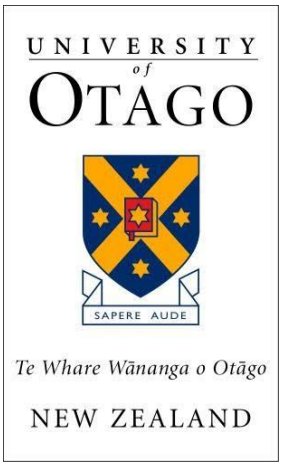
Functional Connectivity Correlates of SSRI Use in Anxiety Disorders: A Resting-State fMRI Study



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Abstract

Background: Anxiety disorders are the most prevalent psychiatric disorders worldwide, responsible for an enormous burden both on the individual and societal levels. Although various effective treatments are now available for these disorders, many people do not respond to any of these treatments. First introduced to the market in the late 1980s, SSRI antidepressants have become the most frequently prescribed first-line pharmaceutical treatment option for anxiety disorders. Despite this, how these medications bring about an improvement in anxiety disorders remains largely unknown, serving as a barrier to the development of new or optimised treatments. Resting-state functional MRI serves as a neuroimaging modality which holds significant potential to reveal new insights into how these medications act upon the human brain. This technique has already been used to gain many insights into various psychiatric disorders and their treatments, however, research focussing on anxiety disorders and the mechanisms of SSRIs is comparatively scarce.

Aims: This research aimed to explore and describe the correlations between brain functional connectivity, SSRI use, and lifetime history of anxiety disorders in a large cohort.

Method: A subset of 488 participants was selected from the UK Biobank, a large-scale prospective study and database. These participants were matched across various demographic and clinical features and categorised into three study groups based on SSRI use at the time of neuroimaging data collection and lifetime history of anxiety disorders. Group-level independent component analysis was utilised to determine networks of resting-state functional connectivity shared among this cohort of participants. A dual regression approach was employed to statistically compare functional connectivity across the three subject groups.

Results: Group-level independent component analysis identified 18 components representing neuronal patterns of resting-state connectivity. None of these components displayed statistically significant differences across subject groups after a conservative correction for multiple comparisons. However, three of these components exhibited results bordering on statistical significance after adjustment. Therefore, exploratory post hoc tests were conducted on these components. These components all consisted of similar brain regions and were all most strongly representative of the sensorimotor resting-state network. Post hoc tests indicated that participants with a history of anxiety disorders who were taking an SSRI at the time of

neuroimaging data collection exhibited widespread reduced functional connectivity within each of these three networks, relative to the group of participants without a history of anxiety disorders who were not taking any medications. Large areas of decreased functional connectivity within these networks were localised within the precentral and postcentral gyri. No significant differences were observed between participants with a lifetime history of anxiety disorders and participants without this history.

Conclusion: The findings of this study suggest that the use of SSRIs is correlated with a decrease in within-network functional connectivity for several networks which correspond most closely to the sensorimotor resting-state network. Further research which focuses on or incorporates this network into analyses is needed to confirm these results, and longitudinal studies are required to determine whether these findings are directly caused by SSRI use, or if they are correlated through other factors.

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List of Abbreviations

5-HT	5-hydroxytryptamine (serotonin)
5-HTT	5-hydroxytryptamine transporter (serotonin transporter)
AB	Asian British
ACC	Anterior cingulate cortex
AG	Agoraphobia
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
APA	American Psychiatric Association
BB	Black British
BDNF	Brain-derived neurotrophic factor
CBT	Cognitive behavioural therapy
CIDI	Composite International Diagnostic Interview
DALY	Disability-adjusted life years
DMN	Default mode network
DSM	Diagnostic and Statistical Manual of Mental Disorders
DTI	Diffusion tensor imaging
DWI	Diffusion weighted imaging
EEG	Electroencephalography
EPI	Echo-planar imaging
FDR	False discovery rate
FIX	FMRIB's ICA-based X-noiseifier
FMRIB	Functional Magnetic Resonance Imaging of the Brain
FSL	FMRIB Software Library

FWER	Family-wise error rate
GABA	Gamma-aminobutyric acid
GAD	Generalised anxiety disorder
GDC	Gradient distortion correction
GLM	Generalised linear model
HPA	Hypothalamic-pituitary-adrenal
IC	Independent component
ICA	Independent component analysis
ICD	International Classification of Diseases
MCFLIRT	Motion Correction using FMRIB's Linear Image Registration Tool
MDD	Major depressive disorder
MELODIC	Multivariate Exploratory Linear Optimized Decomposition into Independent Components
MNI	Montreal Neurological Institute
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NHS	National Health Service
NIfTI	Neuroimaging Informatics Technology Initiative
NM	No medications
NMDA	N-methyl-D-aspartate
OCD	Obsessive-compulsive disorder
PCC	Posterior cingulate cortex
PD	Panic disorder
PET	Positron emission tomography
PFC	Prefrontal cortex
PTSD	Post-traumatic stress disorder

SAD	Social anxiety disorder
SD	Standard deviation
SERT	Serotonin reuptake transporter
SNRI	Serotonin–norepinephrine reuptake inhibitors
SP	Specific phobia
SPECT	Single-photon emission computed tomography
SSRI	Selective serotonin reuptake inhibitor
TFCE	Threshold-free cluster enhancement
WHO	World Health Organisation
WMH	World Mental Health

1. Introduction

This chapter begins by outlining the research background in section 1.1, followed by its purpose, hypotheses, and objectives in section 1.2. This chapter then concludes with an outline of the structure and contents of the following chapters, presented in section 1.3.

1.1. Background

Globally, anxiety disorders are the most prevalent of all mental illnesses. Of these mental disorders, they are the second most significant contributor to disease burden worldwide (1). Furthermore, they are the sixth leading cause of disability, resulting in significant impairments in the lives of hundreds of millions and a substantial economic impact (2). A nationwide survey conducted between 2003 and 2004 estimated that around 15% of the total population and 20% of Māori meet the diagnostic criteria for an anxiety disorder each year in New Zealand, based on 12-month prevalences (3). To estimate these prevalences, this survey utilised the World Mental Health (WMH) Survey Initiative version of the World Health Organization (WHO) Composite International Diagnostic Interview (CIDI) in face-to-face household interviews (4).

Despite a range of treatment options, understanding the mechanisms underlying how these treatments improve anxiety disorder symptoms remains limited. With the availability of new methodologies in recent years comes the opportunity to expand the understanding of these interventions, which would enable clinicians to optimise the use of current treatments and inform the development of safer, more efficacious options.

There are several subtypes of anxiety disorders, including separation anxiety disorder, specific phobia (SP), social anxiety disorder (SAD), panic disorder (PD), agoraphobia, generalised anxiety disorder (GAD) and substance/medication-induced anxiety disorder. Diagnosis of these anxiety disorders is typically based on criteria from the Diagnostic and Statistical Manual of Mental Disorders V (DSM-5) (5). These subtypes are not mutually exclusive and are often comorbid with each other, with overlapping symptoms. Anxiety disorders are also often concurrent with other psychiatric conditions, such as major depressive disorder (MDD) (6). Previous research has found that the comorbidity of various psychiatric conditions with an anxiety disorder significantly reduces a person’s quality of life compared to those who have the same psychiatric condition without an anxiety disorder (7, 8). For example,

a 2017 study found that outpatients with MDD and comorbid GAD experience more severe depressive symptoms, poorer sleep quality, and lower quality of life in both physical and psychological domains compared to outpatients with MDD alone (7). Similarly, a 2008 review found that patients with bipolar disorder and a comorbid anxiety disorder suffer from greater symptom burden, more depressive symptoms, worsened treatment response, higher levels of suicidal ideation, and an overall poorer quality of life compared to patients with bipolar disorder alone (8).

Despite the high prevalence and impact of anxiety disorders, the understanding of their causes and treatments remains limited. Previous research has found that alongside depressive disorders, anxiety disorders are associated with the most significant decrease in quality of life among psychiatric disorders (1, 9). Anxiety disorders are linked to numerous adverse outcomes, including but not limited to increased instances of suicidal ideation and tendencies, elevated school dropout rates, reduced employment prospects, lower socioeconomic status, decreased work productivity, and an elevated risk of coronary disease (5, 10, 11). Therefore, a significant benefit can be gained by building on the current understanding of these disorders and discovering novel or improved treatment options.

Currently, the diagnosis of anxiety disorders is based exclusively on symptomatic presentation and patient history (5). This approach is also common across other psychiatric conditions, which frequently share overlapping clinical features and often occur together (12). This complicates the process of accurate diagnosis and subclassification of anxiety disorders.

The discovery of new objective measures, or “biomarkers”, could revolutionise clinical practice in these areas, leading to improved treatment outcomes and more precise classification in future psychiatric research (12, 13). Additionally, incorporating biomarkers into screening assessments, alongside symptomatic evaluation, could enhance the identification of individuals at a higher risk for anxiety disorders and other psychiatric conditions. This approach may pave the way for the development of preventive measures and earlier interventions (12).

A range of psychotherapies and pharmacological treatments are used to treat anxiety disorders. Cognitive behavioural therapy (CBT) is the most extensively researched psychotherapy for anxiety disorders and is recommended by international guidelines as an effective, safe first-line treatment option (14). CBT may be sufficient alone in treating anxiety disorders; however, it is frequently used in conjunction with pharmacological treatments. There are also a range of other psychotherapies being trialled, such as relaxation and breathing

techniques, mindfulness meditation, psychodynamic therapy, interpersonal therapy, exercise-based interventions, and internet-based psychotherapies (15, 16).

Alongside psychotherapies, pharmacological options hold a crucial role in the treatment of anxiety disorders. Modern pharmacological options have been proven as effective treatments with good safety profiles through a large volume of research over the past decades. These medications are recommended as a first-line treatment option by clinical guidelines internationally, offering an effective standalone or complementary treatment option when combined with psychotherapies (14, 17-20).

Of the pharmacological therapy options available for treating anxiety disorders, international guidelines recommend medications belonging to the selective serotonin reuptake inhibitor (SSRI) class of antidepressants as the first-line pharmacological option in most cases (14, 17-20). This recommendation is based on their proven effectiveness and favourable safety profile relative to other pharmacological options.

SSRIs, along with other classes of medications and psychotherapies, have significantly improved many people's lives. However, previous studies have estimated that the rate of achieving an adequate clinical response to a first-line treatment trial is only between 50-67% among individuals with anxiety disorders (21). This fact along with the substantial worldwide prevalence and impact of anxiety disorders highlight an urgent need for improved treatment options. To inform the development of these new treatment options, it is crucial to understand the underlying pathophysiological mechanisms of anxiety disorders, as well as how the best currently available treatments such as SSRIs can restore these mechanisms in association with an improvement in anxiety disorder symptoms.

SSRIs have now been in use for over 35 years, since the introduction of fluoxetine for the treatment of depression in 1988; however, the exact mechanisms underpinning how this class of medications improves depressive and anxiety disorder symptoms remains largely unknown (22, 23). As a result, there has been significant interest in elucidating the mechanisms behind how SSRIs function, with the use of a wide range of modalities such as receptor binding assays, metabolomics, genomics, proteomics, and notably, a variety of neuroimaging techniques over the years since their introduction. Each of these modalities offer unique insights into how these medications work within the body and offer the potential to uncover why some individuals respond while others do not.

Among these approaches, neuroimaging techniques have rapidly evolved in sophistication, becoming increasingly popular tools for understanding the neural underpinnings of psychiatric conditions. Among these techniques, resting-state functional magnetic resonance imaging (rs-fMRI) enables the investigation of the functional organisation and connectivity of the brain. Rs-fMRI can provide insights into the pathophysiology of various psychiatric conditions as well as the mechanisms associated with their treatments, including why individuals may exhibit vastly different responses to the same treatment.

Rs-fMRI utilises magnetic resonance imaging (MRI) technology to indirectly measure neural activity over time by detecting changes in blood oxygenation levels within various brain regions. In contrast to task-based functional magnetic resonance, which measures neural activity while a subject performs a particular task, rs-fMRI measures the neural activity of a subject during wakeful rest in the absence of any task. From these measures of neural activity, it is possible to derive the "functional connectivity" of the brain, which consists of distinct brain regions which show significant correlations in their activity over time. This includes both positive correlations (when one or more brain regions show an increase in activity, another brain region also has an increase in its activity) and negative correlations (when one or more brain regions show an increase in activity, another brain region has a decrease in activity). Through rs-fMRI investigations, several "resting-state networks" have been determined. These networks represent common functional connectivity patterns which are reproducibly observed across studies and have now become a focal point of many neuroimaging investigations.

Although rs-fMRI has grown in availability and has proven its utility, research into the associations of neural activity with the use of SSRIs remains sparse, particularly in the treatment of anxiety disorders. Studies investigating functional connectivity correlations with SSRI use have typically concentrated on these associations in the context of treating depressive disorders, the original target of these medications, as opposed to anxiety disorders. Additionally, this research gap can be partially explained by the significant cost and time requirements associated with collecting rs-fMRI data from a large number of subjects. These constraints mean rs-fMRI studies often suffer from low participant numbers and statistical power (24, 25).

One cost-effective and time-efficient approach is exemplified by the UK Biobank, a large prospective epidemiological study and database that has collected a vast amount of health-related information on over 500,000 participants since initial data collection began in 2007 (24, 26, 27). Among the data collected is information on demographics, genetics, medical history, medication use, and data collected using various imaging modalities, including rs-fMRI images

and imaging-derived measures. This data is available upon application by researchers internationally from various fields, meaning the same dataset can be utilised to investigate many different aspects of health while providing the benefit of increased sample sizes for imaging studies. The present study aims to utilise the rs-fMRI data collected by the UK Biobank to investigate correlations of the brain's functional architecture with SSRI use at the time of fMRI data collection, with the focus being on people with a lifetime history of one or more anxiety disorders.

1.2. Research Objectives, Hypotheses, and Significance

The primary purpose of this research is to explore resting-state functional connectivity correlations with SSRI use in the context of people with a lifetime history of anxiety disorders utilising resting-state fMRI (rs-fMRI) data. The secondary purpose of this research is to explore resting-state functional correlations with a lifetime history of one or more anxiety disorders using rs-fMRI data.

Specifically, the objectives of this research are:

- Utilise independent component analysis (ICA) to delineate distinct resting-state networks common across subjects.
- Ascertain whether current SSRI use is correlated with alterations in within-network resting-state functional connectivity among participants with a history of one or more anxiety disorders.
- Characterise any correlations between current SSRI use and within-network resting-state functional connectivity, including which resting-state networks differ, brain regions where functional connectivity differs within these networks, and whether SSRI use is associated with increased or decreased functional connectivity.
- Determine if a lifetime history of one or more anxiety disorders is correlated with alterations in within-network resting state functional connectivity.
- Describe any correlations between a lifetime history of an anxiety disorder and within-network resting-state functional connectivity, including which resting-state networks differ, brain regions where functional connectivity differs within these networks, and whether a lifetime history of one or more anxiety disorders is associated with increased or decreased functional connectivity.

Expanding on these objectives, the following hypotheses will be tested:

- **Hypothesis One:** SSRI use at the time of fMRI data collection is correlated with significant differences in within-network resting-state functional connectivity among people with a history of anxiety disorders.
- **Hypothesis Two:** Individuals with a history of anxiety disorders will exhibit significant within-network resting-state functional connectivity differences to those without a history of anxiety disorders.

Information gained in this area can lay the foundation for further research into the neural correlates of anxiety disorders and the mechanism by which SSRIs and other treatment options affect the brain. By expanding the current understanding of how anxiety disorders and their treatments are associated with functional connectivity alterations, the development of new interventions can be informed, in addition to improved diagnosis and monitoring of patients suffering from anxiety disorders.

1.3. Dissertation Outline

In the next chapter (Chapter Two), the context of this research will be set, starting with a discussion of the history, current understanding, consequences, and theorised causes of anxiety disorders. Subsequently, our understanding of present-day treatments for anxiety disorders will be examined, with an emphasis on the first-line pharmacological treatment option: the SSRI class of antidepressants. Finally, this chapter will provide a brief overview and evaluation of some of the findings of studies that have utilised neuroimaging techniques to investigate anxiety disorders and their treatments.

Chapter Three will describe the methodology applied for this research, starting with details on participant selection and grouping before detailing the techniques used to process and analyse participant rs-fMRI data, extract networks of common brain activity, and finally, the statistical analysis used to assess for significant group-level differences.

Chapter Four will cover the results of the analyses, starting with details on the demographic and clinical characteristics of included participants, then presenting the resting-state networks observed at the group level before moving on to the results of the statistical comparison of these networks across subject groups.

Chapter Five will evaluate the findings and implications of the research findings in the context of the current knowledge base. Additionally, areas where further research is required

will be discussed. This chapter will conclude with relevant limitations and important considerations relating to the design of this study and the dataset investigated.

Lastly, Chapter Six will conclude this research, with a brief summary of what it aimed to achieve, the overall methodology employed, an overview of its findings, and recommendations for future research.

2. Literature Review

This chapter begins by covering a brief history of the evolution of our understanding of anxiety disorders, their classification, impacts and epidemiology, and associated risk factors in section 2.1. In section 2.2, the background and current understanding of the first-line pharmacological treatment option, SSRI antidepressants, will be discussed. Finally, section 2.3 evaluates the existing literature surrounding the use of neuroimaging techniques to investigate neural correlations of anxiety disorders as well as SSRIs.

2.1. Anxiety Disorders

Our understanding of anxiety disorders throughout the 20th and 21st centuries saw remarkable advances alongside increasingly sophisticated technology and refined scientific methodologies. With these advances came significant changes in diagnostic criteria and classification of anxiety disorders. An early attempt to classify a broad range of anxiety symptoms was with the term “anxiety neurosis”, introduced by Sigmund Freud in 1895. Symptoms of anxiety neurosis included panic attacks, anxious expectation, general irritability, phobias, and agoraphobia. Freud suggested that these symptoms resulted from “accumulation of sexual excitation that could not find discharge in coitus” (28, 29). Freud’s theories were groundbreaking for their time; however, subsequent research advances replaced these theories with more nuanced and biologically-based understandings of anxiety disorders.

As the understanding of psychiatric disorders evolved throughout the first half of the 20th century, a need for a classification system of these disorders arose. Subsequently, the sixth edition of the International Classification of Diseases (ICD-6) and a variant of the ICD-6, the Diagnostic and Statistical Manual of Mental Disorders (DSM), were published by the World Health Organisation (WHO) and the American Psychiatric Association (APA) respectively (30). This first edition of the DSM, published in 1952, now often referred to as the DSM-I, was the first official manual of psychiatric disorders intended for use within the clinical setting (30). Throughout the latter half of the 20th century and into the 21st century, the definitions of anxiety disorders were modified significantly with each new edition of the DSM, along with additions of new subtypes of anxiety disorders. The most recent edition is the DSM-5-TR (Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision), a revised version of the original DSM-5 published in 2013 (5). This edition is currently used globally as a tool by

clinicians in diagnosing psychiatric disorders and by researchers in classifying these disorders. The DSM-5 details the diagnostic criteria and features, prevalence, development and course, and risk factors of eleven subtypes of anxiety disorders. These are separation anxiety disorder, selective mutism, specific phobia, social anxiety disorder, panic disorder, agoraphobia, generalised anxiety disorder, substance/medication-induced anxiety disorder, anxiety disorder due to another medical condition, other specified anxiety disorder and unspecified anxiety disorder. Obsessive-compulsive disorder (OCD), post-traumatic stress disorder (PTSD), and acute stress disorder were classified as anxiety disorders in the DSM-IV; however, they have now been reclassified within "obsessive-compulsive and related disorders" (OCD) and "trauma- and stressor-related disorders" (PTSD and acute stress disorder) within the DSM-5 and its revisions (5, 31). Therefore, to ensure consistency, it is important to consider which edition of the DSM was used to classify anxiety disorders when interpreting and comparing the results of studies.

Anxiety disorders are the most prevalent psychiatric disorders worldwide, responsible for an enormous cost in quality of life and economic burden (32, 33). A large-scale systematic analysis of the burden of mental disorders, conducted in collaboration with the Global Burden of Disease Study 2019, estimated that the worldwide prevalence of anxiety disorders was around 300 million people in 2019, or approximately 3780 per 100,000 people after age standardisation, the highest among all mental disorders (1). Independent epidemiological studies corroborate these results for the worldwide prevalence of anxiety disorders (33). Among all mental disorders, anxiety disorders rank as the second-largest contributor to the global disease burden, surpassed only by depressive disorders (1, 9). Anxiety disorders are estimated to account for 22.9% of the 125.3 million global age-standardised disability-adjusted life years (DALYs) due to mental disorders (1). There is potential for these results to be biased and not truly representative of the global population, as the studies included in these systematic reviews were primarily conducted in high-income Western countries. Differences in how people are defined as having mental health disorders across different countries are affected by many factors. One factor that may influence the estimated epidemiological measurements is the variation in cultural beliefs surrounding mental health disorders.

Although awareness and acceptance surrounding mental health conditions has increased in many regions, stigma remains a significant barrier, preventing many from seeking help. Consequently, this may lead to an underestimation of the true frequency and impact of anxiety disorders in areas where their understanding and social acceptance remain low. Additionally,

the scarcity of epidemiological studies focussed on low-income nations introduces uncertainty in the validity of observed results, complicating the task of accurately comparing epidemiological measures across different global regions. Another limiting factor of international epidemiological studies is that criteria used to define cases of anxiety disorders, such as the DSM criteria, may not be appropriate for use across all cultural settings (34).

Among those affected by anxiety disorders, there are significant differences in the prevalence and manifestations of these conditions across different demographics. A common finding across studies is that women are approximately twice as likely to have any anxiety disorder relative to men (1, 5, 35-38). Age is also an important variable in the development and presentation of anxiety disorders. Although the median age of onset across all anxiety disorders has been estimated to be around 11 years, the typical age of onset varies greatly between subtypes (30). For example, separation anxiety disorder, selective mutism, specific phobia, and social anxiety disorder typically manifest in childhood or adolescence (5, 39, 40).

In contrast, panic disorder, agoraphobia, and generalised anxiety disorder have a typical onset during adulthood (39, 41). SAD symptoms typically present with less severe symptoms spread over a broader range of specific situations for older adults relative to younger adults, who experience more severe symptoms over a smaller range of situations. GAD typically presents with more severe symptoms for younger adults relative to older adults (42, 43). Due to these significant variations across different demographics, it is important to account for these factors when comparing the results of different studies and during the planning stage of research development.

Several determinants have been identified as risk factors for the development of anxiety disorders. Broadly, these can be categorised as temperamental, environmental, genetic, and physiological risk factors (5, 44-46). While some factors are more specific to certain anxiety disorder subtypes, there is significant overlap between the subtypes. Temperamental risk factors include negative affectivity (the tendency to experience negative emotions), behavioural inhibition, harm avoidance and trait anxiety (an inclination to perceive environmental stimuli as threatening) (47-49). Environmental risk factors common across multiple anxiety disorder subtypes include childhood difficulties and maltreatment, parental overprotection or overcontrol, low emotional warmth from childhood caregivers, and negative experiences such as peer victimisation (5, 50, 51). The risk of developing an anxiety disorder is strongly linked to genetic factors, with previous research identifying several specific genes associated with the various anxiety disorder subtypes (46, 52, 53). Furthermore, individuals with first-degree

relatives have been found to have a significantly higher risk of developing an anxiety disorder (5, 36, 54). The discovery of risk factors such as these provides essential insight into the complex interplay of influences throughout the lifespan that contribute to the onset of these disorders. This knowledge informs the development of improved preventative and targeted treatment strategies.

2.2. Selective Serotonin Reuptake Inhibitors

Currently, a range of treatments of various modalities are used in the management of anxiety disorders. These modalities include psychotherapies, pharmacological treatments, and alternative therapies. This section briefly covers the current treatment options available for anxiety disorders before focusing on the first-line pharmacological treatment option, SSRI antidepressants.

Of the modern treatment options, psychotherapies have the most extensive history. Both Aaron Beck, the creator of CBT, and Albert Ellis, the creator of rational emotive behaviour therapy, have made statements acknowledging the influence of ancient Greek and Roman Stoic philosophies on the development of their respective psychotherapies (55). Various mindfulness psychotherapies, such as mindfulness-based stress reduction and mindfulness-based cognitive therapy, have been developed based on ancient Buddhist spiritual practices (56, 57). Psychotherapies play an extremely important part in the treatment options available for anxiety disorders, having a long history attesting to their effectiveness. CBT is recommended as a first-line treatment option in guidelines around the world.

Compared to psychotherapies, pharmacological treatment options for anxiety disorders are a relatively new development. However, they have been pivotal in treating anxiety disorders by enabling an accessible, effective standalone or complementary treatment option alongside psychotherapies. Currently, there are a few distinct classes of medications which are recommended for the treatment of anxiety disorders. First-line options recommended by international guidelines consist of medicines from the SSRI and serotonin–norepinephrine reuptake inhibitor (SNRI) classes of antidepressants. If a medication from one of these classes fails to adequately treat a patient at a maximum tolerated dose, switching from an SSRI to an SNRI or vice versa is recommended. Alternatively, another medication within the same class can be trialled (14). Second-line medication options include buspirone, benzodiazepines, the antiepileptic pregabalin, and the atypical antipsychotic quetiapine. Third-line pharmacological

treatment options include tricyclic antidepressant medications such as amitriptyline and imipramine.

Despite the significant advances in treatment options throughout the 20th century, there is still an unmet need for improved anxiety disorder interventions. This is demonstrated by the extremely high levels of disability attributable to anxiety disorders worldwide (1, 32, 33) and the low rates of clinical response to the first-line treatment options (21). Additionally, since the introduction of SSRIs in 1988, there has been a lack of major innovations in pharmacological interventions for anxiety disorders (23). Many factors have contributed to this lack of innovation, with one such factor being a relative absence of research specifically targeting the discovery of new or improved treatments for anxiety disorders.

Many of the medications currently used for treating anxiety disorders were serendipitously discovered to be effective for this purpose rather than being developed through an informed approach of understanding and targeting features of their pathophysiology. For example, many of these medicines were developed to treat depressive disorders; however, they were later found to provide a therapeutic benefit in the management of anxiety disorders. Buspirone, a medication used specifically as an anxiolytic today, was initially developed as an antipsychotic; however, it was found to be ineffective for this purpose but effective for treating anxiety disorders (58). To improve the process of developing new medications targeting anxiety disorders, it is vital to uncover the pathophysiological abnormalities associated with anxiety disorders and how current treatment options may function to restore these abnormalities. Currently, however, there remains a comparative lack of understanding of anxiety disorders, as well as how their treatments work.

The development of SSRIs, in addition to older generations of antidepressants, occurred based on the “monoamine hypothesis” of depression, which suggests that depression occurs as a result of decreased levels of the monoamines, i.e., serotonin, norepinephrine, and dopamine within the central nervous system (23). Therefore, it was theorised that medications restoring the levels of these monoamines could improve symptoms of depression. Additionally, in the early years of their use for the treatment of depression, it was observed that SSRIs had the benefit of improving symptoms of anxiety that cooccurred with depression (59-61). This finding inspired subsequent investigations into using SSRIs specifically for treating anxiety disorders, for which good efficacy was observed and eventually led to their widespread adoption in treating anxiety disorders (62-64).

However, since its proposal in the 1950s (65-67), more recent investigations have found that the full mechanism behind depression and how these medicines function is a lot more complex than that postulated by the monoamine hypothesis and that monoamines only play one part in a more intricate mechanism. For example, it has been estimated that at least one-third of those with anxiety disorders do not sufficiently respond to any of the available pharmacological treatments (68). Additionally, recent studies have found pharmacological interventions with targets outside the monoaminergic system, such as ketamine, to be an effective treatment option for depressive disorders. Ketamine acts as an N-methyl-D-aspartate (NMDA) receptor antagonist, influencing glutamatergic neurotransmission, in contrast to most currently used antidepressants, which target the monoamine system (69, 70). The antidepressant effects of ketamine occur rapidly, within a span of hours, a significant contrast to the several weeks required for the onset of action of the monoaminergic-targeted antidepressants (70, 71). Additionally, ketamine has demonstrated efficacy for treatment-resistant depression which has not adequately responded to traditional antidepressants (70). Findings such as these have substantiated the role of alternative neurotransmitter systems in depression and inspired investigations of treatments that target these systems.

Additionally, it remains unclear how both pharmacological and non-pharmacological options, such as psychotherapies, can bring about similar improvements in symptoms of psychiatric conditions such as depression and anxiety disorders. It seems likely that each treatment modality, including psychotherapies and distinct classes of medications, targets different parts of a larger, interconnected mechanism behind anxiety disorders, which is not yet fully understood.

Relative to the quantity of research which has focused on exploring alternative theories of depression, there has been less focus on anxiety disorders. However, results of previous research have indicated ketamine possesses anxiolytic effects in addition to its antidepressive properties (72, 73), and clinical trials investigating its use for the treatment of anxiety disorders are currently underway. Ketamine is just one example of the novel treatments targeting alternative neurotransmitter pathways currently under investigation. There has also been interest in developing new medications targeting alternative pathways involving endocannabinoids, neuropeptides, and neurosteroids, all of which have shown potential as therapeutic agents (74).

Although it is now known that the monoamine hypothesis is not an adequate full explanation of the mechanisms behind the pathophysiology of anxiety and depressive disorders

or the therapeutic mechanisms behind their treatments, it is still important to understand how treatment options, including those targeting monoamine pathways, function. By better understanding these more established medications with a long history of efficacy, such as SSRIs, in addition to discovering new pathways implicated in anxiety disorders, a more comprehensive picture of the underlying mechanisms and their interrelationships can be established.

There are several distinct medications which fall into the SSRI class, including citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine, and sertraline, all with the shared effect of increasing serotonin levels over time within neuronal junctions through inhibition of the serotonin reuptake transporter (SERT, also known as 5-HTT). SSRIs differ from older classes of antidepressants, such as the tricyclic antidepressants and monoamine oxidase inhibitors by being much more selective in their influence over serotonin neurotransmission than norepinephrine and dopamine neurotransmission. SSRIs also have less interaction with muscarinic, cholinergic, and histamine receptors relative to tricyclic antidepressants, therefore avoiding many of the adverse effects associated with these neurotransmitter pathways. In addition, SSRIs have shown similar efficacy to these older antidepressants. When compared to benzodiazepines, which were exceptionally frequently used for the treatment of anxiety disorders, SSRIs are not associated with a significant risk for dependence or addiction and have less severe side effects. The favourable safety and efficacy profile of SSRIs has led to them being considered the optimum first-line pharmacological treatment in most cases of anxiety disorders.

While SSRIs are well-regarded for their safety and efficacy, another important issue to consider is tolerance, which is also frequently referred to as tachyphylaxis in the literature. Tolerance refers to a decrease in the effectiveness of a medication over time with prolonged, repetitive administration. This results in the return of symptoms that initially responded to the medication, with a higher dose of the medication being required to maintain the same therapeutic effects. Tolerance is a common observation across the various classes of antidepressants, including SSRIs. Although there is a notable lack of studies investigating this phenomenon specifically for anxiolytic effects in the treatment of anxiety disorders, some investigations have explored the development of tolerance to the antidepressant effects of these medications. A highly cited 1998 meta-analysis estimated the rate of this occurring to be between 9 and 33% (75), while a more recent 2005 prospective observational study estimated this rate to be 25% during maintenance treatment of depressive episodes with antidepressants

(76). Interestingly, some studies have observed that SSRIs are associated with a higher rate of tolerance to antidepressant effects compared to other classes of antidepressants. One study observed the rate of development of tolerance to TCAs or dual reuptake inhibitors to be 3.7%, which was significantly lower than the rate of 14.1% associated with the use of SSRIs within their cohort (77).

While the exact mechanism behind the development of antidepressant tolerance remains unclear, it has been theorised to be due to either pharmacokinetic or pharmacodynamic processes, or potentially a combination of both (78). Pharmacokinetic tolerance involves a change in the absorption, distribution, metabolism, or excretion of a drug, which results in altered concentration of the drug at its target site in response to long-term repetitive dosing. Pharmacodynamic tolerance involves changes to the sensitivity or quantity of receptors, ion channels, or intracellular signalling molecules in response to long-term repetitive dosing (78).

Another popular theory which may explain the development of tolerance to antidepressants is the oppositional model of tolerance proposed by Fava and Offidani (79). This model theorises that long-term exposure to an antidepressant drug results in neurobiological adaptations which act to counter the initial effects of the drug responsible for its therapeutic benefits, resulting in the development of tolerance. These neurobiological adaptations may also explain the tendency for depressive symptoms to return and for patients to have an elevated vulnerability to relapse upon withdrawal of an antidepressant drug, in which case these oppositional adaptations act unopposed (80). It has also been theorised that these adaptations may contribute to the development of treatment-resistant depression (79). This theory is supported by the results of a 2009 randomised controlled trial, which found there was a 19.9% decrease in likelihood of response to sertraline therapy for every additional prior antidepressant treatment trial (81). Although these results are not directly focused on the treatment of anxiety disorders, they remain an important consideration in this context. This is due to the high comorbidity between depressive disorders and anxiety disorders, as well as the potential for the treatment of anxiety disorders with antidepressants to induce neurobiological adaptations which may be detrimental to the response of emergent depressive episodes.

The full mechanism behind how SSRIs have their anxiolytic effect remains unclear; however, several new theories have been proposed in recent years. It has been known for decades that SSRIs affect serotonergic neurons originating within the raphe nuclei located in the brain stem (82, 83). These serotonergic neurons are the site of almost all serotonin production within the central nervous system (84). The most widely accepted theory is that

SSRI inhibition of SERT, located primarily on somatodendritic regions of serotonergic neurons, reduces serotonin reuptake into these neurons. Consequently, the concentration of serotonin within the somatodendritic areas acutely increases. Through repeated administration of an SSRI daily over several weeks, this somatodendritic serotonin concentration remains elevated, stimulating local 5-HT1A autoreceptors, a subtype of serotonin receptor which functions to inhibit the release of serotonin from neuron terminals. The sustained stimulation of these autoreceptors eventually leads to their downregulation. This downregulation has a disinhibitory effect and ultimately results in an increased rate of impulse firing of the raphe neurons, increased rate of serotonin synthesis, and increased release of serotonin into synaptic clefts (59, 85). This neurochemical theory provides an explanation for the delayed onset of therapeutic effects, as this aligns with the several weeks required for autoreceptor downregulation.

More recent studies have provided many insights into the broader potential mechanisms of SSRIs. Numerous studies have observed that 5-HT7 receptors have a role in influencing non-serotonergic neurotransmission through interaction with neurons responsible for glutamate, gamma-aminobutyric acid (GABA), and dopamine neurotransmission. These interactions are complex and location-dependent (86-88). Concomitantly, recent studies have found evidence that SSRIs may have a therapeutic effect through an ability to influence neuroplasticity. For example, a rodent study by Karpova and colleagues found that long-term administration of fluoxetine results in increased synaptic plasticity, with fear memory neural pathways of adult mice being converted to more closely represent the higher plasticity pathways of juvenile mice. The combination of this elevated plasticity with extinction training significantly increased the erasure of conditioned fear (89). Another study investigated the effects of daily administration of escitalopram in healthy participants over a three-week period (90). This randomised controlled trial involved participants undertaking learning and relearning tasks over this period while taking either escitalopram or a placebo. Concordant with the findings of the rodent study by Karpova et al., this study reported that increases in functional connectivity between various brain regions during the relearning task were significantly potentiated with escitalopram administration. The findings of these studies suggest that medications such as SSRIs may have their effect by facilitating brain adaptability, which occurs due to external factors such as psychotherapies, ultimately leading to their established therapeutic benefit.

Other studies have found that SSRIs have a role in increasing levels of an important neurotrophic factor called brain-derived neurotrophic factor (BDNF) within brain regions, including the hippocampus and prefrontal cortex (91). BDNF has important functions in the

development, growth and survival of neurons and has been observed to be reduced by chronic stress (92, 93). This correlates with the finding that infusion of BDNF into the prefrontal cortex or hippocampus being sufficient to produce antidepressant effects in animal studies (92-94). Another interesting finding is that SSRIs, as well as other anxiolytic medications including tricyclic antidepressants and benzodiazepines, have demonstrated an ability to normalise abnormal hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis often associated with chronic anxiety disorders (95-97). This is theorised to occur through the regulation of genes responsible for various molecules such as glucocorticoid receptors, mineralocorticoid receptors, and corticotropin-releasing hormone (97, 98).

As further research continues into these and other theories, the intricacies and interrelationships of the mechanisms underpinning how SSRIs function will become more apparent. Concurrently, the discovery of new biomarkers will enable better-informed clinical practice and the development of improved therapeutic options for anxiety disorders. The following section will discuss relevant research findings utilising neuroimaging techniques, a modality which enables a higher-level assessment of the mechanism of action of SSRIs alongside the aberrations associated with anxiety disorders.

2.3. Neuroimaging Findings

Neuroimaging techniques have rapidly advanced in their sophistication and are increasingly popular for uncovering neural mechanisms associated with a wide range of psychiatric conditions. These techniques encompass both structural and functional modalities, each of which provides unique and complementary insights into psychiatric disorders. Structural techniques include T1-weighted MRI, diffusion-weighted imaging (DWI), and diffusion tensor imaging (DTI). Functional techniques include positron emission tomography (PET), single-photon emission computed tomography (SPECT), magnetic resonance spectroscopy (MRS), electroencephalography (EEG), and functional magnetic resonance imaging (fMRI). In recent years, fMRI has become extremely popular as a method of choice for investigating functional measures of the brain, and a large proportion of recent studies focus on this modality. This popularity is owed to the versatility of the technique, the good balance of spatial and temporal resolution, and its non-invasive procedure, which avoids the injection of a radioactive tracer required for PET and SPECT. This section covers research findings relevant to anxiety disorders and SSRIs which have been obtained through some of these techniques.

Through the utilisation of the various neuroimaging techniques mentioned, several brain regions have been frequently identified as displaying a range of abnormalities associated with anxiety disorders. The most frequently implicated regions are the amygdala, insula, hippocampus, anterior cingulate cortex (ACC), and areas of the prefrontal cortex.

Due to its well-established central role in fear responses and fear-related neural circuitry (99, 100), the amygdala and its connectivity to other brain regions are the focus of a large proportion of neuroimaging studies investigating anxiety disorders. The amygdala is known to have roles in regulating autonomic and hormonal functions, activation of the flight-or-fight responses, controlling neural plasticity, and in decision-making (101). Numerous fMRI studies have found hyperactivity of the amygdala to be a key feature associated with GAD, SAD, and PD (100-104). As amygdala hyperreactivity is a common finding across multiple anxiety disorder subtypes, and is also consistently observed during fear conditioning of healthy subjects, it is widely recognised as having a central role in the "fear circuitry" of the brain (100). A popular study design used in fMRI investigations of anxiety disorders is a task-based design comprising of the presentation of images of various emotional facial expressions and the measurement of brain activity elicited in response (105). A common result of studies using this design is that patients with anxiety disorders typically have greater amygdala reactivity to presentation of facial expressions of emotion (104, 106).

Another common finding across studies is hyperactivity of the insula and hippocampus in individuals with anxiety disorders (107, 108). The insula has roles in regulating the autonomic nervous system, sensorimotor processing, processing threats, decision making, and processing a range of emotions (100, 109). The hippocampus is known to have roles in learning and memory, cognitive functions, and regulation of emotional behaviours, including regulating anxiety states (107). Importantly, the hippocampus down-regulates the amygdala response in the absence of danger (108).

Abnormal activity of regions of the ACC are also widely reported in association with anxiety disorders. Notably, this varies based on ACC subregions, which differ in their functions. The dorsal ACC (dACC) is involved in processing threats and generating fear responses (108). Hyperactivation of the dACC has been reported as a feature of SP (110), SAD (111) and PTSD (108, 112). In contrast, the rostral ACC (rACC) has a role in downregulating the fear response and emotions (113). The rACC is often found to be hypoactive in individuals with anxiety disorders; however, this finding is less consistent than that of the dACC, with some studies reporting hyperactivity of the rACC in anxiety disorders (108).

The medial prefrontal cortex (mPFC) has been observed to have abnormal activity in individuals with anxiety disorders. The mPFC is known to have a function in regulating threat responses in threat processing regions such as the dACC, insula and amygdala by downregulating their activity. Study findings are mixed, with findings of both hyperactivity and hypoactivity of the mPFC associated with anxiety disorders (114, 115). It seems likely that specific differences in activity levels indicate distinct symptom profiles. One theory is that hyperactivation of the mPFC is indicative of an over-compensatory response to an overactive fear response. In contrast, hypoactivation may suggest a deficit in emotional regulation (108).

In addition to assessing brain region activity levels, neuroimaging studies have also investigated the functional connectivity of brain regions in the context of anxiety disorders. Similarly, fMRI has become a widely employed neuroimaging modality for this purpose. Functional connectivity analysis has provided insights into which brain regions or networks of brain regions have different synchronicity of their activity in association with anxiety disorders relative to individuals without these disorders.

Several frequently reported functional connectivity abnormalities exist across the subtypes of anxiety disorders. Generally, this includes decreased connectivity between brain regions responsible for the generation and processing of emotions, such as the amygdala and insula, with cortical regions which regulate these emotions, such as the mPFC, dorsolateral prefrontal cortex and the rACC (101, 108, 116). Studies which have focused on networks of brain activity have reported intrinsic hyperconnectivity of the limbic system, as well as hyperconnectivity with other networks, including the sensorimotor network, salience network, and default mode network (DMN) (117). The sensorimotor network has been reported to display differences in its functional connectivity and activity in anxiety disorders, which has been speculated to be related to greater muscle tension and hypervigilance (116). Additionally, hypoconnectivity between the amygdala and the DMN, executive control network, and various limbic and subcortical areas have been reported (118-120).

Structural neuroimaging studies have reported various associations of brain volume and density abnormalities with anxiety disorders. However, these results vary considerably between the anxiety disorder subtypes and findings of different studies are often contradictory. For example, the amygdala has been reported to be increased in volume in individuals with GAD and decreased in those with PD and SP in comparison to healthy controls (108). Independent studies have reported reduced amygdala volume, no difference in amygdala volume, and increased amygdala volume in people with SAD relative to controls (108). This inconsistency

in volume differences across studies and across anxiety subtypes is also a feature of the insula, ACC and mPFC (108, 117). These findings suggest that the subtypes may have distinct neural structural differences; however, it is possible that some of these inconsistencies are a result of differences in study populations or due to the frequent comorbidities of anxiety disorders, which are handled differently across independent studies.

Diffusion tensor imaging has been used to investigate structural connectivity of the white matter tracts between brain regions in anxiety disorders. A range of structural integrity deficits of white matter tracts have been observed in association with anxiety disorders, with deficits of the white matter of the left ACC, right postcentral gyrus, corpus callosum, bilateral inferior frontal-occipital fasciculus, and bilateral uncinate fasciculus having been reported (121-124). Notably, the uncinate fasciculus is a white matter fibre tract which connects the orbitofrontal cortex to the ACC, amygdala, and various other limbic regions, so the impaired structural connectivity of this region corroborates the finding of impaired functional connectivity within these same regions. The white matter of the right amygdala has been reported to have increased structural integrity in those with GAD, as indicated by increased fractional anisotropy, which also aligns with functional neuroimaging observations of increased amygdala activity and connectivity (122).

In addition to their application in the investigation of anxiety disorders, neuroimaging techniques can be applied to elucidate how interventions, such as psychotherapies or medications correlate with brain activity (125). This provides information regarding the broader mechanism of action of these interventions, complementary to other methods (126, 127).

Most neuroimaging studies investigating the neural correlates of SSRI use have focused on their use in treating their original target, depressive disorders, with a smaller proportion concentrating on their use specifically for anxiety disorders. Although more studies which specifically target SSRI use in the treatment of anxiety disorders are required, many of the same brain regions are implicated in anxiety disorders as the frequently comorbid depressive disorders (128).

Numerous fMRI studies have found correlations between the use of SSRIs with changes in the levels of activity within certain brain regions. Common findings include reduced activation of the amygdala, hippocampus, parahippocampus and mPFC during fear-inducing stimuli after daily SSRI administration over a period of seven days to several months (129, 130). Hyperactivation of the amygdala in response to negative stimuli, which is associated with anxiety and depressive disorders, has been observed to be normalised after sustained

administration of a therapeutic dose of SSRIs, and has been correlated with an improvement in anxious and depressive symptoms (131-134). Similarly, hyperactivation of the insula in association with anxiety and depressive disorders has been reported to be attenuated in response to either SSRI or CBT treatment, with a greater decrease in insular activity corresponding to a more significant improvement in symptoms (134). The normalisation of levels of brain activity has also been associated with successful treatment with ketamine, indicating a similarity in its mechanism to SSRIs (135).

There have also been neuroimaging studies which have investigated the effects of SSRIs on functional connectivity of the brain. Some studies have reported a reduction in the elevated functional connectivity of the DMN associated with depression in response to SSRI administration, which has been correlated with an improvement in symptoms (136-138). This reduction in functional connectivity has also been observed by studies focused on healthy controls, both acutely after SSRI administration and after long-term continuous treatment (139, 140). Increased functional connectivity between cortical and limbic brain regions has been reported as an effect of SSRI use over several weeks (141). This includes reports of increased functional connectivity between the ACC with both the thalamus and amygdala (141) and increased functional connectivity between the ventrolateral prefrontal cortex and the amygdala (142). A recent randomized controlled trial utilised SPECT imaging to assess SERT occupancy with citalopram and rs-fMRI to evaluate DMN connectivity. The study involved the randomization of forty-five healthy female participants with a mean age of 21.6 into three groups: placebo, low-dose (4mg) citalopram, and high-dose (16mg) citalopram. It measured SERT occupancy with citalopram and assessed resting-state functional connectivity using rs-fMRI. The results showed that increasing occupancy of SERT was negatively associated with DMN connectivity with the ACC, postcentral gyrus, paracingulate gyrus, temporal pole, and superior parietal gyrus. These observations indicate that there is dose-dependent relationship between citalopram use and DMN connectivity (143).

Another recent randomised, placebo-controlled, double-blind crossover study conducted by Klaassens and colleagues investigated the acute effects of citalopram administration on functional connectivity over several hours (144). This study focused on 10 well-defined template resting-state functional networks identified by Smith et al., which are frequently cited throughout the literature (145). At 3.5 hours post-citalopram administration, a decrease in functional connectivity between the right frontoparietal network and Heschl's gyrus, as well as the insula, was reported relative to the placebo group. Following this, 4.5 hours after citalopram

administration, a decrease in functional connectivity between the sensorimotor network and numerous brain regions was observed relative to the placebo. These regions included the precentral and postcentral gyri, supplementary motor area, precuneus, posterior cingulate cortex (PCC), ACC, medial prefrontal cortex, planum temporale, and Heschl's gyrus.

A similar study by the same research group investigated the acute effects of sertraline on functional brain connectivity after a single dose (146). Similarly, this study utilised the 10 resting-state networks defined by Smith et al. as templates. Once again, a decrease in functional connectivity of the precentral and postcentral gyri, supplementary motor area, precuneus, PCC, and ACC with the sensorimotor network was reported within several hours of sertraline administration, relative to placebo. This study also reported decreased functional connectivity of the DMN with the precuneus, PCC, ACC and medial prefrontal cortex, as well as decreased connectivity of the executive control network with the amygdala, cingulate cortex, hippocampus, and thalamus. The results of these studies suggest that SSRIs induce a rapid, widespread change in functional connectivity of various resting-state networks on a scale of several hours. However, the therapeutic effects of SSRIs do not become apparent until several weeks of repeated administration, indicating that the functional connectivity changes reported do not directly correspond to an improvement in symptoms. Future studies could build on the findings of these studies by investigating how functional connectivity differences change over several weeks and relating these changes to symptomatic improvements.

Although many insights into psychiatric conditions and their treatments have been obtained using neuroimaging techniques, a gap remains in the literature surrounding the interventions used to treat anxiety disorders. Many of the neuroimaging studies which have investigated neural correlates of anxiety disorder treatment options, such as those mentioned in the preceding paragraphs, have focused on the use of these interventions in the context of treating depressive disorders, with a relatively small proportion specifically focusing on their application in treating anxiety disorders. This might be attributed to the fact that many of the treatment options for anxiety disorders are identical to those used in depressive disorders, such as SSRIs and CBT, with many of these interventions being initially created for the treatment of depressive disorders before serendipitously being found to be effective for anxiety disorders. Furthermore, due to the very high comorbidity rates of other psychiatric conditions concomitant with anxiety disorders, recruitment of participants without comorbidities and who are taking an SSRI can be challenging. It is also possible that less research effort is focused on anxiety disorders due to the perception of clinicians and the broader public that anxiety disorders are

less prevalent and disabling than they realistically are (74). It has been found that anxiety disorders are severely underdiagnosed and are among the psychiatric disorders with the lowest recognition rates (147, 148). Additionally, there is a belief held by some clinicians that anxiety disorders are adequately treated with the currently available interventions despite the high rate of treatment failure, resulting in less attention and research focused on anxiety disorders compared to other psychiatric conditions like depression (74, 149).

It is also important to acknowledge the limitations typically associated with neuroimaging studies. Many neuroimaging studies suffer from a low number of participants, often around 20-30, resulting in low statistical power (25). This occurs due to practical limitations, such as the high monetary cost of obtaining neuroimaging data for each participant and the significant time required by both participants and researchers in obtaining neuroimaging data suitable for analysis. Furthermore, many steps are typically involved in the preprocessing of neuroimaging data, which contributes to this time requirement. Additionally, in the process of collecting neuroimaging data, there is the risk of some of the data being unusable for numerous reasons, further increasing monetary and time costs. Another limitation of neuroimaging studies, specifically those focused on anxiety disorders, is the potential for comorbidities commonly associated with anxiety disorders to impact study results. These comorbidities can introduce a bias into the findings of neuroimaging studies, resulting in associations of conditions distinct from anxiety disorders being attributed to anxiety disorders if not adequately controlled for. Finally, there is a lack of standardised procedures used for neuroimaging studies; for example, task-based fMRI studies have used many different conditions as the task, making coalescence and comparison of these studies difficult.

3. Methods

This chapter details the research design, beginning with a description of the participant selection and matching procedure in section 3.1. Next, the procedure used in acquiring and preprocessing fMRI data is detailed in section 3.2. Subsequently, the process used in performing group-level independent component analysis (ICA) is explained in section 3.3, before the conclusion of this chapter, with a description of the dual regression procedure employed and the associated statistical analysis in section 3.4.

3.1. Participant Selection and Matching

This research makes use of the dataset collected by the UK Biobank. This large-scale prospective study is collecting a wide range of genotypic and phenotypic details from a cohort of over 500,000 participants (150). At recruitment, participants were aged between 40 to 69 years. The UK Biobank aims to collect imaging data from 100,000 of these participants, with data collection still in progress. The data release used in the present study included rs-fMRI data for a total of 49,128 participants, prior to any exclusions due to confounding diagnoses or "unusable" data (described later in this section).

This research only considered data from the participants' first imaging session, as the available data release had a limited number of participants with data from a repeat imaging session. The present study, therefore, uses a cross-sectional design to investigate the correlations in functional connectivity associated with SSRI use across subject groups. From the participants who had useable rs-fMRI data collected at the first imaging session, two case groups and one control group were selected based on the lifetime presence of any anxiety disorder and SSRI use at the time of the first imaging session.

In order to select participants for each of the three groups, several steps were taken. First, from a total of 502,367 participants included in the data release, 98,018 participants with a history of certain health conditions with the potential to confound results were excluded. The excluded health conditions included cancers, neurological disorders, psychiatric disorders, cardiovascular and cerebrovascular disorders, and respiratory failure. In order to preserve subject numbers and statistical power, participants with a history of depressive disorders were not excluded. Instead, the frequency of depressive symptoms was treated as a covariate to

control for acute depressive effects later in the analysis. The high comorbidity rates between anxiety and depressive disorders informed this decision. Multiple sources were used to assess participants for a history of these conditions, including self-reported conditions at assessment centre appointments, answers given by participants within an online follow-up mental-health questionnaire, hospital diagnoses using both ICD-9 (International Classification of Diseases, Ninth Revision) and ICD-10 diagnosis codes (International Classification of Diseases, Tenth Revision). A full list of the excluded conditions is included in Appendix A. Next, from the remaining 404,349 participants, 370,856 participants who did not have usable rs-fMRI data available for the first imaging session were excluded. This included 367,403 participants without any data and 3,453 with "unusable" data. "Unusable" data refers to data that the UK Biobank determined to be "corrupted, incomplete, missing or otherwise clearly unusable" (150). Of the 33,493 participants with usable data, 14,804 met the case or control inclusion criteria; however, 498 participants were excluded due to missing covariate data, leaving 14,306 participants. These remaining participants were grouped into one of three groups based on lifetime anxiety disorder status, current SSRI use at the imaging session, and other medication use. Details of the criteria used to classify participants as having a lifetime history are provided in Appendix B. At this stage, 122 participants met the inclusion criteria for the Anxiety + SSRI group, 1,499 participants met the Anxiety + No Medications group criteria, and 12,685 participants met the Control group criteria. The Control group consisted of participants who met the same criteria as the Anxiety + No Medications case group but lacked a lifetime history of anxiety disorders.

In selecting participants for the Anxiety + SSRI case group, participants were included if they reported current use of any of six distinct SSRI medications during the verbal interview at the imaging session. These are citalopram, fluoxetine, sertraline, paroxetine, escitalopram, and fluvoxamine. In most cases, the generic names of the SSRIs used by participants were recorded; however, for a smaller proportion of participants, these medications were recorded using various brand names. For example, fluoxetine appeared under three different names: "fluoxetine", "prozac 20mg capsule", and "oxactin 20mg capsule". Each SSRI's generic and brand names were used in the participant grouping process. No information regarding dosing or formulation was available for these medications.

A two-stage matching process was employed to ensure that the participant groups were well-balanced across several covariates. In both stages, propensity scores were estimated using

logistic regression, followed by matching using a genetic algorithm without replacement. This procedure was implemented using the R package "MatchIt", which leverages the "Matching" R package to optimise covariate balance across groups by iteratively evolving the population of participants selected towards an optimal solution. Further details of how these packages work, along with the genetic algorithm they utilise are available online (151-153). A 1:1 matching ratio was used in both matching stages to achieve an equal number of participants across the combined case and Control groups. This avoids the overrepresentation or underrepresentation of subject groups in the group-level functional connectivity maps obtained from independent component analysis (ICA) (154). Matching without replacement ensured that each participant would only be included once in the analysis. Both linear and quadratic covariate terms were incorporated in the matching procedure in order to improve covariate balance.

The covariates used in the first matching stage were age, age squared (age^2), sex, date of scan, head motion, head motion squared (head motion^2), number of self-reported anxiety symptoms, number of self-reported anxiety symptoms squared (number of self-reported anxiety symptoms^2), depressed mood frequency, and depressed mood frequency squared (depressed mood frequency^2). These covariates were chosen to ensure that the baseline characteristics and symptom profiles of the two case groups were similar. Matching case groups based on anxiety and depression symptoms aimed to enable differentiation of any functional activity correlations with SSRIs from functional connectivity correlations with acute anxiety or depression symptoms. The "number of anxiety symptoms" covariate was calculated by summing the number of times each participant answered "Yes" to the following five questions at their imaging session: "Would you call yourself a nervous person?" (Data-field 1970), "Are you a worrier?" (Data-field 1980), "Would you call yourself tense or 'highly strung'?" (Data-field 1990), "Do you worry too long after an embarrassing experience?" (Data-field 2000), and "Do you suffer from 'nerves'?" (Data-field 2010). Possible answers to these questions were: "Yes", "No", "Do not know", and "Prefer not to answer". Participants who had missing data for any of these questions and those that answered "Do not know" for all five questions were excluded. The "depressed mood frequency" covariate was based on participant answers to the imaging session touchscreen question: "Over the past two weeks, how often have you felt down, depressed, or hopeless?" (Data-field 2050). The possible answers were: "Not at all", "Several days", "More than half the days", "Nearly every day", "Do not know", and "Prefer not to

answer". Participants who answered "Do not know" or "Prefer not to answer" were excluded in addition to those with missing data for this data field.

Following the first matching stage, covariate balance was assessed with the use of Love plots and density plots. Two Love plots were created using the "cobalt" package within R, one depicting standardised mean difference and the other depicting the variance ratios of covariates between the two subject groups being matched (155, 156). A maximum absolute standardised mean difference threshold of 0.05 was used, as this has been recommended as a conservative threshold to ensure covariates are well-balanced across groups (157-159). For the assessment of variance ratios, a range from 0.5 to 2.0 is frequently used, with ratios falling within this range being considered well-balanced for that covariate across groups (159, 160). A more conservative range of 0.75 to 1.25 was used for matching subjects to ensure covariates were exceptionally well-balanced across subject groups. As "sex" is a binary covariate, the raw difference in the proportion of each sex was calculated rather than the standardised proportion difference. The raw difference in proportion provides a more intuitive interpretation and better predicts bias when compared to the standardised difference in proportion for binary covariates (161, 162). Additionally, the variance ratio for the "sex" covariate was not calculated, as variance ratios of binary covariates provide no additional information over the difference in proportion and can be misleading (158). Density plots showcasing covariate balance before and after the first matching stage were created using the "plot" function provided by the "MatchIt" package within R (151, 155).

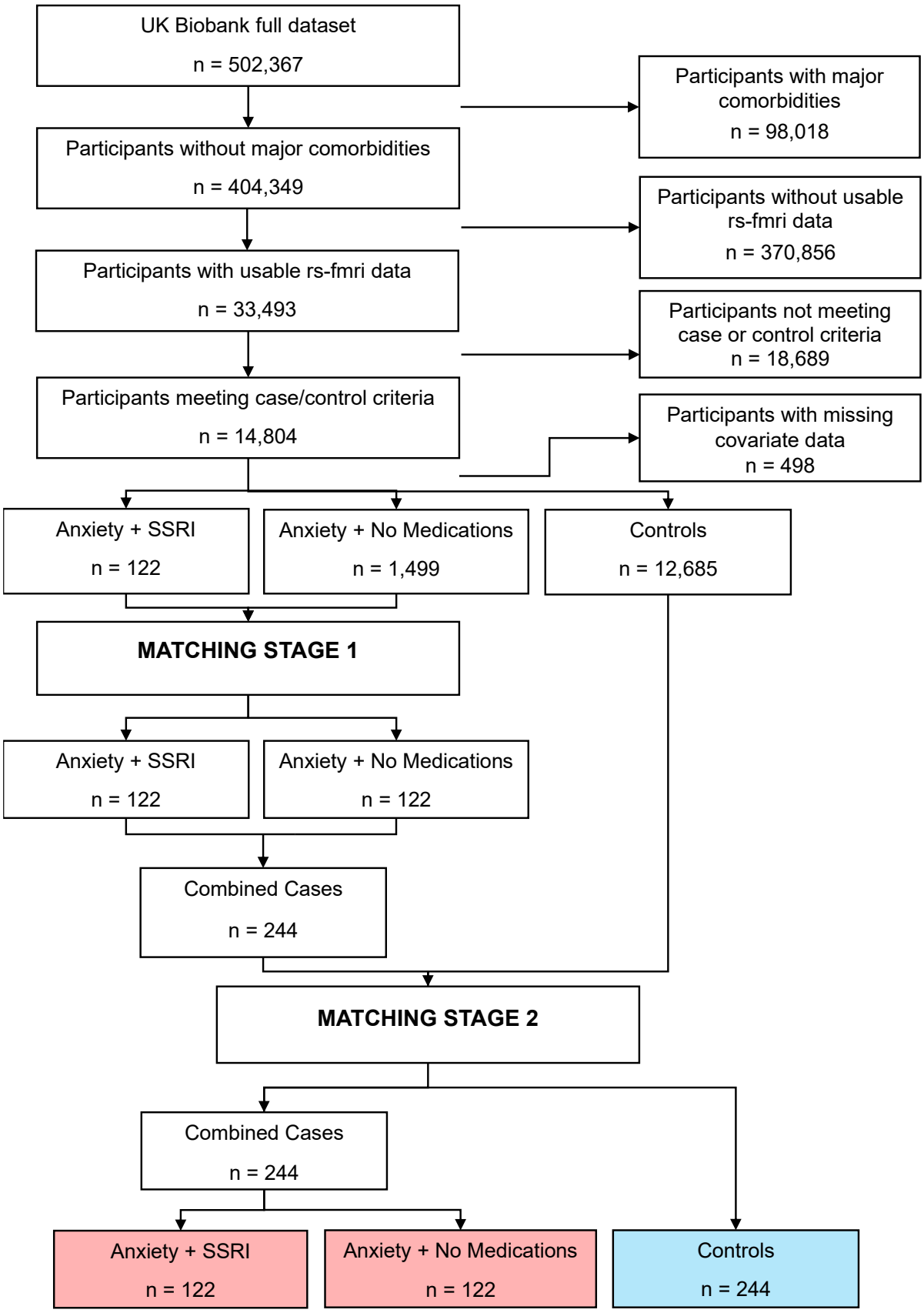
In order to achieve the best possible overall covariate balance, several different matching methods and parameters were trialled and compared. This included nearest neighbour matching, optimal pair matching, and genetic matching methods (163). Each of these methods was trialled using both logit and probit link functions. Of these methods, the genetic method produced the best balance of covariates across groups, using a generalised linear model (GLM) and logit link function to estimate propensity scores and a population size of 200.

The second matching stage involved combining the two case groups into one combined case group of 244 subjects. The covariates used in the second matching stage were age, age^2, sex, date of scan, head motion, and head motion^2. After this matching stage, the balance of these covariates was again assessed using the same method and thresholds as described for the first stage.

After both matching stages, 488 participants were included in the rs-fMRI analysis: 122 in the Anxiety + SSRI group, 122 in the Anxiety + No Medications group, and 244 in the No

Anxiety + No Medications control group. **Error! Not a valid bookmark self-reference.** depicts a flowchart summary of these steps.

Figure 1: *Selection Process for Participant Groups.*



3.2. Resting-State Functional MRI Data Acquisition and Preprocessing

The rs-fMRI data used in the present study was acquired and preprocessed by UK Biobank. Comprehensive details regarding the acquisition and data processing protocol can be accessed online (150, 164). In brief, imaging data was acquired using a standard Siemens Skyra 3T scanner with a standard Siemens 32-channel radiofrequency receive head coil. Rs-fMRI data preprocessing consisted of motion correction using the Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL) linear image registration tool (MCFLIRT) (165), grand-mean intensity normalisation, highpass temporal filtering, EPI (Echo-planar imaging) unwarping, gradient distortion correction (GDC) unwarping, followed by removal of structured artefacts using ICA+FIX processing (Independent Component Analysis followed by FMRIB's ICA-based X-noiseifier) (166, 167). This preprocessing pipeline produced cleaned, filtered functional timeseries data in native fMRI space for each participant with usable data. This cleaned, filtered data for each selected subject was downloaded from the UK Biobank database as "bulk" files in NIfTI format. Following the download of the native space rs-fMRI data, the data for each subject was aligned to standard T1-weighted MNI152 2mm space (specifically, the MNI152 nonlinear 6th generation atlas) using the FSL "applywarp" tool (168, 169). The MNI152 atlas was created by combining the structural T1-weighted images of 152 young, healthy adults into an image representing this population's average brain structure (170). This alignment ensures that every subject's functional data uses a common coordinate system so that a specified coordinate corresponds to the same anatomical location across all subjects. This enables the combination of functional data from multiple subjects and for comparison of local signal intensities across subjects.

3.3. Group-Level Independent Component Analysis

In order to identify functional networks common across all subjects, group-level independent component analysis (ICA) was performed using the cleaned, filtered, standard-space registered functional data from all 488 subjects. This data-driven approach was chosen over an alternative option, which is to use pre-defined atlases of functional connectivity networks. This decision ensured the most accurate representation of the resting-state functional connectivity patterns specific to the included participants. Group-level ICA was conducted by first temporally concatenating the data from all subjects and then feeding the concatenated data into the FSL MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components) tool, with a dimensionality of 25 independent components (166).

This dimensionality was chosen as it is one of the two dimensionalities used by the UK Biobank to derive their correlation matrices of functional connectivity, with the other being a dimensionality of 100. This minimises the difference between the components derived in this study and the UK Biobank, enabling comparison of results with other studies using the UK Biobank-derived components while preserving the functional network structure specific to the included participants. The output consists of a timeseries for each group-level component comprising the concatenation of all subject timeseries and a spatial map for each component. These spatial maps depict brain regions with a high correlation in their timeseries. Spatial maps were visually inspected using FSL's "FSLeyes" image viewer to eliminate non-neuronal noise components (171). All other non-noise components were classified as neuronally-based components.

In order to relate the neuronally-based components to resting states commonly observed and referred to in the literature, the FSL "fslcc" tool was used to correlate these components with a set of 10 well-known and well-defined resting state networks (172). These 10 networks are those described in detail in a widely cited 2009 article by Smith et al. (145). A common practice in ICA studies is the investigation of only the components with correlation strengths with established networks above an arbitrary threshold. However, in this case, it was decided to include all components identified as neuronally-based in the group comparison analysis, regardless of their correlation strengths. This decision ensured that no differences in functional connectivity between subject groups were overlooked due to variations in ICA decomposition.

3.4. Dual Regression and Statistical Analysis

A dual regression analysis was used to statistically compare the three subject groups across all neuronally-based resting-state networks of interest obtained from group-level ICA. This was performed using FSL's dual regression tool, and subsequently, statistical testing was performed using FSL's randomise tool (173-175). This process consisted of three steps, which are described in detail in the remainder of this section.

In the first step, a multivariate spatial regression was conducted using each participant's 4D cleaned, filtered, standard-space functional data as the input (the dependent variable) and the group-ICA spatial maps as the regressors (independent variables). All 25 group-ICA spatial maps were incorporated in this step, including components labelled as artefacts. Including these artefact components was essential, as they explain some of the variance in the data and, therefore, minimise the error term in the General Linear Model (GLM). The output of this first

stage was the estimated timeseries corresponding to each group-ICA spatial map for each participant.

The second step consisted of a multivariate temporal regression. The timeseries data obtained from the first step underwent timeseries normalisation (i.e., each ICA component's timeseries was divided by its standard deviation, scaling each timeseries to have a variance of 1) and were used as the regressors (independent variables) in this step. The output of this regression is a set of 25 spatial maps for each subject. Each spatial map represents a group-ICA spatial map, represented using the data of a single subject. As a result of timeseries normalisation, these spatial maps represent both the shape and strength of the group-ICA component within each subject (154). For each component, the FSL dual regression tool automatically combined the spatial maps representing that component from all subjects into a single output.

In the third step, FSL's randomise tool was used to test for differences in functional connectivity between the three subject groups. From the spatial maps output by the second step (stage two of dual regression), the maps corresponding to the components earlier identified as neuronally-based were tested in this stage, whereas the components labelled as artefacts were ignored. A one-way analysis of covariance (ANCOVA) was conducted on each of the neuronally-based components to determine if there were any significant differences in the mean functional connectivity among the three subject groups. The independent variable for these analyses was the subject group. This was conducted using a general linear model (GLM) design with FSL's randomise tool to perform non-parametric permutation testing. For each component, 10,000 permutations were performed. This involved 10,000 random reassignments of the allocation of subjects between the three subject groups, calculating the threshold-free cluster enhancement (TFCE) adjusted F-statistic of each voxel for each configuration of subject groups, and then constructing a null distribution consisting of the maximum TFCE adjusted F-statistics from each permutation (176). Briefly, TFCE adjusts the F-statistics of each voxel based on the F-statistic values of surrounding voxels. This involves amplifying a voxel's value when it is surrounded by other voxels with relatively large F-statistics and decreasing a voxel's value when voxels with relatively small F-statistics surround it. This means that the values of the voxelwise F-statistics and the spatial extent of "clusters" of voxels are accounted for in the TFCE-adjusted F-statistics (176). Using this method ensures that voxel clusters comprising of moderately large F-statistics distributed over a large spatial region, which is expected of

neuronal signals, are not missed. Additionally, isolated voxels, which are more likely to represent noise, are less likely to be considered statistically significant.

Following the construction of the maximum TFCE-adjusted F-statistic distribution for a particular component, the randomise tool calculated the multiple comparison corrected p-values for each voxel. This was achieved by calculating the F-statistic for every voxel using the subject groups' original, non-permuted configuration. Next, the proportion of maximum F-statistics from the null distribution that was greater than that voxel's F-statistic was calculated for each voxel. This proportion is the multiple comparison corrected p-value, and by setting a significance threshold of less than 0.05, the family-wise error rate was controlled at 5%. In other words, there was a 5% chance of observing one or more false positive results across all voxels within the brain for a particular independent component. To enable convenient display and thresholding, the randomise tool automatically converted the p-values for each voxel into one minus p-values.

Six covariates were controlled for in the ANCOVA tests: age, sex, date of scan, head motion, number of self-reported anxiety symptoms, and frequency of depressed mood within the two weeks preceding the MRI scan. The demeaned values of each of these covariates were entered into the GLM analysis as regressors. These values were calculated by subtracting the overall mean value of the covariate across all three groups from each subject's covariate value. Sex was encoded numerically, with the value "0" corresponding to "Female" and "1" corresponding to "Male". Sex was then demeaned in the same way as the numerical covariates. The "date of scan" covariate was calculated as the number of days elapsed from the first participant's scan to the scan date of each subsequent participant.

In order to assess the statistical significance of the ANCOVA test results, several steps were taken. First, the maximum voxelwise one minus p-values was determined for each of the 18 components using the "fslstats" tool (169). These values were converted into p-values and then corrected for multiple comparisons across the number of neuronally-based independent ANCOVA tests using the "p.adjust" function from the R "stats" software package (155). This function applied a Benjamini–Hochberg correction for the minimum p-values associated with each neuronally-based component (177). These corrected p-values were assessed for significance against the threshold of 0.05, with any components having a minimum p-value less than 0.05 being considered statistically significant. Using the Benjamini–Hochberg method with this threshold controls the false discovery rate (FDR) at 5%, meaning that, on average, up to 5% of components showing a significant group difference may be false positives. This

method was chosen due to its advantage of higher statistical power and reduced chance of making type II errors (false negatives) relative to methods that control the family-wise error rate (FWER), which can become overly conservative when a greater number of independent tests are performed (177). This research is exploratory rather than confirmatory in nature, so greater importance was placed on avoiding false negative results than false positives. However, this correction is conservative relative to many exploratory studies in the neuroimaging literature, where it is not uncommon for no multiple comparison corrections to be performed (154).

To determine which of the three groups were significantly different and the directionality of the group difference (i.e., which group showed higher functional connectivity on average than another group), group-ICA components that showed a statistically significant ANCOVA result were tested further using post hoc pairwise t-tests. These t-tests were also performed using a GLM design, which controlled for the same six covariates as the ANCOVA tests. Six post hoc pairwise t-tests were performed for each group-level ICA component, and their statistical significance was assessed following another correction for multiple comparisons. Similar to the ANCOVA tests, the Benjamini–Hochberg p-value correction method was applied to these post hoc tests using the "p.adjust" R function (155, 177). Post hoc t-tests that returned a corrected p-value of less than 0.05 were considered statistically significant. Again, applying the Benjamini–Hochberg method controlled the false discovery rate at 5%, meaning that up to 5% of the post hoc t-tests for each ICA component may be false positive results.

For each component that differed significantly between subject groups, details regarding clusters of significant voxels were found using the FSL "cluster" tool (178). Only clusters comprising at least 20 voxels were considered for reporting and visualisation.

To further elucidate which specific regions of the brain differed in functional connectivity between subject groups, the FSL "atlasquery" tool (179) was utilised in conjunction with binarised masks of the group-ICA maps, created using the FSL "fslmaths" utility (169, 172), and the TFCE-corrected one-minus p-value maps associated with significant post hoc t-tests. These masks were used to estimate the average probability of a voxel being found within regions defined in the Harvard-Oxford Cortical Structural Atlas and the Harvard-Oxford Subcortical Structural Atlas (180).

4. Results

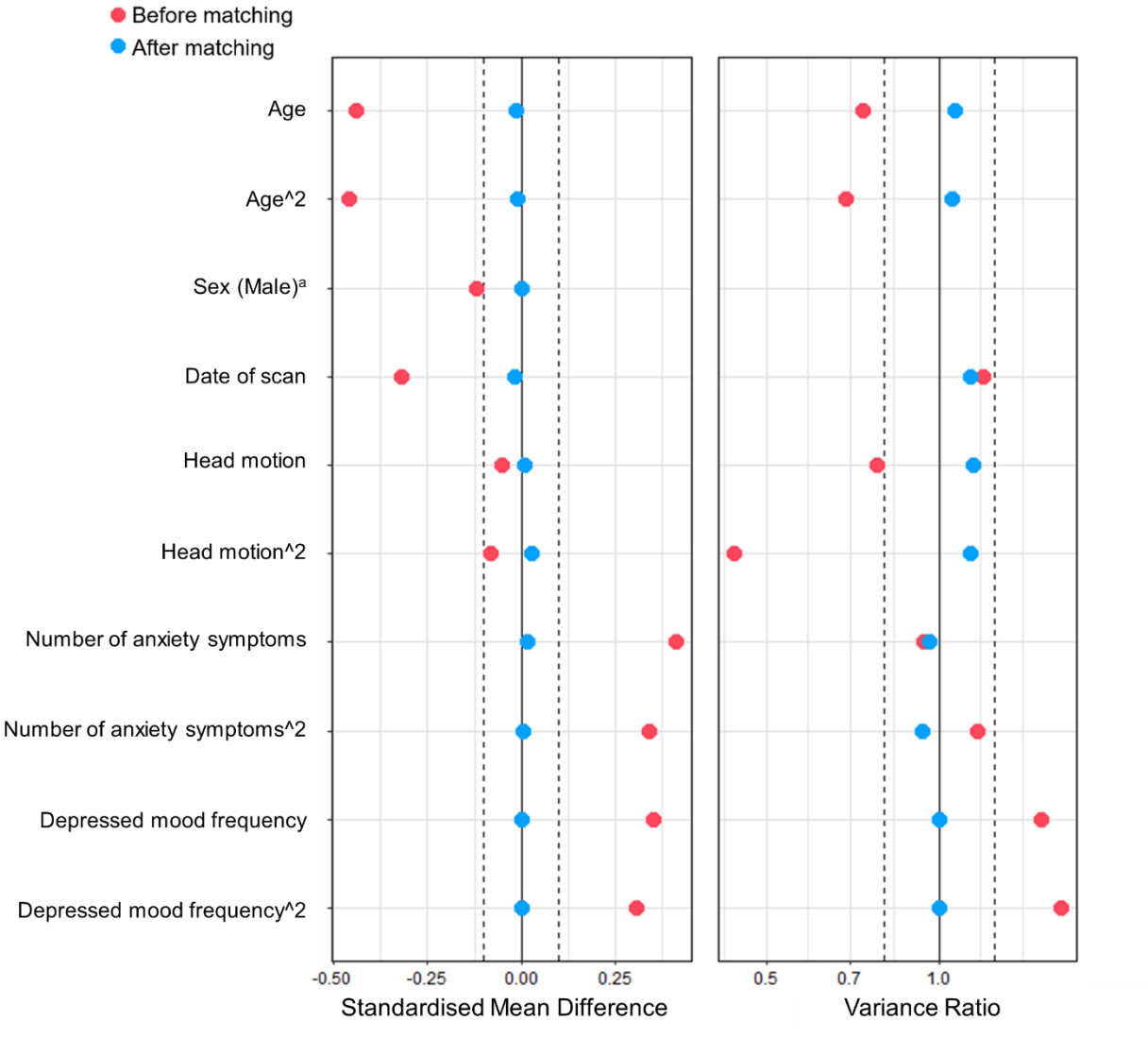
This chapter begins by presenting the results of the covariate balance assessment performed after each matching stage within section 4.1. Next, section 4.2 covers the distribution of the demographics and clinical characteristics of the final participant groups. Finally, the results of the dual regression and associated statistical analysis for differences in functional connectivity within resting-state networks are presented in section 4.3. For each independent component found to be significantly different, a subsection details results specific to that component (sections 4.3.1 to 4.3.3).

4.1. Covariate Balance Assessment

The first stage of matching greatly improved covariate balance, resulting in excellent balance across all covariates between the two case groups. After matching, all continuous covariates' absolute standardised mean differences fell below the conservative threshold of 0.05, with a maximum mean standardised difference of 0.0267 for "head motion^2". Additionally, all covariate variance ratios fell within the conservative target range of 0.75 to 1.25, with the most extreme being 1.15 for head motion. Figure 2 visually depicts these results as Love plots of standardised mean difference and variance ratio for each covariate across the subject groups before and after matching. The improvement in covariate balance across subject groups before and after this first matching stage is also demonstrated in the form of density plots in Figure 3.

The second matching stage also resulted in a well-balanced covariate distribution, this time between the combined case group and Control group. The standardised mean differences of all continuous covariates fell below the conservative maximum absolute value threshold of 0.05, with the most extreme being date with a standardised mean difference of 0.0024. The variance ratios of all continuous covariates fell within the acceptable range of 0.75 to 1.25, with the most extreme being "date of scan" with a variance ratio of 1.06. Figure 4 showcases the Love plots of covariate standardised mean difference and variance ratio across the matched combined case group and Control group before and after the second stage of matching. The improvement in covariate balance before and after the second matching stage is also depicted as density plots in Figure 5.

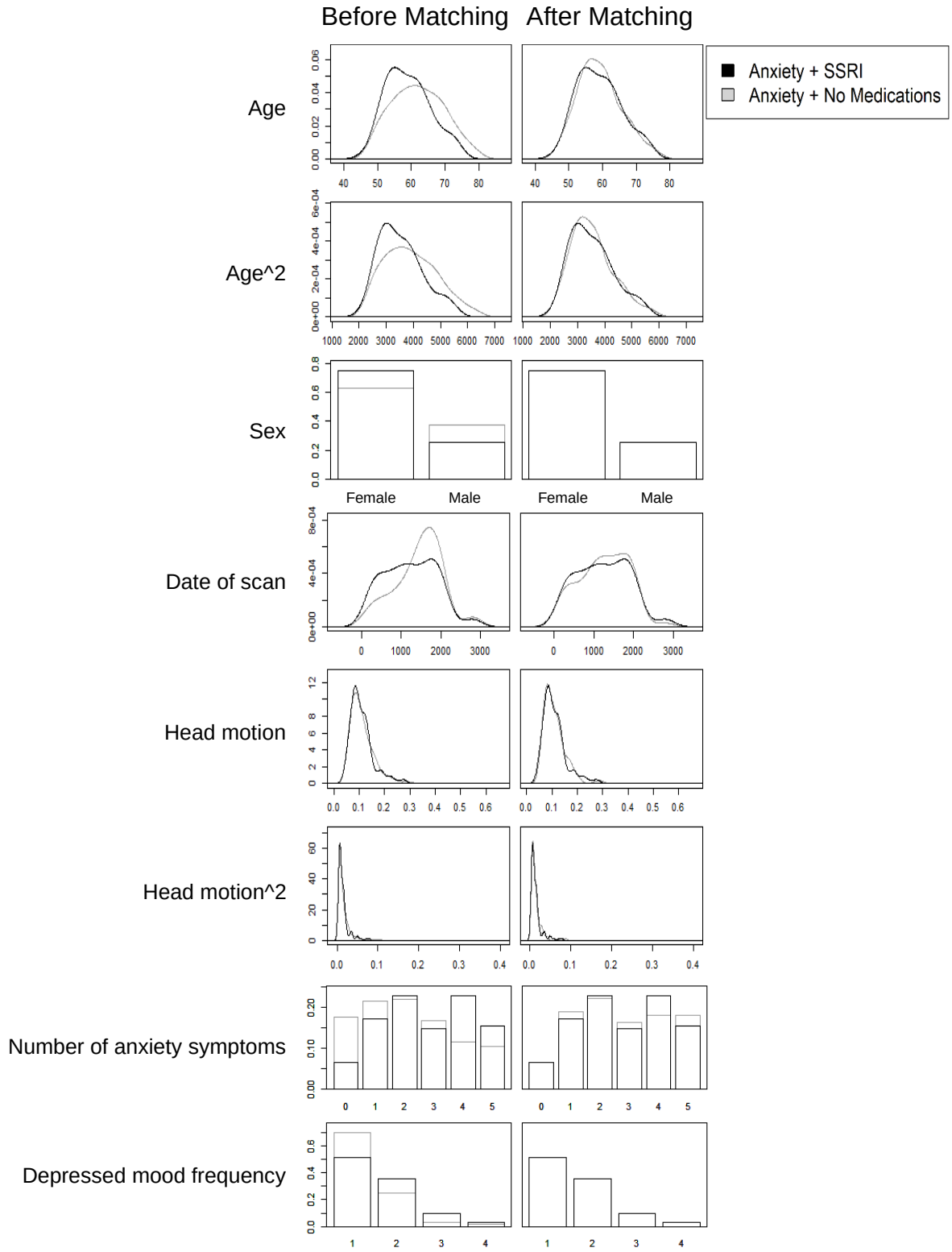
Figure 2: *Love Plots Showcasing Covariate Balance Before and After Stage One of Matching.*



Note. Love plots showcase covariate balance across the two case groups (Anxiety + SSRI, Anxiety + No Medications) before and after stage one of matching. On the standardised mean differences plot (left), dashed lines indicate the range of -0.05 to 1.05. On the variance ratios plot (right), dashed lines indicate the range 0.75 to 1.25. For both plots, data points falling within these ranges indicate an acceptable balance of covariates between participant groups.

[a] Sex (Male) is displayed as a raw, unstandardised mean difference on the left plot and the variance ratio is excluded from the right plot.

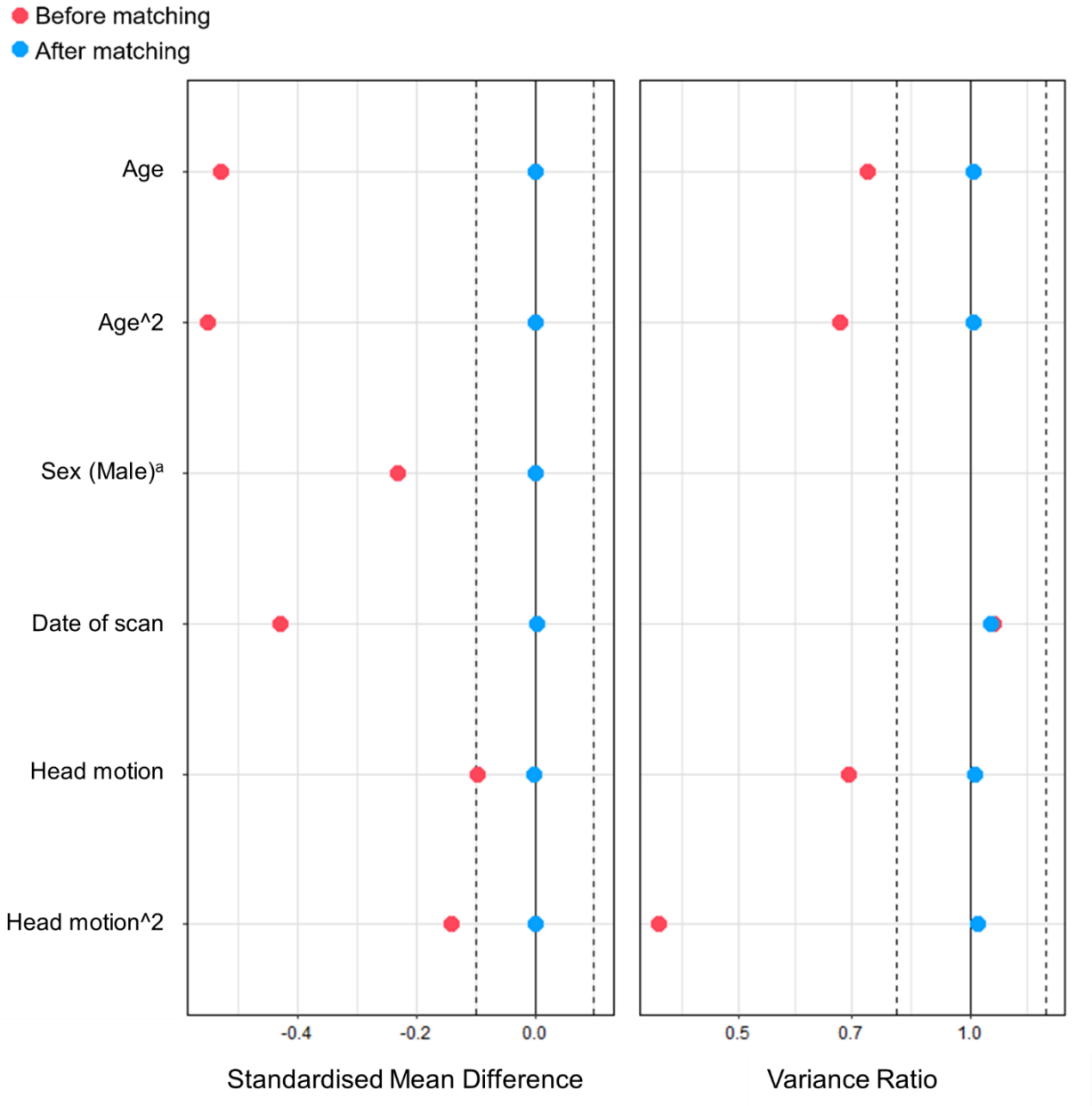
Figure 3: *Density Plots Showcasing Covariate Balance Before and After Stage One of Matching.*



Note. Within each plot, the horizontal axis denotes covariate values, and the vertical axis indicates the density or proportion (for the "sex" plot) of the participants at each covariate value. The black lines represent the Anxiety + SSRI group, and the light grey lines represent the

Anxiety + No Medications group. Greater overlap of the two lines indicates better covariate balance across groups.

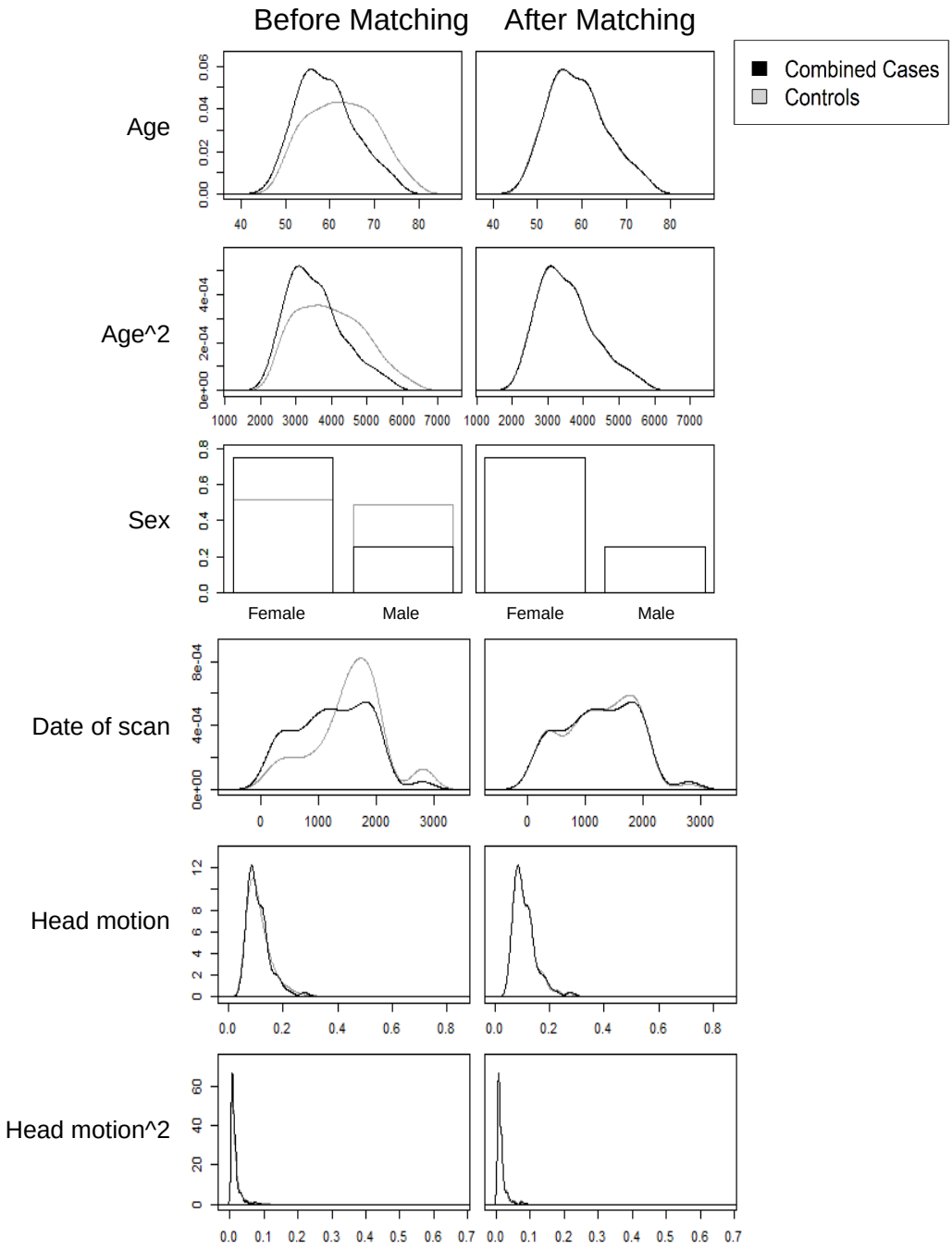
Figure 4: *Love Plots Showcasing Covariate Balance Before and After Stage Two of Matching.*



Note. Love plots showcase covariate balance across the combined case group (including all participants from the Anxiety + SSRI group and the Anxiety + No Medications group) and the Control group (No Anxiety + No Medications) before and after stage two of matching. On the standardised mean differences plot (left), dashed lines indicate the range of -0.05 to 1.05. On the variance ratios plot (right), dashed lines indicate the range 0.75 to 1.25. For both plots, data points falling within these ranges indicate an acceptable balance of covariates between participant groups.

[a] Sex (Male) is displayed as a raw, unstandardised mean difference on the left plot and the variance ratio is excluded from the right plot.

Figure 5: *Covariate Density Plots Before and After Stage Two of Matching.*



Note. Density plots showcasing covariate balance before and after stage two of matching. Within each plot, the horizontal axis denotes covariate values, and the vertical axis indicates the density or proportion (for the "sex" plot) of the participants at each covariate value. The black lines represent the anxiety + SSRI group, and the light grey lines represent the control group (no anxiety + no medications). Greater overlap of the two lines indicates better covariate balance across groups.

4.2. Participant Demographics and Clinical Characteristics

As a result of the matching procedure, the age demographics across all three groups were nearly identical, and the proportions of each sex were identical. As participants were not matched on ethnicity, more variation was observed in this factor across the groups; however, these between-group ethnicity differences were not statistically significant (with p-value of 0.458) using a p-value threshold of less than 0.05.

A statistically significant difference in the distribution of anxiety subtypes was detected between the two anxiety case groups (p-value of 0.011). Further testing indicated that the two case groups only differed significantly in the number of participants with an "unspecified" anxiety disorder and not in any specific named subtypes.

The two anxiety case groups did not differ significantly in the average number of anxiety symptoms, however, both case groups displayed a significantly higher mean number of anxiety symptoms than the control group. Of the five possible anxiety symptoms described in detail within section 3.1, individuals in the Anxiety + SSRI group experienced a mean of 2.8 ± 1.5 symptoms and individuals in the Anxiety + No Medications group experienced a mean of 2.7 ± 1.6 symptoms. The control group was found to have a statistically significant lower mean of 1.2 ± 1.2 out of five anxiety symptoms.

Over the last two weeks, the median depressed mood frequency for all three groups was 1, corresponding to 'Not at all'. This suggests that the typical participant in any of the three groups did not experience any days of depressed mood within this period. The distribution of participants across the four categories of depressed mood frequency was identical between the two case groups. This distribution differed significantly from that of the control group. In each of the case groups, 48.4% of participants reported experiencing some level of depressed mood in the last two weeks, compared to only 18.9% in the control group. Therefore, while the overall frequency of depressed mood was low in all three groups, the two case groups had a significantly higher frequency of depressed mood than the control group.

Details of the demographics and clinical characteristics of the three participant groups are presented in Table 1.

Table 1: *Demographics and Clinical Characteristics of Participant Groups*

Characteristics	Anxiety + SSRI	Anxiety + NM	Controls	p-value
Age (years)				0.982 [a]
Mean ± SD	59.1 ± 6.6	59.2 ± 6.4	59.2 ± 6.5	
Median (Q1, Q3)	59 (54, 63)	58 (54, 63)	58 (54, 63)	
Sex				0.999 [b]
Male	31 (25.4%)	31 (25.4%)	62 (25.4%)	
Female	91 (74.6%)	91 (74.6%)	182 (74.6%)	
Ethnicity				0.458 [c]
White	120 (98.4%)	117 (95.9%)	231 (94.7%)	
Mixed	0 (0%)	2 (1.6%)	6 (2.5%)	
Asian or AB	1 (0.8%)	2 (1.6%)	5 (2.0%)	
Black or BB	0 (0.0%)	0 (0.0%)	3 (1.2%)	
Chinese	0 (0.0%)	1 (0.8%)	0 (0.0%)	
Other	1 (0.8%)	0 (0.0%)	1 (0.4%)	
Anxiety Subtype				0.011 [d]
SAD	8 (6.6%)	4 (3.3%)	0 (0.0%)	
PD	23 (18.9%)	32 (26.2%)	0 (0.0%)	
GAD	83 (68.0%)	91 (74.6%)	0 (0.0%)	
AG	2 (1.6%)	0 (0.0%)	0 (0.0%)	
Unspecified	31 (25.4%)	13 (10.7%)	0 (0.0%)	
Anxiety Symptoms				< 0.001 [a]
Mean ± SD	2.8 ± 1.5	2.7 ± 1.6	1.2 ± 1.2	
Depressed Mood Frequency				< 0.001 [a]
Median (Q1, Q3)	1 (1, 2)	1 (1, 2)	1 (1, 1)	
Not at all (1)	63 (51.6%)	63 (51.6%)	198 (81.1%)	
Several days (2)	43 (35.2%)	43 (35.2%)	38 (15.6%)	
More than half the days (3)	12 (9.8%)	12 (9.8%)	7 (2.9%)	
Nearly every day (4)	4 (3.3%)	4 (3.3%)	1 (0.4%)	

Note. NM = no medications; SD = standard deviation; Q1 = first quartile; Q3 = third quartile; AB = Asian British; BB = Black British; SAD = social anxiety disorder; PD = panic disorder; GAD = generalised anxiety disorder; AG = agoraphobia.

[a] p-value obtained using a Kruskal-Wallis H test comparing all groups.

[b] p-value obtained using a chi-squared test comparing all groups.

[c] p-value obtained using Fisher's exact test comparing all groups.

[d] p-value obtained using Fisher's exact test comparing the "Anxiety + SSRI" and "Anxiety + NM" groups.

At the time of their imaging session, all participants included in the "Anxiety + SSRI" case group reported that they took one of five specific SSRIs, with the most common being citalopram (44.3% of participants) and the least common being escitalopram (3.3% of participants), as shown in Table 2. Notably, due to the relative scarcity of participants taking fluvoxamine (one participant out of 2,474 that attended the first imaging session), no participants taking this particular SSRI were included in this case group. No participants reported taking more than one SSRI concurrently. The distribution of specific SSRIs used by participants in this study group mirrored that of the UK Biobank cohort attending the first imaging session (Table 3).

Table 2*: Distribution of Specific SSRI Usage Among Anxiety + SSRI Group*

SSRI name	Number of participants
Citalopram	54 (44.3%)
Sertraline	34 (27.9%)
Fluoxetine	23 (18.9%)
Paroxetine	7 (5.7%)
Escitalopram	4 (3.3%)
Total	122

Table 3: *Distribution of Specific SSRI Usage Among All Imaging Session One Participants*

SSRI name	Number of participants
Citalopram	990 (40.0%)
Sertraline	741 (30.0%)
Fluoxetine	545 (22.0%)
Paroxetine	126 (5.1%)
Escitalopram	71 (2.9%)
Fluvoxamine	1 (0.0%)
Total	2,474

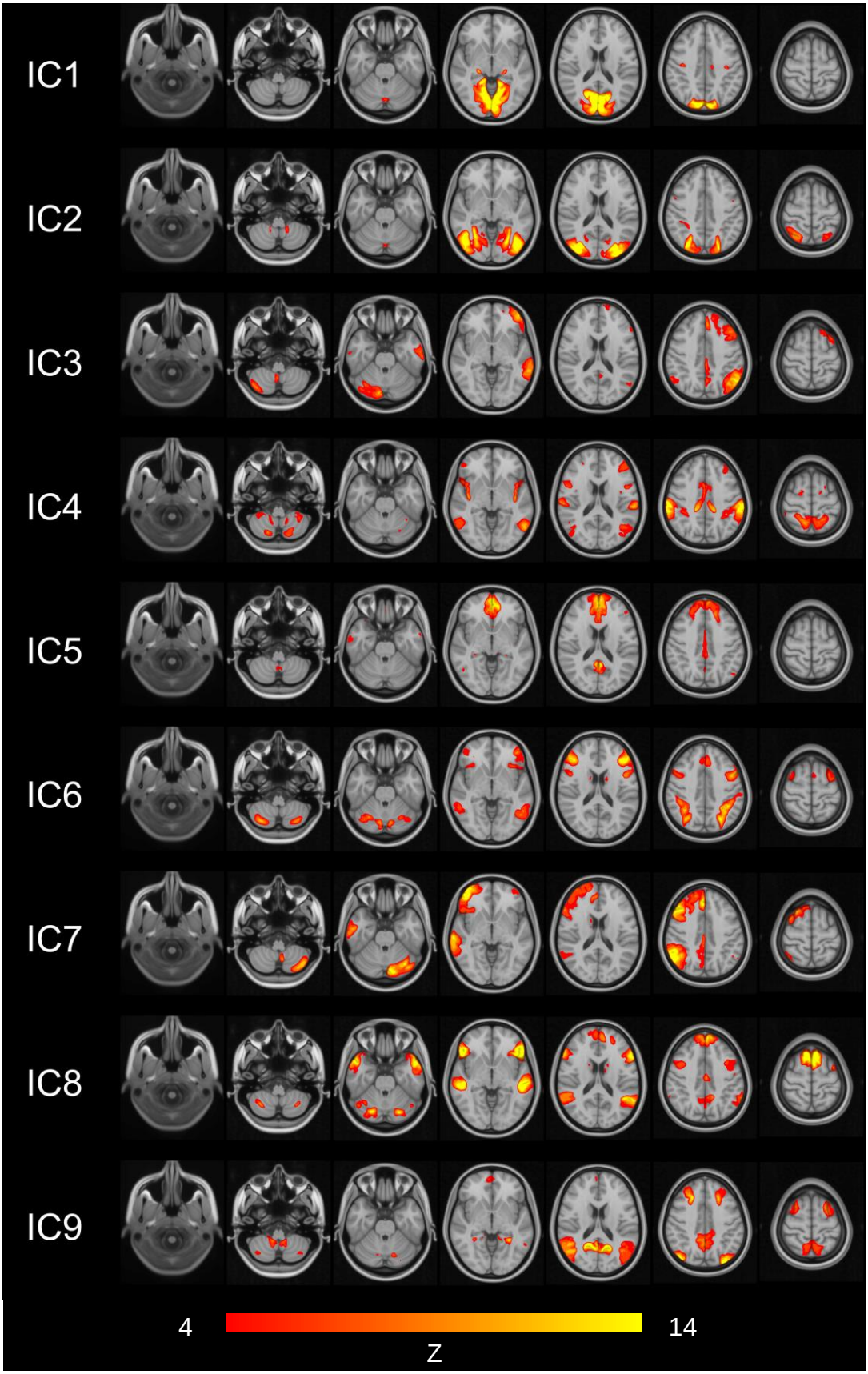
Note. This table includes all participants that attended the first imaging session, irrespective of meeting inclusion criteria for the present study.

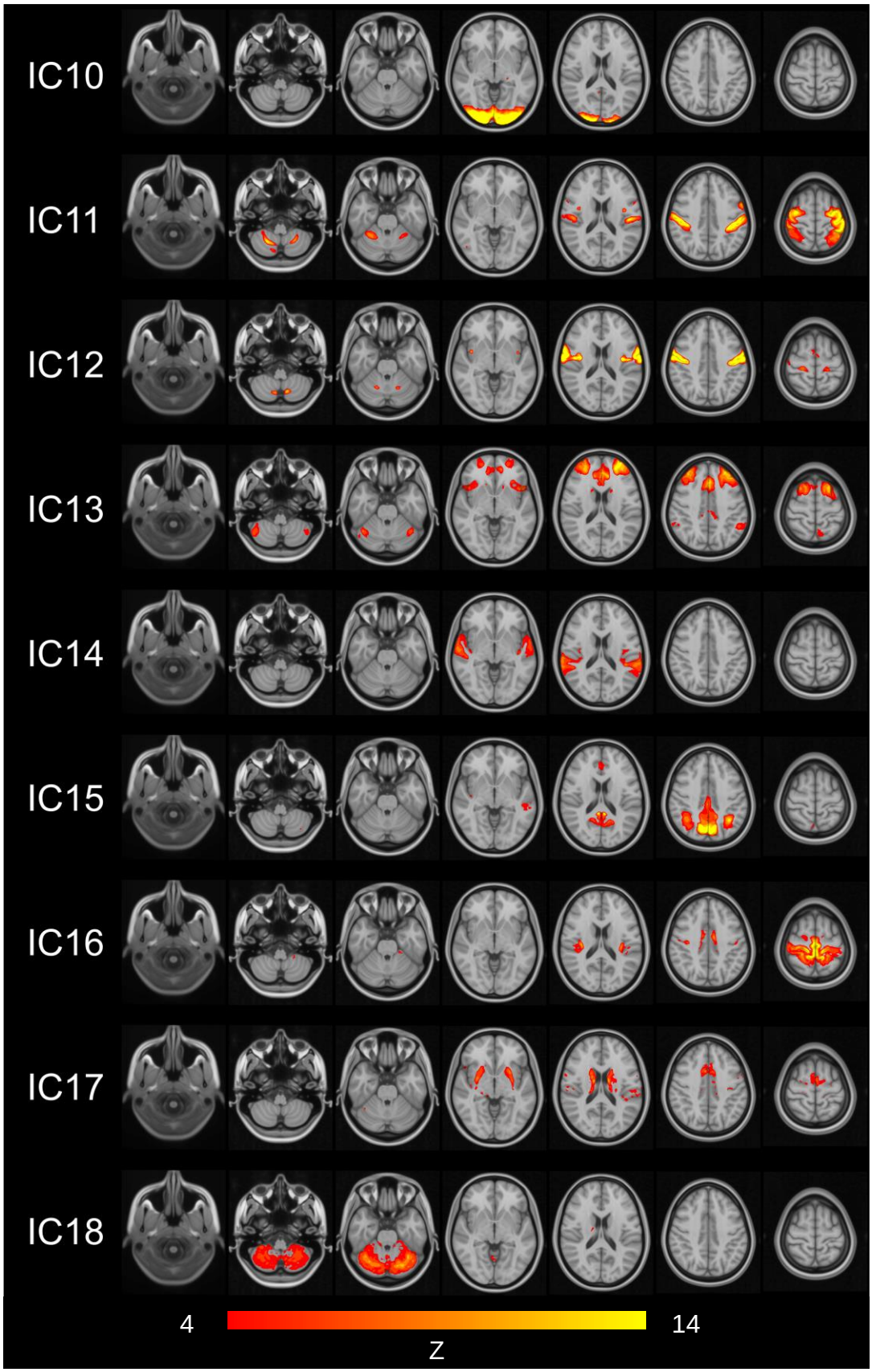
4.3. Group-Level Independent Component Analysis

After conducting group-level ICA and visually inspecting each of the 25 components, 18 were determined to represent neuronally-based resting-state networks of interest (Figure 6). The remaining seven components were labelled as artefactual (Figure 7). Within each figure, the greyscale brain image is the 0.5mm MNI152 non-brain-extracted standard space image, and areas of red and yellow colour depict z-statistics representing the independent component spatial maps, thresholded between 4 and 14.

The results of spatial cross-correlation of these 18 neuronally-based components with the 10 well-matched resting-state networks, as described by Smith et al., are presented in Table 4.

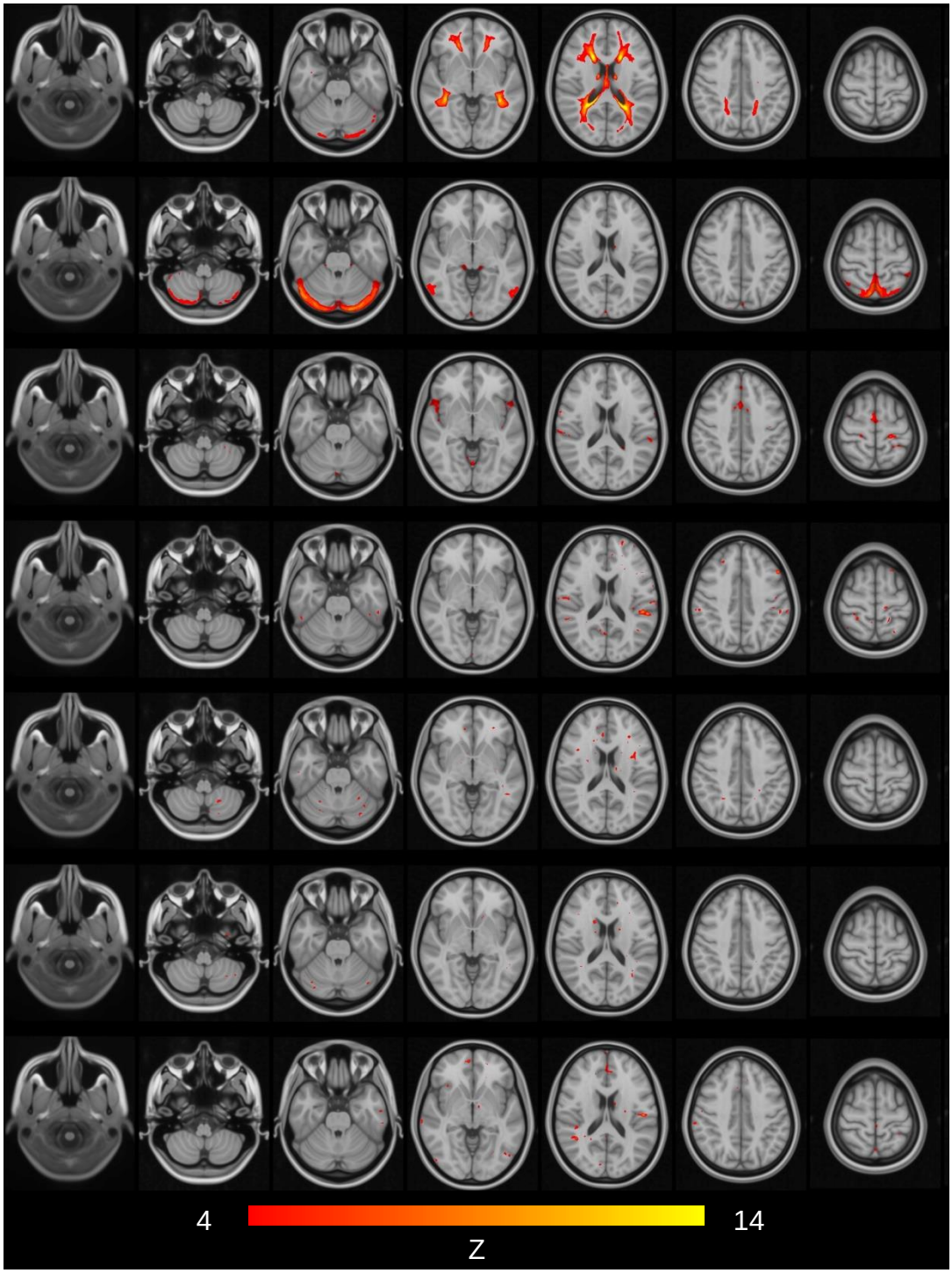
Figure 6: *Neuronally-Based Group-ICA Components.*





Note. All images are displayed using the radiological convention, where the right hemisphere appears on the left side of the image. IC = independent component.

Figure 7: *Artefactual Group-ICA Components.*



Note. All images are displayed using the radiological convention, where the right hemisphere appears on the left side of the image.

Table 4: *Correlation of Neuronally-based Components with Networks Defined by Smith et al.*

Independent component	Smith network name	Correlation coefficient (r)
1	Medial visual	0.80
2	Lateral visual	0.67
3	Left frontoparietal	0.45
4	Auditory	0.25
	Right frontoparietal	0.20
5	Default mode	0.37
	Executive control	0.31
6	Left frontoparietal	0.50
7	Right frontoparietal	0.56
8	Auditory	0.20
9	Default mode	0.39
10	Occipital pole visual	0.76
11	Sensorimotor	0.41
12	Sensorimotor	0.22
13	Executive control	0.53
14	Auditory	0.51
15	Default mode	0.51
16	Sensorimotor	0.30
17	Sensorimotor	0.23
	Auditory	0.27
	Executive control	0.32
18	Cerebellum	0.35

4.4. Dual Regression and Statistical Analysis

Table 5 showcases the results of the 18 independent ANCOVA tests associated with the 18 neuronally-based group-ICA components. Prior to application of the Benjamini–Hochberg correction for multiple comparisons, IC6 (p-value 0.036), IC11 (p-value 0.012), IC12 (p-value 0.003) and IC16 (p-value 0.006) met the significance threshold of p-value less than 0.05. After applying this correction, none of the components remained significant at the 0.05 threshold.

Due to the proximity of the adjusted p-values for the ANCOVA tests associated with IC11, IC12 and IC16 to the threshold of less than 0.05, it was decided to perform exploratory post hoc pairwise t-tests on these three components, despite the lack of statistical significance in the initial analysis. This decision was informed by the conservative approach which was employed, relative to similar studies in this area, which often do not perform multiple comparison corrections (154). In addition, the relatively large number of independent ANCOVA tests performed resulted in a conservative correction, which is associated with an increased risk of type II (false negative) errors. The purpose of these post hoc tests was to assess which specific groups were most likely to significantly differ in their functional connectivity and determine the directionality of functional connectivity differences (i.e., whether functional connectivity was higher or lower in one group than another). Given their exploratory nature, the results of these tests should be interpreted with caution. The findings of these post hoc tests are presented in Table 6.

Before applying the Benjamini–Hochberg method for multiple comparison correction, two out of the six post hoc t-tests for IC11 indicated statistically significant group differences. This included the Controls > Anxiety + No Medications t-test (p-value 0.021) and the Controls > Anxiety + SSRI group (p-value < 0.001). However, after the correction for multiple comparisons, only the Controls > Anxiety + SSRI result remained significant (p-value 0.002). In other words, after correcting for multiple comparisons, post hoc t-tests indicated that, on average, participants in the Control group showed greater functional connectivity than participants in the Anxiety + SSRI group within the network represented by IC11.

IC12 showed statistical significance for two of the six pairwise t-tests. Specifically, the Anxiety + No Medications > Anxiety + SSRI t-test was significant (p-value 0.031), as was the Controls > Anxiety + SSRI t-test (p-value < 0.001). Only the Controls > Anxiety + SSRI t-test remained significant after multiple comparison corrections (adjusted p-value 0.002), again

indicating higher functional connectivity within IC12 for the Control group than the Anxiety + SSRI group.

Similarly, IC16 displayed two significant pairwise t-tests: Controls > Anxiety + No Medications (p-value 0.045) and Controls > Anxiety + SSRI (p-value < 0.001). After correction, only the Controls > Anxiety + SSRI t-test remained statistically significant (adjusted p-value 0.002), mirroring the results observed for IC11 and IC12.

Table 5: *Differences in Functional Connectivity Across Subject Groups: ANCOVA Results for Each Independent Component.*

Independent component	p-value [a]	Adjusted p-value [b]
IC1	0.268	0.689
IC2	0.691	0.970
IC3	0.745	0.970
IC4	0.232	0.689
IC5	0.960	0.970
IC6	0.036*	0.161
IC7	0.970	0.970
IC8	0.535	0.970
IC9	0.266	0.689
IC10	0.583	0.970
IC11	0.012*	0.073 [c]
IC12	0.003*	0.055 [c]
IC13	0.892	0.970
IC14	0.701	0.970
IC15	0.915	0.970
IC16	0.006*	0.055[c]
IC17	0.956	0.970
IC18	0.507	0.970

Note. p-values are presented for analysis of covariance between the three subject groups. IC = Independent component.

[a] p-values are corrected for multiple comparisons across voxels using family-wise error correction; however, they are not corrected for multiple comparisons across independent components.

[b] Adjusted p-values are corrected for multiple comparisons across the 18 independent components using the Benjamini–Hochberg method to control the false discovery rate in addition to multiple comparison correction across voxels.

[c] Adjusted p-values that were marginally above the significance threshold. The independent components associated with these p-values underwent post hoc testing.

* $p < 0.05$.

Table 6: *Post Hoc Pairwise t-Test Results for Notable ANCOVA Components.*

Pairwise t-test	p-value [a]	Adjusted p-value [b]
IC11		
A+NM > A+S	0.055	0.142
A+S > A+NM	0.972	0.972
A+NM > C	0.578	0.868
C > A+NM	0.021*	0.095
A+S > C	0.694	0.961
C > A+S	0.000***	0.002**
IC12		
A+NM > A+S	0.031*	0.111
A+S > A+NM	0.945	0.972
A+NM > C	0.292	0.583
C > A+NM	0.812	0.972
A+S > C	0.829	0.972
C > A+S	0.000***	0.002**
IC16		
A+NM > A+S	0.084	0.189
A+S > A+NM	0.882	0.972
A+NM > C	0.496	0.868
C > A+NM	0.045*	0.134
A+S > C	0.579	0.868
C > A+S	0.000***	0.002**

Note. IC = Independent component; A+NM = Anxiety with no medications; A+S = Anxiety with SSRI treatment; C = Control group.

[a] p-values are corrected for multiple comparisons across voxels using family-wise error correction; however, they are not corrected for multiple comparisons across independent components.

[b] Adjusted p-values are corrected for multiple comparisons across the 18 independent components using the Benjamini–Hochberg method for controlling family-wise error rate in addition to multiple comparison correction across voxels.

*p-value < 0.05. **p < 0.01. ***p < 0.001

The following sections (sections 4.3.1, 4.3.2, and 4.3.3) present detailed results, broken down into the three independent components with significant subject-group differences in functional connectivity (IC11, IC12, and IC16). Figures depicting clusters of significant voxels were created using the FSL tool "FSLeyes" (171). Within each of these figures (Figure 8-12), green voxels denote areas of increased functional connectivity in the Control group relative to the Anxiety + SSRI group, with lighter green voxels signifying greater significance in these differences. Red and yellow voxels represent the underlying independent component which was tested. The blue location cursor is centred on the centre of gravity for each cluster, with the coordinates corresponding to this location in MNI152 2mm space being displayed in millimetres (top) and voxel coordinates (bottom) in the top left corner of the figures. "Centre of gravity" refers to the "weighted average of the coordinates by the intensities within the cluster" (178), with intensities in this case being the one-minus p-value for a voxel within a cluster. In other words, the centre of gravity indicates where the middle of a cluster is located within the brain. All brain images are displayed using the radiological convention, where the right hemisphere of the brain is shown on the left side of the image for coronal and sagittal views.

Following the presentation of these brain images, the names of all brain regions included in the independent component and the average probability of a voxel being found within each brain region are depicted in table form. Additionally, brain regions and probabilities are presented similarly for the voxels found to differ significantly between subject groups. The brain regions in these tables are those defined in the Harvard-Oxford Cortical Structural Atlas and, separately, the Harvard-Oxford Subcortical Structural Atlas (180). These tables indicate the relative distribution of voxels; however, the percentages across all brain regions do not sum to 100, as some voxels are located outside or on the edges of regions of interest included in the structural atlases (179).

4.4.1. Independent Component 11

Two large clusters were observed, indicating areas of increased functional connectivity with the IC11 network in the Control group compared to the Anxiety + SSRI group. Table 7 provides a summary of these clusters. The location of these clusters is presented visually in Figure 8 and Figure 9, with voxels included in each cluster being green in colour.

Table 7: *Clusters of Increased Functional Connectivity with IC11 in Control Group Relative to Anxiety + SSRI Group.*

Region	Voxels	x (mm)	y (mm)	z (mm)	Minimum p-value [a]
L postcentral gyrus	2,265	-39.8	-24.2	53.8	0.000
R postcentral gyrus	1,204	41.8	-21.6	53.2	0.001

Note. Region indicates the location of a cluster's centre of gravity. The x, y, and z columns represent the millimetre coordinates of the cluster's centre of gravity in MNI152 2mm standard space. L = Left; R = Right.

[a] The minimum p-value is the smallest p-value found across all voxels included in the cluster.

Figure 8: *Left Postcentral Gyrus Cluster Associated With IC11.*

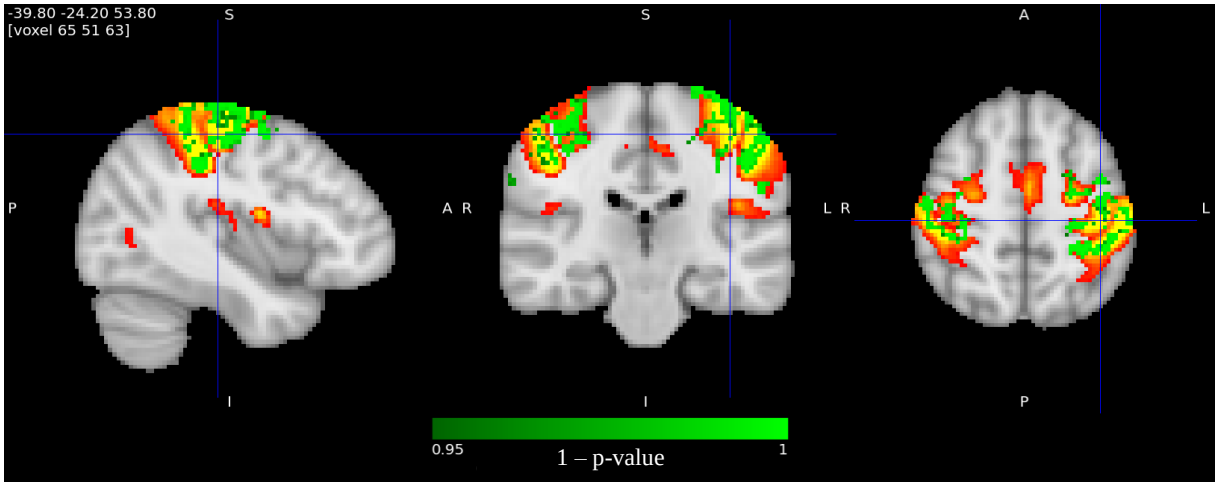
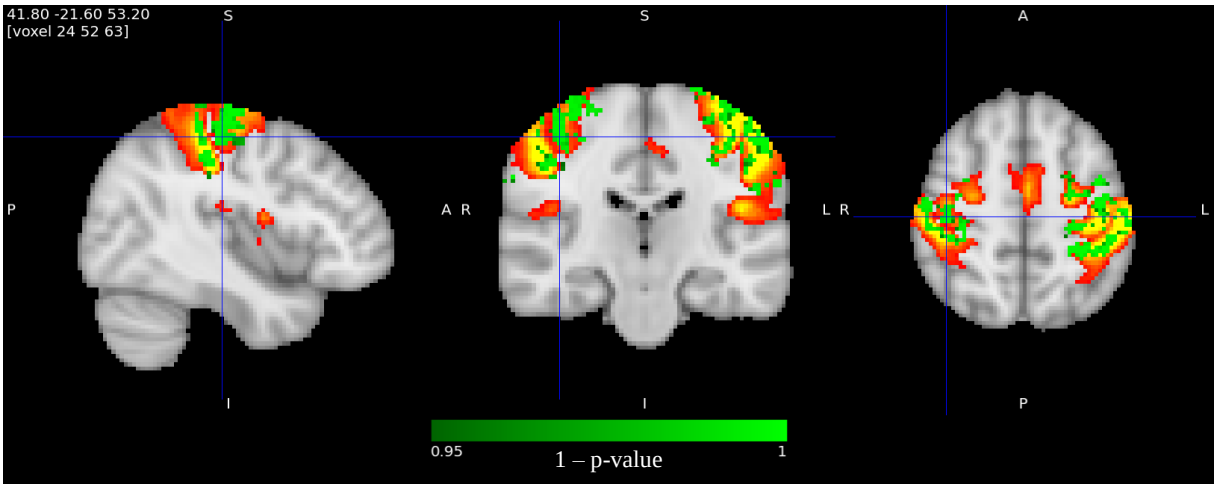


Figure 9: *Right Postcentral Gyrus Cluster Associated With IC11.*



A total of 34 cortical regions were identified as being involved in IC11. The cerebellum was the only subcortical structure involved in IC11. Table 8 details these findings in descending order, from regions with the most voxels to those with the fewest voxels.

Table 8: *Brain Regions Included in IC11.*

Region	Average probability (%) [a]
Cortical [b]	
Postcentral Gyrus	16.1663
Precentral Gyrus	10.6445
Superior Parietal Lobule	7.2793
Supramarginal Gyrus, anterior division	3.9084
Central Opercular Cortex	2.3341
Juxtapositional Lobule Cortex (Supplementary Motor Area)	1.7961
Superior Frontal Gyrus	1.6053
Middle Frontal Gyrus	1.1410
Supramarginal Gyrus, posterior division	1.1399
Lateral Occipital Cortex, inferior division	1.0563
Lateral Occipital Cortex, superior division	0.9787
Parietal Operculum Cortex	0.8044
Inferior Frontal Gyrus, pars opercularis	0.4007
Cingulate Gyrus, anterior division	0.2658
Temporal Occipital Fusiform Cortex	0.2550
Heschl's Gyrus (includes H1 and H2)	0.2131
Insular Cortex	0.1836
Planum Temporale	0.1724
Angular Gyrus	0.1624
Middle Temporal Gyrus, temporooccipital part	0.1380
Cingulate Gyrus, posterior division	0.1034
Lingual Gyrus	0.0758
Paracingulate Gyrus	0.0667
Temporal Pole	0.0544
Temporal Fusiform Cortex, posterior division	0.0325
Inferior Temporal Gyrus, temporooccipital part	0.0293

Region	Average probability (%) [a]
Superior Temporal Gyrus, posterior division	0.0224
Planum Polare	0.0192
Occipital Fusiform Gyrus	0.0113
Precuneus Cortex	0.0108
Middle Temporal Gyrus, posterior division	0.0087
Middle Temporal Gyrus, anterior division	0.0025
Superior Temporal Gyrus, anterior division	0.0018
Inferior Frontal Gyrus, pars triangularis	0.0009
Inferior Temporal Gyrus, posterior division	0.0001
Subcortical [c]	
Cerebellum	10.7561

[a] The average probability of a voxel being found within a particular cortical region, calculated across all significant voxels.

[b] Cortical regions defined using the Harvard-Oxford Cortical Structural Atlas.

[c] Cerebellum defined using the MNI Structural Atlas. All other subcortical regions are defined using the Harvard-Oxford Subcortical Structural Atlas.

Areas of increased functional connectivity with IC11 were observed across 24 cortical regions. No subcortical areas of increased functional connectivity were observed. These results are detailed in Table 9.

Table 9: *Brain Regions Demonstrating Higher Functional Connectivity with IC11 in Control Group Relative to Anxiety + SSRI Group.*

Region	Average probability (%) [a]
Cortical [b]	
Postcentral Gyrus	25.3625
Precentral Gyrus	15.3625
Superior Parietal Lobule	5.9250
Supramarginal Gyrus, anterior division	4.7210
Middle Frontal Gyrus	1.9788
Superior Frontal Gyrus	1.1951
Lateral Occipital Cortex, superior division	0.7914
Supramarginal Gyrus, posterior division	0.4401
Inferior Frontal Gyrus, pars opercularis	0.4152
Lateral Occipital Cortex, inferior division	0.1917
Middle Temporal Gyrus, temporooccipital part	0.0378
Parietal Operculum Cortex	0.0243
Central Opercular Cortex	0.0217
Angular Gyrus	0.0177
Planum Temporale	0.0143
Occipital Pole	0.0134
Cuneal Cortex	0.0103
Supracalcarine Cortex	0.0080
Temporal Occipital Fusiform Cortex	0.0063

Region	Average probability (%) [a]
Inferior Temporal Gyrus, temporooccipital part	0.0057
Inferior Frontal Gyrus, pars triangularis	0.0046
Superior Temporal Gyrus, posterior division	0.0043
Intracalcarine Cortex	0.0043
Occipital Fusiform Gyrus	0.0003
Subcortical [c]	
Cerebellum	0.0649

[a] The average probability of a voxel being found within a particular cortical region, calculated across all significant voxels.

[b] Cortical regions defined using the Harvard-Oxford Cortical Structural Atlas.

[c] Cerebellum defined using the MNI Structural Atlas. All other subcortical regions are defined using the Harvard-Oxford Subcortical Structural Atlas.

4.4.2. Independent Component 12

Two relatively large clusters were observed, indicating areas of increased functional connectivity with the IC12 network in the Control group compared to the Anxiety + SSRI group. Table 10 provides a summary of these clusters. The location of these clusters is presented visually in Figure 10 and Figure 11.

Table 10: *Clusters of Increased Functional Connectivity with IC12 in Control Group Relative to Anxiety + SSRI Group.*

Region	Voxels	x (mm)	y (mm)	z (mm)	Minimum p-value [a]
R postcentral gyrus	540	57.9	-6.69	27.3	0.001
L postcentral gyrus	334	-56.8	-8.39	30.5	0.000

Note. Region indicates the location of a cluster's centre of gravity. The x, y, and z columns represent the millimetre coordinates of the cluster's centre of gravity in MNI152 2mm standard space. L = Left; R = Right.

[a] The minimum p-value is the smallest p-value found across all voxels included in the cluster.

Figure 10: *Right Postcentral Gyrus Cluster Associated With IC12.*

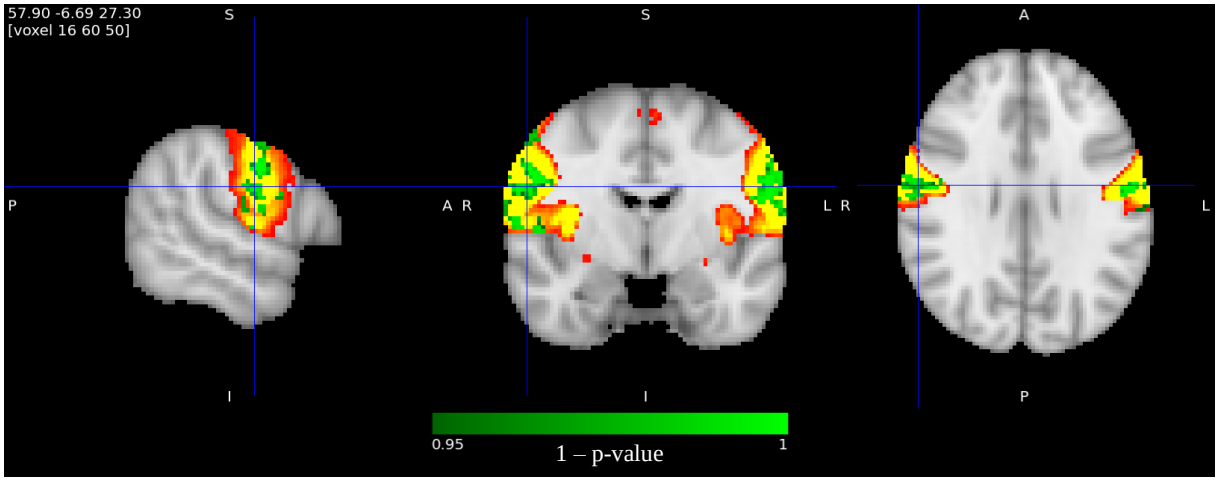
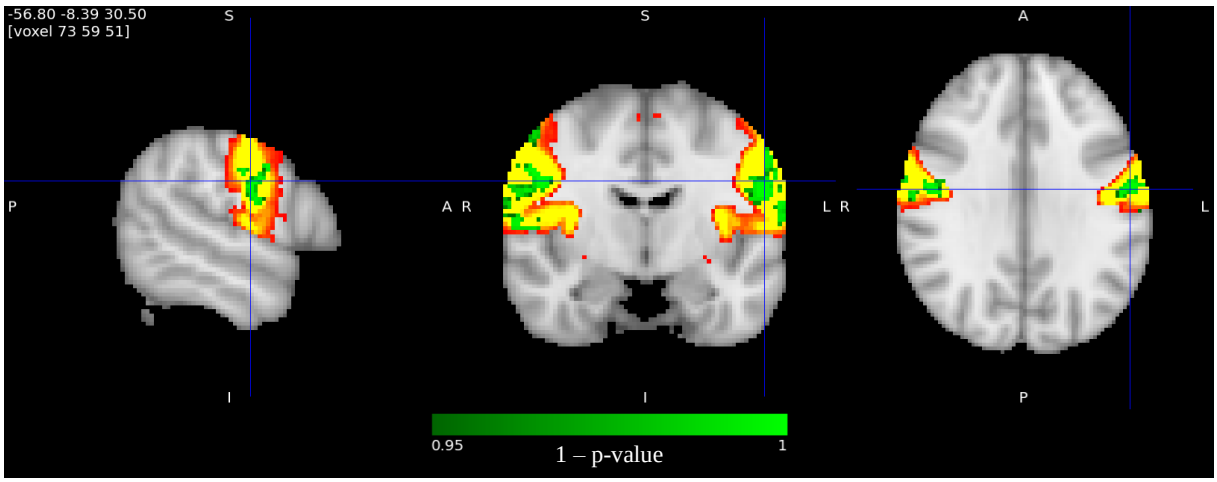


Figure 11: *Left Postcentral Gyrus Cluster Associated With IC12.*



A total of 27 cortical and 10 subcortical regions were identified as being involved in IC12. These findings are detailed in Table 11.

Table 11: *Brain Regions Included in IC12.*

Region	Average probability (%) [a]
Cortical [b]	
Precentral Gyrus	18.4304
Postcentral Gyrus	17.1500
Central Opercular Cortex	7.6282
Insular Cortex	3.1061
Juxtapositional Lobule Cortex (Supplementary Motor Area)	0.8945
Supramarginal Gyrus, anterior division	0.7082
Planum Polare	0.5892
Inferior Frontal Gyrus, pars opercularis	0.5003
Planum Temporale	0.3337
Heschl's Gyrus (includes H1 and H2)	0.2833
Lingual Gyrus	0.2629
Parietal Operculum Cortex	0.2095
Occipital Fusiform Gyrus	0.1656
Superior Temporal Gyrus, anterior division	0.1492
Middle Frontal Gyrus	0.1019
Temporal Occipital Fusiform Cortex	0.0742
Temporal Pole	0.0708
Superior Temporal Gyrus, posterior division	0.0653
Frontal Operculum Cortex	0.0634
Paracingulate Gyrus	0.0349
Frontal Pole	0.0289
Cingulate Gyrus, anterior division	0.0280
Superior Frontal Gyrus	0.0229
Precuneus Cortex	0.0056
Superior Parietal Lobule	0.0018
Middle Temporal Gyrus, anterior division	0.0012
Cingulate Gyrus, posterior division	0.0005
Frontal Orbital Cortex	0.0003

Region	Average probability (%) [a]
Subcortical [c]	
Cerebellum	10.0432
Right Thalamus	0.4112
Left Thalamus	0.3709
Brain-Stem	0.1615
Right Putamen	0.1201
Left Putamen	0.0587
Left Amygdala	0.0126
Right Amygdala	0.0114
Right Pallidum	0.0078
Left Pallidum	0.0018

[a] The average probability of a voxel being found within a particular cortical region, calculated across all significant voxels.

[b] Cortical regions defined using the Harvard-Oxford Cortical Structural Atlas.

[c] Cerebellum defined using the MNI Structural Atlas. All other subcortical regions defined using the Harvard-Oxford Subcortical Structural Atlas.

Areas of increased functional connectivity with IC12 were observed across 12 cortical regions. No subcortical areas of increased functional connectivity were observed. These results are displayed in Table 12.

Table 12: *Brain Regions Demonstrating Higher Functional Connectivity with IC12 in Control Group Relative to Anxiety + SSRI Group.*

Region	Average probability (%) [a]
Cortical [b]	
Postcentral Gyrus	30.7953
Precentral Gyrus	26.3412
Central Opercular Cortex	3.9832
Supramarginal Gyrus, anterior division	0.8736
Planum Polare	0.4183
Planum Temporale	0.3490
Parietal Operculum Cortex	0.2125
Heschl's Gyrus (includes H1 and H2)	0.1756
Inferior Frontal Gyrus, pars opercularis	0.1342
Superior Temporal Gyrus, anterior division	0.0984
Superior Temporal Gyrus, posterior division	0.0749
Middle Frontal Gyrus	0.0537

[a] The average probability of a voxel being found within a certain cortical region, calculated across all significant voxels.

[b] Cortical regions defined using the Harvard-Oxford Cortical Structural Atlas.

4.4.3. Independent Component 16

One large cluster of increased functional connectivity with IC16 in the Control group compared to the Anxiety + SSRI group was observed. This cluster is summarised in Table 13. The location of this cluster is presented visually in Figure 12.

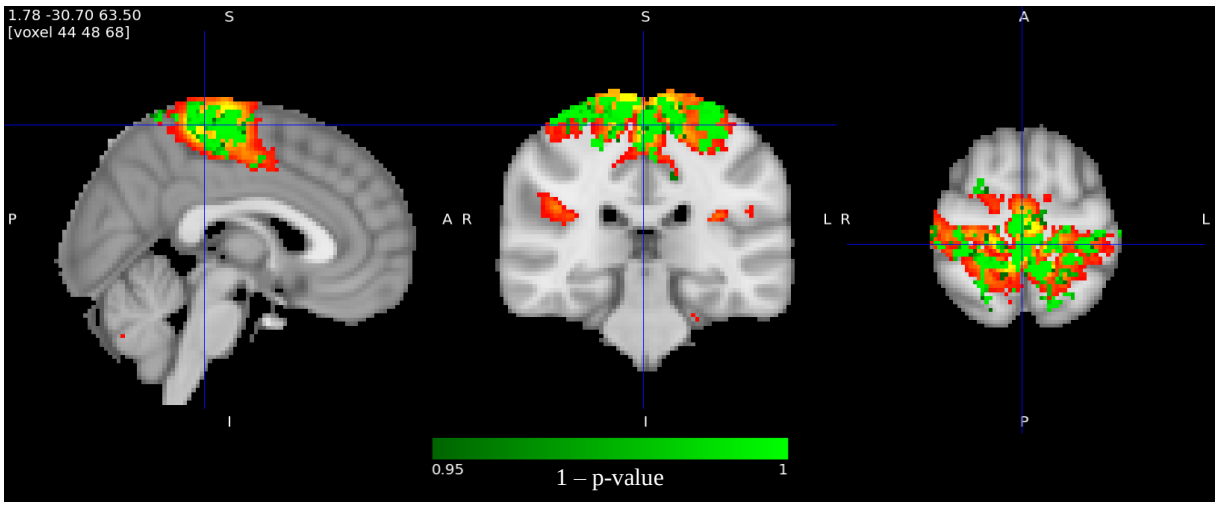
Table 13: *Clusters of Increased Functional Connectivity with IC16 in Control Group Relative to Anxiety + SSRI Group.*

Region	Voxels	x (mm)	y (mm)	z (mm)	Minimum p-value [a]
Precentral gyrus	3,907	1.78	-30.7	63.5	0.000

Note. Region indicates the location of a cluster's centre of gravity. The x, y, and z columns represent the millimetre coordinates of the cluster's centre of gravity in MNI152 2mm standard space. L = Left; R = Right.

[a] The minimum p-value is the smallest p-value found across all voxels included in the cluster.

Figure 12: *Precentral Gyrus Cluster Associated With IC16.*



A total of 23 cortical regions and 4 subcortical regions were identified as being involved in IC16. Table 14 details these findings in descending order, from regions with the most voxels to those with the fewest voxels.

Table 14: *Brain Regions Included in IC16.*

Region	Average probability (%) [a]
Cortical [b]	
Postcentral Gyrus	18.4659
Precentral Gyrus	16.8874
Superior Parietal Lobule	4.6271
Juxtapositional Lobule Cortex (Supplementary Motor Area)	3.9509
Precuneus Cortex	2.8223
Superior Frontal Gyrus	1.9686
Cingulate Gyrus, anterior division	1.6395
Parietal Operculum Cortex	1.5440
Cingulate Gyrus, posterior division	1.0443
Insular Cortex	0.5509
Central Opercular Cortex	0.3953
Lateral Occipital Cortex, superior division	0.3860
Planum Temporale	0.2388
Supramarginal Gyrus, anterior division	0.2311
Supramarginal Gyrus, posterior division	0.1119
Heschl's Gyrus (includes H1 and H2)	0.1006
Paracingulate Gyrus	0.0213
Middle Frontal Gyrus	0.0150
Superior Temporal Gyrus, posterior division	0.0091
Planum Polare	0.0076

Region	Average probability (%) [a]
Parahippocampal Gyrus, posterior division	0.0069
Temporal Fusiform Cortex, posterior division	0.0029
Temporal Occipital Fusiform Cortex	0.0002
Angular Gyrus	0.0001
Subcortical [c]	
Cerebellum	1.1311
Brain-Stem	0.0115
Left Putamen	< 0.0000
Right Putamen	< 0.0000

[a] The average probability of a voxel being found within a certain cortical region, calculated across all significant voxels.

[b] Cortical regions defined using the Harvard-Oxford Cortical Structural Atlas.

[c] Cerebellum defined using the MNI Structural Atlas. All other subcortical regions defined using the Harvard-Oxford Subcortical Structural Atlas.

Areas of increased functional connectivity with IC16 were observed across 13 cortical regions. No subcortical areas of increased functional connectivity were observed. These results are detailed in Table 15.

Table 15: *Brain Regions Demonstrating Higher Functional Connectivity with IC16 in Control Group Relative to Anxiety + SSRI Group.*

Region	Average probability (%) [a]
Cortical [b]	
Postcentral Gyrus	23.3434
Precentral Gyrus	18.2574
Superior Parietal Lobule	4.7124
Precuneus Cortex	2.8411
Juxtapositional Lobule Cortex (Supplementary Motor Area)	2.3168
Lateral Occipital Cortex, superior division	1.3749
Superior Frontal Gyrus	1.0931
Cingulate Gyrus, posterior division	0.8319
Cingulate Gyrus, anterior division	0.7121
Supramarginal Gyrus, anterior division	0.1960
Supramarginal Gyrus, posterior division	0.0415
Middle Frontal Gyrus	0.0294
Angular Gyrus	0.0010

[a] The average probability of a voxel being found within a certain cortical region, calculated across all significant voxels.

[b] Cortical regions defined using the Harvard-Oxford Cortical Structural Atlas.

5. Discussion

This chapter will summarise the main findings of this study, including interpretation and evaluation of these results in association with the current relevant literature. Furthermore, recommendations for future research based on these findings will be discussed.

5.1. Dual Regression and Statistical Analysis

Before correction for multiple comparisons, ANCOVA tests investigating functional connectivity differences across the three subject groups indicated statistically significant group differences associated with four of the 18 neuronally-based components. These were IC6, IC11, IC12, and IC16, all associated with p-values of less than 0.05. Notably, through correlating these components with the 10 resting-state networks defined by Smith et al. (145), IC6 most strongly correlated with the left frontoparietal network, while each of the remaining three (IC11, IC12, and IC16) most strongly correlated with the sensorimotor network. The left frontoparietal network consists of various areas of the frontal and parietal lobes, such as the dorsolateral prefrontal cortex, precuneus, and the PCC; however, the regions included in this network differ across distinct parcellations (145, 181). This network has functions in the coordination of goal-driven behaviours, working memory, and language functions (182-184). The sensorimotor network, as described by Smith and colleagues, consists of the "supplementary motor area, sensorimotor cortex, and secondary somatosensory cortex", having well-established roles in the execution of actions and sensory perception (145).

The association of three distinct components with the sensorimotor network arose due to differences in the dimensionality of the decomposition, acquisition parameters, preprocessing steps, and participant characteristics which distinguish the present study from the study conducted by Smith and colleagues. During the design stage of the present research, a decision was made to use the data obtained from the included participants to derive the independent components rather than use templates of predefined networks to define these components. This data-driven approach ensured that the most accurate representation of the functional networks relevant to the specific selection of participants was obtained, and any unique connectivity patterns were not overlooked. However, as a result, the networks obtained within the present study vary considerably from those observed in prior research. Each of the three significant

networks, although being most strongly correlated with the sensorimotor network described by Smith and colleagues, involved functional connectivity with numerous brain regions not included in the Smith network and overall had a relatively low correlation with this network (145). This complication in comparing data-derived networks to pre-established resting-state networks is a primary disadvantage of ICA-based studies.

Following the correction for multiple comparisons, none of the four components mentioned retained their statistical significance below the p-value threshold of 0.05. Although it is not uncommon for similar ICA-based studies to avoid correction for multiple comparisons across networks (154), it was decided to perform these corrections to mitigate the risk of false positive errors and improve the confidence in results reflecting true findings. Due to the large number of independent ANCOVA tests conducted, this correction was relatively conservative, significantly increasing the observed p-values and the chance of false negative errors. For this reason, it was decided to further investigate components associated with p-values marginally greater than 0.05, namely, IC11, IC12, and IC16. It is, therefore, essential to consider these findings, as well as the findings of subsequent statistical tests, as exploratory, and to interpret them with caution.

Post hoc tests of these three components revealed an interesting pattern. After multiple comparison corrections across the six pairwise t-tests for each component, each component displayed a statistically significant difference for the same contrast, “C > A+S”, or Controls having greater functional connectivity than the Anxiety + SSRI group (Table 6). The adjusted p-values for all significant results were 0.002, indicating only a 0.2% chance of observing results this extreme, or more extreme, assuming that no functional connectivity differences exist between the groups. These were the only statistically significant findings of the 18 total pairwise t-tests conducted over these three components. Overall, these results indicate that participants taking an SSRI at the time of the imaging session had significantly decreased functional connectivity within three of 18 components derived from group-level ICA.

Notably, after multiple comparison correction, no significant differences were observed between the Control group and the group of participants with a lifetime history of anxiety disorder not taking any medications at the time of the imaging appointment. It is important to consider that the effect of acute anxiety symptoms was controlled as a confounding factor within the GLM analyses utilised for the ANCOVA and the subsequent post hoc tests. Therefore, only the functional connectivity correlations of having a history of anxiety disorders were tested for, with any correlations with participant reports of anxiety traits being removed.

The correlation of these reported anxiety traits with functional connectivity was not tested for in this analysis; however, this is an area of interest that future studies could investigate. The finding of no significant differences may also be related to one of the constraints of the UK Biobank dataset, which was the lack of data collected regarding how recently participants met diagnostic criteria for having an anxiety disorder. It is, therefore, possible that participants classified as having a history of an anxiety disorder met the diagnostic criteria many years ago and no longer met the criteria at the time of the imaging session. These participants would be included alongside participants who still met the criteria for diagnosis of one or more anxiety disorders at the time of the imaging session, making it impossible to determine whether the results of this study are relevant to individuals with anxiety disorders in remission, or only relevant to individuals who have not achieved remission.

There were many similarities in the brain regions included in the three components that underwent post hoc testing. The precentral and postcentral gyri were the most prominent regions involved across all three components. The precentral gyrus serves as the location of the primary motor cortex, which is responsible for controlling voluntary motor movement, as well as a part of the supplementary motor cortex, which functions in the planning of voluntary movements (185). The postcentral gyrus is the site of the primary somatosensory cortex, which is responsible for proprioception, or the sense of the location and movement of the body within space, as well as the site where various senses are perceived, such as touch, pressure, temperature and pain (186). Additionally, the secondary somatosensory cortex, which is believed to be involved in integrating sensory information from both halves of the body, as well as in processing and storing memories associated with certain sensory experiences, is located within the postcentral gyrus (187). The somatosensory cortex and the primary motor cortex are highly connected, which enables the conscious awareness of motion (188).

In addition to these more established functions associated with the postcentral gyrus, more recent evidence suggests that this region also has an important function in emotional processing and regulation in concert with other brain regions (189). Previous research suggests that the somatosensory cortex, within the postcentral gyrus, plays a central role in emotional regulation, having functions common across all stages involved in this process. These stages include assessing the emotional significance of a stimulus, producing an appropriate emotional response, and regulating this emotional response (189-191). Previous investigations have revealed that the somatosensory cortex is significantly connected to both the amygdala and the insula, regions which have well-established roles in emotional responses (99, 100, 192). This

finding aligns with the results of the present study, in which the insula was a prominent feature across the networks involving the somatosensory cortex. The amygdala is also featured in the network represented by IC12; however, not to a significant extent. This is most likely due to the majority of amygdala activity being decomposed into other independent components which were not investigated in detail.

Many other brain regions besides the precentral and postcentral gyri were involved in the three significant components and again, these regions were similar between the three components. Several brain areas of particular interest, which have been implicated by numerous studies to have an important role in anxiety disorders, were observed to be involved in these components. Both the ACC and the insular cortex were found to be involved across all three of these components, with the involvement of the insular cortex being an especially prominent feature. The left and right amygdalae, which are the focus of a large proportion of neuroimaging studies investigating anxiety, were identified to be involved in IC12; however, their involvement in this network was extremely limited, with very few voxels corresponding to these structures.

For all three of the components, regions of increased functional connectivity were largely concentrated within the precentral and postcentral gyri. Increased functional connectivity was also observed in many other regions; however, with significantly fewer voxels relative to the postcentral and precentral gyri. Other notable regions which exhibited relatively large areas of increased functional connectivity were the superior parietal lobule with IC11 and IC16, the insular cortex with IC12, the central opercular cortex with IC11 and IC12, and the precuneus cortex, supplementary motor area, and ACC with IC16.

Many previous studies in this area have taken a seed-based correlation approach. These studies use a specific region of interest as a seed and determine the functional connectivity of the entire brain specifically with this seed region. These studies are extremely informative, as they enable new, focused insights into the functional connectivity of brain regions which are known to have an established or suspected role in anxiety disorders or the mechanisms underpinning SSRIs effects.

However, concomitant with the focus on these established regions, there is a relative scarcity of studies which have investigated the functional connectivity associations at the whole-brain level, considering all constituent resting-state networks. In relation to the findings of the present study, very few studies have reported findings of SSRI correlations with the sensorimotor resting-state network, which is most relevant to the findings of this research.

Nonetheless, two similar, well-designed clinical randomised control trials, both conducted by Klaassens and colleagues, reported results congruent with the results of the present study (144, 146). These studies utilised a dual regression-based approach to evaluate the acute effects of sertraline and, separately, citalopram on whole-brain functional connectivity. The same 10 resting-state networks defined by Smith et al., which the present study correlated against, were utilised as template networks. Both studies by Klassens et al. reported an acute decrease in functional connectivity of the precentral and postcentral gyri, supplementary motor area, precuneus, ACC, and PCC with the Smith sensorimotor network within several hours of SSRI administration relative to placebo. The study that focused on citalopram reported decreased functional connectivity within the medial prefrontal cortex, planum temporale, Heschl's gyrus, and cerebellum, in addition to the previously mentioned regions with the Smith sensorimotor resting-state network (144). Both studies reported decreases in functional connectivity predominantly located within the precentral and postcentral gyri, in alignment with the findings of the present study. Other regions of decreased functional connectivity with the sensorimotor network reported by these studies were also concordant with the present study. This includes the supplementary motor area, precuneus, ACC, and PCC, which had decreased functional connectivity with IC16, small areas of the planum temporale with IC11 and IC12, small areas of Heschl's gyrus with IC12 and small areas of the cerebellum with IC11.

Although the studies by Klaassens et al. yielded very similar results to the present study, it is essential to note that methodological differences hinder the direct comparison between these studies and the present study. Firstly, although the three components of interest within this study most strongly correlated with the Smith sensorimotor network, many brain regions not included in this network were also found to be involved across the three components. Therefore, the results of this study indicate a decrease in functional connectivity within most of the same areas as those identified by Klaassens et al.; however, within broader networks of brain activity. Additionally, while the studies by Klaassens et al. focused on the acute effects of SSRIs through repeated measurements over several hours, it is not possible to compare time-based effects with the present study, as no information regarding how long participants had been taking SSRIs was gathered at the UK Biobank assessment centre visits. It is reasonable to assume that participants included in the present study had been taking SSRI medications for significantly longer than participants of the studies by Klassens et al.; however, it is still not possible to make the inference that acute changes in functional connectivity hours after an initial administration of an SSRI are maintained after longer-term maintenance therapy. For example,

the findings of both the studies by Klaassens et al. and the present study may be characteristic of early changes in functional connectivity, which become apparent after several hours and persist for several weeks before attenuating. Future studies could investigate this effect by using the areas of decreased functional activity identified within the present study, such as the precentral and postcentral gyri, as regions of interest in a seed-based analysis and taking a longitudinal approach where fMRI data is collected both acutely after the initial SSRI administration and at predefined intervals over the subsequent weeks and months. The inclusion of these areas as regions of interest would be a departure from many seed-based correlation studies conducted on anxiety disorders and SSRIs, which typically focus on regions of the limbic system. This would enable a more specific classification of the brain regions involved and provide insights into how functional connectivity differences vary over time.

5.2. Limitations

In considering this research's findings and their broader application, it is imperative to acknowledge its limitations. These include the potential non-generalizability of findings to other populations dissimilar from the included participants, necessary assumptions made because of dataset constraints, and the overall features of the study design.

Participants included in this study were selected as a subset of participants recruited for the UK Biobank prospective study. Participants included in the present study had an age range of 45 to 76 years, with a mean age of 59 years at the time of data collection at the first imaging session. This presents a limitation with the generalizability of this study's results, as only middle-aged and older adults were included. The presentation of anxiety disorders varies significantly between age groups and is influenced by many factors. For example, on average, older adults with generalised anxiety disorder (GAD) experience lower symptom severity than younger adults (43). Older adults with SAD experience symptoms that are less severe but are present in a broader range of situations compared to younger adults who experience more severe symptoms in fewer distinct situations (42). SAD in older adults can involve worries about age-related decline in function and health conditions, such as hearing loss, cognitive decline, and incontinence. There are also intergenerational differences in beliefs and behaviours regarding mental health conditions. In the past, a limited understanding of mental health conditions was more prevalent alongside greater stigma. These conditions were often viewed as personal failures or weaknesses rather than legitimate medical ailments. Older generations may, therefore, be less likely to seek help for or report symptoms of psychiatric problems than

younger generations out of fear of discrimination from their friends, family, place of work, or communities and are therefore less likely to receive a diagnosis and appropriate treatment.

As the understanding of mental health has evolved, the diagnostic criteria for various psychiatric conditions have also changed. Consequently, conditions diagnosed in the distant past may not align with current diagnostic standards. In addition, some individuals, despite meeting today's diagnostic criteria for certain conditions, might not have been diagnosed in the past. This is especially relevant to the present research, as some participants were classified as having a history of anxiety disorders based on self-reported diagnosis by a professional, without further information indicating which diagnostic criteria were applied. Some of the participants included in the present study were born prior to the introduction of the first DSM edition in 1952 and may have been diagnosed according to earlier DSM versions or alternative criteria, such as those specified in editions of the ICD. These dissimilarities may manifest with different functional connectivity patterns, complicating the comparison of the present study's results with research focused on a younger or older demographic.

Another limitation of the present study arises from the demographics of the participants recruited for the UK Biobank study. All assessment centres are located within Scotland, Wales, and England. Participants were invited based on the criteria of being registered with a general practitioner through the National Health Service (NHS) and residing within a reasonable commute distance from an assessment centre (193). These localities are reflected in the balance of participants across self-reported ethnicities within the cohort of the present study, with 95.5% of participants across the three study groups reporting their ethnicity as "White". Given that most participants identified with this single ethnic group, there is the potential for biases related to genetic, socio-economic, and cultural factors. These biases may shape measures such as perceived and reported symptoms of anxiety disorders at assessment centres, cultural views and stigma surrounding anxiety disorders and their treatments, and the relationship between genetics and environmental factors. As a result, these biases could limit the external validity of the present study, as the results may not be representative of other populations.

There also exists the potential for bias in the classification of participants as having a lifetime anxiety disorder based on participant-reported symptoms. In general, the onset of anxiety disorders occurs during adolescence or early adulthood. As a result of the age demographic included in this study, participants might not recall details about their anxiety disorder diagnosis or the symptoms they experienced years ago accurately, leading to potential recall bias. Inaccurate recall of self-reported conditions may have resulted in participants being

misclassified during subject group selection. This potential bias introduces a problem when comparing the results of this study with studies that focus on adolescents or younger adults, who are more likely to have more recent onset of anxiety disorders and, therefore, have a more reliable recall of receiving anxiety disorder diagnoses and the symptoms they have experienced.

Some limitations arise due to the inherent constraints of the dataset. The UK Biobank prospective study collects extensive data from a large cohort (over 500,000 participants), with repeat data collected at follow-up sessions. While many measures are captured through diverse modalities, it would not be feasible to gather every specific detail that individual researchers might need for their unique research objectives. As a result, data collection at the imaging session regarding recent and current anxiety symptoms was limited, introducing a challenge in differentiating participants with current anxiety disorders from those who had achieved remission. Therefore, acute anxiety levels at the imaging session needed to be estimated using participant answers to a small number of questions asked at the imaging session. These questions included: “Would you call yourself a nervous person?”, “Are you a worrier?”, “Would you call yourself tense or ‘highly strung’?”, “Do you worry too long after an embarrassing experience?”, and “Do you suffer from ‘nerves’?”. Possible answers to each of these questions were: “Yes”, “No”, “Do not know”, or “Prefer not to answer”. The number of times a participant answered “Yes” across these questions was summed and used to match participants and balance levels of anxiety symptoms across the two case groups. These questions are suboptimal in assessing recent anxiety symptoms, as they are highly subjective and only loosely associated with acute or recent symptoms of anxiety disorders with a lack of specificity to symptoms of these disorders.

Another constraint of the dataset is the limited information collected surrounding past and present medication use. Participants were asked what medications they were taking at the time of the imaging session; however, the doses and formulation of these medications were not gathered. Therefore, a wide range of doses of each SSRI are likely included in the Anxiety + SSRI group, and no dose-related effects could be investigated. Furthermore, the dataset did not include information on how long a participant had been taking each medication and minimal information on past medication use. As a result, the effects of SSRI treatment duration and the effects of past use of SSRI could not be assessed or controlled for.

Although the UK Biobank is a long-term prospective cohort study, a cross-sectional design was chosen for the present study, which brings about several limitations. This design was chosen due to the low number of participants who had attended the repeat imaging session

by the time of the data release used, which would have significantly limited participant group sizes. The primary limitation of the cross-sectional design is that only correlation can be investigated, not causation. Therefore, in the present study, differences in functional connectivity observed between subject groups cannot be interpreted as differences caused by SSRI use compared to no medication use or a history of anxiety disorders. Although participants with potential confounding medical conditions and treatments were excluded and groups of subjects were matched with excellent covariate balance across subject groups, the effects of a participant taking an SSRI cannot be distinguished from functional connectivity associations of a participant requiring administration of an SSRI or the associations with the causes of anxiety disorders.

There are many factors which could explain the true cause of the observed functional connectivity differences. For example, differences in functional activity between participants with and without a history of anxiety disorders may originate from compensatory neural mechanisms which mitigate anxiety symptoms rather than directly from inherent pathophysiological differences. Despite this limitation, the findings of this cross-sectional study still provide a valuable foundation for further investigations by highlighting correlations between functional connectivity differences between subjects taking an SSRI and those not. Subsequent longitudinal studies could focus investigations on the regions of interest identified by the present study to clarify whether functional connectivity differences are caused by SSRI use or an alternative factor. If subsequent investigations find that the functional connectivity differences observed in the present study are a consequence of compensatory neural adaptations in response to the presence of anxiety disorders rather than a consequence of SSRI use, significant value can still be gained in informing the development of new treatments that aim to induce these adaptations.

6. Conclusion

This research aimed to explore and describe correlations of resting-state functional connectivity with the use of SSRI medications, as well as with a lifetime history of anxiety disorders. To achieve this, an ICA-based approach was taken to determine networks of functional connectivity common across a large number of subjects, and dual regression was utilised to statistically compare these networks across groups of subjects.

Overall, the findings of this research suggest a widespread decrease in functional connectivity across several networks of brain connectivity in association with use of SSRI medications. As this study used a cross-sectional design, it is important to note that these observations cannot be interpreted as a causative effect of SSRIs. However, these results highlight regions of interest for further research, as these networks of resting-state functional connectivity, most strongly correlated with the well-defined sensorimotor resting-state network, have not been extensively investigated in prior analyses of SSRI use.

Future longitudinal studies which investigate functional connectivity of these networks and brain regions over time could provide clarity on the time-course of these findings and confirm whether the findings of this cross-sectional study are a direct result of SSRI administration or correlated with their use through other mechanisms. Future studies could also investigate these regions of interest within other distinct, more diverse demographics to determine if the findings of the present study are generalisable beyond the sample cohort of this study.

SSRIs are, however, only one subclass of pharmacological treatments used for the treatment of anxiety disorders. Future studies may focus on different classes of medication, such as the tricyclic antidepressants, benzodiazepines, or even more novel options, such as ketamine. Additionally, future research could focus on correlations of functional connectivity differences relating to various psychotherapies or alternative interventions, such as transcranial magnetic stimulation. Observations of studies focusing on these treatments may be compared to the findings of this study to determine commonalities and dissimilarities across diverse treatment options, which may lead to new insights into the therapeutic mechanisms of these interventions.

Further studies which investigate functional connectivity associated with the use of these various treatment options and anxiety disorders may lead to the discovery of new biomarkers. These could be used to improve current diagnostic procedures, which are currently solely reliant on subjective symptom reporting, enable earlier detection of at-risk individuals, predict which treatment option is most likely to provide optimal benefit to individuals, and pave the way for the development of new, targeted treatment options for anxiety disorders.

References

1. Global, regional, and national burden of 12 mental disorders in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. The Lancet Psychiatry. 2022;9(2):137-50.

2. Baxter AJ, Vos T, Scott KM, Ferrari AJ, Whiteford HA. The global burden of anxiety disorders in 2010. Psychological Medicine. 2014;44(11):2363-74.

3. Wells JE, Oakley Browne MA, Scott KM, McGee MA, Baxter J, Kokaua J, et al. Te Rau Hinengaro: the New Zealand Mental Health Survey: overview of methods and findings. Aust N Z J Psychiatry. 2006;40(10):835-44.

4. Kessler RC, Üstün TB. The World Mental Health (WMH) Survey Initiative version of the World Health Organization (WHO) Composite International Diagnostic Interview (CIDI). International Journal of Methods in Psychiatric Research. 2004;13(2):93-121.

5. American Psychiatric Association. Diagnostic And Statistical Manual Of Mental Disorders, Fifth Edition, Text Revision (DSM-5-TR) 2022. Available from: https://doi.org/10.1176/appi.books.9780890425787.

6. Carter RM, Wittchen HU, Pfister H, Kessler RC. One-year prevalence of subthreshold and threshold DSM-IV generalized anxiety disorder in a nationally representative sample. Depress Anxiety. 2001;13(2):78-88.

7. Zhou Y, Cao Z, Yang M, Xi X, Guo Y, Fang M, et al. Comorbid generalized anxiety disorder and its association with quality of life in patients with major depressive disorder. Sci Rep. 2017;7:40511.

8. El-Mallakh RS, Hollifield M. Comorbid Anxiety in Bipolar Disorder Alters Treatment and Prognosis. Psychiatric Quarterly. 2008;79(2):139-50.

9. Rubio JM, Olfson M, Perez-Fuentes G, Garcia-Toro M, Wang S, Blanco C. Effect of first episode axis I disorders on quality of life. J Nerv Ment Dis. 2014;202(4):271-4.

10. Kanwar A, Malik S, Prokop LJ, Sim LA, Feldstein D, Wang Z, et al. The association between anxiety disorders and suicidal behaviors: a systematic review and meta-analysis. Depress Anxiety. 2013;30(10):917-29.

11. Patel A, Knapp M, Henderson J, Baldwin D. The economic consequences of social phobia. J Affect Disord. 2002;68(2-3):221-33.

12. García-Gutiérrez MS, Navarrete F, Sala F, Gasparyan A, Austrich-Olivares A, Manzanares J. Biomarkers in Psychiatry: Concept, Definition, Types and Relevance to the Clinical Reality. Frontiers in Psychiatry. 2020;11.

13. Shadli SM, Glue P, McIntosh J, McNaughton N. An improved human anxiety process biomarker: characterization of frequency band, personality and pharmacology. Translational Psychiatry. 2015;5(12):e699-e.

14. Bandelow B, Allgulander C, Baldwin DS, Costa DLdC, Denys D, Dilbaz N, et al. World Federation of Societies of Biological Psychiatry (WFSBP) guidelines for treatment of anxiety, obsessive-compulsive and posttraumatic stress disorders – Version 3. Part I: Anxiety disorders. The World Journal of Biological Psychiatry. 2023;24(2):79-117.

15. Byrne GJ. Interventions for generalized anxiety disorder. Curr Opin Psychiatry. 2023;36(2):134-9.

16. Bandelow B, Reitt M, Röver C, Michaelis S, Görlich Y, Wedekind D. Efficacy of treatments for anxiety disorders: a meta-analysis. International Clinical Psychopharmacology. 2015;30(4):183-92.

17. Andrews G, Bell CJ, Boyce P, Gale C, Lampe L, Marwat O, et al. Royal Australian and New Zealand College of Psychiatrists clinical practice guidelines for the treatment of panic disorder, social anxiety disorder and generalised anxiety disorder. Australian & New Zealand Journal of Psychiatry. 2018;52:1109 - 72.

18. Walter HJ, Bukstein OG, Abright AR, Keable H, Ramtekkar U, Ripperger-Suhler J, et al. Clinical Practice Guideline for the Assessment and Treatment of Children and Adolescents With Anxiety Disorders. Journal of the American Academy of Child & Adolescent Psychiatry. 2020;59(10):1107-24.

19. Bandelow B, Zohar J, Hollander E, Kasper S, Möller HJ, Allgulander G, et al. World Federation of Societies of Biological Psychiatry (WFSBP) guidelines for the pharmacological treatment of anxiety, obsessive-compulsive and post-traumatic stress disorders - First revision. World Journal of Biological Psychiatry. 2008;9(4):248-312.

20. Baldwin DS, Anderson IM, Nutt DJ, Bandelow B, Bond A, Davidson JRT, et al. Evidence-based guidelines for the pharmacological treatment of anxiety disorders: Recommendations from the British Association for Psychopharmacology. Journal of Psychopharmacology. 2005;19(6):567-96.

21. Domschke K, Seuling PD, Schiele MA, Bandelow B, Batelaan NM, Bokma WA, et al. The definition of treatment resistance in anxiety disorders: a Delphi method-based consensus guideline. World Psychiatry. 2024;23(1):113-23.

22. Benfield P, Heel RC, Lewis SP. Fluoxetine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in depressive illness. Drugs. 1986;32(6):481-508.

23. Hillhouse TM, Porter JH. A brief history of the development of antidepressant drugs: from monoamines to glutamate. Exp Clin Psychopharmacol. 2015;23(1):1-21.

24. Miller KL, Alfaro-Almagro F, Bangerter NK, Thomas DL, Yacoub E, Xu J, et al. Multimodal population brain imaging in the UK Biobank prospective epidemiological study. Nature Neuroscience. 2016;19(11):1523-36.

25. Grady CL, Rieck JR, Nichol D, Rodrigue KM, Kennedy KM. Influence of sample size and analytic approach on stability and interpretation of brain-behavior correlations in task-related fMRI data. Hum Brain Mapp. 2021;42(1):204-19.

26. UK Biobank. About us: Background [Internet]. 2023 [cited 2023 Aug 02]. Available from: https://www.ukbiobank.ac.uk/learn-more-about-uk-biobank/about-us.

27. UK Biobank. Baseline assessment data collection [Internet]. [cited 2024 Jun 19]. Available from: https://biobank.ndph.ox.ac.uk/ukb/exinfo.cgi?src=baseline_data.

28. Crocq MA. The history of generalized anxiety disorder as a diagnostic category. Dialogues Clin Neurosci. 2017;19(2):107-16.

29. Freud S. OBSESSIONS ET PHOBIES: Leur mécanisme psychique et leur étiologie. GESAMMELTE WERKE: I: WERKE AUS DEN JAHREN 1892–18991991. p. 345-53.

30. American Psychiatric Association. DSM History: APA; n.d. [cited 2023 Aug 5]. Available from: https://www.psychiatry.org/psychiatrists/practice/dsm/about-dsm/history-of-the-dsm#section_5.

31. American Psychiatric Association. Highlights of Changes from DSM-IV-TR to DSM-5 [Internet]. APA; 2013 [cited 2023 Aug 7]. Available from: https://www.psychiatry.org/File%20Library/Psychiatrists/Practice/DSM/APA_DSM_Changes_from_DSM-IV-TR_-to_DSM-5.pdf.

32. Kessler RC, Angermeyer M, Anthony JC, R DEG, Demyttenaere K, Gasquet I, et al. Lifetime prevalence and age-of-onset distributions of mental disorders in the World Health Organization's World Mental Health Survey Initiative. World Psychiatry. 2007;6(3):168-76.

33. Santomauro DF, Mantilla Herrera AM, Shadid J, Zheng P, Ashbaugh C, Pigott DM, et al. Global prevalence and burden of depressive and anxiety disorders in 204 countries and territories in 2020 due to the COVID-19 pandemic. The Lancet. 2021;398(10312):1700-12.

34. Patel V. Cultural factors and international epidemiology. Br Med Bull. 2001;57:33-45.

35. Baxter AJ, Scott KM, Vos T, Whiteford HA. Global prevalence of anxiety disorders: a systematic review and meta-regression. Psychol Med. 2013;43(5):897-910.

36. LeBeau RT, Glenn D, Liao B, Wittchen H-U, Beesdo-Baum K, Ollendick T, et al. Specific phobia: a review of DSM-IV specific phobia and preliminary recommendations for DSM-V. Depression and Anxiety. 2010;27(2):148-67.

37. Ruscio AM, Hallion LS, Lim CC, Aguilar-Gaxiola S, Al-Hamzawi A, Alonso J, et al. Cross-sectional comparison of the epidemiology of DSM-5 generalized anxiety disorder across the globe. JAMA psychiatry. 2017;74(5):465-75.

38. Bandelow B, Michaelis S. Epidemiology of anxiety disorders in the 21st century. Dialogues in Clinical Neuroscience. 2015;17(3):327-35.

39. Lijster JM, Dierckx B, Utens EM, Verhulst FC, Zieldorff C, Dieleman GC, et al. The Age of Onset of Anxiety Disorders. Can J Psychiatry. 2017;62(4):237-46.

40. Kessler RC, Ruscio AM, Shear K, Wittchen H-U. Epidemiology of anxiety disorders. Behavioral neurobiology of anxiety and its treatment. 2010:21-35.

41. Berenz EC, York TP, Bing-Canar H, Amstadter AB, Mezuk B, Gardner CO, et al. Time course of panic disorder and posttraumatic stress disorder onsets. Social psychiatry and psychiatric epidemiology. 2019;54:639-47.

42. Gretarsdottir E, Woodruff-Borden J, Meeks S, Depp CA. Social anxiety in older adults: phenomenology, prevalence, and measurement. Behav Res Ther. 2004;42(4):459-75.

43. Wolitzky-Taylor KB, Castriotta N, Lenze EJ, Stanley MA, Craske MG. Anxiety disorders in older adults: a comprehensive review. Depress Anxiety. 2010;27(2):190-211.

44. Hettema JM, Prescott CA, Myers JM, Neale MC, Kendler KS. The Structure of Genetic and Environmental Risk Factors for Anxiety Disorders in Men and Women. Archives of General Psychiatry. 2005;62(2):182-9.

45. Mohammadi MR, Pourdehghan P, Mostafavi SA, Hooshyari Z, Ahmadi N, Khaleghi A. Generalized anxiety disorder: Prevalence, predictors, and comorbidity in children and adolescents. J Anxiety Disord. 2020;73:102234.

46. Gottschalk MG, Domschke K. Novel developments in genetic and epigenetic mechanisms of anxiety. Curr Opin Psychiatry. 2016;29(1):32-8.

47. Watson D, Clark LA. Negative affectivity: the disposition to experience aversive emotional states. Psychological bulletin. 1984;96(3):465.

48. Gidron Y. Trait Anxiety. In: Gellman MD, Turner JR, editors. Encyclopedia of Behavioral Medicine. New York, NY: Springer New York; 2013. p. 1989-.

49. Rohrbacher H, Hoyer J, Beesdo K, Höfler M, Bittner A, Lieb R, et al. Psychometric properties of the Retrospective Self Report of Inhibition (RSRI) in a representative German sample. International Journal of Methods in Psychiatric Research. 2008;17(2):80-8.

50. Blöte AW, Miers AC, Heyne DA, Westenberg PM. Social anxiety and the school environment of adolescents. Social anxiety and phobia in adolescents: Development, manifestation and intervention strategies. 2015:151-81.

51. Tiet QQ, Bird HR, Hoven CW, Moore R, Wu P, Wicks J, et al. Relationship between specific adverse life events and psychiatric disorders. Journal of abnormal child psychology. 2001;29:153-64.

52. Hettema JM, Neale MC, Kendler KS. A review and meta-analysis of the genetic epidemiology of anxiety disorders. Am J Psychiatry. 2001;158(10):1568-78.

53. Scaini S, Belotti R, Ogliari A. Genetic and environmental contributions to social anxiety across different ages: A meta-analytic approach to twin data. Journal of anxiety disorders. 2014;28(7):650-6.

54. Bögels S, Stein MB. Social phobia. Stress-induced and fear circuitry disorders: Refining the research agenda for DSM–V. 2009:59-75.

55. Cavanna AE, Purpura G, Riva A, Nacinovich R, Seri S. The Western origins of mindfulness therapy in ancient Rome. Neurol Sci. 2023;44(6):1861-9.

56. Marchand WR. Mindfulness-based stress reduction, mindfulness-based cognitive therapy, and Zen meditation for depression, anxiety, pain, and psychological distress. J Psychiatr Pract. 2012;18(4):233-52.

57. Salmon P, Sephton S, Weissbecker I, Hoover K, Ulmer C, Studts JL. Mindfulness meditation in clinical practice. Cognitive and Behavioral Practice. 2004;11(4):434-46.

58. Wilson TK, Tripp J. Buspirone. 2018.

59. Nutt DJ, Forshall S, Bell C, Rich A, Sandford J, Nash J, et al. Mechanisms of action of selective serotonin reuptake inhibitors in the treatment of psychiatric disorders. European Neuropsychopharmacology. 1999;9:S81-S6.

60. Rickels K, Amsterdam J, Clary C, Fox I, Schweizer E, Weise C. A placebo-controlled, double-blind, clinical trial of paroxetine in depressed outpatients. Acta Psychiatrica Scandinavica. 1989;80(S350):117-23.

61. Laws D, Ashford JJ, Anstee JA. A multicentre double-blind comparative trial of fluvoxamine versus lorazepam in mixed anxiety and depression treated in general practice. Acta Psychiatrica Scandinavica. 1990;81(2):185-9.

62. Stein MB, Liebowitz MR, Lydiard RB, Pitts CD, Bushnell W, Gergel I. Paroxetine treatment of generalized social phobia (social anxiety disorder): a randomized controlled trial. Jama. 1998;280(8):708-13.

63. Goodman WK, Price LH, Rasmussen SA, Delgado PL, Heninger GR, Charney DS. Efficacy of fluvoxamine in obsessive-compulsive disorder: a double-blind comparison with placebo. Archives of general psychiatry. 1989;46(1):36-44.

64. Rocca P, Fonzo V, Scotta M, Zanalda E, Ravizza L. Paroxetine efficacy in the treatment of generalized anxiety disorder. Acta Psychiatrica Scandinavica. 1997;95(5):444-50.

65. Hirschfeld RM. History and evolution of the monoamine hypothesis of depression. Journal of clinical psychiatry. 2000;61(6):4-6.

66. Freis ED. Mental depression in hypertensive patients treated for long periods with large doses of reserpine. N Engl J Med. 1954;251(25):1006-8.

67. Schildkraut JJ. The catecholamine hypothesis of affective disorders: a review of supporting evidence. American journal of Psychiatry. 1965;122(5):509-22.

68. Maron E, Nutt D. Biological predictors of pharmacological therapy in anxiety disorders. Dialogues Clin Neurosci. 2015;17(3):305-17.

69. Zanos P, Gould TD. Mechanisms of ketamine action as an antidepressant. Mol Psychiatry. 2018;23(4):801-11.

70. Murrough JW, Iosifescu DV, Chang LC, Al Jurdi RK, Green CE, Perez AM, et al. Antidepressant efficacy of ketamine in treatment-resistant major depression: a two-site randomized controlled trial. Am J Psychiatry. 2013;170(10):1134-42.

71. Strasburger SE, Bhimani PM, Kaabe JH, Krysiak JT, Nanchanatt DL, Nguyen TN, et al. What is the mechanism of Ketamine's rapid-onset antidepressant effect? A concise overview of the surprisingly large number of possibilities. Journal of Clinical Pharmacy and Therapeutics. 2017;42(2):147-54.

72. Whittaker E, Dadabayev AR, Joshi SA, Glue P. Systematic review and meta-analysis of randomized controlled trials of ketamine in the treatment of refractory anxiety spectrum disorders. Therapeutic Advances in Psychopharmacology. 2021;11.

73. Engin E, Treit D, Dickson CT. Anxiolytic- and antidepressant-like properties of ketamine in behavioral and neurophysiological animal models. Neuroscience. 2009;161(2):359-69.

74. Garakani A, Murrough JW, Freire RC, Thom RP, Larkin K, Buono FD, et al. Pharmacotherapy of Anxiety Disorders: Current and Emerging Treatment Options. Frontiers in Psychiatry. 2020;11.

75. Byrne SE, Rothschild AJ. Loss of antidepressant efficacy during maintenance therapy: possible mechanisms and treatments. J Clin Psychiatry. 1998;59(6):279-88.

76. Solomon DA, Leon AC, Mueller TI, Coryell W, Teres JJ, Posternak MA, et al. Tachyphylaxis in unipolar major depressive disorder. J Clin Psychiatry. 2005;66(3):283-90.

77. Posternak MA, Zimmerman M. Dual reuptake inhibitors incur lower rates of tachyphylaxis than selective serotonin reuptake inhibitors: a retrospective study. Journal of Clinical Psychiatry. 2005;66(6):705-7.

78. Targum SD. Identification and treatment of antidepressant tachyphylaxis. Innov Clin Neurosci. 2014;11(3-4):24-8.

79. Fava GA. Can long-term treatment with antidepressant drugs worsen the course of depression? J Clin Psychiatry. 2003;64(2):123-33.

80. Fava GA, Offidani E. The mechanisms of tolerance in antidepressant action. Prog Neuropsychopharmacol Biol Psychiatry. 2011;35(7):1593-602.

81. Amsterdam JD, Williams D, Michelson D, Adler LA, Dunner DL, Nierenberg AA, et al. Tachyphylaxis after repeated antidepressant drug exposure in patients with recurrent major depressive disorder. Neuropsychobiology. 2009;59(4):227-33.

82. Bel N, Artigas F. Fluvoxamine preferentially increases extracellular 5-hydroxytryptamine in the raphe nuclei: an in vivo microdialysis study. European journal of pharmacology. 1992;229(1):101-3.

83. Stahl SM. Mechanism of action of serotonin selective reuptake inhibitors. Serotonin receptors and pathways mediate therapeutic effects and side effects. J Affect Disord. 1998;51(3):215-35.

84. Berger M, Gray JA, Roth BL. The expanded biology of serotonin. Annu Rev Med. 2009;60:355-66.

85. Richelson E, editor Pharmacology of antidepressants. Mayo Clinic Proceedings; 2001: Elsevier.

86. Blattner KM, Canney DJ, Pippin DA, Blass BE. Pharmacology and Therapeutic Potential of the 5-HT7 Receptor. ACS Chemical Neuroscience. 2019;10(1):89-119.

87. Sahu A, Gopalakrishnan L, Gaur N, Chatterjee O, Mol P, Modi PK, et al. The 5-Hydroxytryptamine signaling map: an overview of serotonin-serotonin receptor mediated signaling network. Journal of Cell Communication and Signaling. 2018;12(4):731-5.

88. Andreetta F, Carboni L, Grafton G, Jeggo R, Whyment AD, van den Top M, et al. Hippocampal 5-HT7 receptors signal phosphorylation of the GluA1 subunit to facilitate AMPA

receptor mediated-neurotransmission in vitro and in vivo. British Journal of Pharmacology. 2016;173(9):1438-51.

89. Karpova NN, Pickenhagen A, Lindholm J, Tiraboschi E, Kulesskaya N, Ágústsdóttir A, et al. Fear Erasure in Mice Requires Synergy Between Antidepressant Drugs and Extinction Training. Science. 2011;334(6063):1731-4.

90. Klöbl M, Seiger R, Vanicek T, Handschuh P, Reed MB, Spurny-Dworak B, et al. Escitalopram modulates learning content-specific neuroplasticity of functional brain networks. NeuroImage. 2022;247:118829.

91. Harmer CJ, Duman RS, Cowen PJ. How do antidepressants work? New perspectives for refining future treatment approaches. The Lancet Psychiatry. 2017;4(5):409-18.

92. Castrén E. Neurotrophins and psychiatric disorders. Neurotrophic Factors. 2014:461-79.

93. Björkholm C, Monteggia LM. BDNF–a key transducer of antidepressant effects. Neuropharmacology. 2016;102:72-9.

94. Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. Biological psychiatry. 2006;59(12):1116-27.

95. Risbrough VB, Stein MB. Role of corticotropin releasing factor in anxiety disorders: a translational research perspective. Hormones and behavior. 2006;50(4):550-61.

96. Abelson JL, Khan S, Liberzon I, Young EA. HPA axis activity in patients with panic disorder: review and synthesis of four studies. Depression and anxiety. 2007;24(1):66-76.

97. Tafet GE, Nemeroff CB. Pharmacological Treatment of Anxiety Disorders: The Role of the HPA Axis. Frontiers in Psychiatry. 2020;11.

98. Heydendael W, Jacobson L. Widespread hypothalamic–pituitary–adrenocortical axis-relevant and mood-relevant effects of chronic fluoxetine treatment on glucocorticoid receptor gene expression in mice. European Journal of Neuroscience. 2010;31(5):892-902.

99. Jack M. Gorman, M.D. ,, Justine M. Kent, M.D. ,, Gregory M. Sullivan, M.D. , and, Jeremy D. Coplan, M.D. Neuroanatomical Hypothesis of Panic Disorder, Revised. American Journal of Psychiatry. 2000;157(4):493-505.

100. Amit Etkin MD, Ph.D. ,, Tor D. Wager PD. Functional Neuroimaging of Anxiety: A Meta-Analysis of Emotional Processing in PTSD, Social Anxiety Disorder, and Specific Phobia. American Journal of Psychiatry. 2007;164(10):1476-88.

101. Šimić G, Tkalčić M, Vukić V, Mulc D, Španić E, Šagud M, et al. Understanding Emotions: Origins and Roles of the Amygdala. Biomolecules. 2021;11(6).

102. Buff C, Schmidt C, Brinkmann L, Gathmann B, Tupak S, Straube T. Directed threat imagery in generalized anxiety disorder. Psychological Medicine. 2018;48(4):617-28.

103. Monk CS, Telzer EH, Mogg K, Bradley BP, Mai X, Louro HM, et al. Amygdala and ventrolateral prefrontal cortex activation to masked angry faces in children and adolescents with generalized anxiety disorder. Archives of general psychiatry. 2008;65(5):568-76.

104. Klumpp H, Fitzgerald JM. Neuroimaging Predictors and Mechanisms of Treatment Response in Social Anxiety Disorder: an Overview of the Amygdala. Current Psychiatry Reports. 2018;20(10):89.

105. Hattingh CJ, Ipser J, Tromp S, Syal S, Lochner C, Brooks S, et al. Functional magnetic resonance imaging during emotion recognition in social anxiety disorder: an activation likelihood meta-analysis. Frontiers in human neuroscience. 2013;6:347.

106. Whalen PJ, Johnstone T, Somerville LH, Nitschke JB, Polis S, Alexander AL, et al. A Functional Magnetic Resonance Imaging Predictor of Treatment Response to Venlafaxine in Generalized Anxiety Disorder. Biological Psychiatry. 2008;63(9):858-63.

107. Ghasemi M, Navidhamidi M, Rezaei F, Azizikia A, Mehranfard N. Anxiety and hippocampal neuronal activity: Relationship and potential mechanisms. Cognitive, Affective, & Behavioral Neuroscience. 2022;22(3):431-49.

108. Duval ER, Javanbakht A, Liberzon I. Neural circuits in anxiety and stress disorders: a focused review. Ther Clin Risk Manag. 2015;11:115-26.

109. Uddin LQ, Nomi JS, Hébert-Seropian B, Ghaziri J, Boucher O. Structure and Function of the Human Insula. J Clin Neurophysiol. 2017;34(4):300-6.

110. Caseras X, Giampietro V, Lamas A, Brammer M, Vilarroya O, Carmona S, et al. The functional neuroanatomy of blood-injection-injury phobia: a comparison with spider phobics and healthy controls. Psychol Med. 2010;40(1):125-34.

111. Brühl AB, Rufer M, Delsignore A, Kaffenberger T, Jäncke L, Herwig U. Neural correlates of altered general emotion processing in social anxiety disorder. Brain Res. 2011;1378:72-83.

112. Patel R, Spreng RN, Shin LM, Girard TA. Neurocircuitry models of posttraumatic stress disorder and beyond: a meta-analysis of functional neuroimaging studies. Neurosci Biobehav Rev. 2012;36(9):2130-42.

113. Campbell-Sills L, Simmons AN, Lovero KL, Rochlin AA, Paulus MP, Stein MB. Functioning of neural systems supporting emotion regulation in anxiety-prone individuals. Neuroimage. 2011;54(1):689-96.

114. Blair KS, Geraci M, Otero M, Majestic C, Odenheimer S, Jacobs M, et al. Atypical modulation of medial prefrontal cortex to self-referential comments in generalized social phobia. Psychiatry Res. 2011;193(1):38-45.

115. Sripada CS, Angstadt M, Banks S, Nathan PJ, Liberzon I, Phan KL. Functional neuroimaging of mentalizing during the trust game in social anxiety disorder. Neuroreport. 2009;20(11):984-9.

116. Kolesar TA, Bilevicius E, Wilson AD, Kornelsen J. Systematic review and meta-analyses of neural structural and functional differences in generalized anxiety disorder and healthy controls using magnetic resonance imaging. Neuroimage Clin. 2019;24:102016.

117. Brandl F, Weise B, Mulej Bratec S, Jassim N, Hoffmann Ayala D, Bertram T, et al. Common and specific large-scale brain changes in major depressive disorder, anxiety disorders, and chronic pain: a transdiagnostic multimodal meta-analysis of structural and functional MRI studies. Neuropsychopharmacology. 2022;47(5):1071-80.

118. Briley PM, Webster L, Boutry C, Cottam WJ, Auer DP, Liddle PF, et al. Resting-state functional connectivity correlates of anxiety co-morbidity in major depressive disorder. Neurosci Biobehav Rev. 2022;138:104701.

119. Hilbert K, Lueken U, Beesdo-Baum K. Neural structures, functioning and connectivity in Generalized Anxiety Disorder and interaction with neuroendocrine systems: a systematic review. J Affect Disord. 2014;158:114-26.

120. Hahn A, Stein P, Windischberger C, Weissenbacher A, Spindelegger C, Moser E, et al. Reduced resting-state functional connectivity between amygdala and orbitofrontal cortex in social anxiety disorder. NeuroImage. 2011;56(3):881-9.

121. Tromp DP, Grupe DW, Oathes DJ, McFarlin DR, Hernandez PJ, Kral TR, et al. Reduced structural connectivity of a major frontolimbic pathway in generalized anxiety disorder. Arch Gen Psychiatry. 2012;69(9):925-34.

122. Zhang Y, Li L, Yu R, Liu J, Tang J, Tan L, et al. White matter integrity alterations in first episode, treatment-naive generalized anxiety disorder. J Affect Disord. 2013;148(2-3):196-201.

123. Zhang L, Zhang Y, Li L, Li Z, Li W, Ma N, et al. Different white matter abnormalities between the first-episode, treatment-naive patients with posttraumatic stress disorder and generalized anxiety disorder without comorbid conditions. Journal of affective disorders. 2011;133(1-2):294-9.

124. Wang W, Qian S, Liu K, Li B, Li M, Xin K, et al. Reduced white matter integrity and its correlation with clinical symptom in first-episode, treatment-naive generalized anxiety disorder. Behav Brain Res. 2016;314:159-64.

125. Taylor JM, Whalen PJ. Neuroimaging and Anxiety: the Neural Substrates of Pathological and Non-pathological Anxiety. Curr Psychiatry Rep. 2015;17(6):49.

126. Holzschneider K, Mulert C. Neuroimaging in anxiety disorders. Dialogues in Clinical Neuroscience. 2011;13(4):453-61.

127. Nord CL, Barrett LF, Lindquist KA, Ma Y, Marwood L, Satpute AB, et al. Neural effects of antidepressant medication and psychological treatments: a quantitative synthesis across three meta-analyses. Br J Psychiatry. 2021;219(4):546-50.

128. He C, Gong L, Yin Y, Yuan Y, Zhang H, Lv L, et al. Amygdala connectivity mediates the association between anxiety and depression in patients with major depressive disorder. Brain Imaging and Behavior. 2019;13(4):1146-59.

129. Harmer CJ, Mackay CE, Reid CB, Cowen PJ, Goodwin GM. Antidepressant Drug Treatment Modifies the Neural Processing of Nonconscious Threat Cues. Biological Psychiatry. 2006;59(9):816-20.

130. Meyer BM, Rabl U, Huemer J, Bartova L, Kalcher K, Provenzano J, et al. Prefrontal networks dynamically related to recovery from major depressive disorder: a longitudinal pharmacological fMRI study. Transl Psychiatry. 2019;9(1):64.

131. Rosenblau G, Sterzer P, Stoy M, Park S, Friedel E, Heinz A, et al. Functional neuroanatomy of emotion processing in major depressive disorder is altered after successful antidepressant therapy. J Psychopharmacol. 2012;26(11):1424-33.

132. Godlewska BR, Norbury R, Selvaraj S, Cowen PJ, Harmer CJ. Short-term SSRI treatment normalises amygdala hyperactivity in depressed patients. Psychol Med. 2012;42(12):2609-17.

133. Vai B, Bulgarelli C, Godlewska BR, Cowen PJ, Benedetti F, Harmer CJ. Fronto-limbic effective connectivity as possible predictor of antidepressant response to SSRI administration. Eur Neuropsychopharmacol. 2016;26(12):2000-10.

134. Gorka SM, Young CB, Klumpp H, Kennedy AE, Francis J, Ajilore O, et al. Emotion-based brain mechanisms and predictors for SSRI and CBT treatment of anxiety and depression: a randomized trial. Neuropsychopharmacology. 2019;44(9):1639-48.

135. Kotoula V, Evans JW, Punturieri CE, Zarate CA, Jr. Review: The use of functional magnetic resonance imaging (fMRI) in clinical trials and experimental research studies for depression. Front Neuroimaging. 2023;2:1110258.

136. Goldstein-Piekarski AN, Staveland BR, Ball TM, Yesavage J, Korgaonkar MS, Williams LM. Intrinsic functional connectivity predicts remission on antidepressants: a randomized controlled trial to identify clinically applicable imaging biomarkers. Transl Psychiatry. 2018;8(1):57.

137. Kilpatrick LA, Krause-Sorio B, Siddarth P, Narr KL, Lavretsky H. Default mode network connectivity and treatment response in geriatric depression. Brain Behav. 2022;12(4):e2475.

138. Alexopoulos GS, Hoptman MJ, Kanellopoulos D, Murphy CF, Lim KO, Gunning FM. Functional connectivity in the cognitive control network and the default mode network in late-life depression. J Affect Disord. 2012;139(1):56-65.

139. Matthews SC, Simmons AN, Strigo IA, Arce E, Stein MB, Paulus MP. Escitalopram attenuates posterior cingulate activity during self-evaluation in healthy volunteers. Psychiatry Res. 2010;182(2):81-7.

140. van de Ven V, Wingen M, Kuypers KP, Ramaekers JG, Formisano E. Escitalopram Decreases Cross-Regional Functional Connectivity within the Default-Mode Network. PLoS One. 2013;8(6):e68355.

141. Anand A, Li Y, Wang Y, Wu J, Gao S, Bukhari L, et al. Antidepressant effect on connectivity of the mood-regulating circuit: an FMRI study. Neuropsychopharmacology. 2005;30(7):1334-44.

142. Lu L, Mills JA, Li H, Schroeder HK, Mossman SA, Varney ST, et al. Acute Neurofunctional Effects of Escitalopram in Pediatric Anxiety: A Double-Blind, Placebo-Controlled Trial. J Am Acad Child Adolesc Psychiatry. 2021;60(10):1309-18.

143. Schrantee A, Lucassen PJ, Booij J, Reneman L. Serotonin transporter occupancy by the SSRI citalopram predicts default-mode network connectivity. Eur Neuropsychopharmacol. 2018;28(10):1173-9.

144. Klaassens BL, Rombouts SARB, Winkler AM, van Gorsel HC, van der Grond J, van Gerven JMA. Time related effects on functional brain connectivity after serotonergic and cholinergic neuromodulation. Human Brain Mapping. 2017;38(1):308-25.

145. Smith SM, Fox PT, Miller KL, Glahn DC, Fox PM, Mackay CE, et al. Correspondence of the brain's functional architecture during activation and rest. Proceedings of the National Academy of Sciences. 2009;106(31):13040-5.

146. Klaassens BL, van Gorsel HC, Khalili-Mahani N, van der Grond J, Wyman BT, Whitcher B, et al. Single-dose serotonergic stimulation shows widespread effects on functional brain connectivity. NeuroImage. 2015;122:440-50.

147. Kasper S. Anxiety disorders: under-diagnosed and insufficiently treated. International Journal of Psychiatry in Clinical Practice. 2006;10(sup1):3-9.

148. Wang PS, Berglund P, Olfson M, Pincus HA, Wells KB, Kessler RC. Failure and delay in initial treatment contact after first onset of mental disorders in the National Comorbidity Survey Replication. Archives of general psychiatry. 2005;62(6):603-13.

149. Bystritsky A. Treatment-resistant anxiety disorders. Mol Psychiatry. 2006;11(9):805-14.

150. Smith SM, Alfaro-Almagro F, Miller KL. UK Biobank Brain Imaging Documentation 2022 [cited 2023 Jul 3]. Available from: https://biobank.ctsu.ox.ac.uk/crystal/crystal/docs/brain_mri.pdf.

151. Ho D, Imai K, King G, Stuart EA. MatchIt: Nonparametric Preprocessing for Parametric Causal Inference. Journal of Statistical Software. 2011;42(8):1 - 28.

152. Diamond A, Sekhon JS. Genetic Matching for Estimating Causal Effects: A General Multivariate Matching Method for Achieving Balance in Observational Studies. The Review of Economics and Statistics. 2013;95(3):932-45.

153. Sekhon JS. Multivariate and Propensity Score Matching Software with Automated Balance Optimization: The Matching package for R. Journal of Statistical Software. 2011;42(7):1 - 52.

154. Bijsterbosch J, Smith SM, Beckmann CF. Introduction to Resting State fMRI Functional Connectivity (Oxford Neuroimaging Primers) 2017 [cited 2023 Jul 21].

155. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2023.

156. Greifer N. cobalt: Covariate Balance Tables and Plots. R package version 4.5.1 2023 [cited 2023 Jul 9]. Available from: https://CRAN.R-project.org/package=cobalt.

157. Stuart EA, Lee BK, Leacy FP. Prognostic score-based balance measures can be a useful diagnostic for propensity score methods in comparative effectiveness research. Journal of Clinical Epidemiology. 2013;66(8):S84-S90.e1.

158. Greifer N. Covariate Balance Tables and Plots: A Guide to the cobalt Package [Internet]. 2023 [cited 2023 Aug 11]. Available from: https://cran.r-project.org/web/packages/cobalt/vignettes/cobalt.html.

159. Stuart EA. Matching Methods for Causal Inference: A Review and a Look Forward. Statistical Science. 2010;25(1):1-21, .

160. Rubin DB. Using Propensity Scores to Help Design Observational Studies: Application to the Tobacco Litigation. Health Services and Outcomes Research Methodology. 2001;2(3):169-88.

161. Austin PC. Balance diagnostics for comparing the distribution of baseline covariates between treatment groups in propensity-score matched samples. Statistics in Medicine. 2009;28(25):3083-107.

162. Ali MS, Groenwold RHH, Pestman WR, Belitser SV, Roes KCB, Hoes AW, et al. Propensity score balance measures in pharmacoepidemiology: a simulation study. Pharmacoepidemiology and Drug Safety. 2014;23(8):802-11.

163. Greifer N. Matching Methods [Internet]. 2023 [cited 2023 Jun 16]. Available from: https://cran.r-project.org/web/packages/MatchIt/vignettes/matching-methods.html.

164. Alfaro-Almagro F, Jenkinson M, Bangerter NK, Andersson JL, Griffanti L, Douaud G, et al. Image processing and Quality Control for the first 10,000 brain imaging datasets from UK Biobank. Neuroimage. 2018;166:400-24.

165. Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage. 2002;17(2):825-41.

166. Beckmann CF, Smith SM. Probabilistic independent component analysis for functional magnetic resonance imaging. IEEE Trans Med Imaging. 2004;23(2):137-52.

167. Salimi-Khorshidi G, Douaud G, Beckmann CF, Glasser MF, Griffanti L, Smith SM. Automatic denoising of functional MRI data: combining independent component analysis and hierarchical fusion of classifiers. Neuroimage. 2014;90:449-68.

168. NeuroImaging & Surgical Technologies Lab. MNI ICBM152 non-linear: NIST; 2023 [cited 2023 Aug 9]. Available from: https://nist.mni.mcgill.ca/mni-icbm152-non-linear-6th-generation-symmetric-average-brain-stereotaxic-registration-model/.

169. Woolrich MW, Jbabdi S, Patenaude B, Chappell M, Makni S, Behrens T, et al. Bayesian analysis of neuroimaging data in FSL. Neuroimage. 2009;45(1 Suppl):S173-86.

170. Fonov V, Evans AC, Botteron K, Almli CR, McKinstry RC, Collins DL. Unbiased average age-appropriate atlases for pediatric studies. NeuroImage. 2011;54(1):313-27.

171. McCarthy P. FSLeyes (1.8.1). Zenodo [Internet]. 2023 [cited 2023 Aug 10]. Available from: https://doi.org/10.5281/zenodo.8253783.

172. Fslutils - FslWiki [Internet]. FSL; [updated 2020 18 Apr; cited 2023 Sep 8]. Available from: https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Fslutils.

173. Beckmann CF, Mackay CE, Filippini N, Smith SM. Group comparison of resting-state FMRI data using multi-subject ICA and dual regression. Neuroimage. 2009;47(Suppl 1):S148.
174. Nickerson LD, Smith SM, Ongur D, Beckmann CF. Using Dual Regression to Investigate Network Shape and Amplitude in Functional Connectivity Analyses. Front Neurosci. 2017;11:115.
175. Winkler AM, Ridgway GR, Webster MA, Smith SM, Nichols TE. Permutation inference for the general linear model. NeuroImage. 2014;92:381-97.
176. Smith SM, Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. Neuroimage. 2009;44(1):83-98.
177. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal statistical society: series B (Methodological). 1995;57(1):289-300.
178. Cluster - FslWiki [Internet]. FSL; [updated 2023 Aug 7; cited 2023 Sep 13]. Available from: https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Cluster.
179. Atlasquery - FslWiki [Internet]. FSL; [updated 2013 20 Jan; cited 2023 Sep 14]. Available from: https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlasquery.
180. Atlases - FslWiki [Internet]. FSL; [updated Aug 22 2023; cited 2023 Sep 13]. Available from: https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases.
181. Gong D, He H, Ma W, Liu D, Huang M, Dong L, et al. Functional Integration between Salience and Central Executive Networks: A Role for Action Video Game Experience. Neural Plast. 2016;2016:9803165.
182. Scolari M, Seidl-Rathkopf KN, Kastner S. Functions of the human frontoparietal attention network: Evidence from neuroimaging. Current Opinion in Behavioral Sciences. 2015;1:32-9.
183. Iidaka T, Matsumoto A, Nogawa J, Yamamoto Y, Sadato N. Frontoparietal Network Involved in Successful Retrieval from Episodic Memory. Spatial and Temporal Analyses Using fMRI and ERP. Cerebral Cortex. 2006;16(9):1349-60.
184. Corbetta M. Frontoparietal cortical networks for directing attention and the eye to visual locations: Identical, independent, or overlapping neural systems? Proceedings of the National Academy of Sciences. 1998;95(3):831-8.
185. Banker L, Tadi P. Neuroanatomy, Precentral Gyrus. StatPearls. Treasure Island (FL): StatPearls Publishing

186. DiGuiseppi J, Tadi P. Neuroanatomy, Postcentral Gyrus. StatPearls. Treasure Island (FL): StatPearls Publishing
Copyright © 2023, StatPearls Publishing LLC.; 2023.
187. Chen TL, Babiloni C, Ferretti A, Perrucci MG, Romani GL, Rossini PM, et al. Human secondary somatosensory cortex is involved in the processing of somatosensory rare stimuli: an fMRI study. Neuroimage. 2008;40(4):1765-71.
188. Lee S, Kruglikov I, Huang ZJ, Fishell G, Rudy B. A disinhibitory circuit mediates motor integration in the somatosensory cortex. Nat Neurosci. 2013;16(11):1662-70.
189. Kropf E, Syan SK, Minuzzi L, Frey BN. From anatomy to function: the role of the somatosensory cortex in emotional regulation. Braz J Psychiatry. 2019;41(3):261-9.
190. Adolphs R, Damasio H, Tranel D, Cooper G, Damasio AR. A role for somatosensory cortices in the visual recognition of emotion as revealed by three-dimensional lesion mapping. J Neurosci. 2000;20(7):2683-90.
191. Phillips ML, Drevets WC, Rauch SL, Lane R. Neurobiology of emotion perception II: implications for major psychiatric disorders. Biological Psychiatry. 2003;54(5):515-28.
192. Höistad M, Barbas H. Sequence of information processing for emotions through pathways linking temporal and insular cortices with the amygdala. Neuroimage. 2008;40(3):1016-33.
193. UK Biobank: Protocol for a large-scale prospective epidemiological resource Available from: https://www.ukbiobank.ac.uk/media/gnkeyh2q/study-rationale.pdf.
194. Davis KAS, Cullen B, Adams M, Brailean A, Breen G, Coleman JRI, et al. Indicators of mental disorders in UK Biobank-A comparison of approaches. Int J Methods Psychiatr Res. 2019;28(3):e1796.

Appendices

Appendix A: Excluded Conditions

Online follow-up questionnaire self-reported diagnoses (Data-Field 20544)

- Schizophrenia
- Any other type of psychosis or psychotic illness
- Obsessive compulsive disorder (OCD)
- Mania, hypomania, bipolar or manic-depression
- Prefer not to answer (group A)
- Prefer not to answer (group B)

Self-reported diagnoses (Data-Fields 20001 and 20002)

- All cancers
- Self-reported neurological diagnoses
    - Brain abscess/intracranial abscess
    - Cranial nerve problem/palsy
    - Bell's palsy/facial nerve palsy
    - Trigeminal neuralgia
    - Spinal cord disorder
    - Paraplegia
    - Spina bifida
    - Peripheral nerve disorder
    - Acute infective polyneuritis/Guillain-Barre syndrome
    - Chronic/degenerative neurological problem
    - Epilepsy
    - Migraine
    - Cerebral palsy
    - Other neurological problem
    - Myasthenia gravis
    - Myasthenia gravis (#2)

  - Benign / essential tremor
  - Polio / poliomyelitis
  - Meningioma / benign meningeal tumour
  - Benign neuroma
  - Neurological injury/trauma
  - Head injury
  - Spinal injury
  - Motor neurone disease
  - Multiple sclerosis
  - Parkinsons disease
  - Dementia/Alzheimer's/cognitive impairment
  - Other demyelinating disease (not multiple sclerosis)
- Self-reported psychiatric diagnoses
  - Schizophrenia
  - Mania/bipolar disorder/manic depression
  - Alcohol dependency
  - Opioid dependency
  - Other substance abuse/dependency
  - Post-traumatic stress disorder
  - Obsessive compulsive disorder
- Self-reported cardiovascular diagnoses
  - Stroke
  - Ischaemic stroke
  - Transient ischaemic attack
  - Subdural haemorrhage/haematoma
  - Subarachnoid haemorrhage
  - Heart failure/pulmonary oedema
  - Peripheral vascular disease
  - Heart attack/myocardial infarction
- Self-reported pulmonary diagnoses
  - Respiratory failure

ICD-9 hospital diagnoses

- Mental Disorders
    - Senile and presenile organic psychotic conditions
    - Schizophrenic psychoses
    - Affective psychoses
    - Obsessive-compulsive disorders
    - Specific nonpsychotic mental disorders following organic brain damage
    - Mental retardation
- Diseases of the nervous system and the sense organs
    - Intracranial abscess
    - Hereditary and degenerative diseases of the central nervous system
    - Other disorders of the central nervous system
    - Myasthenia gravis
- Diseases of the circulatory system
    - Cerebrovascular disease

ICD-10 hospital diagnoses

- Mental and behavioural disorders
    - Dementia in Alzheimer's disease
    - Vascular dementia
    - Dementia in other diseases classified elsewhere
    - Unspecified dementia
    - Organic amnesic syndrome, not induced by alcohol and other psychoactive substances
    - Other mental disorders due to brain damage and dysfunction and to physical disease
    - Personality and behavioural disorders due to brain disease, damage and dysfunction
    - Schizophrenia
    - Persistent delusional disorders
    - Acute and transient psychotic disorders
    - Induced delusional disorder

  - Schizoaffective disorders
  - Other nonorganic psychotic disorders
  - Unspecified nonorganic psychosis
  - Manic episode
  - Bipolar affective disorder
  - Obsessive-compulsive disorder
  - Mental retardation

- Diseases of the nervous system
  - Systemic atrophies primarily affecting the central nervous system
  - Parkinson's disease
  - Other degenerative diseases of the nervous system
  - Demyelinating diseases of the central nervous system
  - Epilepsy
  - Status epilepticus
  - Migraine
  - Transient cerebral ischaemic attacks and related syndromes
  - Vascular syndromes of brain in cerebrovascular diseases
  - Cerebral palsy and other paralytic syndromes

- Diseases of the circulatory system
  - Cerebrovascular diseases
  - Pulmonary diagnoses
  - Chronic respiratory failure

Appendix B: Anxiety Case Criteria

Online mental health questionnaire: Self-reported anxiety diagnosis by a professional (Data-Field 20544)

- Social anxiety or social phobia
- Panic attacks
- Anxiety, nerves or generalized anxiety disorder
- Agoraphobia

Online mental health questionnaire: Lifetime GAD cases defined by self-reported symptoms, as defined by Davis et al. (194)

Worried tense or anxious (Data-Field 20421) = Yes

AND

Duration (Data-Field 20420) >= 6 months or All my life

AND

Most days (Data-Field 20538) = Yes

AND

Excessive: More than most (Data-Field 20425) OR Stronger than most (Data-Field 20542)

AND

Number of issues: More than one thing (Data-Field 20543) OR Different worries (Data-Field 20540)

AND

Difficult to control: Difficult to stop worrying (Data-Field 20541) OR Couldn’t put it out of mind (Data-Field 20539) OR Difficult to control (Data-Field 20537)

AND

Functional impairment: Role interference (Data-Field 20418) = Some or A lot

AND

3 somatic symptoms out of:

Restless. (Data-Field 20426); Keyed up or on edge. (Data-Field 20423); Easily tired. (Data-Field 20429); Having difficulty keeping your mind on what you were doing. (Data-Field 20419); More irritable than usual. (Data-Field 20422); Having tense, sore, or aching muscles. (Data-Field 20417); Often having trouble falling or staying asleep. (Data-Field 20427)

Self-reported anxiety disorder or panic attacks at assessment centre visit prior to or at first imaging session (Data-Field 20002)

ICD-9 hospital records of anxiety disorders (Data-Field 41271)

- Anxiety states
    - Anxiety state, unspecified
    - Panic disorder without agoraphobia
    - Generalized anxiety disorder
    - Other anxiety, dissociative, and somatoform disorders

ICD-10 hospital records of anxiety disorders (Data-Field 41270)

- Agoraphobia
- Social phobias
- Panic disorder [episodic paroxysmal anxiety]
- Generalized anxiety disorder