregions as seeds and found no evidence of hypoconnectivity (Yoon et al., 2016). Further research examining amygdala subregion connectivity with the PFC in those with SAD may be needed to clarify this aspect of the fear circuitry in SAD.

An alternative explanation for the inconsistent findings regarding higher and lower connectivity between the amygdala and frontal regions may be due to our grouping of frontal regions as one area. The frontal lobe is thought to be structurally and functionally divided into separate regions with different connectivity patterns, and structural and functional divisions (Stuss et al., 1995). A clearer pattern of connectivity between the amygdala and frontal regions arises when frontal regions are examined as smaller subdivisions. For example, looking specifically at the dorsomedial PFC, there were consistent reports of higher connectivity between this region and the amygdala. Notably, this is shown to be associated with increased self-directed criticism and an increased tendency to exaggerate the significance of potentially self-relevant stimuli from external threat cues in those with SAD (Blair, Shaywitz, et al., 2008; Yuan, Zhu, et al., 2016). Another example of the usefulness of looking at specific frontal regions is by examining the rostral medial PFC, with consistent reports of lower connectivity

between this region and the amygdala in those with SAD compared to controls. Previous research suggests that a decrease in connectivity between these regions is associated with increased social interactional anxiety and decreases in emotion regulation (Dodhia et al., 2014). These findings highlight the importance of considering the various smaller individual subregions of the frontal lobe when examining and interpreting findings, given the number of structurally and functionally different regions that exist. However, discrepancies in how divisions are defined and analysed could lead to difficulty in the synthesis of findings across studies.

3.6.3 Parietal – Frontal Connectivity

Eighteen pairings across 4 studies found evidence of lower connectivity and 4 pairings across 3 studies found evidence of higher connectivity between parietal-frontal regions in those with SAD compared to controls. The most commonly reported pairings were lower connectivity between the bilateral posterior cingulate gyrus and the bilateral superior frontal gyrus, the bilateral precuneus and the right superior frontal gyrus, and the bilateral precuneus and the bilateral gyrus rectus.

3.6.4 Amygdala – Temporal Connectivity

Six pairings across four studies found higher connectivity between these regions in those with SAD compared to controls, and only one pairing from a separate study demonstrating evidence of lower connectivity. The most commonly reported pairings were between the left amygdala and the bilateral fusiform gyrus, and the bilateral amygdala and the bilateral parahippocampal gyrus.

3.6.5 Functional Interpretations

The main connectivity pairings and regions identified in this review have been investigated in previous literature, with tentative interpretations being made of their function. In anxiety disorders, including SAD, it has been thought that the connection between the

amygdala and PFC plays a significant role in controlling attention to salient stimuli and emotion regulation with the presence of disturbed top-down control (inability of the PFC to inhibit the amygdala response) or increased bottom-up processes (hypersensitive amygdala leading to increased activity in the PFC) in maintaining anxiety (Bishop, 2007). The precuneus and posterior cingulate gyrus are known to be important hubs of the default mode network and are involved in self-referential processing, the integration of present and past information, and in allowing for an observer perspective of social interactions (Petrini et al., 2014; Whitfield-Gabrieli et al., 2011). The superior frontal gyrus is thought to be involved in the initiation of novel responses and is activated during shifts of attention (Nagahama et al., 1999; Peraud et al., 2002). Therefore, altered connectivity between these regions and frontal areas may be linked to impairment in socio-cognitive processes that are seen in SAD. The fusiform gyrus is implicated in facial visual processing, and hyperconnectivity between this region and the amygdala may reflect constant hypervigilance to social threats (e.g., angry faces) in people with SAD. This is consistent with Wong and Rapee (2016) recent model of SAD in which they proposed that the constant alertness to social-evaluative threats in the environment serves as a maintenance factor of this disorder. Hyperactivity of the parahippocampal region has been interpreted as being indicative of disruptions to the process of assigning accurate saliency value to a stimulus (Binelli et al., 2014). Therefore, hyperconnectivity between the amygdala and this region may be contributing to dysfunction in post-event processing that also plays a role in the maintenance of this disorder (Wong & Rapee, 2016).

3.6.6 Limitations and Future Directions

Heterogeneity in the sequence parameters and low power are likely to be significantly contributing to the inconsistencies in the findings of this review, and critically needs to be addressed in future studies. Many of the included studies involved a small sample size of participants with SAD (average n = 23; minimum n = 7; maximum n = 53). No study justified

their sample size or included a power calculation. The use of small sample sizes may have contributed to the inconsistent findings (Blackford, 2017) and/or inevitably led to an inflated risk of false positives due to the high number of variables in brain images (Eklund et al., 2016). Supplementary Figure 3.1 demonstrates an increased proportion of uncorrected statistical between-group findings in the studies with smaller sample sizes compared to those with larger sample sizes. Furthermore, previous research shows that sample sizes of 20-30 subjects are likely insufficient to detect reliable relationships between brain and behaviour measures that are reproducible (Grady et al., 2021). It is therefore difficult to confidently conclude if the aforementioned resting-state neuroimaging findings are directly linked to subjective self-reported experiences of social anxiety, and replication is needed in future research. The overlap in participants across four datasets was also another limitation, as it is possible that findings from these samples were inflated due to their recurrent use. Furthermore, there is evidence that physiological confounds, such as differences in respiration and state anxiety, during the scanning process may result in changes in cerebral blood flow and ultimately influence the results (Giardino et al., 2007). For example, there is evidence of a causal role of the amygdala in respiration (Nobis et al., 2018). Whilst the potential confounding effects of state anxiety on fMRI were controlled for by most studies through the use of the State Anxiety Inventory Scale, no studies controlled for the effects of respiration on the findings. Additionally, other important confounders (e.g., medication use, psychiatric comorbidity) and the severity of the disorder (measured by the LSAS) varied widely across samples used and may also have contributed to the variation in the findings observed.

The integration of findings from studies in this review was also hindered by heterogeneity in scanning methods and in the analysis techniques used (see Supplementary Tables 3.2 and 3.3). It can be problematic to integrate results across differing fMRI acquisition settings due to the potential effects that these settings have on results. For example, differences

in repetition time can impact on the discrimination between activated and non-activated brain tissue (Constable & Spencer, 2001). However most notably, the scan time varied between studies (ranging from 200 to 471 seconds) and this likely had an impact on the results. Noble et al. (2017) demonstrated that resting-state scans with a duration of 300 seconds or less were associated with poor test-rest reliability of connectivity. It has been suggested that 10 minutes or more of resting-state data is needed for good intra- and inter-session reliability (Birn et al., 2013), and a higher image sampling rate is unlikely to make up for shorter scan duration (Airan et al., 2016). We support the recommendation of longer scan times to increase the amount of data per subject, which also allows researchers to investigate dynamical brain properties and state changes in functional imaging data (Hutchison et al., 2013). We were unable to draw inference from non-seed analyses given the limited number of studies on these methods (e.g., graph theory, MVPA) to date. Perhaps in future reviews at a later stage, this may be possible and is needed if we are to fully understand the biological mechanisms underlying SAD.

We find it encouraging that the field of resting-state fMRI is moving onto a common data processing framework called *fMRIprep* (Esteban et al., 2019). Streamlined pre-processing frameworks lead to a methodological consistency between studies which will make it easier for scientists to replicate prior research. Differences in the management of common artefacts (such as head motion or breathing effects), the application of global signal regression, and motion thresholds, are susceptible to false-positive results and need to be critically assessed when interpreting findings. Image registration, smoothing, and filtering of resting-state fMRI data also matter in terms of how brain regions are allocated, and differences in this may also influence the interpretation of the findings (Weinberger & Radulescu, 2016).

Given the cross-sectional design of all studies included in this systematic review, it is currently not possible to derive any etiological theories of SAD from this review as causation cannot be implied *(i.e., do neural alterations cause SAD?)*. Whilst having a snapshot of the

neurobiology of SAD in a pre-diagnosed sample is helpful, other brain areas and connections may play a role in the development of this disorder. Longitudinal study designs with sufficient samples from adolescence into adulthood may help increase our understanding of the development of this disorder, and whether neural alterations are unique to or contribute to the development and maintenance of this disorder.

3.6.7 Conclusions

This review suggests that the neurobiology of SAD may differ from previously proposed models that were derived predominantly from the synthesis of task-based neuroimaging studies. In the absence of a task (i.e., resting-state), the literature shows that on average, those with SAD have aberrant connectivity between the amygdala and temporal, parietal, and frontal regions. Additionally, there appear to be differences in the activity of frontal regions in those with SAD compared to controls as shown by a range of neuroimaging analyses. Frontal regions were also found to have significant associations with social anxiety severity. Even amongst the most consistent findings demonstrated across studies, there remained great variation in the direction of activity (hypo- vs. hyperactivity) within regions and connectivity (SAD > HC vs. HC > SAD) between regions. The wide range of analysis methods and seeds used for functional connectivity analyses may have contributed to the presence of mixed findings and led to difficulty in synthesizing results across studies to form strong conclusions regarding the neurobiology of those with SAD at rest. Therefore, this review has led us to provide recommendations to improve methodology to ensure greater rigor for future studies. Further research using studies with larger sample sizes of clinical participants and more consistent analysis methods is necessary to provide further clarification of the resting-state neurobiology of SAD.

3.7 Supplementary Information

3.7.1 Methods

3.7.1.1 Search Strategy

This review was pre-registered with PROSPERO (CRD42020163027) and was conducted following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009). Searches were conducted on the 29th of November, 2020 in the following seven databases: CINAHL, MEDLINE, OVID, PsycINFO, PubMed, Scopus, and Web of Science. Title, abstract, and keyword searches were conducted using the following constructs: "social anxiety" OR "social phobia" OR "socially anxious" AND "resting state" OR "resting-state" OR "at rest" OR "resting". Reference lists of identified studies in this initial search were also examined to ensure no relevant articles were missed.

3.7.1.2 Eligibility Criteria

This review included original studies with the main objective of investigating resting-state neuroimaging in people with SAD. During the screening of abstracts and full texts, studies were included based on the following criteria: i) the full text was published in English, ii) human participants were involved, iii) brain function/activation was measured at rest, and iv) a sample of participants with a diagnosis of SAD was compared to a control group. Reasons for exclusion included being a: i) non-peer-reviewed, non-published or non-empirical paper (e.g., dissertations, corrigendum, editorials, case reports, book chapters, or conference abstracts) or ii) review or a meta-analysis.

3.7.1.3 Data Extraction Process

One reviewer (S.M.) conducted the searches in November 2020, screened for duplicates, and extracted the data. Any uncertainty regarding the eligibility of studies to be included in this review was followed-up with a discussion with the chief investigator (I.L.) for clarification. From each of the included studies in the systematic review, the following

information was extracted: year, author, title, primary aims and hypotheses, study design, results, and conclusions. Details about the participant sample were also extracted, including age, sex, pharmacotherapy use, exposure to psychotherapy, diagnosis (including the severity of symptoms), the method in which diagnosis was made, and the number of participants excluded due to poor quality of the images obtained. Further, information regarding the neuroimaging methodology was extracted, including details of the scanner used, the resting-state acquisition paradigm, and analysis processes.

3.7.1.4 Data Synthesis

Data were synthesized by demographic information (e.g., sample size, sex, age, diagnosis), and neuroimaging information relating to resting-state analysis type (e.g., seed-based, ICA, ReHo, ALFF), the modality of the scan (e.g., fMRI, PET, SPECT), type of connectivity (positive, negative or mixed), and the direction of the contrast (SAD > HC or vice versa). For the main neuroimaging findings, results were grouped by region (frontal, temporal, parietal, basal ganglia/subcortical, amygdala or thalamus) to demonstrate how consistent the findings were across studies within each region (activation findings) and between the regions (connectivity findings). Information on brain-behavior relationships was also reported.

3.7.1.5 Quality Assessment

The articles included in this review were assessed by one reviewer (S.M.) using the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies published by the National Heart, Lung and Blood Institute (National Institutes of Health, 2014). This assessment tool consists of 14 questions to be answered yes, no, not reported, or not applicable. As no cohort studies were included in this review, we adapted the assessment tool to remove questions (i.e., questions 6, 7, 8, 10, and 12) that primarily referred to quality issues in cohort studies.

3.7.2 Results

3.7.2.1 Sample Population

Three pairs of studies used identical samples: Liu et al. (2015); Liu et al. (2015), and Liao et al. (2010); Qiu et al. (2011), and Yuan et al. (2018); Yuan, Zhu, et al. (2016). Four studies had overlapping subjects in both the SAD and control groups (Ding et al., 2011; Liao et al., 2010; Liao et al., 2010; Liao et al., 2011), but the degree of overlap remains unclear. However, given the analyses of the neuroimaging data differed between these studies, they were all considered as separate samples from hereon in. Of the 35 included studies, the Structured Clinical Interview for DSM-IV or DSM-IV-TR (SCID) – Patients Version was the most commonly used validated tool to confirm a diagnosis of SAD (n = 25; First, 1997; First et al., 2002). Other tools used by individual studies included the Clinical International Diagnostic Interview 2.1 (CIDI; World Health Organization, 1997), the Anxiety Interview Schedule for DSM-IV (Grisham, 2004), the Mini-International Neuropsychiatric Interview (Sheehan et al., 1998), and the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime version (Kaufman et al., 1997). One study confirmed diagnosis by clinical interview and three studies did not state how the diagnosis was confirmed. Two studies used the Liebowitz Social Anxiety Scale (LSAS) total score as an additional tool to verify diagnostic status (Dodhia et al., 2014; Prater et al., 2013). In 27 (out of 35) studies, participants were not taking any medications at the time of participation, but five of these 27 studies included participants who were previously on medication (i.e., anti-anxiety or selective serotonin reuptake inhibitor medication) but who undertook a minimum 2-week washing-out period before participation (Manning et al., 2015; Yuan et al., 2018; Yuan, Ren, et al., 2016; Zhang et al., 2015; Zhu et al., 2017). Of the remaining studies, 7 studies had participants with SAD who were taking medications including anti-depressants (Jung et al., 2018; Prater et al., 2013; Rabany et al., 2017) and benzodiazepines (Doruyter et al., 2016; Jung et al., 2018; Rabany et al., 2017; Warwick et al., 2008). Two studies reported that their participants were

taking psychotropic medications but did not specify the class of drug used (Yuan et al., 2018; Yuan et al., 2017).

3.7.2.2 Findings from Study with Paediatric Sample

Dorfman et al. (2016) reported that compared to controls, those with SAD had decreased positive connectivity in the following pairings: right ventral caudate – right SFG, right dorsal caudate – right subgenual ACC, right dorsal caudate – left posterior insula, and left nucleus accumbens – right caudate. Those with SAD were also reported to have decreased connectivity between the left dorsal caudate – left middle frontal gyrus compared to controls, but there were negative correlations between these brain regions in the SAD group and positive correlations in the control group.

3.7.2.3 *Findings from Studies using Other Analyses (n = 8)*

Other methods of analysis used were independent component analysis (ICA; n = 2; Geiger et al., 2016; Liao et al., 2010), regional homogeneity analysis (ReHo; n = 2; Qiu et al., 2011; Zhang et al., 2015), whole-brain functional connectivity analysis (n = 1; Ding et al., 2011), whole-brain functional connectivity strength (n = 1; Liu et al., 2015), Granger causality analysis (n = 1; Liao et al., 2010), and functional connectivity density analysis (n = 1; Cui et al., 2017); see Supplementary Table 3.4. Findings from ICA primarily implicated the frontal (39 regions), parietal (23 regions), and temporal (13 regions) lobes. The aforementioned regions were found across the following networks: executive control, default mode, somatomotor, visual, dorsal attention, central executive, core, and auditory networks. Findings from ReHo primarily implicated frontal (18 regions), parietal (8 regions), and temporal (8 regions) lobes. Findings from Granger causality analysis mostly implicated regions within the frontal lobe (25 regions), followed by the temporal (13 regions) and occipital (12 regions) lobes. Cui et al. (2017) found that those with SAD (vs. controls) had reduced FCD from the right rostral anterior cingulate cortex and the right superior temporal gyrus.

3.7.2.4 Associations Between Brain and Dimensional Measures

3.7.2.4.1 Mood Symptoms (n = 6). Three studies examined the relationship between general anxiety levels and connectivity (Jung et al., 2018; Yuan et al., 2018; Yuan et al., 2017). Three pairings from one study (Jung et al., 2018) reported a positive correlation between general anxiety levels and connectivity between the amygdala and the supramarginal gyrus/precuneus/superior temporal gyrus. Two studies found evidence of negative correlations between general anxiety levels and connectivity between cerebellar regions and the precuneus/dorsal medial prefrontal cortex (Yuan et al., 2018; Yuan et al., 2017). Three studies assessed the relationship between depression and brain activity/connectivity (Doruyter et al., 2018; Jung et al., 2018; Yuan et al., 2018). One study reported a positive association (amygdala and superior temporal gyrus connectivity; Jung et al., 2018) and another study reported a negative association (precuneus and cerebellum connectivity; Yuan et al., 2018). Findings from regional brain metabolism and ALFF analyses consistently reported negative associations between the precuneus and fusiform gyrus and general depression levels (Doruyter et al., 2018; Yuan et al., 2018).

3.6.2.4.2 Illness Duration (n = 2). Illness duration was positively associated with connectivity of the Vermis IX and thalamus (Yuan et al., 2017) and negatively associated with functional connectivity strength of the precuneus and illness duration (Liu et al., 2015).

Supplementary Table 3.1

Results from Non-fMRI Studies

Author	Modality	Analysis	Contrast	Findings
Warwick et al. (2008)	SPECT	Whole-brain	CON > SAD	Pons, L. cerebellum, R. precuneus
			SAD > CON	L. frontal, R. anterior frontal, R. lateral frontal, R. cerebellum
Evans et al. (2009)	PET	ROI & whole-brain	CON > SAD	L. subcallosal cortex, L. dorsal ACC
Doruyter et al. (2016)	SPECT	Seed-based	CON > SAD	L. amygdala – R. SFG/anterior-dorsal ACC[1] Precuneus/PCC – L. cerebellar crus 1[1]
			SAD > CON	R. thalamus – R. MFG and MFG, orbital part [2]
Doruyter et al. (2018)	PET	ROI & whole-brain	SAD > CON	L. FFG, R. temporal pole (mid)

Note. [1] = positive in CON, negative in SAD; [2] = negative in CON, positive in SAD; CON = controls; PET = positron emission tomography; ROI = region-of-interest; SAD = social anxiety disorder; SPECT = single-photon emission computerized tomography.

Supplementary Table 3.2

Scan Parameters of fMRI Studies

Author (year)	Type of Scan	Matrix	Length of scan (seconds)	Eyes open /closed	Phase encode direction	TR (ms)	TE (ms)	Field of view	Flip angle	Voxel size (mm^3)	# of slices	Slice gap	Total volumes	Slice thickness	Analysis type
Anteraper et al. (2014)	Single-shot gradient EPI	NS	384	Open	A > P	6000	30	NS	90	2 x 2 x 2	67	NS	NS	NS	Seed-based
Choi et al. (2016)	EPI	64 x 64 x 30	300	Open	NS	2000	22	24 cm	12	NS	NS	NS	NS	NS	Seed-based
Cui et al. (2017)	EPI	64 x 64	NS	Closed	NS	2000	30	24 cm	90	3.75 x 3.75 x 5 (resampled to 3 x 3 x 3)	30	none	205	NS	Functional connectivity density; Seed-based; ROI-to-ROI; Discriminant analysis
Ding et al. (2011)	Single-shot gradient recalled EPI	64 x 64	NS	Closed	NS	2000	30	24 cm	90	resampled to 3 x 3 x 3	30	none	205	NS	Whole-brain
Dodhia et al. (2014)	Gradient-echo EPI	128 x 128	200	Closed	NS	2000	40	21 cm	90	NS	25	none	NS	NS	Seed-based
Dorfman et al. (2016)	EPI	NS	360	Open	NS	2000	30	19.2 cm	90	3 x 3 x 4	NS	NS	180	NS	Seed-based
Ergul et al. (2019)	EPI	112 x 117	451	Closed	NS	2000	30	224 x 240 mm	90	2 x 2 x 4 (resampled to 2 x 2 x 2)	36	NS	214 + 10 dummy	NS	Seed-based
Geiger et al. (2016)	EPI	210 x 210	300?	Open	NS	2000	30	210 mm	90	3.3 x 3.3 x 3.0 (resampled to 2 x 2 x 2)	34	NS	150	NS	ICA; Seed-based
Hahn et al. (2011)	Gradient-echo EPI	96 x64	360	Open	AC-PC	1000	40	230 x 190 mm	NS	NS	14	1 mm	NS	6 mm	Seed-based
Jung et al. (2018)	EPI	NS	300	NS	NS	2000	30	NS	80	3.4	34	NS	NS	NS	Seed-based
Liao et al. (2010)	Single-shot gradient-recalled EPI	64 x 64	410	Closed	AC-PC	2000	30	24 cm	90	3.75 x 3.75 x 5 (resampled to 3 x 3 x 3)	30	None	205	5 mm	ICA
Liao et al. (2010)	Single-shot gradient-recalled EPI	64 x 64	410	Closed	AC-PC	2000	30	24 cm	90	3.75 x 3.75 x 5 (resampled to 3 x 3 x 3)	30	None	205	5 mm	GCA
Liao et al. (2011)	Single-shot gradient-recalled EPI	64 x 64	410	Closed	NS	2000	30	24 cm	90	3.75 x 3.75 x 5 (resampled to 3 x 3 x 3)	30	None	205	NS	Seed/ROI-based
Liu et al. (2015)	Single-shot gradient-recalled EPI	64 x 64	NS	NS	NS	2000	30	24 cm	90	3.75 x 3.75 x 5 (resampled to 3 x 3 x 3)	30	None	205	5 mm	MVPA
Liu et al. (2015)	Single-shot gradient-recalled EPI	64 x 64	NS	Closed	NS	2000	30	240 x 240 mm	90	3.75 x 3.75 x 5 (resampled to 3 x 3 x 3)	30	None	205	5 mm	Whole-brain FC strength

Manning et al. (2015)	NS	NS	360	Open	NS	6000	30	NS	90	2 x 2 x 2	67	none (interleaved)	NS	1 mm	Seed-based
Pannekoek et al. (2013)	Gradient-echo EPI	NS	471	Closed	NS	2300	30; 28	220 x 220 mm	80	2.3 x 2.3 mm; 3.45 x 3.45 mm	35; 39	None	200	3 mm	Seed-based
Prater et al. (2013)	Gradient-echo EPI	64 x 64	300	Open	NS	2000	25	240 mm	77	3.75 mm^2 inplane	30	NS	NS	5-mm	Seed-based
Qiu et al. (2011)	Single-shot gradient-recalled EPI	64 x 64	410	Closed	AC-PC	2000	30	24 cm	90	3.75 x 3.75 x 5 (resampled to 3 x 3 x 3)	30	None	205	5 mm	ReHo
Qiu et al. (2015)	Single-shot, gradient-recalled EPI	64 x 64	NS	NS	NS	2000	30	24 cm	90	resampled to 3 x 3 x 3	30	None	NS	5-mm	ALFF
Rabany et al. (2017)	EPI sequence	NS	315	Open	NS	1500	27	NS	70	3.4 x 3.4 x 5	29	NS	NS	NS	ROI-to-ROI
Yang et al. (2019)	Gradient EPI	64 x 64	410	Closed	NS	2000	30	240 x 240 mm2	90	3.75 x 3.75 x 5 (resampled to 3 x 3 x 3)	NS	None	205	5 mm	Network-based; graph theory
Yoon et al. (2016)	NS	80 x 79	NS	Closed	NS	3000	30	220 mm	90	NS	NS	0.75 mm	99	3 mm	Seed-based
Yuan et al. (2018)	Gradient EPI	64 x 64	408	Closed	NS	2000	30	NS	90	3.75 x 3.75 x 5 (resampled to 3 x 3 x 3)	NS	None	NS	5 mm	Amplitude of low frequency fluctuations (ALFF); Fractional ALFF; Degree Centrality
Yuan et al. (2017)	Single-shot gradient-recalled EPI	64 x 64	408	Closed	NS	2000	30	240 x 240 mm	90	3.75 x 3.75 x 5 (resampled to 3 x 3 x 3)	30 (per volume)	None	205	5 mm	Seed-based
Yuan, Zhu, et al. (2016)	Single-shot gradient-recalled EPI	64 x 64	408	closed	NS	2000	30	240 x 240 mm	90	3.75 x 3.75 x 5 (resampled to 3 x 3 x 3)	30	None	205	5 mm	Seed-based
Yuan et al. (2018)	Gradient EPI	64 x 64	NS	Closed	NS	2000	30	240 x 240 mm2	90	3.75 x 3.75 x 5 (resampled to 3 x 3 x 3)	30	None	205	5 mm	ALFF; seed-based
Yun et al. (2017)	EPI	64 x 64 x 30	300	Closed	NS	2000	22	240 mm	90	NS	150 (total)	NS	NS	NS	Graph theory
Zhang et al. (2015)	Single-shot gradient-recalled EPI	64 x 64	410	Closed	NS	2000	30	240 mm	90	3.75 x 3.75 x 5 (resampled to 3 x 3 x 3)	30	None	205	5 mm	ALFF
Zhang et al. (2015)	Gradient-echo EPI	64 x 64	NS	Closed	NS	2000	30	240 x 240 mm2	90	3.75 x 3.75 x 5 (resampled to 3 x 3 x 3)	30	None	205	5 mm	MVPA of ReHo maps
Zhu et al. (2017)	Gradient-echo EPI	64 x 64	408	Closed	NS	2000	30	NS	90	3.75 x 3.75 x 5 (resampled to 3 x 3 x 3)	NS	NS	205	5 mm	Graph theory; network analysis; MVPA

Note. # = number; A = anterior; AC = anterior commissure; ALFF = amplitude of low frequency fluctuations; EPI = echo planar imaging; FC = functional connectivity; GCA = Granger causality analysis; ICA = independent component analysis; mm = millimeter; ms = milliseconds; MVPA = multi-voxel pattern analysis; NS = not stated; P = posterior; PC = posterior commissure; ReHo = regional homogeneity; ROI = region of interest; TE = echo time; TR = repetition time.

Supplementary Table 3.3

Pre-Processing and First-Level Analysis Steps of fMRI Studies

Author (year)	Preprocessing						First Level Analysis							
	Program for preprocessing	Discarded data	Motion correction	Slice-time correction	Normalization to template	Smoothing	Program for analysis	Atlas/mask used	Band-pass filtered	Global signal regression (GSR)	Motion threshold	Regression of motion parameters	Regression of physiological measures	Average signal regression
Anteraper et al. (2014)	SPM8	NS	Y	Y	EPI	3-mm Gaussian	CONN	WFU_PickAtlas/ SPM anatomy atlas	0.008 - 0.09 Hz	NS	0.5 mm trans; 0.5 degree rot	NS	NS	Y (WM, CSF)
Choi et al. (2016)	SPM8	First 30 seconds	Y	Y	MNI T1	8-mm Gaussian FWHM	NS	None	Y	NS	NS	NS	NS	NS
Cui et al. (2017)	DPARSF in MATLAB	NS	Y	Y	MNI EPI	8-mm Gaussian FWHM	NS	AAL	0.01 - 0.08 Hz	NS	2mm trans; 2 degrees rot	Y	NS	Y (WM and CSF signals)
Ding et al. (2011)	SPM2	First 5 images	Y	Y	MNI EPI	8-mm Gaussian FWHM	NS	AAL	0.01 - 0.1 Hz	NS	1mm trans; 1 degree rot	Y	NS	Y (ventricles, WM)
Dodhia et al. (2014)	SPM8	NS	Y (motor realignment)	Y	Y	Y	SPM8; CONN toolbox	AAL	NS	NS	3mm trans	Y	NS	Y (CSF, WM)
Dorfman et al. (2016)	AFNI (inc. ANATICOR)	First 4 volumes	NS	Y	Talairach	6-mm Gaussian FWHM	ANFI (inc. ANATICOR)	NS (described in Di Martino et al 2008)	0.01 - 0.1 Hz	NS	3mm gross motion	Y (0.25mm shift from preceding volume censored)	NS	Y (WM)
Ergul et al. (2019)	SPM8	First 10 volumes	Y	NS	MNI	8-mm Gaussian FWHM	CONN toolbox	BA atlas and AAL atlas as per the CONN toolbox	0.01 - 0.1 Hz	NS	3 mm trans; 3 degree rot; mean FD of 0.5mm	Y (from spatial motion correction)	NS	Y (WM, CSF)
Geiger et al. (2016)	SPM12b	NS	Y	Y	MNI	8-mm Gaussian FWHM	ICA: GIFT; Seed: CONN	ICA: Built using AFNI 3dAutomask; Seed: MarsBaR; AAL atlas	0.008 - 0.02 Hz	NS	Y (but do not define threshold)	NS	NS	Y (WM, CSF)
Hahn et al. (2011)	AFNI	First 3 volumes	Y	Y	N27	4-mm Gaussian FWHM	NS	NS	NS	NS	<0.3 mm	Y	Cardiac and respiratory	Y (WM, ventricle)

Jung et al. (2018)	SPM	NS	Y	Y	MNI	9-mm Gaussian	NS	AAL atlas	0.007 – 0.08 Hz	Y	NS	NS	NS	Y (ventricular and WM)
Liao et al. (2010)	SPM2	First 5 volumes	Y	NS	MNI	8-mm Gaussian FWHM	GIFT software	NS	NS	NS	1.5 mm trans; 1.5 degree rot	NS	NS	NS
Liao et al. (2010)	SPM2	First 5 volumes	Y	Y	MNI	8-mm Gaussian FWHM	NS	AAL template	0.01 - 0.08 Hz	NS	1.5 mm trans; 1.5 degree rot	Y	Gross physiological changes	Y (WM, CSF)
Liao et al. (2011)	NS	First 5 volumes	Y	NS	MNI	8-mm Gaussian FWHM	NS	NS	0.01 -0.08 Hz	Y	1.5 mm trans; 1.5 degree rot	Y	NS	Y (WM, CSF)
Liu et al. (2015)	SPM8	First 5 volumes	NS	Y	MNI in SPM8	Not smoothed	NS	AAL atlas	0.01 -0.08 Hz	No	2.0 mm trans; 2.0 degree rot	Y	NS	Y (WM, ventricles)
Liu et al. (2015)	DPARSF	First 5 volumes	Y	Y	MNI in SPM8	Not smoothed	NS	NS	0.01 -0.08 Hz	NS	2.0 mm trans; 2.0 degree rot	Y	NS	Y (WM, ventricles)
Manning et al. (2015)	SPM8	NS	Y	Y	NS	8-mm Gaussian FWHM	NS	NS	0.0008 - 0.083 Hz	No	0.5 mm trans	Y	NS	Y (WM, CSF)
Pannekoek et al. (2013)	FEAT Version 5.98	NS	Y	NS	MNI-152	6-mm Gaussian FWHM	FEAT Version 5.98	NS	< 0.01 Hz	Y	NS	Y	NS	Y (WM, CSF)
Prater et al. (2013)	SPM5	First 8 volumes	Y	Y	MNI	8-mm Gaussian FWHM	NS	NS	0.008 - 0.1 Hz	Y	2.0 mm trans; 2.0 degree rot	Y	NS	NS
Qiu et al. (2011)	SPM8	First five volumes	Y	Y	MNI EPI	4-mm Gaussian FWHM	REST	NS	0.01 - 0.08 Hz	NS	1.5 mm trans; 1.5 degree rot	NS	NS	NS
Qiu et al. (2015)	SPM2	First 5 volumes	Y	Y	MNI	8-mm Gaussian FWHM	REST	NS	0.01 - 0.08 Hz	NS	1.5 mm trans; 1.5 degree rot	NS	NS	NS
Rabany et al. (2017)	CONN toolbox	First 6 images of the scan	Y	Y	MNI	8-mm Gaussian FWHM	CONN toolbox	Stanford Atlas of Functional ROI/Harvard-Oxford structural atlas	0.008 - 0.09 Hz	NS	NS	NS	NS	Y (WM, CSF)
Yang et al. (2019)	SPM8; DPARSF	First 10 volumes	Y	Y	MNI	4-mm Gaussian FWHM	GRETNA	NS	0.01 - 0.1 Hz	With and without	3.0 mm trans; 3.0 degree rot	Y	NS	Y (WM, CSF)
Yoon et al. (2016)	SPM8	First 5 volumes	Y	Y	standard template	6-mm Gaussian FWHM	NS	AnatomyToolbox v.1.8	0.008 - 0.08 Hz	NS	NS	NS	NS	NS

Yuan et al. (2018)	DPARSF	First 10 volumes	Y	Y	EPI template provided by SPM8	4-mm Gaussian FWHM	DPABI	NS	0.01 - 0.08 Hz	Y	3.0 mm trans; 3.0 degree rot	Y	NS	Y (WM, CSF)
Yuan et al. (2017)	DPARSF; SPM8	First 10 volumes	Y	Y	MNI template in SPM8	6-mm Gaussian FWHM	NS	Probabilistic MR atlas	0.01 - 0.08 Hz	No	3.0 mm trans; 3.0 degree rot	Y	NS	Y (WM, CSF)
Yuan, Zhu, et al. (2016)	SPM8; DPARSF	First 10 volumes	NS	Y	EPI template provided by SPM8	4-mm Gaussian FWHM	REST	AAL	0.01 - 0.1 Hz	No	3.0 mm trans; 1.0 degree rot	Y	NS	Y (WM, CSF)
Yuan et al. (2018)	SPM8; DPARSF	First 10 volumes	Y	Y	NS	4x4x4 mm Gaussian FWHM	NS	NS	0.01 - 0.1 Hz	NS	3.0 mm trans; 3.0 degree rot; mean FD of 0.25	Y	NS	Y (WM, CSF)
Yun et al. (2017)	SPM12	First 5 volumes	Y	Y	MNI	8-mm Gaussian FWHM	MATLAB R2014b; brain conn. toolbox	AAL	0.009 - 0.08 Hz	Y	NS	Y	NS	Y (WM, CSF)
Zhang et al. (2015)	SPM8	First 5 volumes	Y	Y	NS	8-mm Gaussian FWHM	REST	NS	0.01 - 0.08 Hz	NS	1.5 mm trans; 1.5 degree rot	NS	NS	NS
Zhang et al. (2015)	DPARSF	First 5 volumes	Y	Y	MNI EPI template	4-mm Gaussian FWHM	PROBID	NS	NS	NS	1.5 mm trans; 1.5 degree rot	NS	NS	NS
Zhu et al. (2017)	DPARSF; SPM8	First 5 volumes	Y	Y	EPI template provided by SPM8	4-mm Gaussian FWHM	GRETNA	NS	0.01 - 0.1 Hz	NS	3.0 mm trans; 3.0 degree rot	Y	NS	Y (WM, CSF)

Note. AAL = automated anatomical labelling; BA = Brodmann's areas; CONN = fMRI functional connectivity toolbox; CSF = cerebrospinal fluid; DPABI = data processing & analysis for brain imaging; DPARSF = data processing assistant for resting-state fMRI; EPI = echo planar imaging; FCD = functional connectivity density; FD = framewise displacement; FWHM = full width half maximum; GIFT = group independent component analysis of fMRI toolbox; GRETNA = graph theoretical network analysis; Hz = hertz; mm = millimeter; ICA = independent component analysis; MNI = Montreal Neurological Institute; MR = magnetic resonance; NS = not stated; REST = resting-state fMRI data analysis Toolkit; ROI = region of interest; rot = rotation; PESTICA = physiologic estimation by temporal ICA; PROBID = pattern recognition of brain image data; SPM = statistical parametric mapping; trans = translation; WFU = Wake Forest University; WM = white matter; Y = yes.

Supplementary Table 3.4

Findings from Studies that Used Other Types of Analyses

Analysis Method	Contrast	Networks/Brain Regions
ICA	CON > SAD	L. executive control network (OFG/SFG, orbital part)[31], R. executive control network[31], ventral default mode network[31], somato-motor network[5], visual network[5], dorsal attention network[5], central executive network[5], default mode network[5], core network[5]
	SAD > CON	L. executive network (MFG)[31], auditory network*[31], dorsal attention network[5], central executive network[5], default mode network[5], core network[5]
ReHo	CON > SAD	R. dorsolateral PFC[40]; R. ACC[40]; L. medial PFC[40]; Bil. medial SFG[23]; L. SFG[23]; Bil. MFG[23]; L. IFG[23]; R. MTG[23]; L. temporal pole[23]; R. FFG[40]; Bil. angular gyrus[40]; R. IPG[40]; R. postcentral gyrus[23]; R. IOG[23]; Bil. cuneus[23]
	SAD > CON	L. SFG[23]; Bil. medial SFG[23]; Bil. MFG[23]; R. IFG[23]; R. pars triangularis[23]; L. medial OFG[23]; R. OFG[23]; Bil. STG[23]; Bil. MTG[23]; R. FFG[23]; Bil. precuneus[23]; R. superior parietal gyrus[23]; L. IPG[23]; R. postcentral gyrus[23]; L. middle occipital gyrus[5,44]; L. cuneus[23]; L. lingual gyrus[23]; R. putamen[40]; L. cerebellum[23]
Whole-brain functional connectivity	CON > SAD; positive connectivity	**Frontal – frontal** - R. MFG (orbital) – bil. IFG (triangular), bil. IFG, opercular; R.MFG - R. SFG (orbital) – bil. IFG (triangular); bil. IFG (opercular) **Parietal-frontal** - R. MFG, orbital - Bil. IPG **Basal ganglia/striatum – frontal** - R. MFG, orbital - R. caudate nucleus **Basal ganglia/striatum – parietal** - L. posterior cingulate gyrus - L. caudate nucleus, L. pallidum **Parietal – temporal** - L. posterior cingulate gyrus - Bil. olfactory cortex **Frontal – temporal** - R. SFG, medial orbital - L. STG, temporal pole - L. SFG, medial orbital - L. STG, temporal pole **Parietal – occipital** L. postcentral gyrus - L. SOG
	SAD > CON; positive connectivity	**Basal ganglia/striatum – frontal** - L. pallidum - L. Rolandic operculum - R. pallidum - L. Rolandic operculum **Basal ganglia/striatum – temporal** - R. pallidum - L. STG **Amygdala – temporal** - L. amygdala - L. Heschl gyrus **Amygdala – frontal** - L. amygdala - L. Rolandic operculum
	CON > SAD; negative connectivity	**Frontal – occipital** - R. MFG (orbital) – bil. calcarine fissure, bil. SOG, bil. cuneus, bil. lingual gyrus, bil. middle occipital gyrus, bil. IOG - R. SFG (orbital) - Bil. calcarine fissure, L. lingual gyrus - R. IFG (orbital) - R. lingual gyrus - R. IFG (opercular) - L. calcarine fissure - R. IFG (triangular) - L. calcarine fissure, Bil. lingual gyrus - L. IFG (triangular) - L. calcarine fissure, Bil. lingual gyrus **Frontal – temporal** - R. IFG, orbital - R. FFG **Thalamus – temporal** - L. thalamus – Bil. STG, L. Heschl gyrus - R. thalamus – Bil. Heschl gyrus, Bil. STG **Thalamus – frontal** - L. thalamus – Bil. Rolandic operculum - R. thalamus – Bil. Rolandic operculum **Basal ganglia/striatum – frontal** - L. caudate nucleus - L. Rolandic operculum **Basal ganglia/striatum – parietal** - L. pallidum - L. supramarginal gyrus **Parietal-frontal** - R. angular gyrus - L. paracentral lobule **Parietal – parietal** - R. angular gyrus - L. postcentral gyrus **Parietal – temporal** - R. IPG - R. Heschl gyrus, R. STG **Parietal – occipital** L. IPG - R. lingual gyrus
	SAD > CON; negative connectivity	**Occipital – temporal** - R. STG- L. middle occipital gyrus - L. STG - L. SOG, R. cuneus **Parietal-frontal** R. MFG, orbital - R. median cingulate gyrus

		- L. olfactory cortex - L. calcarine fissure			
		- L. olfactory cortex - L. calcarine fissure			
Whole brain FCS	CON > SAD	Bil. precuneus			
	SAD > CON	R. FFG			
Granger Causality analysis	CON > SAD	*From other brain regions TO L. amygdala* - L. SFG, medial - Bil. ITG - L. precuneus - Vermis 3	*From L. amygdala TO other brain regions* - L. SFG, medial - R. MTG - Bil. precentral gyrus	*From other brain regions TO R. amygdala* - R. SFG, orbital - Bil. SFG, medial - L. SFG - R. rectus - Bil. ITG - L. FFG - R. precentral gyrus - R. caudate nucleus	*From R. amygdala TO other brain regions* - R. SFG - L. IPG - L. superior parietal gyrus - L. postcentral gyrus - L. precentral - R. SMA - R. paracentral lobule - L. cerebellum_4_5 - Vermis_9
	SAD > CON	*From other brain regions TO L. amygdala* - R. Parahippocampal - R. FFG - R. Lingual gyrus - R. Cuneus - L. Precuneus - R. Precentral gyrus - R. Median cingulate gyrus - L. SMA - Bil. Putamen - Bil. Pallidum - Bil. Cerebelum_8	*From L. amygdala TO other brain regions* - L. MFG, orbital - Bil. ITG - Bil. cuneus - L. calcarine fissure - L. lingual gyrus - R. superior occipital gyrus - R. middle occipital gyrus - L. SMA - R. precentral gyrus - L. precuneus - R. cerebellum_7b - R. cerebellum_8 - Bil. cerebellum_crus2	*From other brain regions TO R. amygdala* - L. MFG, orbital - L. IFG, orbital - L. SFG - L. IFG, triangular - L. parahippocampal - L. FFG - Bil. Lingual gyrus - L. precuneus - Bil. Pallidum - Bil. Thalamus - L. putamen - R. cerebellum_8 - Bil. Cerebellum_9 - L. cerebellum_4_5	*From R. amygdala TO other brain regions* - L. MFG, orbital - L. IFG, orbital - L. IFG, triangular - L. MFG - L. MTG - R. parahippocampal - R. hippocampus - Bil. Calcarine fissure - L. lingual gyrus - Bil. cuneus - L. superior occipital gyrus - L. supramarginal gyrus - L. SMA
ROI	CON > SAD	L. subcallosal cortex and the L. dorsal ACC			
FCDA	CON > SAD	R. rostral ACC and the R. STG			

Note. ACC = anterior cingulate cortex; Bil. = bilateral; CON = control; FCDA = functional connectivity density analysis; FCS = functional connectivity strength; FFG = fusiform gyrus; L. = left; ICA = independent component analysis; IFG = inferior frontal gyrus; IOG = inferior occipital gyrus; IPG = inferior parietal gyrus; ITG = inferior temporal gyrus; MFG = middle frontal gyrus; MTG = middle temporal gyrus; OFG =orbitofrontal gyrus; PFC = prefrontal cortex; R. = right; ReHo = regional homogeneity; ROI = region of interest; SAD = social anxiety disorder; SFG = superior frontal gyrus; SMA = supplementary motor area; SOG = superior occipital gyrus; STG = superior temporal gyrus.

Supplementary Table 3.5

Clusters reported in Seed-Based fMRI

Author (year)	Brain Region 1	Brain Region 2	Correlation	Contrast	P-value
Manning et al. (2015)	Bil. NAcc	Ventral medial PFC, bil. medial anterior PFC, bil. IFG, anterior regions of the dorsal ACC, subgenual ACC, L. temporal pole, L. hippocampus, bil. putamen	Positive	CON > SAD	0.001, corrected
""	Bil. NAcc	bil. somatosensory association cortex, bil. premotor cortex, bil. primary motor cortex, posterior ventral ACC	Negative	SAD > CON	0.006, corrected
""	ventromedial PFC regions	BA25: bil. NAcc, bil. medial anterior PFC, bil. dorsolateral PFC, bil. inferior PFC, ventral ACC, subgenual ACC and dorsal ACC	Positive	CON > SAD	<.001, corrected
""	ventromedial PFC regions	BA5: bil. premotor, bil. primary motor cortex, posterior ventral ACC, dorsal PCC, bil. somatosensory association cortex	Negative	SAD > CON	0.001, corrected
""	ventromedial PFC regions	BA30: bil. somatosensory association cortex, dorsal PCC, bil. secondary visual cortex, bil. associative visual cortex	Negative	SAD > CON	0.002, corrected
""	ventromedial PFC regions	BA37: L. fusiform gyrus, L. STG, L. MTG, L. supramarginal gyrus, L. associative visual cortex	Positive	SAD > CON	0.006, corrected
Choi et al. (2016)	PCC & medial PFC	Orbitofrontal cortex	------	CON > SAD	0.042, FDR corrected
""	PCC & medial PFC	Posterior insula	------	CON > SAD	0.043, FDR corrected
""	PCC & medial PFC	Dorsolateral PFC	------	CON > SAD	0.001, FDR corrected
""	PCC & medial PFC	Inferior occipital gyrus	------	CON > SAD	0.046, FDR corrected
Cui et al. (2017)	R. STG	R. insula and putamen	Positive	CON > SAD	$p < 0.05$, FDR corrected
""	R. STG	R. post central gyrus/precentral gyrus	Positive	CON > SAD	$p < 0.05$, FDR corrected
""	R. STG	L. precentral gyrus/postcentral gyrus	Positive	CON > SAD	$p < 0.05$, FDR corrected
Ergul et al. (2019)	L. retrosplenial cortex	R. fusiform gyrus and posterior cerebellum	Positive	CON > SAD	0.042, FWE corrected

Note. ACC = anterior cingulate cortex; BA = Brodmann's area; bil.= bilateral; CON = controls; FDR = false discovery rate; FWE = family wise corrected; IFG = inferior frontal gyrus; L. = left; MTG = middle temporal gyrus; NAcc = nucleus accumbens; PCC = posterior cingulate cortex; PFC = prefrontal cortex; R. = right; SAD = social anxiety disorder; STG = superior temporal gyrus.

Supplementary Table 3.6

Specific Regions Implicated from Seed-Based fMRI Analyses

Brain Region 1	Brain Region 2	Contrast	Connectivity	Pairings
Frontal	Frontal	SAD > CON	Positive	L. medial SFG - L. medial SFG [41]; L. MFG - R. Rolandic operculum [41]; L. IFG - R. IFG [41]
		CON > SAD	Positive	R. rostral ACC - L. SFG [32]; R. rostral ACC - R. MFG [32]; R. rostral ACC - R. SFG [32]
Frontal	Amyg.	SAD > CON	Positive	Centromedial amyg. - SMA[35]; L. OFG (SFG, orbital part) - L. amyg.[31]; R. amyg. - R. ACC [41]; L. superficial complex - R. IFG[42]; R. superficial complex - R. SFG[42]; R. basolateral complex - L. dmPFC[42]; R. basolateral complex - R. SFG[42]; R. basolateral complex - R. IFG[42]; R. basolateral complex - R. ACC[42]; L. superficial complex - L. dlPFC[42]; L. basolateral complex - L. dlPFC[42]; L. basolateral complex - R. SFG[42]; L. basolateral complex - R. IFG[42]; L. amyg. - L. dmPFC[22]; L. amyg. - R. dorsal medial ACC[22]
		CON > SAD	Positive	L. amyg. - ACC, mPFC[18]; L. amyg. - R. dlPFC[25]; R. amyg. - R. medial frontal gyrus[25]; L. amyg. - L. medial frontal cortex[38]; L. amyg. - L. medial orbitofrontal cortex[38]; L. amygdala - Rostral ACC[19]; R. amyg. - Rostral ACC[19]
			Mixed	R. amyg. - L. SMA[25]
Frontal	Temporal	SAD > CON	Positive	L. medial SFG - L. STG [41]; R. PHG - L. medial SFG [41]; R. PHG - L. medial SFG [41]; R. PHG - L. medial SFG [41]; L. PHG - L. medial SFG [41]; L. PHG - L. medial SFG [41]; L. PHG - L. medial SFG [41]; L. PHG - L. medial orbitofrontal cortex [41]; L. PHG - L. medial SFG [41]; L. PHG - L. medial SFG [41]; L. MFG - L. FFG [41]
		CON > SAD	Positive	L. anterior cingulate and paracingulate gyri - R. ITG[24]; R. STG - L. SMA [32]; R. STG - R. IFG [32]; R. STG - R. precentral gyrus [32]; R. SFG, medial - L. MTG[24]; R. SFG, medial - R. ITG[24]; R. rostral ACC - R. superior temporal gyrus [32]; R. MFG, orbital part - R. FFG[24]; R. SFG, medial orbital - R. ITG[24]; R. SFG, orbital part - R. FFG[24]; R. gyrus rectus - R. MTG[24]; R. gyrus rectus - R. ITG[24]; R. anterior cingulate and paracingulate gyri - R. ITG[24]
Temporal	Temporal	SAD > CON	Positive	L. PHG - L. middle temporal gyrus[11]; R. FFG - L. PHG [41]
		CON > SAD	Positive	L. FFG - R. temporal pole: MTG[24]; L. FFG - R. ITG[24]; R. temporal pole: MTG - L. ITG[24]; R. temporal pole: MTG - R. ITG[24]; R. STG - R. middle temporal pole [32]; R. STG - R. STG [32]; R. STG - L. STG [32]; R. PHG - R. temporal pole: MTG[24]; R. hippocampus - R. temporal pole: MTG[24]; R. FFG - R. temporal pole: MTG[24]; R. FFG - R. ITG[24]
Temporal	Amyg.	SAD > CON	Positive	Centromedial amyg. - L. ITG[35]; L. amyg. - L. FFG[25]; L. amyg. - R. FFG[25]; R. amyg. - L. PHG[25]; L. amygdala - R. PHG [41]; L. centromedial complex - L. temporo - parietal junction[42]
			Negative	R. amyg. - L. MTG[39]
			Mixed	R. amyg. - L. STG[25]
		CON > SAD	Positive	L. amyg. - L. hippocampus[38]
			Mixed	R. amyg. - R. MTG[25]
Parietal	Amyg.	SAD > CON	Positive	Centromedial amyg. - R. angular gyrus[35]; Centromedial amyg. - Precuneus/cingulate gyrus[35]
			Negative	R. amyg. - L. supramarginal gyrus[39]
			Mixed	L. amyg. - PCC[26]
		CON > SAD	Positive	L. amyg. - L. PCC/precuneus[38]
			Mixed	L. amyg. - L. precuneus[25]; L. amyg. - R. supramarginal gyrus[25]
Parietal	Temporal	SAD > CON	Positive	L. PHG - L. PCC [41]
		CON > SAD	Positive	L. precuneus - R. ITG/R. PHG[7]; L. PCG - R. temporal pole: MTG[24]; L. precuneus - R. temporal pole: MTG[24]; R. precuneus - R. temporal pole: MTG[24]; R. precuneus - L. ITG[24]; R. PCG - R. temporal pole: MTG[24]
Occipital	Temporal	CON > SAD	Positive	L. calcarine fissure and surrounding cortex - R. temporal pole: MTG[24]; L. calcarine fissure and surrounding cortex - R. ITG[24]; R. lingual gyrus - R. ITG[24]; R. IOG - R. ITG[24]; R. cuneus - R. temporal pole: MTG[24]; R. calcarine fissure and surrounding cortex - R. temporal pole: MTG[24]; R. calcarine fissure and surrounding cortex - R. ITG[24]; R. middle occipital gyrus - R. temporal pole: MTG[24]
		SAD > CON	Negative	R. posterior ITG - L. IOG[11]
Frontal	Parietal	SAD > CON	Positive	L. supramarginal gyrus - R. Rolandic operculum [41]; R. lateral parietal regions - ACC[26]; PCC – mPFC[26]; Bil. dorsal ACC - L. precuneus[39]
		CON > SAD	Positive	L. gyrus rectus - R. precuneus[24]; L. MFG - R. angular gyrus[31]; L. precuneus - L. mPFC[7]; R. gyrus rectus - L. PCG[24]; R. gyrus rectus - L. precuneus[24]; R. gyrus rectus - R. precuneus[24]; R. SFG, medial - L. PCG[24]; R. SFG, medial - R. PCG[24]; R. SFG, medial - L. precuneus[24]; R. SFG, medial - R. precuneus[24]; L.

				SFG, dorsolateral - R. PCG[24]; L. SFG, medial - L. PCG[24]; L. SFG, medial - R. PCG[24]; L. SFG, medial orbital - L. PCG[24]; R. SFG, medial orbital - L. PCG[24]; R. SFG, medial orbital - R. precuneus[24]; R. rostral ACC - Bil. precuneus/middle cingulate cortex [32]; R. precentral gyrus - R. postcentral gyrus [32]
Frontal	Occipital	SAD > CON	Positive	L. MFG - R. IOG [41]; L. MFG - L. calcarine fissure [41]; R. ACC - R. IOG [41]; Bil. dorsal ACC - L. lateral occipital cortex[39]
		CON > SAD	Positive	R. SFG, orbital part - L. calcarine fissure and surrounding cortex[24]; R. MFG, orbital part - R. cuneus[24]; R. MFG, orbital part - R. superior occipital gyrus[24]; R. SFG, orbital part - R. IOG[24]
Amyg.	Occipital	SAD > CON	Positive	Centromedial amyg. - Associative visual cortex[35]
			Negative	R. amyg. - L. lateral occipital cortex[39]
		CON > SAD	Positive	L. amyg. - L. inferior occipital cortex[38]
Frontal	Basal gang./striatum	SAD > CON	Positive	Striatum/caudate - ACC [35]; Striatum/Bil. putamen – Ventral/subgenual ACC[35]; Striatum/caudate - Medial frontal gyrus (including the SFG, dlPFC, MFG, orbital gyrus, subcallosal gyrus)[35]; Striatum/Bil. putamen - Rectal gyrus[35]; Striatum/Bil. putamen - Premotor cortex[35]; Periaqueductal grey - DLPFC [35]
		CON > SAD	Positive	R. precentral gyrus - R. putamen [32]
Amyg.	Cerebellar	SAD > CON	Positive	L. superficial complex - R. cerebellum[42]; R. basolateral complex - R. cerebellum[42]; L. basolateral complex - R. cerebellum[42]
		CON > SAD	Mixed	L. amyg. - L. cerebellum[25]
Parietal	Basal gang./striatum	SAD > CON	Positive	Striatum/Bil. putamen - L. supramarginal gyrus[35]; Striatum/Bil. putamen - R. supramarginal gyrus[35]; Periaqueductal grey - R. inferior parietal lobule[35]; Periaqueductal grey - Precuneus[35]; Globus pallidus (medial and lateral for internal and external segments) - Bil. precuneus[35]
		CON > SAD	Positive	R. putamen - R. postcentral gyrus [32]
Thalamus	Amyg.	CON > SAD	Positive	R. superficial complex - L. dorsomedial thalamus[42]
Basal gang./striatum	Occipital	SAD > CON	Positive	L. putamen - R. IOG [41]
Insula	Insula	SAD > CON	Positive	L. insula - R. insula [41]
Parietal	Parietal	CON > SAD	Positive	L. PCG - R. angular gyrus[24]
Thalamus	Cerebellar	CON > SAD	Positive	Vermis IX - R. thalamus[29]; Vermis IX - L. thalamus [29]
Parietal	Cerebellar	CON > SAD	Positive	L. precuneus - L. cerebellum posterior lobe[7]; L. precuneus - R. cerebellum posterior lobe[7]
Frontal	Cerebellar	CON > SAD	Positive	Vermis IX - L. dlPFC [29]; Vermis Crus I - L. dorsal lateral prefrontal cortex[29]; L. Crus I - L. lateral prefrontal cortex[29]; L. Crus I - L. dmPFC[29]
Basal gang./striatum	Cerebellar	SAD > CON	Positive	Periaqueductal grey - Cerebellum[35]
Temporal	Basal gang./striatum	SAD > CON	Positive	Striatum/caudate - L. temporal lobe[35]; Striatum/caudate - L. MTG[35]; Periaqueductal grey - L. MTG[35]
Amyg.	Basal gang./striatum	SAD > CON	Mixed	R. amyg. - L. lentiform nucleus[25]
		CON > SAD	Positive	L. amyg. – R. pallidum[38]; L. amyg. – Bil. ventral striatum[38]
Thalamus	Temporal	SAD > CON	Positive	Thalamus - R. STG [35]; Thalamus - L. PHG[35]; Thalamus - L. ITG[35]; Thalamus - R. PHG [35]; Thalamus - R. PHG [35]; Thalamus - R. MTG[35]; Thalamus - L. STG[35]; L. PHG - Thalamus [41]
Thalamus	Frontal	SAD > CON	Positive	Thalamus - R. IFG[35]
Thalamus	Parietal	SAD > CON	Positive	Thalamus - R. inferior parietal lobule[35]; Thalamus - R. superior parietal cortex[35]; Thalamus - Precuneus[35]; Thalamus - PCC[35]
Thalamus	Insula	SAD > CON	Positive	Thalamus - L. insula [41]
Thalamus	Occipital	SAD > CON	Positive	Thalamus - R. IOG [41]

Note. ACC = anterior cingulate cortex; amyg. = amygdala; bil. = bilateral; basal gang. = basal ganglia; dlPFC = dorsolateral prefrontal cortex; dmPFC = dorsomedial prefrontal cortex; mPFC = medial prefrontal cortex; CON = control; FFG = fusiform gyrus; IOG = inferior occipital gyrus; IFG = inferior frontal gyrus; ITG = inferior temporal gyrus; L. = left; MFG = middle frontal gyrus; MTG = middle temporal gyrus; OFG = orbitofrontal gyrus; PCC = posterior cingulate cortex; PCG = posterior cingulate gyrus; PHG = parahippocampal gyrus; R. = right; SAD = social anxiety disorder; SFG = superior frontal gyrus; SMA = supplementary motor area; STG = superior temporal gyrus.

Supplementary Table 3.7

Results from Studies that Used Amplitude of Low-Frequency Fluctuations Analyses

Region	Contrast	ALFF
Frontal	SAD > CON	**1 study; 4 regions;** Bil. SFG, orbital[23]; R. medial frontal gyrus, orbital[23]; R. SFG, medial[23]
	CON > SAD	**3 studies; 17 regions;** Bil. median cingulate gyrus[23]; R. precentral gyrus[23]; L. SMA [32]; Bil. MFG[23]; R. SFG[23]; Bil. IFG, triangular[23]; R. IFG, orbital[23]; Bil. Rolandic operculum[23]; L. SFG, medial[23]; Bil. DLPFC, Bil. Medial PFC[38]
Temporal	SAD > CON	**2 studies; 5 regions;** L. MTG[28]; R. ITG[23]; R. MTG[23]; R. fusiform gyrus[23]; L. PHG[23]
	CON > SAD	**2 studies; 6 regions;** L. MTG[23]; L. Heschl gyrus[23]; R. MTG, pole[23]; L. Fusiform gyrus[23]; L. STG[23]; R. STG[40, 44]
Parietal	SAD > CON	**2 studies; 3 regions;** R. IPL[32]; L. precuneus[28, 32]; R. precuneus[28];
	CON > SAD	**1 study; 8 regions;** Bil. postcentral gyrus[23]; L. inferior parietal gyrus[23]; L. precuneus[23]; Bil. supramarginal gyrus[23]; Bil. superior parietal gyrus[23];
Occipital	SAD > CON	**2 studies; 3 regions;** R. middle occipital gyrus[23,38]; L. middle occipital gyrus[23, 38]; R. IOG[23]
	CON > SAD	**1 study; 1 region;** L. lingual gyrus[28]
Basal ganglia/striatum	SAD > CON	None
	CON > SAD	**1 study; 1 region;** Bil. putamen[32];
Insula	SAD > CON	None
	CON > SAD	**2 studies; 2 regions;** R. insula[23, 38]; L. insula[23]
Cerebellum	SAD > CON	**1 study; 1 region;** R. cerebellum posterior lobe [32]
	CON > SAD	None

Note. ACC = anterior cingulate cortex; Bil. = bilateral; CON = controls; IFG = inferior frontal gyrus; IOG = inferior occipital gyrus; IPL = inferior parietal lobule; ITG = inferior temporal gyrus; L. = left; MFG = middle frontal gyrus; MTG = middle temporal gyrus; PFC = prefrontal cortex; PHG = parahippocampal gyrus; R. = right; SAD = social anxiety disorder; SFG = superior frontal gyrus; SMA = supplementary motor area; STG = superior temporal gyrus.

Supplementary Table 3.8

Citations Corresponding to Numeric Indicators in Supplementary Table 3.6 and Supplementary Table 3.7

7	Yuan et al., (2018)	29	Yuan et al., (2017)
11	Liao et al., (2011)	31	Geiger et al., (2016)
18	Dodhia et al., (2014)	32	Cui et al., (2017)
19	Prater et al., (2013)	35	Anteraper et al., (2014)
22	Yuan, Zhu, et al., (2016)	38	Hahn et al., (2011)
23	Zhang et al., (2015)	39	Pannekoek et al., (2013)
24	Zhu et al., (2017)	40	Qiu et al., (2015)
25	Jung et al., (2018)	41	Yang et al., (2019)
26	Rabany et al., (2017)	42	Yoon et al., (2016)
28	Warwick et al., (2008)	44	Zhang et al., (2015)

Supplementary Table 3.9

Results from Studies that Used Graph Theory Analyses

Author (year)	Global and regional network properties							Findings
	Type	Brain Region	SAD: M (SD)	CON: M (SD)	Contrast	t-value	p-value (Cohen's d)	
Yang et al. (2019)	AUC of normalized shortest path length	n/a	0.93 (0.01)	0.93 (0.01)	CON > SAD	-2.08	0.042 (-0.52)	Decreased AUC λ in SAD (vs. controls) suggests a relatively randomized global topology in SAD. Positive correlation between this and the mean strength of the abnormal connectivity component found by NBS analysis ($r = 0.36$, $p = 0.039$) suggesting that deficits in global topology in SAD may be caused in part by abnormally high connectivity in this network.
	AUC of Nodal degree	L. PHG	16.39 (1.84)	14.33 (2.48)	SAD > CON	3.71	< 0.001[a](0.94)	
		L. PCC	17.10 (2.14)	15.44 (3.04)	SAD > CON	2.55	0.013 (0.64)	
		R. INS	14.31 (2.28)	15.83 (2.38)	CON > SAD	-2.68	0.009 (-0.68)	
		L. CAL	13.38 (2.46)	14.66 (1.90)	CON > SAD	-2.30	0.025 (-0.58)	
		L. MFG	12.94 (2.30)	14.27 (2.24)	CON > SAD	-2.27	0.027 (-0.57)	
	AUC of Nodal efficiency	L. PHG	0.67 (0.03)	0.63 (0.05)	SAD > CON	3.77	< 0.001[1] (0.95)	
		L. PCC	0.68 (0.04)	0.65 (0.06)	SAD > CON	2.61	0.012 (0.66)	
		R. INS	0.63 (0.04)	0.66 (0.04)	CON > SAD	-2.55	0.013 (-0.64)	
		L. CAL	0.60 (0.06)	0.63 (0.04)	CON > SAD	-2.38	0.020 (-0.60)	
		L. MFG	0.60 (0.06)	0.63 (0.05)	CON > SAD	-2.30	0.025 (-0.58)	
	AUC of Nodal participation coefficient	L. PHG	0.73 (0.07)	0.68 (0.07)	SAD > CON	2.69	0.009 (0.68)	
		L. PCC	0.75 (0.05)	0.71 (0.07)	SAD > CON	2.66	0.010 (0.67)	
		L. CAL	0.66 (0.08)	0.71 (0.06)	CON > SAD	-2.57	0.013 (-0.65)	
Yun et al. (2017)	AUC of λ, γ, σ, *Eglob*, *Q*	n/a	n.r.	n.r.	n/a	n.r.	> 0.05	Comparable AUC values for all global network properties between groups (all $p > 0.05$)
	Nodal participation coefficient	Bil. MTG, R. superior temporal cortices, R. inferior frontal cortices, L. SMG, R. MFG, L. angular gyri, R. lingual gyri, R. hippocampus, L. ITG	n.r.	n.r.	n.r.	n.r.	> 0.05	No statistically significant difference between groups for top 10 nodes
	Intra-module degree z-score	L. MTG – bil. insula, superior-middle temporal and inferior frontal cortices, bil. subcortical nuclei of the thalamus, putamen and pallidum	n.r.	n.r.	CON > SAD	2.25	0.029	
Zhu et al. (2017)	λ, γ, σ	n/a	n.r.	n.r.	n/a	n.r.	> 0.05	Comparable values for all global network properties between groups (all $p > 0.05$)
	AUC of L_p	n/a	n.r.	n.r.	SAD > CON	n.r.	< 0.01	
	AUC of C_p	n/a	n.r.	n.r.	SAD > CON	n.r.	< 0.01	
	AUC of nodalDeg and nodalE_{glob}	n/a	n.r.	n.r.	n/a	n.r.	> 0.05	
	AUC of nodalE_{loc}	L. PCG	n.r.	n.r.	CON > SAD	n.r.	< 0.05	
		R. putamen			SAD > CON		< 0.05	

Note. [1] = Bonferroni corrected; λ = normalized clustering coefficient; γ = normalized characteristic path length; σ = smallworldness; *Q* = modularity; AUC = area under the curve; CON = controls; C_p = clustering coefficient; Eglob = normalized global efficiency; FFG = fusiform gyrus; IFG = inferior frontal gyrus; IOG = inferior occipital gyrus; ITG = inferior temporal gyrus; L. = left; L_p = shortest path length; MOG = middle occipital gyrus; MTG = middle temporal gyrus; n/a = not applicable; nodalDeg = nodal centrality; nodalE_{glob} = nodal global efficiency of node; nodalE_{loc} = nodal local efficiency of the node; n.r. = not reported; OFG = orbitofrontal gyrus; PCG = posterior cingulate gyrus; R. = right; ROL = Rolandic operculum; SAD = social anxiety disorder; SMG = supramarginal gyrus; SOG = superior occipital gyrus; STG = Superior temporal gyrus.

Supplementary Table 3.10

Results from Studies that Used Multi-Voxel Pattern Analysis

Author (year)	Number of features in classifier	Features	Brain regions	Efficacy in discriminating between SAD vs CON					
				Accuracy	Sensitivity	Specificity	Significant (Y/N)	z-score	Weight
Zhu et al. (2017)	49	49 decreased FC in SAD vs. CON	As per supp. Table 1	0.667	0.738	0.595	N	1.728	Ranging from 0.032 – 0.650
	1	AUC of L_p	n/a	0.988	0.976	1	Y	12.554	1
	1	AUC of C_p	n/a	0.964	0.929	1	Y	12.783	1
	2	AUC of L_p and C_p	n/a	0.988	0.976	1	Y	12.514	L_p: 0.478 C_p: 0.988
	2	AUC of nodalE_{loc}	L. PCG R. putamen	0.714	0.738	0.691	Y	5.237	L. PCG: 0.228 R. putamen: 0.719
Zhang et al. (2015)	---	ReHo maps	**SAD > CON: frontal:** L. SFG, R. medial SFG, L. medial SFG, R. MFG, L. MFG, R. IFG, R. pars triangularis, L. medial OFG, R. OFG **SAD > CON: temporal:** R. STG, R. MTG, L. STG, L. MTG **SAD > CON: occipital:** L. MOG, R. FFG, L. cuneus, L. lingual gyrus **SAD > CON: parietal:** R. precuneus, R. superior parietal gyrus, L. inferior parietal gyrus, L. precuneus, R. postcentral gyrus **CON > SAD: frontal:** L. medial SFG, R. medial SFG, L. SFG, R. MFG, L. MFG, L. IFG **CON > SAD: temporal:** R. MTG, L. temporal pole, **CON > SAD: parietal:** R. postcentral gyrus **CON > SAD: occipital:** R. IOG, R. cuneus, L. cuneus **CON > SAD: cerebellum:** L. cerebellum	0.7625	0.70	0.825	n.r.	n.r	SAD > CON: ranging from 8.38 to 17.88 CON > SAD: ranging from -19.03 to -8.1
Liu et al. (2015)	148	Consensus features selected from the 250 highest ranked functional connections from whole-brain FC analysis using the F score method	**Regions with sig. higher weight:** R. orbital part of MFG, L. precuneus, L. lingual gyrus, R. orbital part of SFG, R. insula, L. postcentral gyrus, L. middle part of temporal pole, L. SOG, R. superior part of temporal pole, vermis_1&2, L. angular gyrus	0.825	0.85	0.80	Y ($p < 0.001$)	n.r	n.r

Note. CON = controls; FC = functional connectivity; FFG = fusiform gyrus; IFG = inferior frontal gyrus; IOG = inferior occipital gyrus; ITG = inferior temporal gyrus; L. = left; n/a = not applicable; N = no; n.r = not reported; MOG = middle occipital gyrus; MTG = middle temporal gyrus; OFG = orbitofrontal gyrus; PCG = posterior cingulate gyrus; R. = right; ReHo = regional homogeneity; ROL = Rolandic operculum; SAD = social anxiety disorder; SOG = superior occipital gyrus; STG = superior temporal gyrus; Y = yes.

Supplementary Table 3.11

Results from Studies that Reported Associations Between Brain and Dimensional Measures

Author	Results		Sample	Outcome		Statistics	
	Brain Region (name)	Second brain region		Positive correlation	Negative correlation	p	r
Anteraper et al. (2014)	Seed: Striatum/caudate	ACC	n.r	LSAS total	n.a	n.r	n.r
Ding et al. (2011)	R. superior frontal gyrus (medial orbital)	L. superior temporal gyrus (temporal pole)	SAD	n.a	LSAS total LSAS fear LSAS avoidance	< .05[1] < .05, uncorrected < .05[1]	-0.67 -0.49 -0.67
“”	R. inferior frontal gyrus (orbital)	R. fusiform gyrus	SAD	LSAS total LSAS avoidance	n.a	< .05[1] < .05[1]	0.63 0.61
“”	R. middle frontal gyrus (orbital)	L. calcarine fissure	SAD	LSAS total *LSAS fear* LSAS avoidance	n.a	< .05[1] < .05, uncorrected < .05[1]	0.71 0.51 0.73
“”	“”	L. superior occipital gyrus	SAD	LSAS total *LSAS fear* LSAS avoidance	n.a	< .05[1] < .05, uncorrected < .05[1]	0.68 0.49 0.69
“”	“”	R. calcarine fissure	SAD	LSAS total LSAS avoidance	n.a	< .05, uncorrected < .05, uncorrected	0.57 0.57
“”	“”	L. lingual gyrus	SAD	LSAS total LSAS avoidance	n.a	< .05[1] < .05[1]	0.69 0.75
“”	“”	R. lingual gyrus	SAD	LSAS total *LSAS fear* LSAS avoidance	n.a	< .05[1] < .05, uncorrected < .05[1]	0.68 0.51 0.68
“”	“”	L. middle occipital gyrus	SAD	LSAS total LSAS fear LSAS avoidance	n.a	< .05[1] < .05[1] < .05[1]	0.76 0.62 0.70
“”	“”	R. middle occipital gyrus	SAD	LSAS total *LSAS fear* LSAS avoidance	n.a	< .05[1] < .05, uncorrected < .05[1]	0.69 0.48 0.72
“”	“”	L. inferior occipital gyrus	SAD	LSAS total LSAS avoidance	n.a	< .05, uncorrected < .05, uncorrected	0.53 0.51
“”	“”	R. median cingulate gyrus	SAD	n.a	LSAS total LSAS avoidance	< .05, uncorrected < .05, uncorrected	-0.49 -0.51
“”	R. superior frontal gyrus (orbital)	L. calcarine fissure	SAD	LSAS total LSAS fear LSAS avoidance	n.a	< .05[1] < .05[1] < .05[1]	0.88 0.68 0.85

“”	“”	R. calcarine fissure	SAD	LSAS total LSAS fear LSAS avoidance	n.a	< .05[1] < .05[1] < .05[1]	0.82 0.66 0.77
“”	“”	L. lingual gyrus	SAD	LSAS total *LSAS fear* LSAS avoidance	n.a	< .05[1] < .05, uncorrected < .05[1]	0.80 0.59 0.79
Dodhia et al. (2014)	R. amygdala	ACC (rostral), rostral medial PFC	SAD	n.a	LSAS social: with FC in SAD LSAS social: with magnitude of change in connectivity	0.033 0.094	-0.504 -0.407
Doruyter et al. (2018)	L. Fusiform gyrus	--------	SAD	n.a	BDI-II	0.046	n.r
Geiger et al. (2016)	L. orbitofrontal gyrus (superior frontal gyrus, orbital part)	L. amygdala	SAD & CON	LSAS total	n.a	< 0.05, uncorrected	0.398
Jung et al. (2018)	R. amygdala	R. middle temporal gyrus	SAD	n.a	LSAS total SPS	0.008[1] 0.007[1]	-0.436 -0.439
“”	L. amygdala	R. supramarginal gyrus	SAD	HAMA	n.a	0.020, uncorrected	0.406
“”	“”	L. precuneus	SAD	HAMA	n.a	0.017, uncorrected	0.416
“”	R. amygdala	L. superior temporal gyrus	SAD	BDI HAMA	n.a	0.003[1] 0.011, uncorrected	0.488 0.446
“”	L. amygdala	R. insula	SAD	BFNE	n.a	0.044, uncorrected	0.338
“”	“”	R. intraparietal sulcus	SAD	SPS	n.a	0.037, uncorrected	0. 365
Liao et al. (2010)	R. amygdala	L. middle frontal gyrus, orbital	SAD	LSAS avoidance	n.a	0.04 uncorrected	0.45
“”	L. Middle frontal gyrus, orbital	R. amygdala	SAD	LSAS avoidance	n.a	0.03 uncorrected	0.46
“”	L. Inferior temporal gyrus	R. amygdala	SAD	n.a	LSAS avoidance	0.04 uncorrected	-0.45
“”	R. Inferior temporal gyrus	R. amygdala	SAD	n.a	LSAS avoidance	0.02 uncorrected	-0.49
Liao et al. (2010)	Dorsal attention network L. Superior parietal gyrus	----------	SAD	n.a	LSAS total LSAS fear	< 0.05, uncorrected < 0.05[1]	-0.533 -0.639
“”	Visual network: L. Inferior Occipital gyrus	----------	SAD	n.a	LSAS total LSAS fear LSAS avoidance	< 0.05[1] < 0.05[1] < 0.05[1]	-0.621 -0.562 -0.531
“”	Central-executive network: L. superior frontal gyrus	----------	SAD	n.a	LSAS total LSAS fear LSAS avoidance	< 0.05, uncorrected < 0.05, uncorrected < 0.05, uncorrected	-0.594 -0.488 -0.551
“”	Dorsal attention network: R. inferior frontal gyrus, orbital	----------	SAD	LSAS total LSAS fear	n.a	< 0.05, uncorrected < 0.05[1]	0.428 0.618
“”	Default mode network: L. superior frontal gyrus, medial	----------	SAD	LSAS fear	n.a	< 0.05, uncorrected	0.447
“”	Core network: Bil. R. interior cingulate gyrus	----------	SAD	LSAS total LSAS avoidance	n.a	< 0.05, uncorrected < 0.05, uncorrected	0.421 0.444
Liao et al. (2011)	R. posterior inferior temporal gyrus	L. inferior occipital gyrus	SAD	LSAS total LSAS avoidance	n.a	0.012[1] 0.008[1]	0.58 0.60
“”	L. parahippocampal gyrus	L. middle temporal gyrus	SAD	LSAS fear	n.a	0.039, uncorrected	0.54
Liu et al. (2015)	Bil. precuneus	------	SAD	n.a	Illness duration	0.0164, uncorrected	-0.5294
Qiu et al. (2011)	L. inferior parietal gyrus	--------	SAD	LSAS total	n.a	< 0.05, AlphaSim corrected	n.r
“”	R. inferior parietal gyrus	--------	SAD	LSAS total	n.a	< 0.05, AlphaSim corrected	n.r
“”	L. middle occipital gyrus	--------	SAD	LSAS total	n.a	< 0.05, AlphaSim corrected	n.r

“”	L. cuneus	--------	SAD	LSAS total	n.a	< 0.05, AlphaSim corrected	n.r
“”	L. middle prefrontal gyrus	--------	SAD	n.a	LSAS total	< 0.05, AlphaSim corrected	n.r
“”	L. dorsolateral prefrontal cortex	--------	SAD	n.a	LSAS total	< 0.05, AlphaSim corrected	n.r
“”	R. dorsolateral prefrontal cortex	--------	SAD	n.a	LSAS total	< 0.05, AlphaSim corrected	n.r
“”	L. putamen	--------	SAD	n.a	LSAS total	< 0.05, AlphaSim corrected	n.r
Qiu et al. (2015)	R. middle occipital gyrus	--------	SAD	LSAS total LSAS fear	n.a	0.037788, uncorrected 0.021739, uncorrected	0.46724 0.50954
“”	R. inferior occipital gyrus	--------	SAD	LSAS fear	n.a	0.0347, uncorrected	0.4741
“”	R. superior frontal gyrus, medial	--------	SAD	n.a	LSAS avoidance	0.01783, uncorrected	-0.52356
“”	R. superior temporal gyrus	--------	SAD	n.a	LSAS total LSAS fear	0.027697, uncorrected 0.017361, uncorrected	-0.49162 -0.5254
Warwick et al. (2008)	R. lingual	--------	SAD	n.a	LSAS total	< 0.001, uncorrected	0.678
“”	L. frontal	--------	SAD	LSAS total	n.a	< 0.001, uncorrected	0.539
“”	R. fusiform	--------	SAD	n.a	LSAS total	< 0.001, uncorrected	0.693
Yang et al. (2019)	mean strength of the significant connectivity component revealed by NBS analysis		SAD	LSAS total LSAS avoidance	n.a	0.026 0.025	0.40 0.40
Yuan et al. (2017)	L. Crus I	L. dorsal medial prefrontal cortex	SAD	n.a	Spielberger Trait Anxiety Inventory	< 0.05	-0.310
“”	Vermis IX	R. thalamus	SAD	Illness duration	n.a	< 0.05	0.328
Yuan et al. (2018)	L. precuneus	--------	SAD	n.a	LSAS total LSAS fear LSAS avoidance HAMD	0.010[1] 0.016[1] 0.012[1] 0.006[1]	-0.390 -0.366 -0.370 -0.411
“”	L. precuneus	L. cerebellum posterior lobe	SAD	n.a	HAMA HAMD	0.024, uncorrected 0.009, uncorrected	-0.344 -0.393
Zhu et al. (2017)	Area under the curve of the clustering coefficient of the network (Cp) in those with SAD		SAD	n.a	HAMD	0.020, uncorrected	-0.375
“”	AUC of the nodal local efficiency (anodalEloc) of the left posterior cingulate gyrus		SAD	n.a	LSAS avoidance	0.035, uncorrected	-0.326

Note. [1] = Bonferroni-(Holm) corrected; BDI = Beck depression inventory; BFNE = brief fear of negative evaluation; Bil. = bilateral; CON = controls; HAMA = Hamilton Anxiety Scale (Anxiety); HAMD = Hamilton Anxiety Scale (Depression); L. = left; LSAS = Liebowitz social anxiety scale; n.a = not applicable; R. = right; SAD = social anxiety disorder; SPS = social phobia scale.

Supplementary Table 3.12

Risk of Bias Assessment

Author (year)	1	2	3	4	5	6	7	8	9	10	11	12
Anteraper et al. (2014)	Y	Y	Y	Y	N	N	N	Y	Y	Y	N	N
Choi et al. (2016)	Y	Y	Y	Y	N	N	N	Y	Y	Y	N	N
Cui et al. (2017)	Y	Y	Y	Y	N	N	N	N	Y	Y	Y: age, gender, mean head motion	N
Ding et al. (2011)	Y	Y	Y	Y	N	N	N	Y	Y	Y	N	N
Dodhia et al. (2014)	Y	Y	Y	Y	N	N	N	Y	Y	Y	N	N
Dorfman et al. (2016)	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y: sex, age, scanner, IQ	N
Doruyter et al. (2018)	Y	Y	Y	Y	N	N	N	Y	Y	Y	N	N
Doruyter et al. (2016)	Y	Y	Y	Y	N	N	N	Y	Y	Y	N	N
Ergul et al. (2019)	Y	Y	Y	Y	N	N	N	Y	Y	Y	N	N
Evans et al. (2009)	Y	Y	Y	Y	N	N	N	Y	Y	Y	N	N
Geiger et al. (2016)	Y	Y	Y	Y	N	N	N	Y	Y	Y	N	N
Hahn et al. (2011)	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y: age	N
Jung et al. (2018)	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y: medication, comorbid depression	N
Liao et al. (2010)	Y	Y	Y	Y	N	N	N	Y	Y	Y	N	N
Liao et al. (2010)	Y	Y	Y	Y	N	N	N	Y	Y	Y	N	N
Liao et al. (2011)	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y: age, gender, grey matter volume	N
Liu et al. (2015)	Y	Y	Y	Y	N	N	N	Y	Y	Y	N	N
Liu et al. (2015)	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y: gender, age, years of education, head motion	N
Manning et al. (2015)	Y	Y	Y	Y	N	N	N	Y	Y	Y	N	N
Pannekoek et al. (2013)	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y: age, scan location	N
Prater et al. (2013)	Y	Y	Y	Y	N	N	N	Y	Y	Y	N	N
Qiu et al. (2015)	Y	Y	Y	Y	N	N	N	Y	Y	Y	N	N
Qiu et al. (2011)	Y	Y	Y	Y	N	N	N	Y	Y	Y	N	N
Rabany et al. (2017)	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y: gender, age, medication status	N
Warwick et al. (2008)	Y	Y	Y	Y	N	N	N	Y	Y	Y	N	N
Yang et al. (2019)	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y: age, sex	N
Yoon et al. (2016)	Y	Y	Y	Y	N	N	N	Y	Y	Y	N	N
Yuan et al. (2017)	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y: gender,age	N
Yuan et al. (2018)	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y: gender, age, education, mean FD	N
Yuan, Ren, et al. (2016)	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y: gender, age, mean FD, education levels	N
Yuan et al. (2018)	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y: gender, age, mean FD, education levels	N

Yun et al. (2017)	Y	Y	Y	Y	N	N	N	Y	Y	Y	N	N
Zhang et al. (2015)	Y	Y	Y	Y	N	N	N	Y	Y	Y	N	N
Zhang et al. (2015)	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y: gender, age, mean FD	N
Zhu et al. (2017)	Y	Y	Y	Y	N	N	N	Y	Y	Y	N	N

Note. 1 = Was the research question or objective in this paper clearly stated?; 2 = Was the study population clearly specified and defined?; 3 = Was the participation rate of eligible persons at least 50%?; 4 = Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?; 5 = Was a sample size justification, power description, or variance and effect estimates provided?; 6 = For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?; 7 = Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?; 8 = Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?; 9 = Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?; 10 = Was loss to follow-up after baseline 20% or less?; 11 = Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?; 12 = Was the study pre-registered?; FD = framewise displacement; N = no; Y = yes.

Supplementary Table 3.13

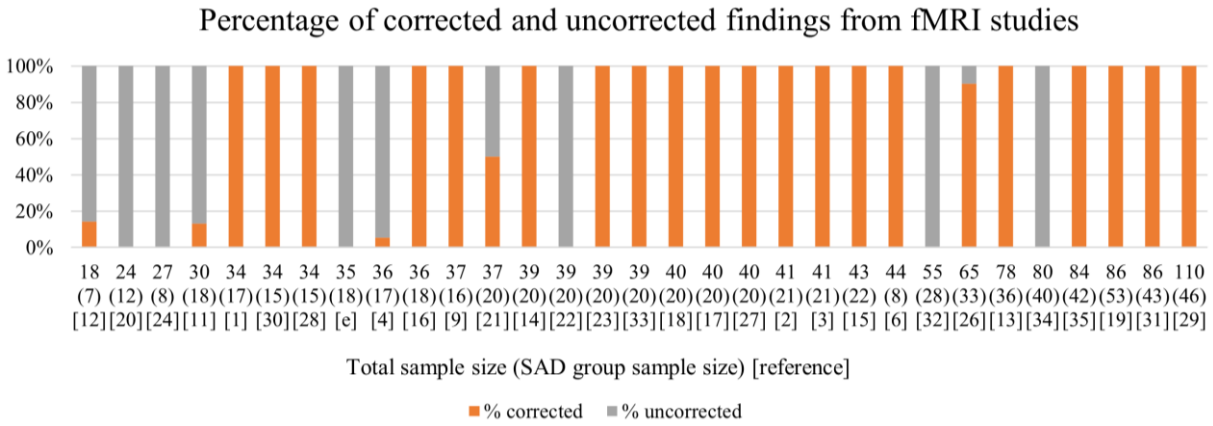
Comparison of Findings from this Review and Brühl et al.'s (2014) Neurobiological Model of SAD

Pairing	Brühl et al. (2014) model	Findings from this review
Frontal - Parietal	CON > SAD	CON > SAD: 4 studies, 18 pairings SAD > CON: 3 studies, 4 pairings
Frontal – Occipital	SAD > CON	CON > SAD: 1 study, 4 pairings SAD > CON: 2 studies, 4 pairings
Frontal – Amygdala	SAD > CON	CON > SAD: 4 studies, 7 pairings SAD > CON: 5 studies, 15 pairings
Frontal – Thalamus	SAD > CON	CON > SAD: none SAD > CON: 1 study, 1 pairing
Amygdala – Parietal	CON > SAD	CON > SAD: 1 study, 1 pairing SAD > CON: 1 study, 2 pairings
Amygdala - Occipital	SAD > CON	CON > SAD: 1 study, 1 pairing SAD > CON: 1 study, 1 pairing
Parietal – Occipital	CON > SAD	None

Note. CON = controls; SAD = social anxiety disorder.

Supplementary Figure 3.1

Relationship between Total Sample Size (SAD Sample Size) and Percentage of Corrected and Uncorrected Between-Group Findings



CHAPTER 4: GENERAL EXPERIMENTAL METHODS

4.1 Chapter Guide

This chapter provides a comprehensive and detailed overview of the experimental methodology for Study 2 (Chapter 5) and Study 3 (Chapter 6). These two studies were written for publication and thus had to comply with word count and other publishing limits implemented by the targeted journals. Therefore, the method sections in both studies are brief. This chapter begins by outlining the general study and participant information followed by a detailed overview of the protocol, including information about assessment measures used and procedures relating to the neuroimaging component of the study. Brief details regarding the statistical analysis plan and software used are also provided.

4.2 General Study Information

4.2.1 Ethics Approval

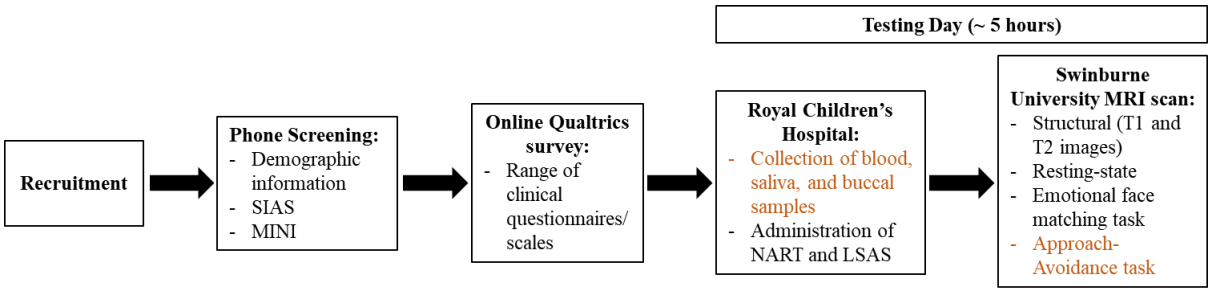
This research study protocol was approved by the Australian Catholic University Human Research and Ethics Committee as meeting the requirements of the National Statement on Ethical Conduct in Human Research (see Appendix B).

4.2.2 Testing Procedure

An overview of the testing procedure can be seen in Figure 4.1. This project was nestled within a larger project that involved the collection of biological samples for epigenetic testing and a range of clinical questionnaires/surveys that were not used as part of this thesis. Further information about each stage of the procedure relevant to this thesis is described in detail below.

Figure 4.1

Overview of Testing Procedure



Note. Orange text signifies data that was not used for this thesis. LSAS = Liebowitz social anxiety scale; MINI = mini-international neuropsychiatric interview; MRI = magnetic resonance imaging; NART = national adult reading test; SIAS = social interaction anxiety scale.

4.3 Participant Information

4.3.1 Recruitment

Both clinical and control participants were primarily recruited using community-based advertising. This included online advertisements on Gumtree and the distribution of flyers across multiple university campuses (i.e., Australian Catholic University, La Trobe University, and Swinburne University of Technology) and local suburbs (e.g., Melbourne and Fitzroy); see Appendix A. Clinical participants were additionally recruited through online advertisements on the Anxiety Disorders Association of Victoria website and Facebook page. A portion of the clinical group were participants who had consented to be contacted regarding participation in further research after completing another study aligned to this project (involving the completion of ecological momentary assessment and the Trier Social Stress Test).

Individuals interested in participating in this study were able to contact the research team via Gumtree or email to obtain further information and to arrange a time to complete the screening process. The initial screening process was conducted over the phone and determined whether the individual was eligible to participate as part of the control or the clinical group. Individuals deemed eligible were accepted into the study. Using email communication, they were provided with a participant information letter (see Appendix A) and were booked in for a

testing session depending on their availability and the availability of the researcher and MRI scanner. All participants provided written informed consent before they participated in the study (see Appendix A).

4.3.2 General Inclusion and Exclusion Criteria

Two participant groups were included in this study: a clinical group of individuals with social anxiety disorder (SAD) and a healthy control group. Participants for both the control and clinical group were included if they were aged between 18 to 55 years, right-handed, non-smokers, fluent English speakers, and were medication free. All participants were required to be available to attend a testing session during business hours in Melbourne, Victoria. Participants for both groups were excluded if they had contraindications to MRI (e.g., metal objects present in the body that could not be removed) or a history or current substance abuse/dependence. Further exclusion criteria for both groups included the presence of a neurological condition, history of head trauma (i.e., having been unconscious for more than five minutes), a neurodevelopmental disorder (e.g., attention deficit hyperactivity disorder), or any other clinically significant medical illness (e.g., cardiovascular disease, cancer, diabetes).

Participants in the control group additionally were required to have no prior or current suspected or current diagnosis of mental illness and did not meet the criteria for any mental illness as assessed in the screening process. Participants in the SAD group were required to meet the diagnostic criteria for SAD based on the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV; American Psychiatric Association, 1994) or the DSM-5 (American Psychiatric Association, 2013) as assessed by the Mini-International Neuropsychiatric Interview (MINI) 6.0.0 English Version (Sheehan et al., 1998). If comorbid mental health issues were reported (specifically, generalised anxiety disorder or depression/low mood), participants were only included if their primary diagnosis was SAD. That is, SAD had to be the condition for which the participant sought help, or which caused the most distress and

impairment in functioning. Participants with a primary diagnosis of SAD who also reported current comorbid acute depressive disorder, bipolar disorder, schizophrenia, post-traumatic stress disorder, and obsessive-compulsive disorder were excluded.

4.3.3 Screening Procedures

Prior to participation, all individuals underwent a thorough phone screening procedure to determine whether they met the inclusion criteria and to rule out any individuals who met any of the exclusion criteria. This included the collection of demographic information relating to the individual's date of birth, sex, education level, handedness, and employment status, and questions regarding health including medical history, past and current medications, and drug and alcohol use. To determine eligibility for either the control or clinical group, the MINI 6.0.0 Screen (English version for the DSM-IV; Sheehan et al., 1998) was administered. Any endorsed items from the MINI 6.0.0 Screen were followed up with the relevant diagnostic module of the full MINI 6.0.0 (Sheehan et al., 1998).

4.3.3.1 The MINI 6.0.0 English Version

The MINI 6.0.0 (Sheehan et al., 1998) is a structured interview which assesses for 17 psychiatric diagnoses based on the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV; American Psychiatric Association, 1994) and the International Statistical Classification of Diseases and Related Health Problems, tenth edition (World Health Organization, 1992). This tool was used to determine whether individuals in the clinical group had a diagnosis of SAD and whether any comorbidities were present. It was also used to ensure that participants in the control group had no current psychiatric diagnoses.

The MINI has two main components: a screener and a follow-up diagnostic module. For the screener, questions are delivered verbally by the assessor to which participants must respond with only 'yes' or 'no' answers. Screener questions that are answered with a 'no' are indicative that the respondent is unlikely to have the corresponding psychiatric disorder.

Screener questions that are answered with a 'yes' are then followed up with further questions from the diagnostic modules of the MINI to determine whether a diagnosis is appropriate. The diagnostic module for SAD in the MINI, as published in Sheehan et al. (2010), is comprised of the following questions:

> *"1: In the past month, did you have persistent fear and significant anxiety at being watched, being the focus of attention, or of being humiliated or embarrassed? This includes things like speaking in public, eating in public or with others, writing while someone watches, or being in social situations.*
>
> *2: Is this social fear excessive or unreasonable and does it almost always make you anxious?*
>
> *3: Do you fear these social situations so much that you avoid them or suffer through them most of the time?*
>
> *4: Do these social fears disrupt your normal work, school, or social functioning or cause you significant distress?"*

An affirmative response to all four questions is indicative of a diagnosis of DSM-IV SAD. The diagnostic criteria for SAD were altered in the DSM-5 whereby the requirement that a person needs to experience the feeling of fear as excessive or unreasonable to receive a diagnosis of SAD was omitted. Instead, the observing clinician is required to recognise the fear as being out of proportion to the actual threat posed by the social situation. Therefore, an affirmative response to questions 1, 3, and 4 and additional information provided suggesting that the fear is out of proportion as evaluated by the interviewer, is also indicative of a diagnosis of DSM-5 SAD. In all cases, evaluations by the interviewer to whether the reported fear was out of proportion to the actual threat was considered within the sociocultural context.

Sheehan et al. (1998) found that the MINI had good specificity (0.86 – 0.88), good sensitivity (0.72 - 0.81), and good negative predictive value (0.94 - 0.97) in diagnosing current

SAD when compared to the Structured Clinical Interview for DSM-IV Axis 1 Disorders and the Composite International Diagnostic Interview. The MINI was also shown to have good interrater (0.94) and test-retest (0.65) reliability in diagnosing current SAD (Sheehan et al., 1998).

4.3.4 Clinical Assessment Measures

Other measures used to characterise the sample included in this study are described below.

4.3.4.1 Liebowitz Social Anxiety Scale

The Liebowitz social anxiety scale (LSAS) is a 24-item scale used to measure the levels of fear and avoidance experienced across a range of social situations (Liebowitz, 1987). It was administered to all participants in the clinical group as an additional measure to confirm their diagnosis of SAD and to assess the level of severity of their symptoms. The scale can be divided into four subscales: fear and avoidance pertaining to performance anxiety (13 items), fear and avoidance pertaining to anxiety in social situations (11 items), total fear (across all 24 items), and total avoidance (across all 24 items). Each item is rated on a 4-point Likert scale ranging from 0 (none) to 3 (severe) to measure fear and 0 (never) to 3 (usually) to measure avoidance. A total score can also be derived, with a maximum raw score of 144 possible. Higher scores are indicative of more severe social anxiety, with scores ranging from 30 to 50 representative of mild social anxiety, 50 to 65 indicating moderate social anxiety, 65 to 80 indicating marked social anxiety, 80 to 95 indicating severe social anxiety, and scores greater than 90 indicating very severe social anxiety. Mennin et al. (2002) demonstrated that a cut-off score of 30 for the LSAS total score was optimal in classifying people with a diagnosis of SAD, and a cut-off score of 60 for the LSAS total score was optimal in classifying people with SAD-generalised subtype (from a sample including non-anxious controls). The LSAS has been shown to have high levels of internal consistency (Cronbach's alpha = 0.96), good convergent validity

(strongly correlated with other measures of social anxiety and avoidance), and good discriminant validity (i.e., can distinguish SAD from general anxiety and depression; Heimberg et al., 1999).

4.3.4.2 Social Interaction Anxiety Scale

The social interaction anxiety scale (SIAS) is a 20-item self-report scale designed to measure fear in situations involving social interaction, such as group conversations (Mattick & Clarke, 1998). It was administered to all participants as an additional measure of social anxiety severity. Each item is rated on a 5-point Likert scale ranging from 0 (not at all characteristic or true of me) to 4 (extremely characteristic or true of me). Three items (5, 9, and 11) are reverse scored before responses of all items are summed to provide a total raw score, with higher total scores representing greater levels of social anxiety. A score greater than 36 (from a total possible 80) on the SIAS is indicative of probable SAD (as opposed to panic disorder; Peters, 2000) and two studies found that a cut-off score of 34 on the SIAS was able to discriminate between those with SAD and community controls without an Axis 1 disorder (Brown et al., 1997; Heimberg et al., 1992). The SIAS has been shown to have high levels of internal consistency (Cronbach's alpha = 0.93), high test-retest reliability (r = 0.92), and good construct validity (strongly correlated with other social anxiety measures; Mattick & Clarke, 1998).

4.3.4.3 National Adult Reading Test

The national adult reading test (NART) is a reading task that estimates premorbid intelligence (Nelson & Willison, 1991). It comprises of 50 irregular words that the participant must read using prior knowledge and phonetic pronunciation. A general intelligence quotient is derived by tallying the number of pronunciation errors made throughout the test. The NART is a well-validated test of general intelligence in the normal adult population and has high levels of inter-rater (r = 0.96) and test-retest reliabilities (Nelson & Willison, 1991). It has also been

shown to have good convergent validity with the Full-Scale Intelligence quotient derived from the Weschler Adult Intelligence Scale (r = 0.72; Schretlen et al., 2005).

4.4 Study Design and Procedures

The following section will briefly detail the overall protocol following participants' recruitment, screening, and inclusion in the study.

4.4.1 General Overview

Participation involved attending a single testing session that had a duration of approximately five hours. Participants were instructed to fast (i.e., no food and beverages, only minimal water) from midnight prior to their testing session until after their samples were taken to avoid the possibility of contaminating substances in the saliva and buccal cell collection. Participants were asked to not consume any caffeine until after the full testing session was complete to not impact on MRI results. Upon completion of the testing session, participants were compensated with a Coles Myer voucher ranging from $50 to $150. The testing session consisted of three parts which will be described below.

Prior to the testing session, participants were asked to complete an online questionnaire using the Qualtrics platform (sent to them via email) that involved completion of the SIAS and were administered the MINI 6.0.0 over the phone. They were then asked to attend the Royal Children's Hospital in Parkville, Victoria. At this location, they completed the consent form, the LSAS, and the NART. Administration of the NART was audio recorded to aid scoring which was completed at a later time. Following this, blood, saliva, and buccal cell samples were collected. These biological samples were not used in this current thesis. Therefore, further details are not provided here. Upon collection of these biological samples, participants were escorted to Swinburne University of Technology for the neuroimaging component of the testing session. They were asked to complete the MRI Safety Sheet and were then instructed to practice two tasks on a laptop that they would be completing in the scanner. Prior to entering

the scanner, the participant was asked to remove any metal objects. An eye test was also conducted and if required, MRI safe glasses were provided by the technician to ensure that they would be able to see the task-related text in the scanner.

Brain scanning was conducted on a Siemens MAGNETOM Tim Trio 3.0 Tesla scanner with a Siemens 12 channel Head Matric Coil (Erlangen, Germany). The participant was provided with a cushion (placed under the knees) and ear plugs to enhance comfort. Padded head foams were also used to minimise their movement throughout the scan. The protocol had a duration of approximately 90 minutes and is described below. A full outline of MRI acquisition parameters for each protocol can be found in Appendix A.

4.4.1.1 Structural Scans

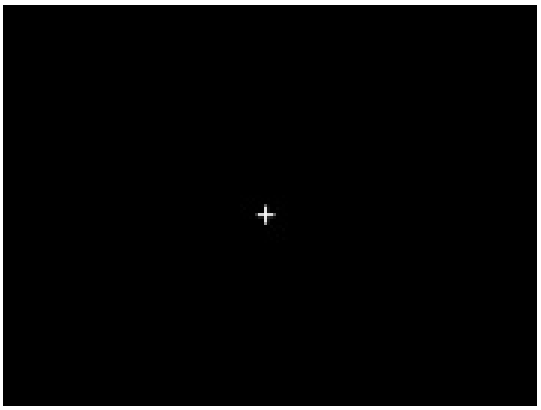
Structural data was first collected for all participants (T1 and T2 images). Participants were played a Planet Earth nature documentary during this time to aid with relaxation and to prevent them from falling asleep (which is thought to be more likely in the absence of any stimuli). At the end of this, an automatic shimming routine was used to optimise magnetic field homogeneity for the subsequent functional runs.

4.4.1.2 Resting-State Scan

All functional runs were acquired using a multiband gradient-echo planar sequence. Resting-state data was collected to study brain activity in the absence of an overt task which allows for a more sensitive measure of pervasive connectivity difficulties. The video was replaced with a full-screen image of a white cross in the middle of a black screen for approximately 8 minutes (see Figure 4.2). Participants were instructed to focus their gaze on the cross, to keep their mind blank, and to remain awake.

Figure 4.2

Resting-State Fixation Cross Displayed During Scan



4.4.1.3 Emotional Face Matching Task

Following this, participants completed the emotional face matching task (EFMT). This task has been designed to robustly activate the amygdala and has been tested in both clinical and healthy samples (Hariri et al., 2002; Labuschagne et al., 2010). Using a block design, this task involves viewing a trio of faces at a time with neutral, happy, angry, or fearful expressions. Face stimuli were selected from the Radboud Faces Database (Langner et al., 2010). The face matching tasks were interspersed with baseline tasks in which participants were required to match shapes (circles, triangles, rectangles) to allow the amygdala to return to baseline activity and to control for perceptual, motor, and cognitive demands not specifically associated with emotion processing. In the scanner, there were two runs of the task that were 5 minutes each in duration. Across the two runs, there were a total of 26 experimental blocks consisting of 12 emotional face blocks (3 blocks for each emotion) and 14 shape blocks, counterbalanced (see Figure 4.3). Each block contained four sequential matching trials presented for 5 seconds. The following instructions are provided:

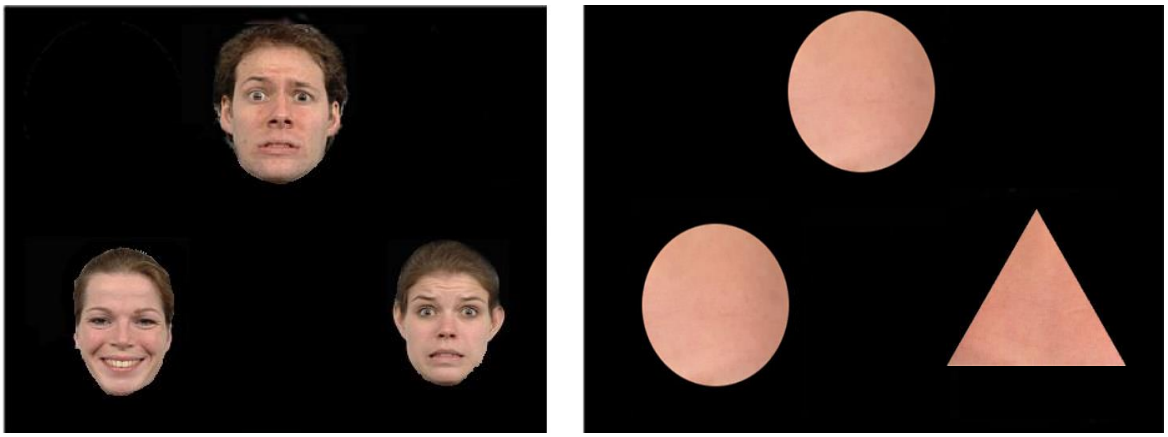
> *"For this task, you will be presented with three pictures per screen, one on the top and two on the bottom. Your task will be to match the top picture with one of the bottom two pictures. You will be presented with either human faces or with shapes.*

[show picture of task] When you see faces, you have to match the top face with one of the bottom two faces in terms of the emotional expression by pressing the left or right button. For example, if you see a happy face on top, then match it with the happy face on the bottom. When you see shapes, you have to match the top, say triangle, with the triangle on the bottom by pressing either the left or right button. You will be presented with alternating blocks of faces and shapes. Ignore the cross-hairs – you do not need to respond to these."

Presentation software (https://neurobs.com) was used to display and record participant responses. For the practice trial version administered prior to the scan, participants were instructed to use the left and right buttons of the mouse. In the scanner, the participants were provided with an MRI compatible button box that was to be held in their right hand. The participants were reminded that the left and right buttons correspond to the left and right buttons on the mouse that was used in the practice task. Button press responses were recorded and analysed for accuracy for each emotion condition.

Figure 4.3

Stimuli from the Emotional Face Matching Task



4.5 General Data and Statistical Analysis

Specific details regarding statistical analyses and software for the two empirical studies will be detailed within their respective chapters (Chapters 5 and 6). Briefly, four software packages were primarily used in this thesis: MATLAB, Statistical Package for the Social Sciences, R using the interface RStudio, and Statistical Parametric Mapping Version 12. For both empirical studies, fMRIprep was used to pre-process the data (Esteban et al., 2019). For the resting-state fMRI study (Chapter 5), Data Processing Assistant for Resting-State fMRI (DPARSF) V5.1 (Chao-Gan & Yu-Feng, 2010; http://rfmri.org/DPARSF) within the Data Processing and Analysis for Brain Imaging (DPABI; http://rfmri.org/dpabi) was used to examine connectivity between regions. For the emotion processing fMRI study (Chapter 6), the CONN toolbox v19.c was used to complete generalized psychophysiological interaction analyses (Whitfield-Gabrieli & Nieto-Castanon, 2012).

CHAPTER 5: RESTING-STATE AMYGDALA SUBREGION AND PRECUNEUS CONNECTIVITY PROVIDE EVIDENCE FOR A DIMENSIONAL APPROACH TO STUDYING SOCIAL ANXIETY DISORDER (STUDY 2)

5.1 Chapter Guide

The following chapter investigates amygdala subregion functional connectivity at rest in those with SAD and in healthy controls. The aims of the study were threefold: i) to test whether resting-state functional connectivity from a range of ROIs or 'seeds' (including the amygdala and its subregions) displayed aberrant connectivity with other brain regions, in SAD compared to controls; ii) to examine a dimensional approach to the study of SAD by exploring the association between resting-state fMRI connectivity and social anxiety severity across all participants (SAD and controls), and iii) to test whether each subregion of the amygdala had functionally distinct connectivity patterns. This paper was prepared with publication in mind, therefore some of the methods and findings are reported in the supplementary material to adhere to publishable manuscript length. It has been published as a preprint on *medRxiv* and has been submitted to the *British Journal of Psychiatry* for review. It has been included in this chapter without any alterations.

Citation:

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5.2 Abstract

Background: Social anxiety disorder (SAD) is a prevalent and disabling mental health condition, characterized by excessive fear and anxiety in social situations. Resting-state functional magnetic resonance imaging (fMRI) paradigms have been increasingly used to understand the neurobiological underpinnings of SAD in the absence of threat-related stimuli. Previous studies have primarily focused on the role of the amygdala in SAD. However, the amygdala consists of functionally and structurally distinct subregions, and recent studies have highlighted the importance of investigating the role of these subregions independently. **Method:** Using multiband fMRI, we analysed resting-state data from 135 participants (42 SAD, 93 healthy controls). By employing voxel-wise permutation testing, we examined group differences of fMRI connectivity and associations between fMRI connectivity and social anxiety symptoms to further investigate the classification of SAD as a categorical or dimensional construct. **Results:** Seed-to-whole brain functional connectivity analysis using multiple 'seeds' including the amygdala and its subregions and the precuneus, revealed no statistically significant group differences. However, social anxiety severity was significantly negatively correlated with functional connectivity of the precuneus - perigenual anterior cingulate cortex and positively correlated with functional connectivity of the amygdala (specifically the superficial subregion) - parietal/cerebellar areas. **Conclusion:** Our findings demonstrate clear links between symptomatology and brain connectivity in the absence of diagnostic differences, with evidence of amygdala subregion-specific alterations. The observed brain-symptom associations did not include disturbances in the brain's fear circuitry (i.e., disturbances in connectivity between amygdala - prefrontal regions) likely due to the absence of threat-related stimuli.

5.3 Introduction

Social anxiety disorder (SAD) is a debilitating mental health condition characterized by a disproportionate level of fear or anxiety in social situations that causes significant distress or functional impairment with a global lifetime estimated prevalence of 4.0% (American Psychiatric Association, 2013; Stein et al., 2017). Accumulating evidence suggests that SAD may not exist as a discrete categorical entity (as defined by the Diagnostic and Statistical Manual of Mental Disorders, fifth edition; DSM-5; American Psychiatric Association, 2013). Instead, it is proposed that symptoms associated with SAD have a dimensional structure (e.g., as a range of severity of anxiety, fear, and avoidance; Boyers et al., 2017; Hyett & McEvoy, 2018; Ruscio, 2010; Skocic et al., 2015). Given the high prevalence and subsequent impairments associated with SAD, there has been increased investigation to further understand the neurobiology of this disorder in the hope that it may improve its identification, classification, and treatment.

Advances in neuroimaging techniques have greatly assisted our understanding of the neurobiological mechanisms implicated in those with SAD. Most often, task-based functional magnetic resonance imaging (fMRI) approaches have elucidated the neural underpinnings of SAD under specific paradigms, such as in response to stimuli of facial expressions. In contrast, resting-state fMRI measures brain activity or connectivity in the absence of stimuli. In SAD, the use of resting-state fMRI allows for the identification of underlying neurobiological changes that are related to the characteristics of the disorder independent of any triggers from socially provoking situations (i.e., is the brain socially anxious outside the context of social threat?).

Findings from systematic reviews investigating task-based and resting-state fMRI in SAD have primarily identified the amygdala as a region of interest. That is, in response to socially relevant stimuli (i.e., threat-related facial expressions), those with SAD are commonly

reported to have hyperactive amygdala responses compared to controls (Binelli et al., 2014; Hattingh et al., 2013). When examining only resting-state fMRI studies, we recently found that the most frequently reported alterations in connectivity were between amygdala-frontal regions in those with SAD compared to controls (Mizzi et al., 2021). However, there were mixed findings with regards to the direction of connectivity, with five studies reporting an increase (Anteraper et al., 2014; Geiger et al., 2016; Yoon et al., 2016; Yuan, Zhu, et al., 2016) and four studies reporting a decrease (Dodhia et al., 2014; Hahn et al., 2011; Jung et al., 2018; Prater et al., 2013).

There are several reasons why these mixed findings may be occurring in the literature. Brain alterations at rest may depend on symptom severity, as supported by evidence of associations between social anxiety severity and resting-state functional connectivity of the amygdala-frontal regions in those with SAD (Dodhia et al., 2014; Liao et al., 2010) and in a combined sample of participants with a diagnosis of SAD and those with no diagnosed psychiatric disorder (Geiger et al., 2016). Additionally, mixed evidence regarding the connectivity patterns of the amygdala with frontal regions in those with SAD may relate to the amygdala having functionally distinct subregions (Balderston et al., 2015; Qiao et al., 2020; Wang et al., 2021). To date, two studies have examined resting-state amygdala subregion connectivity of the centromedial, superficial and basolateral complexes in SAD (Anteraper et al., 2014; Yoon et al., 2016). Both studies consistently found that those with SAD (compared to controls) had increased connectivity between each of the subregions and frontal regions (including the supplementary motor area, inferior frontal gyrus, superior frontal gyrus, dorsolateral prefrontal cortex, dorsomedial prefrontal cortex, and the anterior cingulate cortex). However, these findings and the majority of the previous studies showing that the amygdala and frontal regions are implicated in SAD have been limited in terms of interpretability for various reasons we discuss next.

Most studies to date have used relatively small sample sizes (average of n=23 SAD) which have been shown to detect unreliable brain and brain-behaviour findings that are unlikely to be reproduced (Grady et al., 2021). Moreover, studies have used short resting-state scan lengths (ranging from 200-471 seconds) which have been associated with poor test-retest reliability of connectivity findings (Noble et al., 2017). Then, there has been considerable heterogeneity in scanning acquisition and pre-processing procedures which hinders the identification of consistencies in findings across the literature (Mizzi et al., 2021).

In the current study, we aimed to further elucidate resting-state fMRI connectivity differences in people with SAD compared to controls. We attempted to address the aforementioned limitations by including a larger sample size, a longer scan duration using multiband imaging which reduces the signal-to-noise ratio, and a streamlined and reliable data processing (fMRIPrep) pipeline (to allow for easier replication). Our specific aims were three-fold: i) to test whether resting-state functional connectivity from a range of ROIs or 'seeds' (including the amygdala and its subregions) displayed aberrant connectivity with other brain regions, in SAD compared to controls; ii) to examine a dimensional approach to the study of SAD by exploring the association between resting-state fMRI connectivity and social anxiety severity across all participants (SAD and controls), and iii) to test whether each subregion of the amygdala had functionally distinct connectivity patterns.

5.4 Methods and Materials

5.4.1 Participants

A total of 138 participants were included in this study, 43 of which had SAD and 95 of which were healthy controls. Participants were recruited using community-based advertising, and those with SAD were additionally recruited through online advertisements on the Anxiety Disorders Association of Victoria website and Facebook page.

Participants were included if they were aged between 18 to 55 years, fluent in English, and right-handed. They were excluded if they had a history of or current substance abuse (including smoking), head trauma (defined as being unconscious for ≥ 5 minutes), neurological condition, clinically significant medical illness (e.g., cardiovascular disease, diabetes), and MRI contraindications (e.g., metal objects that cannot be removed or unsafe for MRI. Additionally, the MINI 6.0.0 Screen (English version for the DSM-IV) was used to ensure that those in the control group had no prior or current psychiatric diagnosis. The MINI 6.0.0 English Version was used to determine whether those in the SAD group met the diagnostic criteria for SAD based on the DSM-IV or the DSM-5. The Liebowitz Social Anxiety Scale (LSAS) was used as an additional measure to determine whether participants in the clinical group met the diagnostic criteria for SAD, with a score ≥30 being required for inclusion (Liebowitz, 1987; Mennin et al., 2002). The social interaction anxiety scale (SIAS) was administered to all participants as a measure of social anxiety symptom severity, with higher scores indicating greater levels of social anxiety (Mattick & Clarke, 1998). Written informed consent was obtained from all participants. This study was approved by the Human Research Ethics Committee at Australian Catholic University.

5.4.2 Data Acquisition

Data acquisition was performed on a Siemens MAGNETOM Tim Trio 3.0 Tesla scanner with a Siemens 12 channel head matrix coil (Erlangen, Germany) at Swinburne University of Technology, Australia. Padded foam cushions were used to minimize head movement throughout the scan. All participants were instructed to try to think about nothing in particular (i.e., a resting-state), remain awake, and fixate their gaze on a white crosshair displayed centrally on a black background.

A multiband echo-planar imaging sequence with an acceleration factor of 5 was used to acquire functional MRI data for 8 minutes 38 seconds, along the anterior commissure-

posterior commissure (AC-PC) plane with A > P phase encode direction (voxel size = 2 x 2 x 2 mm; 65 slices; repetition time (TR) = 1020 ms; total volumes = 500, echo time (TE) = 30 ms; flip angle (FA) = 65°). A T_1-weighted sagittal MPRAGE structural image (TR = 1900 ms, TE = 2.52 ms, FA = 9°, 176 slices; voxel size = 1 x 1 x 1mm voxels) and T_2-weighted image (TR = 3200 ms, TE = 402 ms, 176 slices; voxel size = 1 x 1 x 1 mm voxels) were also obtained for anatomical co-registration.

5.4.3 Data Analysis

5.4.3.1 Pre-Processing

T1- and T2-weighted MRI and resting-state fMRI images were converted to Brain Imaging Data Structure (BIDS) format (Gorgolewski et al., 2016). Firstly, the data was pre-processed using fMRIPrep 20.1.1 (Esteban, Markiewicz, et al. (2018); Esteban, Blair, et al. (2018); RRID:SCR_016216), based on Nipype 1.5.0 (Gorgolewski et al. (2011); Gorgolewski et al. (2018); RRID:SCR_002502) using the Ozstar High-Performance Computer (see Supplementary materials for details).

Following this, FSL was used to regress eight parameters out of the fMRI time series (signal from white matter, cerebrospinal fluid in addition to transverse x, y, and z head motion, and rotation x, y, and z head motion). Three participants (SAD = 1, Controls = 2) with excessive head motion, defined as a mean framewise displacement (FD) greater than 0.5 mm, were excluded from the study. This resulted in a total of 42 participants with SAD and 93 control participants. The data were filtered between 0.01 – 0.08 Hz and smoothed to 8 mm full width at half maximum (FWHM). Only fMRI voxels residing within grey matter were used for final analysis.

5.4.3.2 Regions of Interest (ROIs)

Seed ROIs included the subregions of the amygdala. Additionally, regions that were frequently implicated in resting-state fMRI studies of SAD (as identified in the most recent

systematic review on this topic (Mizzi et al., 2021)) were included to observe whether previous findings were replicable. ROIs were defined using two methods. The amygdala subregions were identified using cytoarchitectonic probability maps from the Anatomy Toolbox (Version 2.2b) in SPM12 (Amunts et al., 2005; Eickhoff et al., 2005). All other ROIs (amygdala, subgenual anterior cingulate cortex (ACC), ventromedial prefrontal cortex (vmPFC), and the temporoparietal junction (TPJ)) were identified using NeuroSynth (http://neurosynth.org), which is an online database that uses a meta-analytic approach to synthesize existing neuroimaging literature (Yarkoni et al., 2011). The relevant term was identified and the peak voxel of the region of interest was used as the MNI coordinate in this study (see Table 5.1).

Table 5.1

Coordinates and Size of Regions of Interests (ROIs)

ROI	MNI coordinates	Size of radius sphere (mm)	How it was defined
Amygdala: amygdalostriatal			
Right	27, -11, -11	3	Anatomy toolbox
Left	-27, -11, -11	3	
Amygdala: basolateral			
Right	26, -5, -19	3	Anatomy toolbox
Left	-26, -5, -19	3	
Amygdala: centromedial			
Right	23, -9, -10	3	Anatomy toolbox
Left	-23, -9, -10	3	
Amygdala: superficial			
Right	19, -8, -14	3	Anatomy toolbox
Left	-19, -8, -14	3	
Amygdala: whole amygdala			
Right	24, -4, -18	5	Neurosynth: searched term
Left	-24, -4, -18	5	'emotional' [b]
Precuneus			
Right	4, -58, 38	6	Neurosynth: searched term
Left	-4, -58, 38	6	'default mode' [a]
ACC (subgenual)			
Right	4, 32, -6	6	Neurosynth: searched term
Left	-4, 32, -6	6	'emotional' [b]
vmPFC			
Right	4, 48, -6	6	

TPJ	Left	-4, 48, -6	6	Neurosynth: searched term 'default mode' [a]
	Right	56, -50, 16	6	Neurosynth: searched term 'default mode' [a]
	Left	-56, -50, 16	6	

Note. [a] based on 777 studies and 26256 activations; [b] based on 1708 studies and 58327 activations ACC = anterior cingulate cortex; TPJ = temporoparietal junction; vmPFC = ventromedial prefrontal cortex.

5.4.3.3 fMRI Connectivity Analysis

To assess whether group differences existed between the defined ROIs and the whole-brain, seed-based functional connectivity analysis was completed using Data Processing Assistant for Resting-State fMRI (DPARSF) V5.1 (Chao-Gan & Yu-Feng, 2010; http://rfmri.org/DPARSF) within the Data Processing and Analysis for Brain Imaging (DPABI; http://rfmri.org/dpabi; Yan et al., 2016) implemented in MATLAB R2017b. Pre-processed voxel-wise fMRI time-series were extracted from each seed, and Pearson's correlation coefficients were calculated between the average seed time series, and the time series of all voxels in the brain (total number of grey matter voxels = 173.843). The correlation coefficient was transformed to a *z*-value using Fisher's r-to-z transformation, and the resultant functional connectivity maps for each participant were entered into the two-sample *t*-test.

5.4.3.4 Statistical Analysis

To examine differences in age and mean FD between groups, the non-parametric Mann-Whitney *U* test was used as the data was not normally distributed (as determined by Shapiro-Wilk test; $p < 0.001$). To examine differences in SIAS scores and sex between groups, an independent sample *t*-test, and a chi-square analysis were used respectively.

An independent sample *t*-test was used to analyse group differences in functional connectivity (SAD vs controls) for each ROI (aim i). Two-tailed threshold-free cluster enhancement (TFCE) correction with the Permutation Analysis of Linear Models (PALM) software ($p < 0.05$, 5000 permutations, tail approximation acceleration method) was applied

(Chen et al., 2018; Winkler et al., 2016). Age, sex, and FD (average head movement) were included as covariates of no interest. Given the ongoing debate about the appropriateness of including covariates if they are not matched between groups (Miller & Chapman, 2001), the *t*-tests were run a second time excluding FD as a covariate as head motion differed significantly between groups (control participants had greater head motion than SAD participants).

To examine associations between seeded fMRI connectivity maps and social anxiety severity (as measured by the SIAS) across all participants (aim ii), a voxel-wise Spearman's partial correlation analysis was conducted (due to the non-normal distribution of social anxiety scores). Age and sex were included as covariates. Since TFCE does not support correlation-based permutations, we employed a *p*-min permutation approach to perform multiple comparisons testing (Rempala & Yang, 2013; Westfall & Young, 1993). In total, we randomized SIAS scores 5000 times (i.e., 5000 permutations) while keeping the fMRI connectivity data unchanged for all participants. For each permutation, we extract the minimal *p*-value across all 173,843 voxels, which in turn represents the null distribution (i.e., a 'Bonferroni-like' multiple comparison correction). This procedure asks the question: *what is the strongest correlation any voxel can have 'by chance'*? The average of the 5000 random correlations obtained from the permutation testing was used to generate a statistical threshold. Any voxels with p-values less than this threshold were statistically significant. To avoid interpreting single voxels that may constitute a Type-1 error, we required 10 voxels to be interconnected to reach a statistical significance level. To ensure the functional specificity of amygdala subregions being measured (aim iii), average connectivity maps for each of the subregions (located in the left hemisphere) were compared to one another.

5.5 Results

5.5.1 Demographics

The demographic and clinical characteristics of all participants (aside from 3 participants who were excluded from all further analyses due to excessive head motion) are included in Table 5.2. Five participants in the SAD group had comorbid secondary psychiatric disorders (generalized anxiety disorder (n = 3); post-traumatic stress disorder (n = 1); obsessive-compulsive disorder/attention deficit hyperactivity disorder (n = 1)). There were no significant differences in age and sex between groups. There was a significant difference in mean FD (greater in-scanner head motion in the control group) and SIAS (higher scores in the SAD group). Of note, there was an overlap of SIAS scores between people who were diagnosed with SAD and controls (see the range in Table 5.2).

Table 5.2

Demographic Information of Participants

	SAD	Control	Statistic	*p*-value
n	42	93	-	-
Sex (female/male)	21/21	49/44	$\chi^2 = 0.084$	0.772
Age [a]	27.57 (7.52), 19.52 – 54.96	26.06 (6.50), 18.24 – 49.41	$U = 1718.000$	0.264
SIAS [a]	51.62 (9.80), 28 – 72	18.29 (10.65), 1 – 48	$t = 17.248$	<0.001
LSAS [a]	81.24 (22.58), 41 – 139 [b]	-	-	-
Mean FD [a]	0.16 (0.07), 0.08 – 0.46	0.20 (0.08), 0.07 – 0.46	$U = 1289.000$	0.002

Note . [a] = Mean (standard deviation) and range; [b] = scores were not reported for n=5; FD = framewise displacement; LSAS = Liebowitz Social Anxiety Scale; SIAS = Social Interaction Anxiety Scale; *t* = independent sample *t*-test; $\chi 2$ = chi square analysis; *U* = Mann-Whitney *U* test.

5.5.2 No Between-Group Differences in Functional Connectivity

Seed-based functional connectivity from 18 ROIs showed no statistically significant group differences between SAD and controls, based on 5000 permutations. This was observed when average FD was included and excluded as a covariate of no interest. It is worth noting that we observed moderate, but sub-threshold, effect sizes between groups (see Supplementary Table 5.1 and Supplementary Figure 5.1).

5.5.3 Significant Associations between Functional Connectivity and Social Anxiety Severity

In the combined groups, significant associations ($p < 0.001$, corrected for multiple comparisons using the *p*-min method) were found between SIAS scores and seeded fMRI connectivity of several amygdala subregions and the precuneus (see Table 5.3 and Figure 5.1).

Table 5.3

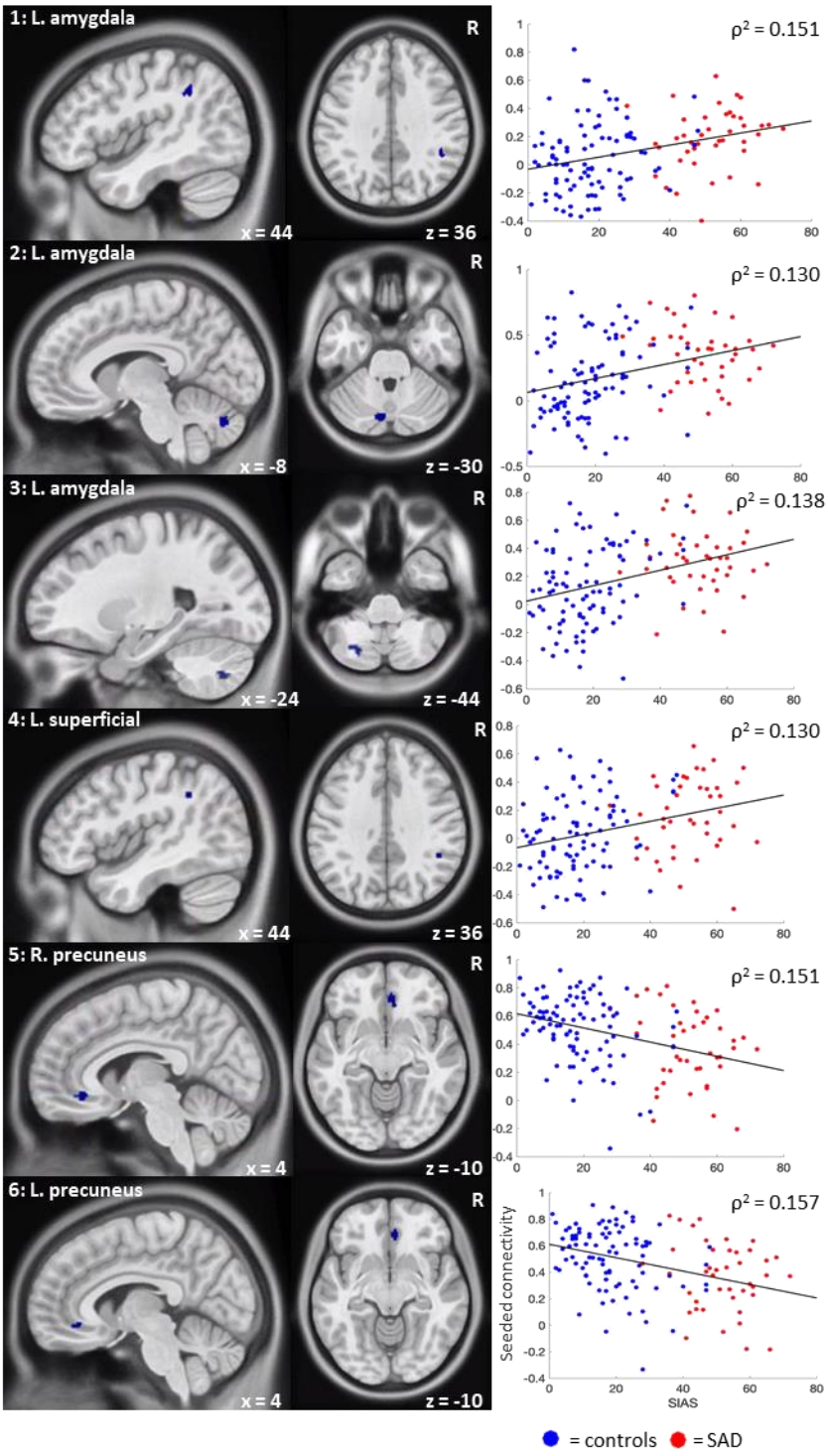
Significant Associations between Resting-State Functional Connectivity and Social Anxiety Severity (SIAS Scores)

Seed region	Regions showing altered connectivity	Peak MNI coordinate	Peak intensity (Spearman ρ value)	p-value	Correlation
L. superficial	R. supramarginal gyrus	44 -46 36	0.361	0.00013	Positive
	L. cerebellum_crus2	-8 -76 -30	0.360	0.00016	Positive
L. amygdala	R. supramarginal gyrus	44 -44 36	0.389	0.00016	Positive
	L. cerebellum_7b	-24 -70 -44	0.372	0.00014	Positive
L. precuneus	R. peri-genu ACC (BA32)	4 38 -10	-0.396	0.00001	Negative
R. precuneus	R. peri-genu ACC (BA32)	4 38 -10	-0.389	0.00013	Negative

Note. N = 135; ACC = anterior cingulate cortex; BA = Brodmann Area; L = left; MNI = Montreal Neurological Institute; SIAS = social interaction anxiety scale; R = right.

Figure 5.1

Connectivity Maps and Spearman's ρ Correlations of the Significant Associations (all p < 0.001) Between Resting-State Functional Connectivity (y-axis) and Social Anxiety Severity (SIAS scores; x-axis)



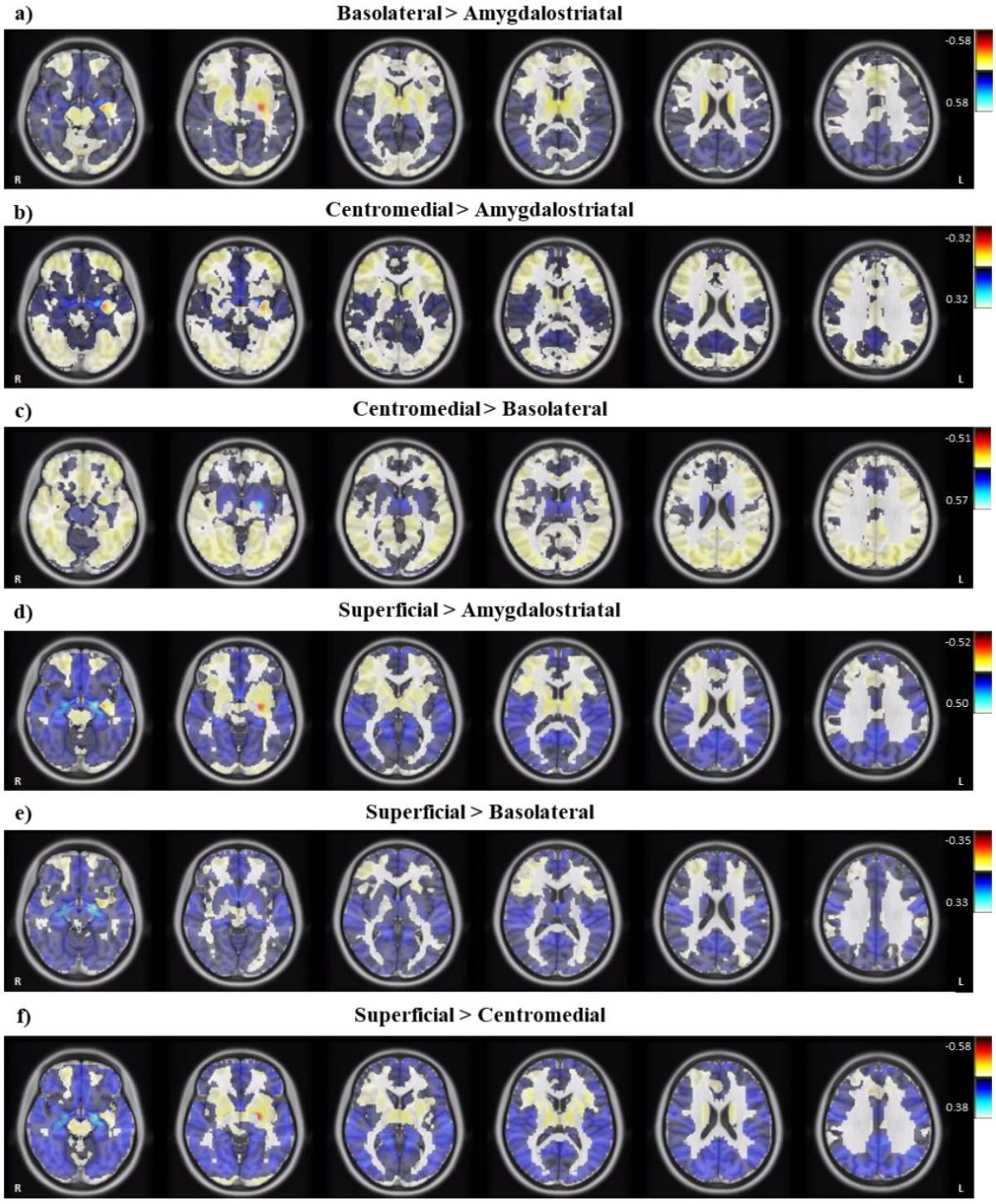
Note. 1 = left amygdala – right supramarginal gyrus; 2 = left amygdala – left cerebellum_crus2; 3 =left amygdala – left cerebellum_7b; 4 = left superficial – right supramarginal gyrus; 5 = right precuneus – right peri-genu ACC ; 6 = left precuneus – right peri-genu ACC.

5.5.4 Amygdala Subregions Display Divergent Functional Connectivity Patterns

In the combined groups, differences in the average connectivity maps when comparing amygdala subregions were observed and presented in Figure 5.2 (for illustration purposes we presented only the left hemispheric maps, but similar patterns were observed with right hemispheric subregions). These images show divergent connectivity patterns depending on the amygdala subregion ROI, thereby providing evidence that the connectivity maps from different amygdala subregions in our study are functionally distinct.

Figure 5.2

Differences in the Average Functional Connectivity Maps of Left Hemispheric Amygdala Subregions



5.6 Discussion

Using multiband fMRI, we found significant associations between social anxiety severity and resting-state functional connectivity across 135 participants (42 with SAD). Specifically, we found positive associations between severity of social anxiety and functional connectivity between the left superficial amygdala – right supramarginal gyrus and the left amygdala – right supramarginal gyrus and left cerebellar regions. We also found negative associations between social anxiety severity and resting-state functional connectivity of the bilateral precuneus and the right peri-genu ACC. These associations were observed in the absence of statistically significant group differences in resting-state functional connectivity (control vs. SAD participants).

5.6.1 Amygdala Subregion-Specific Associations with Social Anxiety

To date, very little is known about the functionality of the amygdala subregions in people with SAD given that no previous resting-state fMRI studies have examined these four commonly classified subregions within this population. Here, we showed that the positive association between social anxiety severity and functional connectivity between the left amygdala and the supramarginal gyrus is driven specifically by the superficial subregion of the amygdala. Increased connectivity between the superficial amygdala and the supramarginal gyrus at rest may indicate enhanced emotional surveillance of socially anxious self-relevant information and an increased tendency for socially anxious people to have negative social-evaluative cognitions, which is thought to maintain the disorder (i.e., negative thoughts/feelings they have about themselves are put onto others thus increasing feelings of fear/anxiety; Jung et al., 2018; Wong & Rapee, 2016). This is because the superficial subregion of the amygdala has been implicated in the processing of socially relevant information (Roy et al., 2009). Additionally, the supramarginal gyrus is thought to play a role in downregulating egocentricity bias (i.e., the tendency to project one's mental state onto others; Silani et al.,

2013), with evidence from a recent meta-analysis finding that those with SAD (compared to controls) had significantly decreased activation in this region when viewing disorder-related scenes (e.g., being in a conference room, harsh faces) compared to neutral scenes (Yu et al., 2021).

There were no significant associations between social anxiety severity and the amygdalostriatal, basolateral and centromedial subregions of the amygdala. This suggests that these subregions may play a role in socio-emotion processing (as measured by task-based fMRI studies) rather than in the absence of any stimuli (i.e., at rest). The centromedial subregion, which is known to be the major output area of the amygdala, sends signals to other neural areas to generate emotional, behavioural, autonomic, and motor responses (Roy et al., 2009). Therefore, aberrant patterns of connectivity in this subregion may only occur in task-based paradigms that require a response (and therefore an output signal). This is supported by findings of greater activation in the centromedial amygdala in response to negatively valenced stimuli compared to positively valenced stimuli in people genetically enriched for SAD (Bas-Hoogendam et al., 2020) and significantly increased activation in the centromedial subregion of the amygdala in those with SAD (compared to controls) when viewing disorder-related scenes compared to neutral scenes (Heitmann et al., 2016). Similarly, the basolateral subregion is involved in the integration of sensory information from the environment (Klumpp & Fitzgerald, 2018). Less is known about the amygdalostriatal subregion, but it is thought to have shared pathways with the basolateral subregion (Wang et al., 2002). The absence of significant findings in these two subregions may reflect the lack of necessity to integrate information in the absence of stimuli during resting-state fMRI.

5.6.2 Precuneus to ACC Associations with Social Anxiety

In addition to the positive associations, we also observed negative associations between the connectivity of the precuneus to the peri-genu ACC and social anxiety severity. This finding

is in line with Brühl et al. (2014) neurobiological model of SAD which posited a decrease in connectivity between the precuneus and ACC in those with SAD compared to controls. The precuneus plays a role in organizing attentional processes such as assessing the context of stimuli (Utevsky et al., 2014) and is a prominent component of the default mode network which is involved in self-referential processing (Raichle, 2015). The peri-genu ACC is involved in emotion regulation and, in a recent meta-analysis, has been identified as having altered functional connectivity to other neural regions across anxiety and affective disorders (Marusak et al., 2016). Disrupted connectivity between the peri-genu ACC and the precuneus is thought to be related to a decreased ability in being able to regulate negative self-relevant emotions which contributes to the maintenance of SAD and has similarly been found in people with major depressive disorder (Peng et al., 2021).

5.6.3 Theoretical Implications

It is of note that, despite observing moderate effect sizes for group differences in functional connectivity, none of these findings remained significant after statistical thresholding ($p < 0.05$, 5000 permutations, TFCE corrected). Voxel-wise permutation testing, which was used in this study, has become an increasingly popular choice to deal with multiple comparisons that may occur in fMRI analyses. This is due to its high sensitivity and its recognition that voxels are not activated independently of their neighbouring voxels (Heller et al., 2006; Smith & Nichols, 2009). However, it has also been found that using such a stringent thresholding approach has its limitations. In addition to fewer degree-of-freedom in TFCE between-group analysis compared to permutation-based correlation analysis, Noble et al. (2020) found that the TFCE approach was not able to detect any medium-sized effects in large sample sizes ranging from 480 to 493 healthy participants using the fMRI data in the Human Connectome Project. They concluded that numerous true effects may have been missed due to the prioritization of controlling family-wise error rates. The link between false-positive errors

(which we can control) and false-negative errors (which we cannot control) is a non-trivial problem in contemporary science, but it remains imperative to minimize the former error type. Therefore, we believe that the differences reported in functional connectivity between groups in this study (but which did not survive thresholding) may be a relevant finding of interest and could be tested in a more hypothesis-driven way in future studies by pre-selecting voxels-of-interest or larger ROI based anxiety-specific a priori hypotheses which will result in fewer multiple comparisons.

Significant associations between functional connectivity and social anxiety severity (in the absence of significant group differences) further contribute to evidence of a dimensional or spectrum conceptualization of SAD, this time from a neurobiological perspective. This is consistent with the National Institute of Mental Health's Research Domain Criteria (RDoC) framework which is advocating for a dimensional approach for the investigation of neurobiological markers of psychiatric disorders (Insel, 2014). Other studies that have used resting-state fMRI in a range of psychiatric and neurodevelopmental disorders (such as attention-deficit hyperactivity disorder, autism spectrum disorder, and major depressive disorder) have similarly found evidence to support the conceptualization of these disorders as being dimensional, with associations between functional connectivity and symptom severity (Chabernaud et al., 2012; Elton et al., 2016; Saris et al., 2020). Findings from taxometric analyses (Ruscio, 2010) and previous fMRI studies in those with SAD also support this approach, with significant positive associations (but no significant group differences when comparing SAD to controls) between social anxiety severity and brain activity (e.g. in the dorsal ACC and right anterior insular cortex) in response to threat stimuli (Savage et al., 2020), and between emotion regulation and amygdala functional connectivity at rest (Rabany et al., 2017).

Our findings of significant associations in the absence of statistically significant group differences (control vs. SAD participants) is also consistent with the most recently proposed integrated etiological and maintenance (IAM) model of SAD (Wong & Rapee, 2016) in which contributing factors (both neurobiological and cognitive) to the aetiology and maintenance of the disorder are identified as being dimensional. However, the most recently proposed neurobiological model of SAD (Brühl et al., 2014) uses a categorical approach to conceptualize changes in neural activity and connectivity occurring in those with SAD compared to controls. Our findings show a similar pattern to the neurobiological model, including decreased connectivity between the precuneus and ACC in those with SAD compared to controls. However, our finding of increased connectivity between the amygdala (including the superficial subregion) and the supramarginal gyrus being associated with increased social anxiety severity is contrary to the model which indicates decreased connectivity between the amygdala and parietal regions in those with SAD compared to controls. Therefore, we provide further insights to this model by highlighting the importance of conceptualizing symptoms associated with SAD dimensionally (by examining associations and not only group comparisons) and the necessity to examine amygdala subregion specific effects that are linked to social anxiety severity. It is therefore critical that both these points are considered in future proposed neurobiological models of SAD.

It is well-known that broader disturbances between the amygdala and frontal regions are strongly implicated in fear processing, and altered connectivity between the amygdala and frontal regions in those with SAD compared to controls has been the most consistently reported across resting-state fMRI studies (reported by 9 of 18 fMRI studies in a systematic review; Mizzi et al., 2021). However, we found no alterations in connectivity between amygdala-frontal regions between groups and no associations between amygdala-frontal connectivity and social anxiety severity. This suggests that people with social anxiety do not have disturbances

in fear processing in the absence of explicit social stimuli (i.e., at rest), perhaps due to a lesser need to be hypervigilant to threat and a reduction in negative cognitions related to being evaluated by others (both factors contributing to the maintenance of social anxiety; Wong & Rapee, 2016).

5.6.4 Conclusion

In conclusion, our study found significant associations between resting-state functional connectivity (with evidence of subregion-specific amygdala effects) and social anxiety severity scores in the absence of significant group differences. Relative to previous resting-state fMRI studies examining SAD, the strengths of this study were the use of a larger sample of participants (n=135) and longer scan length time (518 seconds; known to improve test-retest reliability; Birn et al., 2013). Additionally, our use of multiband fMRI imaging (improving spatial and temporal resolution; Bhandari et al., 2020), stringent fMRI thresholding, and use of *fMRIprep* for preprocessing provides a strong basis for future studies to continue studying and/or replicate these patterns. Based on the current findings, the IAM model of SAD (Wong & Rapee, 2016), and the current RDoC framework, we believe that future studies would benefit from examining changes in brain activity and connectivity in relation to dimensional symptoms (e.g., social anxiety severity) rather than the presence or absence of a diagnosis of SAD (i.e., a categorical approach). This will lead to a more nuanced understanding of the neurobiological mechanisms underlying social anxiety at rest and may contribute to a future dimensional neurobiological model of SAD.

5.7 Supplementary Information

5.7.1 Methods

5.7.1.1 fMRIprep Pre-Processing

5.7.1.1.1 Anatomical Data Preprocessing. T1-weighted (T1w) images were corrected for intensity non-uniformity (INU) with N4BiasFieldCorrection (Tustison et al., 2010), distributed with ANTs 2.2.0 (Avants et al., 2008; RRID:SCR_004757), and used as T1w-reference throughout the workflow. The T1w-reference was then skull-stripped with a Nipype implementation of the antsBrainExtraction.sh workflow (from ANTs), using OASIS30ANTs as the target template. Brain tissue segmentation of cerebrospinal fluid (CSF), white-matter (WM) and gray-matter (GM) was performed on the brain-extracted T1w using fast (FSL 5.0.9, RRID:SCR_002823; Zhang et al., 2001). Brain surfaces were reconstructed using recon-all (FreeSurfer 6.0.1, RRID:SCR_001847; Dale et al., 1999), and the brain mask estimated previously was refined with a custom variation of the method to reconcile ANTs-derived and FreeSurfer-derived segmentations of the cortical gray-matter of Mindboggle (RRID:SCR_002438; Klein et al., 2017).

Volume-based spatial normalization to two standard spaces (MNI152NLin6Asym, MNI152NLin2009cAsym) was performed through nonlinear registration with antsRegistration (ANTs 2.2.0), using brain-extracted versions of both T1w reference and the T1w template. The following templates were selected for spatial normalization: FSL's MNI ICBM 152 non-linear 6th Generation Asymmetric Average Brain Stereotaxic Registration Model [(Evans et al., 2012), RRID:SCR_002823; TemplateFlow ID: MNI152NLin6Asym], ICBM 152 Nonlinear Asymmetrical template version 2009c [(Fonov et al., 2009), RRID:SCR_008796; TemplateFlow ID: MNI152NLin2009cAsym].

5.7.1.1.2 Functional Data Preprocessing. For each of the BOLD runs per subject (across all tasks and sessions), the following preprocessing was performed. First, a reference

volume and its skull-stripped version were generated using a custom methodology of fMRIPrep. Head-motion parameters with respect to the BOLD reference (transformation matrices, and six corresponding rotation and translation parameters) are estimated before any spatiotemporal filtering using mcflirt (FSL 5.0.9; Jenkinson et al., 2002). BOLD runs were slice-time corrected using 3dTshift from AFNI 20160207 (Cox & Hyde, 1997; RRID:SCR_005927).

A deformation field to correct for susceptibility distortions was estimated based on fMRIPrep's fieldmap-less approach. The deformation field is that resulting from co-registering the BOLD reference to the same-subject T1w-reference with its intensity inverted (Huntenburg, 2014; Wang et al., 2017). Registration is performed with antsRegistration (ANTs 2.2.0), and the process regularized by constraining deformation to be nonzero only along the phase-encoding direction, and modulated with an average fieldmap template (Treiber et al., 2016). Based on the estimated susceptibility distortion, a corrected EPI (echo-planar imaging) reference was calculated for a more accurate co-registration with the anatomical reference. The BOLD reference was then co-registered to the T1w reference using bbregister (FreeSurfer) which implements boundary-based registration (Greve & Fischl, 2009).

Co-registration was configured with six degrees of freedom. The BOLD time-series were resampled onto the following surfaces (FreeSurfer reconstruction nomenclature): fsaverage6. The BOLD time-series (including slice-timing correction when applied) were resampled onto their original, native space by applying a single, composite transform to correct for head-motion and susceptibility distortions. These resampled BOLD time-series will be referred to as preprocessed BOLD in original space, or just preprocessed BOLD. The BOLD time-series were resampled into standard space, generating a preprocessed BOLD run in MNI152NLin6Asym space.

Several confounding time-series were calculated based on the preprocessed BOLD: framewise displacement (FD), DVARS and three region-wise global signals. FD was computed using two formulations following Power (absolute sum of relative motions, (Power et al., 2014)) and Jenkinson (relative root mean square displacement between affines, (Jenkinson et al., 2002)). FD and DVARS are calculated for each functional run, both using their implementations in Nipype (following the definitions by (Power et al., 2014)). The three global signals are extracted within the CSF, the WM, and the whole-brain masks. Additionally, a set of physiological regressors were extracted to allow for component-based noise correction (CompCor, (Behzadi et al., 2007)). Principal components are estimated after high-pass filtering the preprocessed BOLD time-series (using a discrete cosine filter with 128s cut-off) for the two CompCor variants: temporal (tCompCor) and anatomical (aCompCor). tCompCor components are then calculated from the top 5% variable voxels within a mask covering the subcortical regions. This subcortical mask is obtained by heavily eroding the brain mask, which ensures it does not include cortical GM regions. For aCompCor, components are calculated within the intersection of the aforementioned mask and the union of CSF and WM masks calculated in T1w space, after their projection to the native space of each functional run (using the inverse BOLD-to-T1w transformation). Components are also calculated separately within the WM and CSF masks. For each CompCor decomposition, the k components with the largest singular values are retained, such that the retained components' time series are sufficient to explain 50 percent of variance across the nuisance mask (CSF, WM, combined, or temporal). The remaining components are dropped from consideration.

The head-motion estimates calculated in the correction step were also placed within the corresponding confounds file. The confound time series derived from head motion estimates and global signals were expanded with the inclusion of temporal derivatives and quadratic terms for each (Satterthwaite et al., 2013). Frames that exceeded a threshold of 0.5 mm FD or

1.5 standardised DVARS were annotated as motion outliers. All resamplings can be performed with a single interpolation step by composing all the pertinent transformations (i.e. head-motion transform matrices, susceptibility distortion correction when available, and co-registrations to anatomical and output spaces). Gridded (volumetric) resamplings were performed using antsApplyTransforms (ANTs), configured with Lanczos interpolation to minimize the smoothing effects of other kernels (Lanczos, 1964). Non-gridded (surface) resamplings were performed using mri_vol2surf (FreeSurfer).

5.7.2 Results

Supplementary Table 5.1

Group Differences in Resting-State Functional Connectivity between those with SAD (n = 42) and Controls (n = 93)

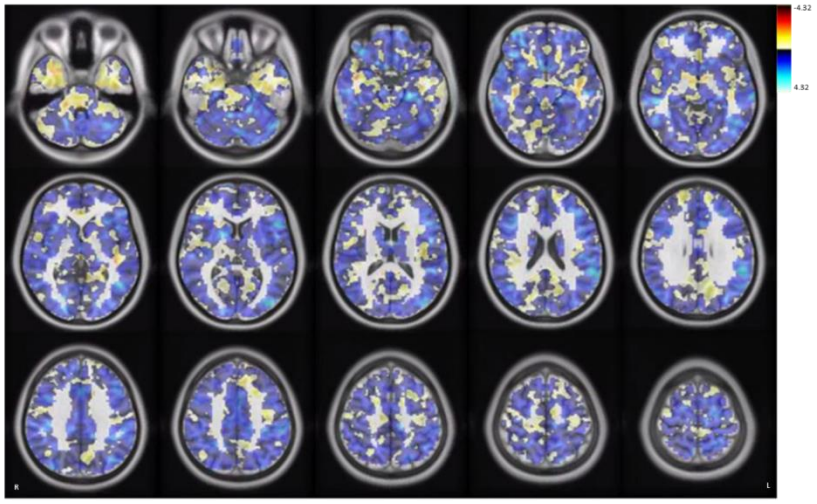
Seed #	Seed region	SAD vs. Controls		
		Regions showing peak altered connectivity	**Peak MNI coordinate**	**Peak intensity**
1	L. amygdalostriatal	R. calcarine gyrus	20 -90 4	3.892
2	R. amygdalostriatal	R. superior frontal gyrus	18 56 12	3.960
3	L. basolateral	R. supramarginal gyrus (inferior parietal lobule)	52 -38 42	4.1655
4	R. basolateral	L. cerebellum (IV-V)	-18 -28 -26	4.5144
5	L. centromedial	R. medial temporal pole	46 16 -42	3.7061
6	R. centromedial	R. superior frontal gyrus	18 56 12	3.6414
7	L. superficial	R. supramarginal gyrus (inferior parietal lobule)	58 -38 34	4.0305
8	R. superficial	L. anterior agranular insula complex	-30 -62 0	3.4051
9	L. amygdala	R. supramarginal gyrus (inferior parietal lobule)	52 -40 42	4.1854
10	R. amygdala	L. cerebellum (IV-V)	-20 -28 -26	3.7871
11	L. precuneus	R. calcarine gyrus	22 -90 2	4.0954
12	R. precuneus	R. frontal opercular area 2	20 -60 42	3.9529
13	L. ACC (subgenual)	R. cerebellum (IX)	2 -50 -42	-4.1908
14	R. ACC (subgenual)	R. cerebellum (IX)	4 -48 -42	-3.7414
15	L. vmPFC	L. cingulate gyrus, frontal opercular area 1	-16 8 46	-4.294
16	R. vmPFC	L. cingulate gyrus, frontal opercular area 1	-16 8 46	-3.8349
17	L. TPJ	R. anterior agranular insula complex	-42 -26 0	-4.3152
18	R. TPJ	L. precuneus, frontal opercular area 3	-18 -52 56	3.8643

Note. Region names were identified using the Automatic Anatomical Labelling Atlas and Glasser et al. (2016) parcellation map. ACC = anterior cingulate cortex; L = left; R = right; MNI = Montreal Neurological Institute; vmPFC = ventromedial prefrontal cortex; TPJ = temporoparietal junction.

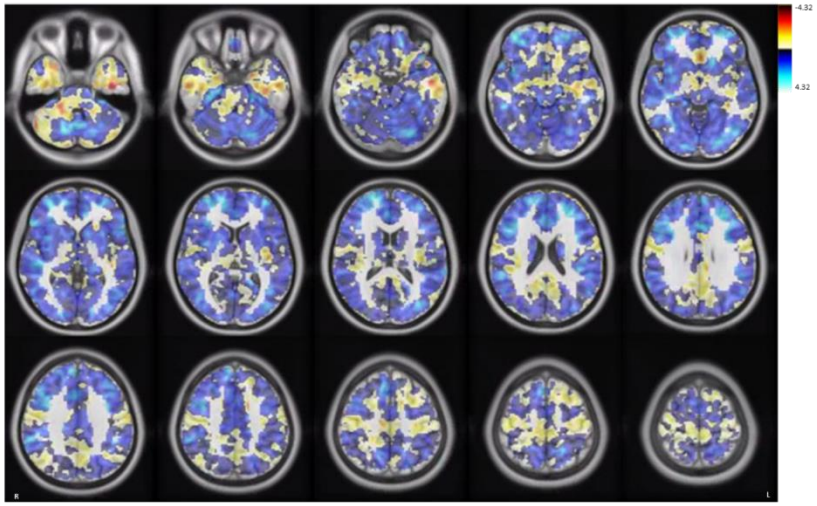
Supplementary Figure 5.1

Seed-based Functional Connectivity Maps of 18 seed regions of 135 Participants (42 SAD)

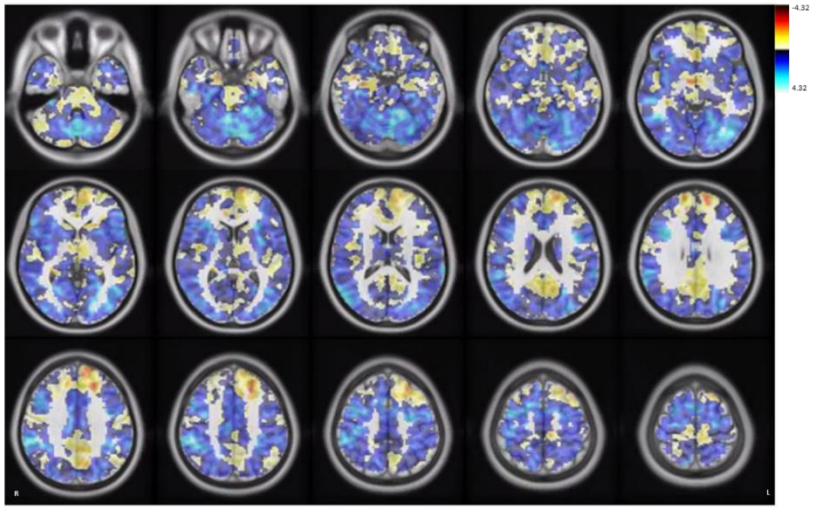
1. Left amygdalostriatal



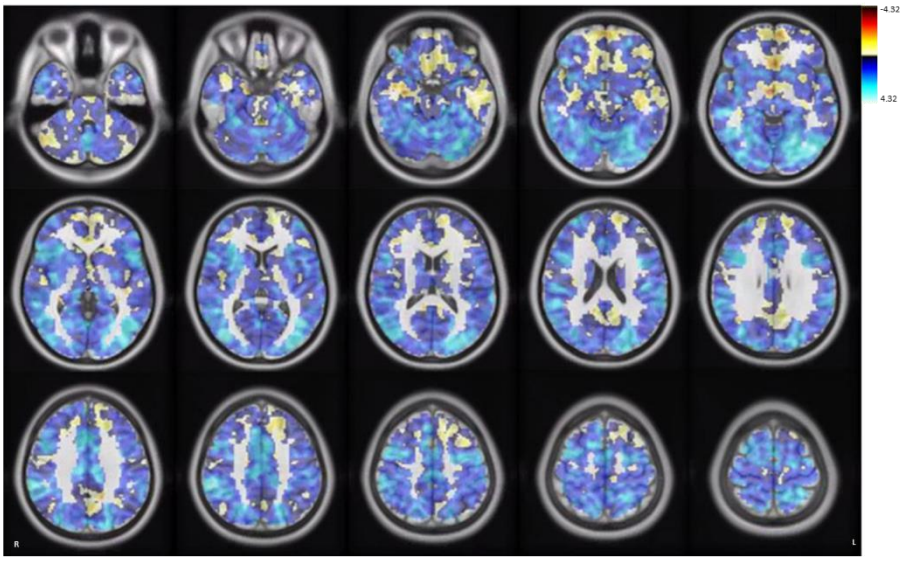
2. Right amygdalostriatal



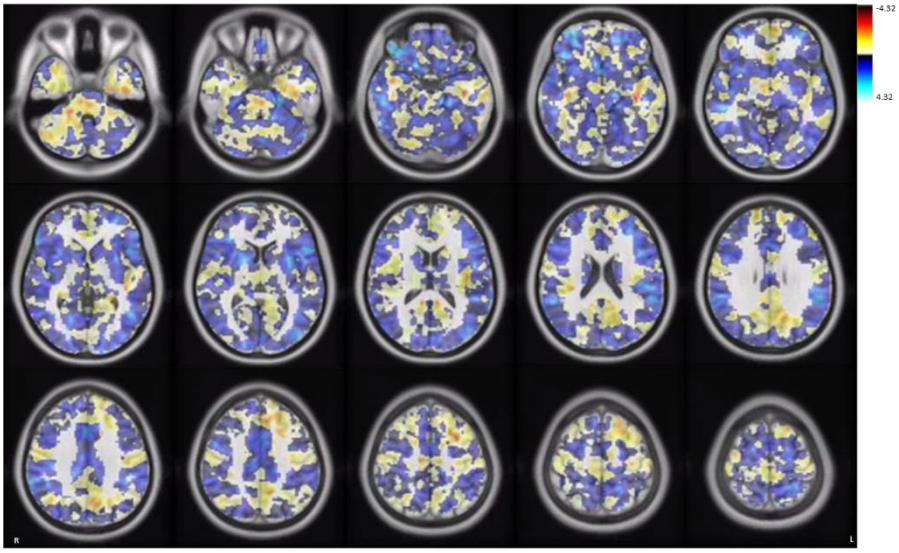
3. Left basolateral



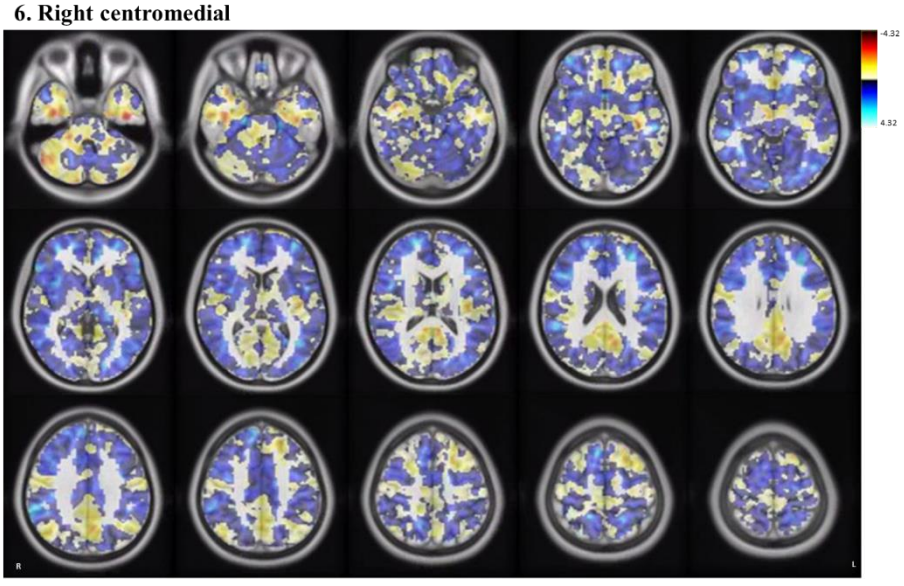
4. Right basolateral



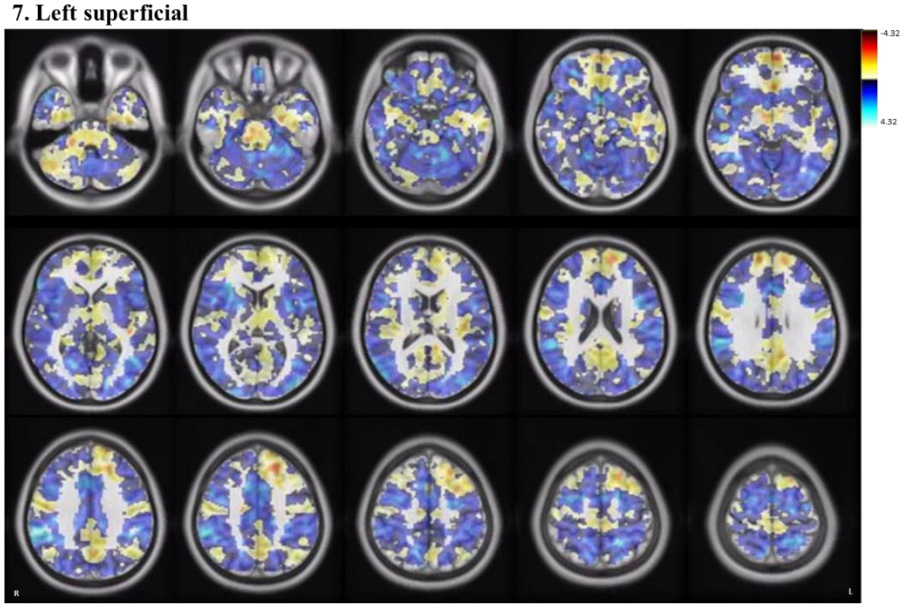
5. Left centromedial



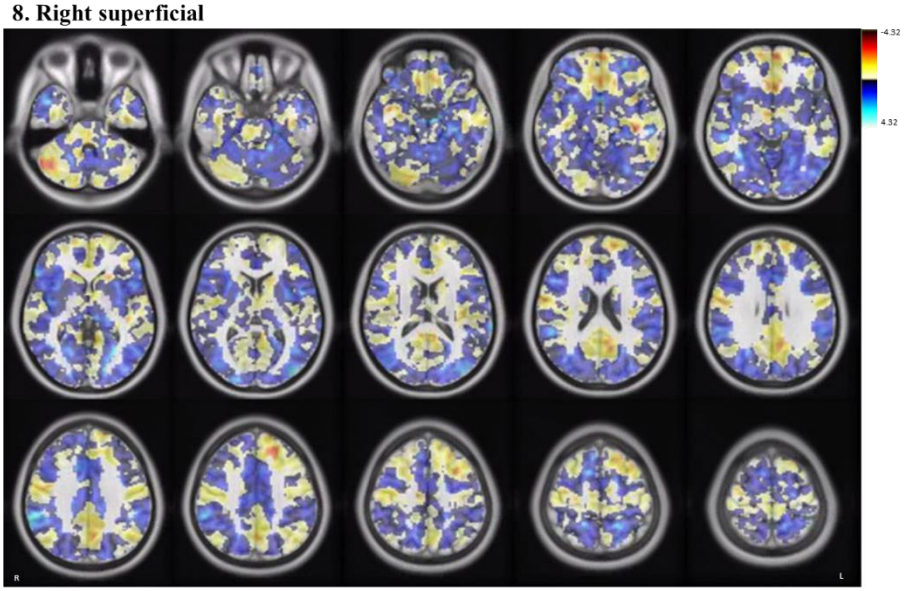
6. Right centromedial



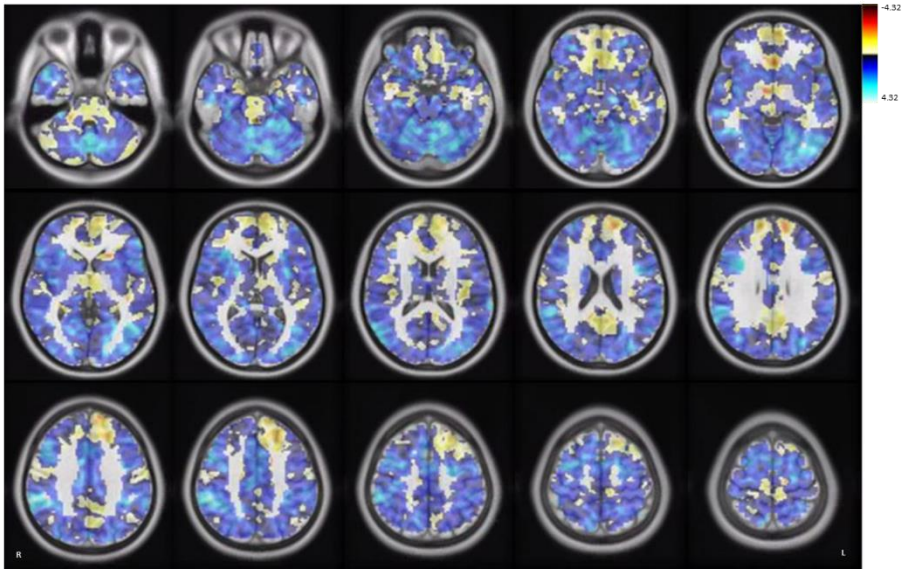
7. Left superficial



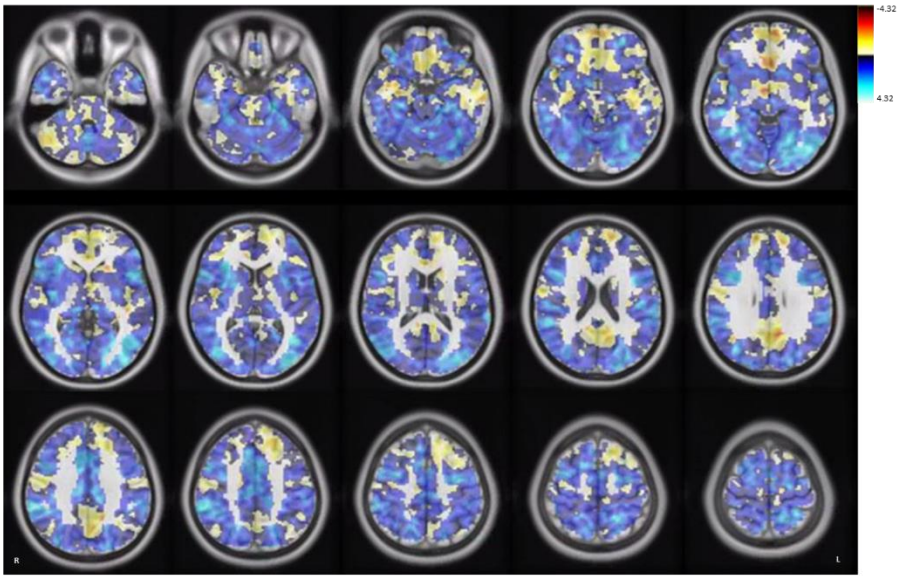
8. Right superficial



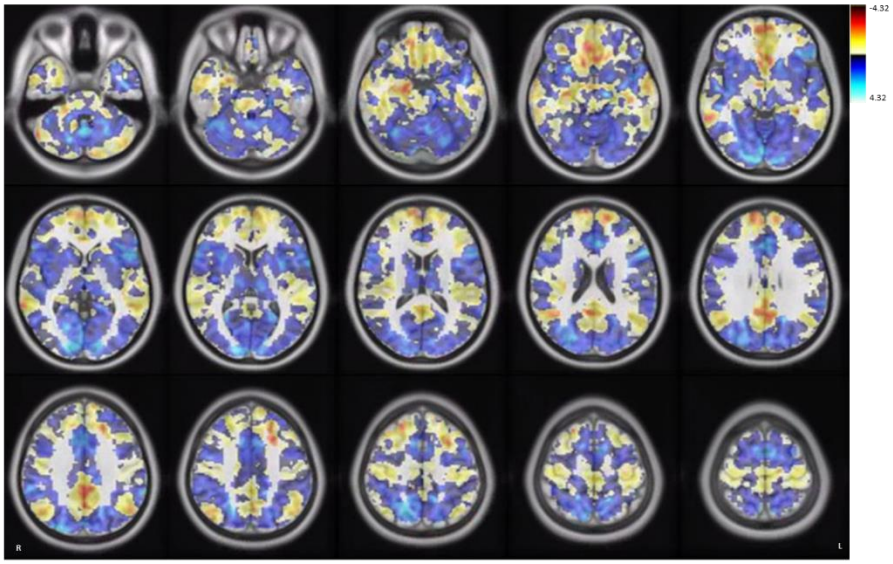
9. Left amygdala



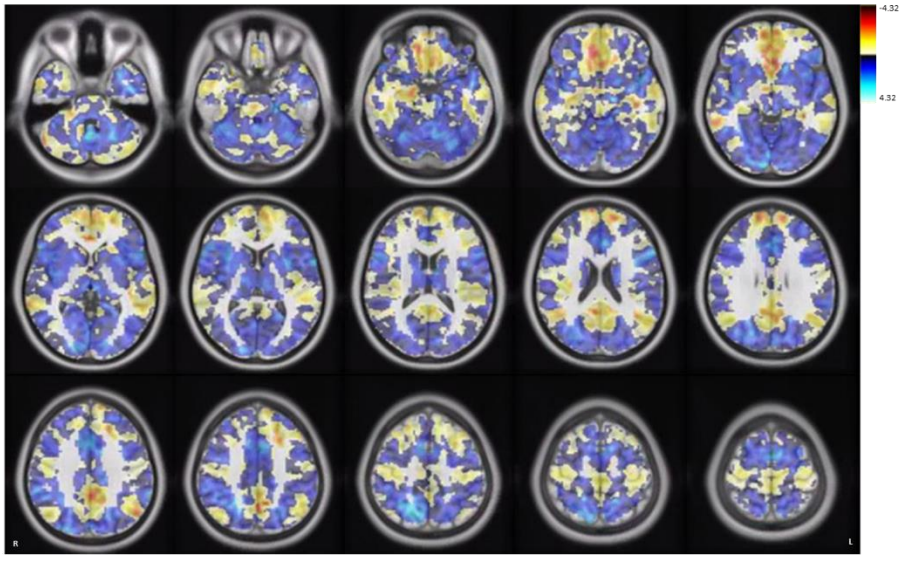
10. Right amygdala



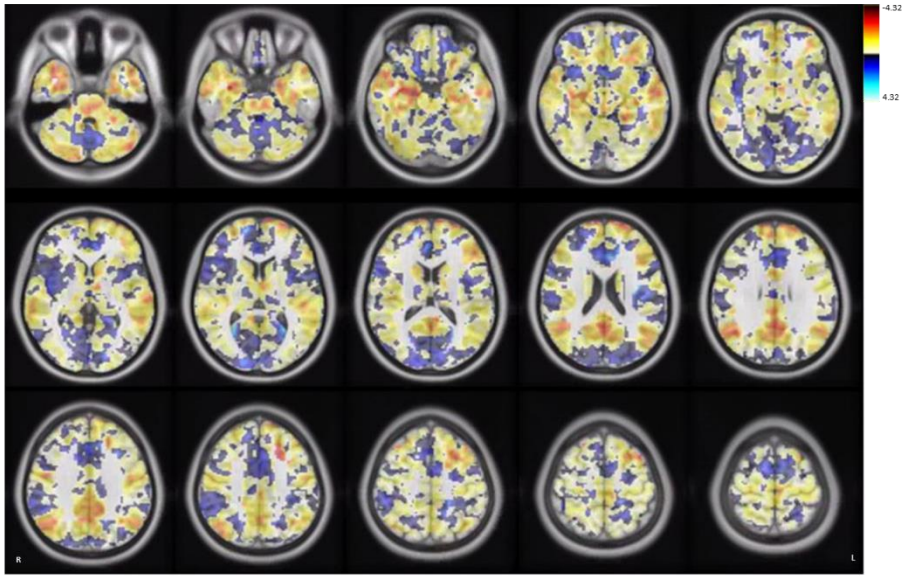
11. Left precuneus



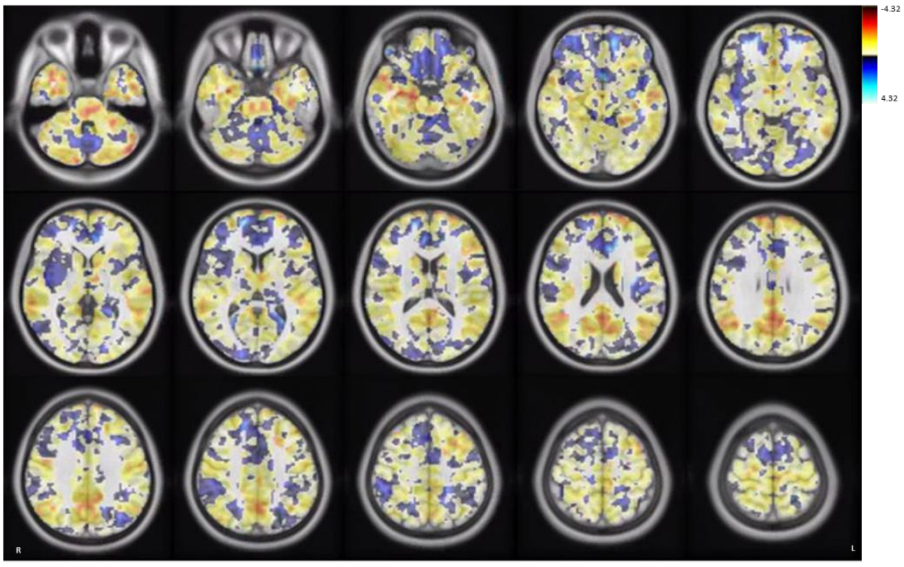
12. Right precuneus



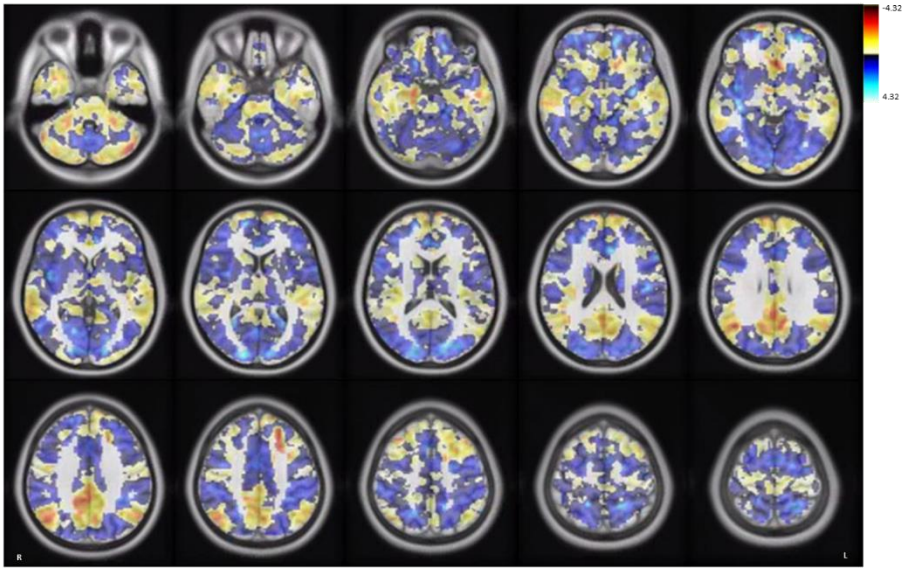
13. Left anterior cingulate cortex (subgenual)



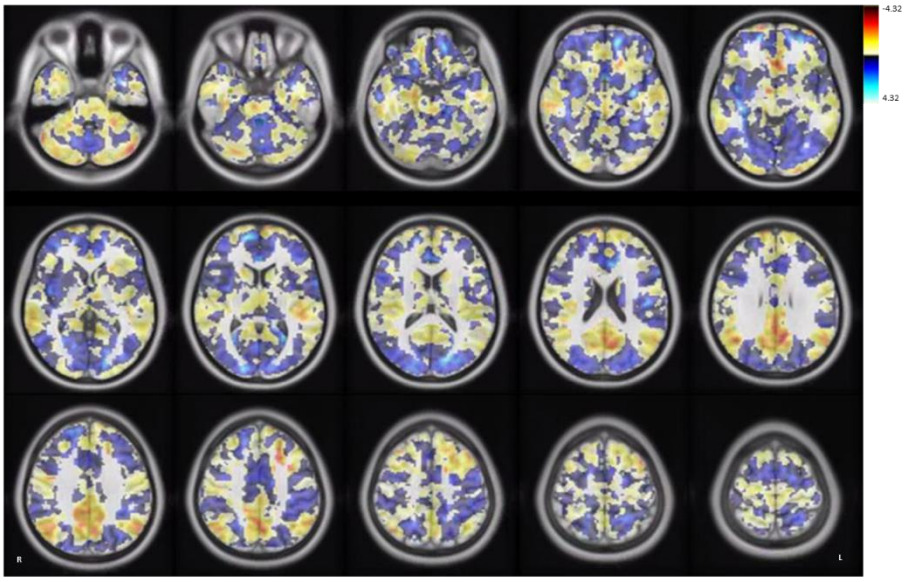
14. Right anterior cingulate cortex (subgenual)



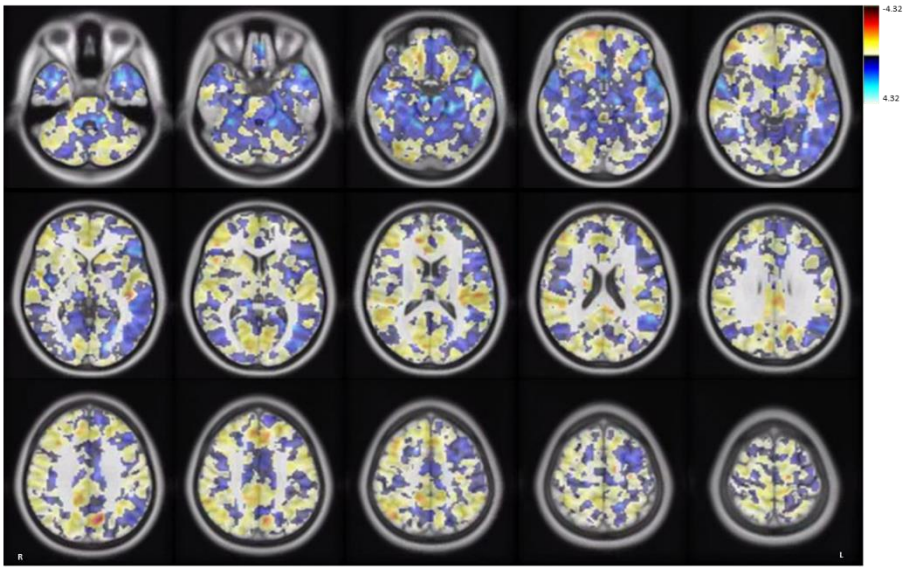
15. Left ventromedial prefrontal cortex



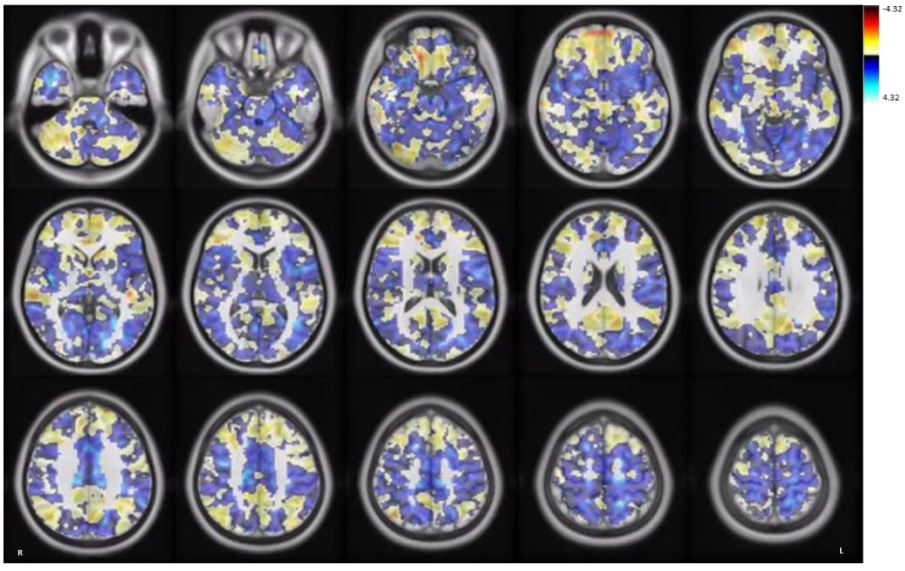
16. Right ventromedial prefrontal cortex



17. Left temporoparietal junction



18. Right temporoparietal junction



CHAPTER 6: AMYGDALA SUBREGION-SPECIFIC DYSFUNCTION DURING EMOTION PROCESSING IN SOCIAL ANXIETY DISORDER (STUDY 3)

6.1 Chapter Guide

This chapter presents Study 3 which examined brain function in the context of emotion processing in those with SAD and healthy controls. Given the evidence of aberrant neural functioning during emotion processing in SAD but the discrepancies across the literature (see Chapter 2 for review), and the role of the amygdala subregions in those with SAD as identified in Study 2 (Chapter 5), this study aimed to further clarify the neural alterations occurring in those with SAD compared to controls by examining amygdala subregion-specific activity and connectivity patterns in response to emotional facial expressions. As a secondary aim and similar to Study 2, this study also investigated whether subregion activity and connectivity patterns were associated with social anxiety severity across all participants to investigate the neurobiological correlates of dimensional symptoms. This paper was prepared with publication in mind, therefore some of the methods and findings are reported in the Supplementary Material presented at the end to adhere to publishable manuscript length.

6.2 Abstract

Background: Social anxiety disorder (SAD) is characterised by fear and avoidance of social situations. Increased attention to social threat in the environment is implicated in this disorder, including hypervigilance and/or avoidance of others' facial expressions. Altered activity and connectivity of the amygdala are frequently reported in neuroimaging studies examining face emotion processing in SAD, although with mixed findings. This study aimed to clarify the amygdala's role in emotion processing in SAD by examining the activity and connectivity patterns of four structurally and functionally distinct amygdala subregions. **Method:** Using multiband functional magnetic resonance imaging (fMRI), we assessed brain responses to Happy, Fearful, and Angry faces for 42 individuals with SAD and 84 controls. Group differences in fMRI activation and connectivity (using generalised psychophysiological interactions (gPPI) of the bilateral amygdala and four subregions (amygdalostriatal, basolateral, centromedial, and superficial) were examined as well as associations between connectivity and activity of amygdala subregions and social anxiety severity. **Results:** Compared to controls, those with SAD showed increased superficial subregion activation regardless of emotions. gPPI analyses revealed decreased connectivity (SAD < controls) between the superficial subregion and the precuneus to Angry faces, and increased connectivity (SAD > controls) between the basolateral subregion and precentral/postcentral and supramarginal gyrus to Fearful faces. Social anxiety severity was positively correlated with superficial and centromedial subregion activation (but not with whole amygdala activation) to Happy faces. **Conclusion:** The findings demonstrate amygdala subregion-specific alterations in SAD that may help explain the discrepancies in the literature regarding the amygdala's role in emotion processing in SAD. Future research should examine the effects of treatment for SAD on the alterations of the specific amygdala subregions to elucidate optimal efficacious treatments.

***Keywords**:* Social Phobia, fMRI, Functional Connectivity, Precuneus, Superficial, Basolateral

6.3 Introduction

Social anxiety disorder (SAD) is characterized by several symptoms, including anxiety and/or avoidance in social situations (American Psychiatric Association, 2013). Several factors contribute to the maintenance of this disorder, including increased sensitivity to perceived social threats (e.g., angry faces) and a negative bias in the processing of social information (e.g., increased likelihood of interpreting a neutral face as threatening; Wong & Rapee, 2016). Due to the relevance of face emotion processing in this disorder, there have been continuing attempts to elucidate the neurobiological underpinnings of such differences in those with SAD.

The most recently proposed neurobiological model of SAD suggests an amplified fear circuitry, most commonly involving the amygdala and frontal regions, that is evident during emotion processing in those with SAD compared to controls (Brühl et al., 2014). This model is supported by a meta-analysis of 23 studies (including unpublished data) reporting increased activation in several brain regions, including the bilateral amygdala in those with SAD compared to controls when completing a face-processing task (regardless of emotional expression; Gentili et al., 2016). However, a more recent meta-analysis of 15 studies that used emotional faces as stimuli found no differences in amygdala activation in those with SAD compared to controls (Yu et al., 2021). Additionally, a meta-analysis investigating the effects of pharmacotherapy and psychotherapy on functional neuroimaging in adults with SAD reported no changes in the amygdala across studies despite its hypothesised role in SAD (Li et al., 2016). These divergent imaging findings of the amygdala and the absence of evidence that current treatment options target the amygdala suggests that emotion processing dysfunction in SAD individuals may not be that clear cut, with possible explanations for the conflicting results discussed next.

All of the studies included in the aforementioned meta-analyses examined the amygdala as a single homogenous unit. However, it is well known that the amygdala is a complex structure composed of anatomically and functionally distinct nuclei; in humans, commonly

classified into the basolateral, centromedial, superficial, and amygdalostriatal subregions (Balderston et al., 2015; Wang et al., 2021). Since common brain imaging analyses often extract signals from neural regions with peak activation, findings are likely obtained from different amygdala subregions that may contribute to the discrepancies in results.

The basolateral subregion is the main input area of the amygdala, receiving information from sensory cortical regions, the medial prefrontal cortex, and subcortical regions, including the hippocampus and thalamus. The basolateral subregion of the amygdala is implicated in the process of fear conditioning and reward-related learning and the integration of this information with relevant self-cognitions (Bzdok et al., 2013). The centromedial region is thought to be the significant output region of the amygdala and is known for generating responses, including autonomic and motor responses (Kerestes et al., 2017). The superficial amygdala is bilaterally connected to multiple brain regions, is sensitive to social information, and plays a role in social cognition due to its strong connections with the insula and the inferior frontal gyrus (Bzdok et al., 2013). The amygdalostriatal subregion sends projections to the striatum which is thought to be one of the brain's main coordination centres due to its inputs from the cerebral cortex (Berendse et al., 1992; Wang et al., 2002). Therefore, the amygdalostriatal subregion contributes associative emotional information to the other details coordinated by the striatum (Wouterlood et al., 2018).

There is preliminary evidence of amygdalar subregion differences in those with SAD from resting-state functional magnetic resonance image (fMRI) studies (Anteraper et al., 2014; Yoon et al., 2016). Findings from these studies demonstrate increased connectivity between the basolateral, superficial, and centromedial subregions and frontal regions (e.g., superior frontal gyrus, dorsolateral prefrontal cortex, the anterior cingulate cortex) in those with SAD compared to controls. However, there is limited evidence of amygdala subregion differences in response to emotion processing in those with SAD, with only one study demonstrating increased activation in the central and lateral amygdala in response to relevant disorder-related

scenes in the clinical group (including people with SAD, panic disorder, dental phobia, and post-traumatic stress disorder) compared to controls (Feldker et al., 2017).

The current study primarily aimed to build on the literature by examining group differences (SAD vs. controls) in activation and connectivity of the amygdala and four of its subregions (amygdalostriatal, basolateral, centromedial, and superficial) in response to emotional facial expressions involving fear, angry, and happy faces. Our secondary aim was to test for associations between brain (activity and connectivity) and social anxiety severity across all participants. This was to test for the existence of brain-symptom associations along a dimensional spectrum (aligned with the Research Domain Criteria project which aims to classify psychopathology based on dimensions of observable behaviours and neurobiological measures (Insel, 2014)). This is an exploratory study due to limited research investigating the amygdala subregions in SAD to date.

6.4 Methods

6.4.1 Participants

We recruited 43 participants for the SAD group and 95 participants for the healthy control group. The following exclusion criteria for the control group was applied: left-handed, history of/current psychiatric or neurological disorder, taking psychotropic medication, head trauma (unconscious for > 5 minutes), substance abuse, and the usual MRI contraindications, including the presence of metal objects in the body that cannot be removed or made safe for MRI. Of the recruited participants, 12 had an incomplete scan or experienced problems during MRI data acquisition. Thus, the final sample comprised 42 participants in the clinical group and 84 participants in the control group. This study was approved by the Human Research Ethics Committee at Australian Catholic University.

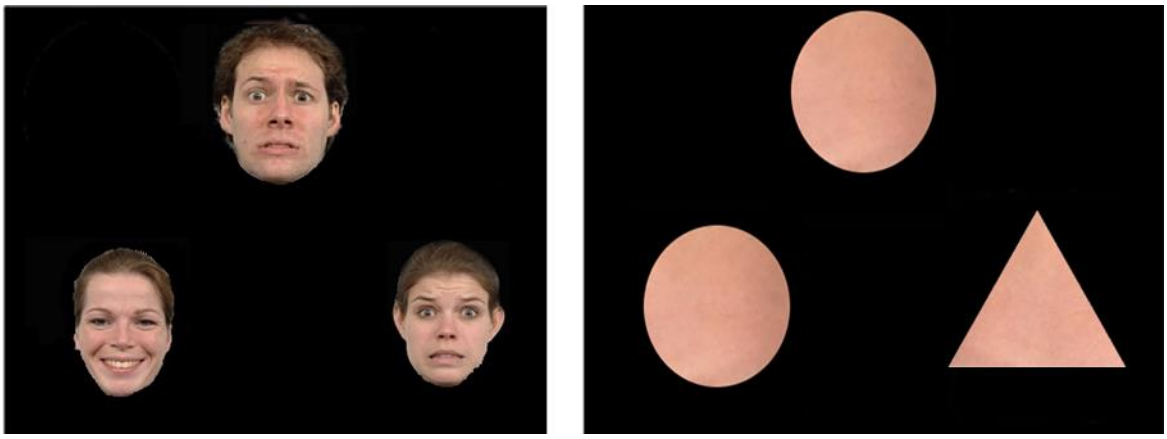
6.4.2 Emotional Face Matching Task

Before testing, participants were trained on a modified version of a widely used emotional face-matching task (EFMT), an explicit emotion classification task designed to

robustly activate the amygdala across clinical and healthy populations (Hariri et al., 2002; Labuschagne et al., 2010). Participants were shown a trio of faces for each trial and were instructed to select which of two faces (displayed on the bottom of the screen) expressed the same emotion as the target face (displaced on the top of the screen). Face stimuli were selected from the validated Radboud Faces Database (Langner et al., 2010) and expressed fear, anger, or happiness. Participants were instructed to complete a similar shape-matching task (including circles, triangles, and rectangles; see Figure 6.1).

Figure 6.1

Face and Shape Stimuli used in the Emotional Face Matching Task



Face-matching trials were arranged into blocks interspaced between blocks of shape-matching trials. In total, there were 26 experimental blocks consisting of 12 face-matching ones (4 for each emotion) and 14 shape-matching blocks, counterbalanced across two experimental runs. Each block contains four sequential matching trials presented for 5 seconds. Participants were asked to use their right hand to press one of two buttons on an MRI-compatible button box. Button-box responses were recorded and analysed for accuracy for each emotion condition. Presentation software (https://neurobs.com) displayed the stimuli and recorded participants' responses. Task presentation and recording of behavioural responses were performed with Presentation software (Neurobehavioural Systems, Albany, CA, USA).

6.4.3 fMRI Methods

T1-weighted MRI and task-based functional data were acquired on a Siemens MAGNETOM Tim Trio 3.0 T Scanner (Erlangen, Germany) at Swinburne University of Technology, Australia. Padded foam cushions were used to minimize head movement throughout the scan. A multiband echo-planar imaging sequence was used to acquire functional images [repetition time (TR) = 1.02s; echo time (TE) = 30ms; flip angle (FA) = 65^{o}; multiband acceleration factor (MB) = 5; 65 transversal slices with 96x96 voxels at 2mm in-plane resolution; 294 volumes for each of the two runs]. A T1-weighted sagittal MP-RAGE structural image was also obtained for anatomical reference (TR=1.9 s, TE=2.52ms, FA=9°, 176 slices with 1x1x1mm voxels).

6.4.4 fMRI Data Pre-Processing

Pre-processing and subsequent analyses were performed on MASSIVE HPC (www.massive.org.au - see Supplementary materials for more details). The imaging data were pre-processed using fMRIPrep 20.2.3 (Esteban, Markiewicz, et al. (2018); Esteban, Blair, et al. (2018); RRID:SCR_016216), based on Nipype 1.6.1 (Gorgolewski et al. (2011); Gorgolewski et al. (2018); RRID:SCR_002502). Following this, the data were smoothed to 6mm FWHM using FSL. All output images from fMRIprep underwent manual quality assurance for each participant.

6.4.5 Region-of-Interest (ROI) Activation Analysis

6.4.5.1 First-Level fMRI Activation Analysis

First-level analysis was conducted using SPM12 implemented in Matlab 2018a. A general linear model (GLM), including the onsets and durations of each condition (happy, angry, fear, shapes), were modelled using the classical Restricted Maximum Likelihood (ReML) estimation method. Six realignment parameters (translational x y z; rotational x y z) were included as nuisance regressors, and low-frequency artifacts were removed using a high-pass filter at 128 s. The following contrasts were generated for each subject at the first level: happy > shapes, fear > shapes, and angry > shapes.

6.4.5.2 Second-Level fMRI Activation Analysis

Spherical ROIs of subregions of the amygdala and the amygdala as a whole were constructed using the MarsBar toolbox in SPM12 (3mm radius spheres for each amygdala subregion; 5mm radius sphere for the whole amygdala). Coordinates of each ROI were defined a priori using cytoarchitectonic probability maps from the Anatomy Toolbox (Version 2.2b) in SPM12 (Amunts et al., 2005; Eickhoff et al., 2005). The mean activation within each ROI for each contrast was then extracted for each participant using the MarsBaR toolbox.

6.4.6 fMRI Connectivity Analysis

Task-dependent seed-to-voxel functional connectivity analysis (using the amygdala and its subregions as seeds) was conducted using the generalised psychophysiological interactions (gPPI) method using the CONN toolbox v19.c (Whitfield-Gabrieli & Nieto-Castanon, 2012). This method involves the calculation of general linear models which include psychological predictors (i.e., the hemodynamic response function convolved with the task condition time series), physiological predictors (the mean time series for each seed), and their interaction. Compared to standard PPI approaches, gPPI has been shown to have greater sensitivity and specificity by reducing the probability of false negative and false positive findings (McLaren et al., 2012). The pre-processed and smoothed data were imported into the toolbox and further band-pass filtered between 0.01 and 0.08 Hz. Stimuli onsets and duration for each condition (i.e., happy, angry, fearful, shapes) were specified. To correct physiological noise and confounds, denoising was conducted to regress the principal components of white matter, cerebrospinal fluid, and 12 realignment parameters (6 motion parameters and 6 first-order temporal derivatives). The BOLD time course for each ROIs was then extracted for each participant and condition. Seed-to-voxel beta maps were computed with connectivity measures calculated as bivariate correlations for each bilateral ROIs.

6.4.7 Statistical Analyses

To examine differences in age between groups, the non-parametric Mann-Whitney *U* test was used due to the non-normal distribution of the data (as determined by the Shapiro-Wilk test; $p < 0.001$). To examine differences in sex and SIAS scores between groups, chi-square analysis and an independent sample *t*-test were used, respectively. The response times and accuracy of responses were compared between groups using the Mann-Whitney *U* test for all comparisons except for the reaction times in the anger condition where *t*-tests were utilised due to the normal distribution of the data.

To explore activation differences between groups, a 2 (group: SAD, controls) x 3 (emotion contrast: happy faces vs shapes, fearful faces vs shapes, angry faces vs shapes) repeated measures analysis of variance (rmANOVA) was conducted for each bilateral ROI. To determine whether laterality had an impact on findings, 2 (group: SAD, con) x 2 (laterality: left, right) x 3 (emotion contrast: happy faces vs shapes, fearful faces vs shapes, angry faces vs shapes) rmANOVAs were conducted for each bilateral ROI. Findings from analyses investigating laterality are reported in the Supplementary Material. Spearman's rank correlations were performed for each contrast of interest to assess the relationship between bilateral ROI activation and social anxiety severity (as measured by the social interaction anxiety scale, or SIAS) across all participants.

For the connectivity findings, multiple ANCOVAs examining interactions between seed region BOLD time series, the group contrast (SAD > CON), and the condition contrasts (fear/anger/happy faces > shapes) were computed. Additionally, multiple linear regression models were conducted for each bilateral ROI and each contrast to evaluate associations between connectivity and social anxiety severity across all participants. Age and sex were included as covariates of no interest for the connectivity analyses. Random field theory was used at a cluster threshold of $p < 0.05$ cluster-size *p* False Discovery Rate (FDR) corrected and a voxel threshold of $p < 0.001$ uncorrected. Findings from analyses investigating laterality are reported in the supplementary.

6.5 Results

6.5.1 Demographics

The characteristics for all participants (except for the 12 participants who were excluded) are included in Table 6.1. There were no significant differences in age and sex between groups. The SAD group had significantly higher SIAS scores compared to the control group. Five participants in the SAD group had comorbid secondary psychiatric disorders (generalized anxiety disorder (n = 3); post-traumatic stress disorder (n = 1); obsessive-compulsive disorder/attention deficit hyperactivity disorder (n = 1)).

Table 6.1

Demographic Information for Participants

	SAD	Control	Statistic	*p*-value
n	42	84	-	-
Sex (f/m)	21/21	44/40	$\chi^2 = 0.801$	0.851
Age [a]	27.18 (7.39), 19.52 – 54.96	25.72 (6.45), 18.24 – 49.41	$U = 1548.000$	0.264
SIAS [a]	51.33 (9.81), 28 – 72	18.77 (10.78), 1 – 48	$t = 16.461$	<0.001
LSAS [a]	81.73 (22.24), 41 – 139 [b]	-	-	-

Note. [a] Mean (standard deviation) and range; [b] scores were not reported for n=5; LSAS = Liebowitz Social Anxiety Scale; SIAS = Social Interaction Anxiety Scale; *t* = independent sample *t*-test; $\chi 2$ = chi-square analysis; *U* = Mann-Whitney *U* test.

6.5.2 Behavioural Data

There was a significant difference in reaction-time in response to all emotional faces, with the SAD group having significantly slower reaction times than the control group (see Table 6.2). There was no difference in reaction time in response to shapes (the control condition; see Supplementary Figure 6.1 for distribution of reaction times). There was no significant difference in the percentage of correct responses between groups in all conditions ($p > 0.05$).

Table 6.2

Reaction Times and Accuracy in the Emotional Face Matching Task

	Reaction time (seconds): mean (SD)	Percentage correct: mean (SD)	Statistic (reaction time)	*p*-value
Happy				
SAD	1.293 (0.306)	82.74 (28.55)	*U* = 1296.000	0.015
CON	1.155 (0.245)	88.24 (17.61)		
Fear				
SAD	1.527 (0.366)	85.12 (30.83)	*U* = 1228.000	0.006*
CON	1.356 (0.324)	91.74 (19.74)		
Angry				
SAD	1.763 (0.343)	78.57 (25.53)	*t* = 2.646	0.009*
CON	1.584 (0.365)	85.27 (14.86)		
Shapes				
SAD	0.773 (0.137)	87.54 (32.46)	*U* = 1507.000	0.184
CON	0.735 (0.166)	93.05 (23.58)		

Note. * = significant after Bonferonni correction; CON = controls; SAD = social anxiety disorder; SD = standard deviation; *t* = independent sample *t*-test; *U* = Mann-Whitney *U* test.

6.5.3 Brain Imaging Findings

6.5.3.1 Task Validation

The expected patterns of activations in prefrontal and limbic regions were observed when examining overall task activation collapsed across emotions and groups (i.e., emotion > shapes contrast for all participants; see Supplementary Figure 6.2).

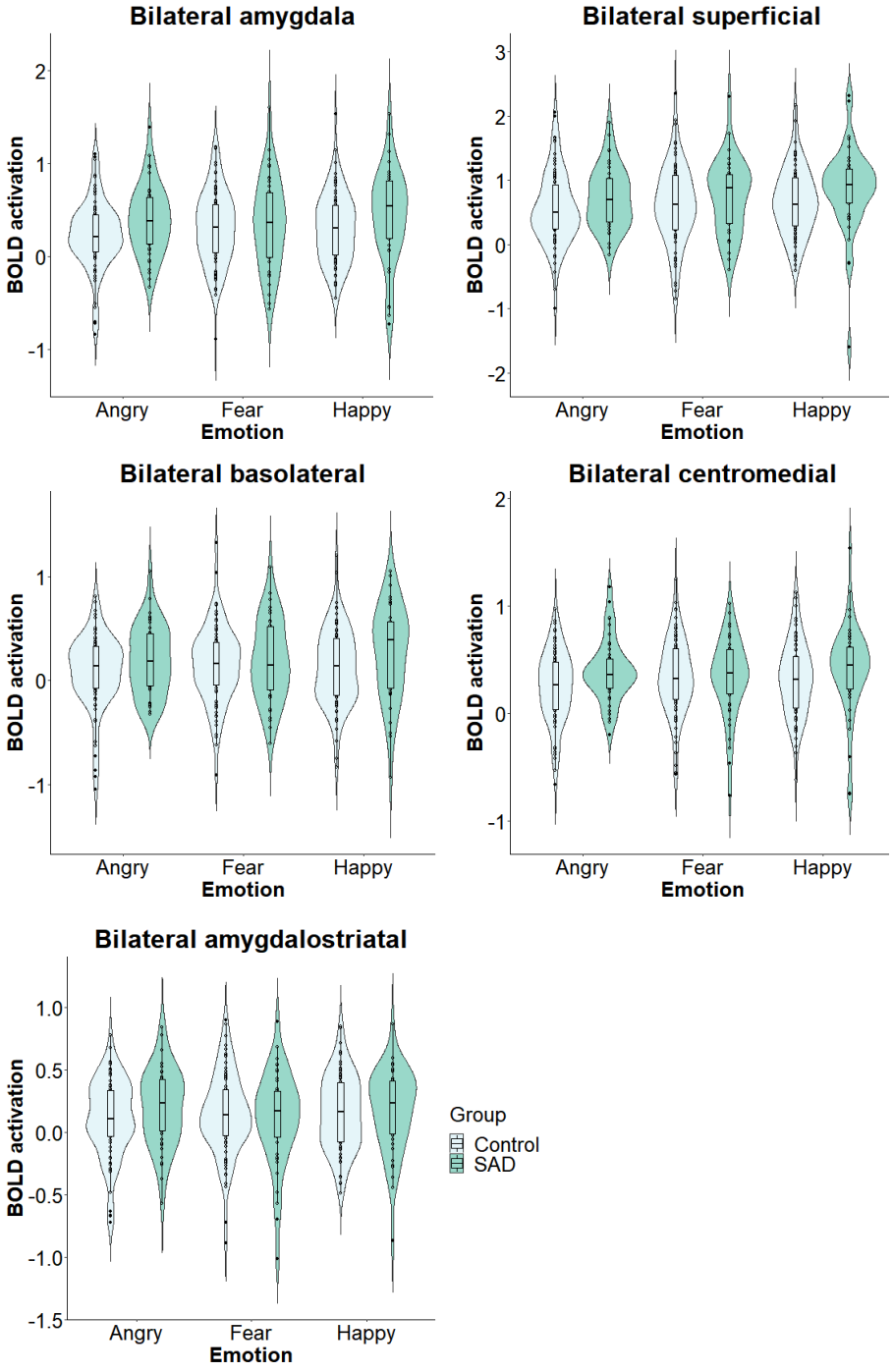
6.5.3.2 Activation Findings

There was a significant main effect of Group observed in the rmANOVAs for the bilateral amygdala ($F(1) = 4.119$, $p = 0.045$, $\eta 2 = 0.032$) and the bilateral superficial amygdala ($F(1) = 4.324$, $p = 0.040$, $\eta 2 = 0.034$); the SAD group had significantly higher activation in these two regions than controls across the three emotions (see Figure 6.2). There was no significant main effect of Group for the bilateral amygdalostriatal and centromedial subregions, and no significant main or interaction effects of Emotion across any of the regions of interest.

Trend-level findings involving the basolateral subregion are reported in the Supplementary Material.

Figure 6.2

Violin Plots Showing Spread of BOLD Signal for the Amygdala and Subregions Across Each Emotion

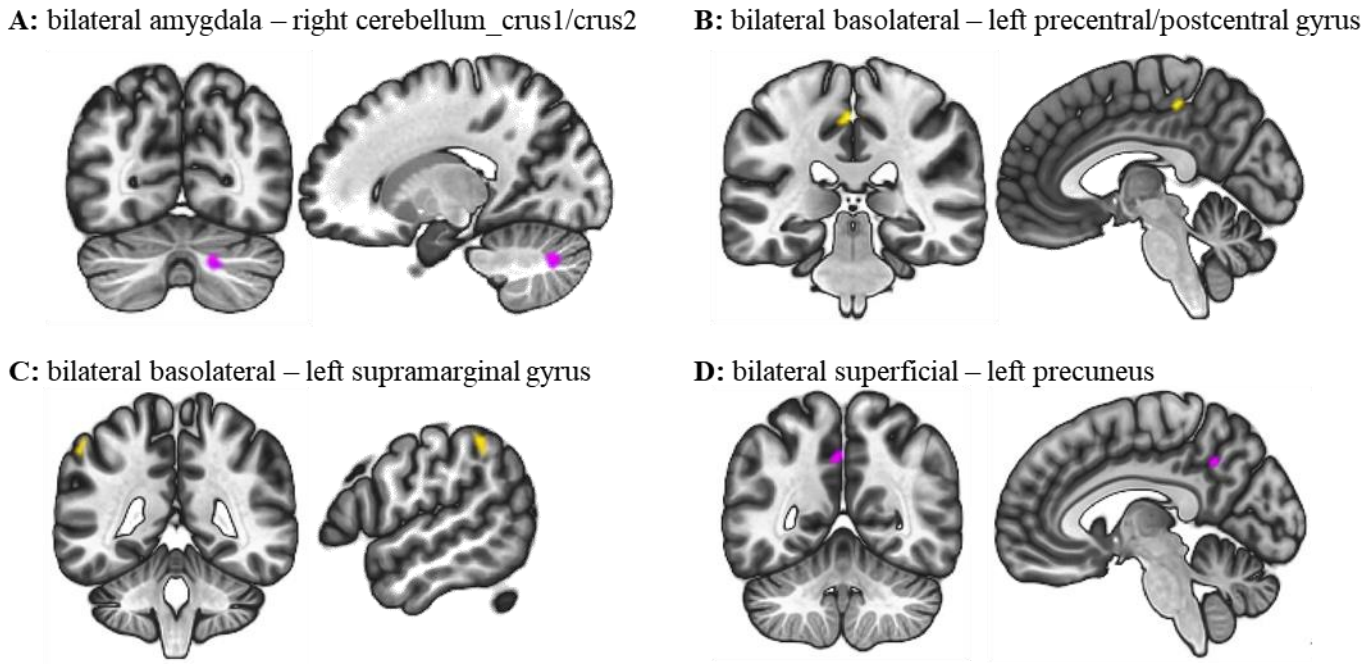


6.5.3.3 Connectivity Findings

For the whole amygdala, those with SAD, compared to controls, had significantly lower connectivity between the bilateral amygdala and right cerebellum (crus1-2) in response to Happy faces (p = 0.016, FDR corrected). There were no significant connectivity findings for the amygdala subregions when using the centromedial and amygdalostriatal subregions as seeds. Compared to controls, those with SAD had significantly increased connectivity between the bilateral basolateral subregion – pre/postcentral gyrus (p = 0.004, FDR corrected) and between the bilateral basolateral subregion – left supramarginal gyrus (p = 0.032, FDR corrected) in response to Fearful faces. Additionally, the SAD group had significantly decreased connectivity between the bilateral superficial subregion – left precuneus in response to Angry faces compared to controls (p = 0.049, FDR corrected; see Figure 6.3).

Figure 6.3

Regions that show Alterations in Connectivity with the Bilateral Seeds



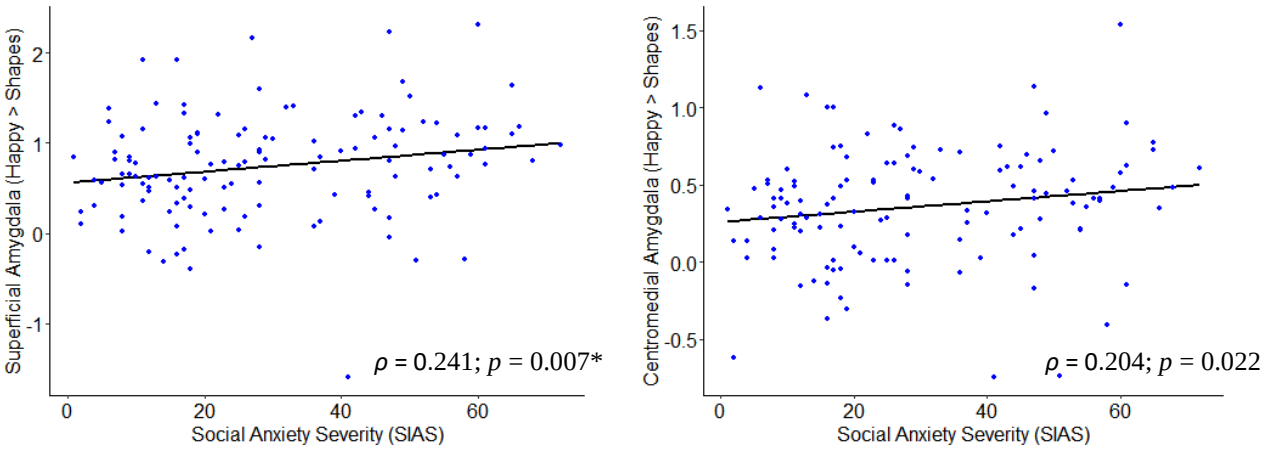
Note. A = MNI peak coordinate: 14 -72 -34; cluster size of 87 voxels; SAD < CON; happy > shapes. B = MNI peak coordinate: -4 -32 50; cluster size of 135 voxels; SAD > CON; fear > shapes. C = MNI peak coordinate: -56 -44 50; cluster size of 79 voxels; SAD > CON; fear > shapes. D = MNI peak coordinate: -6 -54 40; cluster size of 75 voxels; SAD < CON; angry > shapes.

6.5.4 Brain-Behaviour Associations: fMRI Activity and Connectivity Analysis

There were significant positive correlations between social anxiety severity (SIAS) and activation of the bilateral centromedial and superficial amygdalar subregions in response to Happy faces across all participants (see Figure 6.4). This was not evident for basolateral or amygdalostriatal subregions, and no associations were found in response to Anger and Fearful faces. There were no significant associations between gPPI functional connectivity and social anxiety severity across the seed regions that survived cluster correction (*p*-FDR corrected < 0.05). The uncorrected findings ($p < 0.001$ voxel-threshold, $p < 0.05$ cluster-threshold) can be found in Supplementary Table 6.1.

Figure 6.4

Associations between BOLD response and Social Anxiety Severity Across All Participants (n = 126)



Note. * = survived Bonferroni corrected; SIAS = social interaction anxiety scale.

6.6 Discussion

Using multiband fMRI, we found subregion-specific group differences in amygdala activity and connectivity across 126 participants (42 with SAD). The superficial subregion was most commonly implicated across our findings, consistent with evidence demonstrating the superficial amygdala being attuned to social information and being pronounced in the selective processing of social stimuli, more so than the basolateral and centromedial subregions (Bzdok

et al., 2013; Goossens et al., 2009). When examining activation of the amygdala subregions, the superficial complex and the whole amygdala demonstrated hyperactivity in those with SAD compared to controls, irrespective of emotion. No significant group activation differences were observed for the centromedial and amygdalostriatal subregions, and only trend-level significance was observed for the basolateral subregion. When examining functional connectivity, subregion-specific group differences in connectivity patterns were observed for the basolateral and superficial complexes (no group differences for the centromedial and amygdalostriatal subregions). Specifically, those with SAD (vs. controls) had hyperconnectivity between the basolateral amygdala and the pre/postcentral and supramarginal gyrus in response to Fearful faces, and hypoconnectivity between the superficial amygdala and precuneus in response to Angry faces. SAD participants also exhibited hypoconnectivity of the whole amygdala with the cerebellum in response to Happy faces. Regarding brain-symptom associations across all participants, we found positive associations between superficial and centromedial subregion activation and social anxiety severity in response to Happy faces. Collectively, the results provide evidence of subregion-specific amygdala alterations and evidence to support the consideration of social anxiety as a dimensional construct.

6.6.1 Subregion-Specific Amygdala Alterations in SAD

Collectively, the activation and connectivity findings from this study demonstrate three main findings: i) subregion-specific (i.e., superficial) hyperactivity to faces irrespective of emotion in SAD (vs. controls); ii) aberrant subregion- and emotion-specific connectivity patterns involving hypoconnectivity between the superficial amygdala and the precuneus to Angry faces, and hyperconnectivity of the basolateral subregion with the supramarginal gyrus and pre- and post-central gyrus to Fearful faces in those with SAD (vs. controls); and iii) that the specificity of the amygdala's subregional (i.e., basolateral) response to Fear was not uniquely reflected in its *localised* activity, but was evident in the *hyperconnectivity* it had

between the basolateral subregion to broader brain regions (i.e., supramarginal and pre- and post-central gyri).

Our finding of hyperactivation of the whole amygdala and the superficial subregion (with some trend-level evidence also for the basolateral subregion) in those with SAD (vs. controls) was observed in response to all emotional stimuli, with no significant differences between angry, fearful, and happy faces. This is consistent with previous findings demonstrating hyperactivation of the amygdala in response to faces regardless of valence and provides support for the amygdala being involved more broadly in the detection of socially relevant information (rather than only in threat detection and fear processing; Sander et al., 2003; Sergerie et al., 2008). However, the findings from our study suggest that such hyperactivation may be driven by two (superficial and basolateral) of four amygdala subregions studied in the current study. This differential subregional response to emotional face stimuli may explain why there have been mixed findings in the literature to date, with studies reporting increased or no change in amygdala activation in those with SAD compared to controls when completing facial emotion processing tasks (see meta-analyses for review of studies: Gentili et al. (2016) and Yu et al. (2021)). These previous studies examined the amygdala as a homogenous region, thereby discounting the subregion-specific differences in activation patterns demonstrated in this study.

Unlike the activation findings, threat-specific (Angry/Fear) subregion effects were observed in the connectivity findings and were different from the connectivity patterns of the whole amygdala seed that were observed only in response to positive (Happy) emotions. The basolateral subregion appeared to be uniquely implicated in Fear emotion processing, as indicated by the hyperconnectivity observed between the basolateral subregion and supramarginal as well as the pre/post-central gyri to Fearful faces in those with SAD (vs. controls). No other subregions (or the amygdala as a whole) had altered connectivity patterns in response to Fearful faces. The supramarginal gyrus has been implicated in attentional control

to emotionally salient information and emotion regulation (Loeffler et al., 2019; Modi et al., 2015). Therefore, hyperconnectivity between the basolateral subregions and the supramarginal gyrus in those with SAD may be indicative of an increased focus on fearful stimuli in the environment which is implicated in maintenance of the disorder (Wong & Rapee, 2016). Additionally, our finding of hyperconnectivity between the basolateral subregion and the pre- and postcentral gyri in those with SAD may be related to the slower reaction time they had in matching faces with threatening emotional expressions (of Fear and Anger) compared to controls. The basolateral subregion and the pre-and postcentral gyri are functionally and structurally connected, with the latter regions thought to receive socio-emotional inputs specifically from the basolateral subregion of the amygdala (Grèzes et al., 2014; Roy et al., 2009). It is possible that slower reactions in response to Fear faces may reflect greater post-event processing of threat stimuli that is implicated as a maintenance factor of SAD (Wong & Rapee, 2016).

Hypoconnectivity between the superficial amygdala and precuneus to Angry faces was observed in those with SAD (vs. controls). The precuneus is involved in self-focused perception, and voluntary attention shifting and is a hub of the default mode network, which has been more widely implicated in social cognition and self-referential processing (Cavanna & Trimble, 2006; Davey & Harrison, 2018; Raichle, 2015). Increased connectivity between the amygdala and precuneus has been associated with the down-regulation of emotion and directing attention to non-arousing regions of unpleasant images (Ferri et al., 2016). Therefore, the findings of hypoconnectivity between these regions in SAD as reported in this study may be linked to the difficulty that those with SAD experience in being able to decrease and/or minimise the intensity of the fear and anxiety they experience when encountering social stimuli (Jazaieri et al., 2015).

When examining the amygdala region as a whole, our finding of decreased connectivity with the cerebellum in response to only Happy faces is supported in part from results from a

recent meta-analysis concerning emotional face processing in SAD that found lower activation of the cerebellar region in SAD compared to controls in response to emotional faces (vs. neutral faces/stimuli; Yu et al., 2021). The posterior cerebellum (Crus I/II) that was implicated in the current study is thought to be specialized in making social judgments that require 'mentalising' (i.e., being able to infer what is occurring in another person's mind; Van Overwalle et al., 2020). Therefore, decreased connectivity between the amygdala and cerebellum regions may be indicative of the post-event processing that occurs when people with SAD encounter social stimuli (e.g., inferring what someone is thinking based on their facial emotional expression) and the performance deficits (as evident by slower reaction times) they can experience as a result (Wong & Rapee, 2016).

6.6.2 Subregion-Specific Associations with Social Anxiety Severity

We found subregion-specific and emotion-specific associations between activation and symptom severity which were not detected when examining the amygdala as a single homogenous unit. The superficial and centromedial subregions were positively associated with social anxiety severity in response to Happy faces. This was aligned with the recent findings of Crane et al. (2021) who found a positive association between amygdala activation in response to Happy (vs. Angry) faces and social anxiety severity. The centromedial subregion is the primary output centre of the amygdala, and it integrates information from the brain to mediate behavioural responses to stimuli. The superficial amygdala is involved in processing socially relevant information and reward-related processes in the context of social stimuli (approach-avoidance behaviours). As per Crane et al. (2021) interpretation, it could be that greater activation of these subregions may be indicative of the conflict that those with higher levels of social anxiety have in wanting social interaction but also fearing it (i.e., wanting to approach people with friendly/happy faces but having concurrent experiences fear and anxiety). Moreover, it is possible that associations with Happy faces were more salient to all respondents, as was evident by quicker reaction times in the face matching task than Fearful

and Angry faces. Interestingly, these subregion-specific severity associations were found in the absence of any such associations involving the whole amygdala, thereby highlighting the importance of considering amygdala subregions in the neurobiology of SAD.

6.6.3 Limitations and Future Directions

This study is not without limitations. Functional connectivity is a correlative measure and thus cannot be interpreted as causal/directional effects between regions. Through analysis techniques measuring effective connectivity, future studies may provide further insight into the causal nature of the subregion-specific connectivity patterns observed in this study to further understand the underlying neurobiology of those with SAD during emotion processing. Furthermore, our use of seed-to-voxel functional connectivity analyses did not implicate the prefrontal cortex. Given the prominence of findings across the literature of aberrant amygdala – prefrontal connectivity in those with SAD, ROI-to-ROI analyses using the amygdala subregions and a priori defined prefrontal seeds may allow for a better understanding of the relationship between these two neural regions.

Given the subregion-specific effects observed in this study, we believe that future studies investigating the impact of treatment on brain functioning in SAD would benefit from examining activation and connectivity patterns of amygdala subregions to determine which treatment option best attenuates the hyperactivity observed in the basolateral and superficial subregions and normalizes connectivity patterns between subregions and default mode regions. This recommendation is aligned with Klumpp and Fitzgerald (2018) suggestion for future research to investigate the role of amygdala regions as potential treatment targets in those with SAD and may lead to better treatment outcomes for this clinical group.

Additionally, DMN regions such as the precuneus have been implicated in task-based fMRI paradigms involving emotional faces, however not as consistently as the amygdala and frontal areas (see Lucherini Angeletti et al. for review). This may be due to the contrasts used in studies, with many studies examining emotion face processing in SAD using happy or

neutral faces as a baseline control condition as opposed to the differences used in this study of faces > shapes (Binelli et al., 2016; Fonzo et al., 2015; Klumpp et al., 2012; Prater et al., 2013). Studies using happy or neutral faces as a baseline are predicated on the assumption that people with SAD process faces the same way as controls. However, there is evidence that those with SAD evaluate happy and neutral faces as more negative and threatening than controls (Birbaumer et al., 1998; Cooney et al., 2006). Additionally, facial expressions communicate more self-relevant information, recruiting more medial brain regions (i.e., the precuneus and the supramarginal gyrus). Thus, studies that contrast between facial expressions may be missing out on this effect (Loeffler et al., 2019) and as such, it is suggested that other baseline measurements are used instead in addition to and/or as a replacement for happy or neutral faces (Filkowski & Haas, 2017).

6.6.4 Conclusion

This study presents novel findings of amygdalar subregion-specific alterations in neural activation and connectivity during face emotion processing in individuals with SAD. We found hyperactivation of the basolateral and superficial subregions across all emotions. Emotion-specific (threat) effects were observed only when examining the broader connections of the amygdala subregions to broader regions of the brain, and hyperconnectivity of the basolateral subregion was uniquely implicated in fear processing in SAD. Lastly, brain-symptom associations were also only evident when examining activation of the subregions (with no associations observed for the whole amygdala and no connectivity associations). These findings contribute significantly to the understanding of the underlying neurobiology of SAD, highlighting the importance of investigating subregion-specific activation and connectivity patterns, and provide further evidence to support the relationship between neural activity and 'social anxiety' as a dimensional construct. Overall, these findings have significant implications for future research investigating current and novel treatments of SAD in highlighting the basolateral and superficial subregions as potential specific treatment targets.

6.7 Supplementary Information

6.7.1 Methods

6.7.1.1 fMRIprep Pre-Processing

Results included in this manuscript come from preprocessing performed using fMRIPrep 20.2.3 (Esteban et al., 2019; Esteban et al., 2019; RRID:SCR_016216), which is based on Nipype 1.6.1 (Gorgolewski et al., 2011; Gorgolewski et al., 2018; RRID:SCR_002502).

6.7.1.1.1 Anatomical Data Preprocessing. A total of 1 T1-weighted (T1w) images were found within the input BIDS dataset. The T1-weighted (T1w) image was corrected for intensity non-uniformity (INU) with N4BiasFieldCorrection (Tustison et al., 2010), distributed with ANTs 2.3.3 (Avants et al., 2008; RRID:SCR_004757), and used as T1w-reference throughout the workflow. The T1w-reference was then skull-stripped with a Nipype implementation of the antsBrainExtraction.sh workflow (from ANTs), using OASIS30ANTs as a target template. Brain tissue segmentation of cerebrospinal fluid (CSF), white matter (WM), and gray matter (GM) were performed on the brain-extracted T1w using fast (FSL 5.0.9, RRID:SCR_002823, Zhang et al., 2001).

Volume-based spatial normalization to one standard space (MNI152NLin2009cAsym) was performed through nonlinear registration with antsRegistration (ANTs 2.3.3), using brain-extracted versions of both T1w reference and the T1w template. The following template was selected for spatial normalization: ICBM 152 Nonlinear Asymmetrical template version 2009c [(Fonov et al., 2009; RRID:SCR_008796; TemplateFlow ID: MNI152NLin2009cAsym].

6.7.1.1.2 Functional Data Preprocessing. For each of the 2 BOLD runs found per subject (across all tasks and sessions), the following preprocessing was performed. First, a reference volume and its skull-stripped version were generated using a custom methodology of fMRIPrep. A deformation field to correct for susceptibility distortions was estimated based on fMRIPrep's field map-less approach. The deformation field is that resulting from co-

registering the BOLD reference to the same-subject T1w-reference with its intensity inverted (Huntenburg, 2014; Wang et al., 2017).

Registration is performed with antsRegistration (ANTs 2.3.3), and the process is regularized by constraining deformation to be nonzero only along the phase-encoding direction and modulated with an average field map template (Treiber et al., 2016). Based on the estimated susceptibility distortion, a corrected EPI (echo-planar imaging) reference was calculated for a more accurate co-registration with the anatomical reference. The BOLD reference was then co-registered to the T1w reference using flirt (FSL 5.0.9, (Jenkinson et al., 2002) with the boundary-based registration (Greve & Fischl, 2009) cost-function.

Co-registration was configured with nine degrees of freedom to account for distortions remaining in the BOLD reference. Head-motion parameters with respect to the BOLD reference (transformation matrices, and six corresponding rotation and translation parameters) are estimated before any spatiotemporal filtering using mcflirt (FSL 5.0.9, (Jenkinson et al., 2002). BOLD runs were slice-time corrected using 3dTshift from AFNI 20160207 (Cox & Hyde, 1997; RRID:SCR_005927). The BOLD time-series (including slice-timing correction when applied) were resampled onto their original, native space by applying a single, composite transform to correct for head-motion and susceptibility distortions. These resampled BOLD time-series will be referred to as preprocessed BOLD in original space, or just preprocessed BOLD. The BOLD time-series were resampled into standard space, generating a preprocessed BOLD run in MNI152NLin2009cAsym space. First, a reference volume and its skull-stripped version were generated using a custom methodology of fMRIPrep.

Several confounding time-series were calculated based on the preprocessed BOLD: framewise displacement (FD), DVARS, and three region-wise global signals. FD was computed using two formulations following Power (absolute sum of relative motions, (Power et al., 2014)) and Jenkinson (relative root mean square displacement between affines, (Jenkinson et al., 2002)). FD and DVARS are calculated for each functional run, both using

their implementations in Nipype (following the definitions by (Power et al., 2014)). The three global signals are extracted within the CSF, the WM, and the whole-brain masks. Additionally, a set of physiological regressors were extracted to allow for component-based noise correction (CompCor; Behzadi et al., 2007). Principal components are estimated after high-pass filtering the preprocessed BOLD time-series (using a discrete cosine filter with 128s cut-off) for the two CompCor variants: temporal (tCompCor) and anatomical (aCompCor). tCompCor components are then calculated from the top 2% variable voxels within the brain mask. For aCompCor, three probabilistic masks (CSF, WM, and combined CSF+WM) are generated in anatomical space. The implementation differs from that of Behzadi et al. (2007) in that instead of eroding the masks by 2 pixels on BOLD space, the aCompCor masks are subtracted from a mask of pixels that likely contain a volume fraction of GM. This mask is obtained by thresholding the corresponding partial volume map at 0.05, and it ensures components are not extracted from voxels containing a minimal fraction of GM. Finally, these masks are resampled into BOLD space and binarized by thresholding at 0.99 (as in the original implementation). Components are also calculated separately within the WM and CSF masks. For each CompCor decomposition, the k components with the largest singular values are retained, such that the retained components' time series are sufficient to explain 50 percent of variance across the nuisance mask (CSF, WM, combined, or temporal). The remaining components are dropped from consideration.

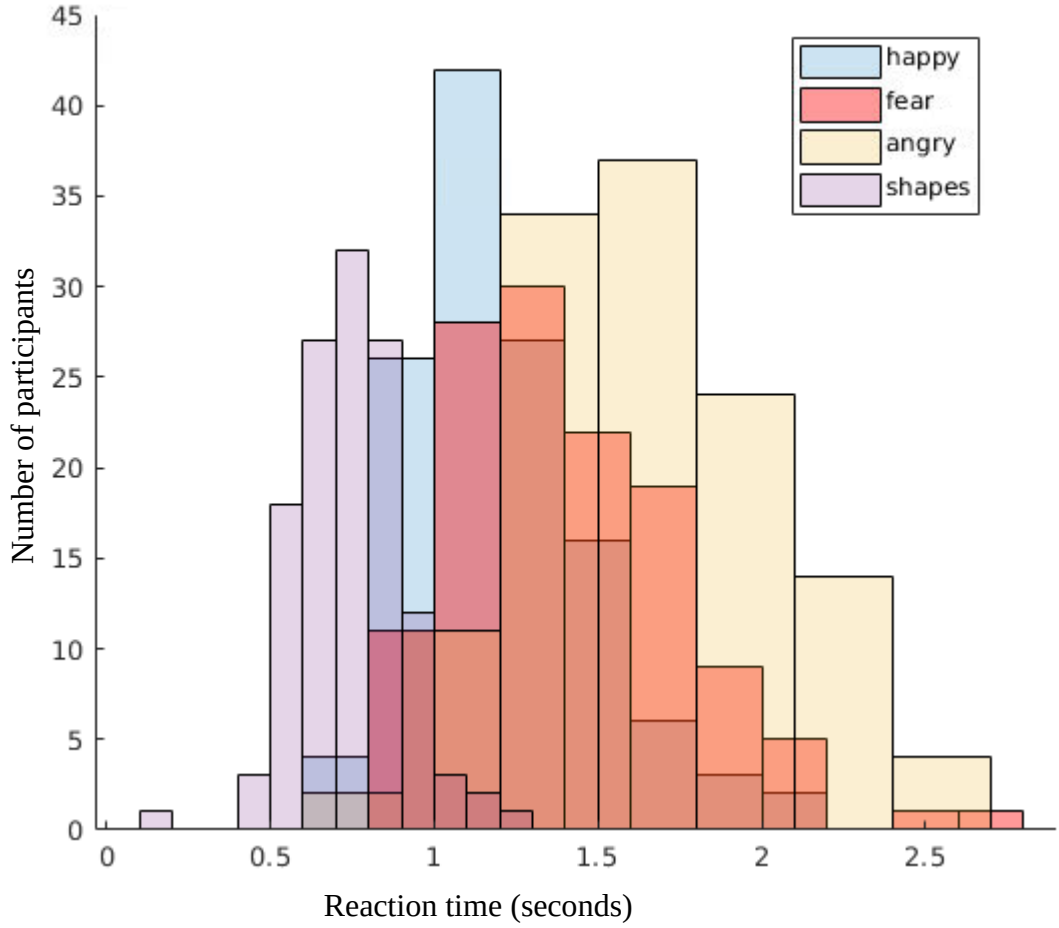
The head-motion estimates calculated in the correction step were also placed within the corresponding confounds file. The confound time series derived from head motion estimates and global signals were expanded with the inclusion of temporal derivatives and quadratic terms for each (Satterthwaite et al., 2013). Frames that exceeded a threshold of 0.5 mm FD or 1.5 standardised DVARS were annotated as motion outliers. All resamplings can be performed with a single interpolation step by composing all the pertinent transformations (i.e. head-motion transform matrices, susceptibility distortion correction when available, and co-

registrations to anatomical and output spaces). Gridded (volumetric) resamplings were performed using antsApplyTransforms (ANTs), configured with Lanczos interpolation to minimize the smoothing effects of other kernels (Lanczos, 1964). Non-gridded (surface) resamplings were performed using mrivol2surf (FreeSurfer).

6.7.2 Results

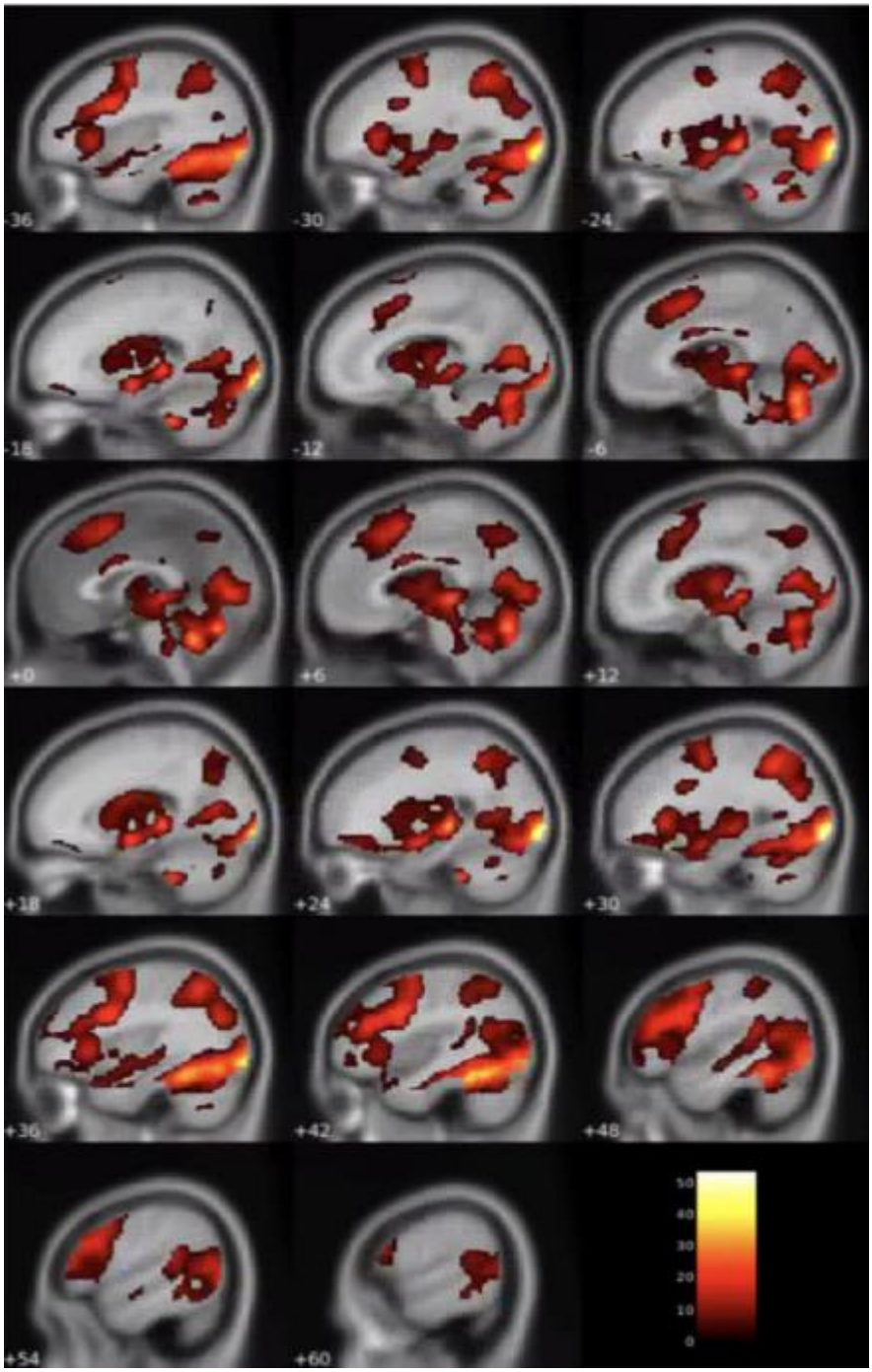
Supplementary Figure 6.1

Distribution of Reaction Times Across Conditions



Supplementary Figure 6.2

Patterns of Activation when Examining Overall Task Activation (Emotion > Shapes) Across All Participants



6.7.2.1 Uncorrected and Exploratory Finding

The SAD group demonstrated greater activation than the control group in the bilateral basolateral subregion seed ($F(1) = 3.794$, $p = 0.054$, $\eta 2 = 0.030$) across the three emotion conditions.

6.7.2.2 Laterality Effects

There was a significant main effect of laterality for the amygdala ($p < 0.001$), amygdalostriatal ($p = 0.038$), centromedial ($p < 0.001$) and superficial subregion seeds ($p = 0.001$), with greater activation in the right hemisphere compared to the left. A significant main effect of group (SAD > CON) was also observed for the amygdala ($p = 0.045$) and the superficial subregion ($p = 0.040$). There were no significant main or interaction effects for the basolateral subregion. No laterality effects in the connectivity data were observed.

6.7.2.3 Brain-Behaviour Associations

Supplementary Table 6.1

Associations between Connectivity and Social Anxiety Severity

Seed	Contrast	Cluster	Coordinate	Cluster Size	Cluster *p* value	T-statistic
Bil. Basolateral	Angry > Shapes	Paracingulate gyrus/ superior frontal gyrus	8 30 40	38	0.012	4.40
		Frontal pole	-10 56 44	32	0.019	-3.78
		Frontal orbital cortex	-44 30 -12	32	0.019	-4.11
Bil. Centromedial	Fear > Shapes	Temporal fusiform cortex/parahippocampal gyrus	-30 -8 -38	48	0.006	4.55
		Central Opercular Cortex	46 -4 16	30	0.026	5.61
Bil. Amygdalostriatal	Fear > Shapes	Temporal fusiform cortex/parahippocampal gyrus	-30 -6 -38	34	0.014	4.47
Bil. Superficial	Fear > Shapes	Cerebellum_Crus1	38 -56 -36	46	0.026	-4.65
		Supramarginal gyrus	-64 -40 32	41	0.026	4.17
		Frontal medial cortex	6 54 -16	31	0.026	-4.10
Bil. Amygdala	Fear > Shapes	Temporal pole/middle temporal gyrus	56 6 -36	30	0.029	-4.80
	Happy > Shapes	Cerebellum_8/Crus2	14 -72 -36	35	0.021	-4.43

Note. Cluster *p* values are uncorrected (did not survive FWE correction); cluster threshold set at 30 voxels; bil = bilateral.

CHAPTER 7: GENERAL DISCUSSION

7.1 Chapter Guide

The overall objective of this thesis was to better understand the neurobiological mechanisms implicated in SAD, with a focus on the amygdala and its subregions, to allow for more targeted treatment approaches and better outcomes for those with SAD. The following section will discuss the key findings from the three separate research investigations, comprising a systematic review on resting-state neuroimaging studies in SAD and two experimental studies that examined the neural underpinnings of the socially anxious brain at rest and in response to emotional faces. Taken together, the findings from all three studies within this thesis make a significant contribution to four significant areas in this field, including i) the current research literature on the neurobiological mechanisms implicated in SAD, ii) neurobiological models of SAD, iii) the classification of SAD, and iv) the potential identification of new treatment targets for those with SAD. This chapter will also identify the general strengths and limitations that arose from this thesis, and directions for future research will be suggested before an overall conclusion is provided.

7.2 Summary of the Main Findings

7.2.1 Study 1: Systematic Review

The systematic review performed in Study 1 (Chapter 3) sought to synthesise the findings of the literature in resting-state neuroimaging in SAD by summarising differences in the activity and connectivity of brain regions in SAD compared to controls and examining associations between brain functioning and social anxiety severity. Of the 35 studies that were included in this review, there were limited studies that investigated differences in localised activity of neural regions between groups (n = 6) thus limiting interpretation and highlighting the paucity of research in this area. The majority of studies (n = 31) use fMRI to investigate brain functioning in SAD. Of these 31 studies, the most common analysis was ROI-to-ROI or

seed-based functional connectivity analysis (n = 18) and findings from these studies were the focus of the review.

The systematic review demonstrated that those with SAD (vs. controls) had aberrant connectivity involving the amygdala to temporal, frontal, and parietal regions in the absence of overt stimuli (i.e., at rest), although with variations in the direction of alterations between groups. Across all studies in the review, frontal regions were most implicated and were most associated with social anxiety severity. The discrepancies in the direction of the connectivity findings were thought to be a result of various limitations in the literature to date (see Discussion of Chapter 3) along with the deficiency of studies investigating the subregions of the amygdala that were addressed by the subsequent studies of this thesis. Of the 35 studies included, only two studies investigated subregions of the amygdala highlighting the lack of research in this area. Results from these studies, however, preliminary implicated the amygdala subregions in SAD reporting increased connectivity between the centromedial, basolateral and superficial subregions and a range of neural regions (including the dorsolateral prefrontal cortex (PFC), anterior cingulate cortex (ACC), supplementary motor area, and cerebellum) and decreased connectivity between the superficial subregion and the dorsomedial thalamus (Anteraper et al., 2014; Yoon et al., 2016).

7.2.2 Study 2: Resting-State fMRI

The primary aims of Study 2 (Chapter 5) were to investigate whether resting-state functional connectivity using fMRI differed in those with SAD compared to controls (N = 135) and whether functional connectivity was associated with social anxiety. The connectivity patterns of the four amygdala subregions (amygdalostriatal, basolateral, centromedial, and superficial) were of focus in this study. No significant group differences were observed in the connectivity involving the amygdala or its four subregions, or any of the other ROIs (i.e., the precuneus, ACC, vmPFC, and the temporoparietal junction). However, there were statistically

significant associations between resting-state functional connectivity and social anxiety severity across all participants. Specifically, the results showed a significant negative association between social anxiety severity and resting-state functional connectivity involving the bilateral precuneus to the right peri-genu ACC. Additionally, significant positive associations were found between social anxiety severity and resting-state functional connectivity of the whole amygdala and superficial subregion with the supramarginal gyrus. Taken together, this study demonstrates clear links between symptomatology and resting-state brain connectivity (in the absence of diagnostic group differences), with evidence of amygdala subregion-specific involvement.

7.2.3 Study 3: Task-Based fMRI

Study 3 (Chapter 6) examined differences in activity and connectivity during emotion face processing using fMRI in those with SAD relative to controls (N = 126), and examined associations between brain functioning and social anxiety severity across all participants. Subregion-specific group differences in activity and connectivity were observed in both the basolateral and superficial subregions of the amygdala. Compared to controls, those with SAD demonstrated hyperactivation of these two subregions, hyperconnectivity of the basolateral amygdala subregion with the pre- and postcentral gyrus and supramarginal gyrus, and hypoconnectivity of the superficial amygdala subregion with the precuneus. Additionally, the amygdala seed was hyperactive in those with SAD compared to controls and demonstrated hypoconnectivity with cerebellar regions. Across all participants, social anxiety severity was positively associated with activation of the superficial and centromedial amygdala subregion however there were no significant associations between connectivity and social anxiety severity. These findings demonstrate amygdala subregion-specific dysfunction in emotion processing in those with SAD (compared to controls) that is also linked to symptomatology.

7.3. Thesis Contributions to the Current Understanding of SAD

7.3.1 Implications for Research

Collectively, the findings from the systematic review (Chapter 3), the resting-state fMRI study (Chapter 5), and the emotional face processing task-based fMRI study (Chapter 6), have made significant and unique contributions to the neurobiological understanding of SAD in several ways. Four main contributions to research will be discussed next.

Firstly, the findings from this thesis contribute to research by demonstrating that high-quality research methodology and analysis approaches in neuroimaging are critical to the outcomes and therefore the understanding of SAD. The systematic review (Study 1) demonstrated the discrepancies within the neuroimaging literature in SAD to date, with limited replication of brain regions being implicated and mixed findings across studies when narrowing in to more commonly implicated regions (i.e., frontal-amygdala functional connectivity was the most reported finding across studies (n = 9) with 5 studies reporting hyperconnectivity and 4 studies reporting hypoconnectivity in those with SAD compared to controls). Studies 2 and 3 addressed many of the limitations identified in the literature to date (see Section 2.5.7 of Chapter 2) which are known to lead to inflated risks of false positives, decreased reproducibility, and poor test-retest reliability (Birn et al., 2013; Blackford, 2017; Eklund et al., 2016; Noble et al., 2017). This was done through the use of a relatively larger sample size of clinical participants (n = 42 compared to the average n = 23 of studies in the systematic review), having a longer scan time for the resting-state study (518 seconds compared to the average of 369 seconds of studies in the systematic review), using multiband EPI during acquisition (rather than single-shot EPI), and stringent thresholds for statistical analyses.

Through addressing these limitations, Studies 2 and 3 provided novel insights into the neurobiology of SAD. Unlike the findings from the systematic review which demonstrated group differences (SAD vs controls) in neural functioning at rest, the resting-state fMRI study (Study 2) demonstrated no statistically significant group differences. This would suggest that

there are no aberrant connectivity patterns in those with SAD compared to controls in the absence of social stimuli (i.e., at rest). However, Study 3 demonstrated group differences in connectivity patterns in the presence of social stimuli (i.e., emotional faces).

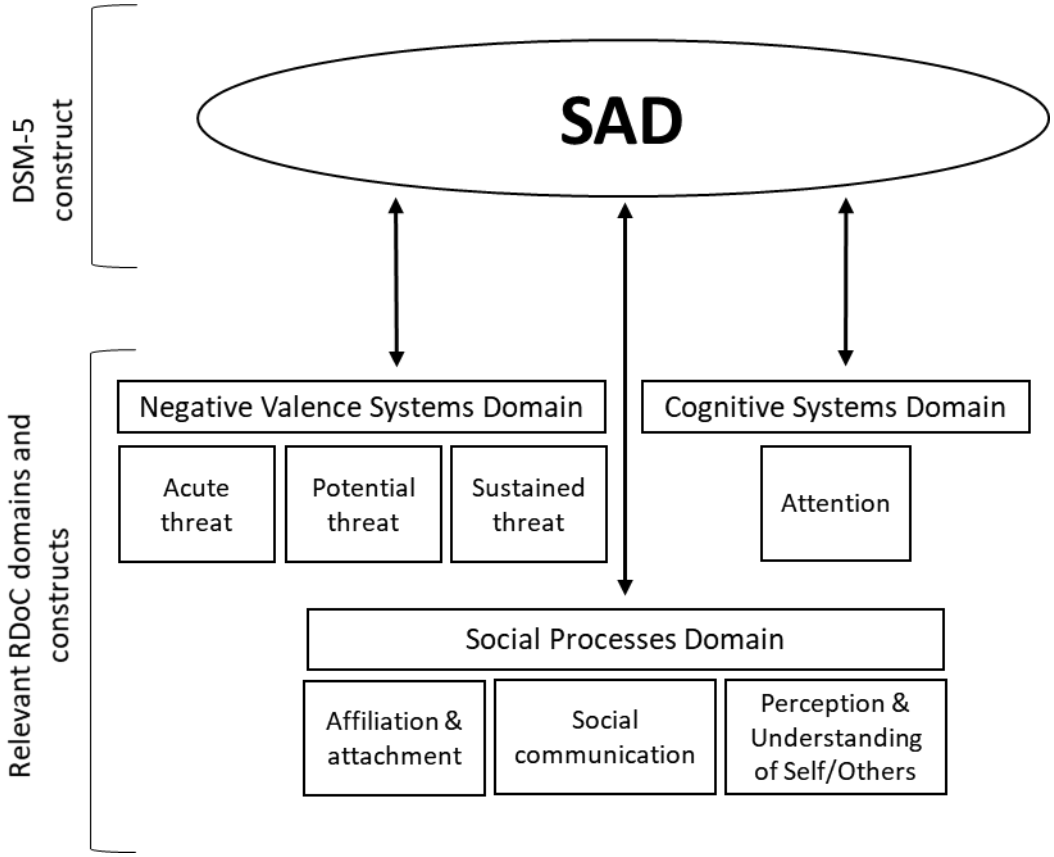
Additionally, brain-behaviour associations across all participants in support of a dimensional conceptualisation of social anxiety were observed in all three studies of this thesis. This was evident in the alterations in functional connectivity that were associated with social anxiety severity at rest (Study 2) and during emotion processing (Study 3). There was limited evidence of this in Study 1. Of the 18 studies included in the systematic review that used ROI-to-ROI or seed-based functional connectivity analysis, only 7 reported associations between connectivity and social anxiety severity. Of these 7 studies, only one investigated this association across all participants involved in the study which reported a positive association between social anxiety severity and connectivity between the amygdala-orbitofrontal gyrus (Geiger et al., 2016). All other studies investigated the association between brain-behaviour within the participants who had a diagnosis of SAD, thus limiting the interpretation of social anxiety being aligned with the RDoC approach of investigating neural mechanisms with symptoms as a dimensional construct that exists across both clinical and non-clinical participants.

Future neuroimaging studies investigating SAD should continue to use methods that improve the reliability of the findings as done in Studies 2 and 3, to allow for better consensus across the literature with regards to the neurobiological underpinnings of this disorder. Additionally, the significant brain-social anxiety severity associations found in this thesis highlight the importance of investigating associations between brain and behaviour dimensionally, consistent with the RDoC framework (Hyett & McEvoy, 2018). This was particularly relevant for Study 2 in which no group differences were observed. While this thesis investigated associations between brain and social anxiety severity, future studies should

examine associations between brain (function and structure) and the various RDoC constructs thought to be related to SAD (see Figure 7.1) to determine whether the purported transdiagnostic nature of such constructs is supported (Insel, 2014). In the context of SAD, constructs under the 'negative valence system' domain and the 'attention' construct are thought to reflect the fear of negative evaluation, attentional biases, and the misinterpretation of ambiguous/neutral social cues that are implicated in the theoretical model of the aetiology and maintenance of this disorder (Wong & Rapee, 2016). Constructs under the 'social processes' domain are thought to be relevant due to the disrupted social relationships that occur in SAD.

Figure 7.1

Overview of the Relationship Between DSM-5 Categorisation of SAD and Related RDoC Constructs

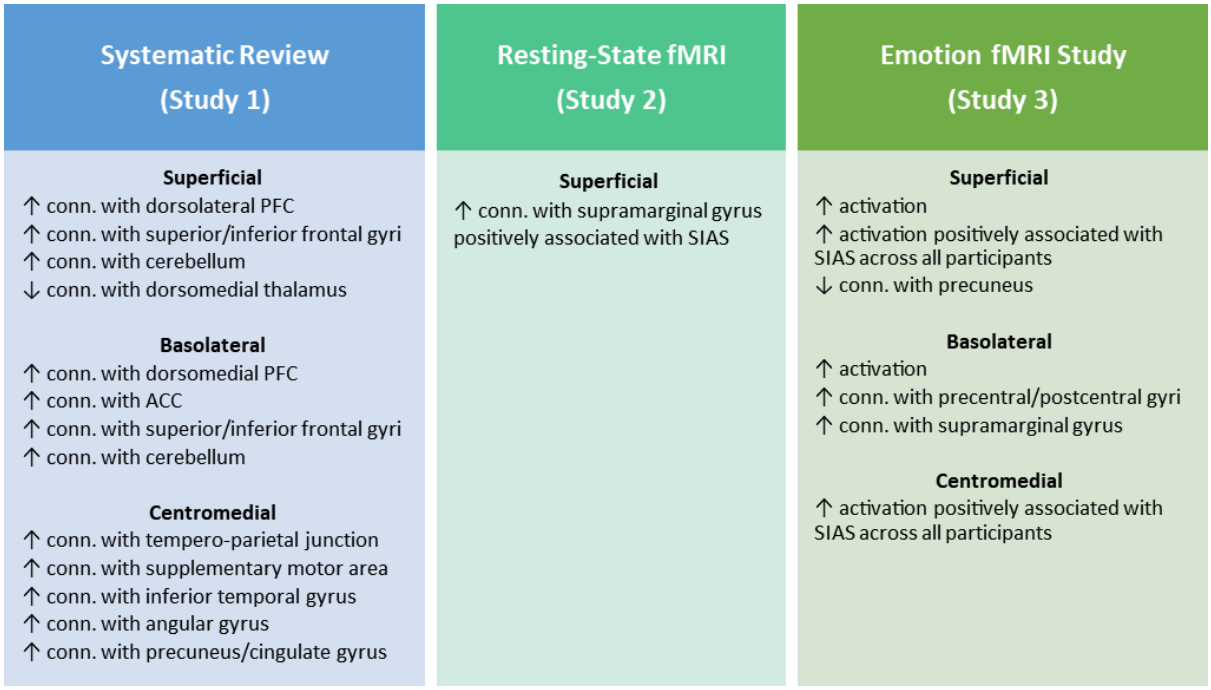


Note. Bi-directional arrows represent the relationship that these two constructs have with one another; SAD = social anxiety disorder.

Secondly, the findings from this thesis contribute significantly to the research by demonstrating that the amygdala subregions are important in elucidating the neurobiology of SAD. Specifically, subregion-specific alterations in connectivity and activity were observed in three of four (basolateral, centromedial, and superficial) subregions across the three studies included in this review, with both hyper- and hypoconnectivity observed in those with SAD compared to controls (see Figure 7.2).

Figure 7.2

Summary of the Amygdala Subregions Implicated in the Three Studies of this Thesis



Note. ↑ = social anxiety disorder > controls; ↓ = social anxiety disorder < controls; ACC = anterior cingulate cortex; conn. = connectivity; PFC = prefrontal cortex; SIAS = social anxiety severity.

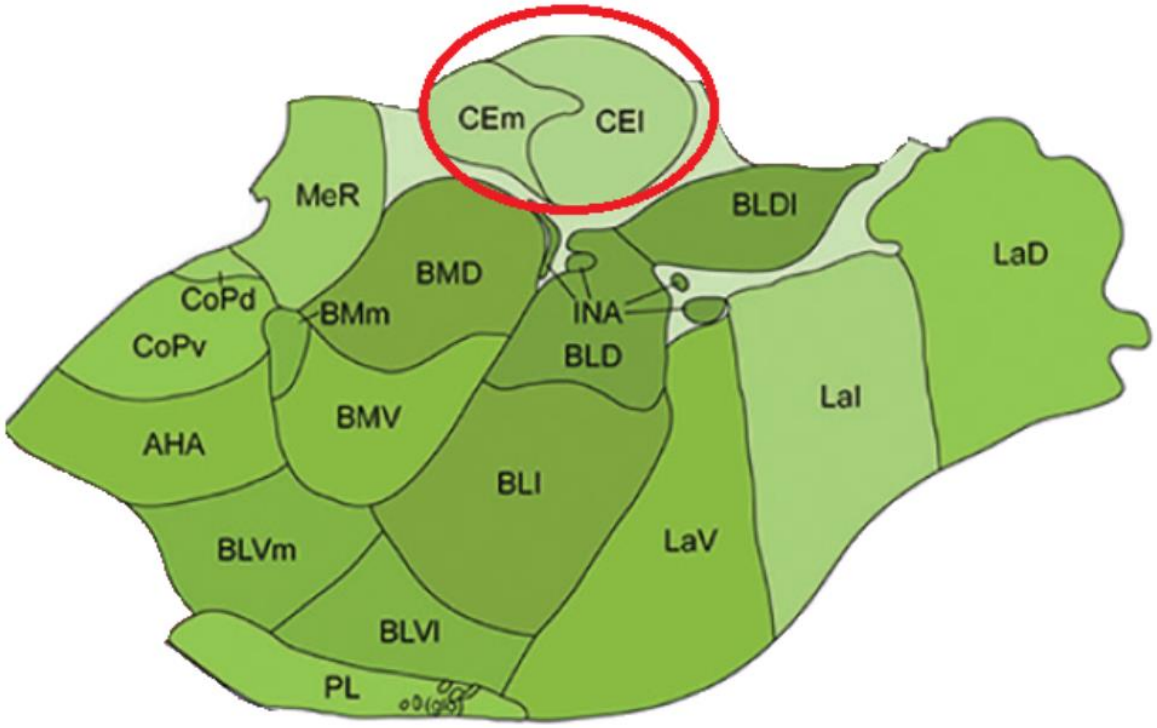
It is likely that previous studies investigating the amygdala as a whole have unknowingly extracted BOLD data from different subregions of the amygdala (depending on where peak voxels/clusters were identified), thus contributing to discrepancies across the literature as was evident in the systematic review (Study 1) and the broader literature (see Chapter 2 for review of neuroimaging literature). Given that the amygdala is commonly

investigated in the SAD neuroimaging literature, future research should continue to examine it as a region made of functionally and structurally distinct subregions rather than as a single homogenous unit. This has broader implications for research in other mental health disorders where the amygdala has been implicated, including other anxiety disorders (e.g., generalised anxiety disorder, panic disorder, and specific phobia; Rauch et al., 2003), mood disorders (e.g., major depressive disorder, bipolar disorder; Anand & Shekhar, 2003; Garrett & Chang, 2008), and psychotic disorders (Makowski et al., 2017; Mukherjee et al., 2016).

Moreover, with advances in neuroimaging, future research should also improve the reliability of extracting information from these small subregions and perhaps even consider additional subregions than covered in this thesis. Findings from animal and human studies suggest that the amygdala consists of approximately 13 subnuclei (Amaral, 1992; Ding et al., 2016; Pitkänen et al., 1997). While the four subregions in this thesis are indicative of major groupings of these subnuclei, each group can be subdivided into smaller areas. For example, the centromedial subregion includes the central amygdala and the medial amygdala (see Figure 7.3; Aerts & Seuntjens, 2021). As evident in this thesis, current neuroimaging techniques have allowed for the investigation of only larger amygdala subregions using fMRI. In structural MRI, current methods have allowed for the investigation of nine amygdala subnuclei including the anterior amygdala area, the cortico-amygdaloid transition area, the lateral nucleus, the basal nucleus, the paralaminar nucleus, the accessory basal, the medial, the central, and the cortical subnuclei (Saygin et al., 2017). However, research should continue to seek to improve the acquisition and analysis of fMRI brain data in order to be able to further elucidate the role of these subnuclei in SAD and other psychiatric disorders.

Figure 7.3

Structural Parcellation of the Amygdalar Complex Based on One Section of the Human Brain



Note. Figure adapted from original, see Ding et al. (2016). Subregions were identified through the use of high-resolution MRI imaging and histological sampling. AHA = amygdalohippocampal area; BLDl = dorsal lateral subdivision of the basolateral nucleus; BLl = intermediate division of the basolateral nucleus; BMD = dorsal (magnocellular) division of the basolateral nucleus; BMm = medial division of the basomedial nucleus; BMV = ventral division of the basomedial nucleus; BLVl = ventral lateral subdivision of the basolateral nucleus; BLVm = ventral medial subdivision of the basolateral nucleus; CEI = lateral subdivision of central nucleus; CEm = medial subdivision of central nucleus; CoPd = dorsal subdivision of the posterior cortical nucleus; CoPv = ventral subdivision of the posterior cortical nucleus; INA = intercalated nucleus of the amygdala; LaD = dorsal division of the lateral nucleus; Lal= intermediate division of the lateral nucleus; LaV = ventral division of the lateral nucleus; MeR = rostral subdivision of the medial nucleus; PL = parilaminar nucleus.

The third contribution of this thesis relates to the precuneus, a brain region that was consistently implicated across the three studies in this thesis. In the review (Study 1), alterations in precuneus connectivity with the amygdala, temporal and frontal regions were observed in those with SAD compared to controls, although with mixed findings (both hyper- and hypoconnectivity). In the empirical studies, both studies implicated decreased precuneus connectivity with social anxiety. In Study 2, decreased connectivity between the precuneus-

peri-genu ACC was associated with increased social anxiety severity at rest, and in Study 3, those with SAD had decreased connectivity between the precuneus-superficial subregion during emotion processing compared to controls.

The precuneus is known to be a hub of the DMN which is implicated in self- and other-referential (social) processing that has been shown to become less activated during performance of demanding emotional/cognitive tasks (Davey & Harrison, 2018; Harrison et al., 2008). It is thought that there is a positive association between deactivation of the DMN and the attention required to perform a task (i.e., more deactivation as a task becomes more difficult; McKiernan et al., 2003). Therefore the findings from this thesis of hypoconnectivity involving the precuneus may be reflective of the greater attentional demands that are required during emotion processing and increased self- and other-referential processing that is ongoing even in the absence of overt stimuli (i.e., at rest). Future studies should continue to investigate the role of precuneus in SAD, with limited research focusing on this brain region thus far. This is evident in Study 1 whereby only two of 18 studies using seed-based or ROI-to-ROI functional connectivity analysis included the precuneus as an ROI (Ergul et al., 2019; C. Yuan et al., 2018). Additionally, a review of the literature on face emotion processing in SAD found no studies that had investigated the precuneus as a seed region (Yu et al., 2021).

Lastly, the fourth contribution of this thesis relates to the cerebellum as it was consistently implicated across the three studies in this thesis. In the review (Study 1), five fMRI studies reported alterations in connectivity involving cerebellar regions (Anteraper et al., 2014; Jung et al., 2018; Yoon et al., 2016; M. Yuan et al., 2018; Yuan et al., 2017). Of note, two of these five studies specifically reported alterations in connectivity between the amygdala and cerebellar regions. Jung et al. (2018) reported hypoconnectivity between the whole amygdala and the cerebellum and Yoon et al. (2016) reported hyperconnectivity between the superficial and basolateral amygdala subregions and the cerebellum in those with SAD compared to

controls. In Study 2, increased connectivity between the amygdala-cerebellum_crus2/7b was associated with increased social anxiety severity at rest, and in Study 3, hypoconnectivity between the amygdala-cerebellum_crus1/crus2 in response to Happy faces was observed in SAD compared to controls.

Of note, the cerebellar subregion, cerebellum_crus2, was consistently implicated across empirical studies in this thesis. The role of the cerebellum_crus2 in responding to Happy faces (Study 3) is further supported by findings of increased connectivity between this region and the amygdala in response to happy (vs. disgust and neutral faces) in a sample of healthy adults with no psychiatric disorder (Schienle & Scharmüller, 2013). The cerebellum_crus2 has been implicated in the attribution of emotions to the self and others (Van Overwalle et al., 2020). Thereby, the hyperconnectivity between amygdala-cerebellum_crus2 observed at rest (Study 2) may be indicative of the tendency for people with SAD to engage in excessive and maladaptive self-focused attention to their own emotions (Clark & Wells, 1995). Furthermore, the hypoconnectivity observed in Study 3 between the amygdalar and cerebellar_crus2 in response to Happy faces may be indicative of the difficulties that those with SAD have in processing emotions of a positive valence in others (Silvia et al., 2006). This is consistent with the literature emphasising fear of positive evaluation that occurs in those with SAD due to Happy faces potentially indicating a social interaction that one must engage with (Weeks et al., 2008). These preliminary findings and interpretations of the cerebellum_crus2 involvement in SAD should further be investigated by ensuring acquisition methods are used that have high spatial resolution and a slice angle to achieve optimal coverage of the whole cerebellum (van der Zwaag et al., 2013).

7.3.2 Implications for Theoretical Models

To date, there have only been a small number of theoretical models proposed to describe the possible underlying neurobiological dysfunctions implicated in SAD (Brühl et al.,

2014; Etkin & Wager, 2007). However, these models had limitations. Both task-based and resting-state studies were combined to form the model proposed by Brühl et al. (2014), and Etkin and Wager (2007) included a small number of task-based (emotion processing) studies on SAD as part of a larger review and meta-analysis to propose a common neurobiological model of emotion processing across SAD, post-traumatic stress disorder and specific phobia (Etkin & Wager, 2007). While some consistencies can be observed in the findings from this thesis and the most recently proposed neurobiological model of SAD model (see Figure 7.4; Brühl et al., 2014), the findings from the systematic review (Study 1) and the differing connectivity patterns at rest (Study 2) and during emotion processing (Study 3) included in this thesis refutes the proposition of one unifying neurobiological model for SAD. Instead, the findings of differing neural processes at rest and during emotion processing provide support for distinct neurobiological models to be developed.

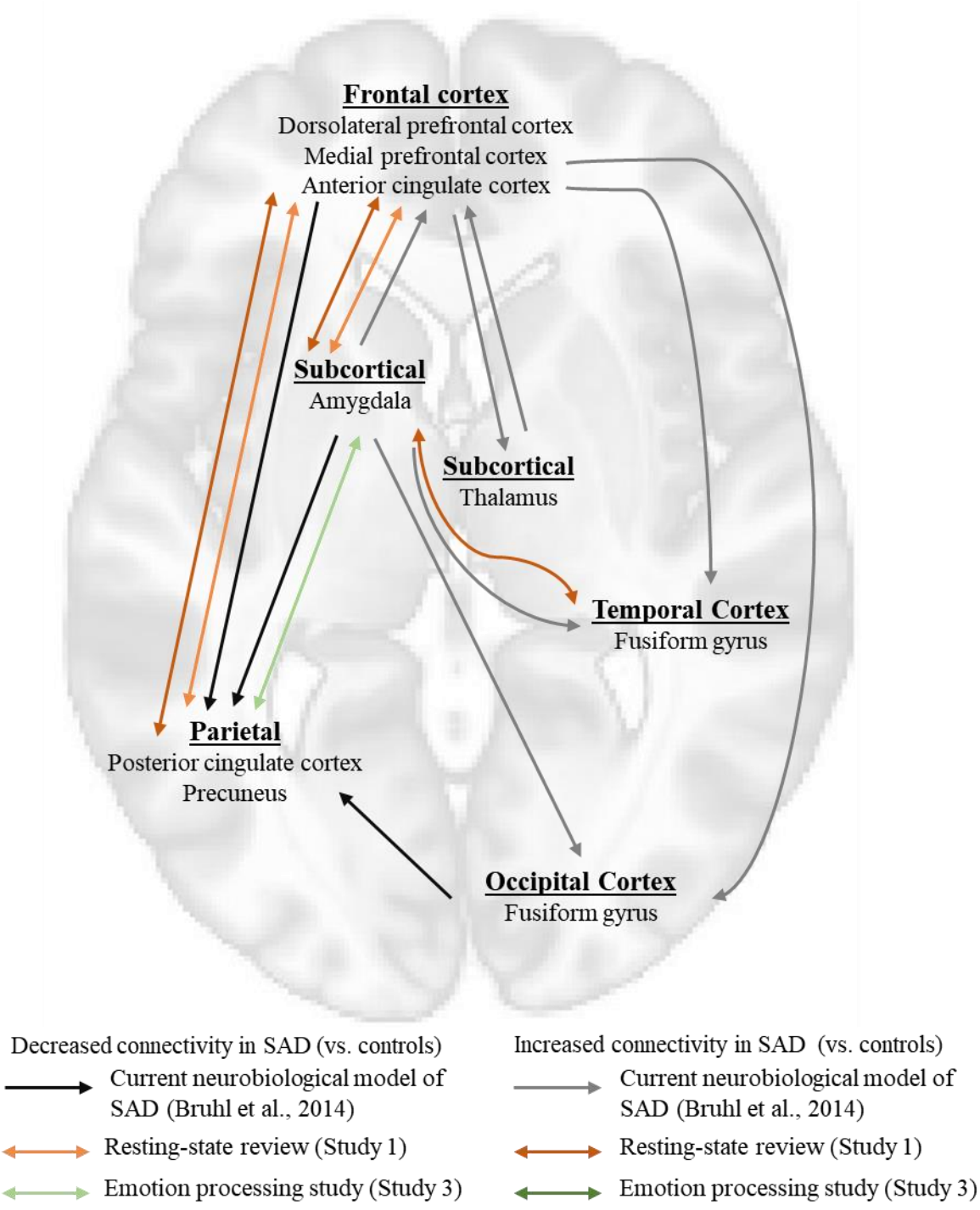
The systematic review from this thesis (Study 1) involved a review of the largest number of resting-state studies in SAD to date. Consistent findings between this review and Brühl et al. (2014) neurobiological model of SAD included hypoconnectivity between frontal-parietal regions and hyperconnectivity between amygdala-frontal regions and amygdala-temporal regions (i.e., fusiform gyrus) in those with SAD compared to controls. However, inconsistent with the model were findings of hyperconnectivity between frontal-parietal regions and hypoconnectivity between amygdala-frontal regions in those with SAD compared to controls. Additionally, the review found both hyper- and hypoconnectivity of regions within the temporal lobe (such as the parahippocampal gyrus, the temporal pole, the inferior, middle, and superior gyrus) in those with SAD compared to controls which were not included in Brühl et al. (2014) model. Additionally, the findings from Study 2 implicated the amygdala, precuneus, and ACC, all of which were consistent with regions included in Brühl et al.'s (2014) neurobiological model of SAD. However, inconsistent with the model was the finding of no

group differences in resting-state functional connectivity. Lastly, the findings from Study 3 were consistent with the model in demonstrating hypoconnectivity between the amygdala-parietal regions in those with SAD compared to controls. However, findings of the supramarginal gyrus and pre- and post-central gyri were novel findings that had not been conceptualised in previous theoretical models.

Collectively, the two empirical studies (Study 2 and 3) along with the systematic review (Study 1) demonstrated some consistencies with Brühl et al. (2014) theoretical model of SAD, but also highlighted novel findings and discrepancies with the existing model. The findings collectively provide evidence to support the need for further development of neurobiological models of SAD. While our findings provide preliminary data that will inform such models, further research using improved methods (as stated in section 7.3.1 of this chapter) is needed to gather more data. Additionally, the differences in findings between Study 2 and Study 3 support the need for models to distinguish between neural processing at rest (in the absence of social stimuli) and in the face of feared social stimuli. Additionally, findings of brain-behaviour associations across all three studies of this thesis provide justification to conceptualise models of neural functioning in relation to social anxiety severity across people who had a diagnosis of SAD and those with no psychiatric disorder.

Figure 7.4

Connectivity Findings from this Thesis in Comparison to the Most Recent Proposed Neurobiological Model of SAD



Note. No findings from the empirical fMRI resting-state study (Study 2) are present due to a lack of any significant group difference. Additionally, no findings of hyperconnectivity in the emotion processing study (Study 3) were consistent with the model. Figure adapted from original, see Brühl et al. (2014).

Moreover, this thesis consistently demonstrated the involvement of selective amygdala subregions in the brain dysfunctions of people with SAD, with these subregions of the amygdala not previously considered in any of the theoretical models of SAD. Of the four subregions investigated in this thesis, the superficial subregion was implicated in SAD across both resting-state and emotion processing paradigms and was also the subregion that was consistently and selectively associated with social anxiety severity. At rest (Study 2), social anxiety severity was found to be positively associated with connectivity between the superficial subregion-supramarginal gyrus. During emotion processing (Study 3), those with SAD (vs. controls) were observed to have hyperactivity of the superficial amygdala and hypoconnectivity between the superficial subregion-precuneus. Additionally, there was a positive association between activation of the superficial subregion and social anxiety severity. While none of the other subregions were implicated at rest, the basolateral and centromedial subregions were uniquely identified to play a role in emotion processing in social anxiety. Compared to controls, those with SAD had hyperconnectivity of the basolateral subregion with the pre- and postcentral gyrus and the supramarginal gyrus. Across all participants, there was a positive association between social anxiety severity and activation of the centromedial amygdala subregion. To date, no theoretical models of the neurobiology of SAD have included amygdala subregions. These findings of selective subregion involvement depending on the context (i.e., in the absence of social stimuli or during emotion processing) are critical in providing preliminary evidence for the need for amygdala subregions to be included in future theoretical models that distinguish between neural functioning of those with SAD in the absence of presence of social stimuli.

7.3.3 Clinical (Diagnostic and Treatment) Implications

7.3.3.1 Towards a Dimensional Approach

The identification of significant associations between functional connectivity and social anxiety severity and the lack of significant group differences reported in the resting-state study (Study 2) demonstrates the importance of integrating dimensional approaches in the assessment of SAD to supplement the categorical classification that is typically used. This would allow for better identification of subthreshold presentations, which is advantageous in providing the opportunity for early intervention. The use of dimensional measures as part of the diagnostic process was recognised by the American Psychiatric Association in the most recently published DSM (American Psychiatric Association, 2013). To supplement the assessment of SAD, the DSM-5 included the Social Anxiety Disorder Dimensional Scale (SAD-D) which is a 10-item dimensional assessment of SAD symptoms corresponding to the DSM-5 criteria (LeBeau et al., 2016). As a measure, it has been shown to have good psychometric properties including good internal consistency, convergent validity with other social anxiety assessment measures, and good test-retest reliability (Lebeau et al., 2012). Other validated measures to assess social anxiety as a dimensional construct include the Social Phobia Inventory or the Liebowitz Social Anxiety Scale (Connor et al., 2000; Liebowitz, 1987).

7.3.3.2 Use of Neuroimaging in Diagnostic Assessments

To date, the assessment of SAD typically involves the completion of a self-report measure (see examples listed above) and a clinical interview by a psychologist or psychiatrist (National Institute for Health and Care Excellence [NICE], 2013). Thus, the nature of assessment involves bias that can occur in the context of self-report measures and engagement in a 'social' encounter with a professional, which can be anxiety-provoking for individuals with SAD. Given this, many people with SAD may never seek assessment or treatment or may only do so when they reach a crisis point or intense severity of symptoms. Evidence from a sample of US adults reported that only 20% of adults with SAD had received treatment for this disorder, with a 12-year delay (on average) between onset and first treatment, and with those

reporting more impairment in daily living being more likely to seek treatment (Grant et al., 2005). For this reason, it would be beneficial to develop further assessment measures that are less confronting than the clinical interview. While assessment questionnaires offer such comfort, the level of subjectivity involved in such measures may be affecting diagnostic accuracy.

Although still too early for the topic of SAD, neuroimaging has the potential to offer a pathway that is less confronting to those with SAD in commencing the assessment process and engaging in services. The use of a biological test as part of the assessment procedure may also lead to increased diagnostic accuracy due to bypassing clinician subjectivity and the current difficulties in defining and diagnosing mental disorders due to the overlapping nature of symptoms and high levels of comorbidities. Using neuroimaging as part of the diagnostic process has been investigated in other disorders, with a machine learning algorithm showing promising results in using fMRI scans to distinguish people with ASD or ADHD from people with no psychiatric disorders (Eslami et al., 2021; Saeed, 2018). Evidence from meta-analyses also suggests that the accuracy of classifications for depression and psychosis using multi-voxel pattern analysis of fMRI data being approximately 70-80% (Arbabshirani et al., 2017; Kambeitz et al., 2017; Kambeitz et al., 2015). Findings from this thesis contribute to evidence of the basolateral, centromedial, and superficial subregions as being potential regions of interest that may be used as potential classifiers for SAD. However further research using machine learning analyses is required to verify this. Additionally, such research must also examine whether findings can distinguish between psychiatric disorders (especially if they have high rates of comorbidities such as SAD and major depressive disorder).

As the literature moves to a more dimensional approach to diagnosis, it may also be that neuroimaging findings may be used to identify individual deficits to allow for targeted treatment based on one’s profile of deficits (Canario et al., 2021). Individual deficits may be

defined within the six major domains of human functioning as proposed by the RDoC framework: negative valence systems, positive valence systems, cognitive systems, systems for social processes, arousal/regulatory systems, and sensorimotor systems (Insel, 2014). This is aligned with the concept of precision medicine which is an individualised approach to understanding psychopathology (Quinlan et al., 2020). There are currently no objective and accurate methods to determine prognoses within the first few years after the onset of a mental illness, and neuroimaging (along with machine learning tools) may be used to enhance prognostic predictions by using a baseline scan to predict illness outcomes (e.g., quality of life, social and occupational functioning). Using machine learning to predict the transition from risk state to more serious illness has been used with accuracies of 70% and above for psychosis (Koutsouleris et al., 2014). Detection and identification of people with a poor prognosis may allow for earlier intervention and the development of treatments that are better suited to the individual's needs.

7.3.3.3 Early Intervention

It is clear from both the resting-state (Study 2) and emotion processing (Study 3) fMRI studies that there are changes in functional connectivity occurring in people with subthreshold SAD who are still experiencing high levels of social anxiety. Findings across both studies predominantly demonstrated a positive association between social anxiety severity and brain functioning. This demonstrates that changes in brain functioning occur even before a person meets criteria for diagnosis of SAD, and highlights the need to consider early intervention for these people who are experiencing moderate-to-severe levels of social anxiety but who may not be experiencing a level of distress and impairment required for diagnosis. The identification of this subthreshold population in the community will allow for early intervention with the aim to improve prognosis and slow any progression in the increasing of social anxiety severity and the subsequent impact that this would have on daily functioning, behaviour, and brain function.

Additionally, early intervention may also help in improving response rates and decreasing dropout rates to treatment as treatment-seekers would be less socially anxious and would have fewer alterations in their brain functioning relative to those with severe social anxiety.

The argument for early intervention is supported by findings from a naturalistic longitudinal study in adolescents and young adults (Beesdo-Baum et al., 2012). They found that people with subthreshold symptomatic SAD (defined as those who met only 4 of 5 diagnostic criteria for SAD as defined by the DSM-IV) led to increased persistence of the disorder in later years and that subthreshold SAD conditions were associated with increased likelihood of SAD diagnosis and/or increased severity of symptoms, thus indicating the likelihood to progress within the spectrum of social anxiety over time (Beesdo-Baum et al., 2012). Furthermore, a systematic review on studies investigating the course of this disorder identified that those with SAD from the general population and primary care settings who have less severe symptoms had a better longitudinal course (77% remission rate after three years) than those with SAD from clinical samples who have more severe symptoms (40% remission rate after five years; Steinert et al., 2013).

7.3.3.4 Current Treatments

Treatment options for SAD have been extensively researched (see Section 2.4 in Chapter 2 for a review). While there is evidence that these treatments can be efficacious, response to treatment remains varied and many do not report clinically meaningful improvement (Klumpp & Fitzgerald, 2018). Findings from meta-analyses have indicated moderate to large effect sizes for symptom remission in those with SAD when undergoing pharmacological treatment compared to pill placebo (Hedges' g = 0.41) and for those who undergo CBT compared to waitlist (Hedges' g = 0.80; Cuijpers et al., 2016; Curtiss et al., 2017). However, there remains significant dropout rates for people undergoing treatment. People with SAD are up to 2.76 times more likely to drop out of pharmacotherapy treatment

compared to pill placebo (Williams et al., 2020), and those with anxiety disorders had a dropout rate of 19.6% when undergoing CBT treatment (Fernandez et al., 2015). Additionally, a significant proportion of individuals do not improve with CBT, with remission rates of 40.4% post-treatment (Springer et al., 2018). This is despite CBT being the first-line treatment for SAD (National Institute for Health and Care Excellence [NICE], 2013).

The use of neuroimaging as an avenue to better understand neural mechanisms of change during treatment and neural predictors of clinical improvement may lead to an optimisation of clinical outcomes and the potential discovery of more efficacious treatments for SAD. Preliminary evidence of this was provided in Whitfield-Gabrieli et al. (2016) study which demonstrated that neuromarkers from resting-state fMRI and diffusion-weighted MRI predicted response to CBT treatment for those with SAD better than a current clinician-administered measure of disease severity (i.e., the LSAS).

Studies have primarily investigated the effects of psychotherapy on alterations in amygdala activity and connectivity in people with SAD, additionally demonstrating how functions of the amygdala can predict treatment response to SAD (Burklund et al., 2017; Whitfield-Gabrieli et al., 2016; Young et al., 2019). However, a review on this topic and a replication study both demonstrated how the amygdala features identified in predicting treatment response continue are varied, with both increased and decreased baseline amygdala activity to emotional faces corresponding to greater to symptom improvement following CBT (Ashar et al., 2021; Klumpp et al., 2014). With regards to connectivity findings, improvement following CBT was predicted by both reduced and increased functional connectivity between the amygdala and the ACC (Klumpp et al., 2014). These findings suggest that amygdala activity and connectivity pathways are affected by CBT and can predict prognosis, but the discrepancies in findings between studies hinder the clinical applicability of such findings.

There is limited research investigating the effects of treatment on amygdala subregion functioning to date, with only one study investigating this in SAD. Faria et al. (2012) demonstrated increased activation in basolateral, basomedial, and ventrolateral amygdala subregions in those with SAD who responded to placebo or SSRI treatment (as determined by the response on the Clinical Global Impression improvement scale) compared to non-responders. The effects of SSRIs on amygdala subregion functioning have also been investigated in major depressive disorder, with a 12-week course of SSRIs demonstrated to 'normalise' the decrease in effective connectivity from the basolateral subregion to the opercular part of the inferior frontal gyrus and from the superficial subregion to the posterior cingulate cortex to a level that was observed at baseline in those with major depressive disorder (vs. controls; Xiao et al., 2021). The effects of oxytocin on amygdalar subregion functioning during resting-state fMRI have also been preliminarily investigated in other populations. In a sample of healthy participants, oxytocin (vs. placebo) was shown to decrease connectivity between the centromedial amygdala and the precuneus, lingual gyrus, fusiform gyrus, and middle temporal gyrus. Increased connectivity was also observed between three amygdala subregions (basolateral, centromedial, and superficial) and regions of the brain including the cerebellum, middle frontal gyrus, inferior parietal lobule, and the medial PFC/superior frontal gyrus (Eckstein et al., 2017). Additionally, oxytocin was found to 'normalise' differential amygdala-subregion connectivity patterns observed in people with post-traumatic stress disorder (vs. trauma-exposed controls) in a placebo condition (Koch et al., 2016). Specifically, the hypoconnectivity observed between the centromedial subregion-ventromedial PFC in males with post-traumatic stress disorder and the hyperconnectivity observed between the basolateral subregion-dorsal ACC in females with post-traumatic stress disorder were 'normalised' after oxytocin administration to a level similar to what was observed in the trauma-exposed controls (Koch et al., 2016).

Given the lack of studies examining the effects of treatment on amygdala subregion functioning, it is important that future studies continue to investigate this. Findings from this project implicate the basolateral, centromedial, and superficial amygdala subregions in SAD and future research investigating the effects of psychotherapy and pharmacotherapy on the brain may be able to reach further consensus by investigating these subregions as different therapies may engage specific subregions of the amygdala (Li et al., 2016).

7.4 Strengths and Limitations of the Research in this Thesis and Future Directions

7.4.1 Comorbidities

Most participants in the clinical group reported the absence of current comorbidities during the screening process as assessed by the MINI (Sheehan et al., 1998). This is advantageous in that it allows for a greater understanding of the neurobiology of SAD in isolation without the potential confounders that other disorders may have on the findings. However, conclusions drawn from this sample with minimal comorbidities may have limited the ecological validity and generalisability of the findings due to not being representative of the high comorbidity rates that exist in the SAD population. Those with SAD are known to have high comorbidity rates with other psychiatric disorders including mood disorders (e.g., major depression, bipolar disorder), other anxiety disorders (e.g., generalised anxiety disorder, specific phobia), obsessive-compulsive and related disorders, and alcohol use disorder (American Psychiatric Association, 2013; Koyuncu et al., 2019). Therefore, research such as this that has a clinical sample with only a small portion having comorbidities limits the translatability and clinical utility of such findings, and it remains unclear whether such findings would still exist in people with SAD with co-existing psychiatric disorders. Future research could make use of sensitivity analyses in clinical samples with comorbidities to determine how unique the findings are to the disorder of interest (Hu & Shi, 2010; Wilke, 2012), thus also increasing the ecological validity and generalisability.

The lack of comorbidities in this sample may be indicative of the lack of sensitivity of the MINI as a screening device to rule out the presence of other psychiatric disorders in the screening process. The gold standard for making diagnoses is the Structured Clinical Interview for DSM disorders (First et al., 2015) and although it is lengthy to administer, it may be important to better characterise the sample used particularly for case-control study designs.

7.4.2 Diagnosis

The sample of people with SAD in this thesis was considered as one homogenous group. However, there is evidence of considerable intra-group variability within people who have a diagnosis of SAD. In a study involving two independent groups of people with SAD and a baseline control group, Talmon et al. (2021) identified variability within SAD participants. They identified a group with positive self-beliefs and a group with negative self-beliefs and noted that the former group remained distinct from the control group. This variability extended to neural findings. Compared to people with SAD with positive self-beliefs, those with negative self-beliefs had greater DMN activation during negative trait judgements, however, there was no difference between those with SAD who had positive self-beliefs and controls. Thus, this indicates that there is heterogeneity in self-referential processing within people with a diagnosis of SAD which impacts neuroimaging findings.

Several subtypes of SAD have been proposed throughout the years with the generalised subtype of SAD being introduced in the DSM-III-R as a description of those who fear most social situations as opposed to the residual non-generalised subtype which describes those who fear only a few social situations. In the current DSM-5, only one specifier, *'performance only'* is available which describes those with SAD whose fears are restricted to speaking or performing in public. There have been numerous investigations into subtyping SAD with a continuing lack of consensus (Binelli et al., 2015; Costache et al., 2020; Hofmann et al., 2004;

Perugi et al., 2001). Further investigation into the subtypes of this disorder is recommended as long as categorical classifications are used in both clinical practice and research.

7.4.3 Power (Sample Size)

The sample size used in this study addresses a limitation in the previous literature, being relatively larger than most sample sizes (approximately 23-24) of the most-cited fMRI literature (Szucs & Ioannidis, 2020). Small sample sizes within the neuroimaging literature have been shown to be related to poor replicability and reproducibility, thus confounding the results, making consensus difficult across studies, and consequentially limiting the derivation of theories from these results (Bossier et al., 2020; Button et al., 2013; Turner et al., 2018).

It is promising to see that neuroimaging is heading in the direction of larger studies, including the growth of multi-site cohort imaging studies which are being used to investigate neuroimaging in psychiatric disorders. Such collaborations include the Human Connectome Project ($N \approx 1200$), the UK Biobank ($N \approx 100{,}000$), the Imagen consortium ($N \approx 2000$), and the ENIGMA consortium ($N \approx 12{,}000$; Schumann et al., 2010; Sudlow et al., 2015; Thompson et al., 2014; Van Essen et al., 2013). Additionally, initiatives such as NeuroVault and Neurosynth allow for data to be pooled into meta- or mega-analyses (Gorgolewski et al., 2015; Yarkoni et al., 2011). Whilst larger samples allay the limitations associated with underpowered studies, some limitations arise that must be considered going forward. Given the geographical diversity of consortium datasets, ethnic differences must be considered as potential confounders in statistical analyses. Additionally, most studies from these datasets are cross-sectional rather than longitudinal which prevents observations of brain changes that are occurring over time to allow better identification of prognosis and onset (Thompson et al., 2014).

7.4.4 Structural Differences in Amygdalar Subregions

Findings from this thesis provide evidence of amygdalar subregional differences in localised activity and functional connectivity in SAD as demonstrated through the use of fMRI. However, due to limitations relating to the scope and timelines of this thesis, differences in the volume and density of these subregions were unable to be explored. In studies that examined structural differences of the amygdala as a whole in SAD, there have been contrasting findings reported. Compared to controls, those with SAD have shown decreased (Irle et al., 2010; Meng et al., 2013; Syal et al., 2012), increased (Machado-de-Sousa et al., 2014), and no differences (Månsson et al., 2016; Brühl et al., 2014; Kawaguchi et al., 2016) in grey matter volume of the bilateral amygdala. Therefore, future studies may be better able to elucidate volumetric changes of the amygdala in SAD by investigating differences at a subregional level. Amygdalar subregion-specific volumetric changes have been implicated across psychiatric disorders. Compared to controls, people with major depressive disorder, schizophrenia and post-traumatic stress disorder have been shown to have decreased volumes in several amygdalar subregions including the lateral nucleus, anterior amygdaloid area, basal nucleus, accessory basal nucleus, central nucleus, medial nucleus, and the paralaminar nucleus (Kim et al., 2021; Zhang et al., 2021; Zheng et al., 2019).

7.4.5 fMRI analysis

The abundance of analysis methods within the fMRI literature has contributed to heterogeneity in findings across studies, with increased calls for streamlined and consistent approaches within the field. A data pre-processing pipeline, *fMRIprep*, was developed to address such concerns and is being increasingly used as a pre-processing tool in the neuroimaging literature (Esteban et al., 2019). *fMRIprep* was used in this project to pre-process all imaging data in alignment with the neuroimaging field. However, there remains great variability and little consensus in methods to deal with motion in the scanner and multiple comparisons that frequently occur in fMRI analyses. Ongoing research suggests new methods

and provides comparisons of different techniques used across the field, however, there is concern that methods are becoming too stringent. As indicated in the resting-state fMRI study in this thesis (Study 2) in which the increasingly popular voxel-wise permutation testing was used to deal with multiple comparisons (Smith & Nichols, 2009), such stringent thresholding may result in the failure to detect true effects (Noble et al., 2020). Future research should continue to investigate methods that are best used to analyse fMRI data with an aim for streamlined approaches to be developed across all steps of the process to reduce heterogeneity in the literature and to allow for further replicability and validation of results.

7.5. Conclusion

In conclusion, this thesis provides significant advances in the understanding of the neurobiology of SAD with a focus on the amygdala and its subregions. Findings from the three studies in this thesis consistently implicated the superficial amygdala in having altered activity and connectivity patterns relating to SAD or social anxiety severity. The basolateral and centromedial subregions were additionally implicated, with hyperconnectivity and hyperactivity of these regions relating to SAD or social anxiety severity. Additionally, findings from this thesis provide support for the existence of classification of this disorder as a categorical entity (i.e., SAD) and for social anxiety as a dimensional construct that exists across the general population. Particularly in the absence of social stimuli, it appears that the neurobiological functionality of the amygdala and its subregions are more related to social anxiety severity as opposed to being implicated in the disorder itself.

Overall, findings from this thesis provide support for future research to continue examining the amygdala subregions as distinct regions in SAD and across the neuroimaging literature in general. The importance of using high-quality research methods and analysis approaches in neuroimaging is also highlighted throughout this thesis, particularly when examining smaller subregions that require higher spatial resolution. Ongoing research should

continue to examine both current and novel pharmaco- and psychotherapeutic treatments that can ‘normalise’ neural connectivity and connectivity patterns of these amygdala subregions to improve the efficacy of treatment options for SAD. Moreover, the differences in findings between the resting-state and emotion processing study highlight the need for future theoretical models of the neurobiology of SAD to distinguish between neural processes in the absence and presence of social stimuli. This is in contrast to the current existing model which combines task-based and resting-state findings. While a new model cannot be proposed at this stage due to the preliminary nature of these findings, this thesis makes important contributions to the current knowledge of the underlying neurobiology of SAD, which will serve to inform future models, research and clinical practices, and treatment targets.

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Appendices

Appendix A – Study Material

Appendix A – 1. Recruitment Flyers

Appendix A – 2. Participant Information Letter

ACU
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PARTICIPANT INFORMATION LETTER

PROJECT TITLE: The Neurodevelopmental Brain Imaging Study
PRINCIPAL INVESTIGATORS: Dr Izelle Labuschagne (ACU), Dr Darren Hocking (La Trobe University), Prof Susan Rossell (Swinburne University of Technology), Prof Peter Rendell (ACU)
RESEARCH ASSISTANTS: Ms Rachel Pelly (ACU)
STUDENT RESEARCHER: Ms Britney Jane Keech (La Trobe University), Ms Caitlin Grace (ACU), Mr Kenneth Kwok Wai Yu (La Trobe University)

Dear Participant,

You are invited to participate in the research project described below.

What is the project about?
The research project investigates the biological processes, including brain and hormonal responses, to emotional and anxiety related information, in young people with varying social emotional profiles. We will recruit people for four different groups: i) Williams syndrome (WS), ii) Autism spectrum disorder (ASD), iii) Social anxiety disorder (SAD), and iv) typically healthy young people. The project aims to examine; a) whether hormones known to be involved in social and emotional behaviours, i.e., *oxytocin* and *vasopressin*, will show different concentration levels in the body for each of the four participant groups, and b) whether there is a relationship between the hormonal concentration levels and how the brain response to social tasks in each group. The research project will be a brain imaging study during which a MRI scan will be completed and we will also collect samples including blood, saliva and some cells from the inside of the cheek from all participants.

Oxytocin and *vasopressin* are two hormones that are closely related and produced in the same area in the brain. Oxytocin is best known for its role in pregnancy, and it is commonly administered to pregnant women to induce labour. Our research is not interested in the pregnancy effects of these hormones, but instead how these hormones play a role in our everyday social and emotional behaviours. Recent evidence has suggested that oxytocin and vasopressin play a key role in our everyday social and emotional behaviours including emotion recognition and anxiety, which are behaviours closely associated with WS, ASD and SAD.

This research is novel and will provide answers to questions such as whether hormonal levels implicated in social and emotional behaviours are different between WS, ASD and SAD compared to the typical group, and whether these hormonal levels relate to variations in brain responses in WS, ASD and SAD compared to the typical group.

Who is undertaking the project?
This project is being conducted by **Dr Izelle Labuschagne, Prof Peter Rendell, Ms Caitlin Grace**, and **Ms Rachel Pelly**, from the Australian Catholic University, **Dr Darren Hocking, Ms Britney Jane Keech**, and **Mr Kenneth Kwok Wai Yu** from La Trobe University, and **Prof Susan Rossell**, from Swinburne University. Dr Labuschagne is a neuroscientist with a strong interest in anxiety and emotion related mental health problems including understanding how these behaviours manifest in the brain. Dr Hocking is a developmental cognitive neuroscientist with a strong background in neurodevelopment disorders, in particular, that of WS and ASD. Prof Rossell is a neuropsychologist and neuroscientist with strong expertise in setting up neuroimaging protocols. Prof Peter Rendell is registered psychologist and director of the Cognition & Emotion Research Centre at ACU.

Prof Rendell will provide invaluable oversight of the project and support of the statistical protocols. Ms Rachel Pelly is an experienced research assistant who has an Honours degree in Psychology. There will be three student researchers on this project. Ms Britney Jane and Mr Kenneth Kwok Wai Yu are both currently completing their Masters in psychology at La Trobe University. Ms Caitlin Grace is currently completing her combined psychology Masters and PhD at ACU.

Are there any risks associated with participating in this project?

The potential risks associated with this project are minimal. However, it is possible that you may experience some discomfort during the following procedures:

Collection of blood, saliva and buccal cells from the mouth: We will be measuring oxytocin and vasopressin through blood, saliva and buccal cell measures. We anticipate that the collection of these samples from you will produce minimal risk. The *blood* will be collected by an experienced nurse and will involve inserting a small needle into your arm and collecting a sample of blood into a tube. The collection of *saliva* will be small and involve drooling into a tube. The collection of *buccal cells* involves using a small cotton swab and rubbing this against the inside of your cheek for about 5-10 s. We are collecting these cells as it provides us with an excellent source to measure DNA that will be used in our genetic analyses. All these procedures are well common to research involving humans and are considered safe and harmless. However, to ensure we are not causing any distress to you or make you feel uncomfortable, we will carefully explain the procedures and will allow you to ask any questions. In the event that you are not comfortable with the procedures, we will not continue with the collection and there will be no consequence to you or your participation in the study.

Brain scan (MRI): The MRI procedure does not involve any exposure to any ionizing radiation, and there is no known health risks associated with the MRI procedure. It is possible, however, that some discomfort will be felt during scanning due to the small cavity of the MRI scanner which could restrict your body movements, and also due to the noise from the MRI scanner. To minimise and manage any risks posed by the study, a number of steps will be put in place. Firstly, you will be thoroughly screened for MRI clearance prior to participating by both the research staff and the MRI radiographer. This will involve questioning you and/or your parents/guardians about metal implants or accidents whereby metal may have become lodged in the body, and about potential pregnancies. Secondly, physical discomfort inside the scanner will be reduced by cushioning and earplugs (standard procedure for MRI facilities). Thirdly, you will be able to communicate with the MRI radiographer and the researcher at all times during scanning; they will also provide you with a 'panic' button which you may press at any stage to stop the scanner if you are not comfortable to continue. Fourthly, you will also be made aware prior to participation of your rights to stop the scanning and/or cease participation at any stage.

What will I be asked to do?

You and/or your parents will be required to complete an initial phone screening session (phase 1) to determine your suitability for the MRI scan session which will follow on a separate day (phase 2).

Phase 1 – Phone screening and demographic session (approx. 15-20 mins): If you are interested in participating in the study, we will conduct a phone screening session with you. This will involve a phone call from us during which you and/or your parents/guardians will need to answer demographic questions, and we will also ask about any history of head injury, the presence of any metal objects in the body, etc., to determine your suitability for the MRI scan. We will also be asking questions about your health which will involve questions relating to medical history, past and current medications, drug and alcohol use, etc. This is to ensure that you are fit and suitable to participate in this research project. Once the screening has been passed, you will then be provided with a Consent form and Explanatory statement. We will also arrange a time for the next session that is most suitable for you to attend the brain scanning session.

Phase 2 – Hormone sample and Brain scan (MRI) assessment (approx. 3.5 hrs): **The hormone samples will be collected at the Royal Children's Hospital (Flemington Road, Parkville). The MRI scan session will be conducted at The Brain and Psychological Sciences Research Centre (Swinburne University of Technology, Burwood Hwy, Hawthorn).**

i) Upon arrival – you will meet the researcher at the Royal Children's Hospital (RCH) in a specified location. Here a nurse of the RCH will take the blood sample. You and the researcher will then go to a private testing room where the researcher will take the buccal cheek cell sample and instruct you on how to provide the saliva sample. We are doing this because we want to measure the concentration levels of key hormones (oxytocin and vasopressin) in your body so that we can see how these hormones relate to your thinking processes and brain activity. You will also complete a MRI screening form. You will then be asked to complete a series of questionnaires and computerised tasks which will assess various aspects relating to behaviour and your thinking processes (i.e., cognition) such as rating pictures of faces expressing different emotions, and answering questions about your behaviour in social and other situations.

ii) Before the MRI – the researcher will accompany you to Swinburne University where the MRI will take place.

iii) For the MRI scan, you will be screened again by a radiographer to ensure you are ready to enter the scanner. You will be in the scanner for approximately 60 mins during which we will talk to you throughout the session via a microphone. During the scan, you will be asked to complete two computerised tasks and you will be given a button box in your hands to make your responses – this will be similar to playing a computer game, only this time you will be doing this inside an MRI scanner. Both tasks involve viewing pictures of human faces with different emotional expressions. You will have a chance to practice these tasks prior to entering the scanner to make sure you are familiar with how it all works. For more information, you can also visit the website for Swinburne's neuroimaging facility: http://www.swinburne.edu.au/lss/bpsyc/facilities.html

How much time will the project take?

This research project will require a phone screening session of approximately 15-20 mins during a convenient time. If you are suitable for the MRI scan, the sample collection at the Royal Children's Hospital and MRI session at The Brain and Psychological Sciences Research Centre will take approximately 4 hours which includes the 60 min MRI scan.

What are the benefits of the research project?

There are no direct benefits to individuals who choose to participate in this research. However, the findings from this study have significant implications for Williams syndrome, autism and social anxiety disorder which may also further implicate other psychiatric disorders characterised by abnormal emotion processing.

Can I withdraw from the study?

Participation in this study is completely voluntary. You are not under any obligation to participate. If you agree to participate, you can withdraw from the study at any time without adverse consequences. If you are currently getting treatment, there will also be no consequences to your treatment in the future if you choose to withdraw.

Will anyone else know the results of the project?

A report of the study may be submitted for publication, but individual participants will not be identifiable in such a report. That is, all the results will be published as group results. The data collected will be stored in accordance with the ACU's regulations, kept on University premises, in a locked filing cabinet, or on a password protected

hard-drive for 5 years. All participants will be assigned a participant ID and only the ID code will be use in all data collection procedures. Therefore, no individual participant results will be identifiable during the reporting of the project results.

Will I be able to find out the results of the project?
We will provide all the participants with a copy of the study report or publication upon request.

Who do I contact if I have questions about the project?
Any questions regarding this project can be directed to the main researchers: Dr Izelle Labuschagne (ph: 03 9953 3816 or email: izelle.labuschagne@acu.edu.au) and Dr Darren Hocking (ph: 03 9479 5462 or email: d.hocking@latrobe.edu.au).

What if I have a complaint or any concerns?
The study has been reviewed by the Human Research Ethics Committee at Australian Catholic University (review number 2015 104H). If you have any complaints or concerns about the conduct of the project, you may write to the Manager of the Human Research Ethics Committee care of the Office of the Deputy Vice Chancellor (Research).

Manager, Ethics
c/o Office of the Deputy Vice Chancellor (Research)
Australian Catholic University
North Sydney Campus
PO Box 968
NORTH SYDNEY, NSW 2059
Ph.: 02 9739 2519
Fax: 02 9739 2870
Email: resethics.manager@acu.edu.au

Any complaint or concern will be treated in confidence and fully investigated. You will be informed of the outcome.

I want to participate! How do I sign up?
If you are interested in the study, you can contact the researchers by phone or email and they will then arrange a time to do the initial phone call. You will be sent a copy of the consent form and information document which you will need to read and sign. There will be two copies of the consent and you will be required to bring the consent form to the MRI session.

Yours sincerely,

[redacted]
Dr Izelle Labuschagne

[redacted]
Dr Darren Hocking

[redacted]
Prof Susan Rossell

[redacted]
Prof Peter Rendell

MAP

The Brain and Psychological Sciences Research Centre
Swinburne University of Technology
Burwood Hwy, Hawthorn

The MRI facility is at the bottom/basement level of the ACT building.

Appendix A – 3. Consent Form

AUSTRALIAN CATHOLIC UNIVERSITY

For those 18 and older:

CONSENT FORM

TITLE OF PROJECT: The Neurodevelopmental Brain Imaging Study

NAME OF PRINCIPAL INVESTIGATORS: Dr Izelle Labuschagne, Dr Darren Hocking, Prof Susan Rossell, Prof Peter Rendell, Ms Hannah Thomson, Ms Britney Jane Keech, Ms Rebecca Bobin

I ..... *(the participant)* have read (*or, where appropriate, have had read to me)* and understood the information provided in the Letter to Participants. Any questions I have asked have been answered to my satisfaction. I agree to participate in this this study, which will involve approximately 2 hours of completing questionnaires and tasks, including a 40-min MRI scan and providing some samples (of blood, hair and some cells from my cheek), realising that I can withdraw my consent at any time without adverse consequences. I agree that research data collected for the study may be published or may be provided to other researchers in a form that does not identify me in any way.

NAME OF PARTICIPANT: .....

SIGNATURE ..... **DATE** .....

SIGNATURE OF PRINCIPAL INVESTIGATOR: .....

DATE:.....

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Appendix A – 4. Pre-Testing Phone Screening and Demographics Questionnaire

Phone Screening and Demographics Questionnaire

Name: _ **Age:** _ _ _ _ _ **D.O.B**. _ _ _ _ _ _ _ _ _ _ _ _

Gender: _ _ _ _ _ _ **Eng. First Language:** Y / N **Cultural Background:** _ _ _ _ _ _ _ _ _ _ _ _ _

Email address: _

Phone: (h)_ _ _ _ _ _ _ _ _ _ _ _ _ (m) _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ **Date:**_ _ _ _ _ _ _ _ _ _ _ _

Instructions: *Now I will need to go through several questions with you. These questions are designed to help us understand any medical problems that you may have and to determine your suitability for the study. All information given will be treated in the strictest confidence.*

1. **Are you a NON-smoker** ☐ Yes ☐ No

2. **Do have any <u>metal objects</u> in your body which can't be removed or not MRI safe?** ☐ Yes ☐ No
   - A surgical clip ☐ Yes ☐ No
   - Have you at any time held a job in a metal-working industry or one in which you may have been exposed to metallic dust or splinters ☐ Yes ☐ No
   - Have you suffered a shrapnel wound ☐ Yes ☐ No

   Make note of objects which will need to be removed: _____

3. **Do you take any hormonal <u>contraceptives</u>?** ☐ Yes ☐ No

4. **Are you RIGHT handed?** ☐ Yes ☐ No

5. **Do you take any <u>medications</u> (prescription or over-the counter)?** ☐ Yes ☐ No

   If yes, please fill in the details in the table below:

Name of medication	Dose	Number of times taken each day	Date of commencement

   - Criteria: Psychotropic medication free for min 2 wks (8wks for fluoxetine, 4 wks for MOIs)

6. **Do you have history of <u>psychiatric illness</u> or current diagnosis ***<u>PERSONALLY</u>***?** ☐ Yes ☐ No
   Anxiety or depression (in last 6 months)? ☐ Yes ☐ No
   Any other psychological problem? ☐ Yes ☐ No

Details:

_____

_____

Do you experience social anxiety: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

7. Do you have history of psychiatric illness or current diagnosis in your *FAMILY*? ☐Yes ☐ No

Bipolar	☐Yes	☐ No
Schizophrenia	☐ Yes	☐ No
Obsessive Compulsive Disorder (OCD)	☐ Yes	☐ No
Post-Traumatic Stress Disorder (PSTD)	☐ Yes	☐ No
Anxiety or depression?	☐ Yes	☐ No
Any other psychological problem?	☐ Yes	☐ No

Details: (type and who)

_____

_____

8. Do you have any of the following medical problems? *If yes, please give details.*

High or low blood pressure?	☐ Yes	☐No
Respiratory problems?	☐ Yes	☐ No
Stomach or intestinal problems?	☐ Yes	☐ No
Liver problems?	☐ Yes	☐ No
Kidney or urinary problems?	☐ Yes	☐ No
Diabetes?	☐ Yes	☐ No
Anaemia or blood disorders?	☐ Yes	☐ No
Eyesight problems or colour blindness?	☐ Yes	☐ No
Cancer?	☐ Yes	☐ No
Skin disorders?	☐ Yes	☐ No

9. Do you currently suffer from any cold or flu symptoms? ☐ Yes ☐ No

If yes, when did it start: _____ and are you taking medications_____

10. Do you or have you used any illegal drugs/substances before? ☐ Yes ☐ No

If yes, what type(s): _____

How often: _____

When was the last time you've used these? _____

Have you used anything in the last 6 months? ☐ Yes ☐ No → **X**

11. Do you drink alcohol? ☐ Yes ☐ No

If yes, # of glass = _____ / day / week. Main type: _____

When was the last time you had a drink: _____ Amount: _____

12. Do you follow any special diet? ☐ Yes ☐ No

If yes, what type? _____

13. When did you last consult a doctor? And for what reason?

Date of last doctor's appointment: _____ Reason: _____

14. Have you ever suffered a head injury and/or loss of consciousness? ☐ Yes ☐ No

If yes,

Did you have to go to hospital? ☐ Yes ☐ No

Were you unconscious for longer than 5 minutes? ☐ Yes ☐ No

15. Other:

Do you drink coffee? ☐ Yes ☐ No → # of cups /day = _____

Do you use glasses? ☐ Yes ☐ No → Contact lenses? ☐ Yes ☐ No

16. Education:

a) At what age did you begin your education (e.g., Primary school)? _____

b) At what age did you finish your education? _____

c) What is the highest education standard you have completed? (e.g. primary, secondary, tertiary course, trade course, apprenticeship)

_____

17. With regards to the hormone collection, are you comfortable with providing a

- **a. Blood sample** ☐ Yes ☐ No
- **b. Saliva sample** ☐ Yes ☐ No
- **c. Buccal cell sample** ☐ Yes ☐ No
  - i. Please note; a buccal cell sample involves having a swab/cotton tip run along the inside of your cheek for 5-10 seconds

18. Are you willing to fast from midnight until after hormone samples are collected? (approximately 10-11am) _ _ _ _ _ _ _ _ _ _ _ _

19. Are you comfortable using public transport and do you have a MYKI? _ _ _ _ _ _

Appendix A – 5. MRI Data Acquisition Parameters

SIEMENS MAGNETOM TrioTim syngo MR B17

\\USER\Specific trials\ACU-development\Social-Anx\localizer

TA: 0:13 PAT: Off Voxel size: 1.1×1.0×7.0 mm Rel. SNR: 1.00 SIEMENS: gre

Properties	
Prio Recon	Off
Before measurement	
After measurement	
Load to viewer	On
Inline movie	Off
Auto store images	On
Load to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	Off
Start measurement without further preparation	Off
Wait for user to start	Off
Start measurements	single

Routine	
Slice group 1	
Slices	1
Dist. factor	20 %
Position	L0.0 A20.0 H0.0
Orientation	Sagittal
Phase enc. dir.	A >> P
Rotation	0.00 deg
Slice group 2	
Slices	1
Dist. factor	20 %
Position	L0.0 A24.1 H0.0
Orientation	Transversal
Phase enc. dir.	A >> P
Rotation	0.00 deg
Slice group 3	
Slices	1
Dist. factor	20 %
Position	L0.0 A24.1 H0.0
Orientation	Coronal
Phase enc. dir.	R >> L
Rotation	0.00 deg
Phase oversampling	0 %
FoV read	250 mm
FoV phase	100.0 %
Slice thickness	7.0 mm
TR	8.6 ms
TE	4.00 ms
Averages	2
Concatenations	3
Filter	Prescan Normalize, Elliptical filter
Coil elements	HEA;HEP

Contrast	
TD	0 ms
MTC	Off
Magn. preparation	None
Flip angle	20 deg
Fat suppr.	None
Water suppr.	None
Averaging mode	Short term
Reconstruction	Magnitude
Measurements	1
Multiple series	Each measurement

Resolution	
Base resolution	256
Phase resolution	90 %
Phase partial Fourier	Off
Interpolation	On
PAT mode	None
Matrix Coil Mode	Auto (CP)
Image Filter	Off
Distortion Corr.	Off
Unfiltered images	Off
Prescan Normalize	On
Normalize	Off
B1 filter	Off
Raw filter	Off
Elliptical filter	On
Mode	Inplane

Geometry	
Multi-slice mode	Sequential
Series	Interleaved
Saturation mode	Standard
Special sat.	None
Tim CT mode	Off

System	
Body	Off
HEP	On
HEA	On
Positioning mode	REF
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Save uncombined	Off
Coil Combine Mode	Adaptive Combine
AutoAlign	---
Auto Coil Select	Default
Shim mode	Tune up
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Silicone	Off
? Ref. amplitude 1H	0.000 V
Adjustment Tolerance	Auto
Adjust volume	
Position	Isocenter
Orientation	Transversal
Rotation	0.00 deg
R >> L	350 mm
A >> P	263 mm
F >> H	350 mm

Physio	
1st Signal/Mode	None
Segments	1
Dark blood	Off
Resp. control	Off

Inline	
Subtract	Off
Liver registration	Off
Std-Dev-Sag	Off
Std-Dev-Cor	Off

1/+

SIEMENS MAGNETOM TrioTim syngo MR B17

Std-Dev-Tra	Off
Std-Dev-Time	Off
MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On
Wash - In	Off
Wash - Out	Off
TTP	Off
PEI	Off
MIP - time	Off

Sequence

Introduction	On
Dimension	2D
Phase stabilisation	Off
Asymmetric echo	Allowed
Contrasts	1
Bandwidth	320 Hz/Px
Flow comp.	No
Allowed delay	0 s
RF pulse type	Normal
Gradient mode	Normal
Excitation	Slice-sel.
RF spoiling	On

SIEMENS MAGNETOM TrioTim syngo MR B17

\\USER\Specific trials\ACU-development\Social-Anx\t1_mpr_ns_sag_p2_1mm_iso

TA: 8:08 PAT: Off Voxel size: 1.0×1.0×1.0 mm Rel. SNR: 1.00 SIEMENS: tfl

Properties

Prio Recon	Off
Before measurement	
After measurement	
Load to viewer	On
Inline movie	Off
Auto store images	On
Load to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	Off
Start measurement without further preparation	On
Wait for user to start	Off
Start measurements	single

Routine

Slab group 1	
Slabs	1
Dist. factor	50 %
Position	L0.0 A30.7 F8.0
Orientation	S > C-1.3
Phase enc. dir.	A >> P
Rotation	0.00 deg
Phase oversampling	0 %
Slice oversampling	27.3 %
Slices per slab	176
FoV read	256 mm
FoV phase	100.0 %
Slice thickness	1.00 mm
TR	1900 ms
TE	2.52 ms
Averages	1
Concatenations	1
Filter	Prescan Normalize
Coil elements	HEA;HEP

Contrast

Magn. preparation	Non-sel. IR
TI	900 ms
Flip angle	9 deg
Fat suppr.	None
Water suppr.	None
Averaging mode	Long term
Reconstruction	Magnitude
Measurements	1
Multiple series	Each measurement

Resolution

Base resolution	256
Phase resolution	100 %
Slice resolution	100 %
Phase partial Fourier	Off
Slice partial Fourier	Off
Interpolation	Off
PAT mode	None
Matrix Coil Mode	Auto (CP)
Image Filter	Off
Distortion Corr.	Off
Unfiltered images	Off
Prescan Normalize	On
Normalize	Off
B1 filter	Off
Raw filter	Off
Elliptical filter	Off

Geometry

Multi-slice mode	Single shot
Series	Ascending

System

Body	Off
HEP	On
HEA	On
SP4	Off
SP2	Off
SP8	Off
SP6	Off
SP3	Off
SP1	Off
SP7	Off
SP5	Off
Positioning mode	REF
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Save uncombined	Off
Coil Combine Mode	Adaptive Combine
AutoAlign	---
Auto Coil Select	Default
Shim mode	Tune up
Adjust with body coil	On
Confirm freq. adjustment	Off
Assume Silicone	Off
? Ref. amplitude 1H	0.000 V
Adjustment Tolerance	Auto
Adjust volume	
Position	Isocenter
Orientation	Transversal
Rotation	0.00 deg
R >> L	350 mm
A >> P	263 mm
F >> H	350 mm

Physio

1st Signal/Mode	None
Dark blood	Off
Resp. control	Off

Inline

Subtract	Off
Std-Dev-Sag	Off
Std-Dev-Cor	Off
Std-Dev-Tra	Off
Std-Dev-Time	Off
MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On

Sequence

Introduction	On
Dimension	3D

3/+

SIEMENS MAGNETOM TrioTim syngo MR B17

Elliptical scanning	Off
Asymmetric echo	Allowed
Bandwidth	170 Hz/Px
Flow comp.	No
Echo spacing	7.5 ms
RF pulse type	Normal
Gradient mode	Normal
Excitation	Non-sel.
RF spoiling	On

SIEMENS MAGNETOM TrioTim syngo MR B17

\\USER\Specific trials\ACU-development\Social-Anx\t2_spc_sag_p2_iso_1.0

TA: 4:43 PAT: 2 Voxel size: 1.0×1.0×1.0 mm Rel. SNR: 1.00 SIEMENS: tse_vfl

Properties

Prio Recon	Off
Before measurement	
After measurement	
Load to viewer	On
Inline movie	Off
Auto store images	On
Load to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	Off
Start measurement without further preparation	On
Wait for user to start	Off
Start measurements	single

Routine

Slab group 1	
Slabs	1
Position	Isocenter
Orientation	Sagittal
Phase enc. dir.	A >> P
Rotation	0.00 deg
Phase oversampling	0 %
Slice oversampling	0.0 %
Slices per slab	176
FoV read	256 mm
FoV phase	100.0 %
Slice thickness	1.00 mm
TR	3200 ms
TE	402 ms
Averages	1.0
Concatenations	1
Filter	Raw filter, Prescan Normalize
Coil elements	HEA;HEP

Contrast

MTC	Off
Magn. preparation	None
Fat suppr.	None
Water suppr.	None
Restore magn.	Off
Reconstruction	Magnitude
Measurements	1
Multiple series	Each measurement

Resolution

Base resolution	256
Phase resolution	100 %
Slice resolution	100 %
Phase partial Fourier	Allowed
Slice partial Fourier	Off
Interpolation	Off
PAT mode	GRAPPA
Accel. factor PE	2
Ref. lines PE	24
Accel. factor 3D	1
Matrix Coil Mode	Auto (Triple)
Reference scan mode	Integrated
Image Filter	Off
Distortion Corr.	Off
Unfiltered images	Off
Prescan Normalize	On
Normalize	Off
B1 filter	Off
Raw filter	On
Intensity	Weak
Slope	25
Elliptical filter	Off

Geometry

Special sat.	None

System

Body	Off
HEP	On
HEA	On
Positioning mode	FIX
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Save uncombined	Off
Coil Combine Mode	Adaptive Combine
AutoAlign	---
Auto Coil Select	Default
Shim mode	Tune up
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Silicone	Off
? Ref. amplitude 1H	0.000 V
Adjustment Tolerance	Auto
Adjust volume	
Position	Isocenter
Orientation	Transversal
Rotation	0.00 deg
R >> L	350 mm
A >> P	263 mm
F >> H	350 mm

Physio

1st Signal/Mode	None
Dark blood	Off
Resp. control	Off

Inline

Subtract	Off
Std-Dev-Sag	Off
Std-Dev-Cor	Off
Std-Dev-Tra	Off
Std-Dev-Time	Off
MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On

Sequence

Introduction	On
Dimension	3D
Bandwidth	751 Hz/Px
Flow comp.	No
Allowed delay	30 s
Echo spacing	3.36 ms

5/+

SIEMENS MAGNETOM TrioTim syngo MR B17

Adiabatic-mode	Off
Define	Echo trains
Turbo factor	141
Slice turbo factor	2
Echo trains per slice	1
Echo train duration	870
RF pulse type	Normal
Gradient mode	Fast
Excitation	Non-sel.
Flip angle mode	T2 var

SIEMENS MAGNETOM TrioTim syngo MR B17

\\USER\Specific trials\ACU-development\Social-Anx\MB_FC-iso_fixation

TA: 8:38 PAT: Off Voxel size: 2.0×2.0×2.0 mm Rel. SNR: 1.00 USER: cmrr_mbep2d_bold

Properties

Prio Recon	Off
Before measurement	
After measurement	
Load to viewer	On
Inline movie	Off
Auto store images	On
Load to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	On
Start measurement without further preparation	On
Wait for user to start	On
Start measurements	single

Routine

Slice group 1	
Slices	65
Dist. factor	0 %
Position	R1.2 A9.7 H1.2
Orientation	T > C-30.8
Phase enc. dir.	A >> P
Rotation	0.00 deg
Phase oversampling	0 %
FoV read	192 mm
FoV phase	100.0 %
Slice thickness	2.00 mm
TR	1020 ms
TE	30.0 ms
Multi-band accel. factor	5
Filter	Prescan Normalize
Coil elements	HEA;HEP

Contrast

MTC	Off
Magn. preparation	None
Flip angle	65 deg
Fat suppr.	Fat sat.
Averaging mode	Long term
Reconstruction	Magnitude
Measurements	500
Delay in TR	0 ms
Multiple series	Off

Resolution

Base resolution	96
Phase resolution	100 %
Phase partial Fourier	6/8
Interpolation	Off
PAT mode	None
Matrix Coil Mode	Auto (CP)
Distortion Corr.	Off
Unfiltered images	Off
Prescan Normalize	On
Raw filter	On
Elliptical filter	Off
Hamming	Off

Geometry

Multi-slice mode	Interleaved
Series	Interleaved
Special sat.	None

System

Body	Off
HEP	On
HEA	On
SP4	Off
SP2	Off
SP8	Off
SP6	Off
SP3	Off
SP1	Off
SP7	Off
SP5	Off
Positioning mode	FIX
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Coil Combine Mode	Sum of Squares
AutoAlign	---
Auto Coil Select	Default
Shim mode	Advanced
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Silicone	Off
? Ref. amplitude 1H	0.000 V
Adjustment Tolerance	Auto
Adjust volume	
Position	R1.2 A9.7 H1.2
Orientation	T > C-30.8
Rotation	0.00 deg
R >> L	192 mm
A >> P	192 mm
F >> H	130 mm

Physio

1st Signal/Mode	None

BOLD

GLM Statistics	Off
Dynamic t-maps	Off
Starting ignore meas	0
Ignore after transition	0
Model transition states	On
Temp. highpass filter	On
Threshold	4.00
Paradigm size	20
Meas[1]	Baseline
Meas[2]	Baseline
Meas[3]	Baseline
Meas[4]	Baseline
Meas[5]	Baseline
Meas[6]	Baseline
Meas[7]	Baseline
Meas[8]	Baseline
Meas[9]	Baseline
Meas[10]	Baseline
Meas[11]	Active
Meas[12]	Active
Meas[13]	Active
Meas[14]	Active
Meas[15]	Active

7/+

SIEMENS MAGNETOM TrioTim syngo MR B17

Meas[16]	Active
Meas[17]	Active
Meas[18]	Active
Meas[19]	Active
Meas[20]	Active
Motion correction	Off
Spatial filter	Off

Sequence

Introduction	Off
Contrasts	1
Bandwidth	1860 Hz/Px
Flow comp.	No
Free echo spacing	Off
Echo spacing	0.69 ms
EPI factor	96
Gradient mode	Fast
RF spoiling	Off
Excite pulse duration	4600 us
Single-band images	Off
MB LeakBlock kernel	Off
MB dual kernel	Off
MB RF phase scramble	Off
SENSE1 coil combine	Off
Invert RO/PE polarity	Off
PF omits higher k-space	Off
Online multi-band recon.	Online
FFT scale factor	0.90
Physio recording	Off
Triggering scheme	Standard

SIEMENS MAGNETOM TrioTim syngo MR B17

\\USER\Specific trials\ACU-development\Social-Anx\MB-SE-fc-AP

TA: 0:22 PAT: Off Voxel size: 2.0×2.0×2.0 mm Rel. SNR: 1.00 USER: cmrr_mbep2d_se

Properties	
Prio Recon	Off
Before measurement	
After measurement	
Load to viewer	On
Inline movie	Off
Auto store images	On
Load to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	Off
Start measurement without further preparation	On
Wait for user to start	Off
Start measurements	single

Routine	
Slice group 1	
Slices	65
Dist. factor	0 %
Position	R1.2 A9.7 H1.2
Orientation	T > C-30.8
Phase enc. dir.	A >> P
Rotation	0.00 deg
Phase oversampling	0 %
FoV read	192 mm
FoV phase	100.0 %
Slice thickness	2.00 mm
TR	1220 ms
TE	42.8 ms
Multi-band accel. factor	5
Filter	Prescan Normalize
Coil elements	HEA;HEP

Contrast	
MTC	Off
Magn. preparation	None
Flip angle	65 deg
Refocus flip angle	180 deg
Fat suppr.	Fat sat.
Grad. rev. fat suppr.	Disabled
Averaging mode	Long term
Reconstruction	Magnitude
Measurements	10
Delay in TR	0 ms
Multiple series	Off

Resolution	
Base resolution	96
Phase resolution	100 %
Phase partial Fourier	6/8
Interpolation	Off
PAT mode	None
Matrix Coil Mode	Auto (CP)
Distortion Corr.	Off
Unfiltered images	Off
Prescan Normalize	On
Raw filter	On
Elliptical filter	Off
Hamming	Off

Geometry	
Multi-slice mode	Interleaved
Series	Interleaved
Special sat.	None

System	
Body	Off
HEP	On
HEA	On
SP4	Off
SP2	Off
SP8	Off
SP6	Off
SP3	Off
SP1	Off
SP7	Off
SP5	Off
Positioning mode	FIX
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Coil Combine Mode	Sum of Squares
AutoAlign	---
Auto Coil Select	Default
Shim mode	Advanced
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Silicone	Off
? Ref. amplitude 1H	0.000 V
Adjustment Tolerance	Auto
Adjust volume	
Position	R1.2 A9.7 H1.2
Orientation	T > C-30.8
Rotation	0.00 deg
R >> L	192 mm
A >> P	192 mm
F >> H	130 mm

Physio	
1st Signal/Mode	None

BOLD	
GLM Statistics	Off
Dynamic t-maps	Off
Starting ignore meas	0
Ignore after transition	0
Model transition states	On
Temp. highpass filter	On
Threshold	4.00
Paradigm size	15
Meas[1]	Baseline
Meas[2]	Baseline
Meas[3]	Baseline
Meas[4]	Baseline
Meas[5]	Baseline
Meas[6]	Baseline
Meas[7]	Baseline
Meas[8]	Baseline
Meas[9]	Baseline
Meas[10]	Baseline
Meas[11]	Active
Meas[12]	Active
Meas[13]	Active
Meas[14]	Active

9/+

SIEMENS MAGNETOM TrioTim syngo MR B17

Meas[15]	Active
Motion correction	Off
Spatial filter	Off

Sequence

Introduction	Off
Contrasts	1
Bandwidth	1736 Hz/Px
Free echo spacing	Off
Echo spacing	0.72 ms
EPI factor	96
Gradient mode	Fast
Excite pulse duration	5760 us
Refocus pulse duration	6400 us
Single-band images	Off
MB LeakBlock kernel	Off
MB dual kernel	Off
MB RF phase scramble	Off
Time-shifted MB RF	Off
SENSE1 coil combine	Off
Invert RO/PE polarity	Off
Online multi-band recon.	Online
FFT scale factor	1.00
Physio recording	Off
Triggering scheme	Standard

SIEMENS MAGNETOM TrioTim syngo MR B17

\\USER\Specific trials\ACU-development\Social-Anx\MB-SE-fc-PA

TA: 0:22 PAT: Off Voxel size: 2.0×2.0×2.0 mm Rel. SNR: 1.00 USER: cmrr_mbep2d_se

Properties	
Prio Recon	Off
Before measurement	
After measurement	
Load to viewer	On
Inline movie	Off
Auto store images	On
Load to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	Off
Start measurement without further preparation	On
Wait for user to start	Off
Start measurements	single

Routine	
Slice group 1	
Slices	65
Dist. factor	0 %
Position	R1.2 A9.7 H1.2
Orientation	T > C-30.8
Phase enc. dir.	A >> P
Rotation	0.00 deg
Phase oversampling	0 %
FoV read	192 mm
FoV phase	100.0 %
Slice thickness	2.00 mm
TR	1200 ms
TE	41.6 ms
Multi-band accel. factor	5
Filter	Prescan Normalize
Coil elements	HEA;HEP

Contrast	
MTC	Off
Magn. preparation	None
Flip angle	65 deg
Refocus flip angle	180 deg
Fat suppr.	Fat sat.
Grad. rev. fat suppr.	Disabled
Averaging mode	Long term
Reconstruction	Magnitude
Measurements	10
Delay in TR	0 ms
Multiple series	Off

Resolution	
Base resolution	96
Phase resolution	100 %
Phase partial Fourier	6/8
Interpolation	Off
PAT mode	None
Matrix Coil Mode	Auto (CP)
Distortion Corr.	Off
Unfiltered images	Off
Prescan Normalize	On
Raw filter	On
Elliptical filter	Off
Hamming	Off

Geometry	
Multi-slice mode	Interleaved
Series	Interleaved
Special sat.	None

System	
Body	Off
HEP	On
HEA	On
SP4	Off
SP2	Off
SP8	Off
SP6	Off
SP3	Off
SP1	Off
SP7	Off
SP5	Off
Positioning mode	FIX
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Coil Combine Mode	Sum of Squares
AutoAlign	---
Auto Coil Select	Default
Shim mode	Advanced
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Silicone	Off
? Ref. amplitude 1H	0.000 V
Adjustment Tolerance	Auto
Adjust volume	
Position	R1.2 A9.7 H1.2
Orientation	T > C-30.8
Rotation	0.00 deg
R >> L	192 mm
A >> P	192 mm
F >> H	130 mm

Physio	
1st Signal/Mode	None

BOLD	
GLM Statistics	Off
Dynamic t-maps	Off
Starting ignore meas	0
Ignore after transition	0
Model transition states	On
Temp. highpass filter	On
Threshold	4.00
Paradigm size	15
Meas[1]	Baseline
Meas[2]	Baseline
Meas[3]	Baseline
Meas[4]	Baseline
Meas[5]	Baseline
Meas[6]	Baseline
Meas[7]	Baseline
Meas[8]	Baseline
Meas[9]	Baseline
Meas[10]	Baseline
Meas[11]	Active
Meas[12]	Active
Meas[13]	Active
Meas[14]	Active

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SIEMENS MAGNETOM TrioTim syngo MR B17

Meas[15]	Active
Motion correction	Off
Spatial filter	Off

Sequence

Introduction	Off
Contrasts	1
Bandwidth	1736 Hz/Px
Free echo spacing	Off
Echo spacing	0.72 ms
EPI factor	96
Gradient mode	Fast
Excite pulse duration	5760 us
Refocus pulse duration	5120 us
Single-band images	Off
MB LeakBlock kernel	Off
MB dual kernel	Off
MB RF phase scramble	Off
Time-shifted MB RF	Off
SENSE1 coil combine	Off
Invert RO/PE polarity	On
Online multi-band recon.	Online
FFT scale factor	1.00
Physio recording	Off
Triggering scheme	Standard

SIEMENS MAGNETOM TrioTim syngo MR B17

\\USER\Specific trials\ACU-development\Social-Anx\MB_face_match-1

TA: 5:08 PAT: Off Voxel size: 2.0×2.0×2.0 mm Rel. SNR: 1.00 USER: cmrr_mbep2d_bold

Properties

Prio Recon	Off
Before measurement	
After measurement	
Load to viewer	On
Inline movie	Off
Auto store images	On
Load to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	On
Start measurement without further preparation	On
Wait for user to start	On
Start measurements	single

Routine

Slice group 1	
Slices	65
Dist. factor	0 %
Position	R1.2 A9.7 H1.2
Orientation	T > C-30.8
Phase enc. dir.	A >> P
Rotation	0.00 deg
Phase oversampling	0 %
FoV read	192 mm
FoV phase	100.0 %
Slice thickness	2.00 mm
TR	1020 ms
TE	30.0 ms
Multi-band accel. factor	5
Filter	Prescan Normalize
Coil elements	HEA;HEP

Contrast

MTC	Off
Magn. preparation	None
Flip angle	65 deg
Fat suppr.	Fat sat.
Averaging mode	Long term
Reconstruction	Magnitude
Measurements	294
Delay in TR	0 ms
Multiple series	Off

Resolution

Base resolution	96
Phase resolution	100 %
Phase partial Fourier	6/8
Interpolation	Off
PAT mode	None
Matrix Coil Mode	Auto (CP)
Distortion Corr.	Off
Unfiltered images	Off
Prescan Normalize	On
Raw filter	On
Elliptical filter	Off
Hamming	Off

Geometry

Multi-slice mode	Interleaved
Series	Interleaved
Special sat.	None

System

Body	Off
HEP	On
HEA	On
SP4	Off
SP2	Off
SP8	Off
SP6	Off
SP3	Off
SP1	Off
SP7	Off
SP5	Off
Positioning mode	FIX
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Coil Combine Mode	Sum of Squares
AutoAlign	---
Auto Coil Select	Default
Shim mode	Advanced
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Silicone	Off
? Ref. amplitude 1H	0.000 V
Adjustment Tolerance	Auto
Adjust volume	
Position	R1.2 A9.7 H1.2
Orientation	T > C-30.8
Rotation	0.00 deg
R >> L	192 mm
A >> P	192 mm
F >> H	130 mm

Physio

1st Signal/Mode	None

BOLD

GLM Statistics	Off
Dynamic t-maps	Off
Starting ignore meas	0
Ignore after transition	0
Model transition states	On
Temp. highpass filter	On
Threshold	4.00
Paradigm size	20
Meas[1]	Baseline
Meas[2]	Baseline
Meas[3]	Baseline
Meas[4]	Baseline
Meas[5]	Baseline
Meas[6]	Baseline
Meas[7]	Baseline
Meas[8]	Baseline
Meas[9]	Baseline
Meas[10]	Baseline
Meas[11]	Active
Meas[12]	Active
Meas[13]	Active
Meas[14]	Active
Meas[15]	Active

SIEMENS MAGNETOM TrioTim syngo MR B17

Meas[16]	Active
Meas[17]	Active
Meas[18]	Active
Meas[19]	Active
Meas[20]	Active
Motion correction	Off
Spatial filter	Off

Sequence

Introduction	Off
Contrasts	1
Bandwidth	1860 Hz/Px
Flow comp.	No
Free echo spacing	Off
Echo spacing	0.69 ms
EPI factor	96
Gradient mode	Fast
RF spoiling	Off
Excite pulse duration	4500 us
Single-band images	Off
MB LeakBlock kernel	Off
MB dual kernel	Off
MB RF phase scramble	Off
SENSE1 coil combine	Off
Invert RO/PE polarity	Off
PF omits higher k-space	Off
Online multi-band recon.	Online
FFT scale factor	0.90
Physio recording	Off
Triggering scheme	Standard

SIEMENS MAGNETOM TrioTim syngo MR B17

\\USER\Specific trials\ACU-development\Social-Anx\MB_face_match-2

TA: 5:08 PAT: Off Voxel size: 2.0×2.0×2.0 mm Rel. SNR: 1.00 USER: cmrr_mbep2d_bold

Properties

Prio Recon	Off
Before measurement	
After measurement	
Load to viewer	On
Inline movie	Off
Auto store images	On
Load to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	On
Start measurement without further preparation	On
Wait for user to start	On
Start measurements	single

Routine

Slice group 1	
Slices	65
Dist. factor	0 %
Position	R1.2 A9.7 H1.2
Orientation	T > C-30.8
Phase enc. dir.	A >> P
Rotation	0.00 deg
Phase oversampling	0 %
FoV read	192 mm
FoV phase	100.0 %
Slice thickness	2.00 mm
TR	1020 ms
TE	30.0 ms
Multi-band accel. factor	5
Filter	Prescan Normalize
Coil elements	HEA;HEP

Contrast

MTC	Off
Magn. preparation	None
Flip angle	65 deg
Fat suppr.	Fat sat.
Averaging mode	Long term
Reconstruction	Magnitude
Measurements	294
Delay in TR	0 ms
Multiple series	Off

Resolution

Base resolution	96
Phase resolution	100 %
Phase partial Fourier	6/8
Interpolation	Off
PAT mode	None
Matrix Coil Mode	Auto (CP)
Distortion Corr.	Off
Unfiltered images	Off
Prescan Normalize	On
Raw filter	On
Elliptical filter	Off
Hamming	Off

Geometry

Multi-slice mode	Interleaved
Series	Interleaved
Special sat.	None

System

Body	Off
HEP	On
HEA	On
SP4	Off
SP2	Off
SP8	Off
SP6	Off
SP3	Off
SP1	Off
SP7	Off
SP5	Off
Positioning mode	FIX
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Coil Combine Mode	Sum of Squares
AutoAlign	---
Auto Coil Select	Default
Shim mode	Advanced
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Silicone	Off
? Ref. amplitude 1H	0.000 V
Adjustment Tolerance	Auto
Adjust volume	
Position	R1.2 A9.7 H1.2
Orientation	T > C-30.8
Rotation	0.00 deg
R >> L	192 mm
A >> P	192 mm
F >> H	130 mm

Physio

1st Signal/Mode	None

BOLD

GLM Statistics	Off
Dynamic t-maps	Off
Starting ignore meas	0
Ignore after transition	0
Model transition states	On
Temp. highpass filter	On
Threshold	4.00
Paradigm size	20
Meas[1]	Baseline
Meas[2]	Baseline
Meas[3]	Baseline
Meas[4]	Baseline
Meas[5]	Baseline
Meas[6]	Baseline
Meas[7]	Baseline
Meas[8]	Baseline
Meas[9]	Baseline
Meas[10]	Baseline
Meas[11]	Active
Meas[12]	Active
Meas[13]	Active
Meas[14]	Active
Meas[15]	Active

15/+

SIEMENS MAGNETOM TrioTim syngo MR B17

Meas[16]	Active
Meas[17]	Active
Meas[18]	Active
Meas[19]	Active
Meas[20]	Active
Motion correction	Off
Spatial filter	Off

Sequence

Introduction	Off
Contrasts	1
Bandwidth	1860 Hz/Px
Flow comp.	No
Free echo spacing	Off
Echo spacing	0.69 ms
EPI factor	96
Gradient mode	Fast
RF spoiling	Off
Excite pulse duration	4500 us
Single-band images	Off
MB LeakBlock kernel	Off
MB dual kernel	Off
MB RF phase scramble	Off
SENSE1 coil combine	Off
Invert RO/PE polarity	Off
PF omits higher k-space	Off
Online multi-band recon.	Online
FFT scale factor	0.90
Physio recording	Off
Triggering scheme	Standard

Appendix B – Ethics Approval and Other Relevant Documentation

Appendix B – 1. Original Ethics Approval

Principal Investigator: Dr Izelle Labuschagne
Co-Investigator: Dr Skye McLennan, Dr Darren Hocking, Prof Peter Rendell, Dr Peter Koval
Student Researcher: Caitlin Grace (HDR Student)
Research Assistant: Ms Rachel Pelly

Ethics Register Number: 2016-217H
Project Title: Daily emotional functioning in Social Anxiety Disorder
Risk Level: Low Risk
Date Approved: 24/11/2016
Ethics Clearance End Date: 31/12/2017

This email is to advise that your application has been reviewed by the Australian Catholic University's Human Research Ethics Committee and confirmed as meeting the requirements of the National Statement on Ethical Conduct in Human Research.
The data collection of your project has received ethical clearance but the decision and authority to commence may be dependent on factors beyond the remit of the ethics review process and approval is subject to ratification at the next available Committee meeting. The Chief Investigator is responsible for ensuring that outstanding permission letters are obtained, interview/survey questions, if relevant, and a copy forwarded to ACU HREC before any data collection can occur. Failure to provide outstanding documents to the ACU HREC before data collection commences is in breach of the National Statement on Ethical Conduct in Human Research and the Australian Code for the Responsible Conduct of Research. Further, this approval is only valid as long as approved procedures are followed.

If your project is a Clinical Trial, you are required to register it in a publicly accessible trials registry prior to enrolment of the first participant (e.g. Australian New Zealand Clinical Trials Registry http://www.anzctr.org.au/) as a condition of ethics approval.

If you require a formal approval certificate, please respond via reply email and one will be issued.

Appendix B – 2. Ethics Extension Request Approval from HREC

End of 2018.

Ethics Register Number: 2016-217H
Project Title: Daily emotional functioning in Social Anxiety Disorder
Data Collection Date Extended: 31/12/2018
Thank you for returning the Ethics Progress Report for your project.
The Deputy Chair of the Human Research Ethics Committee has approved your request to extend the project. The new expiry date for the project is the 31/12/2018.
We wish you well in this ongoing project.
Kind regards,
Ms Pratigya Pozniak
Research Ethics Officer | Office of the Deputy Vice-Chancellor (Research)
Australian Catholic University
T: 02 9739 2646 E: res.ethics@acu.edu.au
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End of 2019.
Ethics Register Number: 2016-217H
Project Title: Daily emotional functioning in Social Anxiety Disorder
Data Collection Date Extended: 31/12/2019
Thank you for returning the Ethics Progress Report for your project.
The Deputy Chair of the Human Research Ethics Committee has approved your request to extend the project.
The new expiry date for the project is the 31/12/2019.
We wish you well in this ongoing project.
Kind regards,
Ms Kylie Pashley
Research Ethics Officer | Office of the Deputy Vice-Chancellor (Research)
on behalf of ACU HREC Chair, Assoc Prof. Michael Baker
Australian Catholic University
T: 02 9739 2646 E: res.ethics@acu.edu.au
THIS IS AN AUTOMATICALLY GENERATED RESEARCHMASTER EMAIL

Appendix B – 3. Preregistration of Study 2

NIHR | National Institute for Health Research

PROSPERO
International prospective register of systematic reviews

To enable PROSPERO to focus on COVID-19 submissions, this registration record has undergone basic automated checks for eligibility and is published exactly as submitted. PROSPERO has never provided peer review, and usual checking by the PROSPERO team does not endorse content. Therefore, automatically published records should be treated as any other PROSPERO registration. Further detail is provided here.

Citation

Simone Mizzi, Izelle Labuschagne, Valentina Lorenzetti, Sally Grace, Markus Heinrichs, Gill Terrett, Hannah Thompson. A systematic meta-analysis of resting-state fMRI in social anxiety disorder (SAD). PROSPERO 2020 CRD42020163027 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020163027

Review question

To determine whether there are patterns of abnormal intrinsic connectivity (as demonstrated by resting state functional magnetic resonance imaging) in individuals with SAD compared to those without SAD

Searches

The following electronic databases will be searched: CINAHL, MEDLINE, OVID, PsycINFO, PubMed, Scopus, and Web of Science. Search terms include ("social anxiety" OR "social phobia" OR "socially anxious") AND ("resting-state" OR "resting state" OR "rest"). We will also search reference lists of eligible studies. Duplications will be removed.

Types of study to be included

Studies will be included if they meet the following eligibility criteria:

- Full-text is published in English and is a peer-reviewed journal article

- Human participants are involved

- Brain function is measured using resting-state fMRI

- Participants with a diagnosis of SAD are involved

Studies will be excluded if:

- It is a single case-report, book chapter, or conference abstract only

- It is a review or meta-analysis of the literature

- There is no control comparison group

For the meta-analysis, studies will additionally be required to report specific peak foci of activation in either Talairach or Monteral Neurological Institute (MNI) space. If needed, we will contact the corresponding author to request additional data so that we are able to include the study in the review.

Condition or domain being studied

Resting-state neural activity

Participants/population

People with a diagnosis of social anxiety disorder

Intervention(s), exposure(s)

None

Comparator(s)/control
Healthy control comparison group

Main outcome(s)
Resting state brain activity correlates

Measures of effect
Not applicable

Additional outcome(s)
None

Measures of effect
Not applicable

Data extraction (selection and coding)
The process of selecting articles to be included in this review will be mapped using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher, Liberati, Tetzlaff, & Altman, 2009). To determine which studies will be included, two reviewers will screen the titles and abstracts and then the resultant full-text articles that have not been excluded. Any discrepancies during either stage of the review process will be resolved through discussion between the two reviewers under the supervision of a senior staff member.

The following information will be extracted from the included studies: year of publication, participant details (including number, mean age, sex distribution, psychiatric comorbidities, and medication status), data analysis method, MRI related parameters (including procedure and design of the scan), and brain regions of interest (including voxel coordinates) that were reported in the findings.

Risk of bias (quality) assessment
Risk of bias will be assessed using the Cochrane Handbook which allows us to assess each study as having 'low', 'high' or 'unclear' risk on the following domains: (a) sequence generation, (b) allocation concealment, (c) blinding of participants and personnel, (d) blinding of outcome assessment, (e) incomplete outcome data, (f) selective reporting, and (g) other biases.

Strategy for data synthesis
As part of the systematic review, a summary of the findings from all included studies will be presented in a table. For the meta-analysis, we will group the results from studies based on the approach that was used to analyse the resting-state fMRI data (e.g., whole brain seed based approach, independent components analysis, graph theory) and will use the Activation Likelihood Estimation approach to synthesise the coordinates within each group (given there is a sufficient number of studies).

Analysis of subgroups or subsets
If enough data points exist, we will perform sub-analyses on gender and on resting-state condition (eyes closed or open).

Contact details for further information
Simone Mizzi
simonemizzi1@gmail.com

Organisational affiliation of the review
Australian Catholic University

Review team members and their organisational affiliations
Ms Simone Mizzi. Australian Catholic University
Dr Izelle Labuschagne. Australian Catholic University
Dr Valentina Lorenzetti. Australian Catholic University
Dr Sally Grace. Australian Catholic University
Professor Markus Heinrichs. University of Freiburg

Assistant/Associate Professor Gill Terrett. Australian Catholic University
Ms Hannah Thompson. Australian Catholic University

Type and method of review
Meta-analysis, Systematic review

Anticipated or actual start date
28 November 2019

Anticipated completion date
19 December 2020

Funding sources/sponsors
Not applicable

Conflicts of interest
None known

Language
English

Country
Australia

Stage of review
Review Ongoing

Subject index terms status
Subject indexing assigned by CRD

Subject index terms
MeSH headings have not been applied to this record

Date of registration in PROSPERO
10 July 2020

Date of first submission
19 December 2019

Stage of review at time of this submission

Stage	Started	Completed
Preliminary searches	Yes	No
Piloting of the study selection process	Yes	No
Formal screening of search results against eligibility criteria	No	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

The record owner confirms that the information they have supplied for this submission is accurate and complete and they understand that deliberate provision of inaccurate information or omission of data may be construed as scientific misconduct.

The record owner confirms that they will update the status of the review when it is completed and will add publication details in due course.

Versions
10 July 2020

Appendix B – 4. Permission to Reproduce SAD Criteria from the APA

Dear Simone,

Permission is granted for use of the material as outlined in the request below for your dissertation only. Permission is granted under the following conditions:

- Material must be reproduced without modification, with the exception of style and format changes
- Permission is nonexclusive and limited to this one-time use
- Use is limited to English language only; print and website use only
- Permission must be requested for additional uses (including subsequent editions, revisions, and any electronic use)
- Commercial use is not permitted

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Please let me know if you have any questions.

Sincerely,

Robin

Robin Allan

Permissions Coordinator

American Psychiatric Association Publishing
Phone: 800-368-5777
Email: rallan@psych.org
www.appi.org; www.psychiatryonline.org, www.psychiatry.org

Appendix B – 5. Statement of Contribution by Others

The following statement of contribution is made regarding Chapter 3 (Study 1) of this thesis, which was published as:

Mizzi, S., Pedersen, M., Lorenzetti, V., Heinrichs, M., & Labuschagne, I. (2021). Resting-state neuroimaging in social anxiety disorder: a systematic review. Molecular Psychiatry. https://doi.org/10.1038/s41380-021-01154-6.

First author: Simone Mizzi

I acknowledge that my contribution to the above paper is 70%.

Extent of contribution: S.M. was involved in the conceptualisation of the work, performed literature searches, article screening, data extraction and checking, synthesised the data for reporting, wrote manuscript drafts, and finalised the manuscript for publication.

Signature:  Date: 20/03/2022

Second author: Mangor Pedersen

I acknowledge that my contribution to the above paper is 10%.

Extent of contribution: M.P. was involved in providing comments and edits on manuscript drafts.

Signature:  Date: 21/03/2022

Third author: Valentina Lorenzetti

I acknowledge that my contribution to the above paper is 5%.

Extent of contribution: V.L. provided feedback on manuscript drafts.

Signature:  Date: 21/03/2022

Fourth author: Markus Heinrichs

I acknowledge that my contribution to the above paper is 5%.

Extent of contribution: M.H. provided feedback on manuscript drafts.

Signature: [redacted] Date: 21/03/2022

Last author: Izelle Labuschagne

I acknowledge that my contribution to the above paper is 10%.

Extent of contribution: I.L. contributed significantly to the conceptualisation of the work, discussion of ideas and revisions, and provided comments and edits on all manuscript drafts.

Signature: [redacted] Date: 21/03/2022

The following statement of contribution is made regarding Chapter 5 (Study 2) of this thesis, which was published as a preprint:

Mizzi, S., Pedersen, M., Rossell, S., Rendell, P.G., Terrett, G., Heinrichs, M., & Labuschagne, I. (2022). Resting-State Amygdala Subregion and Precuneus Connectivity Provide Evidence for a Dimensional Approach to Studying Social Anxiety Disorder. medRxiv.

First author: Simone Mizzi

I acknowledge that my contribution to the above paper is 71%.

Extent of contribution: S.M. was involved in the conceptualisation of the work, collection of data, data extracting and checking, data analysis, wrote manuscript drafts, and finalised the manuscript for submission.

Signature: [redacted] Date: 20/03/2022

Second author: Mangor Pedersen

I acknowledge that my contribution to the above paper is 9%.

Extent of contribution: M.P. was involved in conceptualisation of the work, data analysis and providing comments and edits on manuscript drafts.

Signature:  Date: 21/03/2022

Third author: Susan Rossell

I acknowledge that my contribution to the above paper is 3%.

Extent of contribution: S.L provided feedback on manuscript drafts.

Signature:  Date: 21/03/2022

Fourth author: Peter Rendell

I acknowledge that my contribution to the above paper is 2%.

Extent of contribution: P.R. provided feedback on manuscript drafts.

Signature:  Date: 22/03/2022

Fifth author: Gill Terrett

I acknowledge that my contribution to the above paper is 2%.

Extent of contribution: G.T. provided feedback on manuscript drafts.

Signature:  Date: 20/03/2022

Sixth author: Markus Heinrichs

I acknowledge that my contribution to the above paper is 3%.

Extent of contribution: M.H. provided feedback on manuscript drafts.

Signature:  Date: 21/03/2022

Last author: Izelle Labuschagne

I acknowledge that my contribution to the above paper is 10%.

Extent of contribution: I.L. contributed significantly to the conceptualisation of the work, discussion of ideas and revisions, data analysis and provided comments and edits on all manuscript drafts.

Signature:  Date: 21/03/2022