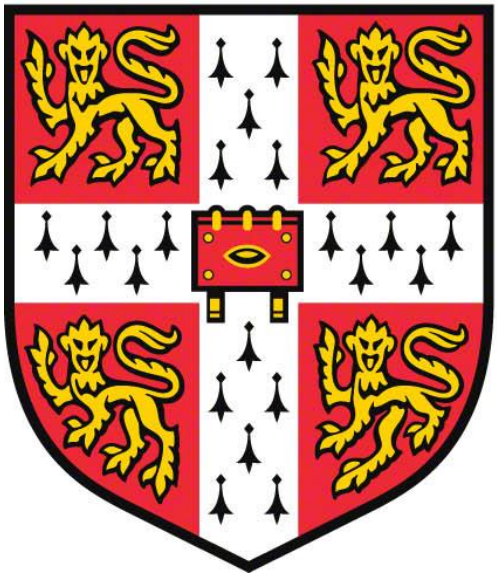
A neuroimaging study to characterise adolescent major depressive disorder, the effects of cognitive behavioural therapy, and potential subtypes based on familial loading



Luca Matthew Villa

Department of Psychiatry

University of Cambridge

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Hughes Hall June 2020

Lasciate ogne speranza, voi ch'intrate

Declaration

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the preface and specified in the text. It is not substantially the same as any work that has already been submitted before for any degree or other qualification except as declared in the preface and specified in the text. It does not exceed the prescribed word limit of 60,000 words.

The work detailed in **Chapters 2**, **3**, and **4** is revised from material that has been published. This work was primarily designed and conducted by me, and the publication alongside the co-authors' contributions are detailed below:

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Luca Matthew Villa

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Abstract

A neuroimaging study to characterise adolescent major depressive disorder, the effects of cognitive behavioural therapy, and potential subtypes based on familial loading

Luca Matthew Villa

Adolescent major depressive disorder (MDD) can have devastating consequences that remain into adulthood. Although the severe effects on those that suffer from MDD are well understood, the neural mechanisms underpinning the illness are still unclear, as the past literature exploring adolescent MDD has suffered greatly from a lack of consistency. In particular, it is still unclear as to what deviations of brain structure and function actually occur in adolescent MDD. Furthermore, though much of the past literature has claimed that cognitive behavioural therapy (CBT) has a normalising effect on disrupted brain function in adolescent MDD, this has not been directly tested. Additionally, past literature has found that adult MDD patients with a high familial loading for MDD, defined as having at least one first degree relative with MDD, show different symptom profiles to MDD patients with a low familial loading for the illness. However, it is not yet clear whether these symptom differences also occur in adolescent MDD patients with differing familial loadings, nor is it clear whether these two patient groups also differ with respect to brain structure and function. If so, familial loading for MDD could potentially be used to subtype adolescent MDD patients.

This thesis aimed to further elucidate the neural processes that may be unfolding within the depressed adolescent brain by investigating pre-treatment differences in cortical thickness, white matter volume, and resting-state functional connectivity, using the largest studied sample of adolescent MDD patients under the age of 18 years. This thesis further aimed to investigate CBT's effects on brain function, and whether adolescent MDD patients could be subtyped based on familial loading for MDD.

Before receiving treatment, adolescent MDD patients showed structural deviations in the form of greater cortical thickness and white matter volume within frontal and limbic regions of the brain. Furthermore, adolescent MDD patients also showed extensive pre-treatment overconnectivity within multiple functional brain networks, being the fronto-limbic, default mode, central executive, and salience networks.

Moreover, when investigating the CBT-related changes to resting-state functional connectivity, in adolescent MDD patients, and how they relate to regions showing pre-treatment functional disruption, it was found that regions showing the greatest pre-treatment functional disruption actually showed the weakest CBT-related changes in resting-state functional connectivity. This suggests that CBT does not have a normalising effect on brain function in adolescent MDD but instead may have a rehabilitative effect on resting-state functional connectivity.

Finally, when separating adolescent MDD patients into those with a high or low familial loading for MDD, the two patient groups differed with respect to the structure and function of the default mode network, as well as differing in rumination symptoms.

Together, this work demonstrates the need for larger sample sizes to be used when investigating an illness as heterogenous as adolescent MDD, as well as direct investigations into the potential mechanisms of CBT. Moreover, it appears that some of this heterogeneity found in adolescent MDD may be partly addressed by subtyping patients, which may have implications for how the illness is viewed and treated in the future.

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And to Dad, I have to thank you for everything and more.

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1. Major Depressive Disorder

1.1. Introduction

Major depressive disorder (MDD) has been viewed by some as a form of sadness (KTHopkins, 2015), or as an emotional state that individuals can simply "snap out of" (Reavley & Pilkington, 2014; Robinson, Turk, Jilka, & Cella, 2019). However, this concept of depression does not sufficiently capture the sheer devastation caused by the illness. Suffering from MDD can affect all aspects of life as its debilitating effects not only infringe upon emotion and mood but also decay one's ability to regulate sleeping and eating patterns, movement, and abilities to concentrate, pay attention, and make decisions.

Nearly 80% of individuals who suffer from MDD report experiencing discrimination for simply having the illness (Lasalvia et al., 2013), with around 60% of individuals experiencing discrimination within the workplace (Brouwers et al., 2016). Moreover, major depression has been related to reduced income and increased unemployment (Fergusson, Boden, & Horwood, 2007; Jefferis et al., 2011; Rizvi et al., 2015; Whooley et al., 2002), with recurrent MDD being related to a greater likelihood of being unemployed for two years or more (Zimmerman et al., 2010). Major depression can also hinder interpersonal relationships (Geller, Zimerman, Williams, Bolhofner, & Craney, 2001; Lam, Schuck, Smith, Farmer, & Checkley, 2003; Zlotnick, Kohn, Keitner, & Della Grotta, 2000) with those suffering from the illness being more likely for their marriages to end in divorce or separation (Bulloch, Williams, Lavorato, & Patten, 2009), and to have poor quality friendships (Goodyer, Herbert, Tamplin, Secher, & Pearson, 1997). Most troubling of all, the illness has been associated with a 50% increased mortality risk (Cuijpers, Vogelzangs, et al., 2014), and having recurrent major depression has been found to reduce life expectancy by as much as 10 years (Chang et al., 2011; Chesney, Goodwin, & Fazel, 2014). Correspondingly, MDD is strongly related to suicidal ideation and suicide attempts (Angst, Angst, & Stassen, 1999; Oquendo et al., 2004; Otte et al., 2016; Sokero et al., 2005), with individuals suffering from the illness being 20 times more likely to die by suicide than

the general population (Chesney et al., 2014), and some even estimate that of the 788,000 suicides that occur per year (World Health Organization, 2017), nearly 50% of them are related to an episode of major depression (Otte et al., 2016). With these effects in mind, it is not so surprising that MDD is currently the world's leading cause of disability (World Health Organization, 2017), affecting around 322 million people worldwide (World Health Organization, 2017).

Clearly, the view that MDD is simply a form of sadness that can be "snapped out of" with ease, is a gross misunderstanding of the illness.

1.2. Adolescent major depressive disorder

Developing MDD during adolescence can be particularly harmful as its consequences can be long lasting and remain into adulthood. Rates of major depression increase sharply from childhood to adolescence, with the prevalence of MDD being between 1-2% in children (Maughan, Collishaw, & Stringaris, 2013; Wichstrøm et al., 2012) and increasing to 4-8% in adolescents (Avenevoli, Swendsen, He, Burstein, & Merikangas, 2015; Jane Costello, Erkanli, & Angold, 2006; Merikangas, Nakamura, & Kessler, 2009; Thapar, Collishaw, Pine, & Thapar, 2012; Valuck, Libby, Sills, Giese, & Allen, 2004).

The devastating effects of developing MDD appear to be exacerbated in adolescent MDD. Individuals who first experience the illness during adolescence are more likely to suffer recurrent episodes of depression than adult-onset patients (Zisook et al., 2007), and these recurrent episodes are likely to persist into adulthood (Johnson, Dupuis, Piche, Clayborne, & Colman, 2018; Rohde, Lewinsohn, Klein, Seeley, & Gau, 2013; Thapar et al., 2012). Adolescent MDD patients also experience reduced educational achievement (Esch et al., 2014; Fergusson et al., 2007; Fergusson & Woodward, 2002; Ronald C. Kessler, Foster, Saunders, & Stang, 1995), with depressed adolescents performing worse academically at school (Jonsson, Goodman, Von Knorring, Von Knorring, & Koupil, 2012), being 30%

more likely to drop out from high school in the United States (Fletcher, 2008, 2010; Quiroga, Janosz, Bisset, & Morin, 2013), and accounting for around 5% of university dropouts (Kessler et al., 1995). Most alarmingly, adolescent MDD patients are also at an increased risk of suicide (Avenevoli et al., 2015; Zisook et al., 2007), and are more likely to attempt suicide than adult patients (Rohde et al., 2013), with as many as 11% of adolescent MDD patients attempting suicide (Avenevoli et al., 2015) compared to around 8% of adult MDD patients (Dong et al., 2019). Clearly, experiencing MDD during adolescence can bring a cavalcade of desolation to the lives of those that suffer from it, which demonstrates the urgent need to understand the illness.

1.3. Diagnosis

MDD is often diagnosed using one of the two main diagnostic manuals for mental illnesses, being the Diagnostic and Statistical Manual of Mental Disorders (DSM-5; American Psychiatric Association, 2013), and the International Statistical Classification of Diseases and Related Health Problems (ICD-10; World Health Organization, 2016).

Under the DSM-5 criteria (American Psychiatric Association, 2013), to be diagnosed with MDD, an individual must experience at least 5 of 9 potential symptoms, out of which one must be either a depressed mood or a loss of interest or pleasure, with the other symptoms being insomnia, hypersomnia, loss of appetite, increased appetite, psychomotor agitation or retardation, loss of energy, reduced concentration, feelings of worthlessness or guilt, and suicidal ideation. However, when diagnosing adolescents, the criteria differ slightly, whereby in adolescents, depressed mood can instead be replaced with irritability.

Under the DSM-5 (American Psychiatric Association, 2013), individuals must have experienced these symptoms almost every day over a period of at least two weeks, with the exception of suicidal ideation, which only needs to be recurrent, with no specified frequency of occurrence, suicide

attempts, which only need to have occurred once, as well as weight changes, which need to have changed by 5% over one month. Furthermore, to be considered for MDD, symptoms cannot be attributed to the physiological effects of substance abuse, another medical condition, or psychosis, and nor can there be a history of mania (where individuals experience extreme elation and energy) or hypomania (a less extreme form of mania). Finally, the clinician assessing the individual must make a judgement on whether the symptoms being experienced are a normal response to a significant loss or grief, or whether they are pathological in nature.

The ICD-10 criteria (World Health Organization, 2016) for MDD are relatively similar to those of the DSM-5, having 10 overall symptoms, with there being three key symptoms of depression, being depressed mood, loss of interest and enjoyment, and reduced energy. Other diagnostic symptoms are also included, being reduced concentration, reduced self-esteem, feelings of guilt and worthlessness, a pessimistic view of the future, suicidal ideation and acts of self-harm, disturbed sleep, and a diminished appetite that must have occurred over a period of at least 2 weeks. The ICD-10 also subdivides MDD into mild, moderate, and severe depression. For mild depression, only two key symptoms and two other symptoms are needed, whereas for moderate depression, two key symptoms and at least three other symptoms are required, and for severe depression, all three key symptoms and at least three other symptoms must be present (Gruenberg, Goldstein, & Pincus, 2008). Furthermore, the ICD-10 explicitly excludes the inclusion of individuals who are experiencing bereavement, which the DSM-5 controversially does not (Pies, 2014).

1.3.1. DSM-IV vs DSM-5

The DSM-5 (American Psychiatric Association, 2013) was introduced relatively recently, in May 2013, meaning that the vast majority of the past literature has identified MDD patients using older diagnostic criteria. Moreover, many studies currently being published, including the entirety of the work within this thesis, have used the older diagnostic criteria of the DSM-IV (American Psychiatric

Association, 1994). Therefore, a brief comparison of the criteria for MDD between the slightly older DSM-IV, used by this thesis, and the newer DSM-5 is warranted.

The vast majority of the criteria for MDD is the same between the DSM-IV and the DSM-5 (Uher, Payne, Pavlova, & Perlis, 2014). One difference between them is that the DSM-5 now includes feelings of hopelessness, being a very pessimistic view of the future, as a descriptor of depressed mood, whereas the DSM-IV did not. Though this is a small change, some have argued that feelings of hopelessness are distinct from depressed mood and can occur independently in MDD patients, with some MDD patients suffering from depressed mood but not experiencing feelings of hopelessness, or vice-versa (Greene, 1989; Joiner et al., 2001). Therefore, as depressed mood is one of the key symptoms required for the diagnosis of MDD, it has been argued that the inclusion of hopelessness as a descriptor of depressed mood, in the DSM-5, may lead to more diagnoses of MDD as the criteria has been broadened (Uher et al., 2014).

It has also been argued that this inclusion may also reduce the reliability of MDD diagnoses as some clinicians will accept feelings of hopelessness as evidence of depressed mood, whereas others may not, based on their understanding of past research claiming that depressed mood and hopelessness are independent of each other (Uher et al., 2014). An investigation into the diagnostic validity and reliability of the DSM-5 did find that the reliability estimates for MDD diagnoses were relatively poor compared to the DSM-IV (Regier et al., 2013), potentially supporting this claim, though it has been suggested that this discrepancy may be due to methodological differences between studies of the DSM-IV's and DSM-5's reliability, rather than being an inherent issue with the DSM-5 itself (Chmielewski, Clark, Michael Bagby, & Watson, 2015).

The other main, and most controversial, difference between the DSM-IV and the DSM-5's criteria for MDD is their approach to bereavement. In the older DSM-IV criteria, individuals who had experienced recent bereavement could only be diagnosed with MDD if their symptoms meeting the criteria lasted for longer than two months and if their symptoms were considered as not being

typical characteristics of grief, such as being suicidal (Pies, 2014; Uher et al., 2014). The DSM-5, however, does not include these extra criteria for individuals who have experienced bereavement and instead asks clinicians to make a judgement on whether an individual's observed symptoms are related to a normal response of grief or to a significant loss. This change has led some to argue that the DSM-5 medicalises normal grief and reduces the diagnostic validity of MDD, as it may include individuals experiencing normal reactions to grief and loss, though whether this is actually the case is still highly debated (Pies, 2014; Uher et al., 2014; Wakefield & First, 2012a, 2012b).

It should therefore be noted that the work within this thesis used the diagnostic criteria of the DSM-IV (American Psychiatric Association, 1994), which did not include feelings of hopelessness as a descriptor of depressed mood, and also included more stringent criteria for individuals experiencing bereavement. This means that the characteristics of MDD patients included within this body of work may differ slightly to those that have been studied more recently using the DSM-5 (American Psychiatric Association, 2013).

1.4. Characteristics of major depressive disorder

1.4.1. Comorbidity

MDD is highly comorbid with other illnesses, with up to 70% of MDD patients suffering from at least one other mental illness (Avenevoli et al., 2015; Kessler et al., 2003). MDD is highly comorbid with anxiety disorders, with around 50% of MDD patients also suffering from an anxiety disorder (Fava et al., 2000; Kessler et al., 2015; Kessler et al., 2003; Kessler, Merikangas, & Wang, 2007), and the presence of anxiety with MDD is so common that most studies attempting to investigate MDD exclude comorbidities with other mental illnesses but not anxiety, as their MDD patient samples would likely be unrepresentative.

MDD patients are also likely to suffer from addiction and substance abuse, with around 30% of MDD patients suffering from substance use disorders (Davis et al., 2006; Davis et al., 2005; Kessler et al., 2003). MDD is also strongly related to obsessive compulsive disorder (OCD) as 30-40% of OCD patients also suffer from MDD (Quarantini et al., 2011; Torres et al., 2006; Tükel, et al., 2002; Viswanath et al., 2012), with antidepressant medications even being a first-line treatment for OCD symptoms (Hirschtritt, Bloch, & Mathews, 2017). Other disorders that are often comorbid with MDD include eating disorders, such as bulimia and anorexia, as well as borderline personality disorder (American Psychiatric Association, 2013; Hasin et al., 2018).

1.4.2. Gender

MDD is more prevalent in females across multiple cultures worldwide (World Health Organization, 2017). This gender imbalance in diagnoses appears to occur during adolescence (Merikangas et al., 2009; Thapar et al., 2012), with females being between 1.4-2 times more likely to develop the illness than males (Kessler et al., 2005; Romans, Tyas, Cohen, & Silverstone, 2007; Seedat et al., 2009; Weissman & Klerman, 1977; World Health Organization, 2017). However, though this is one of the most consistent findings within the MDD literature, the cause of this gender difference in prevalence is still unknown, though many explanations have been proposed.

Three types of explanations exist: artefactual, whereby there is no real gender difference in the prevalence of MDD but that certain behavioural, social, and methodological factors bias and misrepresent the prevalence rates of MDD; social, whereby societal and cultural norms affect the two genders differently and cause the gender differences in MDD prevalence; and biological, where inherent biological differences exist between the two genders and cause females to be more likely to develop MDD (Parker & Brotchie, 2010).

Artefactual explanations for this gender difference in MDD prevalence include the suggestion that males are more likely to underreport their experience of depressive symptoms, which then biases the prevalence rates of MDD (Chevron, Quinlan, & Blatt, 1978). Another argument claims that males

and females show their symptoms of depression differently, with males supposedly showing more "acting out" reactions to their symptoms of depression compared to females who are more likely to react to their symptoms in the form of crying (Nolen-Hoeksema, 1987), which leads to different clinical interpretations of their behaviours (Hammen & Peters, 1977; Winokur & Clayton, 1967). Other artefactual explanations include the argument that as MDD has historically been associated with females, clinicians are therefore biased towards diagnosing females with depression and underdiagnosing males, biasing MDD prevalence rates (Potts, Burnam, & Wells, 1991). Others argue that methodological biases exist in the instruments used to detect MDD that diminish the experiences of males and inaccurately assess them as not being clinically depressed (Martin, Neighbors, & Griffith, 2013).

Social explanations for the gender imbalance in MDD diagnoses often argue that the different social roles and cultural norms, experienced by males and females, place more environmental stress and trauma upon females, which then makes them more likely to develop MDD than males (Aneshensel, Frerichs, & Clark, 1981; Bebbington, 1996; Gore & Mangione, 1983).

Biological arguments for this gender imbalance vary greatly but argue that there are inherent biological differences between males and females that predispose females to developing MDD. Some explanations claim that females have a greater biological reactivity to stress (Nolen-Hoeksema, 2001), which may make them more vulnerable to developing MDD, whereas others argue that males and females differ in their exposure to hormones, whereby oestrogen and progesterone are greater in females, and that this greater exposure in females leads to differences in brain structure and function that increase the reactivity of limbic regions of the brain towards negative stimuli, which predisposes females to developing MDD (Parker & Brotchie, 2004; Young & Korszun, 1998). Unsurprisingly, such biological explanations are controversial.

Moreover, whether this gender difference in MDD diagnoses also manifests itself at the symptom level, with male and female MDD patients having different subjective experiences of the illness, is

also a contentious topic. Some have found that females develop the illness earlier in life (Marcus et al., 2005; Schuch, Roest, Nolen, Penninx, & De Jonge, 2014; Smith et al., 2008) however many others have failed to find any differences regarding age of onset (Carter, Joyce, Mulder, Luty, & McKenzie, 2000; Kessler, McGonagle, Swartz, Blazer, & Nelson, 1993; Kim et al., 2015; Weissman et al., 1993). Furthermore, regarding differences in symptom profiles, multiple studies report that females are more likely to show sleep and appetite disturbances (Carter et al., 2000; Kim et al., 2015; Romans et al., 2007; Schuch et al., 2014; Silverstein, 1999), and yet others have argued that these differences may be caused by males being more likely to forget their symptoms, rather than actually experiencing fewer of these disturbances (Angst & Dobler-Mikola, 1984; Wilhelm & Parker, 1994), and that changes to weight and appetite are more likely to be salient to females, due to societal and cultural norms, meaning that they are more likely to report these symptoms than males (Romans et al., 2007).

Therefore, although it is clear that there are more females diagnosed with MDD than males, both the supposed causes for this, and the existence of symptomatic differences between male and female MDD patients, remain controversial.

1.4.3. Recurrence

Major depression is often recurrent in nature, meaning that those suffering from the illness can become trapped in cycles of depressive episodes and experience multiple debilitating episodes throughout their lives. For example, first-episode MDD patients have a 40-60% chance of developing a second episode in the future (Eaton et al., 2008; Kessing, Hansen, Andersen, & Angst, 2004; Monroe & Harkness, 2011; Solomon et al., 2000), and MDD patients who have experienced two episodes of depression have a 60-70% chance of experiencing another episode (Bockting, Hollon, Jarrett, Kuyken, & Dobson, 2015; Monroe & Harkness, 2011), and once patients have experienced three depressive episodes, they have up to a 90% chance of experiencing another (Bockting et al., 2015; Kessing et al., 2004; Monroe & Harkness, 2011). Furthermore, even after receiving successful

treatment, MDD patients are likely to experience relapses in the future (Ramana et al., 1995), with 70-85% of patients eventually relapsing after initially responding to treatment (Kovacs, Obrosky, & George, 2016; Mueller et al., 1999).

One explanation for this level of recurrence in MDD is known as the kindling hypothesis (Kendler & Gardner, 2016; Kendler, Thornton, & Gardner, 2000; Post, 1992). The hypothesis claims that extremely stressful life events are needed to initially develop a major depressive episode but, with each successive episode, individuals become more sensitive to life stress. This means that with time, less stress is required to trigger an episode of depression until stressful life events are no longer required to produce them, with them occurring almost spontaneously (Kendler & Gardner, 2016; Monroe & Harkness, 2005). An example of this hypothesis would be that an extreme life stress, such as the death of a loved one, would initially be required for an individual to experience their first major depressive episode. However, after having experienced multiple episodes of depression, a much less traumatic event, such as being insulted by a friend, would eventually be able to trigger a major depressive episode.

Other explanations for the high levels of recurrence in MDD include cognitive scarring hypotheses (Lewinsohn, Allen, Seeley, & Gotlib, 1999; Teasdale & Dent, 1987), whereby individuals who have previously suffered from MDD are biased towards interpreting their experiences as being negative, even after remitting. These negative thought biases lower the mood of the individuals and experiencing a low mood makes these negative thought biases more accessible and more likely to occur, which leads to a cycle of negative interpretations creating low mood, which leads to more negative interpretations which then lowers mood even further. This cycle eventually lowers mood to such an extent that the individuals experience another episode of depression.

Environmental explanations have also been used to explain recurrence in MDD. Environmental factors such as familial disruption, work-related stress, or criticism and hostility from relatives and partners, can trigger the development of major depressive episodes (Hooley, Orley, & Teasdale,

1986; Vaughn & Leff, 1976). If these environmental stressors are not addressed once MDD patients have remitted, they are likely to contribute to the patients' stress and will eventually trigger new episodes of depression – resulting in recurrence.

1.5. Treatments for major depressive disorder

Multiple forms of treatment exist for MDD and they are often divided into psychotherapies and pharmacotherapies, with successful treatment response usually being defined as patients showing at least a 50% reduction in symptoms (Al-Harbi, 2012; Cuijpers, Karyotaki, et al., 2014; Lin, Chen, Wang, & Lane, 2013; Mayberg, Lozano, Voon, McNeely, et al., 2005).

1.5.1. Psychotherapies

Of the psychotherapies, cognitive behavioural therapy (CBT) is one of the most common that MDD patients receive during treatment. CBT is based on the premise that major depression is caused by overly negative beliefs and thought processes that bias patients towards extremely negative interpretations of their experiences (Beck, 1987; Beck, Rush, Shaw, & Emery, 1979; Driessen & Hollon, 2010). These biased thought processes occur in the form of negative schema, psychological representations of concepts such as events, people, social roles, and tasks, that influence beliefs and expectations of these concepts. For example, the schema of a birthday party may include the belief that they are enjoyable and include the expectation of balloons, presents, and music; whereas the schema of a doctoral thesis may include the belief that they are daunting to undertake, and include the expectation of stress, criticism, and missed deadlines. Negative schema in major depression bias MDD patients towards holding negative and overly pessimistic beliefs and expectations about themselves (e.g. "I am a terrible person"), the world around them (e.g. "everyone hates me"), and their future (e.g. "I'm always going to be a failure), known as the negative triad (Clark & Beck, 2010). This approach argues that these negative schema and other negatively biased thought processes

generate and maintain episodes of major depression. CBT therefore attempts to change these thought processes to make them less negatively biased, which should ameliorate symptoms of major depression (Driessen & Hollon, 2010). This is done through strategies such as increasing exposure to pleasurable experiences, attempting to rationalise and modify negatively biased beliefs to make them become more positive, developing various skills and routines such as organising, planning, and social skills, and through developing coping strategies for stressful experiences.

Another common psychotherapy used to treat MDD is psychoanalytic therapy. Psychoanalytic therapy primarily focuses on the unconscious, attempting to understand unconscious thoughts, feelings, and desires that patients may have and that might be contributing towards their experience of major depression. Generally, psychoanalytic theories of depression argue that the illness is triggered when an individual experiences an extreme loss, which can include (but is not limited to) the loss of a loved one or important relationship (Freud, 1957; Milrod, 1988; Newman & Hirt, 1983; Rustin, 2009). This loss creates feelings of anger and aggression that are unconsciously redirected from the source of the loss towards the individual themselves, causing feelings of worthlessness, self-loathing, low self-esteem, and the experience of major depression (Freud, 1957; Milrod, 1988; Newman & Hirt, 1983; Rustin, 2009). Psychoanalytic therapy therefore aims to uncover these unconscious feelings of anger and aggression and tries to amend them to alleviate symptoms of depression. It also attempts to explore the patient's attachments to loved ones, and their conscious and unconscious feelings towards these attachments, attempting to help the patient understand how these attachments may be contributing towards their symptoms of depression (Goodyer et al., 2017). The therapy addresses these issues through therapeutic sessions between MDD patients and their therapist, where the therapist attempts to help the patient verbalise their conscious and unconscious thoughts and feelings (Goodyer et al., 2017). During therapy, they may discuss the patient's experiences such as their memories, dreams, thoughts, and desires, to help uncover their unconscious thoughts and feelings towards themselves and others (Goodyer et al., 2017), which should help them address their emotions and behaviour, and lessen their feelings of depression.

Interpersonal psychotherapy is another form of treatment that is used for MDD. Interpersonal psychotherapy focuses on the interpersonal problems that patients with MDD may face, and attempts to identify and ameliorate interpersonal factors in an individual's life that may be contributing to their symptoms of depression (Markowitz & Weissman, 2004). The underlying principle of the therapy is that problematic interpersonal factors, such as the death of a loved one, ending of a relationship, struggles with significant others, or changes in their interpersonal roles, contribute to the onset of MDD and can also deteriorate after the onset of the illness, further exacerbating the illness' effects (Markowitz & Weissman, 2004). It proposes that many individuals with MDD do not identify the link between their interpersonal problems and their illness, and instead blame themselves for the illness. The therapy therefore aims to help the patient first acknowledge that their symptoms of MDD are not their fault, and that they are linked to their current interpersonal situation. Once the interpersonal factors that may be contributing to the patient's symptoms have been identified, strategies are then employed to ameliorate the interpersonal factors. For example, a therapist may encourage a patient to resolve interpersonal conflicts the patient may be experiencing with a significant other, or decrease social isolation and improve interpersonal skills if the patient is lacking interpersonal relationships. Other strategies may include helping the patient in allowing themselves to mourn the loss of a loved one, or mourn the loss of an interpersonal role they once had and accepting the transition to their new interpersonal role.

Another form of treatment that is used for adolescent MDD is a brief psychosocial intervention. Unlike other psychotherapies, brief psychosocial interventions have no overarching psychological theory attached to their creation, and do not include cognitive or reflective aspects in their approach. Instead, they attempt to directly modify and enhance behavioural and environmental aspects of an MDD patient's life. This is done by improving the education of patients and their families about major depression, enhancing interpersonal skills and relationships, improving personal and mental hygiene, increasing the amount of pleasurable activities being done by the

patient, improving problem solving skills, and by improving the patients' awareness evaluation of their own mental states (Goodyer et al., 2017; National Institute for Health and Care Excellence, 2019).

Both CBT and psychoanalytic therapy have been shown to be clinically effective in the treatment for MDD in both adults (Clarke, Mayo-Wilson, Kenny, & Pilling, 2015; DeRubeis, Siegle, & Hollon, 2008b; Driessen & Hollon, 2010; Fonagy et al., 2015; Hofmann, Asnaani, Vonk, Sawyer, & Fang, 2012) and adolescents (Abbass, Rabung, Leichsenring, Refseth, & Midgley, 2013; Clarke et al., 2005; Goodyer et al., 2017; Melvin et al., 2006; Weersing, Jeffreys, Do, Schwartz, & Bolano, 2017), as has interpersonal psychotherapy (Cuijpers et al., 2011; Mufson et al., 2004; Santor & Kusumakar, 2001), whereas brief psychosocial interventions are mainly targeted towards adolescent MDD patients (National Institute for Health and Care Excellence, 2019). Furthermore, when comparing the efficacy of psychotherapies, the majority of the literature has found that they are equally as effective as each other in treating adult MDD (Cuijpers, Berking, et al., 2013; Cuijpers, Karyotaki, et al., 2014; Cuijpers, van Straten, Andersson, & van Oppen, 2008; Linde et al., 2015), with around 50-60% of MDD patients showing a clinical response to them (Cuijpers, Karyotaki, et al., 2014), as well as being equally as effective in adolescent suffering from MDD (Goodyer et al., 2017).

This apparent therapeutic equivalence between various different psychotherapies, known as the dodo bird effect, has been observed across psychiatric disorders and has led to the debate of how psychotherapies actually treat psychiatric illnesses. Two explanations generally emerge from this debate, either most or all of the different psychotherapies work through differing psychological mechanisms that by chance are equally as effective as each other, or that most or all of the psychotherapies share a common mechanism, which is why their therapeutic effects appear equivalent (González-Blanch & Carral-Fernández, 2017; Rosenzweig, 1936). Multiple meta-analyses have been conducted to investigate this effect and whether there are indeed no discernible differences between psychotherapies, with some claiming that CBT may show slight superiority to

other therapies (Marcus, O'Connell, Norris, & Sawaqdeh, 2014; Tolin, 2010), whilst most others have found no differences between individual therapies (Baardseth et al., 2013; Barth et al., 2016; Cuijpers, Karyotaki, de Wit, & Ebert, 2020; Wampold et al., 1997). Ultimately, it could be argued that if it has been so difficult to detect any discernible differences in the overall effectiveness of psychotherapies after nearly 80 years of investigation, then the differences either do not exist or are negligible. Instead, it could be argued that a greater focus should be placed on understanding why these diverse sets of therapies show relative equivalence, as identifying the different or similar psychological mechanisms that cause these similar therapeutic effects may lead to a more efficient delivery of psychotherapy.

1.5.2. Pharmacotherapies

MDD can also be treated using pharmacotherapies, with the most common type of antidepressant medications prescribed for MDD being selective serotonin reuptake inhibitors (SSRIs; Ilyas & Moncrieff, 2012; Olfson & Marcus, 2009). During typical synaptic transmission, a variety of neurotransmitters, including serotonin, are released into the synaptic cleft by the presynaptic neuron. These neurotransmitters travel across the synaptic cleft, via diffusion, towards the postsynaptic neuron, where they bind to their corresponding receptors. Any remaining neurotransmitters within the synaptic cleft that have not bound to a receptor are then reabsorbed by the presynaptic neuron so they can be reused, known as reuptake. SSRIs function by blocking the process of reuptake of serotonin, meaning that there is more serotonin left within the synaptic cleft that can then bind to receptors in the postsynaptic neuron.

Although the prescription of SSRIs for adolescent MDD is particularly controversial due to their potential for increasing suicidality twice as much as CBT (Barbui, Esposito, & Cipriani, 2009; March, 2004; March et al., 2007), SSRIs have consistently been shown to be effective at reducing symptoms of MDD in both adult (Cipriani et al., 2018; Papakostas, Charles, & Fava, 2010; Von Wolff, Hölzel,

Westphal, Härter, & Kriston, 2013) and adolescent MDD patients (Goodyer et al., 2007; Locher et al., 2017; March, 2004; March et al., 2007; Usala, Clavenna, Zuddas, & Bonati, 2008), with around 50-60% of both adult and adolescent MDD patients showing a clinical response to SSRIs (Goodyer et al., 2007; Hirschfeld, 1999; March et al., 2007; Papakostas et al., 2010).

Antidepressant medications have also generally been found to be equally as effective at treating MDD as psychotherapies in adult MDD patients (Casacalenda, Perry, & Looper, 2002; Cuijpers, Hollon, et al., 2013; Cuijpers, Sijbrandij, et al., 2013), with the combination of both psycho-and pharmacotherapies being the most effective form of treatment for adult MDD patients (de Maat, Dekker, Schoevers, & de Jonghe, 2007; Karyotaki et al., 2016; Those et al., 1997; Zobel et al., 2011). With respect to adolescent MDD, the literature is less clear as to the superiority of either pharmacotherapies or psychotherapies, though the combination of pharmacotherapies with psychotherapies does appear to be the most suitable form of treatment for adolescent MDD (Brent et al., 2008; Dubicka et al., 2010; March, 2004; March et al., 2007), with some claiming that this combination may protect adolescent patients from the increased suicidality that is associated with SSRIs (March, 2004; March et al., 2007).

1.6. Major depressive disorder as an illness of the brain?

There is a substantial amount of evidence suggesting that MDD has a strong biological component. A multitude of genes have been associated with MDD, and the illness is heritable, with its heritability estimate being around 38% (Flint & Kendler, 2014) – though it should be noted that the heritability estimate for MDD is substantially lower than other mental illnesses such as bipolar disorder and schizophrenia, which have heritability estimates of around 80% (Gordovez & McMahon, 2020; Hilker et al., 2018). Studies have also observed neurochemical differences in those with MDD, such as differences in the synthesis of various neurotransmitters within the brain, including serotonin and monoamine oxidise A (Barton et al., 2008; Kaufman et al., 2015; Meyer et al., 2006; Rosa-Neto et al.,

2004), and that modulation of neurotransmitters within the brain can induce depressive symptoms in MDD patients (Ruhé, Mason, & Schene, 2007). Moreover, pharmacological interventions, such as the use of SSRIs and more recently ketamine (Katalinic et al., 2013; Krystal, Abdallah, Sanacora, Charney, & Duman, 2019) have consistently been shown to improve symptoms of depression by directly affecting the brain's neurochemistry.

Further highlighting the importance of biological factors in MDD, a growing body of work has shown that inflammation may play an important role in MDD. Studies have consistently shown that individuals with MDD have elevated peripheral pro-inflammatory markers (Dowlati et al., 2010; Liu, Ho, & Mak, 2012; Smith, Au, Ollis, & Schmitz, 2018), and some studies indicate that pro-inflammatory agents may induce symptoms of depression (Kohler, Krogh, Mors, & Eriksen Benros, 2016), with trials having found that anti-inflammatory medications can reduce depressive symptoms in MDD patients (Köhler-Forsberg et al., 2019). Therefore, as multiple biological markers, mechanisms, and interventions have been identified in MDD it is clear that, whilst environmental factors play an enormous role in the development and maintenance of the illness, MDD must have a strong biological component to its genesis. Therefore, a neurobiological understanding of the depressed adolescent brain may lead to a greater insight into the underpinnings of the illness, which may eventually lead to the development of new and more effective treatments and interventions.

However, although there is an abundance of evidence suggesting that biological factors play a strong role in the development and experience of MDD, there is a considerable amount of opposition towards viewing MDD, and mental illnesses in general, as being biologically based or as having neurological underpinnings, with some even claiming that neuroscientific investigations of mental illnesses are "intellectually trivial" (Kinderman, 2014).

The main premise of this argument is that although the brain is ultimately involved in psychological processes, reducing psychological problems such as major depression to the neural level is redundant as psychological phenomena are far too complex to be explained biologically, and even if

there are biological aspects to mental illnesses, they will ultimately have been caused by the environment (Cooke & Kinderman, 2018; Kinderman, 2014; Kingdon & Young, 2007). For example, it would be difficult to neurobiologically explain why an individual may choose a certain outfit for an interview, but it would be far easier to explain it psychologically, based on psychological concepts such as conformity; and although there may be a neurobiological aspect to choosing an outfit, that choice was ultimately caused by the environmental context of an interview. Therefore, it is argued that the same logic applies to major depression and other mental health problems, whereby it is impossible to neurobiologically explain why an individual experiences low mood, but it is possible to explain when examining their thought processes, emotions, and environmental experiences.

There is merit to aspects of this line of thought, such as highlighting the importance of environmental and psychological factors that play an extremely important role in the genesis of MDD, especially as studies have demonstrated the effects of environmental adversity on the brain's structure and function (Andersen & Teicher, 2008; Andersen et al., 2008; Teicher, Anderson, Ohashi, & Polcari, 2014; Teicher, Samson, Anderson, & Ohashi, 2016). However, this line of thought would therefore lead to the flawed conclusion that Alzheimer's disease, which is almost universally accepted to have neurobiological causes (Kingdon & Young, 2007; Scheltens et al., 2016; Soria Lopez, González, & Léger, 2019; Weller & Budson, 2018), is in fact purely a psychological problem with a trivial or unimportant biological component to it – demonstrating a critical flaw in this view of mental illnesses. Thus, although biological approaches to understanding major depression have not yet found a neurobiological "cause" of the illness (Kinderman, 2014), and any biological components of MDD will be intertwined with environmental and psychological processes, it would be unwise to dismiss this approach as "trivial", especially given the evidence that biological interventions, such as antidepressant medications, do successfully treat 50-60% of individuals suffering from MDD (Goodyer et al., 2007; Hirschfeld, 1999; March et al., 2007; Papakostas et al., 2010). Consequently, neurobiological investigations of MDD are warranted and may be beneficial to understanding the illness.

1.7. Brain regions and mechanisms implicated in major depressive disorder

Being a mood disorder that is characterised by disturbances to emotion and its regulation, many of the neuroanatomical brain regions that have been implicated in MDD are those thought to underpin these core functions. Brain regions most commonly associated with aspects of MDD are regions within the limbic system, including the hypothalamus, amygdala, and hippocampus, as well as regions within the prefrontal and frontal cortex, including the orbitofrontal cortex, medial frontal gyrus, and anterior cingulate cortex.

The hypothalamus is involved in the regulation of the endocrine system and is a key component of the hypothalamic-pituitary-adrenal (HPA) axis. It is responsible for the release of stress hormones known as glucocorticoids, such as cortisol, whereby in the event of an environmental stressor the HPA axis is activated and triggers the release of cortisol in the bloodstream.

The amygdala is typically thought to be involved in learning and implicit memory formation, such as fear conditioning, threat detection, and the triggering of stress responses and activation of the HPA axis. It is thought to be involved in symptoms of anxiety that many individuals with MDD experience, as well as the biases towards interpreting environmental stimuli as being negatively valenced (Dannlowski et al., 2007), and negative biases in memory recall (Hamilton & Gotlib, 2008).

The hippocampus is classically thought to be involved in learning, the consolidation of new episodic memories, and spatial navigation (Anand & Dhikav, 2012; Campbell & MacQueen, 2004). Moreover, the hippocampus is also involved in the regulation of the hypothalamus, whereby it has an inhibitory effect on the HPA axis' stress responses. However, the hippocampus contains a high concentration of glucocorticoid receptors, making it highly sensitive to their release, and overexposure to glucocorticoids has been found to cause disruption in hippocampal function and eventually atrophy within the region (MacQueen & Frodl, 2011).

Prefrontal and frontal regions of the brain are thought to have a regulatory effect on the limbic system and HPA axis. The orbitofrontal cortex is thought to be involved in reward processing, which

can be divided into the lateral orbitofrontal cortex and medial orbitofrontal cortex. The lateral orbitofrontal cortex is thought to be involved in processing aversive stimuli and punishments, particularly a lack of reward, and it has been hypothesised that overactivity within the region may lead to symptoms of MDD such as depressed mood (Rolls, Cheng, Du, et al., 2020; Rolls, Cheng, & Feng, 2020). Conversely, the medial orbitofrontal cortex is thought to be involved in the processing of pleasant stimuli and rewards, and it is therefore thought that underactivity within the region may underlie symptoms of anhedonia in MDD (Rolls, Cheng, & Feng, 2020).

The medial frontal cortex is thought to be partially involved in self-referential processing, as well as the appraisal of emotionally valenced stimuli, and downregulation of limbic regions such as the amygdala (Johnstone, Van Reekum, Urry, Kalin, & Davidson, 2007; Lemogne et al., 2009). It is therefore thought to be involved in the experience of rumination symptoms in MDD, as well as deficits in emotion regulation.

The anterior cingulate cortex is hypothesised to mediate between the emotional reactivity of the limbic system and emotion regulation of the frontal system (Mayberg, 1997; Stevens, Hurley, & Taber, 2011), and can be divided into two separate parts, the pregenual anterior cingulate and the subgenual anterior cingulate, with the pregenual anterior cingulate receiving inputs predominantly from other emotion regulating frontal regions, and the subgenual anterior cingulate receiving inputs predominantly from limbic regions. It is hypothesised that dysfunction of the anterior cingulate cortex may lead to an imbalance between the emotionally reactive limbic system and the emotion regulating frontal systems of the brain, leading to symptoms of MDD (Mayberg, 1997; Stevens et al., 2011).

Together, based on the functions of these brain regions, it has been hypothesised that MDD is characterised by issues revolving the communication between predominantly frontal and limbic brain regions. More ventral brain regions, including the amygdala, hypothalamus, subgenual anterior cingulate, and hippocampus, are hypothesised to be responsible for disturbances to emotional

reactivity, depressed mood, and vegetive and somatic symptoms – such as disturbances to sleep and appetite – whereas the more dorsal brain regions, including the medial frontal gyrus, orbitofrontal cortex, and parts of the middle frontal and superior frontal gyri, are hypothesised to be responsible for disturbances to cognition, attention, emotion regulation, and reward processing – though striatal and limbic brain regions, including the putamen, caudate, nucleus accumbens and thalamus are also considered to be heavily involved in reward processing issues in MDD (Mayberg, 1997, 2003). It has been hypothesised that the anterior cingulate cortex, particularly the pregenual anterior cingulate, is responsible for mediating between these subsystems within the brain, and that its dysfunction may give way to disturbances in the communication between these systems and ultimately give rise to the onset of MDD.

How disruption of brain networks subserving emotion and its regulation originates is unclear. One of the most influential hypotheses for the aetiology of MDD is HPA axis overactivity (Stokes, 1995). The hypothesis states that in individuals who develop MDD, the HPA axis is overactive, which leads to an overproduction and release of cortisol in the body and brain in response to perceived environmental stress or threats. The cause of an overactive HPA axis is unclear, but it could potentially be partly due to overactive amygdala function, over-triggering the stress response of the HPA axis. The overactivity of the HPA axis leads to large amounts of glucocorticoids, such as cortisol, being released into the bloodstream, and overexposure to these glucocorticoids within the brain eventually have detrimental effects on brain regions involved in regulating the HPA axis and in emotion regulation. A primary example of these detrimental effects is hypothesised to occur within the hippocampus as due to the region's sensitivity to glucocorticoids, its overexposure to glucocorticoids leads to its diminished function and eventual atrophy. Compounding these detrimental effects is that as the hippocampus has an inhibitory effect on the HPA axis, its gradual dysfunction reduces its ability to further regulate the axis, leading to even greater HPA axis activity. Moreover, overexposure of frontal brain regions to glucocorticoids is thought to hinder their ability

to regulate emotion and the HPA axis itself, eventually giving rise to underregulated emotion and symptoms of depression.

The overactive HPA axis hypothesis has found substantial support in the literature. Firstly, many studies in adult MDD have shown that individuals with MDD show greater levels of cortisol (Vreeburg et al., 2009), supporting the existence of an overactive HPA axis in MDD. Neuroimaging studies of adults with MDD (which will be discussed in the following sections) have also found evidence for amygdala overactivity during tasks involving emotion processing, supporting the hypothesis that the amygdala may be overactive and over-triggering stress responses that in turn lead to HPA axis overactivity. Moreover, neuroimaging studies have also found indications for reduced hippocampal volume in adults with MDD, which support the hypothesis' suggestion that the hippocampus may undergo atrophy after overexposure to glucocorticoids. Finally, more indirect evidence supporting the HPA axis hypothesis comes from the observation that individuals suffering from illnesses characterised by overproduction of glucocorticoids, such as Cushing's syndrome, are at a substantially greater risk of suffering from MDD (Pereira, Tiemensma, & Romijn, 2010).

However, whilst overactivity of the HPA axis in MDD has received substantial support, it is unclear whether it ultimately causes MDD, or is instead caused by the illness itself. Firstly, greater levels of cortisol have also been reported in individuals with schizophrenia and bipolar disorder (Girshkin, Matheson, Shepherd, & Green, 2014), suggesting that HPA axis overactivity may either not be specific to MDD or that it may occur as a result of suffering from a severe psychiatric illness. Furthermore, a greater rate of MDD in individuals suffering from illnesses characterised by overproduction of glucocorticoids may simply be a result of the difficulties and stresses that these individuals experience due to their illnesses. Moreover, whilst overactivity of the amygdala and potential atrophy of the hippocampus have been reported in adults with MDD, findings in adolescent MDD have been far less consistent (as will be discussed in **Chapters 2** and **3**), which could indicate that these supposed mechanisms of HPA axis overactivity are not present during the

developmentally earlier stages of the illness, and may instead arise later in adulthood as a result of MDD.

Given the enormous role that the structure and function of specific brain regions and brain network have been hypothesised to play in MDD, their assessment through neuroimaging techniques may be crucial in developing a greater understanding of the illness.

1.8. Magnetic resonance imaging

Although the advent of magnetic resonance imaging (MRI) was undoubtably a great advance in medicine, neuroimaging has so far had little impact in the development of treatments for MDD - apart from the proposal of deep brain stimulation for treatment resistant depression (Mayberg, Lozano, Voon, Mcneely, et al., 2005), which is yet to be proven as effective in randomised control trials (Dougherty et al., 2015; Holtzheimer et al., 2017).

MRI functions by acting upon the hydrogen atoms in water molecules within the body. Hydrogen atoms consist of one proton and one electron, both of which have a charge (protons being positively charged and electrons being negatively charged). Furthermore, protons within the hydrogen nuclei also spin on an axis, like a spinning top; and due to the law of electromagnetic induction (Faraday, 1833), when a charged particle moves it generates its own magnetic field, creating both a North and South pole for that particle – shown in *section B* of **Figure *1.1***. As each of the positively charged hydrogen protons in water spin on an axis, they each create their own individual magnetic fields – where each proton's North and South poles are aligned randomly with respect to each other – shown in *section C* of **Figure *1.1***. If these hydrogen protons are then placed inside a much stronger magnetic field, their North and South poles will align in the direction of that magnetic field.

An MRI scanner creates its own strong magnetic field and when an individual is placed inside the MRI scanner, the North and South poles of the hydrogen protons in their body align to be either

parallel with the scanner's magnetic field (where the North and South poles of the protons are in the same direction as the North and South poles of the MRI scanner) or anti-parallel to the scanner's magnetic field (where the protons' North and South poles are in the opposite direction to those of the scanner) – shown in *section D* of **Figure *1.1***. During this process, the majority of the hydrogen protons will be parallel to the scanner's magnetic field, as most hydrogen protons in the body do not have enough thermal energy to align themselves against the MRI scanner's magnetic field. Hydrogen protons that have enough energy to align anti-parallel are known as high energy protons, whereas those that only have enough thermal energy to align parallel to the scanner's magnetic field are known as low energy protons.

Apart from realigning the North and South poles of hydrogen protons, being inside the scanner's magnetic field also adds an extra level of spin, or wobble, to the body's hydrogen protons known as precession (Westbrook, Kaut Roth, 2011) – which is a circular path, similar to the wobble of a spinning top that is about to fall over, shown in *section A* of **Figure *1.2***. The speed at which it takes one proton to complete this circular path is known as the precessional frequency (Westbrook, Kaut Roth, 2011). When this induction of precession occurs, although each of the hydrogen atoms begin to wobble in the same circular manner, they are out of phase with each other – meaning that they are each at different points of the circular path – shown in *section B* of **Figure *1.2***.

To generate images of the body, radio waves are then emitted in pulses towards the body part of interest, being the individual's brain. These radio waves are at the same frequency as the precessional frequency of the brain's hydrogen protons, which causes the protons to resonate.

Resonance has two key effects on the brain's hydrogen protons. First, it gives the protons more energy, which allows more low energy protons to reach the high energy state and align anti-parallel to the MRI scanner's magnetic field – shown in *section E* of **Figure *1.2***.

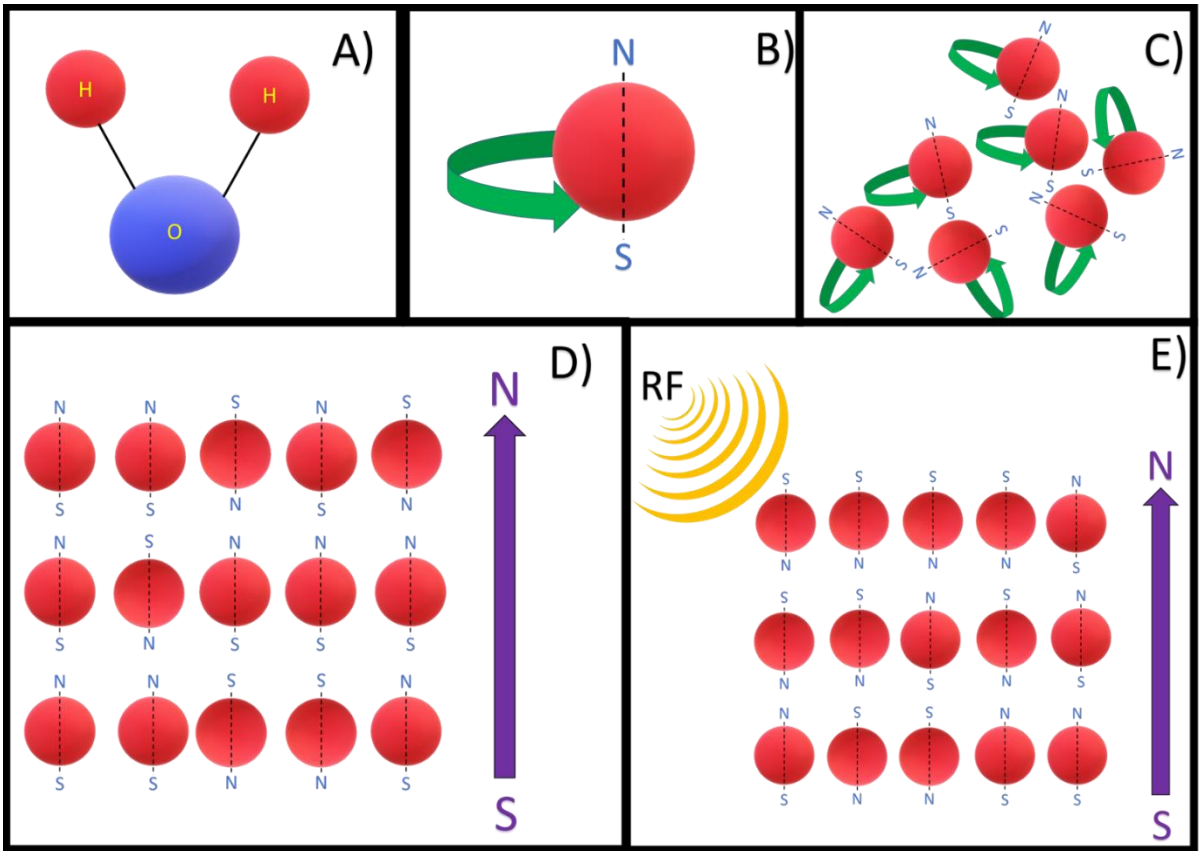


Figure 1.1. Shows the processes that lead to the generation of structural images in MRI. A) Shows a water molecule, made of two hydrogen atoms and one oxygen atom. B) Shows the spin of a hydrogen proton that causes it to produce its own electromagnetic field, generating a North and South pole. C) Shows how the North and South poles of multiple hydrogen atoms are aligned randomly to each other. D) Shows how the North and South poles of the hydrogen atoms align either parallel or anti-parallel to the MRI scanner's magnetic field, with more protons being aligned parallel. E) Shows how a radio frequency pulse causes more hydrogen atoms to align anti-parallel to the MRI scanner's magnetic field.

The second effect that resonance has on the brain's hydrogen protons is that it causes them to wobble in phase with each other, meaning that they are all at the same point in the circular path – shown in *sections C* and *D* of **Figure *1.2***. As protons move in phase with each other, an electrical signal is generated and detected by the MRI scanner.

When the radio wave pulses are stopped, these two effects are undone. Therefore, the new high energy hydrogen protons lose their energy and realign parallel to the scanner's magnetic field. This loss of energy, emitted from the brain's hydrogen protons, is detected by the MRI scanner and is used to create structural images of the brain known as T1 weighted images.

Meanwhile, stopping the radio wave pulses also causes the hydrogen protons to start to wobble out of phase with each other, leading to a loss in the electrical signal that they initially produced. This loss of electrical signal, and the time it takes to lose it, is detected by the MRI scanner. Deoxygenated blood causes nearby hydrogen protons to go out of phase with each other at a faster rate, due to the magnetic properties of iron in the blood interacting with the protons' magnetic fields – shown in *section E* of **Figure *1.2***. Therefore, hydrogen protons near a blood vessel containing deoxygenated blood will go out of phase faster causing their electrical signal to decay faster (Greve, 2011), which is detected by the MRI scanner. This is known as the blood oxygen level dependent (BOLD) effect.

Regions of the brain that increase their function will require more oxygen, leading to greater amounts of deoxygenated blood around those regions. Thus, the MRI scanner can detect brain regions containing deoxygenated blood, which allows images inferring brain function to be generated, and is used during functional magnetic resonance imaging (fMRI).

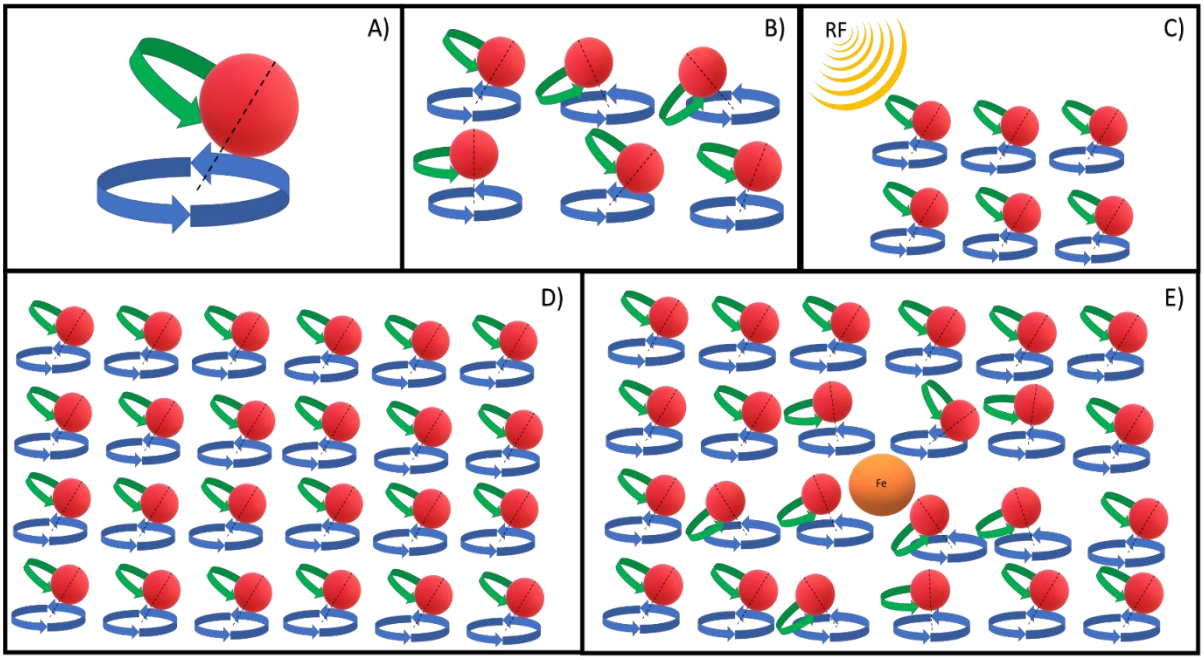


Figure 1.2. Shows the processes that allow the bold effect to be measured. A) Shows the precession of a hydrogen atom. B) Shows that different hydrogen atoms in the brain are at separate points of their precessional paths. C and D) Show hydrogen atoms precessing in phase with each other, after a radio frequency pulse is applied. E) Shows how the presence of an iron atom, from deoxygenated blood, causes the surrounding hydrogen protons to go out of phase faster than those further away.

1.9. Measures of brain structure and function

1.9.1. Grey and white matter volume

The brain is comprised of neurons and can be segmented into grey and white matter, whose properties are analysed in neuroimaging studies. Grey matter is comprised of the cell bodies, dendrites and axon terminals of neurons, involved in signal processing, whereas white matter is comprised of myelin that surrounds the neuronal axons, involved in signal transmission, shown in **Figure *1.3***.

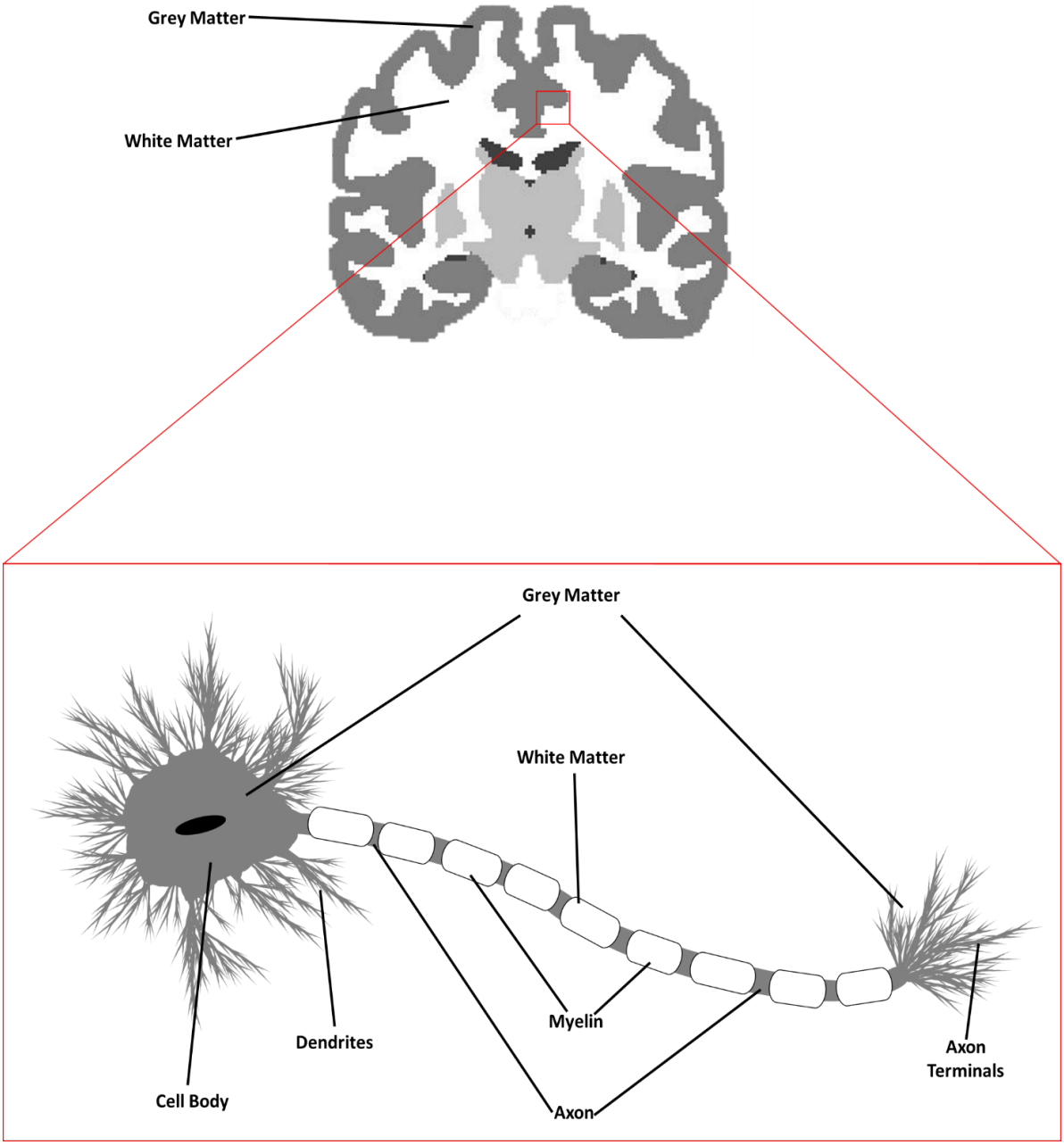


Figure 1.3. Shows the constituent parts of the neuron that make up grey and white matter divisions within the brain.

Grey matter volume is a metric often used in neuroimaging studies, and is defined as the amount of grey matter between the inner grey/white matter boundary and the outer pial surface (Winkler et al., 2010), and is a function of both the thickness and surface area of the brain, shown in **Figure *1.4***. White matter volume can be defined as the amount of white matter in a given region, within the grey/white matter border.

Grey matter volume is often measured using voxel-based morphometry (VBM), which breaks the brain down into thousands of individual three-dimensional pixels (known as voxels) and attempts to measure the amount of grey matter in a given voxel of the brain (Winkler et al., 2010). This is done by segmenting T1 weighted brain images into different tissue types, often being grey matter, white matter, and cerebrospinal fluid. The grey matter volume images are then normalised to a standard template brain, to ensure that any set of brain images being compared are within the same coordinate space and are aligned with each other. The segmentations of grey matter volume are then spatially smoothed to enhance the signal-to-noise ratio of the images. To measure white matter volume, the same process is applied but pre-processing procedures focus on estimating white matter rather than grey matter.

Generally, adult MDD patients tend to show grey matter volume reductions within predominantly frontal and limbic brain regions, compared to healthy controls, with grey matter volume reductions within the anterior cingulate cortex being the most consistent finding, though hippocampal volume reductions have also frequently been reported (Bora, Fornito, Pantelis, & Yücel, 2012; Du et al., 2012; Grieve, Korgaonkar, Koslow, Gordon, & Williams, 2013; Kempton et al., 2011; Koolschijn, Van Haren, Lensvelt-Mulders, Hulshoff Pol, & Kahn, 2009; Lai, 2013; Peng, Chen, Yin, Jia, & Gong, 2016; Schmaal et al., 2016; Wise et al., 2017; Zhao et al., 2014). Studies investigating white matter volume in adult MDD are far fewer, with greater white matter volumes in frontal regions of the brain being found in adult MDD patients (Zeng et al., 2012), though others have struggled to find differences (Abe et al., 2010; Kim, Hamilton, & Gotlib, 2008). With respect to adolescent MDD, the literature has

been far less consistent and it is still unclear as to what deviations in grey and white matter volume actually occur in adolescent MDD.

1.9.2. Cortical thickness

Cortical thickness is defined by the distance between the inner grey/white matter boundary and the outer pial surface along the cortex (Fischl & Dale, 2000; Winkler et al., 2010) – shown in **Figure *1.4***. Multiple methods of estimating cortical thickness exist, which can be divided into surface-based methods and volume-based methods. Surface based methods attempt to reconstruct the inner grey/whitematter boundary and outer pial surface using surface meshes (Fischl, 2012; Fischl & Dale, 2000; Righart et al., 2017; Tustison et al., 2014), which then measure the distances between the two surfaces to estimate cortical thickness. Volume-based methods instead attempt to estimate cortical thickness within the image itself, at the voxel level, rather than attempting to reconstruct the inner and outer surfaces of the brain (Avants, Tustison, & Song, 2009; Das, Avants, Grossman, & Gee, 2009; Righart et al., 2017; Tustison et al., 2014).

Regarding cortical thickness in MDD, studies have often found that adult MDD patients show cortical thinning within predominantly frontal regions of the brain, with thinning within the orbitofrontal cortex most often being reported in adult MDD patients, though cortical thinning in parietal and posterior regions such as the posterior cingulate cortex have also been reported (Grieve et al., 2013; Järnum et al., 2011; Rajkowska et al., 1999; Schmaal et al., 2017; Suh et al., 2019; Won, Choi, Kang, Lee, & Ham, 2016). The adolescent MDD literature investigating cortical thickness, like that investigating grey and white matter volume, has been inconsistent and no consensus has yet been reached over whether deviations in cortical thickness occur in adolescent MDD.

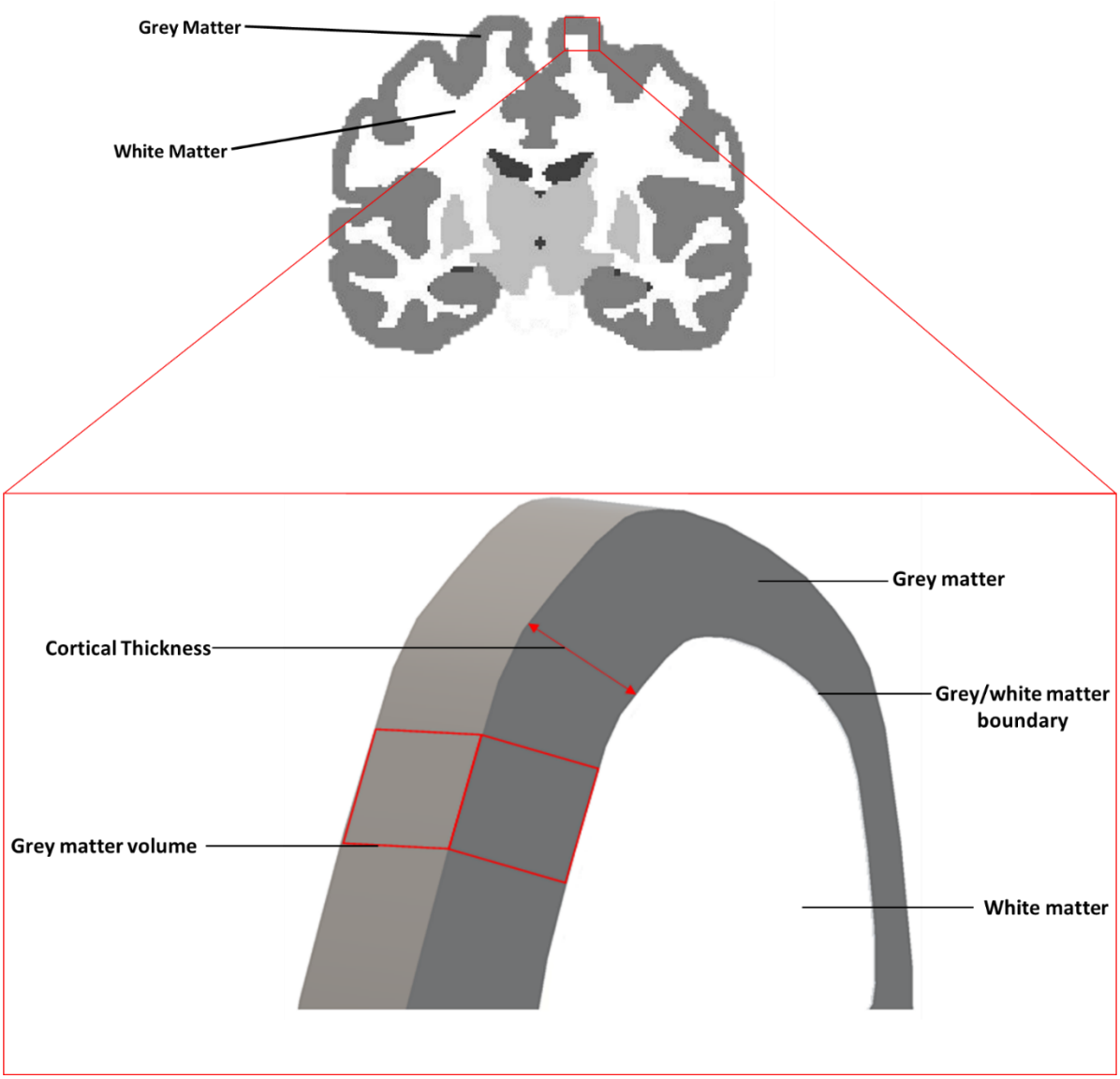


Figure 1.4. Shows the measurement of cortical thickness and grey matter volume, commonly used in neuroimaging analyses.

1.9.3. Resting-state functional connectivity

Although much can be inferred from the brain's activity during a task, known as task-based fMRI paradigms, observing the brain's function when at rest can also lead to intriguing and illuminating findings, through the measure of resting-state functional connectivity. Functional connectivity is derived by correlating a brain region's activity pattern over time, known as a timeseries, with the activity pattern of one or more other regions of the brain – shown in **Figure *1.5***. When the functional connectivity of a specific region is the primary interest of a study, the timeseries of that region, known as a seed region, is extracted and correlated to the timeseries of multiple other brain regions, which is known as a seed-based approach.

The reasoning behind estimating functional connectivity can be understood if one were to imagine viewing a silent video of a group of singers. As the video is silent, the exact words that are being sung by each individual cannot necessarily be discerned, but by observing the mouth movements of each singer throughout the video, and comparing them to the mouth movements of the other singers, those whose mouths move at the same time can be presumed to be singing the same song. The same reasoning applies when studying resting-state functional connectivity, in that regions of the brain that activate at the same time as each other are likely to be working within the same brain network and potentially on the same function. This technique has led to the discovery of multiple functional brain networks, related to aspects of both cognition and emotion, and some of which have been implicated in MDD.

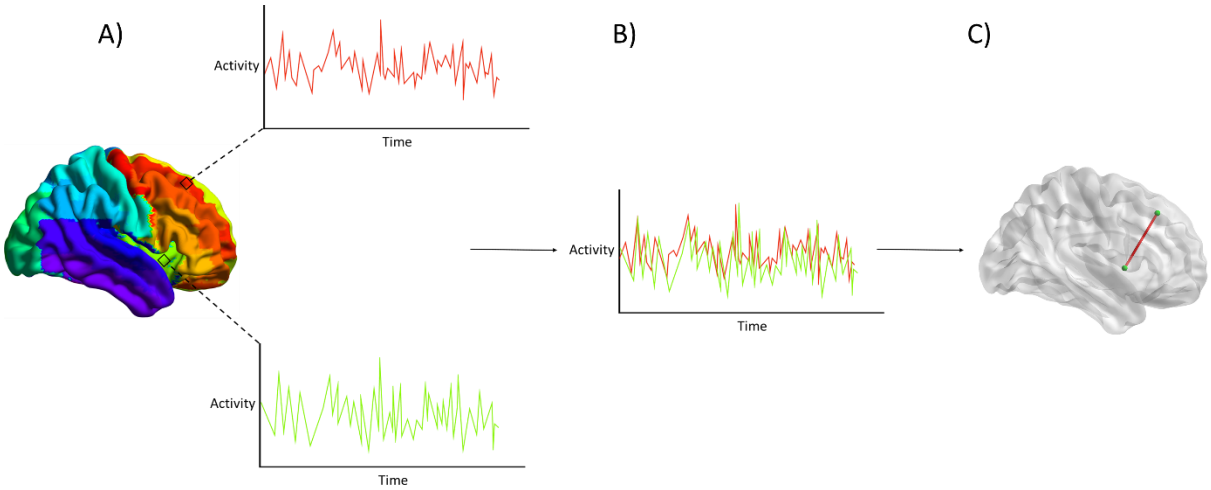


Figure 1.5. Shows the steps in investigating resting-state functional connectivity. A) Shows the timeseries extraction of multiple brain regions. B) Shows the correlation between the timeseries of two brain regions. C) Shows the inference made that the two regions are functionally connected.

1.10. Functional networks implicated with major depressive disorder

Four key brain networks have been implicated with MDD, being the fronto-limbic network, default mode network, central executive network, and salience network, shown in **Table 1.1.** Each of these networks can be observed when examining functional connectivity, and each are thought to subserve various aspects of cognitive and emotional processing that may underlie the experiences of MDD. However, whilst functional disruption to these key networks has been observed in adult MDD patients, it is still unclear as to what extent these findings apply to adolescent MDD, as the literature has suffered from inconsistencies.

1.10.1. Fronto-limbic network

The fronto-limbic network is involved in both emotional reactivity and emotion regulation, and is comprised of both limbic and frontal regions of the brain, which include the pregenual anterior cingulate cortex, subgenual anterior cingulate cortex, hippocampus, thalamus, medial orbitofrontal cortex, and medial frontal gyrus (Graham et al., 2013; Suckling, 2012; Zhong, Pu, & Yao, 2016). Past research studying the functional activity of regions within this network have led to the highly influential fronto-limbic hypothesis (Mayberg, 1997, 2003), which argues that there is an imbalance or poor communication between the emotionally reactive limbic regions and the emotional regulatory frontal regions, whereby the limbic regions are underregulated by the frontal regions. This imbalance within the fronto-limbic network, leading to underregulated emotional responses, is thought to give rise to the symptoms of major depression (Casey et al., 2010; Mayberg, 1997, 2003; Roiser & Sahakian, 2013; Suckling, 2012). This hypothesis has been supported by research into adult MDD, with past studies finding that adult MDD patients show reduced activity within frontal regions and increased activity in limbic regions, when at rest and when conducting emotional processing tasks (Lai, 2014; Steele, Currie, Lawrie, & Reid, 2007; Zhong et al., 2016), as well as showing disrupted resting-state functional connectivity within the network (Wang, Hermens, Hickie, & Lagopoulos, 2012).

1.10.2. Default mode network

The default mode network is active when an individual is at rest and is doing nothing, hence it being known as the brain's "default mode" (Raichle, 2015; Raichle et al., 2001; Shulman et al., 1997). The network is thought to be involved in multiple cognitive and emotional processes, with some of the most prominent being internal thought generation, and self-referential processing (Kaiser, Andrews-Hanna, Wager, & Pizzagalli, 2015; Mantini & Vanduffel, 2013; Mulders, van Eijndhoven, Schene, Beckmann, & Tendolkar, 2015; Raichle, 2015) and is comprised of the inferior parietal lobule, posterior cingulate cortex, precuneus, hippocampus, parahippocampus, subgenual anterior cingulate cortex, and medial frontal gyrus (Graham et al., 2013; Kaiser et al., 2015; Mulders et al., 2015). It is thought that, due to its roles in thought generation and self-referential processing, disruption within the default mode network occurs in MDD patients, that leads to the production and maintenance of negative thoughts and rumination symptoms that are so characteristic of MDD (Graham et al., 2013; Zhu et al., 2012). In line with this premise, multiple studies have shown that adult MDD patients show disrupted resting-state functional connectivity of the default mode network (Kaiser et al., 2015; Mulders et al., 2015; Zhong et al., 2016), and this disruption has even been related to rumination symptoms in adult MDD (Berman et al., 2011; Hamilton, Farmer, Fogelman, & Gotlib, 2015; Hamilton et al., 2011; Zhu et al., 2012).

1.10.3. Central executive network

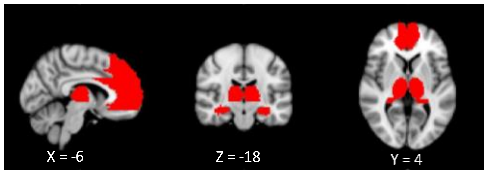
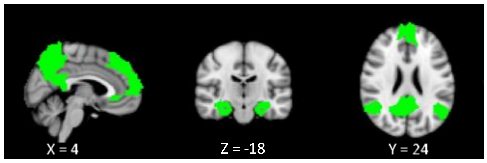
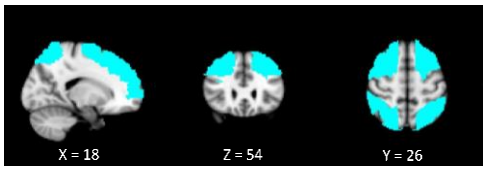
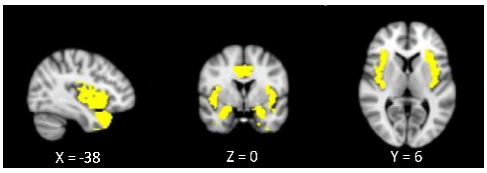
Unlike the default mode network, the central executive network is most active when an individual is taking part in a cognitively demanding task, and is therefore thought to be involved in processes such as executive function, cognitive control, and attention (Goulden et al., 2014; Kaiser et al., 2015; Mulders et al., 2015; Wang, Yang, Sun, Shi, & Duan, 2016). The central executive network is comprised of frontal and parietal regions of the brain, being the superior frontal gyrus, middle frontal gyrus, and posterior parietal cortex (Goulden et al., 2014; Kaiser et al., 2015; Mulders et al., 2015; Sherman et al., 2014). Due to its roles with cognition, it has been suggested that disruption

within the central executive network may be involved in the development of cognitive deficits that patients with MDD often experience. Disruption to the central executive network's functional connectivity has indeed been found in adult MDD patients (Kaiser et al., 2015; Mulders et al., 2015; Wang et al., 2016), suggesting that it may play a role in experiencing the illness.

1.10.4. Salience network

The salience network is considered to be involved in directing attention towards emotional stimuli (Kaiser et al., 2015; Mulders et al., 2015; Seeley et al., 2007), particularly those that are stressful and threatening (Hermans, Henckens, Joëls, & Fernández, 2014; Mulders et al., 2015), and is also thought to be involved in switching between the default mode network and the central executive network (Goulden et al., 2014). The salience network includes the amygdala, insula, midcingulate, and temporal pole. Due to its role in orienting attention to emotionally salient and threatening stimuli, it is thought that disruption within the salience network may account for the heightened response towards negative stimuli, and negative biases when interpreting emotional information that are often displayed by patients with MDD (Hamilton et al., 2012, 2016). In line with this hypothesis, adult MDD patients have shown disruption in the functional connectivity of the salience network (Mulders et al., 2015) and the amygdala often shows hyperactivity in adult MDD patients towards emotional stimuli (Abler, Erk, Herwig, & Walter, 2007; Davey, Allen, Harrison, & Yucel, 2011; Groenewold, Opmeer, de Jonge, Aleman, & Costafreda, 2013).

Table 1.1. Shows four key functional networks implicated in adult depression.

Network	Functions	Regions	Network Map
Fronto-Limbic	Emotional reactivity, emotional regulation	Medial frontal gyrus, medial orbitofrontal cortex, hippocampus, thalamus, pregenual anterior cingulate, subgenual anterior cingulate	
Default Mode	Internal thought generation, self-referential processing	Medial frontal gyrus, posterior cingulate, parahippocampus, hippocampus, precuneus, subgenual anterior cingulate cortex, inferior parietal lobule	
Central Executive	Executive function, cognitive control	Superior frontal gyrus, middle frontal gyrus, posterior parietal cortex	
Salience	Switching between the default-mode and central executive networks, directing attention towards emotionally salient stimuli	Insula, midcingulate, amygdala, temporal pole	

1.11. Neurobiological subtypes of major depressive disorder?

The existence of clinically meaningful subtypes of MDD is a topic that has perplexed the field. MDD is a highly heterogenous illness and individual patients vary greatly between each other across multiple domains, such as in their age of onset, symptoms, comorbidities, depressive episode duration and recurrence, and in their response to treatment. This heterogeneity within the illness can even be observed from the criteria used to diagnose it – whereby opposing symptoms such as insomnia or hyposomnia, increased appetite or decreased appetite, weight gain or weight loss, are present (American Psychiatric Association, 2013). This has led many to question whether there are subtypes of MDD and whether these can be discerned neurobiologically.

Multiple studies have attempted to use measures of brain structure and function to identify clinically meaningful subtypes of MDD patients. Most of these investigations have attempted to predict response to various treatments for MDD by using a variety of neural correlates (Chen et al., 2007; Downar et al., 2014; Fox, Buckner, White, Greicius, & Pascual-Leone, 2012; Fu, Steiner, & Costafreda, 2013; Kennedy et al., 2007; Konarski et al., 2009; Salvadore et al., 2009; Williams et al., 2015) that tend to implicate fronto-limbic regions of the brain and could potentially identify the subtypes of patients that respond best to certain treatments (Fonseka, MacQueen, & Kennedy, 2018). However, whilst this approach has shown promise, many of these findings are yet to be reliably replicated and exact predictions of treatment response are inconsistent (Fonseka et al., 2018).

Newer data-driven approaches have attempted to identify neurobiological subtypes of MDD based on aspects of brain structure and function, rather than just treatment response, with some claiming to find distinct biological subtypes of MDD patients that even partially map onto variations in symptom profiles (Drysdale et al., 2017; Feder et al., 2017; Grosenick et al., 2019; Haroon et al., 2018). However, these studies have again been inconsistent and suffer from a lack of replication (Beijers, Wardenaar, van Loo, & Schoevers, 2019; Dinga et al., 2019), making it unclear as to whether clinically meaningful neurobiological subtypes of MDD actually exist.

1.12. Motivation for thesis

MDD clearly has a strong neurobiological component, which has mostly been studied in adults suffering from the illness. However, much less is known about the structural and functional deviations that may occur within the brain in adolescent MDD patients, and it is still unclear how generalisable findings from adult MDD patients are to adolescents. Moreover, whilst it is understood that psychological therapies are effective at treating adolescent MDD, and is in some cases preferred over pharmacotherapies (National Institute for Health and Care Excellence, 2019), the mechanisms of how they act upon the brain are relatively unknown. These three issues alone highlight a large gap in our understanding of adolescent MDD, and if improved, could help illuminate the aetiology of the illness and potentially provide insights into new and more effective treatments and interventions for the illness. Furthermore, when viewing the diagnostic criteria of MDD and its potential variations in its symptoms, levels of recurrence, and treatment outcomes, it is clear that the illness is heterogenous and within this heterogeneity may lie neurobiologically distinct subtypes that, if identified, could help clarify any inconsistencies within the field and help provide better predictions for patient prognoses.

This thesis will therefore attempt to provide a better understanding of the neurobiology of adolescent MDD. This will be achieved by attempting to establish whether structural differences within the brain are indeed present in adolescent MDD, by assessing differences in both cortical thickness and white matter volume between a large group of adolescent MDD patients and healthy adolescent controls. The thesis will also attempt to identify the nature of pre-treatment functional disruption to brain networks in adolescent MDD, by examining differences in resting-state functional connectivity between adolescents with MDD and healthy adolescent controls. This thesis will also attempt to identify how CBT may act upon disrupted brain function in adolescent MDD, by assessing changes in resting-state functional connectivity before and after treatment with CBT in a sample of adolescent MDD patients. The thesis will next attempt to unravel aspects of heterogeneity in MDD

by investigating whether distinguishing adolescent MDD patients based on their familial loading for the illness may have any clinically and neurobiologically meaningful effects.

A breakdown of each individual chapter is detailed below:

In **Chapter 2**, the thesis will investigate structural deviations in adolescent MDD, comparing the cortical thickness and white matter volume of adolescent MDD patients to those of healthy adolescent controls, hypothesising that adolescent MDD patients will show cortical thickness deviations within the subgenual anterior cingulate and medial orbitofrontal cortex, as well as white matter volume differences within frontal brain regions. It will also hypothesise that adolescent MDD patients will show age-related differences in these two measures of brain structure, compared to healthy controls.

In **Chapter 3**, the thesis will focus on resting-state functional connectivity in adolescent MDD, and how the functional networks of the brain may be disrupted in patients before receiving treatment. It will be hypothesised that adolescent MDD patients will show disruption to functional brain networks, being the fronto-limbic, default mode, central executive, and salience networks.

Chapter 4 will investigate the potential effects of CBT on resting-state functional connectivity in adolescent MDD patients, and how it may act upon functionally disrupted brain regions. It will hypothesise that CBT will have a normalising effect on disrupted brain function, and that regions showing the greatest pre-treatment functional disruption will show the greatest CBT-related changes in resting-state functional connectivity.

Chapter 5 will investigate the potential for neurobiologically subtyping adolescent MDD patients based on their familial loading for the illness, investigating differences in both brain structure and function between these potential subtypes. It will hypothesise that adolescent MDD patients with a high familial loading for the illness will show lower grey matter volume, cortical thickness, and white matter volume in regions of the default mode network, compared to adolescent MDD patients with

a low familial loading for the illness, as well as showing overconnectivity within the default mode network compared to those with a low familial loading.

Chapter 6 will investigate whether subtyping adolescent MDD patients based on familial loading for the illness has any clinical significance, by studying any differences in symptoms experienced by the two subtypes and whether they have a relationship to the neurobiology of the illness. It will hypothesise that adolescent MDD patients with a high familial loading for the illness will show differences in their level of rumination symptoms, compared to those with a low familial loading, and that the two familial loading groups will differ on how aspects of grey matter volume and resting-state functional connectivity relate to these rumination symptoms.

Finally, in **Chapter 7**, general conclusions, debates, limitations, and future directions for this work and the field will be discussed.

2. Brain structure in adolescent major depressive disorder

2.1. Introduction

Most of the literature surrounding brain structure in adolescent MDD has focused on grey matter volume, and whilst many studies have attempted to investigate it, many of them conflict with regard to which structural deviations are actually characteristic of the illness and as to the clinical significance of these deviations.

Much of the early work investigating grey matter volume in adolescent MDD focused on the structure of the amygdala and hippocampus. At the time, based on work investigating adult MDD, it was thought that the amygdala was hyperactive in MDD, which would lead to increased grey matter volume within the region (Frodl et al., 2003, 2002), and that the hippocampus was exposed to hypersecretion of glucocorticoids in MDD, which would lead to its atrophy (Bremner et al., 2000; Steffens et al., 2000). Early neuroimaging studies then tried to apply these hypotheses to adolescent MDD patients with some initial success. For example, MacMillan et al. (2003) found that child and adolescent MDD patients showed greater amygdala:hippocampus volume ratios than controls, arguing that the ratio in patients may be related to hyperactivity of the amygdala, whilst MacMaster and Kusumakar (2004) also found reduced hippocampal volumes in adolescent MDD patients, supporting the concept of adolescent MDD mirroring the neurobiological characteristics of adult MDD.

However, further studies focusing on grey matter volume in adolescent MDD later cast doubt on the presence of structural deviations within the amygdala and hippocampus. Rosso et al. (2005) found that child and adolescent MDD patients showed reduced grey matter volume within the amygdala, rather than the previously hypothesised increased grey matter volume, and also failed to find any deviations to hippocampal structure in their patients, arguing that amygdala volume reductions were a predisposing factor for MDD. Conversely, Caetano et al. (2007) later found that child and adolescent MDD patients showed reduced hippocampal volumes and no deviations to the amygdala

when compared to controls. They argued that reduced hippocampal volumes were characteristic of child and adolescent MDD and that any previously reported amygdala deviations in paediatric MDD were instead caused by the accidental inclusion of bipolar disorder patients. More recent studies have only contributed to the inconsistencies surrounding the amygdala and hippocampus' structure in adolescent MDD, with some still claiming that reduced hippocampal grey matter volume is a neurobiological characteristic of adolescent MDD (Kim, Suh, Lee, Lee, & Lee, 2019; MacMaster, Carrey, Langevin, Jaworska, & Crawford, 2014; Whittle et al., 2014) and with others finding no deviations to either structures (Hagan et al., 2015a; Pannekoek et al., 2014; Shad, Muddasani, & Rao, 2012). Such conflicting results and conclusions are typical within the adolescent MDD literature, making it difficult to discern which brain structures are actually afflicted by the illness.

Other work investigating grey matter volume in adolescent MDD has attempted to shift away from focusing solely on the amygdala and hippocampus but again has been marred by inconsistencies. For example, Matsuo et al. (2008) centred their investigations on the striatum, due to it having been implicated in the past adult MDD literature (Husain et al., 1991; Krishnan et al., 1992; Parashos, Tupler, Blitchington, & Krishnan, 1998) and because of its involvement in emotion regulation, executive function, and motivation (Alexander, 1986; Mega & Cummings, 1994; Soares & Mann, 1997). They found that child and adolescent MDD patients showed reduced grey matter volume within the caudate and suggested that its development may deviate in MDD patients during adolescence. However, other studies have since either not found grey matter volume reductions within the striatum in adolescent MDD patients (Kim et al., 2019; MacMaster et al., 2014) or have found that they dissipate when controlling for socioeconomic status (Shad et al., 2012), shrouding the true extent of the striatum's involvement in adolescent MDD.

Prefrontal regions of the brain have also been implicated in adult MDD, which has led others to investigate its grey matter volume in adolescent MDD. Chen et al. (2008), for example, investigated the orbitofrontal cortex in adolescent MDD, based on its role in cognition, emotion and reward

processing (Damasio, 1994), as well as having been previously implicated in adult MDD (Bremner et al., 2002; Lacerda et al., 2004; Lai, Payne, Byrum, Steffens, & Krishnan, 2000; Rajkowska et al., 1999). They, like many other past studies, expected findings from adult MDD patients, in this case being reduced grey matter volume within the orbitofrontal cortex, to be replicated in adolescent MDD patients. However, they failed to find any orbitofrontal grey matter volume deviations in their child and adolescent MDD patients and suggested that they may occur later in the illness' progression. Conversely, Shad et al. (2012) later found that adolescent MDD patients did show reduced grey matter volume within the orbitofrontal cortex, though this difference did not survive controlling for socioeconomic status, again demonstrating the literature's inconsistency regarding grey matter volume in adolescent MDD.

Moreover, the anterior cingulate cortex, the region that has been most consistently reported to show grey matter volume reductions in adult MDD patients (Bora et al., 2012; Koolschijn et al., 2009), has also been implicated in adolescent MDD but again the findings surrounding its structure have been inconsistent. Pannekoek et al. (2014) investigated the grey matter volume of the anterior cingulate cortex and did find that adolescent MDD patients showed reduced grey matter volume within the region, matching findings from the adult MDD literature. They argued that the neuronal maturational processes of the anterior cingulate cortex may be affected in adolescent MDD, causing deviating developmental trajectories within the region. However, as is the case with the majority of the adolescent MDD literature investigating grey matter volume, this finding has been both supported (MacMaster et al., 2014), and also disputed by subsequent studies failing to find overall grey matter volume reductions within the anterior cingulate cortex in adolescent MDD (Hagan et al., 2015a; Whittle et al., 2014). Some have suggested that deviating developmental trajectories of the anterior cingulate are more prominent in adolescent MDD, rather than overall group differences, which may emerge later in the illness' progression (Hagan et al., 2015a).

Clearly, the literature surrounding grey matter volume in adolescent MDD is far from consistent, which makes it difficult to draw conclusions about the neurobiological nature of the illness. However, grey matter volume is a factor of the thickness and surface area of the brain (Winkler et al., 2010), with cortical thickness being particularly affected by white matter development (Sowell et al., 2003; Whitaker et al., 2016). Therefore, the investigation of other structural measurements of the brain, such as cortical thickness and white matter volume, may provide more clarity to the neurobiological characteristics of adolescent MDD.

2.2. Cortical thickness and white matter volume in adolescent major depressive disorder

Unlike the literature investigating grey matter volume in adolescent MDD, studies focusing on cortical thickness are relatively few in number, but like the grey matter volume literature, they are often hindered by the use of small sample sizes and vary greatly in their findings.

The first study to investigate cortical thickness in adolescent MDD was conducted by Fallucca et al. (2011), who compared 24 child and adolescent MDD patients to 24 OCD patients, and 30 healthy controls, expecting to find reduced cortical thickness within prefrontal and frontal regions of the brain – as had been previously found in the adult literature (Peterson et al., 2009; Rajkowska et al., 1999). However, they found no significant differences in frontal regions of the brain and instead found that MDD patients showed greater cortical thickness in temporal regions and also reduced thickness in parietal regions, compared to both OCD patients and controls. They concluded that deviations in cortical thickness do indeed occur in child and adolescent MDD, but that focusing only on frontal regions may be too narrow as structural deviations appear to occur in other regions.

Reynolds et al. (2014) later attempted to build upon Fallucca et al.'s (2011) work, and investigated the surprising lack of cortical thickness deviations within fronto-limbic regions in adolescent MDD. Using 30 adolescent MDD patients and 16 adolescent controls, they focused on the fronto-limbic

network, expecting to find cortical thinning within the middle frontal gyrus and anterior cingulate cortex. Instead, they found that the adolescent MDD patients actually showed greater cortical thickness within both regions, and argued that these structural deviations may be indicative of a deviating developmental trajectory of neuronal pruning. Moreover, they speculated that the greater anterior cingulate thickness may have also been caused by the regions' overactivity in MDD patients that would lead to a greater neuronal number or density – though this claim was based on previous work using adult MDD patients (Botvinick, Nystrom, Fissell, Carter, & Cohen, 1999; Knutson, Bhanji, Cooney, Atlas, & Gotlib, 2008; Smoski et al., 2009) and they lacked any functional data of their own to support this claim.

Jaworska et al. (2014) later investigated cortical thickness in a sample of 36 adult MDD patients and 18 healthy adult controls, whereby 20 of the MDD patients had first experienced the illness before the age of 24 years. They found that when comparing healthy controls to the entire MDD patient group, patients showed greater cortical thickness within the frontal pole. Interestingly, this greater cortical thickness was driven by the early onset patients, further suggesting that adolescent-onset MDD may be related with greater cortical thickness in frontal regions. They also claimed that this cortical thickening in MDD patients may be part of a neurocompensatory or adaptive mechanism within the brain, though the workings of this mechanism were not explained.

Identifying the potential for deviations in cortical thickness to be used as biomarkers for MDD, Foland-Ross et al. (2015) attempted to investigate whether the cortical thickness of multiple brain regions could be used to predict the onset of depression in adolescent females. They collected cortical thickness estimates from 33 adolescents and followed them up over a period of 5 years, after which, 18 had developed a depressive disorder. Using machine learning methods, they used average cortical thickness estimates, from the baseline scans, to see if cortical thickness could be used to accurately predict who had developed depression. In total, they were around 70% accurate in their predictions and found that the thickness of the medial orbitofrontal cortex was the best

predictor for developing depression, followed by the precentral gyrus, anterior cingulate cortex, and the insula. They further compared the baseline cortical thickness of those who later developed depression to those who did not, but in contrast with past work relating greater frontal cortical thickness to adolescent MDD (Jaworska et al., 2014; Reynolds et al., 2014), they found that individuals who would later develop depression showed reduced cortical thickness within the medial orbitofrontal cortex. They argued that cortical thickness may potentially become a useful biomarker for identifying those at the greatest risk for developing depression and that deviations in cortical thickness appear to occur before the onset of MDD and may even contribute to the development of the illness.

The largest investigation of cortical thickness in MDD was conducted by Schmaal et al. (2017) who, in a collaborative worldwide study, compared the cortical thickness between MDD patients and healthy controls, in both adults and adolescents. Their study included data from 1902 adult MDD patients, and 7658 healthy adult controls, and data from 213 adolescent MDD patients and 294 healthy adolescent controls. When focusing on adults, MDD patients showed reduced cortical thickness within multiple regions such as the medial orbitofrontal cortex, anterior cingulate cortex, posterior cingulate cortex, and insula. However, when they focused on adolescents, they found no significant differences, leading to the potential conclusion that there may actually be no detectable deviations in cortical thickness in adolescent MDD. However, 70% of their adolescent patients were between the ages of 18-21 years, meaning that their study may not have been able to capture the extent of structural deviations in younger adolescent MDD patients, who would be in an earlier stage of brain development and may be affected differently by the illness than the developmentally more mature sample used by Schmaal et al. (2017).

More recently, identifying the inconsistencies within the literature, Kim et al. (2019) investigated cortical thickness in adolescent MDD and attempted to address this inconsistency by homogenising their patient sample. They ensured that their patients were all medication-naïve and had only

experienced one episode of depression, claiming that the inclusion of patients taking antidepressant medication as well as the inclusion of patients with recurrent MDD may have contributed to the heterogeneity of previous studies. They compared the cortical thickness of 27 adolescent MDD patients to 27 healthy adolescent controls but instead of bringing clarity and cohesion to the literature, they further contributed to its inconsistency as they found that their adolescent MDD patients showed reduced cortical thickness within the left occipital regions of the brain, claiming to be the only study to ever report cortical thinning within the precuneus and cuneus in adolescent MDD patients.

The literature investigating cortical thickness in adolescent MDD is far from consistent, with studies designed to bring clarity to the literature only further contributing to its inconsistency (Kim et al., 2019; Schmaal et al., 2017). The reasons for these inconsistencies may in part be methodological, such as differences in scanning parameters or pre-processing methods between studies, or be demographic in nature, such as differences in the ages, medication status, or chronicity of patients. However, what is clear is that the vast majority of studies investigating cortical thickness in adolescent MDD have had relatively small sample sizes. Even the largest study, which used data from 213 adolescent patients, only had 64 adolescent MDD patients below the age of 18 years (Schmaal et al., 2017). Therefore, studies utilising large samples of adolescent MDD patients are vital in order to bring clarity to the cortical thickness literature.

Regarding the literature surrounding white matter volume in adolescent MDD, whilst studies investigating it are extremely sparse, they are relatively consistent in what they have found. (Steingard et al., 2002) were the first to study white matter volume in adolescent MDD, comparing 19 adolescent MDD patients to 38 healthy controls. They found that adolescent MDD patients showed reduced white matter volumes within the frontal lobes and that it was positively correlated to age, arguing that adolescent MDD may be characterised by a delayed maturational process in either myelination or synaptic pruning of the frontal lobes. This was later supported by two other

studies that also found reduced white matter volume within prefrontal regions in adolescent MDD patients (Gabbay et al., 2012; MacMaster et al., 2014), though both used relatively small sample sizes. However, whilst the sparse literature investigating white matter volume in adolescent MDD is relatively consistent, it does conflict with findings showing adult MDD patients having greater white matter volumes in frontal and prefrontal regions of the brain (Zeng et al., 2012). This contrast may be a distinct neurobiological difference between adolescent and adult MDD patients but due to the small sample sizes previously used to investigate white matter volume in adolescent MDD, it cannot be argued with confidence.

We therefore conducted the present study to investigate both cortical thickness and white matter volume in adolescent MDD, using a highly powered sample previously reported by Hagan et al. (2015a). We investigated case-control differences in cortical thickness of the subgenual anterior cingulate cortex and medial orbitofrontal cortex as they have previously been implicated in both adult (Grieve et al., 2013; Peterson et al., 2009; Schmaal et al., 2017) and adolescent MDD (Foland-Ross et al., 2015; Jaworska et al., 2014). We also investigated white matter volume of frontal brain regions, being the medial frontal gyrus, middle frontal gyrus, superior frontal gyrus, and medial orbitofrontal cortex, to further investigate the claims that adolescent MDD may be characterised by deviating white matter development (Gabbay et al., 2012; MacMaster et al., 2014; Steingard et al., 2002). However, as the literature on cortical thickness and white matter volume in adolescent MDD has either been inconsistent or hindered by small sample sizes we did not predict the directions of any potential case-control differences. We did expect to find a group-by-age interaction in both cortical thickness and white matter volume within the anterior cingulate cortex, as had previously been found in grey matter volume using our sample (Hagan et al., 2015a).

2.3. Methods

2.3.1. Participants

Participants were recruited by the Improving Mood with Psychoanalytic and Cognitive Therapies (IMPACT) study (Goodyer et al., 2017, 2011). IMPACT was a multicentre, single-blind, randomised controlled superiority trial, spanning across the areas of North West England, East Anglia, and North London, assessing the medium-term effects of CBT, short-term brief psychoanalytical therapy, and a brief psychosocial intervention. Patients (*n* = 465) met diagnostic requirements for MDD using the DSM-IV (American Psychiatric Association, 1994) and were randomised to one of the three treatment arms. Patients were assessed over an 86-week period, with assessments being conducted at baseline, prior to randomization, then at 6, 12, 36, 52, and 86 weeks after randomization.

A subset of patients enrolled in IMPACT were later recruited for the MR-IMPACT study (*n* = 128; Hagan et al., 2015a; Hagan et al., 2013), which recruited patients from East Anglia (*n* = 110) and North London (*n* = 18). Patients in MR-IMPACT were assessed using structural and functional MRI at an initial baseline scan, prior to starting treatment. The Short Moods and Feelings Questionnaire (SMFQ; Angold et al., 1995) and State-Trait Anxiety Inventory (STAI; Spielberger et al., 1970) were administered on the day of scanning as a measure of immediate depression and anxiety symptoms.

Additionally, healthy adolescent controls (*n* = 40), with no personal history of MDD and no first-degree relatives with MDD, were recruited for MR-IMPACT (Hagan et al., 2015a), and received the same assessments as patients.

The study received favorable ethical opinion by the National Research Ethics Service Committee East of England (reference: 09/H0308/168).

Other components of the MR-IMPACT study, that investigated pre-treatment brain function and the potential longitudinal effects of CBT on brain function are detailed in **Chapter 3** and **Chapter 4** respectively.

Exclusion criteria for MR-IMPACT were the presence of a developmental disorder or generalised learning difficulties, alcohol or drug dependence or abuse, the use of medications that may interact with SSRI medication, the presence of MRI contraindication or brain abnormality, or intolerance to the MRI environment (Hagan et al., 2015a).

Out of the 128 adolescent MDD patients, structural data from 19 patients were excluded: 2 for brain abnormalities, 11 for MRI artefacts, and 6 for having started psychological treatment before the scan. Of the 40 controls, structural data from 4 controls were excluded: 1 for a pre-existing medical condition, 1 for a brain abnormality, and 2 for pre-existing medical conditions.

This left a sample of 109 adolescent MDD patients and 36 healthy adolescent controls with structural data. The patient group had a mean age of 15.6 years and were comprised of 81 females and 28 males, and 36 patients were taking antidepressant medication at the time of scanning. The control group had a mean age of 15.7 years and were comprised of 26 females and 10 males. Further participant demographics are shown in **Table 2.1**.

Statistical testing for demographic measures was done with SPSS (version 25; IBM, 2017) using a statistical threshold of $p = 0.05$. Independent samples *t*-tests were used for comparing differences in age, and symptom measures between patients and controls, and a chi-square test was used to compare differences in gender proportions between patients and controls.

2.3.2. Self-report measures

As previously mentioned, the SMFQ (Angold et al., 1995) and STAI (Spielberger et al., 1970) were administered to assess symptoms of depression and anxiety.

The SMFQ is a freely available self-report questionnaire, containing 13 items that measure symptoms of depression in the past two weeks, and is often used for its ease of use and for its brevity (Thabrew, Stasiak, Bavin, Frampton, & Merry, 2018). The questionnaire is rated on a three-point scale, (0 = *not true*, 1 = *sometimes true*, 2 = *true*) and has a clinical cut-off of ≥ 8. It has been well-validated for use with both children (Angold et al., 1995; Sharp, Goodyer, & Croudace, 2006) and adolescents (Rhew et al., 2010; Thabrew et al., 2018; Turner, Joinson, Peters, Wiles, & Lewis, 2014) and also use in both clinical (Kuo, Stoep, & Stewart, 2005) and non-clinical samples (Sharp et al., 2006).

The STAI is a self-report questionnaire, containing 20 items, aimed at measuring trait anxiety (STAI-T), a stable personality trait of one's general tendency to experience anxiety, and state anxiety (STAI-S), a temporary subjective measure of one's current feelings of anxiety (Balsamo et al., 2013). The questionnaire uses a 4-point scale (1 = *almost never*, 2 = *sometimes*, 3 = *often*, 4 = *almost always*) and has been validated for use in adolescents (Hishinuma et al., 2001).

2.3.3. MRI acquisition

Images were acquired using a 3.0 Tesla Magnetom Trio Tim scanner fitted with a quadrature birdcage head coil, based at the Wolfson Brain Imaging Centre, University of Cambridge, UK. T1-weighted structural images were collected in the sagittal plane, using a three-dimensional magnetically prepared rapid acquisition gradient echo sequence (3D-MPRAGE; Hagan et al., 2015a). 176 slices, 1.00mm in thickness, were acquired, using an echo time of 2.98ms, a repetition time of 2.30s, an inversion time of 900ms, a flip angle of 9^{o}, a field of view of 240 x 256 mm^{2}, a voxel size of 1.00 x 1.00 x 1.00mm^{3}, and an interleaved series (Hagan et al., 2015a).

2.3.4. Cortical thickness image pre-processing

Multiple methods of estimating cortical thickness exist, with the Freesurfer pipeline (Dale, Fischl, & Sereno, 1999; Fischl, 2012; Fischl & Dale, 2000; Fischl et al., 2002; Fischl, Sereno, & Dale, 1999; Fischl et al., 2004) being the most commonly used programme (Tustison et al., 2014). It utilises a surface-based method that estimates cortical thickness by segmenting T1 weighted images of the brain into the different tissue types, being grey matter, white matter, and cerebrospinal fluid, in order to identify the grey/white matter boundary. The grey/white matter boundary is reconstructed into a mesh comprising of minute triangles known as vertices. The mesh is then expanded out towards the grey matter/cerebrospinal fluid boundary, until each point of the mesh reaches an area of high contrast – which is used to form a model of the outer pial surface. Cortical thickness is then estimated at each vertex by measuring the closest point from the inner grey/white matter surface to the outer pial surface.

However, other methods of estimating cortical thickness exist and may provide some advantages over those used by the Freesurfer pipeline. A well-established volume-based pipeline is the Advanced Normalisation Tools (ANTs) Cortical Thickness pipeline (Avants et al., 2009; Tustison et al., 2013). The ANTs pipeline segments the T1 weighted brain images into six different tissue types, being cortical grey matter, subcortical grey matter, white matter, cerebrospinal fluid, the cerebellum, and the brain stem. Based on this segmentation, a one voxel thick sheet is applied along the grey/white matter boundary and another one voxel thick sheet is applied along the outer grey matter/cerebrospinal fluid boundary. The inner boundary is then expanded out and registered to align with the outer boundary. This registration generates a correspondence field that details which regions of the expanded inner boundary relate to the outer boundary, and the distance between these points is then used to estimate cortical thickness.

Although both methods are well-established and have been shown to be reliable, a large-scale direct comparison between Fresurfer and ANTs showed that ANTs' volume-based method performed

better than Freesurfer's surface-based method at predicting both age and gender based on cortical thickness estimations (Tustison et al., 2014), suggesting that its measurements may provide greater biological validity. Moreover, Freesurfer's closest point algorithm, whereby the closest distance from the inner grey/whitematter boundary to the outer pial surface is measured to generate a cortical thickness measurement, is likely to underestimate the thickness of curved regions of the cortex, as in certain circumstances the curved outer surface will not be the closest point to the inner surface and its thickness will therefore not be estimated. The ANTs cortical thickness pipeline is less likely to suffer from this issue due to its use of the correspondence field (Das et al., 2009), and for these reasons, the ANTs cortical thickness pipeline was used for the present study to estimate cortical thickness.

Images were initially reoriented by 90, 180, or 270^{o} of rotation to match the alignment of the Montreal Neurological Institute template (MNI152; Grabner et al., 2006), using FMRIB Software Library (FSL; Smith et al., 2004).

Images were then pre-processed using the ANTs cortical thickness pipeline (Avants et al., 2009). During the pipeline, images were bias field corrected, which corrects for inhomogeneities in the magnetic field that can lead to artefacts within the images. They were then skull stripped, where skull tissue is removed from the image, and segmented into cortical grey matter, subcortical grey matter, white matter, cerebrospinal fluid, the cerebellum, and the brain stem, producing cortical thickness maps. Cortical thickness maps were then linearly registered to the MNI152 template (Grabner et al., 2006) and then non-linearly registered to it. Spatial smoothing was applied with a Gaussian kernel of 3mm. This is where the intensity of each voxel is replaced by the average intensity of the voxels surrounding it, which is done to improve the signal to noise ratio as it reduces the variability between voxels.

2.3.5. White matter volume image pre-processing

Structural images were oriented in the same manner as the cortical thickness images. FSL-VBM (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012) was then used to remove non-cerebral tissues and bias field correct the images.

The images were then segmented into the different tissue types, being grey matter, white matter, and cerebral spinal fluid. For a T1 weighted image, each tissue type will produce different signal intensities. Under perfect conditions, the signal intensities of each voxel can be used to identify which tissue type the voxel represents. However, typical scanning sequences may contain artefacts that can then lead to errors in the voxel intensities, causing misclassifications. Sources of artefacts include inhomogeneities in the scanner's magnetic field, signal noise, head motion, and poor resolution. Therefore, simply using a voxel's signal intensity is likely to lead to misclassifications of tissue types. Instead, FSL's FMRIB's Automated Segmentation Tool (FAST) algorithm uses Hidden Markov Random Field Models, which identify the signal intensity of a voxel and compare it to the signal intensities of neighbouring voxels (Zhang, Brady, & Smith, 2001). It then estimates the percentage of grey matter volume that is contained in each voxel, known as partial volume estimation.

Once the white matter volume maps were generated, a study-specific template was created using 72 participant images (36 patients and 36 controls). The white matter volume maps were then non-linearly registered to the study-specific template. Images were subsequently linearly registered and then non-linearly registered to the MNI152 template (Grabner et al., 2006). The white matter volume estimates of each voxel were then corrected for the warping that occurred from the registration process, using the Jacobian determinants of the warps. Spatial smoothing was then applied with a Gaussian kernel of 5mm.

2.3.6. Case-control analyses

To investigate case-control differences in cortical thickness, a mask covering the subgenual anterior cingulate and medial orbitofrontal cortex was used. These regions were chosen based on previous findings in the literature that had associated them with MDD (Grieve et al., 2013; Peterson et al., 2009; Schmaal et al., 2017). The Automated Anatomical Labelling (AAL) atlas (Tzourio-Mazoyer et al., 2002) was used to identify the medial orbitofrontal cortex. To identify the subgenual anterior cingulate, the anterior cingulate regions of the AAL template were divided into the pregenual anterior cingulate and subgenual anterior cingulate – shown in **Figure *2.1***. An exploratory whole-brain case-control analysis was also conducted to identify other non-hypothesised regions potentially showing case-control differences in cortical thickness.

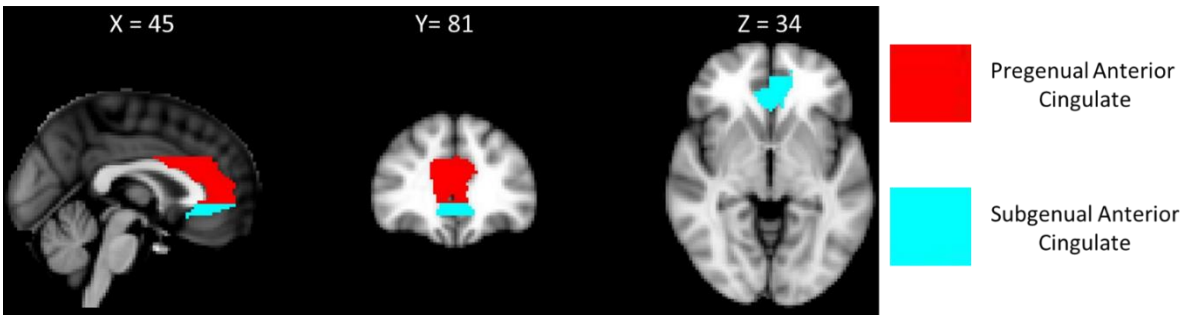


Figure 2.1. Shows the division of the anterior cingulate cortex into the pregenual and subgenual anterior cingulate.

To investigate case-control differences in white matter volume a mask encompassing the medial frontal gyrus, middle frontal gyrus, superior frontal gyrus, and medial orbitofrontal cortex was used, based on previous findings within the literature (Gabbay et al., 2012; MacMaster et al., 2014; Steingard et al., 2002; Zeng et al., 2012), and were identified using the AAL atlas (Tzourio-Mazoyer et al., 2002). An exploratory whole-brain case-control analysis was also conducted to identify other regions potentially showing case-control differences in white matter volume.

For analyses in both cortical thickness and white matter volume, an ANCOVA was used to investigate case-control differences at each voxel within the masks, with group (patient or control) as the independent variable and age (years) and gender values as covariates. For inference of the results of the general linear model, permutation-based methods were used with FSL Randomise (Jenkinson et al., 2012). This procedure conducted 100,000 permutations per statistical test, using threshold-free cluster enhancement, with a Family-wise error (p<0.05) correction to account for multiple comparisons, generating clusters of significant case-control differences. Cohen's *d* (Cohen, 1988) was used as a measure of effect size.

2.3.7. Post-hoc correlations

To investigate the relationship between any deviations in brain structure and symptoms of depression, the mean values from significant clusters showing case-control differences in cortical thickness and white matter volume were correlated with mean SMFQ scores and STAI scores. A Shapiro-Wilk test of normality (Shapiro & Wilk, 1965) identified symptom scores as not being normally distributed, and thus Spearman's Rho (Kendall, 1957; Spearman, 1904) was used for these correlations.

The mean values of significant clusters showing case-control differences in cortical thickness were correlated to the mean values of significant clusters showing case-control differences in white matter volume.

2.3.8. Analyses for structural group-by-age interactions

A mask of the entire anterior cingulate cortex was used to investigate group-by-age interactions in both cortical thickness and white matter volume. The AAL template (Tzourio-Mazoyer et al., 2002) was used to identify the anterior cingulate cortex.

An ANCOVA was applied to investigate group-by-age interactions, with gender as a covariate, using the same permutation-based inference as previously described. Exploratory whole-brain group-by-age interaction analyses, in both cortical thickness and white matter volume, were also conducted.

2.3.9. Analyses of medication use

As it has been claimed that the inclusion of adolescent MDD patients taking antidepressant medication may interfere with analyses of brain structure (Kim et al., 2019), we ran additional case-control analyses in both cortical thickness and white matter volume, excluding the 36 patients taking antidepressant medication, therefore comparing medication-free MDD patients to controls. We also ran analyses comparing medication-free MDD patients to MDD patients taking antidepressant medications.

These analyses were conducted on the same regions of interest as those used for the previously mentioned cortical thickness and white matter volume case-control analyses and were also conducted as whole-brain analyses.

2.4. Results

2.4.1. Demographics

There were no differences between patients and controls in age, $t(143) = 0.361$, $p = 0.718$, or gender, $\chi^2(1) = 0.062$, $p = 0.805$. Participant demographics are shown in **Table 2.1**.

Compared to controls, patients had significantly higher SMFQ scores, $t(143) = 13.758$, $p = 5.79 \times 10^{-28}$, STAI-S scores, $t(143) = 8.356$, $p = 5.15 \times 10^{-14}$, and STAI-T scores, $t(143) = 20.048$, $p = 2.22 \times 10^{-43}$.

Table 2.1. Shows participant characteristics with standard deviations (SD) in parentheses.

	Mean Age (SD), range	**Gender Proportion (% Female)**	**Mean SMFQ Score**	**Mean STAI-T Score**	**Mean STAI-S Score**	**Antidepressant Medication use (% taking)**
Patients, *n* = 109	15.56 (1.27), 11.83-17.96	74.31	19.20 (6.95)	60.12 (7.89)	45.62 (10.91)	31.8
Controls, *n* = 36	15.65 (1.45), 12.14-17.73	72.22	3.03 (1.93)	31.03 (6.39)	29.56 (6.43)	0

2.4.2. Cortical thickness

2.4.2.1. Case-control analyses

Patients showed significantly greater cortical thickness within the bilateral subgenual anterior cingulate and medial orbitofrontal cortex, shown in **Figure *2.2*** and **Table 2.2**.

There were no significant relationships between mean cortical thickness in the significant cluster and mean SMFQ scores, r_S = 0.130, p_{corr} = 0.735, mean STAI-S scores, r_S = -0.007, p_{corr} = 0.944, or mean STAI-T scores, r_S = 0.075, p_{corr} = 0.735.

No significant differences were found in cortical thickness, when conducting a whole-brain analysis.

2.4.2.2. Group-by-age interaction analyses

In agreement with our hypothesis, we found a significant group-by-age interaction in cortical thickness, within the left and right pregenual anterior cingulate cortex, shown in **Figure *2.2*** and **Table 2.2**, where cortical thickness decreased with age in controls but increased with age in MDD patients.

There were no significant correlations, in patients, between the significant cortical thickness cluster showing a group-by-age interaction and mean SMFQ scores, r_S = 0.079, p_{corr} = 0.414, mean STAI-S scores, r_S = 0.145, p_{corr} = 0.399, or mean STAI-T scores, r_S = 0.100, p_{corr} = 0.414.

A whole-brain analysis also found a small but significant cluster within the midcingulate, showing a group-by-age interaction, where cortical thickness decreased with age in controls but showed no relationship with age in MDD patients, shown in **Figure *2.2*** and **Table 2.2**.

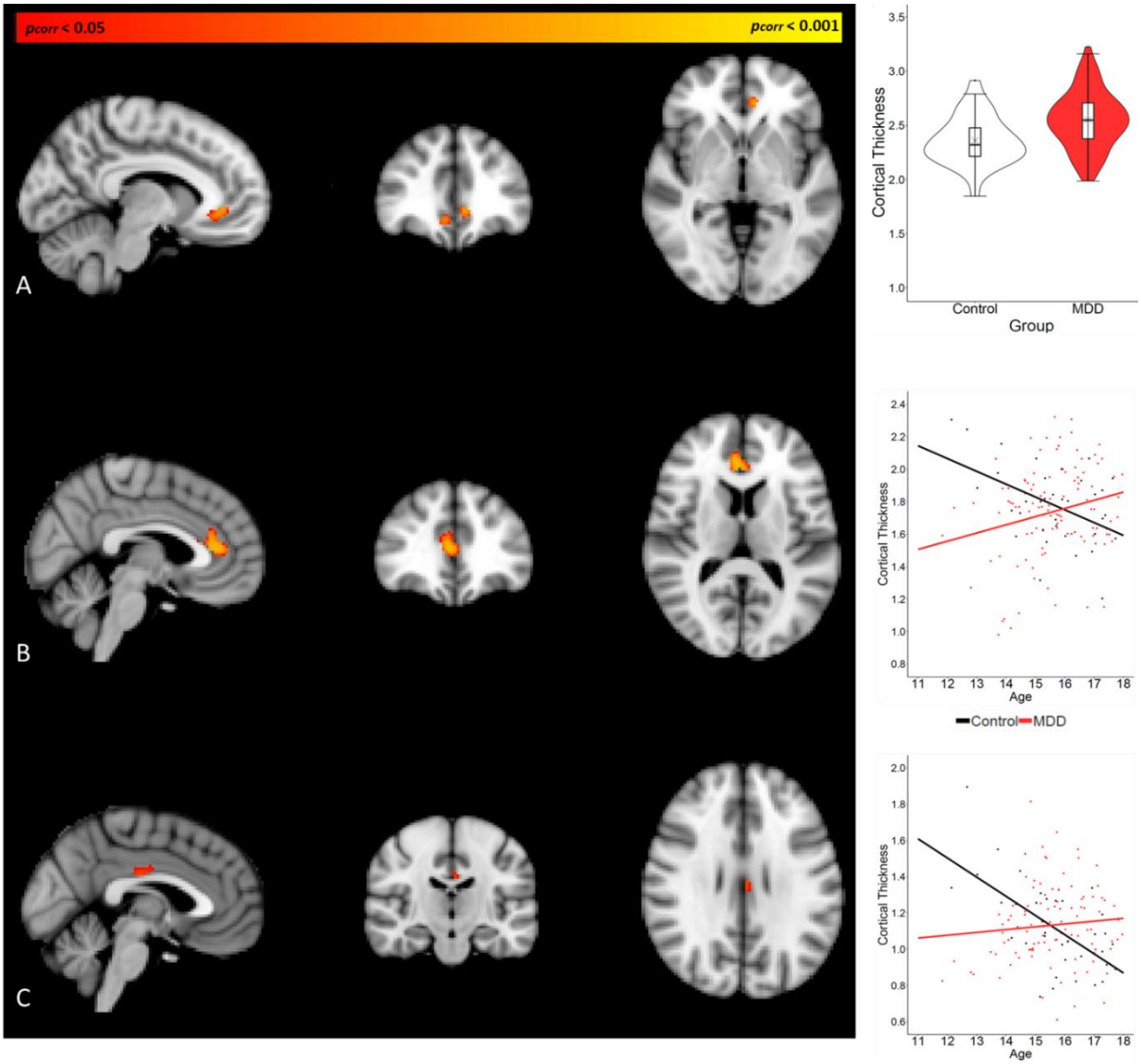


Figure 2.2. Shows the case-control differences and group-by-age interactions in cortical thickness. A) The greater cortical thickness, within the bilateral subgenual anterior cingulate cortex and medial orbitofrontal cortex, found in adolescent MDD patients. B) The significant group-by-age interaction in cortical thickness within the pregenual anterior cingulate cortex. C) The group-by-age interaction within the midcingulate, found by a whole-brain analysis.

Table 2.2. Shows details of significant cortical thickness clusters.

Cortical thickness cluster	Number of voxels	*Peak pcorr*-value	Peak *t*-score	Peak MNI Coordinates	Cohen's *d*	Regions involved (peak region in bold)
A: Case-control	179	0.022	3.58	49, 82, 35	0.771	**Left subgenual anterior cingulate**, left medial orbitofrontal cortex, right anterior cingulate, right medial orbitofrontal cortex
B: Group-by-age interaction	412	0.014	3.56	47, 81, 42		Left pregenual anterior cingulate, **right pregenual anterior cingulate**
C: Group-by-age interaction	29	0.037	4.60	46, 54, 50		**Left midcingulate**

2.4.3. White matter volume

2.4.3.1. Case-control analyses

Patients showed greater white matter volume within the bilateral medial frontal gyri, bilateral superior frontal gyri and right middle frontal gyrus, shown in **Figure *2.3*** and **Table 2.3**.

There were no significant correlations between the mean white matter volume in the significant cluster and mean SMFQ scores, r_s = -0.211, p_{corr} = 0.0767, mean STAI-S scores, r_s = -0.206, p_{corr} = 0.0767, or mean STAI-T scores, r_s = -0.192, p_{corr} = 0.0767.

There was also no significant correlation between the two significant clusters of mean white matter volume and mean cortical thickness, r = -0.017, p_{corr} = 0.861.

When conducting an exploratory whole-brain analysis of white matter volume, patients showed two clusters of greater white matter volume, one near the right hippocampus and the other near the left hippocampus, shown in **Figure *2.3*** and **Table 2.3**.

2.4.3.2. Group-by-age interaction analyses

In agreement with our hypothesis, we found a group-by-age interaction in white matter volume, within the left and right pregenual anterior cingulate cortex and the right subgenual anterior cingulate cortex, shown in **Figure *2.3*** and **Table 2.3**, where white matter volume decreased with age in controls but increased with age in MDD patients.

There were no significant correlations, in patients, between the significant white matter volume cluster showing a group-by-age interaction and mean SMFQ scores, r_s = -0.056, p_{corr} = 0.562, mean STAI-S scores, r_s = -0.099, p_{corr} = 0.457, or mean STAI-T scores, r_s = -0.107, p_{corr} = 0.457.

A whole-brain analysis found no significant group-by-age interactions in white matter volume.

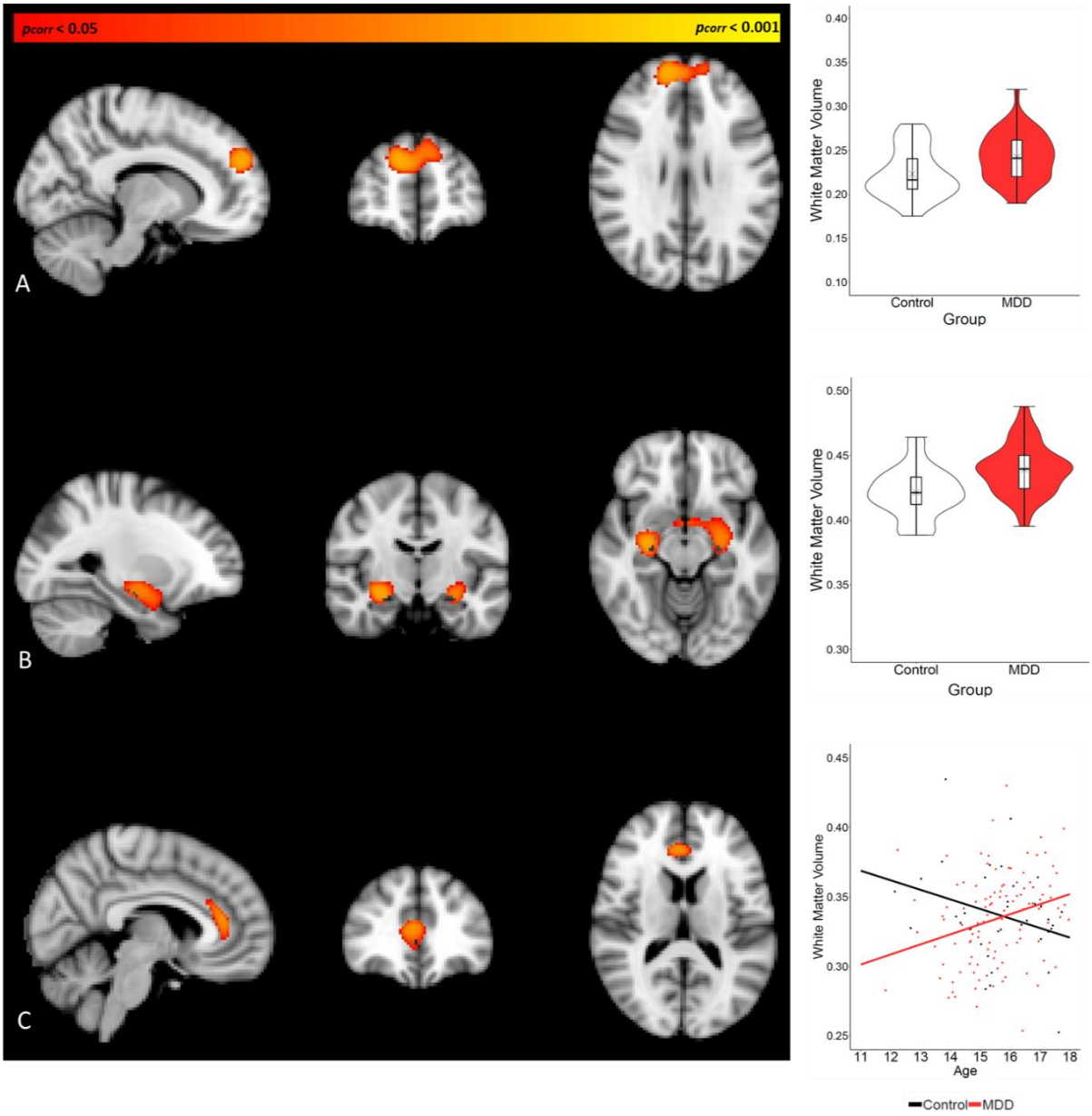


Figure 2.3. Shows the case-control differences and group-by-age interaction in White matter volume. A) Shows the greater white matter volume, within frontal brain regions, found in adolescent MDD patients. B) Shows the greater white matter volume in MDD patients, found by a whole-brain analysis, within limbic and striatal regions. C) Shows the significant group-by-age interaction in white matter volume within the pregenual and subgenual anterior cingulate cortex.

Table 2.3. Shows details of significant white matter volume clusters.

White matter volume cluster	Number of voxels	*Peak pcorr*-value	Peak *t*-score	Peak MNI Coordinates	Cohen's *d*	Regions involved (peak region in bold)
A: Case-control	828	0.016	3.62	51, 89, 50	0.71	Left superior frontal gyrus, right superior frontal gyrus, right middle frontal gyrus, left medial frontal gyrus, **right medial frontal gyrus**
B: Case-control – cluster 1	435	0.025	3.83	58, 57, 30	0.84	Right insula, **right hippocampus**, right amygdala, right putamen, right pallidum
B: Case-control – cluster 2	1004	0.017	4.46	31, 56, 30	0.92	**Left hippocampus, left** parahippocampus, left amygdala, left caudate, right caudate, left putamen, left pallidum, left olfactory cortex, right olfactory cortex
C: Group-by-age interaction	322	0.22	3.17	48, 81, 43		Left pregenual anterior cingulate, **right pregenual anterior cingulate,** right subgenual anterior cingulate

2.4.4. Medication use

2.4.4.1. Demographics

Medication-free patients did not significantly differ to patients taking antidepressant medication in age, $t(112) = 0.015$, $p = 0.988$, or gender, $\chi^2(1) = 0.047$, $p = 0.828$.

Medication-free patients did not differ to patients taking antidepressant medication in SMFQ scores, $t(112) = 1.628$, $p = 0.106$, STAI-S scores, $t(112) = 1.335$, $p = 0.184$, or STAI-T scores, $t(112) = 0.989$, $p = 0.325$.

There were no significant differences between medication-free patients and controls in age, $t(114) = 0.409$, $p = 0.684$, or gender, $\chi^2(1) = 1.090$, $p = 0.296$.

Compared to controls, medication-free patients showed greater SMFQ scores, $t(114) = 13.434$, $p = 3.047 \times 10^{-25}$, STAI-S scores, $t(114) = 8.712$, $p = 2.718 \times 10^{-14}$, and STAI-T scores, $t(114) = 20.006$, $p = 4.2969 \times 10^{-43}$.

2.4.4.2. Cortical thickness

2.4.4.2.1. Medication-free vs controls

When excluding the 36 MDD patients that were taking antidepressant medication, medication-free MDD patients still showed greater cortical thickness within the subgenual anterior cingulate cortex and medial orbitofrontal cortex, though the size of the clusters was reduced, shown in **Figure *2.4*** and **Table 9.1** of the **Appendix**.

When conducting a whole-brain analysis of cortical thickness, comparing medication-free MDD patients to controls, patients showed lower cortical thickness within the precentral gyrus, shown in **Figure *2.4* and Table 9.1** of the **Appendix**.

2.4.4.2.2. Group-by-age interaction analyses

There was still a significant group-by-age interaction within the pregenual anterior cingulate cortex, and a whole-brain analysis still showed a significant group-by-age interaction within the midcingulate, shown in **Figure *2.4*** and **Table 9.1** of the **Appendix**.

2.4.4.3. White matter volume

2.4.4.3.1. Medication-free vs controls

When comparing medication-free MDD patients to controls, MDD patients still showed greater white matter volume within frontal brain regions, though the size of the significant clusters were reduced; shown in **Figure *2.5*** and **Table 9.2** of the **Appendix**.

Using a whole-brain analysis, patients still showed greater white matter volume within the hippocampal and striatal regions, shown in **Figure *2.5*** and **Table 9.2** of the **Appendix**.

2.4.4.3.2. Group-by-age interactions

A significant group-by age interaction was still present within the pregenual anterior cingulate cortex, shown in **Figure *2.5*** and **Table 9.2** of the **Appendix**, but a whole-brain analysis found no significant group-by-age interactions.

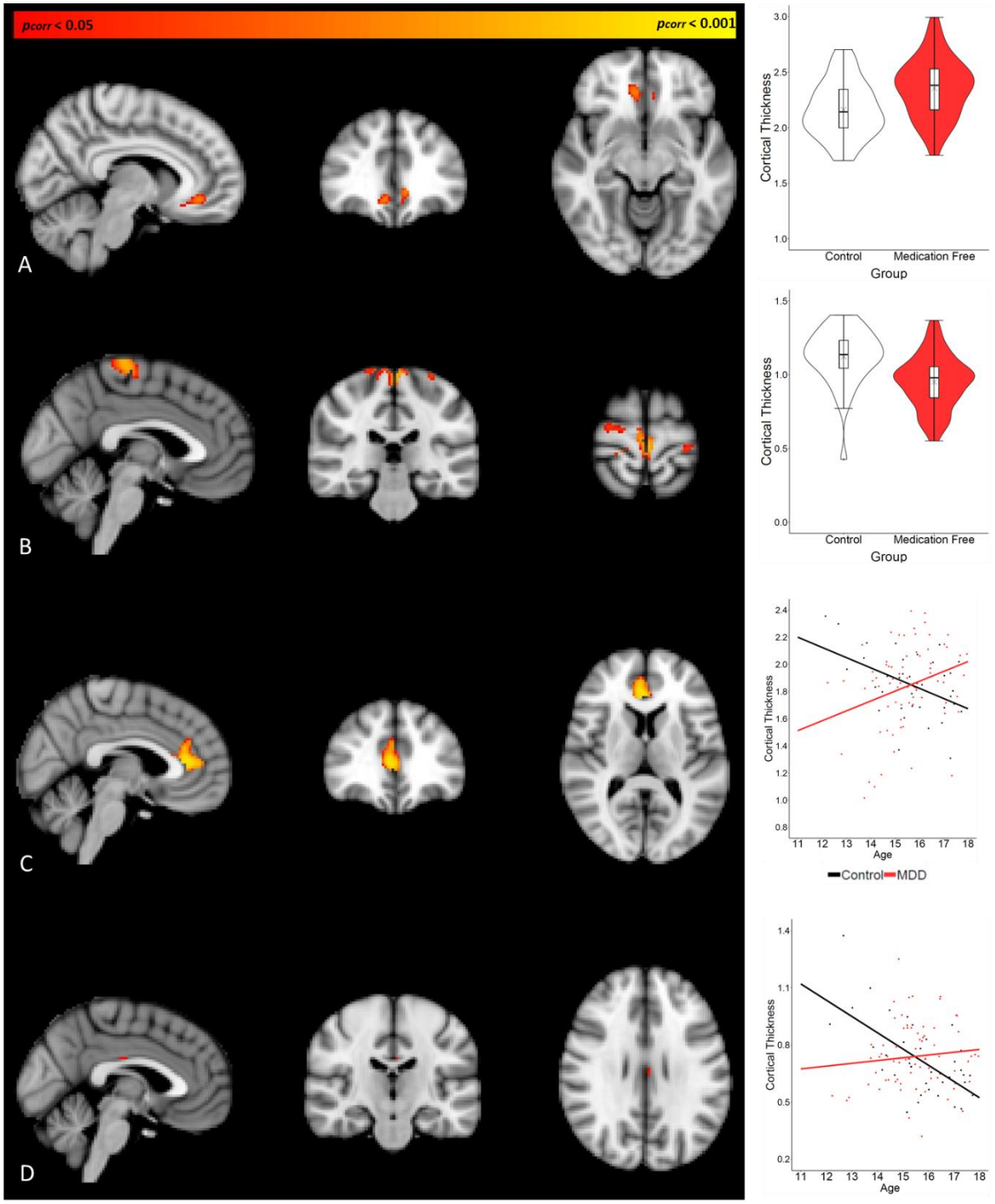


Figure 2.4. Shows the significant group differences and group-by-age interactions in cortical thickness, when excluding MDD patients taking antidepressant medications. A) Shows the greater cortical thickness, in medication-free MDD patients, within the subgenual anterior cingulate cortex and medial orbitofrontal cortex. B) Shows the lower cortical thickness, in medication-free MDD patients, within the precentral gyrus – found using a whole-brain analysis. C) Shows the group-by-age interaction in cortical thickness, within the pregenual anterior cingulate. D) Shows the group-by-age interaction in cortical thickness, within the midcingulate cortex, found using a whole-brain analysis.

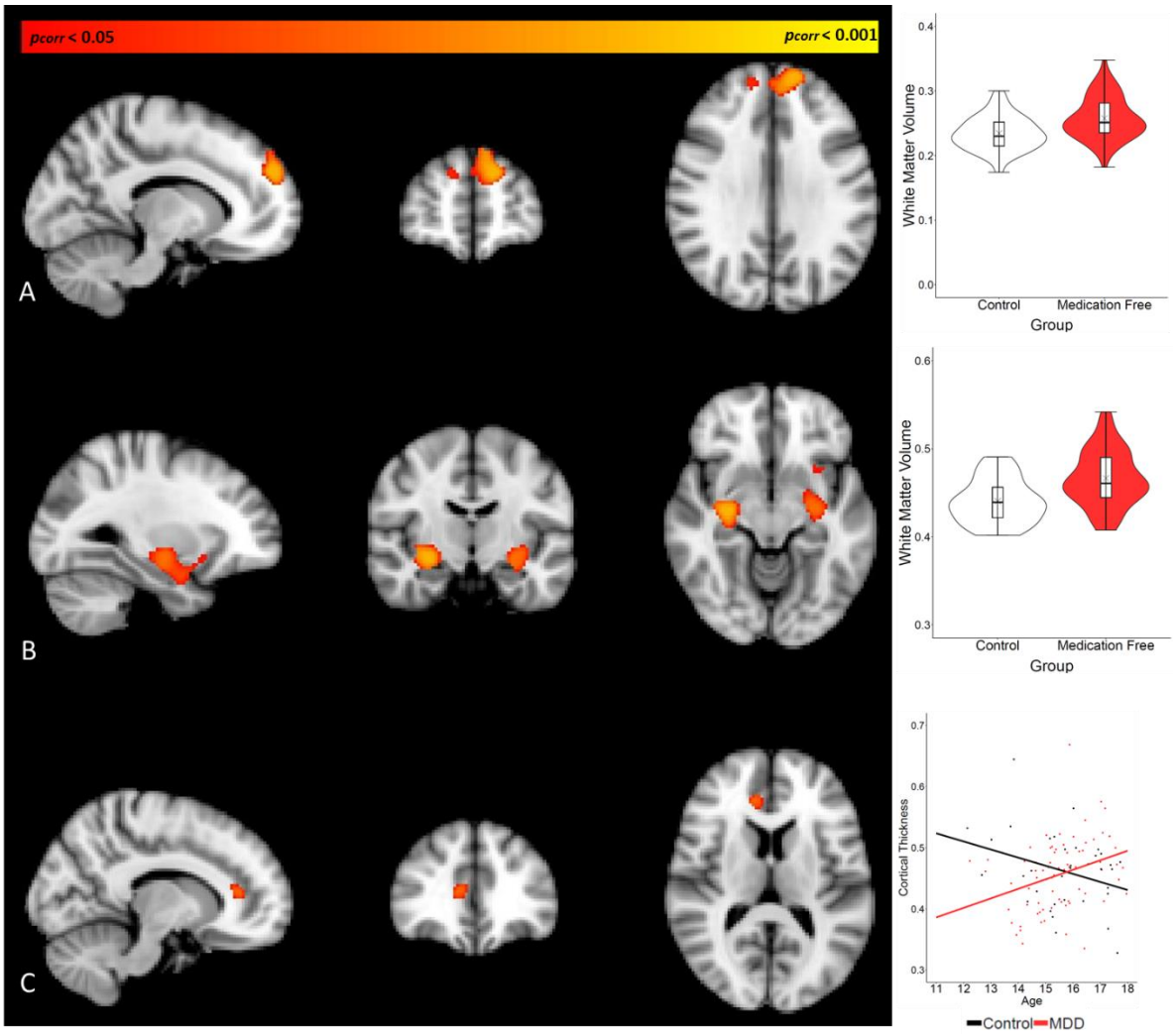


Figure 2.5. Shows the significant group differences and group-by-age interactions in white matter volume, when excluding MDD patients taking antidepressant medications. A) Shows the greater white matter volume within frontal brain regions, found in medication-free MDD patients. B) Shows the greater white matter volume within limbic and striatal regions, found in medication-free patients using a whole-brain analysis. C) Shows the group-by-age interaction in white matter volume, within the pregenual anterior cingulate cortex.

2.4.4.4. Medication-free vs patients taking antidepressant medications

No significant differences in brain structure were found when comparing medication-free MDD patients to those taking antidepressant medications.

2.5. Discussion

Our results show that deviations in brain structure do appear to occur in adolescent MDD, with patients showing both greater cortical thickness within the subgenual anterior cingulate cortex and medial orbitofrontal cortex, and also showing greater white matter volume within the medial frontal gyri, superior frontal gyri, and the middle frontal gyri.

Patients in our sample showed greater cortical thickness within the subgenual anterior cingulate cortex and the medial orbitofrontal cortex bilaterally. Both the medial orbitofrontal cortex and subgenual anterior cingulate cortex have previously been implicated in adolescent MDD (Foland-Ross et al., 2015; Jaworska et al., 2014), and cortical thickness within these regions has even been found to be some of the most useful for predicting the onset of MDD in adolescence (Foland-Ross et al., 2015). Therefore, it appears that these two regions have some role in the neurobiology of adolescent MDD but whether they directly contribute to the development of the illness itself or are instead structurally altered by the illness is unclear. The lack of a correlation between the significant cortical thickness cluster and symptoms of depression, in patients, is not surprising when given that past research has also struggled to find a relationship between MDD-related structural deviations and depressive symptoms (Kim et al., 2019; MacMaster & Kusumakar, 2004; Pannekoek et al., 2014; Reynolds et al., 2014; Rosso et al., 2005; Schmaal et al., 2017; Shad et al., 2012; Whittle et al., 2014), but it does suggest that deviations to cortical thickness within these regions are not directly related to current depressive state. Previous longitudinal investigations into MDD-related structural deviations suggest that deviations within these regions may occur prior to the onset of the illness (Foland-Ross et al., 2015; Vulser et al., 2015; Whittle et al., 2014), so it may be that deviations to cortical thickness and white matter volume, within frontal and limbic regions of the brain, are part of a deviating developmental process of brain structure, which may be a predisposing factor for the illness.

Furthermore, the finding of greater cortical thickness in adolescent MDD patients, within the subgenual anterior cingulate and medial orbitofrontal cortex, is in conflict with the majority of the adult MDD literature, where studies have often found reduced cortical thickness within these regions in patients reported (Grieve et al., 2013; Järnum et al., 2011; Rajkowska et al., 1999; Schmaal et al., 2017; Suh et al., 2019; Won et al., 2016). However, other work investigating cortical thickness in adolescent MDD has indicated that patients can show greater cortical thickness within these regions. Greater cortical thickness within frontal regions has previously been found in MDD patients whose illness onset was before the age of 24 years (Jaworska et al., 2014), as has greater cortical thickness within the anterior cingulate cortex (Reynolds et al., 2014). This discrepancy in cortical thickness between adolescent and adult MDD patients may demonstrate a difference between adult-onset and adolescent-onset MDD. On the other hand, this discrepancy may be the marker of a more dynamic process within the brain, where cortical thickness is initially greater in patients, during the earlier stage of adolescent MDD's progression, which then becomes thinner as the illness progresses (Schmaal et al., 2017). Longitudinal structural studies, examining the development of cortical thickness in MDD patients may address this issue.

Additionally, greater cortical thickness in adolescent MDD has previously been explained as possibly being due to increased neuronal number or neural density (Reynolds et al., 2014). In addition to increased grey matter, several longitudinal studies have linked cortical thickness reductions during brain maturation to myelination across the grey-white matter boundary (Sowell et al., 2003; Whitaker et al., 2016). Therefore, the increased cortical thickness within the subgenual anterior cingulate cortex and medial orbitofrontal cortex, found in our patients, may partly reflect a delay or premature curtailment of this myelination process, as well as an increase of neurons or synaptic connections. Furthermore, the increased white matter volume within the frontal lobes and limbic regions, in patients, does indeed suggest that deviating white matter development is taking place but, as we did not find a correlation between the significant cortical thickness and white matter

volume clusters, it is unclear whether they are directly related and contributing towards one another.

The largest study investigating cortical thickness in adolescent MDD, comparing 213 adolescent MDD patients to 294 controls, found no group differences in cortical thickness, instead finding differences in cortical surface area (Schmaal et al., 2017). Conversely, we found that adolescent MDD patients showed both case-control differences and a group-by-age interaction in cortical thickness. The reason for this discrepancy may be due to the substantial age differences between these two patient samples. In Schmaal et al.'s (2017) study, 70% of their adolescent sample were between the ages of 18-21 years, with only 64 of their adolescent MDD patients below 18 years. Contrastingly, with a mean age of 15.56 years, none of our 109 MDD patients used for the structural analyses were older than 18 years, and thus were substantially younger than those in Schmaal et al. (2017). Furthermore, and as conceded by Schmaal et al. (2017), early adolescence may be a more sensitive period for structural deviations to occur in adolescent MDD, and their use of a substantially older adolescent sample may have hindered their ability to detect deviations in cortical thickness.

Moreover, the greater cortical thickness and white matter volume in patients appear to contradict each other as the increased cortical thickness suggests a lack of myelination, whereas the increased white matter volume suggests heightened white matter development. It may be that white matter development is affected differently within different regions of the fronto-limbic network, where the subgenual anterior cingulate cortex and medial orbitofrontal cortex are under-myelinated, whereas other frontal and limbic regions are over-myelinated. On the other hand, these seemingly different structural deviations may be part of the same process whereby myelination across the grey-white matter boundary may be hindered and instead increases within the white matter tracts, giving the appearance of greater cortical thickness whilst also appearing to show greater white matter volume. Other measures of white matter and myelination, such as magnetisation transfer, may help clarify these results.

We also found that patients showed greater white matter volume within the medial frontal, superior frontal, and the middle frontal gyri, as well as showing greater white matter volume within the hippocampus, amygdala, and thalamus when using an exploratory whole-brain analysis. This finding of greater white matter volume in adolescent MDD patients is surprising when comparing it to past research in adolescent MDD as, with the exception of Shad et al. (2012), the majority of studies investigating white matter volume in adolescent MDD have found that patients show reduced white matter volume compared to healthy controls (Gabbay et al., 2012; MacMaster et al., 2014; Steingard et al., 2002). Instead, our finding of adolescent MDD patients showing greater white matter volume is more similar to the adult literature which has found greater white matter volume in adult MDD patients, compared to controls, particularly within frontal regions of the brain (Zeng et al., 2012). The reason for this divergence with the majority of the adolescent MDD literature is unclear but may be due to the small sample sizes used by past studies. With a sample size of 109 patients, our sample is the largest sample of adolescent MDD patients under the age of 18 years that has been used to study white matter volume – whereas no previous study of white matter volume in adolescent MDD has had a medically diagnosed patient sample size above 32. It may therefore be that when studied in large samples, adolescent MDD patients are similar to adult MDD patients (Zeng et al., 2012), and generally show greater white matter volume compared to healthy controls.

We found group-by-age interactions, within the anterior cingulate cortex, in both cortical thickness and white matter volume. With respect to cortical thickness, controls showed reduced cortical thickness with age, whereas MDD patients showed increased cortical thickness with age. The same pattern was found in white matter volume, with controls showing white matter volume decreases with age but adolescent MDD patients showing increases in white matter volume with age. These results support Hagan et al.'s (2015) previous results, who found similar group-by-age interactions within the anterior cingulate cortex and thalamus, and further support the notion that adolescent MDD is characterised by deviating developmental trajectories in brain structure. They also demonstrate that the anterior cingulate cortex, a region that consistently shows grey matter volume

reductions in adult MDD (Bora et al., 2012; Koolschijn et al., 2009), may be a key region that is affected by the progression of the illness in adolescents.

Although it has been claimed that the inclusion of adolescent MDD patients taking antidepressant medication may confound analyses into brain structure (Kim et al., 2019), we found that the inclusion of patients taking antidepressant medication had a minimal effect on our results. When we excluded the 36 patients taking antidepressant medications, and compared medication-free MDD patients to controls, we found that the patients still showed greater cortical thickness within the subgenual anterior cingulate cortex and medial orbitofrontal cortex, and still showed greater white matter volume within frontal and limbic regions. However, when conducting a whole-brain analysis of cortical thickness, comparing medication-free patients to controls, we did find that the medication-free patients also showed reduced cortical thickness within the precentral gyrus – which was not found in the whole-brain analysis that included the patients taking antidepressant medication. Multiple studies have previously identified the precentral gyrus to be affected in MDD. Schmaal et al. (2017) found that adolescent MDD patients showed reduced surface area of the left precentral gyrus, and in their meta-analysis Bora, Fornito, Pantelis, and Yücel (2012) found that 25-50% of studies on adult MDD find that adult patients show grey matter volume reductions within the right precentral gyrus. Moreover, Foland-Ross et al. (2015) even found that cortical thickness of the precentral gyrus was one of the regions that best predicted the onset of MDD in adolescence. Therefore, it may be that the precentral gyrus is implicated in adolescent MDD, and perhaps the inclusion of patients taking antidepressant medication may mask structural deviations occurring within this area. However, when comparing medication-free MDD patients to those taking antidepressant medications, we found no significant differences between the two patient groups, and it is therefore possible that this finding within the precentral gyrus may be a statistical artifact caused by removing nearly a third of the patient sample.

In summary, when studied using large sample sizes, adolescent MDD patients do appear to show deviations in cortical thickness and white matter volume. These deviations may be caused by deviating developmental trajectories of white matter, in particular disrupted myelination may be contributing towards the greater cortical thickness and greater white matter volume found in patients. The next chapter will further investigate the extent of the neurobiological differences in adolescent MDD, by focusing on whether this sample of adolescent MDD patients also demonstrate disruption to resting-state functional connectivity.

3. Resting-state functional connectivity in adolescent major depressive disorder

3.1. Introduction

Research of resting-state functional connectivity in adolescent MDD has predominantly focused on four key functional networks, the fronto-limbic network, involved in emotional reactivity and regulation (Casey et al., 2010; Mayberg, 1997; Roiser & Sahakian, 2013; Wang et al., 2012), the default mode network, involved in internal thoughts and self-referential processing (Kaiser et al., 2015; Mantini & Vanduffel, 2013; Mulders et al., 2015; Raichle, 2015), the central executive network, involved in executive function, cognitive control, and attention (Goulden et al., 2014; Kaiser et al., 2015; Mulders et al., 2015; Wang et al., 2016), and the salience network, involved in switching between the default mode and central executive networks, and in directing attention towards emotionally salient stimuli (Goulden et al., 2014; Hermans et al., 2014; Manoliu et al., 2014; Mulders et al., 2015; Ramasubbu et al., 2014; Seeley et al., 2007). However, although these networks have been implicated with adolescent MDD, the exact nature in how they may be disrupted is unclear when examining the literature.

Although the fronto-limbic hypothesis of MDD (Mayberg, 1997, 2003) is the most prominent neurobiological explanation for the development of the illness, research investigating the resting-state functional connectivity of the fronto-limbic network in adolescent MDD has been far from definitive. The first study to examine the resting-state functional connectivity of the fronto-limbic network focused on the subgenual anterior cingulate cortex as a seed region (Cullen et al., 2009), using a sample of 14 adolescent MDD patients and 14 healthy adolescent controls. Supporting the fronto-limbic hypothesis, they found that MDD patients showed weaker resting-state functional connectivity between the subgenual anterior cingulate seed and frontal regions of the brain, including the medial frontal gyrus, suggesting that adolescent MDD may be characterised by underconnectivity within the fronto-limbic network, and that this underconnectivity may lead to underregulated emotion and gives rise to the symptoms of major depression.

However, this conclusion was contradicted by two later studies focusing on the fronto-limbic network in adolescent MDD. Davey, Harrison, Yücel, and Allen (2012), who studied 18 adolescent MDD patients, comparing them to 20 healthy controls, found that MDD patients showed greater resting-state functional connectivity between the subgenual anterior cingulate and the dorsomedial frontal cortex, and also showed greater functional connectivity between the pregenual anterior cingulate and the left dorsolateral prefrontal cortex. Whereas Connolly et al. (2013), who used a sample of 23 adolescent MDD patients and 36 healthy adolescent controls, also found greater resting-state functional connectivity between the subgenual anterior cingulate cortex and the inferior frontal gyri, insula, and amygdala, in adolescent MDD patients. These two studies contradicted the notion of adolescent MDD being a result of underconnectivity within the fronto-limbic network, making the exact nature of fronto-limbic disruption in adolescent MDD unclear.

Inconsistencies have not solely affected research focusing on the fronto-limbic network, with the implication of other key functional networks being disputed in the literature. Ho et al. (2015) focused on the functional connectivity of the default mode network, using a sample of 26 adolescent MDD patients and 37 healthy adolescent controls, with the medial prefrontal cortex and posterior cingulate cortex being used as seed regions. They found that adolescent MDD patients showed greater resting-state functional connectivity between the two seeds and other regions of the default mode network, including the precuneus, also finding that the default mode network was less able to deactivate in patients when changing from resting-state to an emotional task. Their findings suggested that the default mode network is overconnected and overactive in adolescent MDD, which may lead to the deficits in emotional processing that are characteristic of the illness. However, Pannekoek et al. (2014), who studied a sample of 26 adolescent MDD patients and 26 healthy adolescent controls, later disputed the claim of an overconnected default mode network in adolescent MDD, as when they used the posterior cingulate cortex as a seed region in their resting-state functional connectivity analyses, they found no differences in default mode network functional connectivity, casting doubt on the default mode network's involvement in adolescent MDD.

The central executive network's implication with adolescent MDD has also suffered from similar inconsistencies. Peters, Burkhouse, Feldhaus, Langenecker, and Jacobs (2016) studied aspects of resting-state functional connectivity that may confer a greater risk for developing a new episode of depression in remitted MDD patients. Using the left dorsolateral prefrontal cortex as a seed region to examine the functional connectivity of the central executive network, they compared 23 adolescent remitted MDD patients to 10 healthy adolescent controls. They found that patients showed greater resting-state functional connectivity between the dorsolateral prefrontal cortex seed and the middle frontal and inferior frontal gyri, and that this overconnectivity was positively correlated to residual symptoms of depression. They argued that this overconnectivity within the central executive network in remitted MDD patients was indicative of leftover functional disruption that may lead to a greater risk for developing future major depressive episodes, through a weakened ability of the network to regulate emotions.

However, other work examining the central executive network in adolescent MDD has conflicted with the notion that the network is overconnected in adolescent patients suffering from the illness. Sacchet et al. (2016) investigated functional disruption to resting-state networks in adolescent MDD, comparing 55 adolescent MDD patients to 56 adolescent healthy controls, and found that the patients showed reduced resting-state functional connectivity within the central executive network. Specifically, they found that MDD patients showed reduced functional connectivity between the medial prefrontal cortex and parietal regions of the brain, as well as showing reduced functional connectivity with other frontal regions such as the middle frontal gyrus. They therefore argued that adolescent MDD may be characterised by underconnectivity within the central executive network, rather than the overconnectivity suggested by Peters et al. (2016), which further obscured the network's role in adolescent MDD.

Further inconsistencies arise when examining functional disruption to the salience network in adolescent MDD. Jacobs et al. (2016) attempted to understand whether disruption to resting-state

functional connectivity in adolescent MDD was a state-marker for the illness, meaning that functional disruption would occur only when a patient were actively depressed, or whether it was more indicative of a trait-marker for the illness, meaning that the functional disruption would be present regardless of a patient's current depressed state. To examine this, they compared the resting-state functional connectivity of 17 actively depressed MDD patients to 34 remitted MDD patients, and to 26 healthy controls. Using the amygdalae as seeds, they found that MDD patients showed greater resting-state functional connectivity between the left amygdala and the right insula, regardless of whether they were actively depressed or remitted, and that this overconnectivity within the salience network was most prominent in patients who had only experienced a single episode of depression. They suggested that overconnectivity within the salience network may be a fixed characteristic of adolescent MDD, being a trait-marker for the illness, which is most prominent during the early stages of the illness' progression.

Yet, other work has contradicted the suggestion that overconnectivity within the salience network is a fixed characteristic of adolescent MDD. Firstly, Davey et al. (2012) used regions of the midcigulate, a key component of the salience network, as seed regions in their resting-state functional connectivity analyses. However, they failed to find any differences in midcingulate functional connectivity between adolescent MDD patients and healthy controls, even though 50% of their patients were experiencing their first episode of MDD, arguing that disruption to the salience network may occur later in the course of the illness and may not necessarily be a primary characteristic of MDD, therefore completely contradicting the claims of Jacobs et al. (2016). Secondly, Connolly et al. (2017) focused on the resting-state functional connectivity of the amygdala, comparing 48 adolescent MDD patients to 53 healthy adolescent controls. They found no overall group differences in the functional connectivity of the salience network and even found that greater connectivity between the right amygdala and bilateral insula predicted symptom improvements in patients three months later – a finding that goes against Jacobs et al.'s (2016) claim that overconnectivity within the network is indicative of aberrant functional disruption in MDD.

Similar to the literature examining brain structure in adolescent MDD (discussed in **Chapter 2**), the literature examining disruption to functional brain networks in adolescent MDD is mixed, with studies failing to replicate and even directly conflicting in their findings and conclusions about the nature of this supposed functional disruption. Though differences in the methods used and sample demographics cannot be discounted as part of the reason for such inconsistencies within the literature, one key reason for the lack of clarity within the literature may be due to the small sample sizes used. We therefore conducted the present study to investigate pre-treatment functional disruption that may be present in adolescent MDD, using a highly powered sample of 82 adolescent MDD patients and 34 healthy controls. We aimed to investigate the resting-state functional connectivity of the fronto-limbic, default mode, central executive and salience networks, using a seed-based approach. We expected to find differences within these networks, between adolescent MDD patients and controls, though due to the lack of consistency within the literature, we did not hypothesise whether these differences would be manifested in the form of overconnectivity or underconnectivity. We also aimed to investigate whether the deviations in cortical thickness and white matter volume, detailed in **Chapter 2**, were related to any disruption to resting-state functional connectivity.

3.2. Methods

3.2.1. Participants

Participants were recruited from the MR-IMPACT study (Hagan et al., 2015a; Hagan et al., 2013), as previously detailed in **Chapter 2**. In brief, 128 adolescent MDD patients, who met the diagnostic requirements for MDD using the DSM-IV (American Psychiatric Association, 1994), were recruited and were assessed at a baseline structural and resting-state functional scan, prior to having received any psychological treatment. On the day of scanning, they were administered the SMFQ (Angold et al., 1995) as a measure of depressive symptoms, and also the STAI (Spielberger et al., 1970) as a measure of anxiety symptoms. Forty healthy adolescent controls, who had no history of MDD and no first-degree relatives with MDD, were also recruited and received the same assessments as the MDD patients.

Patients were then randomised to receive one of three psychological therapies, 20 of whom received CBT. These 20 patients were invited to a follow-up assessment where resting-state functional MRI was assessed, six months later, after patients had received at least six sessions of CBT. Thirty-three of the healthy controls were also assessed using resting-state functional MRI six months after their first MRI assessment.

The 128 patients were therefore separated into two independent samples: patients who only took part in the pre-treatment scan (n = 108) and those that that took part in both the pre-treatment and post-treatment functional scans (n = 20). This separation was done to allow independence of the two patient groups when comparing any pre-treatment functional disruption to any post-treatment CBT-related changes in resting-state functional connectivity. Data from healthy controls were used with both cross-sectional and longitudinal analyses, due to small sample sizes.

The remainder of this chapter will focus on the cross-sectional analyses of patients who only took part in the pre-treatment scan, and investigations of using the longitudinal sample are detailed in **Chapter 4**.

Therefore, for cross-sectional analyses of pre-treatment functional disruption in adolescent MDD, there were 108 adolescent MDD patients and 40 adolescent healthy controls. Of the 108 adolescent MDD patients who took part in the pre-treatment scan, data from 26 were excluded: 13 for excessive head motion, 3 for co-morbid psychosis, 3 for leaving the study, 6 for missing data, and 1 for not meeting the study's inclusion criteria. Of the 40 healthy controls who took part in the initial functional scan, data from 6 were excluded: 2 for leaving the study, and 4 for excessive head motion.

This left a final sample of 82 MDD patients, and 34 healthy controls for the pre-treatment analyses. Of these 82 patients, 75 also had structural data, which was used to investigate the relationship between deviations in brain structure and pre-treatment functional disruption.

As in **Chapter 2**, statistical testing for demographic measures was done with SPSS (version 25; IBM, 2017) using a statistical threshold of $p = 0.05$. Independent samples *t*-tests were used for comparing differences in age, SMFQ, STAI-T, and STAI-S scores between patients and controls, and a chi-square test was used to compare differences in gender proportions between patients and controls.

3.2.2. MRI acquisition

Images were acquired using a 3.0 Tesla Magnetom Trio Tim scanner fitted with a quadrature birdcage head coil, based at the Wolfson Brain Imaging Centre, University of Cambridge, UK. Whole-brain, BOLD-sensitive echo-planar images were collected with participants in resting wakefulness with eyes closed. Scans lasted 8 minutes and 56 seconds, collecting 256 T2* weighted images. Images were acquired using a repetition time of 2s, a flip angle of 78˚, an echo time of 30ms, an echo spacing time of 0.47ms, and an interleaved series. The voxel field of view was 192 × 192 mm^2 and the voxel size was 3.00 x 3.00 x 3.00mm^3.

3.2.3. Functional image pre-processing

BOLD-sensitive images were first reoriented by 90, 180, or 270^{o} to match the alignment of the MNI152 template (Grabner et al., 2006), using FSL (Smith et al., 2004). The images were then corrected for head motion using the speedypp algorithm from the BrainWavelet Toolbox (www.brainwavelet.org), according to the protocol by Patel et al. (2014). During this process, slice-timing correction was applied, as images were acquired using an interleaved series, followed by rigid body alignment to the first frame of data to correct for head motion between slices. Next, BOLD-sensitive images were linearly transformed to their corresponding skull-stripped structural images and were then linearly and non-linearly registered to the MNI152 template (Grabner et al., 2006). Spatial smoothing was then applied, using a Gaussian kernel of 6mm, followed by an intensity normalisation to a whole-brain median of 1000 (Patel et al., 2014). Next, timeseries wavelet despiking was applied, followed by the confound signal regression of six motion parameters, their first-order temporal derivatives, and cerebrospinal fluid signal. A high-pass frequency filter of above 0.01Hz was used, as was prewhitening to correct for temporal autocorrelation.

For every functional scan, the mean time-series of each seed region was extracted. Then, at every intracerebral voxel, a general linear model was regressed using FSL's FMRIB Expert Analysis Tool (FEAT; version 6; Jenkinson et al., 2012; Woolrich, Behrens, Beckmann, Jenkinson, & Smith, 2004; Woolrich, Ripley, Brady, & Smith, 2001) with the voxel time-series as the dependent variable and the seed time-series as the independent variable.

3.2.4. Analyses for case-control differences in seed-based resting-state functional connectivity

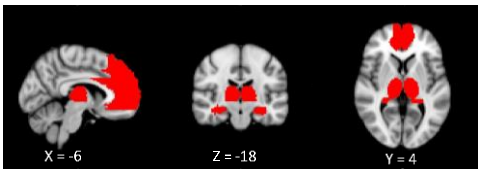
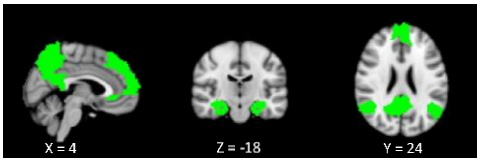
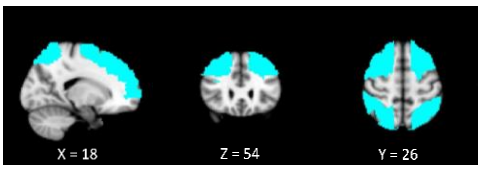
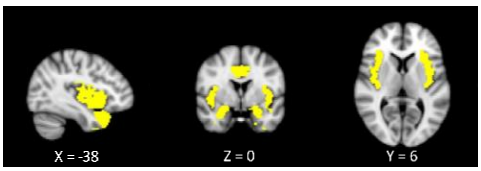
Seed-based methods were used to investigate resting-state functional connectivity of functional brain networks, with seeds located bilaterally. For the fronto-limbic network, the medial orbitofrontal cortex, hippocampi, and medial frontal gyri were used as seed regions. For the default mode network, the precuneus and the subgenual anterior cingulate cortex were used. For the central executive network, the middle frontal gyri and superior frontal gyri were seed regions. For the salience network, the amygdalae were used. The boundaries of the seed regions were defined from the AAL atlas (Tzourio-Mazoyer et al., 2002), except for the subgenual anterior cingulate seeds, which were identified by dividing the anterior cingulate cortex into the pregenual and subgenual regions, detailed in **Chapter 2**.

For each seed, an ANCOVA was used to investigate case-control differences at each voxel across the mask, excluding voxels within the seed region. As multiple seed regions, such as the hippocampus and subgenual anterior cingulate, are involved in multiple brain networks, the mask covered regions of all four networks. Age and gender were included as covariates. Statistical inferences were conducted using permutation-based methods via FSL Randomise (Jenkinson et al., 2012). This procedure conducted 100,000 permutations per statistical test, using threshold-free cluster enhancement, with a Family-wise error ($p < 0.05$) correction to account for multiple comparisons, generating clusters of significant case-control differences. Cohen's *d* (Cohen, 1988) was used as a measure of effect size.

Regions of the fronto-limbic network were defined as the medial frontal gyrus, medial orbitofrontal cortex, hippocampus, thalamus, pregenual anterior cingulate cortex, and subgenual anterior cingulate cortex. Regions of the default mode network were the medial prefrontal gyrus, posterior cingulate cortex, parahippocampus, hippocampus, inferior parietal lobule, precuneus, and subgenual anterior cingulate cortex. Regions of the central executive network were defined as the superior

frontal gyrus, middle frontal gyrus, superior parietal lobule, and the inferior parietal lobule. Regions of the salience network were defined as the insula, midcingulate, amygdala, superior temporal pole, and the middle temporal pole, shown in **Table 3.1**. Exploratory whole-brain analyses were also conducted.

Table 3.1. Shows four key functional networks implicated in adult depression.

Network	Functions	Regions	Network Map
Fronto-Limbic	Emotional reactivity, emotional regulation	Medial frontal gyrus, medial orbitofrontal cortex, hippocampus, thalamus, pregenual anterior cingulate, subgenual anterior cingulate	
Default Mode	Internal thought generation, self-referential processing	Medial frontal gyrus, posterior cingulate, parahippocampus, hippocampus, precuneus, subgenual anterior cingulate cortex, inferior parietal lobule	
Central Executive	Executive function, cognitive control	Superior frontal gyrus, middle frontal gyrus, posterior parietal cortex	
Salience	Switching between the default-mode and central executive networks, directing attention towards emotionally salient stimuli	Insula, midcingulate, amygdala, temporal pole	

3.2.5. Post-hoc correlations

To investigate the relationship between pre-treatment functional disruption in resting-state functional connectivity to symptoms, mean values from significant clusters showing case-control differences in resting-state functional connectivity were correlated with mean SMFQ scores and STAI

scores. A Shapiro-Wilk test of normality (Shapiro & Wilk, 1965) identified symptom scores as not being normally distributed, and thus Spearman's Rho (Kendall, 1957; Spearman, 1904) was used for these correlations. All correlations were corrected using the false discovery rate (Hochberg, 1995).

3.2.6. Use of medication

As antidepressant medications have been shown to affect brain function and resting-state functional connectivity (Brakowski et al., 2017; Chau, Fogelman, Nordanskog, Drevets, & Hamilton, 2017; Chen et al., 2008; Delaveau et al., 2011; Dichter, Gibbs, & Smoski, 2015; Li et al., 2013; McCabe & Mishor, 2011; Wang et al., 2015), we ran additional case-control analyses that excluded the 34 patients taking antidepressant medications, therefore comparing medication-free MDD patients to controls.

We also directly compared the resting-state functional connectivity of MDD patients taking antidepressant medications to medication-free MDD patients.

These analyses were conducted using the same seed-based methods as previously mentioned, focusing on both the four key functional networks and exploratory whole-brain analyses.

3.2.7. Relationship between deviations in brain structure and pre-treatment functional disruption

The mean cortical thickness, white matter volumes, and resting-state functional connectivity values of significant structural and functional clusters were extracted for each of the 75 patients who had both functional and structural data. Structural mean values were then correlated with the functional mean values of only the largest functional clusters, using Pearson's *r* (Lee Rodgers & Alan Nice Wander, 1988; Pearson, 1896). Correlations were corrected using the false discovery rate (Hochberg, 1995).

3.3. Results

3.3.1. Demographics

There were no differences between patients and controls in age, $t(114) = 0.175$, $p = 0.861$; and gender, $\chi^2(1) = 0.026$, $p = 0.871$. Patients had significantly higher SMFQ scores, $t(114) = 13.781$, $p = 5.01 \times 10^{-26}$, STAI-S scores, $t(114) = 9.112$, $p = 3.24 \times 10^{-15}$, and STAI-T scores, $t(114) = 18.760$, $p = 1.204 \times 10^{-36}$. Participant demographics are shown in **Table 3.2**.

Table 3.2. Shows the participant demographics, with standard deviations in parentheses.

	Mean Age (SD), range	Gender Proportion (% Female)	Mean SMFQ Score	Mean STAI-T Score	Mean STAI-S Score	Antidepressant Medication use (% taking)
Patients, *n* = 82	15.69 (1.12), 13.48-17.96	78.05	19.98 (7.18)	59.78 (8.02)	45.93 (10.01)	41.46
Controls, *n* = 34	15.73 (1.44), 12.14-17.73	79.41	2.71 (1.99)	30.59 (6.58)	28.85 (6.75)	0

3.3.2. Analyses across the four key networks

In agreement with our hypothesis that there would be pre-treatment case-control differences, various regions showed greater resting-state functional connectivity with the right superior frontal gyrus, right subgenual anterior cingulate cortex, and right amygdala seeds, in patients; shown in **Figure *3.1*** and **Table 3.3**.

There were no significant correlations between mean clusters of resting-state functional connectivity and mean SMFQ, STAI-S and STAI-T scores, shown in **Table 3.3**.

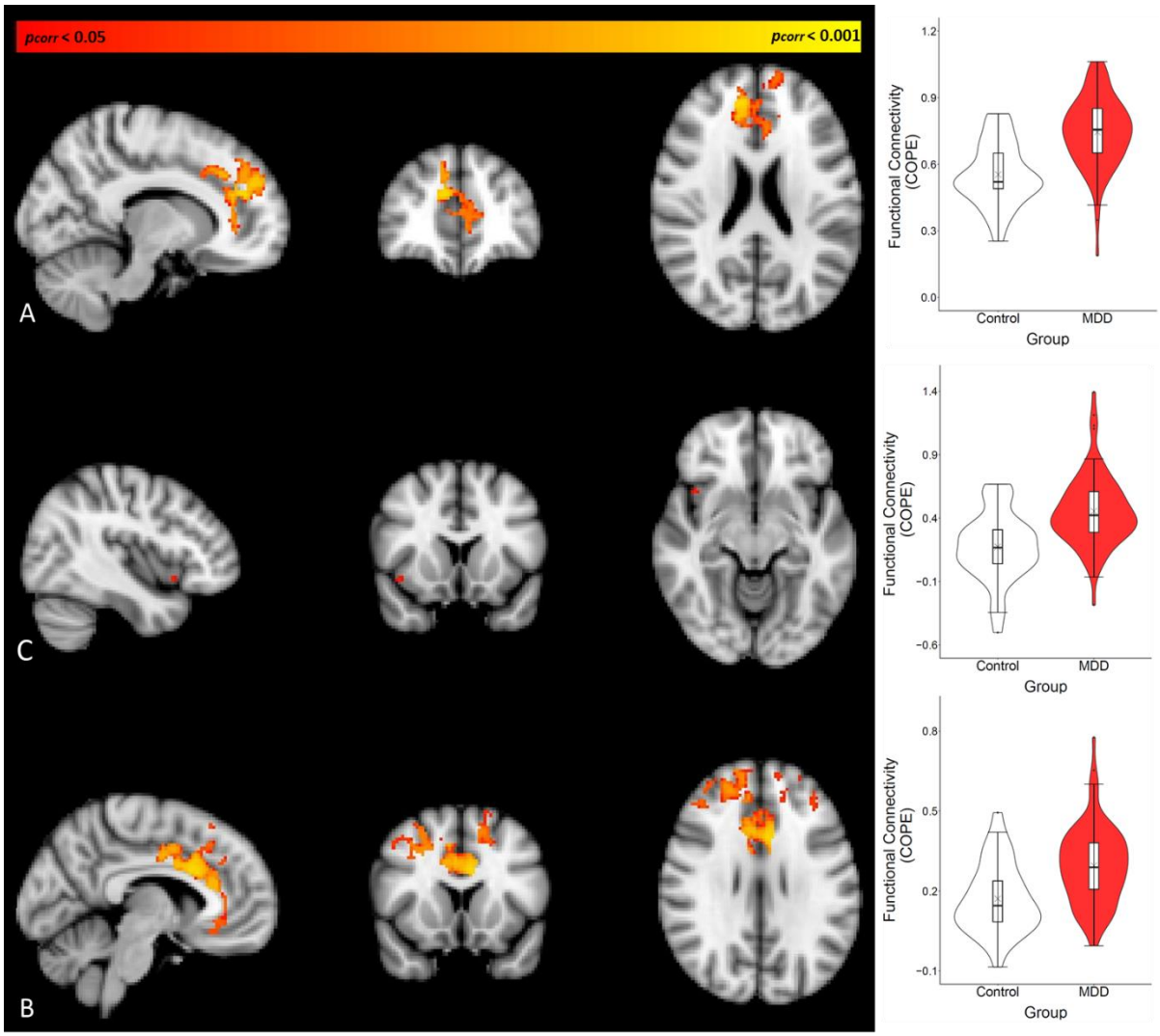


Figure 3.1. Shows the clusters of greater resting-state functional connectivity found in adolescent MDD patients, when focusing on the four key functional networks. A) Right Superior Frontal Gyrus Seed. B) Right Amygdala Seed. C) Right Subgenual Anterior Cingulate Seed.

Table 3.3. Showing details of the significant differences in resting-state functional connectivity between adolescent MDD patients and controls.

Seed Region	Number of voxels	*Peak p*-value	Peak *t*-score	Peak MNI Coordinates	Cohen's *d*	Regions showing greater connectivity (peak region in bold)	Correlation with SMFQ scores	Correlation with STAI-S scores	Correlation with STAI-T scores
Right Superior Frontal Gyrus - Cluster 1	2	0.049	3.33	43, 90, 46	0.55	**Left medial frontal gyrus**	r_s = 0.046, p = 0.68	r_s = -0.028, p = 0.81	r_s = 0.00079, p = 0.99
Right Superior Frontal Gyrus - Cluster 2	93	0.029	3.79	39, 93, 47	0.58	**Left superior frontal gyrus**, left medial frontal gyrus,	r_s = 0.12, p = 0.27	r_s = -0.084, p = 0.45	r_s = 0.088, p = 0.43
Right Superior Frontal Gyrus - Cluster 3	1306	0.0073	4.38	51, 85, 47	0.80	Left superior frontal gyrus, left medial frontal gyrus, right medial frontal gyrus, left midcingulate, right midcingulate, left pregenual anterior cingulate, left subgenual anterior cingulate, **right pregenual anterior cingulate**, right subgenual anterior cingulate	r_s = 0.13, p = 0.25	r_s = 0.089, p = 0.42	r_s = 0.11, p = 0.33
Right Amygdala	7	0.046	4.95	66, 70, 31	0.85	**Right insula**	r_s = 0.047, p = 0.67	r_s = -0.099, p = 0.38	r_s = -0.056, p = 0.61
Right Subgenual Anterior Cingulate - Cluster 1	26	0.039	4.26	55, 38, 44	0.51	**Right precuneus**	r_s = 0.047, p = 0.68	r_s = -0.029, p = 0.79	r_s = -0.014, p = 0.90
Right Subgenual Anterior Cingulate - Cluster 2	83	0.016	5.356	52, 59, 35	0.80	**Right thalamus**	r_s = -0.035, p = 0.76	r_s = -0.0085, p = 0.94	r_s = 0.043, p = 0.70
Right Subgenual Anterior Cingulate - Cluster 3	5589	0.0079	4.53	42, 70, 50	0.79	Left superior frontal gyrus, right superior frontal gyrus, left middle frontal gyrus, right middle frontal gyrus, left medial frontal gyrus, right medial frontal gyrus, left medial orbitofrontal gyrus, left midcingulate, right midcingulate, **left pregenual anterior cingulate**, left subgenual anterior cingulate, right pregenual anterior cingulate	r_s = 0.028, p = 0.80	r_s = 0.068, p = 0.54	r_s = 0.12, p = 0.29

3.3.3. Whole-brain analyses

Whole-brain analyses also showed similar results, with multiple regions of the brain showing greater resting-state functional connectivity, in patients, to the right subgenual anterior cingulate cortex and right amygdala seeds, shown in **Figure *3.2*** and **Table 9.3** of the **Appendix**. The right superior frontal gyrus seed showed no differences in resting-state functional connectivity in the whole-brain analyses.

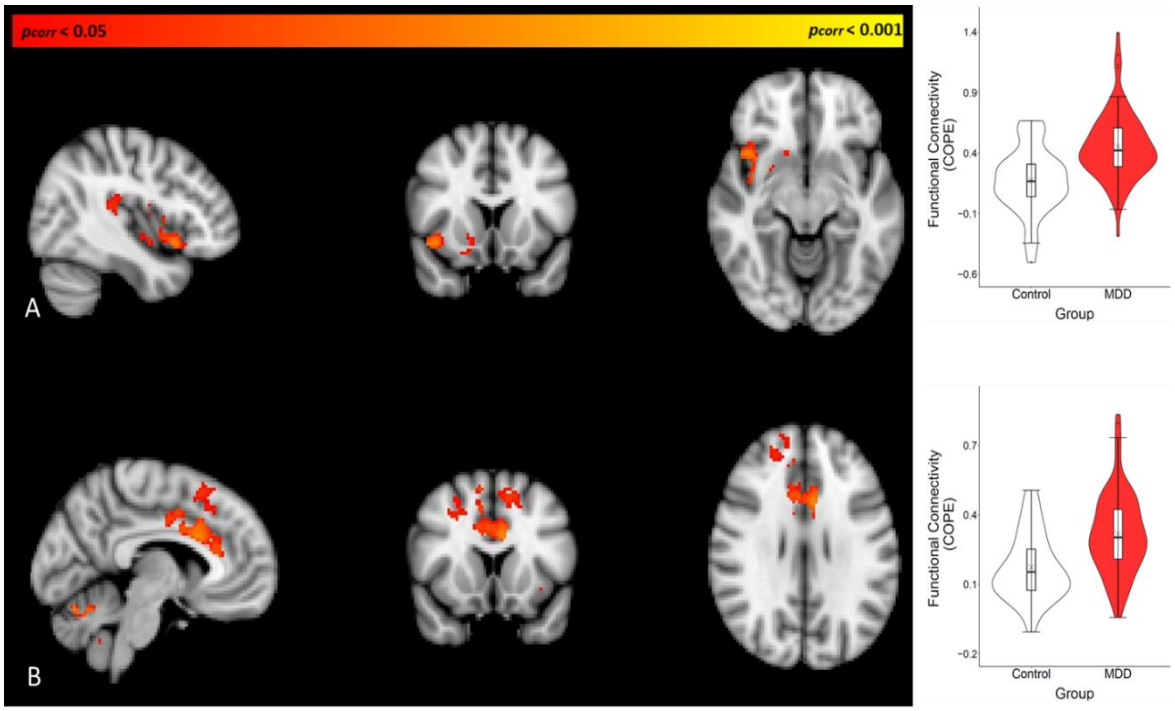


Figure 3.2. Shows clusters where adolescent MDD patients showed greater resting-state functional connectivity compared to controls, when using whole-brain analyses whole-brain level. A) Right Amygdala Seed. B) Right Subgenual Anterior Cingulate Seed.

3.3.4. Relationship between structural and functional differences

There were no significant correlations between any significant functional clusters of the four key networks and structural clusters.

3.3.5. Use of antidepressant medication

3.3.5.1. Demographics

Medication-free MDD patients did not significantly differ to MDD patients taking antidepressant medication in age, $t(80) = 0.359$, $p = 0.721$, or gender, $\chi^2(1) = 2.578$, $p = 0.108$.

Medication-free patients did not differ to patients taking antidepressant medication in SMFQ scores, $t(80) = 0.062$, $p = 0.950$, STAI-S scores, $t(80) = 0.542$, $p = 0.589$, or STAI-T scores, $t(80) = 0.120$, $p = 0.905$.

There were also no significant differences between medication-free MDD patients and healthy controls in age, $t(86) = 0.281$, $p = 0.779$, or gender, $\chi^2(1) = 0.216$, $p = 0.642$.

Compared to controls, medication-free patients showed greater SMFQ scores, $t(86) = 16.849$, $p = 5.567 \times 10^{-29}$, STAI-S scores, $t(86) = 9.014$, $p = 4.600 \times 10^{-14}$, and STAI-T scores, $t(86) = 17.467$, $p = 5.029 \times 10^{-30}$.

3.3.5.2. Medication-free patients vs controls

When comparing medication-free MDD patients to healthy controls, and focusing on the four key functional networks, multiple regions of the brain showed greater resting-state functional connectivity to the right superior frontal gyrus, right amygdala, and right subgenual anterior cingulate cortex seeds, shown in **Figure *3.3***. Exploratory whole-brain analyses also found clusters of greater resting-state functional connectivity between multiple brain regions and the right amygdala and right subgenual anterior cingulate cortex seeds, in medication-free patients, shown in **Figure *3.4***.

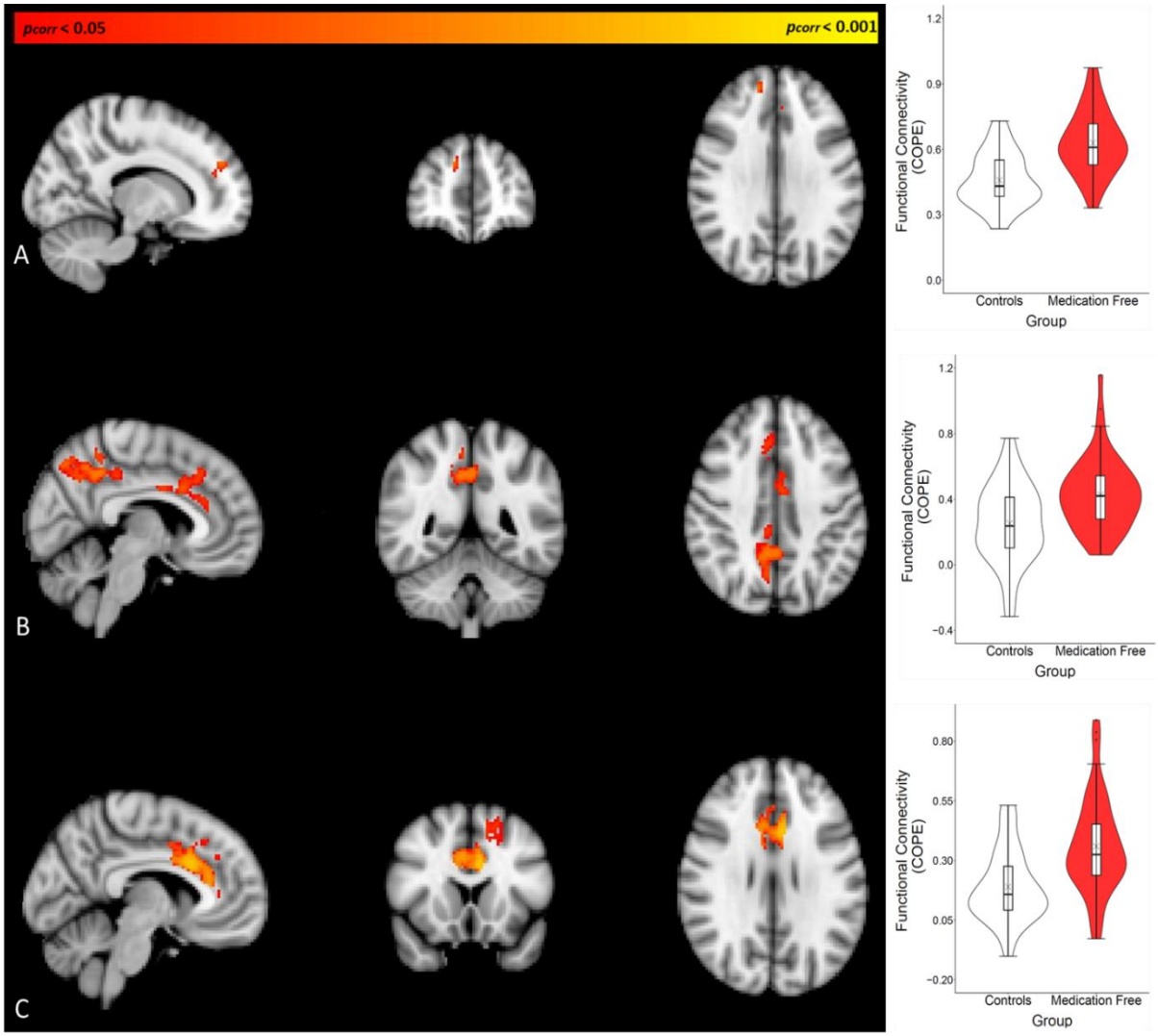


Figure 3.3. Shows clusters where medication-free adolescent MDD patients showed greater resting-state functional connectivity compared to controls, when focusing on the four key networks. A) Right superior frontal gyrus seed. B) Right amygdala seed. C) Right subgenual anterior cingulate cortex seed.

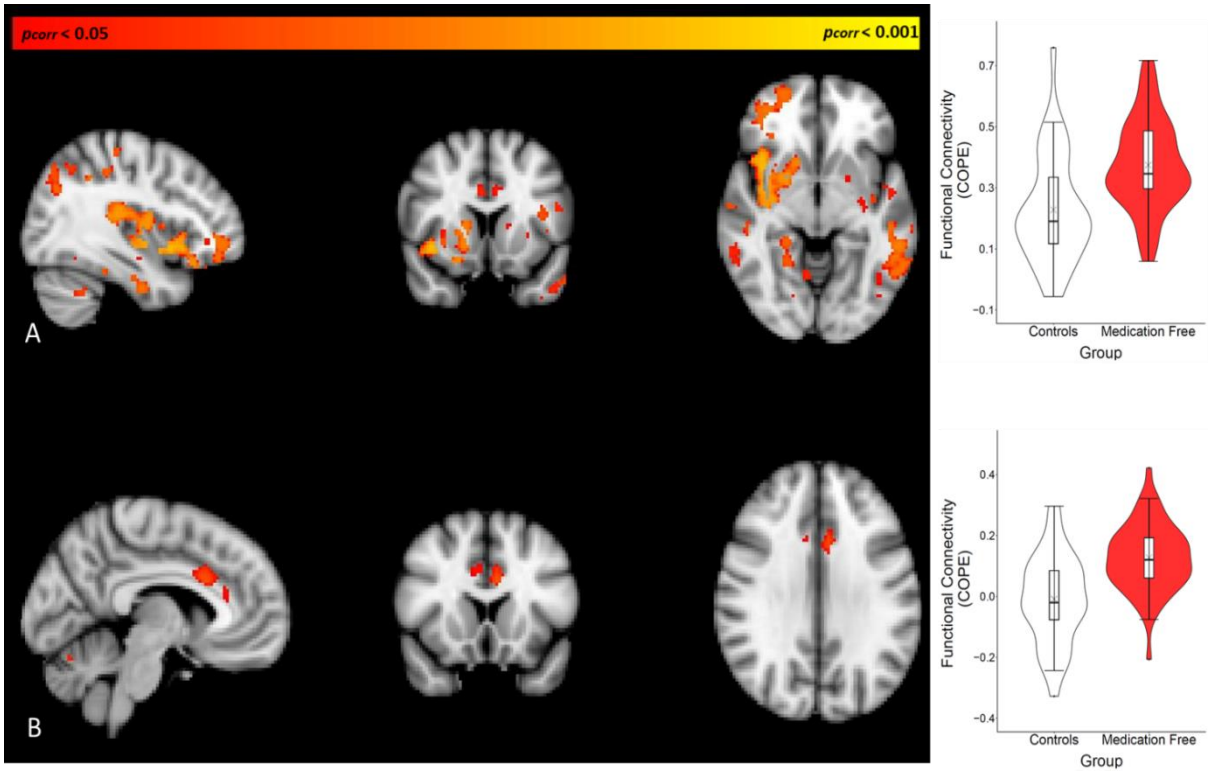


Figure 3.4. Shows clusters where medication-free adolescent MDD patients showed greater resting-state functional connectivity compared to controls, when using whole-brain analyses. A) Right amygdala seed. B) Right subgenual anterior cingulate cortex seed.

3.3.5.3. Medication-free patients vs patients taking antidepressants

No significant differences were found when comparing medication-free MDD patients to MDD patients taking antidepressant medications.

3.4. Discussion

Our results demonstrate that pre-treatment functional disruption does indeed occur within multiple resting-state functional networks in adolescent MDD. In particular, adolescent MDD patients showed greater resting-state functional connectivity, compared to controls, between regions of the fronto-limbic network, default mode network, central executive network, and salience network.

A key finding of this work is that the subgenual anterior cingulate cortex showed extensive overconnectivity with multiple regions that span across all four functional networks, rather than just being confined to the fronto-limbic or default mode networks. In patients, the right subgenual anterior cingulate seed showed greater resting-state functional connectivity with fronto-limbic regions, such as the pregenual anterior cingulate cortex and medial orbitofrontal cortex, and also showed greater functional connectivity with regions of the default mode network, such as the precuenus and medial frontal gyrus. Furthermore, in MDD patients, the right subgenual anterior cingulate seed also showed greater resting-state functional connectivity with regions such as the midcingulate, superior frontal gyrus and middle frontal gyrus, implicating its overconnectivity with the salience and central executive networks. These findings suggest that adolescent MDD is characterised by overconnectivity of the fronto-limbic network rather than underconnectivity, and that it is also characterised by overconnectivity within the default mode network. These results also demonstrate that the subgenual anterior cingulate cortex is involved in the functional disruption of all four key resting-state networks, supporting the claim that the subgenual anterior cingulate cortex plays a pivotal role in the functional disruption that occurs in adolescent MDD (Connolly et al., 2013; Greicius et al., 2007; Wang et al., 2012).

It was also found that, in MDD patients, the right amygdala seed showed greater resting-state functional connectivity with the right insula. The cluster showing this effect was small when the case-control analyses were constrained to defined regions of the four key functional networks, but whole-brain analyses did show that the right amygdala was extensively overconnected to the right

insula, suggesting that overconnectivity within the salience network does indeed occur in adolescent MDD. Although this result is in line with some of the previous adolescent MDD literature, it does oppose research focusing on salience network functional connectivity in adult MDD. In adult MDD, it has predominantly been found that the amygdala shows underconnectivity with the insula (Mulders et al., 2015; Tang et al., 2018) rather than overconnectivity. The reason for this contradiction in direction between our findings and those of the adult MDD literature is unclear and it may be due to differences in methodology or perhaps be a defining difference between adolescent MDD and adult MDD. It may be that salience network connectivity is initially overconnected in adolescent MDD and, as the illness progresses, this functional disruption converts into underconnectivity, as has previously been suggested by Jacobs et al. (2016). Longitudinal tracking of the amygdala's resting-state functional connectivity in adolescent MDD would clarify this issue.

The right superior frontal gyrus showed greater resting-state functional connectivity with predominantly frontal regions of the brain including the left superior frontal gyrus and medial frontal gyrus, in MDD patients. This overconnectivity in MDD patients suggests that adolescent MDD is also characterised by overconnectivity within the central executive network, rather than underconnectivity, which was previously suggested by Sacchet et al. (2016). However, this functional disruption only occurred within the anterior parts of the central executive network, and the adolescent patients did not show any disruption between frontal and parietal regions of the network, which has been reported in adult MDD patients (Mulders et al., 2015). It may therefore be that functional disruption to the posterior central executive network requires more time to develop and our sample may have been too early in that development. Furthermore, in MDD patients, the right superior frontal gyrus seed showed greater resting-state functional connectivity with the pregenual anterior cingulate cortex, subgenual anterior cingulate cortex, and midcingulate, again demonstrating that the functional connectivity between the four key networks themselves is disrupted in adolescent MDD.

Although not hypothesised, using whole-brain analyses, we also found that the right subgenual anterior cingulate cortex showed overconnectivity with regions of the cerebellum in MDD patients. Functional disruption between the cerebellum and regions of the default network has been previously implicated with MDD. Liu et al. (2012) found that the cerebellum showed decreased resting-state functional connectivity to regions within the default mode network in adult MDD patients, and the cerebellum has also shown decreased functional responses in reward processing tasks in adult MDD patients (Zhang, Chang, Guo, Zhang, & Wang, 2013), as well as showing grey matter volume reductions in adult patients (Peng et al., 2011). Together with past research, our findings indicate that the cerebellum may play a role in more complex higher-order processes that are involved with regions of the default mode network, which may be disrupted in adolescent MDD. If this were the case, it would be expected that further analyses using regions of the cerebellum as seed regions would find disrupted resting-state functional connectivity between the cerebellum and multiple regions of the default mode network.

With respect to the right subgenual anterior cingulate seed, the exclusion of adolescent MDD patients taking antidepressant medication did not greatly affect the results of the case-control analyses when comparing medication-free MDD patients to controls. The right subgenual anterior cingulate seed still showed greater resting-state functional connectivity with frontal and limbic regions that were similar to those identified when comparing all MDD patients to controls. With respect to the right superior frontal gyrus, clusters showing greater resting-state functional connectivity in patients were greatly reduced when excluding patients taking antidepressant medications, though the clusters were still significant and still within the anterior cingulate and frontal regions. This effect is likely due to a loss in power, after excluding data from 34 MDD patients rather than demonstrating the effects of antidepressant medication – especially as when we compared the resting-state functional connectivity of medication-free patients to patients taking antidepressant medications there were no significant differences regarding any seed region.

However, when focusing on the right amygdala seed's resting-state functional connectivity, the exclusion of MDD patients taking antidepressant medications did lead to medication-free MDD patients showing large clusters of greater functional connectivity between the right amygdala and the midcingulate and precuneus, compared to controls. It may be that antidepressant medications have a normalising effect on the amygdala's resting-state functional connectivity within the salience network and to regions of the default mode network. However, this again seems unlikely as we failed to find any significant differences between medication-free patients and MDD patients taking antidepressant medications. Instead the exclusion of data from 34 patients in this analysis makes the results of the medication-free vs controls analysis more susceptible to outliers and type I errors, which may explain our finding.

Moreover, the lack of any differences between medication-free MDD patients and MDD patients taking antidepressant medications, in resting-state functional connectivity, may at first seem surprising given the well documented effects that antidepressant medications have on brain function and resting-state functional connectivity (Brakowski et al., 2017; Chau, Fogelman, Nordanskog, Drevets, & Hamilton, 2017; Chen et al., 2008; Delaveau et al., 2011; Dichter, Gibbs, & Smoski, 2015; Li et al., 2013; McCabe & Mishor, 2011; Wang et al., 2015). However, all of our patients were actively depressed, due to the inclusion criteria of the IMPACT and MR-IMPACT studies (Goodyer et al., 2017; Hagan et al., 2015a), meaning that the patients taking antidepressant medications were unlikely to be experiencing their positive effects. Therefore, it may be that the patients taking antidepressant medications were either not responding to their medications or had simply not had enough time for their antidepressant effects to develop, which may explain the lack of differences in resting-state functional connectivity.

Interestingly, there were no significant correlations between any of the significant clusters showing overconnectivity in MDD patients and SMFQ, STAI-S, or STAI-T scores, though this is not uncommon as multiple previous studies have failed to find relationships between functional disruption and

symptoms in MDD (Cullen et al., 2009; Kaiser et al., 2015; Pannekoek et al., 2014; Sacchet et al., 2016). It may be that our measures of general depression and anxiety were not sensitive enough or were too broad to be related to the pre-treatment functional disruption that we found. Moreover, it could also be that the observed pre-treatment functional disruption is instead a trait marker of adolescent MDD rather than being a marker of current depressed state, as has previously been suggested by Jacobs et al. (2016). The use of more precise measures of depressive symptoms may help clarify this issue.

The spatial convergence between seeds that showed pre-treatment functional disruption and regions that showed structural deviations in cortical thickness and white matter volume, being the subgenual anterior cingulate and superior frontal gyrus, hints towards an underlying process that might be causing both structural and functional deviations within the same regions. However, the lack of correlation between the seed-based functional clusters and structural clusters contradicts this possibility and is striking given that the past literature has previously found strong relationships between structural deviations and functional disruption in adult MDD (De Kwaasteniet et al., 2013; Ma et al., 2012; Späti et al., 2015; Van Tol et al., 2014). As the pre-treatment functional clusters were large and spanned across multiple regions and networks of the brain, simply correlating clusters of deviating cortical thickness and white matter volume with such large functional clusters may have been too imprecise to find a significant relationship. Conversely, it may instead be that in adolescent MDD, although some brain regions show both structural and functional deviations, they have independent causes and do not interact with each other. Longitudinal tracking of structural deviations in adolescent MDD and how they relate to longitudinal changes in resting-state functional connectivity may help explain this issue.

In conclusion, adolescent MDD appears to be characterised by overconnectivity within regions of the fronto-limbic, default mode, central executive, and salience networks and also by overconnectivity between the networks themselves. This pre-treatment functional disruption does not appear to be

directly linked to pre-treatment deviations in brain structure or symptoms of depression and anxiety. The question that now remains is whether these regions are amenable to psychological treatment and in what way they may be affected by it, which will be studied in the next chapter.

4. Relationship between pre-treatment functional disruption and cognitive behavioural therapy

4.1. Introduction

We have established that, before receiving psychological treatment, adolescent MDD appears to be characterised by overconnectivity within the fronto-limbic, default mode, central executive and salience networks. However, although the longitudinal effects of antidepressant medications are well documented (Chau et al., 2017; Chen et al., 2008; Delaveau et al., 2011; Li et al., 2013; McCabe & Mishor, 2011; Wang et al., 2015), a question still remains on how CBT acts upon the depressed adolescent brain and, specifically, how it may act upon brain regions and networks showing pre-treatment functional disruption.

Although relatively few studies have examined the effects of CBT on brain function in MDD, and even fewer have focused on adolescent MDD, those that have appear to suggest that it targets regions of the fronto-limbic and default mode networks. The first study to investigate the effects of CBT on brain function in MDD was conducted by Goldapple et al. (2004), who used positron emission tomography (PET) to study the resting-state glucose metabolism of 14 adult MDD patients, who received an average of 17.7 sessions of CBT over a period of around 26 weeks. Comparing whole-brain pre-treatment to post-treatment PET scans, they found that receiving CBT was associated with increased glucose metabolism within limbic regions, such as the hippocampus, and decreased metabolism within frontal and other cortical regions such as the dorsolateral prefrontal cortex, ventromedial frontal cortex, and posterior cingulate cortex – indicating that CBT may act upon the fronto-limbic and default mode networks. They also compared the effects of CBT to those of antidepressant medication and found that the changes in glucose metabolism within the ventromedial frontal cortex and posterior cingulate cortex appeared to be unique to CBT. They suggested that the increases in glucose metabolism within limbic regions may relate to CBT-related increases in attention to emotionally salient stimuli whilst the decreases in metabolism, within

frontal and other cortical regions, may be indicative of CBT-related reductions in rumination and other maladaptive thought processes, though they had no behavioural or psychometric data to support this.

Furthermore, Kennedy et al. (2007) investigated the effects of CBT on brain function in adult MDD, again implicating regions of the fronto-limbic and default mode networks with response to CBT. Using pre- and post-treatment PET scans, they studied changes in glucose metabolism of 12 adult MDD patients, who received an average of 14 sessions of CBT over a 16-week period. They also found that response to CBT was associated with decreases in glucose metabolism within predominantly frontal and other cortical regions such as the orbitofrontal cortex, dorsomedial prefrontal cortex, and posterior cingulate cortex, and also associated with increased metabolism within the subgenual anterior cingulate cortex. They also compared the effects of CBT to those of antidepressant medication and found that increased metabolism within the subgenual anterior cingulate cortex appeared to be unique to CBT, as was decreased metabolism within the posterior cingulate cortex. Their findings further suggested that CBT's effects occur most prominently within regions of the fronto-limbic and default mode network. However, although their work demonstrates which regions may potentially be affected by CBT, both Kennedy et al. (2007) and Goldapple et al. (2004) did not investigate pre-treatment functional disruption in their MDD patients and therefore could not investigate how the therapy may act upon initially disrupted brain regions.

Later studies focusing on the longitudinal effects of CBT on brain function did also include analyses investigating pre-treatment functional disruption in MDD, although almost all of these used task-based paradigms instead of resting-state designs. Ritchey, Dolcos, Eddington, Strauman, and Cabeza, (2011) investigated the potential effects of CBT on brain function, and how they may be related to pre-treatment functional disruption, using task-based fMRI. They collected data from 22 adult MDD patients and 14 healthy adult controls, all of whom took part in a task-based fMRI paradigm where participants viewed images and had to evaluate their emotional valence. All participants were

assessed at a baseline scan and then scanned again after patients had received an average of 21 sessions of CBT, over a 30-week period. Ritchey, Dolcos, Eddington, Strauman, and Cabeza (2011) found that, before receiving treatment, adult MDD patients showed an overall reduced activity within the ventromedial prefrontal cortex when exposed to emotional stimuli in general, but also showed greater activity within the insula, dorsolateral prefrontal cortex, and temporal lobe that was specific to negatively valenced stimuli. After receiving CBT, these pre-treatment functional deviations appeared to be reversed in patients, as activity within these regions matched that of controls in the post treatment scan. Ritchey, Dolcos, Eddington, Strauman, and Cabeza (2011) argued that their results demonstrated that regions showing pre-treatment functional disruption are sensitive to treatment, suggesting that CBT has a normalising effect on these predominantly fronto-limbic regions.

Furthermore, this potential normalising effect of CBT was also noted by Yoshimura et al. (2014), who longitudinally compared the functional activity of 23 adult MDD patients and 15 healthy controls, on an fMRI task that involved self-referential processing. Before receiving CBT, MDD patients showed reduced activity within the midcingulate and medial prefrontal cortex during positive self-referential processing, and also showed greater activation within these regions during negative self-referential processing. After MDD patients had received 12 weekly sessions of CBT, the functional deviations during self-referential processing were reduced compared to their pre-treatment scans. In line with Ritchey, Dolcos, Eddington, Strauman, and Cabeza (2011), they argued that CBT may attenuate the over- and underactivity of functionally disrupted regions in MDD, producing a normalising effect on brain function.

These supposed normalising effects of CBT have also been observed when examining resting-state functional connectivity in MDD. Shou et al. (2017) investigated CBT-related changes in resting-state functional connectivity in both MDD patients and also patients suffering from post-traumatic stress disorder (PTSD). Using a sample of 17 adult MDD patients, 18 adult PTSD patients, and 18 healthy

adult controls, they compared pre-treatment functional connectivity between the three groups and also investigated the longitudinal changes that occurred in patients' resting-state functional connectivity after they had received 12 sessions of CBT over a 12-week period. They found that before treatment, both adult MDD and PTSD patients showed underconnectivity between the amygdala and the inferior frontal gyrus, compared to controls, and that once patients had received CBT, the functional connectivity between these two regions increased in patients to match the levels of healthy controls. This effect occurred in both MDD and PTSD patients, and again, in line with the previous literature, suggested that CBT does indeed have a normalising effect on pre-treatment functional disruption, and that this normalising effect may occur across multiple psychiatric illnesses.

Moreover, although almost the entirety of the literature examining the neural effects of CBT in MDD have focused on adult samples, some have claimed that the normalising effects of CBT also occur in adolescent MDD patients, though these studies have been limited by their designs. One of the few studies to focus on adolescent MDD was conducted by Jacobs et al. (2016), who investigated the effects of rumination-focused CBT (Watkins et al., 2011) on resting-state functional connectivity. They studied a group of 17 adolescent MDD patients who received 8 sessions of the therapy over an 8-week period, whilst also including a separate group of 16 adolescent MDD patients who did not receive treatment for the study's duration. In patients that received CBT, they found that the resting-state functional connectivity between the left posterior cingulate cortex, and the orbitofrontal cortex, right posterior cingulate cortex, inferior frontal gyrus, inferior temporal gyrus, and midcingulate, decreased after the 8-week period. They argued that these results demonstrated the restorative effects of rumination-focused CBT on the functional connectivity of the default mode and central executive networks, helping normalise initially disrupted brain function. However, the validity of their conclusions is questionable, as although they included a separate group of adolescent MDD patients who did not receive treatment, they never ran any direct statistical comparisons between the two patient groups. This flaw makes it impossible to discern whether the

changes in functional connectivity observed in the patients who received treatment were actually related to receiving rumination-focused CBT or simply due to aging effects, or even scan variability.

Straub et al. (2017) also investigated CBT-related changes in resting-state functional connectivity in adolescent MDD, using a sample of 19 adolescent MDD patients and 19 healthy adolescent controls. Adolescent MDD patients and controls were compared at a pre-treatment scan, and then patients were later scanned again after receiving 5 sessions of group CBT over a 5-week period. Before receiving treatment, MDD patients showed overconnectivity within the fronto-limbic and default mode networks, compared to controls, whereas after receiving CBT, this connectivity within the fronto-limbic and default mode networks appeared to decrease in the adolescent MDD patients. Straub et al. (2017) therefore argued that these CBT-related changes in resting-state functional connectivity, in MDD patients, showed that CBT was ameliorating disrupted functional connectivity and was normalising the functional connectivity of the fronto-limbic and default mode networks back to similar levels of the healthy controls. However, their conclusions were speculative and potentially flawed as although they did run statistical pre-treatment comparisons between their patient and control groups, they did not longitudinally follow-up their control group and therefore could not establish whether the longitudinal changes in resting-state functional connectivity, observed in their patient group, were actually related to receiving CBT.

Overall, when examining the literature, studies investigating the effects of CBT on brain function in MDD appear to mostly argue that CBT has a normalising effect on pre-treatment functional disruption – which is a finding that spans across PET and fMRI studies, as well as task-based and resting-state paradigms. Additionally, although the literature examining CBT in adolescent MDD has argued that this supposed normalising effect also occurs in adolescent MDD, this claim is unclear due to the design limitations of previous research (Jacobs et al., 2016; Straub et al., 2017). However, the precise relationship between pre-treatment functional disruption and later CBT-related changes in adolescent MDD has not been explored, as although the majority of the literature claims CBT has

a normalising effect on disrupted brain function in MDD, this has not been directly tested by investigating whether regions showing the greatest pre-treatment functional disruption actually show the greatest CBT-related changes, highlighting a fundamental gap in our understanding of how the treatment may act upon the brain.

We therefore investigated the longitudinal effects of CBT on resting-state functional connectivity, in adolescent MDD patients, and investigated the relationship between pre-treatment functional disruption and later CBT-related changes in resting-state functional connectivity. We hypothesised that the regions showing pre-treatment functional disruption in MDD patients, identified by our pre-treatment functional analyses in **Chapter 3**, would also show CBT-related changes in resting-state functional connectivity. We also hypothesised that if CBT does have a normalizing effect on disrupted brain function, as has previously been suggested (Franklin, Carson, & Welch, 2016; Jacobs et al., 2016; Ritchey et al., 2011; Shou et al., 2017; Straub et al., 2017; Yoshimura et al., 2017), then regions of the brain showing the greatest pre-treatment functional disruption would also receive the greatest impact from CBT and therefore show the greatest CBT-related changes in resting-state functional connectivity.

4.2. Methods

4.2.1. Participants

The participants used to investigate potential CBT-related changes in resting-state functional connectivity were from the same MR-IMPACT sample (Hagan et al., 2015a; Hagan et al., 2013) as mentioned in **Chapter 2** and **Chapter 3**. In this sample, 128 adolescent MDD patients took part in a pre-treatment resting-state functional MRI scan, as did all 40 adolescent healthy controls. Symptoms of depression and anxiety were assessed on the day of scanning, using the SMFQ (Angold et al., 1995) and STAI (Spielberger et al., 1970) respectively. The 40 healthy controls had no personal history of MDD nor a first-degree relative with MDD.

After the pre-treatment scan, MDD patients were then randomised to receive one of three therapies, one of which being CBT. To be eligible to take part in the follow-up MRI assessment, MDD patients must have attended at least 6 out of 20 scheduled CBT appointments. Twenty of these MDD patients were allocated to receive CBT, where they received a mean of 12.85 sessions of therapy over a period of six months, with a median of 8 sessions and a mean interval of 16 days between sessions, and were subsequently invited for a follow-up resting-state functional MRI scan. Thirty-three of the healthy controls were also invited to a follow-up functional MRI scan, 6 months after their initial baseline scan. At this follow-up assessment, the SMFQ and STAI were again administered.

Data from the 128 MDD patients were therefore separated into two independent samples: a *cross-sectional* sample of 108 MDD patients who were only assessed before receiving treatment, and a *longitudinal* sample of 20 MDD patients who took part in both pre-treatment and post-treatment scans. This separation was done to allow independence of the two patient groups when comparing any pre-treatment functional disruption to any post-treatment CBT-related changes in resting-state functional connectivity. Data from healthy controls were used with both cross-sectional and longitudinal analyses, due to small sample sizes.

As has been detailed in **Chapter 3**, data from 26 MDD patients and 6 controls were excluded from the *cross-sectional* sample, leaving a final *cross-sectional* sample of 82 adolescent MDD patients and 34 healthy adolescent controls.

Data from 3 MDD patients were excluded from the *longitudinal* sample: 2 for excessive head motion, and 1 for missing functional data. Data from 3 healthy controls were also excluded from the *longitudinal* sample, due to excessive head motion. This left a final *longitudinal* sample of 17 adolescent MDD patients and 30 healthy adolescent controls, which was used to investigate CBT-related changes in resting-state functional connectivity.

Similar to **Chapter 2** and **Chapter 3**, statistical testing for demographic measures was done with SPSS (version 25; IBM, 2017) using a statistical threshold of $p = 0.05$. Independent samples *t*-tests were used for comparing differences in age, SMFQ, STAI-T, and STAI-S scores between patients and controls, and a chi-square test was used to compare differences in gender proportions between patients and controls. A repeated measures ANOVA was used to test for longitudinal changes in symptoms, between MDD patients and controls.

4.2.2. Functional MRI acquisition and pre-processing

Image acquisition parameters for the baseline and follow-up functional MRI scans were identical to those mentioned in **Chapter 3**. Images were acquired using a 3.0 Tesla Magnetom Trio Tim scanner fitted with a quadrature birdcage head coil, based at the Wolfson Brain Imaging Centre, University of Cambridge, UK. Whole-brain, BOLD-sensitive echo-planar images were collected with participants in resting wakefulness with eyes closed. Scans lasted 8 minutes and 56 seconds, collecting 256 T2* weighted images. Images were acquired using a repetition time of 2s, a flip angle of 78˚, an echo time of 30ms, an echo spacing time of 0.47ms, and an interleaved series. The voxel field of view was 192 × 192 mm^2 and the voxel size was 3.00 x 3.00 x 3.00mm^3.

4.2.3. Functional image pre-processing

Functional image preprocessing methods were identical to those detailed in **Chapter 2**. In brief, functional images were reoriented by 90, 180, or 270°, using FSL (Smith et al., 2004), in order to match the alignment of the MNI152 template (Grabner et al., 2006). Head motion correction of functional images was conducted using the speedypp algorithm from the BrainWavelet Toolbox (www.brainwavelet.org; Patel et al., 2014), and functional images were then non-linearly transformed to the MNI152 template (Grabner et al., 2006) using ANTs (Avants et al., 2009).

The mean time-series of each seed region was extracted from every functional scan, and at every intracerebral voxel, a general linear model was regressed using FSL FEAT (Jenkinson et al., 2012; Woolrich, Behrens, Beckmann, Jenkinson, & Smith, 2004; Woolrich, Ripley, Brady, & Smith, 2001).

4.2.4. Analyses for CBT-related changes in resting-state functional connectivity

Seed-based methods were used to investigate CBT-related changes in resting-state functional connectivity, using the *longitudinal* sample, with seeds located bilaterally. The chosen seed regions were those that had shown pre-treatment functional disruption within the *cross-sectional* sample, being the subgenual anterior cingulate cortex, the amygdala, and the superior frontal gyrus.

For each seed, an ANCOVA was used to investigate case-control differences at each voxel across a mask that covered regions of the fronto-limbic, default mode, central executive, and salience networks, excluding voxels within the seed region. Regions that were included within this mask are detailed in **Chapter 3.**

Seed-based resting-state functional connectivity maps from each participant's post-treatment functional scan were subtracted from their respective pre-treatment resting-state functional connectivity maps and then between-group comparisons were made, controlling for age changes and gender, using the same permutation-based methods as previously mentioned in **Chapter 2** and **Chapter 3,** via FSL Randomise (Jenkinson et al., 2012). This is equivalent to testing for a group-by-time interaction in a 2x2 design.

Although there was no placebo group in this study, due to the ethical issues of restricting treatment to some patients, we refer to group-by-time interactions as CBT-related changes.

4.2.5. Post-hoc correlations

Significant functional clusters showing CBT-related changes in patients were correlated with changes in symptoms. The change in resting-state functional connectivity, relative to baseline resting-state functional connectivity, was correlated with the slope of symptom changes in SMFQ and STAI scores. All correlations were corrected using the false discovery rate (Hochberg, 1995).

4.2.6. Analyses investigating the relationship between pre-treatment functional disruption and later CBT-related changes

Each case-control and group-by-time interaction seed analysis produced a *z*-score map for their respective effects. In other words, the case-control *z*-score maps show regions of MDD-related pre-treatment functional disruption within the *cross-sectional* sample. Similarly, group-by-time interaction *z*-score maps show regions exhibiting CBT-related changes within the *longitudinal* sample. We aimed to investigate whether regions showing the greatest pre-treatment functional disruption would also show the greatest CBT-related changes by investigating the relationship between case-control *z*-scores and group-by-time interaction *z*-scores.

For this approach, we parcellated the brain into 118 regions; each region was a parcel of the AAL template (Tzourio-Mazoyer et al., 2002), except for the anterior cingulate cortex which was sub-divided into the pregenual anterior cingulate and subgenual anterior cingulate using the regions previously described in **Chapter 2**. We conducted two sets of 118 seed-based resting-state functional connectivity analyses, one set of the case-control analyses – using the *cross-sectional* sample - and another set of the group-by-time analyses – using the *longitudinal* sample.

Each seed analysis produced a voxel-wise *z*-score map across the brain for case-control and group-by-time effects from which 117 mean *z*-scores were obtained (i.e. number of parcels minus the seed region). For each seed and each corresponding parcel ("target region"), correlations were calculated between the case-control mean *z*-scores with the corresponding mean group-by-time *z*-scores across the voxels of the region. The procedure is illustrated in **Figure *4.1***.

As almost all of these correlations involved thousands of individual voxels, we did not conduct significance testing as very small effects are significant even at small *p*-value thresholds. Instead, correlation coefficients were converted into r^2 as a measure of effect size.

To investigate the relationship between pre-treatment functional disruption and CBT-related changes at the network level, seed regions were grouped into the default mode, fronto-limbic, central executive, and salience networks, and the effect sizes of their target regions were averaged across their network. Effect sizes of positive and negative correlations were treated separately when averaging. We also averaged the target region effect sizes across all 118 seed regions to identify the relationship between pre-treatment functional disruption and CBT-related changes across the whole brain.

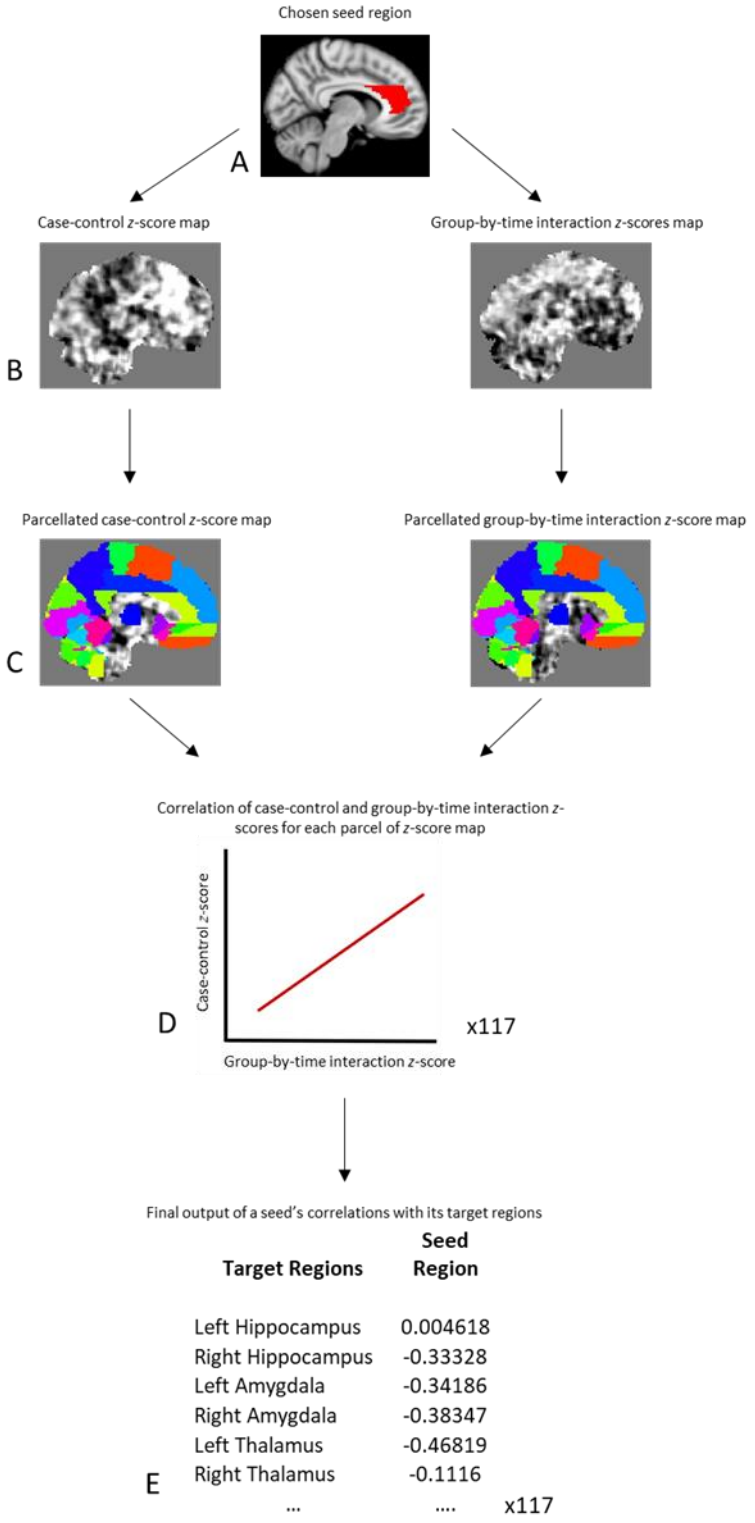


Figure 4.1. Demonstrates the method used to investigate the relationship between pre-treatment functional disruption and cognitive behavioural therapy-related changes in resting-state functional connectivity. A) Shows an example of a seed region used. B) Shows the case-control and group-by-time interaction z-score maps generated by the seed analysis. C) Shows both of the z-score maps being parcellated into the 117 target regions. D) Demonstrates a correlation between the case-control and group-by-time z-scores of a certain target region. E) Shows the final results of a seed regions correlations corresponding to each target region.

4.3. Results

4.3.1. Demographics

Demographic details of the *longitudinal* sample are shown in **Table 4.1**.

At baseline, there were no differences between patients and controls, in the *longitudinal* sample, in age, $t(45) = 0.436$, $p = 0.665$; and gender, $\chi^2(1) = 0.039$, $p = 0.844$. Patients had significantly higher SMFQ scores, $t(45) = 12.414$, $p = 3.923 \times 10^{-16}$, STAI-S scores, $t(45) = 7.280$, $p = 3.928 \times 10^{-9}$, and STAI-T scores, $t(45) = 13.423$, $p = 2.427 \times 10^{-17}$.

At follow-up, there was no difference between patients and controls, in the *longitudinal* sample, in age, $t(45) = 0.385$, $p = 0.702$. Patients had significantly higher SMFQ scores, $t(45) = 6.258$, $p = 1.538 \times 10^{-7}$, STAI-S scores, $t(45) = 3.796$, $p = 4.560 \times 10^{-4}$, and STAI-T scores, $t(45) = 6.075$, $p = 7.846 \times 10^{-7}$, at follow-up.

There were no differences between the patients in the *cross-sectional* sample and patients at baseline in the *longitudinal* sample, in age, $t(97) = 0.858$, $p = 0.393$; SMFQ scores, $t(97) = 0.987$, $p = 0.326$; STAI-S scores, $t(97) = 0.846$, $p = 0.400$; STAI-T scores, $t(97) = 0.268$, $p = 0.789$; or gender, $\chi^2(1) = 0.156$, $p = 0.693$.

There were no differences between the controls in the *cross-sectional* sample and controls at baseline in the *longitudinal* sample, in age, $t(62) = 0.334$, $p = 0.740$; SMFQ scores, $t(62) = 0.223$, $p = 0.824$; STAI-S scores, $t(62) = 0.047$, $p = 0.963$; STAI-T scores, $t(62) = 0.052$, $p = 0.958$; or gender, $\chi^2(1) = 0.003$, $p = 0.953$.

After receiving CBT, all adolescent MDD patients improved in symptoms of depression, $F(1, 43) = 29.24$, $p = 3.00 \times 10^{-6}$, state anxiety, $F(1, 43) = 12.23$, $p = 0.0011$, and trait anxiety, $F(1, 43) = 30.73$, $p = 2.00 \times 10^{-6}$, shown in **Figure *4.2***.

Table 4.1. Shows demographic details of the longitudinal sample.

Group	First Mean Age (SD), range	Gender Proportion (% Female)	First Mean SMFQ Score	First Mean STAI-T Score	First Mean STAI-S Score	Second Mean Age (SD), range	Second Mean SMFQ Score	Second Mean STAI-T Score	Second Mean STAI-S Score
Longitudinal Patients, *n* = 17	15.42 (1.37), 12.89-17.56	82.35	18.12 (6.48)	60.35 (8.02)	48.24 (11.33)	16.07 (1.34), 13.41-18.17	9.00 (5.28)	44.17 (10.48)	35.71 (10.14)
Longitudinal Controls, *n* = 30	15.59 (1.47), 12.14-17.73	80.00	2.60 (1.77)	30.50 (6.91)	28.93 (6.89)	16.24 (1.46), 12.92-18.30	2.21 (1.81)	28.68 (6.62)	26.43 (6.30)

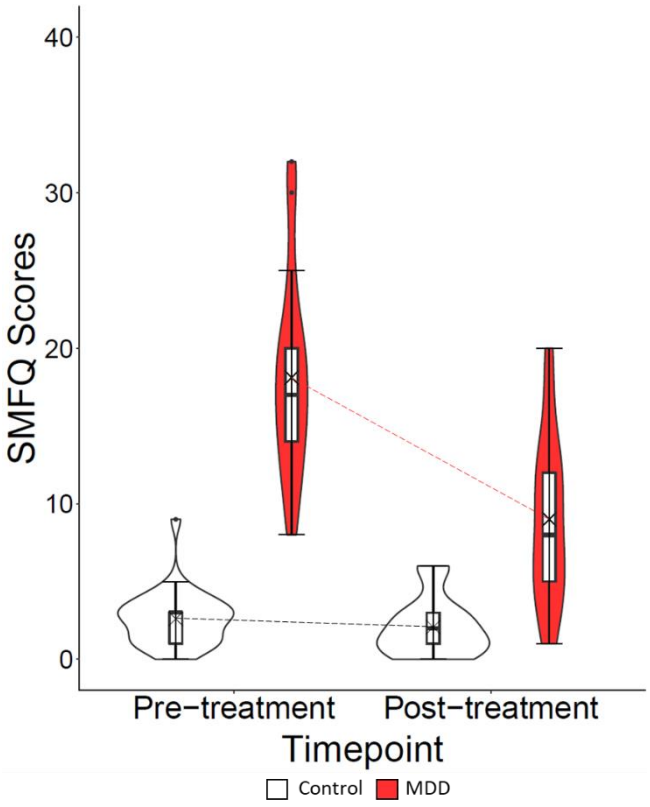


Figure 4.2. Shows the changes in SMFQ scores before and after patients received cognitive behavioural therapy.

4.3.2. CBT-related changes in resting-state functional connectivity

Associated with the left amygdala and the left subgenual anterior cingulate cortex seeds, a variety of regions showed significant group-by-time interactions in resting-state functional connectivity, shown in **Figure *4.3*** and **Table 4.2**. In all significant interactions, resting-state functional connectivity with the seed region had increased in MDD patients after treatment.

There was no relationship between any of the CBT-related changes in resting-state functional connectivity and changes in symptoms, shown in **Table 4.2**.

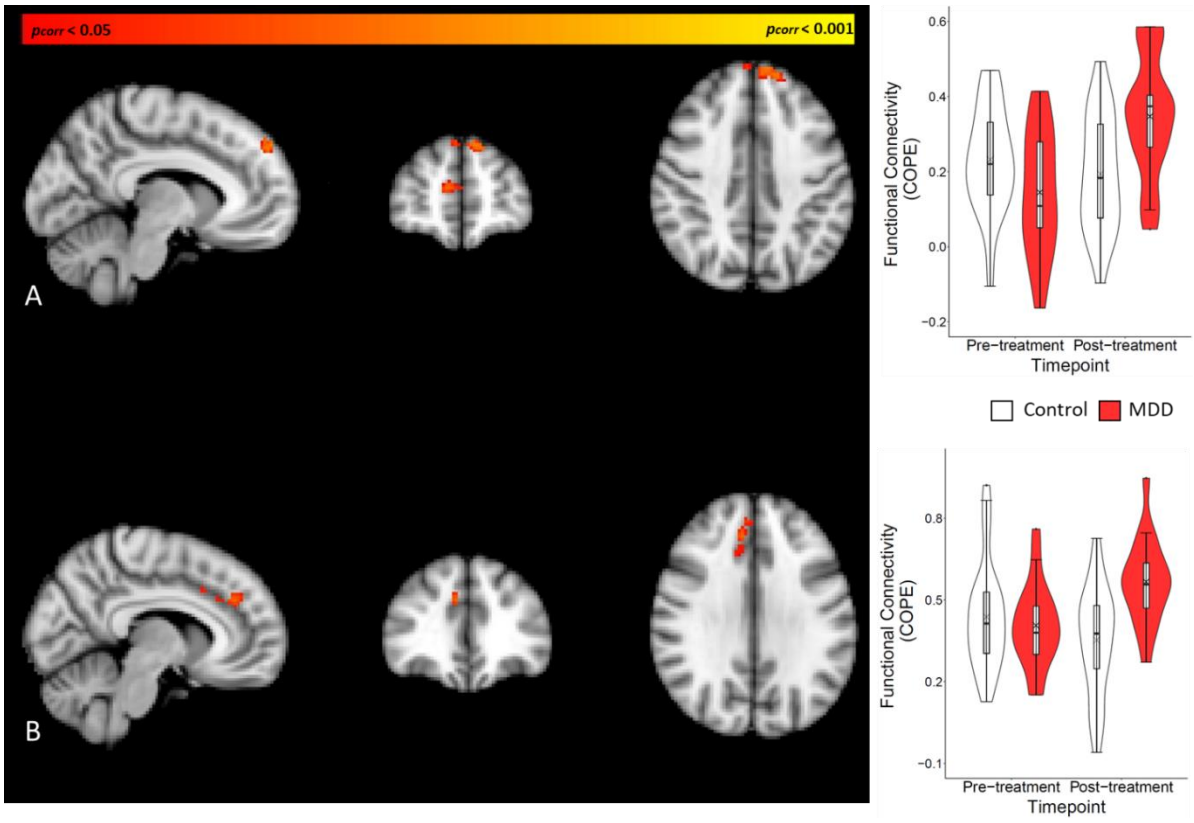


Figure 4.3. Shows the group-by-time interactions in resting-state functional connectivity. A) Left Amygdala Seed. B) Left Subgenual Anterior Cingulate Cortex Seed.

Table 4.2. Shows details of significant clusters showing CBT-related changes in resting-state functional connectivity.

Seed Region	Number of voxels	*Peak p*-value	Peak *t*-score	Peak MNI Coordinates	Regions showing increased connectivity (peak region in bold)	Correlation with Change in SMFQ scores	Correlation with Change in STAI-S scores	Correlation with Change in STAI-T scores
Left Amygdala - Cluster 1	3	0.048	3.65	46, 79, 50	**Right pregenual anterior cingulate,** left pregenual anterior cingulate	r_s = 0.078, p = 0.76	r_s = 0.23, p = 0.37	r_s = -0.17, p = 0.52
Left Amygdala - Cluster 2	7	0.040	4.69	47, 90, 56	Left medial frontal gyrus, **right medial frontal gyrus**	r_s = 0.051, p = 0.84	r_s = 0.069, p = 0.79	r_s = -0.27, p = 0.29
Left Amygdala - Cluster 3	69	0.031	4.57	50, 90, 42	Left medial frontal gyrus, **right medial frontal gyrus, l**eft pregenual anterior cingulate, right pregenual anterior cingulate	r_s = 0.34, p = 0.178	r_s = 0.51, p = 0.038	r_s = 0.23, p = 0.38
Left Amygdala - Cluster 4	180	0.026	4.48	41, 90, 56	**Left medial frontal gyrus**, right medial frontal gyrus, left superior frontal gyrus	r_s = 0.12, p = 0.64	r_s = 0.012, p = 0.96	r_s = -0.16, p = 0.53
Left Subgenual Anterior Cingulate - Cluster 1	4	0.048	4.16	46, 90, 42	Left medial frontal gyrus, **right pregenual anterior cingulate**	r_s = 0.015, p = 0.96	r_s = 0.27, p = 0.29	r_s = -0.066, p = 0.80
Left Subgenual Anterior Cingulate - Cluster 2	46	0.034	4.55	51, 73, 54	**Right midcingulate**	r_s = 0.12, p = 0.65	r_s = 0.16, p = 0.55	r_s = -0.17, p = 0.52
Left Subgenual Anterior Cingulate - Cluster 3	73	0.032	4.69	49, 81, 51	Left medial frontal gyrus, right medial frontal gyrus, **right midcingulate**, right pregenual anterior cingulate	r_s = 0.076, p = 0.77	r_s = 0.12, p = 0.65	r_s = -0.25, p = 0.34

4.3.3. Relationship between pre-treatment functional disruption and CBT-related changes

Strikingly, although we hypothesised that regions showing the greatest pre-treatment functional disruption would show the greatest CBT-related changes in resting-state functional connectivity, producing positive correlations between case-control and group-by-time interaction *z*-scores, few correlations were actually positive. Of the 13,806 correlations only 2025 (14.67%) were positive; **Figure *4.4***.

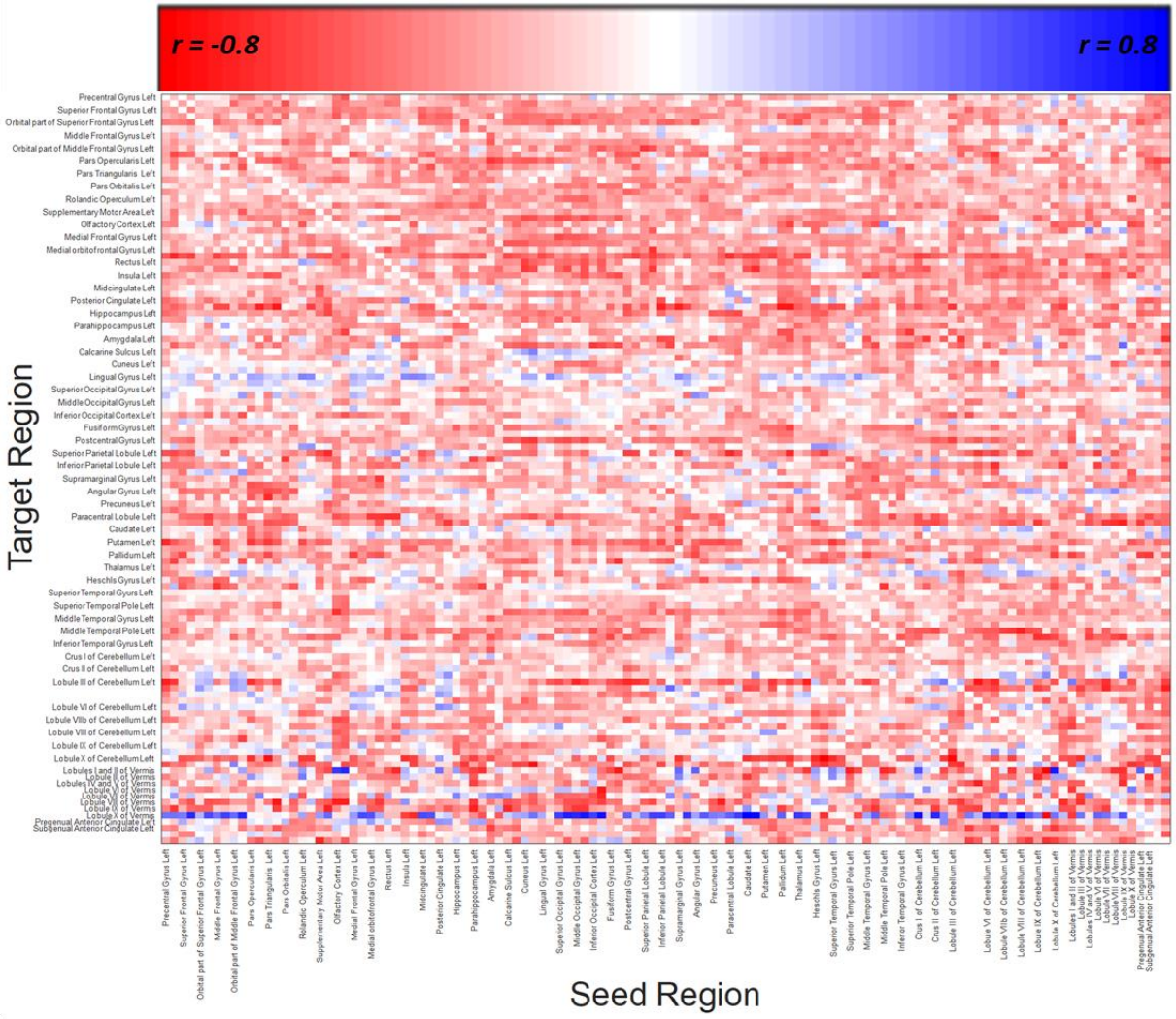


Figure 4.4. Shows the correlation matrix, between case-control *z*-scores and group-by-time *z*-scores, of all 118 seed regions and their 117 target regions.

The r^2 values of the positive correlations, between pre-treatment case-control *z*-scores and group-by-time interaction *z*-scores, ranged between 5.979×10^{-9} - 0.8177, although almost all were weak, with the median r^2=0.0082. Only 5% of the positive correlations had $r^2 > 0.15$, shown in **Figure *4.5***. The r^2 values of positive correlations, averaged across functional networks and the whole brain are shown in **Figure *4.6***.

The r^2 values of the negative correlations, between pre-treatment case-control *z*-scores and group-by-time interaction *z*-scores, ranged between 8.3244×10^{-10} - 0.8062, with a median r^2 of 0.0716. The distribution of these negative correlations is shown in **Figure *4.5***, with 25% of the correlations having an $r^2 > 0.15$. The r^2 values of negative correlations, averaged across functional networks and the whole brain are shown in **Figure *4.7***.

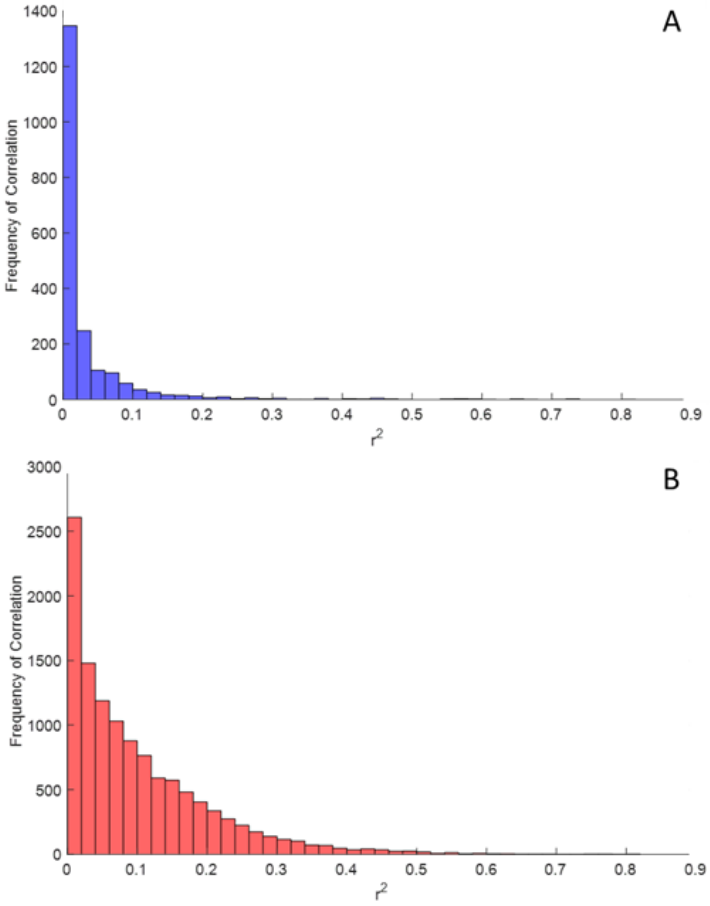


Figure 4.5. A) Shows the frequency distribution of positive correlations. B) Shows the frequency distribution of negative correlations.

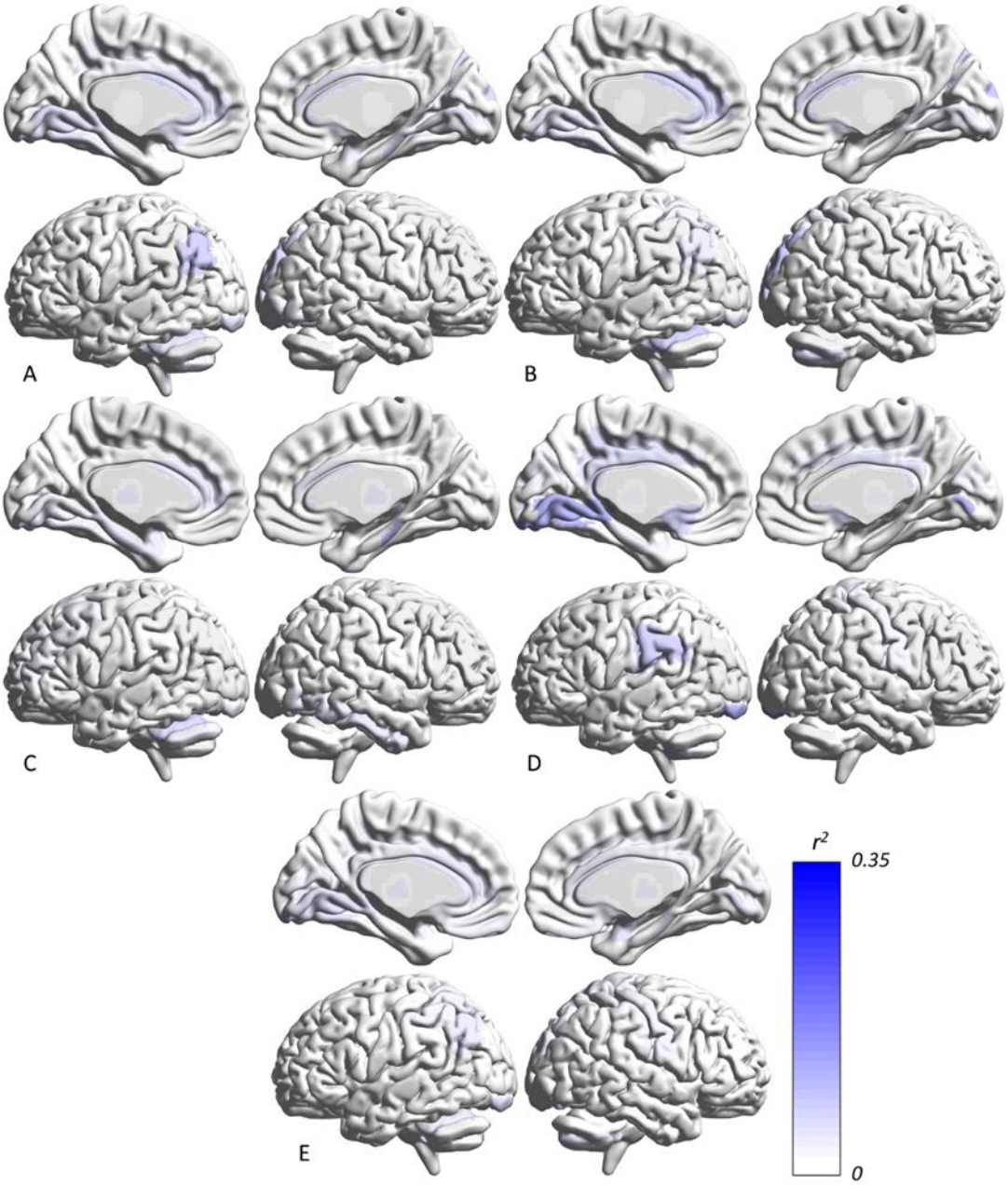


Figure 4.6. Shows the averaged r^2 values for positive correlations for each seed grouping. A) Fronto-limbic network. B) Default mode network. C) Central executive network. D) Salience network. E) Average across all 118 seed regions.

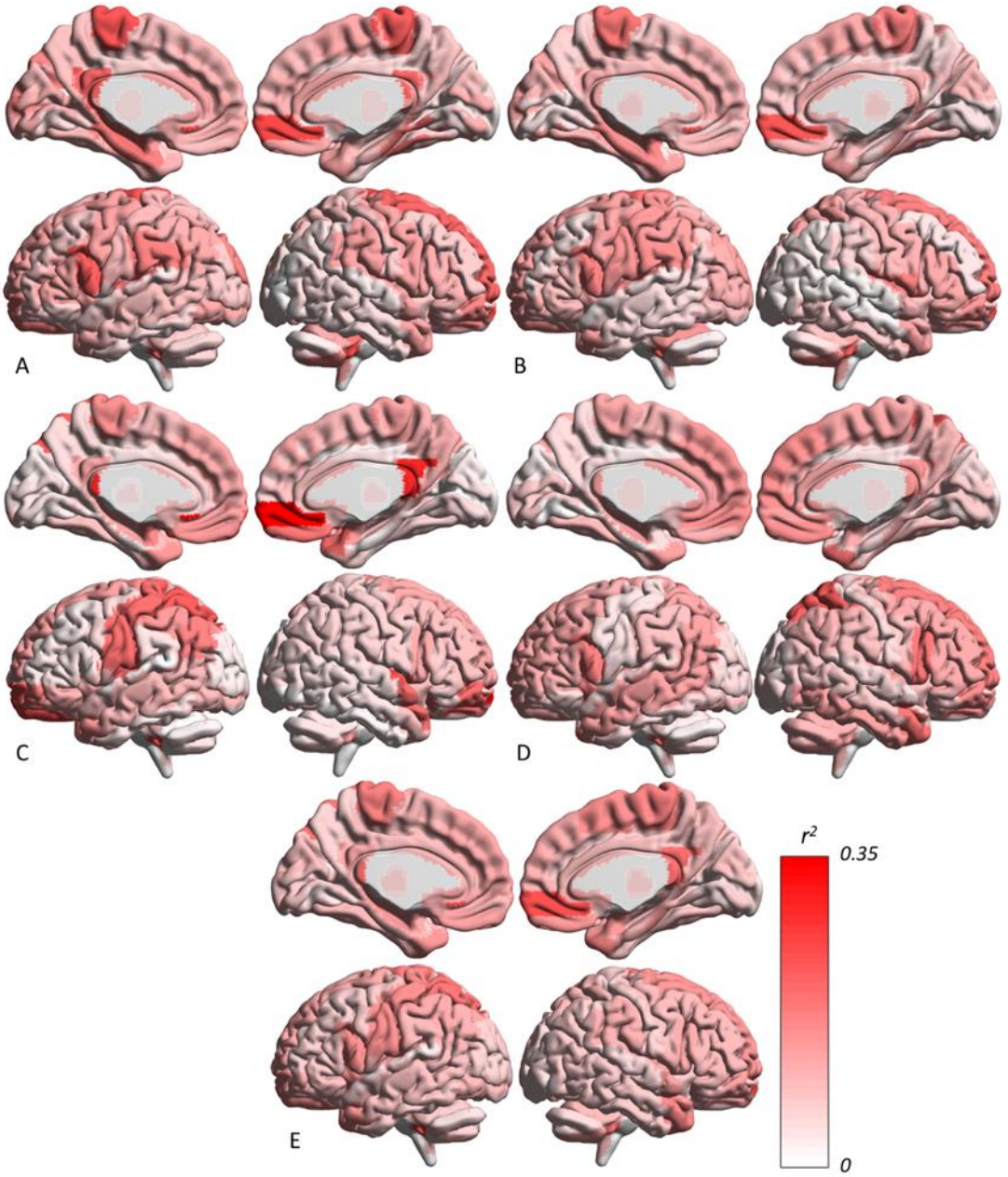


Figure 4.7. Shows the averaged r^2 values for negative correlations for each seed grouping. A) Fronto-limbic network. B) Default mode network. C) Central executive network. D) Salience network. E) Average across all 118 seed regions.

The top 10% of target regions, showing the strongest averaged effect sizes for negative correlations, between pre-treatment case-control *z*-scores and group-by-time interaction *z*-scores, across the seed regions grouped into networks and the whole brain, are shown in **Figure *4.8***. Rankings of all target regions, showing the strongest averaged effect sizes for negative correlations, across the seed regions grouped into networks, are shown in **Table 9.4** of the **Appendix**.

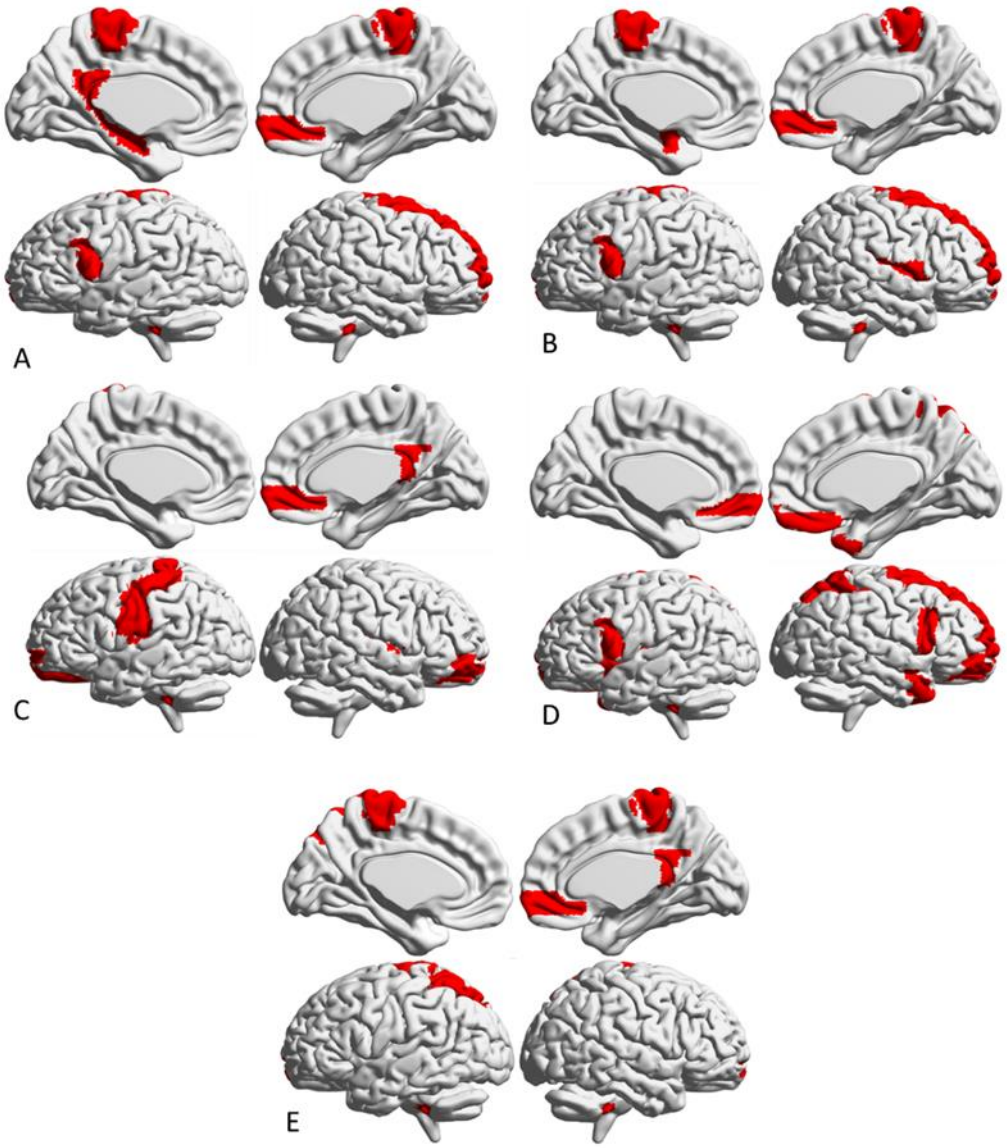


Figure 4.8. Shows the top 10% strongest negative correlations for each seed grouping. A) Fronto-limbic network. B) Default mode network. C) Central executive network. D) Salience network. E) Average across all 118 seed regions.

4.4. Discussion

Patients showed CBT-related changes (group-by-time interactions) between predominantly frontal regions and the left subgenual anterior cingulate cortex and left amygdala seeds, with resting-state functional connectivity increasing following CBT. Regions showing the greatest functional disruption often later showed the weakest CBT-related changes in resting-state functional connectivity, with this effect being most prominent within the medial orbitofrontal cortex.

Firstly, in adolescent MDD patients, the left subgenual anterior cingulate seed showed increased resting-state functional connectivity with regions of the fronto-limbic network, including the medial frontal gyrus and the pregenual anterior cingulate cortex, as well as increased resting-state functional connectivity with the midcingulate, implicating the salience network, after they had received CBT. Furthermore, the left amygdala seed also showed increased resting-state functional connectivity with predominantly fronto-limbic regions, such as the medial frontal gyrus and the pregenual anterior cingulate cortex, after MDD patients had received CBT. These findings indicate that the functional connectivity of the fronto-limbic network may be a key target of CBT's effects, amplifying the connectivity between limbic and frontal regions of the brain, which is in line with previous work on the effects of CBT in adult MDD (Goldapple et al., 2004; Kennedy et al., 2007). Previous work has made inferences as to what these functional changes within the fronto-limbic network mean with regards to the psychological functioning of MDD patients, such as CBT-related improvements in attention to emotionally salient stimuli (Goldapple et al., 2004), or improvements in "top-down" emotional regulation, whereby CBT resets disrupted activity of frontal brain regions and improves their ability to regulate emotion (DeRubeis, Siegle, & Hollon, 2008a). However, it is difficult to discern how these CBT-related changes in resting-state functional connectivity translate to changes in patients' psychological functioning as we lack sufficient data to make such claims.

A key focus of this work was the relationship between the magnitude and location of pre-treatment functional disruption and CBT-related changes in resting-state functional connectivity. The vast

majority of the correlations between pre-treatment case-control *z*-scores and group-by-time interaction *z*-scores were negative with this effect often being strongest within the medial orbitofrontal cortex as, when examining the four major functional networks and the whole brain, it consistently showed some of the strongest negative correlations. These results suggest that CBT-related changes in resting-state functional connectivity are weaker within regions that show the greatest pre-treatment functional disruption and do not show normalizing effects, as if normalization were occurring, CBT-related changes in resting-state functional connectivity would be greatest in regions showing the greatest functional disruption. This is a striking finding given that much of the literature examining CBT's effects on brain function has claimed that CBT normalises pre-treatment functional disruption (Franklin et al., 2016; Jacobs et al., 2016; Ritchey et al., 2011; Shou et al., 2017; Straub et al., 2017; Yoshimura et al., 2014).

Multiple reasons exist for why our work examining CBT deviates from that of the literature. Firstly, we separated MDD patient into those that only took part in the cross-sectional pre-treatment resting-state functional scan and those that were longitudinally scanned. This separation allowed us to keep our analyses investigating pre-treatment functional disruption independent of our analyses investigating CBT-related changes. On the other hand, much of the previous literature that claims CBT normalises disrupted brain function in MDD has used the same patients for both their investigations of pre-treatment functional disruption and later CBT-related changes (Franklin et al., 2016; Jacobs et al., 2016; Ritchey et al., 2011; Shou et al., 2017; Straub et al., 2017; Yoshimura et al., 2014), often identifying initially disrupted regions before treatment and then examining those same regions after treatment. This means that past literature, which has used the same patients for both pre- and post-treatment analyses, is likely to be vulnerable to the effects of regression to the mean – whereby an initial statistically extreme measurement is unlikely to be so extreme at a later measurement simply due to the statistical probability of achieving such an extreme measurement being low in general. An example of this is if one wins the lottery on a given week, that same person

is unlikely to win it again the next week, not because they have become worse at playing the lottery but simply because winning the lottery in general is highly unlikely.

Another potential reason for why our results go against the assertion that CBT has a normalizing effect on brain function is that we examined the precise relationship between pre-treatment functional disruption and later CBT-related changes in resting-state functional connectivity. Conversely, past literature has mainly investigated whether regions are functionally disrupted in MDD patients, and then whether these overall levels of functional disruption change after receiving treatment to match the level of healthy controls. Therefore, although past studies may have noted that regions showing pre-treatment functional disruption also appear to change after receiving CBT, they have not investigated whether regions of the greatest functional disruption also show the greatest CBT-related changes, which means they were unable to precisely examine how CBT's effects may vary within regions showing pre-treatment functional disruption.

Why regions with strongly disrupted resting-state functional connectivity might be less amenable to CBT is uncertain. Regions showing the greatest functional disruption may simply take more time to demonstrate CBT-related changes, and a six-month interval, although accompanied by large symptom improvements, was insufficient to see the full effects of CBT. Alternatively, regions showing the greatest functional disruption may become functionally hindered and less responsive to CBT. It has previously been claimed that a kindling effect may occur in MDD (Kendler & Gardner, 2016; Kendler et al., 2000; Post, 1992), whereby an aspect of brain structure or function becomes impaired in some manner that remains even after patients have received treatment, and makes them more likely to develop a future major depressive episode. According to this line of thought, our finding that regions showing the greatest pre-treatment functional disruption also show the weakest CBT-related changes, would be indicative of aspects of brain function becoming hindered in some form that makes them less sensitive to treatment and remain functionally disrupted. Though plausible, this kindling explanation would require that regions showing pre-treatment functional

disruption to be causing the subjective experience of a depressive episode, whereas, in **Chapter 3**, we found no relationship between pre-treatment functional disruption and symptoms of depression or anxiety. The maintenance of this effect by long-term observation of resting-state functional connectivity would resolve these possibilities.

Another explanation is that CBT functionally enhances brain regions that are less affected by the illness, instead of normalizing affected regions. CBT aims to help patients achieve remission by modifying cognitive and behavioral aspects of adolescent MDD. This is done by increasing exposure to pleasurable behaviors, building skills, and by identifying cognitive biases, such as attentional and interpretation bias, and changing them to become less negative. This development of new skills, routines, and changes to thought processes may lead to functional compensation within the brain. This could explain why the observed CBT-related changes led to increased resting-state functional connectivity between the left subgenual anterior cingulate cortex seed and frontal regions, rather than leading to normalizing effects, and more generally that regions of pre-treatment functional disruption did not overlap with the regions showing CBT-related changes. In future it may be more appropriate to focus on the relationship between treatment-related functional changes and potential CBT-related enhancements in specific cognitive or emotional domains, such as emotional regulation, rather than focusing solely on their relationship to a reduction in symptoms of depression.

A disruptive trend in resting-state functional connectivity as MDD progresses cannot be discounted. There was no placebo group within the study, meaning that the observed group-by-time interactions in resting-state functional connectivity may not actually demonstrate the direct effects of CBT. Regions of significant group-by-time interactions may in fact reflect patients' resting-state functional connectivity becoming further disrupted, rather than demonstrating CBT-related changes, which may account for the lack of correlations between changes in resting-state functional connectivity and changes in symptoms. Negative correlations between pre-treatment functional disruption and

later CBT-related changes in resting-state functional connectivity may be due to a ceiling or non-linear effect in functional disruption, with some functionally disrupted regions being at maximum and incapable of further alteration. Such regions would then show the weakest changes over time compared to initially less affected regions with more scope for disruption. However, there is little evidence to support the existence of this ceiling effect, and the absence of a correlation between changes in resting-state functional connectivity and changes in symptoms may simply be due to the small sample size in the *longitudinal* sample.

Moreover, the lack of a placebo group of MDD patients in this study limits the conclusions that can be made. A longitudinally assessed placebo group of MDD patients was not included due to the ethical issue of delaying treatment, and instead, the comparison group for our longitudinal analyses were healthy adolescent controls assessed longitudinally over the same time period. The absence of a placebo group means that any putative CBT-related changes in resting-state functional connectivity in MDD patients cannot be uniquely ascribed to receiving treatment and may instead be interpreted as being due to aging or maturational processes that are specific to adolescent MDD, or may even be part of the progression of MDD-related functional disruption.

Furthermore, although the size of the *longitudinal* patient sample is similar to those of other studies investigating CBT, its small size means our patient sample is potentially unrepresentative of the general adolescent MDD population and the results more susceptible to statistical outliers. Our sample is also likely to be affected by attritional bias, with those patients whose symptoms did not improve being more likely to withdraw from the study and not attend the follow-up assessment.

In conclusion, the relationship between pre-treatment functional disruption and CBT-related changes are mostly negative, with brain regions having the greatest pre-treatment functional disruption showing the weakest CBT-related changes in resting-state functional connectivity. With the rehabilitative nature of CBT in mind, this finding suggests that there is more to the

neurobiological effects of CBT on adolescent MDD than a simple transition from functional disruption to normalization.

5. Subtyping adolescent major depressive disorder patients based on familial loading

5.1. Introduction

Throughout the previous chapters, a common theme has emerged when examining the past literature on the neurobiology of adolescent MDD, inconsistency. Although the lack of adequately powered studies investigating brain structure and function in adolescent MDD has undoubtably contributed to this, as have differences in methodology, this level of inconsistency may also demonstrate the neurobiological heterogeneity of the illness. Indeed, the struggles of the neuroimaging literature are not so surprising given that under the DSM-5 criteria (American Psychiatric Association, 2013), and the older DSM-IV criteria (American Psychiatric Association, 1994), there are nearly 1000 possible combinations of symptoms of MDD (Fried & Nesse, 2015; Østergaard, Jensen, & Bech, 2011), with it being possible for two individual MDD patients to show no symptom overlap.

Clearly this heterogeneity is an issue when trying to identify the neurobiological characteristics of MDD, as aggregating potentially neurobiologically dissimilar patients into one “depressed” group for classic case-control analyses is likely to lead to inconsistent and sometimes confusing results that may implicate separate and unrelated regions and functions of the brain. Therefore, identifying neurobiological subtypes of MDD should be a pressing concern for neuroimaging research, as it could help bring some clarity and cohesion to the literature.

Although complex methods to neurobiologically subtype MDD patients already exist, through the advent of newer machine learning algorithms (Drysdale et al., 2017; Feder et al., 2017; Grosenick et al., 2019; Haroon et al., 2018), such approaches suffer from a lack of reproducibility (Beijers et al., 2019; Dinga et al., 2019) and a more straightforward approach may lie in separating patients into those who have a high or low familial loading for the illness, which is done by identifying those who have at least one first degree relative with MDD. The reason for making the distinction between those with a high and low familial loading for MDD is that the two patient groups are likely to differ

both genetically and also in their environmental experiences, which may lead to differences in both their brain structure and function.

Studies focusing on neurobiological differences between individuals with differing familial loadings for MDD have found intriguing results. MacMaster et al. (2008) found that individuals with a high familial loading for MDD showed reduced grey matter volume within the left and right hippocampi compared to healthy controls with a low familial loading for MDD. This was further supported by Rao et al. (2010) who found that both MDD patients and healthy controls with a high familial loading for MDD showed reduced hippocampal volumes, compared to healthy controls with a low familial loading, suggesting that having an increased familial loading for MDD may lead to structural deviations within the brain, regardless of whether individuals actually suffer from the illness.

Moreover, the most influential study to investigate the relationship between familial loading for MDD and brain structure was conducted by Peterson et al. (2009), who studied the cortical thickness of 66 high familial loading and 65 low familial loading individuals. Both samples were comprised of adults, adolescents, and children, and both groups also contained patients diagnosed with MDD. When comparing the two groups, they found that individuals with a high familial loading for MDD showed lower cortical thickness within multiple regions of the default mode network, such as the left subgenual anterior cingulate cortex, left posterior cingulate cortex and left precuneus, but also showed greater cortical thickness within the right subgenual anterior cingulate cortex, right medial orbitofrontal cortex, and right posterior cingulate cortex. These structural differences between the two familial loading groups remained once controlling for age and diagnoses of MDD, and many were still present within the same sample 8 years later (Hao et al., 2017). Additionally, using the same sample as Peterson et al. (2009), Dubin et al. (2012) compared the white matter volumes between the two familial loading groups and found that individuals with a high familial loading for MDD also showed reduced white matter volume within both frontal and parietal regions of the

brain. The findings from these studies demonstrated that structural deviations may be present between individuals with differing familial loadings for MDD and appear to be stable over time.

It has also been suggested that aspects of brain function, particularly related to the default mode network, may also differ between individuals with varying familial loadings for MDD. Posner et al. (2016) investigated differences in resting-state functional connectivity between individuals with differing familial loadings for MDD, focusing on the functional connectivity of the default mode network. Using the same sample as Peterson et al. (2009), they found that individuals with a high familial loading showed greater resting-state functional connectivity between regions of the default mode network, including the precuneus and posterior cingulate cortex. They also found that individuals with a high familial loading for MDD showed underconnectivity between regions of the default mode network and central executive network. Furthermore, they investigated the structural connectivity of the default mode network, through tractography, and found that individuals with a high familial loading showed reduced structural connectivity between the precuneus and the dorsolateral prefrontal cortex. Their results demonstrated that deviations in brain function also appear to be present between individuals with differing familial loadings for MDD, particularly within the default mode network.

These findings were later supported by Chai et al. (2016), who also compared the resting-state functional connectivity between healthy children with differing familial loadings for MDD. When comparing the two familial loading groups, they found that children with a high familial loading showed overconnectivity between the posterior cingulate cortex, medial prefrontal cortex, and the subgenual anterior cingulate cortex. High familial loading children also showed weaker connectivity between the left dorsolateral prefrontal cortex and bilateral subgenual anterior cingulate cortex, compared to low familial loading individuals. Moreover, these deviations between healthy individuals with differing familial loadings for MDD were later used by Shapero et al. (2019) to try and predict the onset of the illness. They studied their participants over a 4-year period to identify

who had developed MDD, and investigated whether the initial differences in resting-state functional connectivity between the two groups, initially observed by Chai et al. (2016), would predict the later development of MDD during adolescence. They found that the underconnectivity between the default mode network and the central executive network were relatively good predictors of the development of adolescent MDD in high-familial loading individuals, achieving a 67% accuracy rate. These results further suggested that having a high familial loading for MDD may be related to deviations within the default mode network, and its connectivity to the central executive network, and that they may contribute towards the development of MDD during adolescence.

The past literature appears to show that both brain structure and function vary between individuals with differing familial loadings for MDD. However, these studies investigating familial loading are often mostly or even completely comprised of participants who have not been diagnosed with MDD, and therefore do not directly compare MDD patients with differing familial loadings. This lack of a comparison between patients with either high or low familial loadings makes it difficult to establish whether the differences that have been found in brain structure and function are part of a common trajectory towards developing MDD, which will eventually occur in all individuals that develop the illness regardless of their familial loading (Peterson et al., 2009; Posner et al., 2016), or whether they are unique to those with an increased familial loading for MDD. If the latter were the case, then it may eventually help to distinguish neurological subtypes of MDD.

The present exploratory study therefore investigated whether adolescent MDD patients would show differences in both the structure and function of the default mode network depending on their familial loading, investigating the cortical thickness, white matter volume, grey matter volume and resting-state functional connectivity of regions within this network. Based on previous findings (Dubin et al., 2012; Hao et al., 2017; Peterson et al., 2009; Posner et al., 2016), we hypothesised that high familial loading MDD patients would show lower white matter volume, cortical thickness, and

grey matter volume, but show greater resting-state functional connectivity within the default mode network, when compared to low familial loading MDD patients.

5.2. Methods

5.2.1. Participant samples

Participants were recruited from the IMPACT study (Goodyer et al., 2017), which was a randomised controlled superiority trial assessing the medium-term effects of psychological therapies in adolescent MDD, using patients (N=465) who met the diagnostic requirements for MDD of the DSM-IV (American Psychiatric Association, 1994).

Patients were also asked the two following questions:

"*Does any family member currently suffer from any medical, emotional or behavioural problems which affect their daily life? If yes, please give details (age, diagnosis, treatment)*"

"*Has any family member suffered any medical, emotional or behavioural problems in the past? If yes, please give details (age, diagnosis, treatment)*"

The responses to these questions were used to assess the familial loading of patients.

Participants who stated that a first degree relative had depression were classed as having a high familial loading for MDD. Participants who did not list depression as an illness that their first-degree relatives suffered from were classed as having a low heritability loading for MDD.

Any participants who mentioned a first degree relative who suffered from another mental illness or behavioural problems but did not specifically mention depression were excluded from the analyses due to the potential confounds of unknown comorbid MDD.

Participants who stated having any other relative with depression, that was not a first degree relative, were excluded from the analyses.

Participants who mentioned a relative having depression but not explicitly how they were related were excluded from the analyses. Participants who did not answer either of the questions were also excluded from the analyses.

This produced a sample of 94 patients who were classified as having a high familial loading for MDD, and 126 patients who were classified as having a low familial loading.

Of the 94 high loading and 126 low loading patients, 34 high loading and 34 low loading patients were enrolled in the MR-IMPACT study (Hagan et al., 2015a; Hagan et al., 2013), where structural and functional MRI were assessed. On the day of scanning, participants completed the SMFQ (Sharp, Goodyer, & Croudace, 2006) as a measure of depressive symptoms and the STAI (Spielberger, Gorsuch, & Lushene, 1970) as a measure of anxiety symptoms.

The structural MRI data of 1 high loading and 3 low loading patients were excluded and the functional MRI data of 6 high loading and 4 low loading patients were also excluded.

This left a sample of 33 high loading and 31 low loading patients with structural MRI data, and 28 high loading and 30 low loading patients with functional MRI data.

5.2.2. MRI acquisition

Scanning parameters are identical to those detailed in **Chapters 2** and **Chapter 3**. Imaging data were acquired using a 3.0 Tesla Magnetom Trio Tim scanner fitted with a quadrature birdcage head coil, based at the Wolfson Brain Imaging Centre, University of Cambridge, UK. T1-weighted images depicting brain structure were collected in the sagittal plane, using a 3D-MPRAGE sequence. Whole-brain, BOLD-sensitive echo-planar images were collected with participants lying awake, at rest, with eyes closed. Functional scans lasted 8 minutes and 56 seconds, collecting 256 images.

5.2.3. Image pre-processing

The standardized pre-processing procedures detailed in **Chapter 2** and **Chapter 3** were conducted, using Advanced Normalization Tools (ANTs; Avants, Tustison, & Song, 2009) for cortical thickness

preprocessing, FSL VBM (Jenkinson et al., 2012) for grey matter volume and white matter volume pre-processing, and the speedypp algorithm from the BrainWavelet Toolbox (www.brainwavelet.org), alongside ANTs and FSL FEAT (Jenkinson et al., 2012; Woolrich, Behrens, Beckmann, Jenkinson, & Smith, 2004; Woolrich, Ripley, Brady, & Smith, 2001), for resting-state functional connectivity pre-processing.

5.2.4. Statistical analyses of structural images

A mask of the default mode network was created using the AAL atlas (Tzourio-Mazoyer et al., 2002). Regions included within this mask were the medial frontal gyrus, posterior cingulate cortex, parahippocampus, hippocampus, precuneus, subgenual anterior cingulate cortex, and inferior parietal lobule.

An ANCOVA was used investigate differences in grey matter volume, white matter volume and cortical thickness between high and low familial loading patients, at each voxel within the default mode network mask. Age in years, gender and current symptoms of depression (SMFQ scores) were used as covariates. Permutation-based methods, using FSL Randomise (Jenkinson et al., 2012), were conducted for inference testing, using 100,000 permutations per statistical test, threshold-free cluster enhancement, and a Family-wise error correction to account for multiple comparisons. Cohen's *d* (Cohen, 1988) was used as a measure of effect size. A separate analysis was also conducted, comparing high and low familial loading patients, but also controlling for antidepressant medication use.

5.2.5. Seed-based correlation analyses of functional images

To investigate resting-state functional connectivity of the default mode network, we utilised seed-based methods, using the left and right hippocampi, left and right precuneus, and left and

right subgenual anterior cingulate cortex as seed regions. These were identified using the AAL atlas (Tzourio-Mazoyer et al., 2002).

To investigate differences in resting-state functional connectivity between high and low loading patients, an ANCOVA was used, with age in years, gender and SMFQ scores as covariates. For each seed-based analysis, the general linear model was applied to every voxel within the default mode network mask, excluding the seed region itself. The same permutation-based methods as previously described were used for statistical inferences. Separate analyses were also conducted, comparing high and low familial loading patients, but also controlling for antidepressant medication status.

5.3. Results

5.3.1. Demographics

Participant demographics are shown in **Table 5.1**.

For those with structural data, there were no differences between high familial loading patients and low familial loading patients in age, $t(62) = 0.058$, $p = 0.954$, or medication status, $\chi^2(1) = 1.56$, $p = 0.212$. However, only for patients used in the structural analyses, there was a slight gender imbalance with high familial loading patients being comprised of more males compared to low familial loading patients, $\chi^2(1) = 4.20$, $p = 0.040$, $\Phi = 0.256$.

For those with structural data, there were no differences in SMFQ scores, $t(62) = 0.755$, $p = 0.453$, STAI-S scores, $t(62) = 1.129$, $p = 0.263$, and STAI-T scores $t(62) = 1.317$, $p = 0.193$.

For those with functional data, there were no differences between high familial loading patients and low familial loading patients in gender, $\chi^2(1) = 2.28$, $p = 0.131$, age, $t(56) = 0.466$, $p = 0.643$, or medication status, $\chi^2(1) = 2.92$, $p = 0.087$.

For those with functional data, there were no differences in SMFQ scores, $t(56) = 0.166$, $p = 0.869$, STAI-S scores, $t(56) = 0.772$, $p = 0.443$, and STAI-T scores $t(56) = 1.188$, $p = 0.240$.

Table 5.1. Shows participant demographics with standard deviations in brackets.

	Mean Age (Years)	Gender proportion (% female)	Percentage Taking Antidepressant Medication	Mean Depression Score (SMFQ)	Mean State Anxiety Score (STAI-S)	Mean Trait Anxiety Score (STAI-T)
Structure						
Low, *n* = *31*	15.46 (1.04)	90.32	25.81	20.23 (6.77)	47.52 (11.23)	61.94 (7.87)
High, *n* = 33	15.44 (1.46)	69.70	39.39	18.91 (7.15)	44.33 (11.32)	59.30 (8.11)
Function						
Low, *n* = 30	15.43 (1.10)	90.00	20.00	19.93 (7.69)	47.73 (10.23)	61.57 (7.93)
High, *n* = 28	15.57 (1.27)	75.00	39.29	19.61 (7.28)	45.50 (11.77)	58.93 (8.98)

5.3.2. Brain structure

Contrary to our initial hypothesis that high familial loading patients would show lower cortical thickness and white matter volume in regions of the default mode network, we found no significant differences in either measure of brain structure.

However, in line with our hypothesis, we found that high familial loading MDD patients showed lower grey matter volume within the precuneus, bilaterally, compared to low familial loading MDD patients, shown in **Figure 5.1** and **Table 5.2**.

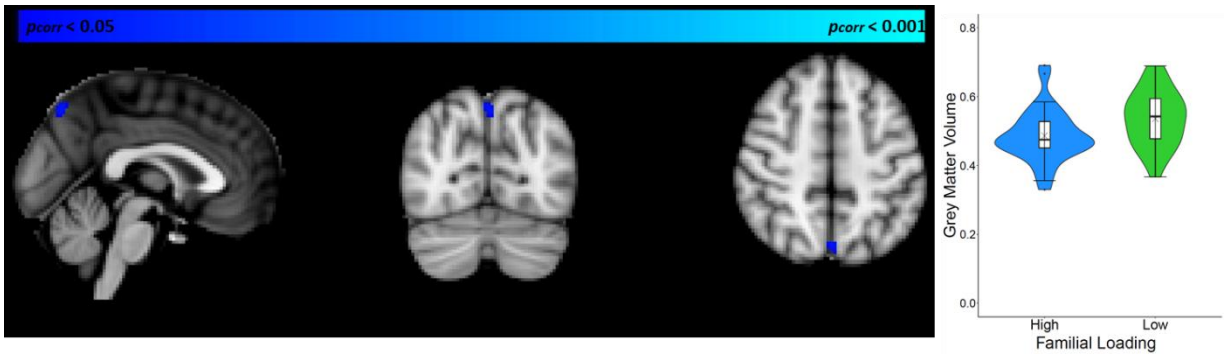


Figure 5.1. Shows the significant cluster in the precuneus where patients with a high familial loading for depression showed lower grey matter volume, compared to patients with a low familial loading.

Table 5.2. Shows details about the cluster within the precuneus that showed reduced grey matter volume in high familial loading patients, compared to low familial loading patients.

	Number of Voxels	***Peak pcorr*-value**	**Peak *t*-score**	**Cohen's *d***	**Peak MNI Coordinates**	**Regions involved (peak region in bold)**
Grey Matter Volume Cluster	39	0.046	3.42	0.63	45, 27, 61	**Left precuneus**, right precuneus

5.3.3. Brain function

Contradicting our hypothesis that high familial loading MDD patients would show greater resting-state functional connectivity within the default mode network, we instead found that high familial loading patients showed weaker resting-state functional connectivity compared to low familial loading patients. The precuneus showed weaker resting-state functional connectivity, in high familial loading MDD patients, with the left and right hippocampus seeds and the right subgenual anterior cingulate seed, shown in **Figure 5.2** and **Table 5.3**.

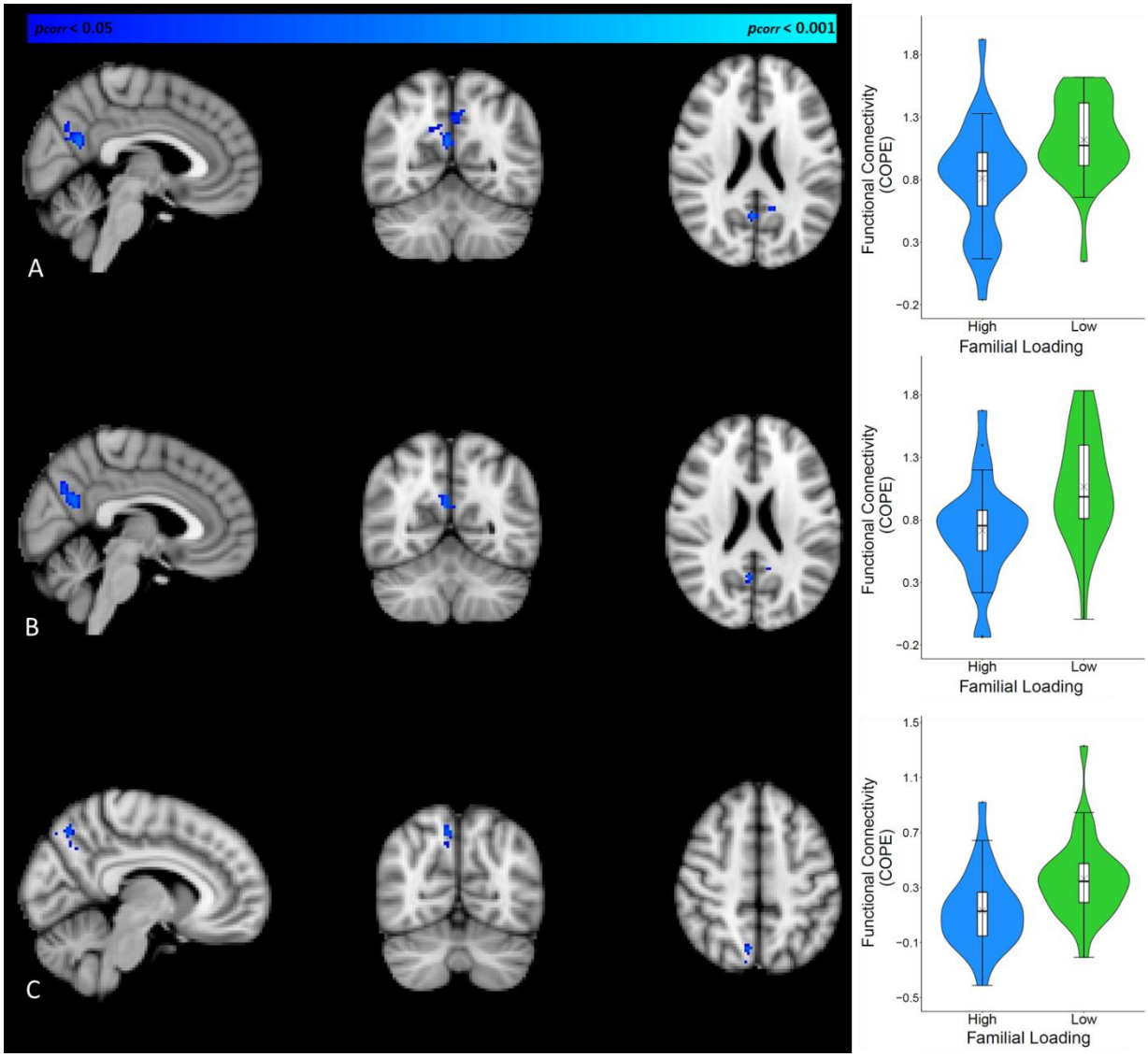


Figure 5.2. Shows the significant clusters within the precuneus where patients with a high familial loading for depression showed decreased resting-state functional connectivity, compared to patients with a low familial loading. A) Left hippocampus seed. B) Right hippocampus seed. C) Right subgenual anterior cingulate seed.

Table 5.3. Shows details about the clusters within the precuneus that showed reduced resting-state functional connectivity in high familial loading patients, compared to low familial loading patients.

	Number of Voxels	*Peak pcorr*-value	Peak *t*-score	Cohen's *d*	Peak MNI Coordinates	Regions involved (peak region in bold)
Left Hippocampus Seed	234	0.027	3.97	0.72	47, 34, 47	Left precuneus, **right precuneus**
Right Hippocampus Seed	191	0.030	3.58	0.69	47, 34, 47	Left precuneus, **right precuneus**
Right Subgenual Anterior cingulate Seed	28	0.033	4.14	0.62	49, 30, 61	**Right precuneus**

5.3.4. Controlling for antidepressant medication status

Both structural and functional differences between high and low familial loading patients remained after controlling for medication status, though functional clusters were smaller, shown in **Figure 5.3**, **Table 5.4**, and **Figure 5.4**, and **Table 5.5**.

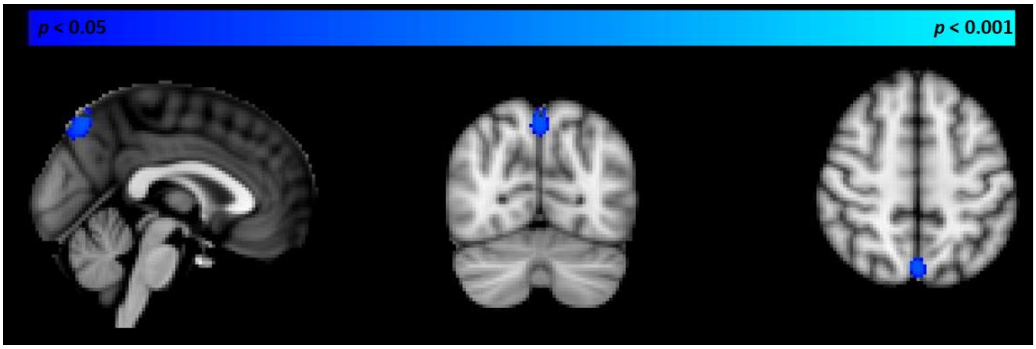


Figure 5.3. Shows the cluster where MDD patients with a high familial loading had lower grey matter volume than low familial loading patents, after controlling for antidepressant medication status.

Table 5.4. Shows details of the significant grey matter volume cluster where high familial loading MDD patients had lower grey matter volume than low familial loading patents, after controlling for antidepressant medication status.

Cluster	Number of Voxels	*Peak pcorr*-value	Peak *t*-score	Peak MNI Coordinates	Regions involved (peak region in bold)
Grey matter volume cluster	175	0.033	3.60	45, 28, 62	**Left precuneus**, right precuneus

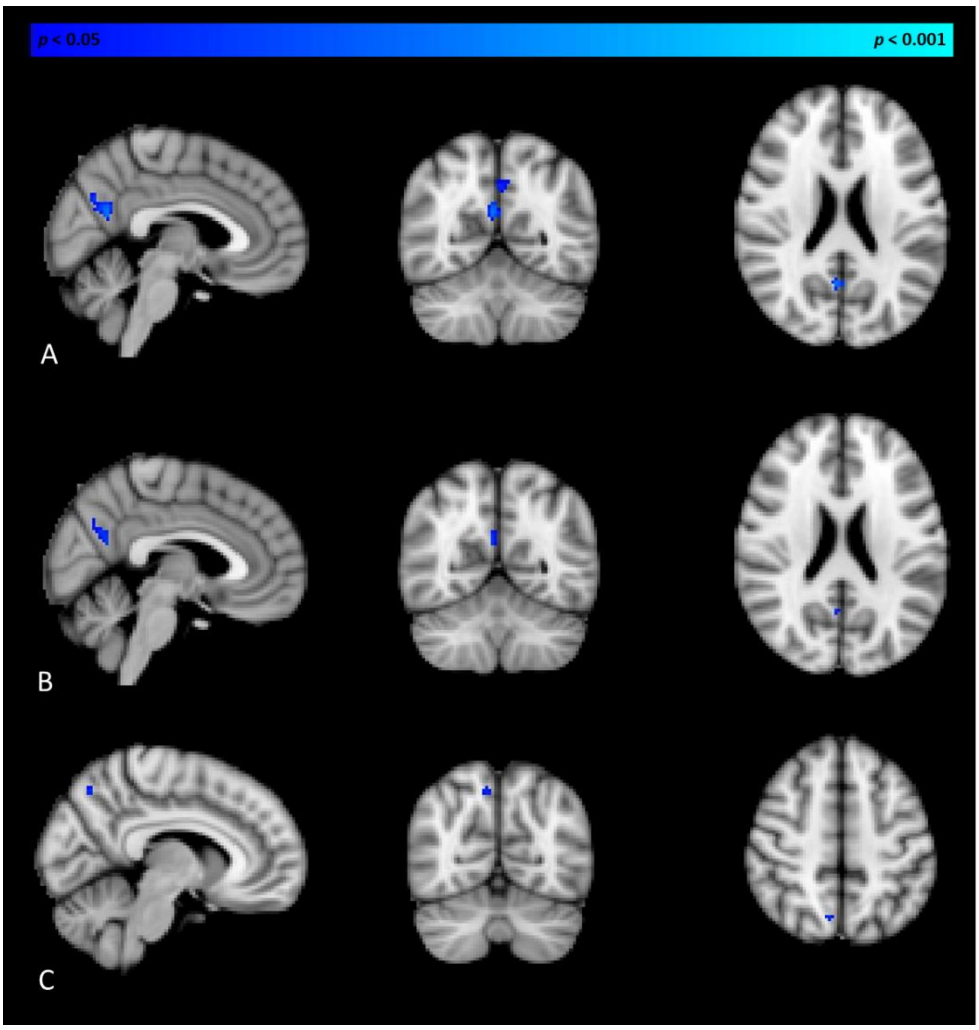


Figure 5.4. Shows the significant clusters after controlling for antidepressant medication status, where high familial loading MDD patients showed lower resting-state functional connectivity. A) Left hippocampus seed. B) Right hippocampus seed. C) Right subgenual anterior cingulate seed.

Table 5.5. Shows details on significant clusters where high familial loading MDD patients showed lower resting-state functional connectivity compared to low familial loading patients, after controlling for antidepressant medication status.

Seed Region	Number of Voxels	*Peak pcorr*-value	Peak *t*-score	Peak MNI Coordinates	Regions involved (peak region in bold)
Left Hippocampus Seed – Cluster 1	35	0.043	3.32	44, 33, 56	**Left precuneus**
Left Hippocampus Seed – Cluster 2	47	0.038	3.48	41, 31, 52	**Left precuneus**
Left Hippocampus Seed – Cluster 2	85	0.027	3.96	47, 34, 47	**Left precuneus**, right precuneus
Right Hippocampus Seed -Cluster 1	1	0.049	3.41	43, 30, 51	**Left precuneus**
Right Hippocampus Seed – Cluster 2	46	0.040	3.45	47, 34, 47	**Right precuneus**
Right Subgenual Anterior cingulate Seed	10	0.042	4.07	49, 30, 61	**Right precuneus**

5.4. Discussion

Adolescent MDD patients with a high familial loading for the illness showed lower grey matter volume bilaterally within the precuneus, compared to low familial loading MDD patients. Additionally, mirroring the structural findings, high familial loading patients also showed reduced resting-state functional connectivity between the precuneus, and the hippocampus seeds, and right subgenual anterior cingulate cortex seed, indicating weaker default mode network connectivity. These results suggest that the structure and function of the default mode network may be affected by patients' familial loading for MDD, suggesting that these two groups of MDD patients may neurobiologically differ from each other.

Our results demonstrate that a common neurobiological endpoint in MDD may be unlikely to be the case and instead there may be neurobiological subtypes of the illness. The cause for these differences between patients with differing familial loadings is unclear. One potential explanation may be that the contributing factors for MDD are different between the two patient groups, with high familial loading patients having a greater genetic contribution and low familial loading patients having a greater environmental contribution toward the development of the illness. However, this is purely speculative as we lack sufficient data to substantiate this claim and other explanations are possible. For example, with respect to the high familial loading group, having a first degree relative with MDD will undoubtably affect the environment, which may then contribute towards the development of the illness. Moreover, we did not conduct thorough genetic comparisons between the two patient groups, and it is therefore possible that patients within the low familial loading group may still carry similar genes, that predispose them to developing MDD, to the high familial loading group. It should also be noted that, with its heritability estimate being around 38% (Flint & Kendler, 2014), MDD likely has a relatively low genetic component to its genesis compared to other psychiatric illnesses such as bipolar disorder and schizophrenia, which both have heritability estimates of around 80% (Gordovez & McMahon, 2020; Hilker et al., 2018). Therefore, while genetic

factors may have been responsible for some of the neurobiological differences observed between the two familial loading groups, environmental factors will likely have had a strong contribution to these findings.

Alternatively, the reason for the differences in the default mode network's structure and function between the two patient groups may be due to their sensitivity to environmental stress. Individuals with an increased familial loading for MDD do appear to be more sensitive to environmental stress, evidenced by previous research finding that high familial loading individuals are more likely to develop an episode of depression after experiencing stressful life events than those with a low familial loading for MDD (Kendler et al., 1995; Monroe, Slavich, & Gotlib, 2014). Furthermore, the default mode network also appears to be susceptible to environmental stress, shown by past research finding that adults who experienced childhood maltreatment had greater grey matter volume within the precuneus (Jensen et al., 2015) and that the precuneus also showed greater centrality within structural networks of the brain (Teicher et al., 2014), which was claimed to be indicative of greater functional connectivity within the default mode network (Teicher et al., 2016). Together, these past findings may help explain the differences between our two patient groups. As low familial loading patients appear to be less sensitive to life stress, they may have experienced comparatively more environmental stress in order to develop MDD, which in turn may have led to greater changes to the structure and function of the default mode network, which may explain why they showed both greater grey matter volume and greater resting-state functional connectivity within the default mode network. Future research that includes detailed environmental and genetic data may address this potential explanation.

Moreover, whilst adolescent MDD patients with differing familial loadings for the illness may deviate in both in the structure and function of the default mode network, it is unclear whether distinguishing MDD patients based on familial loading has any clinical significance. Within this neuroimaging sample, the two familial loading groups did not differ on symptoms of depression or

anxiety, as measured by the SMFQ and STAI. However, these symptom measures are relatively general and may not have the precision to capture variations in individual symptoms of depression or anxiety. Additionally, investigating differences in rumination symptoms between these two familial loading groups may be warranted, as the default mode network appears to deviate in these two patient groups. The default mode network has been associated to rumination symptoms (Berman et al., 2011; Hamilton et al., 2015, 2011; Zhu et al., 2012) and the deviations in both its structure and function, as seen in the two familial loading groups, may also manifest themselves in deviations to rumination symptoms. Therefore, more specific symptom measures, particularly those investigating rumination symptoms, using a large sample of MDD patients with differing familial loadings, may be required in order to understand whether distinguishing MDD patients based on their familial loading for the illness has any clinical significance.

The main limitation of this work is that the MR-IMPACT study (Hagan et al., 2013; Hagan et al., 2015a) was not initially designed to investigate familial loading in MDD. Therefore, these exploratory secondary analyses that we have conducted should be viewed with caution, as they require further investigation using a study that has been specifically designed to investigate familial loading in MDD. Secondly, the method of identifying which patients had a first degree relative with MDD was conducted by simply asking whether they had any relatives with medical, emotional, or behavioural problems. This reliance on self-report, instead of verifying family history through medical history checks, means that the method is vulnerable to participants misremembering or forgetting whether a relative of theirs had MDD.

In conclusion, MDD patients with either a high or low familial loading for the illness appear to be neurobiologically different, specifically regarding both the structure and function of the default mode network, and may have implications for neurobiologically subtyping adolescent MDD. However, whether distinguishing these two patient groups has any clinical significance is yet to be understood and will be investigated in the next chapter.

6. Familial loading subtypes and symptoms of major depressive disorder

6.1. Introduction

In the previous chapter, it was argued that separating adolescent MDD patients into those who have a high or low familial loading for the illness – defined by having at least one first-degree relative with MDD – may be a simple and useful method of subtyping adolescent MDD patients. Using this method, we showed that patients with high and low familial loadings differed in both the structure and function of the default mode network. However, whilst there may be neurobiological differences between these two subtypes, it is still unclear whether distinguishing MDD patients based on their familial loading for MDD has any clinical significance.

Past research does appear to indicate that there may be some clinical, as well as neurobiological, differences between MDD patients with differing familial loadings for the illness. Firstly, this issue of familial loading for MDD may be pertinent to adolescents as it has been found that individuals with an increased familial loading for the illness are more likely to have earlier age of onsets of MDD (Kendler, Gatz, Gardner, & Pedersen, 2006; Lohoff, 2010; Thapar et al., 2012; Weissman et al., 1987; Williamson, Birmaher, Axelson, Ryan, & Dahl, 2004), particularly before the age of 20 years (Tozzi et al., 2008). This may mean that adolescence is a crucial period where differences in familial loading begin to manifest themselves.

Using this approach, there is also evidence that individuals with a high familial loading for MDD have different distinguishable characteristics compared to those with a low familial loading. For example, children with a high familial loading for MDD are around three times more likely to develop the illness (Williamson et al., 2004) and, as previously mentioned, likely to experience the illness earlier in life (Kendler et al., 2006; Lohoff, 2010; Thapar et al., 2012; Tozzi et al., 2008; Weissman et al., 1987; Williamson et al., 2004). Furthermore, those with a high familial loading for MDD appear to be more sensitive to the detrimental effects of stress as they are more likely to develop an episode of MDD after experiencing stressful life events than those with a low familial loading (Kendler et al.,

1995; Monroe et al., 2014). Additionally, once individuals with a high familial loading for MDD have developed the illness, they are more likely to suffer from recurrent major depressive episodes than individuals with a low familial loading (Colvin, Richardson, Cyranowski, Youk, & Bromberger, 2014; Lieb, Isensee, Höfler, Pfister, & Wittchen, 2002).

Moreover, MDD patients with varying familial loadings for the illness not only appear to differ on when and for how long they suffer with the illness but may also differ on how they experience its symptoms. For example, Tozzi et al. (2008) found that adult MDD patients with a high familial loading for MDD showed greater levels of anxiety and pathological guilt, as well as greater impairments to their daily activities suggesting that familial loading for MDD may lead to differences in the symptoms of the illness. Additionally, further supporting this claim, (Wang et al., 2015) also found that in adult MDD patients, those with a high familial loading for the illness showed greater symptoms of anxiety and anhedonia, and Serretti et al. (2014) found that in adult MDD patients who were not responsive to antidepressant medication, those with a high familial loading for MDD showed greater levels of core symptoms, such as guilt, anxiety, depressed mood, psychomotor retardation, and disruption to daily activities.

MDD patients with differing familial loadings for the illness do therefore appear to differ on key aspects, such as age of onset, recurrence, and symptomology. However, the entirety of the literature investigating differences in symptoms between MDD patients with varying familial loadings for the illness have solely focused on adult MDD, which is surprising given its significance to adolescent MDD in the form of earlier illness onset before the age of 20 years (Tozzi et al., 2008). We therefore conducted the present study to investigate differences in symptomology between adolescent MDD patients with either a high or low familial loading for MDD. We focused our investigations on 7 different measures of symptoms related to MDD, being general symptoms of depression, general symptoms of anxiety, general obsessive-compulsive symptoms, self-criticism, self-efficacy, dependency on others, and rumination symptoms. The previous work in **Chapter 5** demonstrated

that these two patient groups differed on the structure and function of the default mode network, which has been associated with rumination symptoms in MDD (Berman et al., 2011; Hamilton et al., 2015, 2011; Zhu et al., 2012). We therefore particularly focused our investigations on rumination symptoms between these two patient groups, as the neurobiological differences within the default mode network may manifest themselves in differences in rumination, and also investigated whether the relationship between rumination symptoms and aspects of brain structure and function would differ between the two familial loading groups.

6.2. Methods

6.2.1. Participants and self-report measures

Participants were recruited from the IMPACT study (Goodyer et al., 2017). In brief, IMPAPCT was a randomized controlled superiority trial assessing medium-term effects of psychological therapies in adolescent MDD, using patients (*n* = 465) who met the diagnostic requirements for MDD of the DSM-IV (American Psychiatric Association, 1994). Patients completed a variety of different self-report questionnaires, to assess various symptoms and characteristics of MDD.

The Moods and Feelings Questionnaire (MFQ; Angold et al., 1995) was administered to all patients as a measure for general symptoms of depression, and a subsample of the patients (*n* = 381) completed the Ruminative Response Scale (RRS; Nolen-Hoeksema, 1991), as a measure of rumination symptoms.

The Depressive Experiences Questionnaire (DEQ; Blatt, Schaffer, Bers, & Quinlan, 1992) was also administered. It contains three subscales, which were completed by a subsample of patients, that measure self-efficacy (*n* = 379), dependency on others (*n* = 375), and self-criticism (*n* = 373).

Furthermore, the Revised Children's Manifest Anxiety Scale (RCMAS; Reynolds & Richmond, 1997) was administered as a measure of general anxiety symptoms and was completed by almost all patients (*n* = 464), and the Short Leyton Obsessional Inventory (LOI; Bamber, Tamplin, Park, Kyte, & Goodyer, 2002) was also administered to a subsample of patients (*n* = 443), as a measure of obsessive compulsive symptoms.

As detailed in **Chapter 5,** patients were assessed as either having a high or low familial loading for MDD, based on self-reported family history of MDD – with those reporting having at least one first degree relative with MDD being classed as having a high familial loading.

This produced a sample of 94 MDD patients who were classified as having a high familial loading for MDD, and 126 patients who were classified as having a low familial loading. As not all patients

completed every symptom measure, there were different numbers of high and low familial loading patients for each measure.

For the MFQ, the was data from 94 high familial loading patients and 126 low familial loading patients.

For the DEQ, there was data from 75 high and 107 low familial loading patients for the self-efficacy subscale, 77 high and 107 low familial loading patients for the dependency subscale, and data from 76 high and 104 low familial loading patients on the self-criticism subscale.

For the RCMAS, data was collected from 94 high and 126 low familial loading patients.

For the LOI, data was obtained from 88 high and 124 low familial loading patients.

For the RRS, there was data from 76 high familial loading patients and 103 low loading patients. Of these patients, 28 high and 24 low familial loading patients had structural MRI data, and 25 high and 23 low familial loading patients had functional MRI data.

6.2.2. Statistical analyses of demographic and symptom measures

Statistical testing for demographic and symptom measures was done with SPSS (version 25; IBM, 2017) using a statistical threshold of $p = 0.05$. Independent samples *t*-tests were used for comparing differences in age between high and low familial loading groups, and a chi-square test was used to compare differences in gender proportions, and medication status between the two patient groups.

As the number of MDD patients who completed each symptom assessment varied for each measure, we conducted individual univariate analyses on each symptom measure. This was done using an ANCOVA, controlling for age, gender, and medication status. Each univariate analysis was Bonferroni corrected to control for multiple comparisons. Cohen's *d* (Cohen, 1988) was used as a measure of effect size.

6.2.3. Neuroimaging analyses

To investigate differences between the high and low familial loading groups in the relationship between rumination symptoms and brain structure and function, familial loading-by-rumination symptom interactions in both grey matter volume and resting-state functional connectivity were conducted.

The same methods as previously described in **Chapters 2, 3, 4,** and **5** were used for the pre-processing of structural and functional images. For structural analyses, familial loading-by-rumination interactions in grey matter volume were conducted using a mask encompassing the default mode network, controlling for age, gender, and SMFQ scores.

For analyses investigating resting-state functional connectivity, seed-based methods were used with the left and right hippocampus, left and right precuneus, and left and right subgenual anterior cingulate cortex used as seed regions. Additional analyses were conducted to control for antidepressant medication status.

6.3. Results

6.3.1. Demographics

There were no differences between high and low familial loading MDD patients in age, $t(218) = 1.704$, $p = 0.0899$; or gender, $\chi^2(1) = 1.143$, $p = 0.285$. The two patient groups did however differ on medication status, with high familial loading patients being more likely to be taking antidepressant medications, $\chi^2(1) = 6.639$, $p = 0.00998$, $\Phi = 0.175$, shown in **Table 6.1**.

Table 6.1. Shows the demographic details of high and low familial loading MDD patients, for each self-report measure.

Group	Mean Age (SD), range	Gender Proportion (% Female)	Antidepressant Medication use (% taking)
Dependency on Others (DEQ)			
Low, *n* = 107	15.44 (1.26), 12.00-17.92	81.3	18.7
High, *n* = 77	15.14 (1.52), 11.42-17.76	70.1	31.2
Self-Efficacy (DEQ)			
Low, *n* = 107	15.41 (1.26), 12.00-17.92	81.3	18.7
High, *n* = 75	15.15 (1.51), 11.42-17.76	72.0	32.0
Self-Criticism (DEQ)			
Low, *n* = 104	15.42 (1.28), 12.00-17.92	82.7	19.2
High, *n* = 76	15.16 (1.50), 11.42-17.76	72.4	31.6
General Obsessive-Compulsive Symptoms (LOI)			
Low, *n* = 124	15.35 (1.32), 11.71-17.92	79.0	16.9
High, *n* = 88	15.12 (1.51), 11.30-17.76	72.7	33.0
General Depression Symptoms (MFQ)			
Low, *n* = 126	15.34 (1.31), 11.71-17.92	78.6	16.7
High, *n* = 94	15.02 (1.53), 11.30-17.76	72.3	30.9
General Anxiety Symptoms (RCMAS)			
Low, *n* = 126	15.34 (1.31), 11.71-17.92	78.6	16.7
High, *n* = 94	15.01 (1.53), 11.30-17.76	72.3	30.9
Rumination Symptoms (RRS)			
Low, *n* = 103	15.40 (1.25), 11.71-17.92	81.6	18.4
High, *n* = 76	15.03 (1.54), 11.42-17.76	75.0	30.3

6.3.2. Symptom measures

There were no significant differences in general symptoms of anxiety, $F(1, 213) = 7.204$, $p_{corr} = 0.0549$, or general symptoms of depression, $F(1, 213) = 6.169$, $p_{corr} = 0.0964$.

There were also no differences between high and low familial loading MDD patients on self-efficacy, $F(1, 175) = 0.256$, $p_{corr} = 1.000$, dependency on others, $F(1, 177) = 0.130$, $p_{corr} = 1.000$, self-criticism, $F(1, 173) = 0.766$, $p_{corr} = 1.000$, or obsessive-compulsive symptoms, $F(1, 205) = 1.448$, $p_{corr} = 1.000$.

The two patient groups did however differ on symptoms of rumination, with high familial loading MDD patients showing lower rumination symptoms than the low familial loading patients, $F(1, 172) = 9.340$, $p_{corr} = 0.0182$, $d = 0.50$, shown in **Figure 6.1** and **Figure 6.2**.

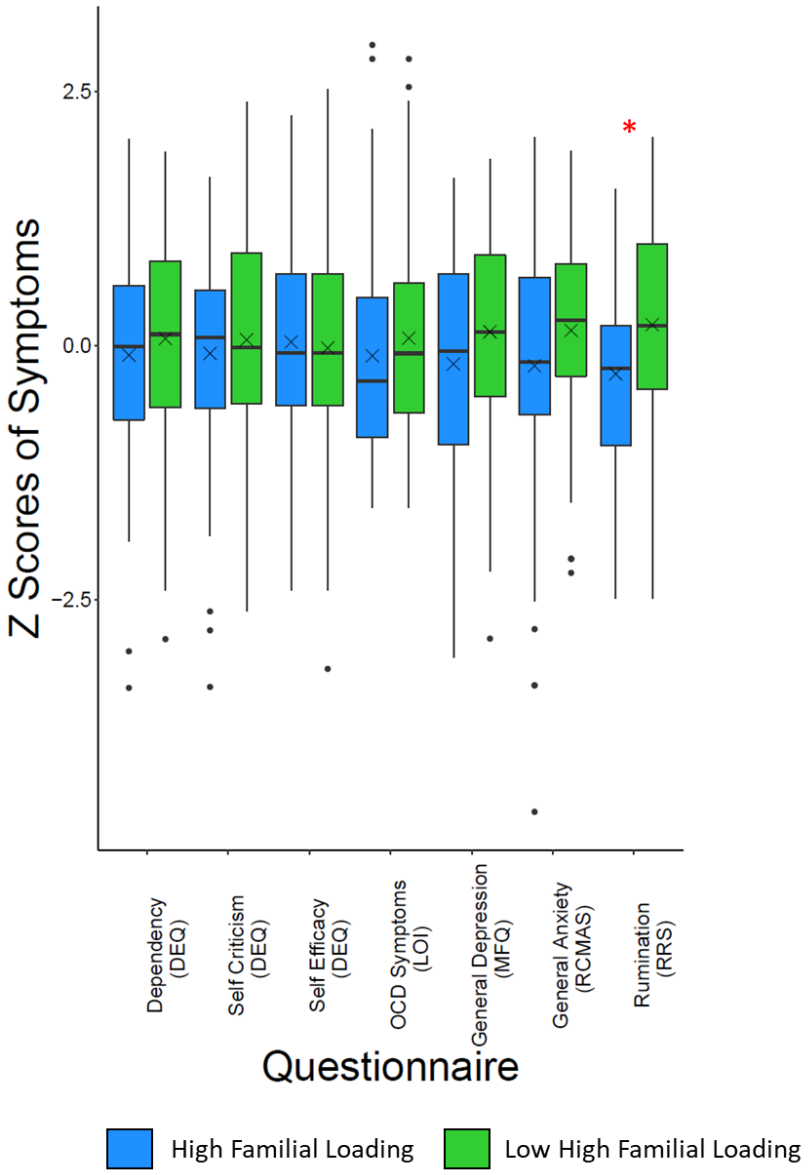


Figure 6.1. Shows the symptom profiles of the two familial loading MDD patient groups.

* *pcorr* < 0.05

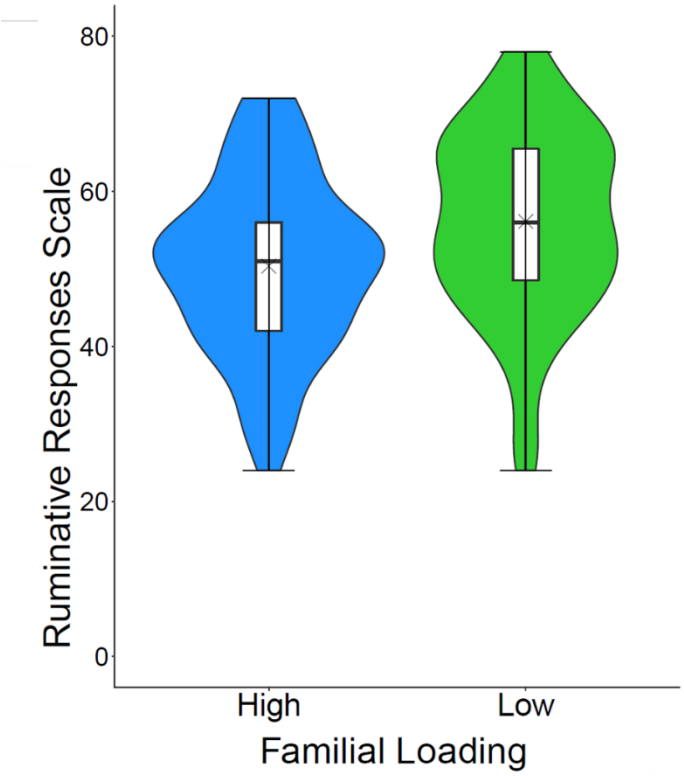


Figure 6.2. Shows the lower rumination symptoms in high familial loading MDD patients.

6.3.3. Neuroimaging analyses

For those with both structural MRI there were no significant differences between high or low familial loading MDD patients in rumination symptoms, $F(1, 48) = 0.128$, $p = 0.722$, age, $t(50) = 0.061$, $p = 0.952$, or medication status $\chi^2(1) = 0.745$, $p = 0.388$. There were also no differences between the two patient groups in SMFQ scores, $t(50) = 0.675$, $p = 0.503$, and STAI-S scores, $t(50) = 1.797$, $p = 0.0780$. However high hereditary loading patients showed lower STAI-T scores, $t(50) = 2.033$, $p = 0.047$, $d = 0.570$, and had a higher proportion of males, $\chi^2(1) = 4.309$, $p = 0.038$, $\Phi = 0.288$.

For those who had functional data, there were no significant differences between high and low familial loading MDD patients in rumination symptoms, $F(1, 44) = 0.0210$, $p = 0.885$, age, $t(46) = 0.515$, $p = 0.609$, gender, $\chi^2(1) = 3.714$, $p = 0.0540$, or medication status $\chi^2(1) = 2.146$, $p = 0.143$. There were also no differences between the two patient groups in SMFQ scores, $t(46) = 0.749$, $p = 0.458$, or STAI-S scores, $t(46) = 1.702$, $p = 0.0960$, but again high familial loading patients had lower STAI-T scores, $t(46) = 2.147$, $p = 0.0370$, $d = 0.623$.

6.3.3.1. Grey matter volume

There was a familial loading-by-rumination interaction in grey matter volume, with the heritable loading groups showing the opposite relationship between grey matter volume and rumination symptoms within the left medial frontal gyrus, shown in **Figure 6.3** and **Table 6.2**.

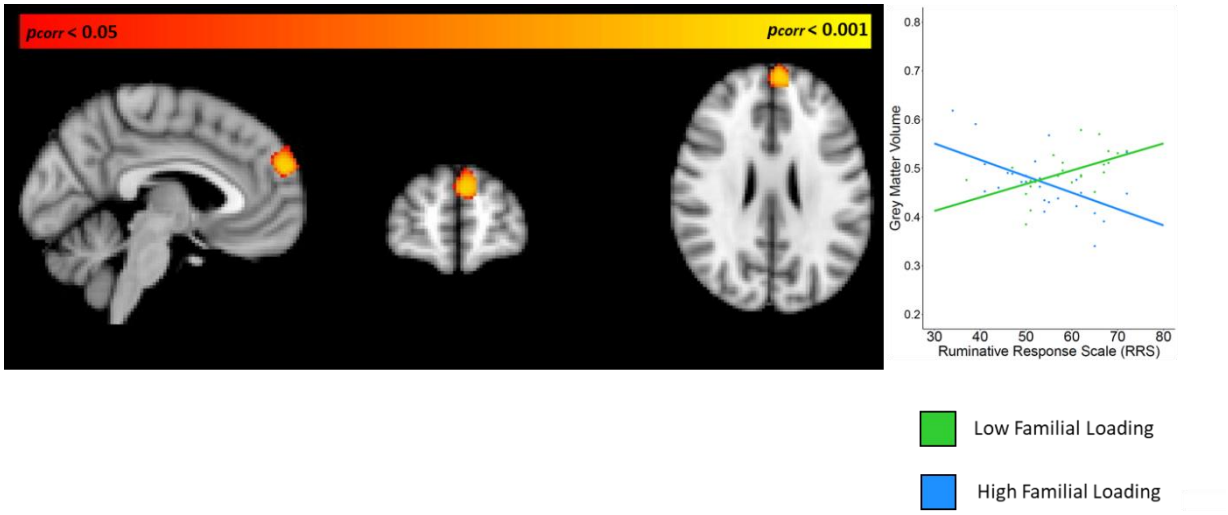


Figure 6.3. Shows the familial loading-by-rumination interaction in grey matter volume, within the medial frontal gyrus.

Table 6.2. Shows details about the cluster within the precuneus that showed a familial loading-by-rumination interaction in grey matter volume.

	Number of Voxels	***Peak pcorr*-value**	**Peak *t*-score**	**Peak MNI Coordinates**	**Regions involved (peak region in bold)**
Grey Matter Volume Cluster	281	0.009	4.74	43, 92, 49	**Left medial frontal gyrus**

6.3.3.2. Resting-state functional connectivity

There were familial loading-by-rumination interactions in resting-state functional connectivity, with the heritable loading groups showing the opposite relationship between resting-state functional connectivity and rumination symptoms, found in the connectivity between the medial frontal gyri and the left and right precuneus seeds, shown in **Figure 6.4** and **Table 6.3**.

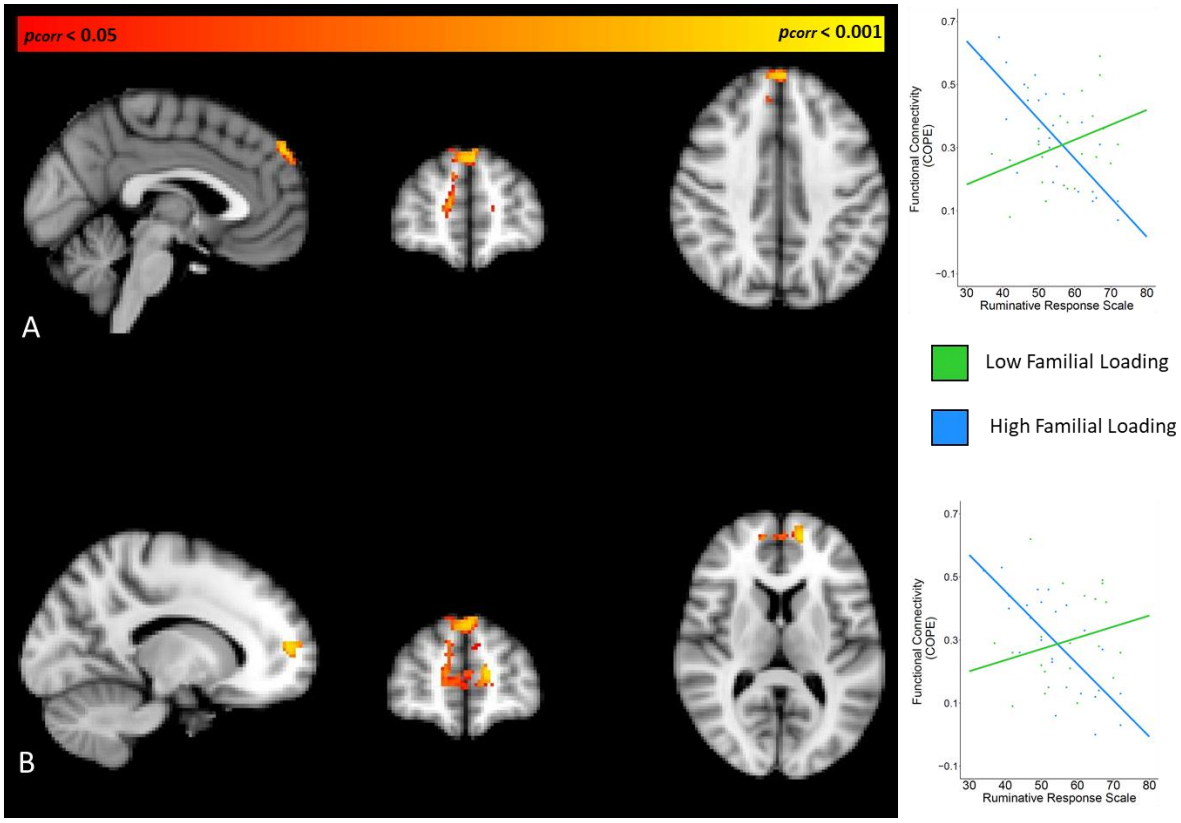


Figure 6.4. Shows the familial loading-by-rumination interactions in resting-state functional connectivity, within the medial frontal gyrus. A) Left precuneus seed. B) Right precuneus seed.

Table 6.3. Shows details about the clusters within the precuneus that showed a familial loading-by-rumination interactions in resting-state functional connectivity.

Seed region	Number of Voxels	*Peak pcorr*-value	Peak *t*-score	Peak MNI Coordinates	Regions involved (peak region in bold)
Left Precuneus Seed	254	0.008	4.35	44, 90, 56	**Left medial frontal gyrus**, right Medial frontal gyrus
Right Precuneus Seed	500	0.006	4.56	39, 90, 41	**Left medial frontal gyrus**, right medial frontal gyrus

6.3.4. Controlling for antidepressant medication status

The significant familial loading-by-rumination interactions in both grey matter volume and resting-state functional connectivity remained after controlling for antidepressant medication status, shown in **Figure 6.5** and **Table 6.4**, and **Figure 6.6** and **Table 6.5**.

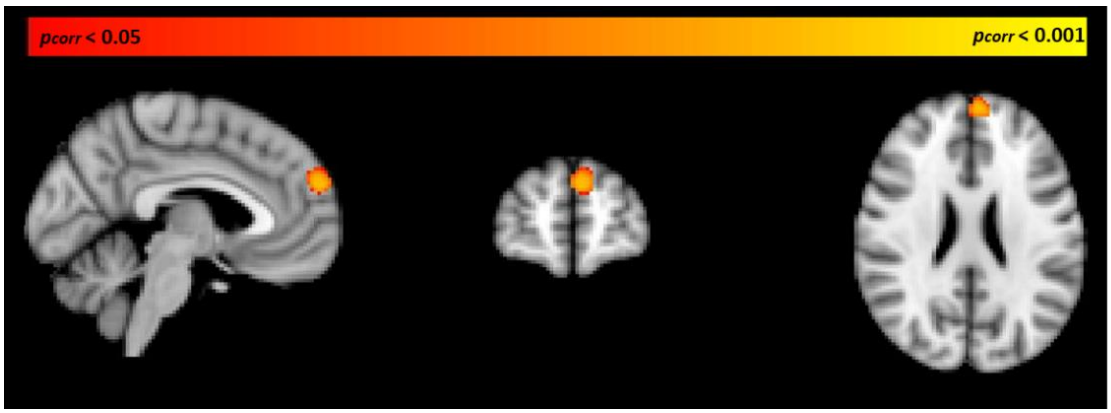


Figure 6.5. The grey matter volume cluster that showed still showed a significant familial loading-by-rumination interaction after controlling for anti-depressant medication status.

Table 6.4. Shows the cluster details of the significant familial loading-by-rumination cluster in grey matter volume, after controlling for anti-depressant medication status.

	Number of Voxels	*Peak pcorr*-value	Peak *t*-score	Peak MNI Coordinates	Regions involved (peak region in bold)
Grey Matter Volume Cluster	204	0.013	4.52	43, 92, 48	**Left medial frontal gyrus**

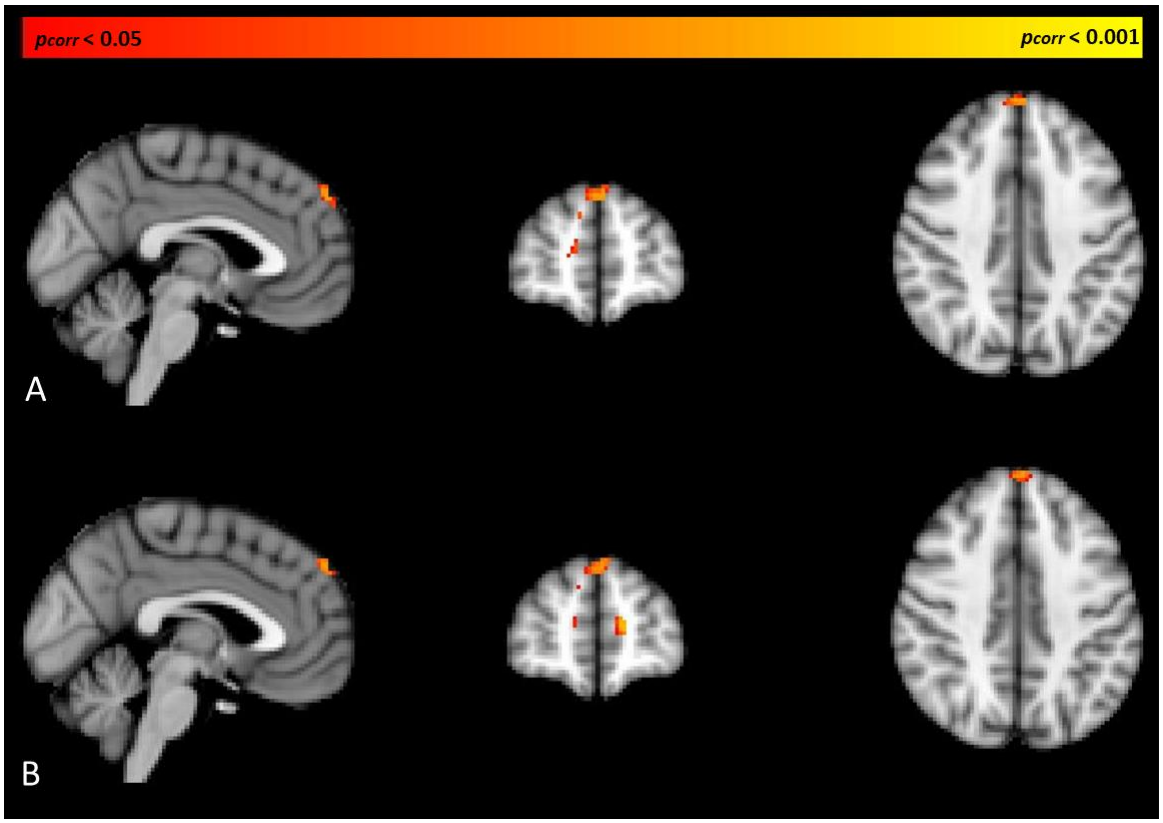


Figure 6.6. The significant clusters in resting-state functional connectivity that showed still showed a significant familial loading-by-rumination interaction after controlling for medication status. A) Left precuneus Seed. B) Right precuneus seed.

Table 6.5. Shows the cluster details of the significant familial loading-by-rumination cluster in resting-state functional connectivity, after controlling for anti-depressant medication status.

Seed region	Number of Voxels	*Peak pcorr*-value	Peak *t*-score	Peak MNI Coordinates	Regions involved (peak region in bold)
Left Precuneus Seed – Cluster 1	3	0.048	4.35	38, 91, 40	**Left medial frontal gyrus**
Left Precuneus Seed – Cluster 2	15	0.033	4.10	51, 90, 43	**Right medial frontal gyrus**
Left Precuneus Seed – Cluster 3	24	0.040	3.85	60, 61, 20	**Right Hippocampus**
Left Precuneus Seed – Cluster 4	38	0.013	4.58	51, 86, 51	**Right medial frontal gyrus**
Left Precuneus Seed – Cluster 5	54	0.018	4.16	44, 90, 56	**Left medial frontal gyrus**, right medial frontal gyrus
Right Precuneus Seed – Cluster 1	5	0.045	3.52	51, 90, 42	**Right medial frontal gyrus**
Right Precuneus Seed – Cluster 2	11	0.040	3.74	48, 89, 38	**Right medial frontal gyrus**
Right Precuneus Seed – Cluster 3	16	0.041	3.72	50, 88, 49	**Right medial frontal gyrus**
Right Precuneus Seed – Cluster 4	59	0.013	4.48	38, 91, 40	**Left medial frontal gyrus**
Right Precuneus Seed – Cluster 5	59	0.020	4.13	44, 90, 57	**Left medial frontal gyrus**, right medial frontal gyrus

6.4. Discussion

Patients with different familial loadings for MDD differed on rumination symptom severity, with high familial loading patients having lower rumination symptoms than low familial loading patients. High loading patients were also more likely to have been prescribed antidepressant medication at the beginning of the study, though this difference was relatively small and did not affect the rumination results when controlling for medication status. Furthermore, there were significant familial loading-by-rumination interactions in both grey matter volume and resting-state functional connectivity, suggesting that the relationship between rumination symptoms and the brain may differ between the two patient groups.

Although the two patient groups did not differ in overall symptoms of depression, MDD patients with a high familial loading showed lower rumination symptoms compared to patients with a low familial loading. This was the only symptom measure that the two familial loading groups varied on, potentially indicating that it may be a distinguishing feature of having a high familial loading for MDD. This does differ from the previous literature focusing on adult MDD, where patients with a high familial loading have generally shown greater symptoms of MDD, such as anhedonia, guilt, and psychomotor retardation (Serretti et al., 2014; Tozzi et al., 2008; Wang et al., 2015). The reason for this contrast between our findings and those of the adult MDD literature are unclear but may demonstrate a difference in the effects of familial loading in adult and adolescent MDD.

One potential reason for the contrast between our findings and those of the adult literature may be due to the duration of the illness between our adolescent sample and those of other samples studying adult MDD patients. MDD patients with a high familial loading have previously been shown to have earlier ages of onset of the illness (Kendler et al., 2006; Lohoff, 2010; Thapar et al., 2012; Tozzi et al., 2008; Weissman et al., 1987; Williamson et al., 2004) and greater levels of recurrence (Colvin et al., 2014; Lieb et al., 2002) than MDD patients with a low familial loading. It may therefore be that adult MDD patients with a high familial loading will have experienced more episodes of

depression and suffered from the illness for a longer period of time, than MDD patients with a low familial loading, and this more extensive experience of MDD may lead to their symptoms becoming worse over time, which may explain why in adulthood, MDD patients with a high familial loading for the illness have previously shown greater symptoms of depression. Alternatively, past research has not previously investigated differences in rumination symptoms in MDD patients with differing familial loadings for the illness, and it may be that whilst high familial loading patients show lower rumination symptoms, they may still show greater levels of symptoms, such as guilt, anhedonia or psychomotor retardation (Serretti et al., 2014; Wang et al., 2015) that were not directly measured in this study. Longitudinal investigations of the symptom profiles of MDD patients with high and low familial loadings for the illness, using more detailed symptom measures, may address this issue.

We also found that adolescent MDD patients with a high familial loading were more likely to have been prescribed antidepressant medication at the beginning of the IMPACT study (Goodyer et al., 2017). A difference in medication prescription between patients with differing familial loadings has previously been observed by Wang et al. (2015), who found that high familial loading MDD patients were more likely to have been prescribed benzodiazepines than those with a low familial loading. Although the effect size was relatively small, this finding may indicate that high familial loading MDD patients present differently to clinicians, leading to differences in medication prescription.

The finding of lower rumination symptoms in high familial loading MDD patients is strikingly in line with the neuroimaging results investigating differences in brain structure and function between the two patient groups, discussed in **Chapter 5**. In those analyses, it was found that high familial loading patients showed both lower grey matter volume within the precuneus, a key region of the default mode network, as well as showing lower resting-state functional connectivity between the precuneus and other regions of the default mode network. As the default mode network has been related to rumination symptoms (Berman et al., 2011; Hamilton et al., 2015, 2011; Zhu et al., 2012), the findings of lower rumination symptoms alongside these neurobiological differences may indicate

that the deviations to the default mode network may manifest themselves in symptom differences between the two patient groups. Furthermore, not only did the two familial loading groups differ in their brain structure and function, and rumination symptoms, but they also differed in how rumination symptoms were related to the structure and function of the medial frontal gyrus, evidenced by the familial loading-by-rumination interactions found in both grey matter volume and resting-state functional connectivity. Together, these differences in brain structure, brain function, rumination symptoms, and the relationship between rumination symptoms and the brain, all indicate that subtyping adolescent MDD patients based on their familial loading for the illness may be both neurologically and clinically meaningful.

In conclusion, adolescent MDD patients with varying familial loadings for the illness show differences in their symptoms of the illness, differences in their brain structure and function, and in how these symptoms relate to the brain. Combined, these findings suggest that adolescent MDD patients can be subtyped based on their familial loading for the illness, which has implications for both how the illness is studied and how it is viewed clinically.

7. General discussion

7.1 Overview of thesis

Overall, the studies in this thesis attempted to clarify the inconsistencies found in the neuroimaging literature investigating adolescent MDD, and explore the nature of CBT's potential effects on disrupted brain function. Much of the past literature had been unclear as to whether structural deviations within the brain are present in adolescent MDD, and if so, in what direction they occur with respect to healthy controls. Moreover, the nature of the disruption to functional brain networks in adolescent MDD was unclear when examining past research, as studies had reported both underconnectivity and overconnectivity within key brain networks implicated with the illness. Additionally, the manner in which CBT may act upon this disrupted brain function in adolescent MDD was relatively poorly described by past research that suffered from multiple methodological issues that hindered its ability to detail the potential mechanisms of CBT. Furthermore, relatively few past studies had attempted to identify neurobiological subtypes of MDD that, given the heterogeneity of symptoms presented in MDD, are vital for addressing the inconsistencies within the neuroimaging literature. The work within this thesis attempted to partially address these issues that had hindered the field's ability to provide a consensus on the neurobiology of adolescent MDD and its treatment. The manner in which this was attempted, the implications for the future of the field, and the weaknesses in the studies within this thesis will be discussed.

7.2. Summary of findings

In **Chapter 2**, we examined structural deviations in adolescent MDD, by focusing on cortical thickness and white matter volume, using the largest studied neuroimaging sample of adolescent MDD patients under the age of 18 years – comparing 109 adolescent MDD patients to 36 healthy adolescent controls. We found that adolescent MDD patients showed greater cortical thickness

within the subgenual anterior cingulate cortex and medial orbitofrontal cortex, which was in contrast to the adult MDD literature, which has predominantly found that adult MDD patients show reduced cortical thickness within these regions. We also found that adolescent MDD patients showed greater white matter volume within predominantly frontal regions of the brain, which contrasted with past work on adolescent MDD patients but was in line with previous findings in adult MDD. These two findings suggested that white matter development, particularly myelination, may deviate in adolescent MDD, which was further supported by the group-by-age interactions found in both the cortical thickness and white matter volume of the anterior cingulate cortex.

We then studied resting-state functional connectivity in **Chapter 3**, comparing 82 adolescent MDD patients, who were scanned before receiving any psychological treatment, to 34 healthy adolescent controls. Using seed-based analyses, we found that adolescent MDD patients showed overconnectivity within the fronto-limbic, default mode, central executive, and salience networks, compared to controls, and that the functional connectivity between the networks themselves was also disrupted in MDD patients. These results were partly in line with the adult MDD literature, particularly regarding overconnectivity within the default mode network, but did contrast with the adult literature, in that adolescent MDD patients showed overconnectivity within the salience network, whereas adult patients have predominantly shown underconnectivity. Surprisingly, we found no relationship between this functional disruption and symptoms of depression or anxiety in patients, nor was the functional disruption related to the structural deviations found when studying cortical thickness and white matter volume.

In **Chapter 4** we focused on CBT and its potential effects on resting-state functional connectivity in adolescent MDD. Using a separate patient sample of 17 adolescent MDD patients who were scanned before and after receiving CBT, and 30 healthy adolescent controls who were also scanned longitudinally, we investigated group-by-time interactions to identify CBT-related changes in resting-state functional connectivity. We found that CBT increased the resting-state functional connectivity

within predominantly the fronto-limbic and default mode networks. We then investigated the relationship between MDD-related pre-treatment functional disruption and CBT-related changes in functional connectivity, to identify whether CBT has a normalising effect on disrupted brain function in adolescent MDD. Instead of showing a normalising effect, we found that CBT-related changes in resting-state functional connectivity were greatest in regions that showed less pre-treatment functional disruption – suggesting that CBT may have a rehabilitative effect on functional connectivity in adolescent MDD, rather than a normalising effect, which had previously been reported in the literature.

We then focused on potential neurobiological subtypes of adolescent MDD in **Chapter 5**, comparing 33 adolescent MDD patients who had a high familial loading for MDD, by having at least one first-degree relative who also suffered from MDD, to 31 patients who did not have a first-degree relative with the illness, and therefore had a low familial loading. We found that MDD patients with a high familial loading showed lower grey matter volume within the precuneus and also showed reduced resting-state functional connectivity within the default mode network. These findings suggested that adolescent MDD patients may differ neurobiologically based on their familial loading for the illness, and that the default mode network in particular may differ both in its function and structure between the two groups, which could eventually lead to their subtyping in future studies.

In **Chapter 6**, to investigate whether this potential subtyping of adolescent MDD patients, based on familial loading for the illness, had any clinically meaningful implications, we compared the symptom profiles of 94 adolescent MDD patients with a high familial loading to 126 patients with a low familial loading. We found that high familial loading adolescent MDD patients had lower rumination symptoms, compared to low familial loading patients, and were more likely to be prescribed antidepressant medications than low familial loading patients. We also investigated the relationship between rumination symptoms and aspects of brain structure and function, finding a familial loading-by-rumination interaction in the functional connectivity and grey matter volume of the

default mode network. These findings demonstrated that adolescent MDD patients with varying familial loadings for the illness also differed on their symptomology and how these symptoms were related to the brain, further supporting the notion of subtyping adolescent MDD patients based on their familial loadings for the illness.

7.3. Brain structure in adolescent major depressive disorder

The literature attempting to identify structural deviations in adolescent MDD, by comparing adolescent MDD patients to healthy adolescent controls, has been consistently unreliable in its findings. Throughout the literature, studies have found greater grey and white matter levels in adolescent MDD patients, compared to controls (Fallucca et al., 2011; Jaworska et al., 2014; Reynolds et al., 2014), others have found reduced levels of these tissue types (Foland-Ross et al., 2015; Gabbay et al., 2012; Kim et al., 2019; Steingard et al., 2002), and others have argued that no structural differences occur in the early stages of the illness' emergence during adolescence (Schmaal et al., 2017; Shad et al., 2012). Moreover, multiple hypotheses and explanations have been proposed attempting to address whether structural deviations actually occur in adolescent MDD and why they may emerge. Some have argued that in adolescent MDD, regions of the brain show atrophy caused by excessive exposure to glucocorticoids (Bremner et al., 2002; MacMaster & Kusumakar, 2004), whilst others have claimed that neurobiological compensatory or protective mechanisms occur in adolescent MDD, leading to increased grey matter within various brain regions (Jaworska et al., 2014), whilst others have even argued that brain function in adolescent MDD is so disrupted that it causes the structural deviations observed in adolescent MDD (MacMillan et al., 2003; Reynolds et al., 2014). One of the largest contributors to this inconsistency and conflict within the literature is that, up until this thesis, no investigation of cortical thickness or white matter volume in adolescent MDD patients under the age of 18 years, has ever surpassed a patient sample size of 64. Moreover, when the final samples of all studies investigating cortical thickness in

adolescent MDD are totalled, excluding this thesis, only 193 adolescent MDD patients under the age of 18 have been used, and only 93 have been used to study white matter volume. This means that the sample used in this thesis contains 36% of all adolescent MDD patients under the age of 18 to have ever been studied in an analysis of cortical thickness focusing on adolescent MDD, and contains 54% of adolescent MDD patients to have ever been studied in an analysis of white matter volume. After acknowledging the extent of how underpowered previous studies of cortical thickness and white matter volume have been in the past, it is not surprising that the literature focusing on these measures has been so inconsistent. In light of this critical flaw within the literature, far more highly powered studies are needed if the field is to move forward in its understanding of brain structure in adolescent MDD.

Given the weaknesses and inconsistency in the past literature, what can actually be concluded regarding brain structure in adolescent MDD? Firstly, based on the findings of this thesis, deviations in cortical thickness and white matter volume do appear to be present within frontal and limbic brain regions in adolescent MDD patients – at least in those under the age of 18 years. This suggestion can be posited with a relative degree of confidence given that the patients used in this sample make up a large proportion of adolescent MDD patients to have ever been studied with respect to cortical thickness and white matter volume – though it should be noted that whilst this sample is large with respect to the past literature, it is still relatively small when attempting to understand an illness as heterogenous as adolescent MDD. However, whether these structural deviations are clinically meaningful and actually play a role in the genesis or experience of MDD during adolescence is unclear. The work within this thesis was unable to link the deviations in cortical thickness and white matter volume to symptoms of depression or anxiety, and much of the past literature has also struggled to find a relationship between adolescent MDD-related structural deviations and symptoms of the illness (Kim et al., 2019; MacMaster & Kusumakar, 2004; Pannekoek et al., 2014; Reynolds et al., 2014; Rosso et al., 2005; Schmaal et al., 2017; Shad et al., 2012; Whittle et al., 2014). Therefore, whilst it can be claimed that structural deviations are present in adolescent

MDD, it is uncertain whether these structural deviations in fronto-limbic regions are pernicious in nature or have a more complex basis.

Secondly, what does appear to be clearer, is that adolescent MDD is characterised by deviating developmental trajectories of brain structure. Although the existence and direction of any overall case-control structural differences in adolescent MDD have been highly debated, the literature has been markedly consistent in suggesting and evidencing deviating developmental trajectories of brain structure in adolescent patients suffering from MDD (Foland-Ross et al., 2015; Hagan et al., 2015a; Matsuo et al., 2008; Pannekoek et al., 2014; Rosso et al., 2005; Steingard et al., 2002; Whittle et al., 2014). We found that patients showed group-by-age interactions in both cortical thickness and white matter volume, alongside (Hagan et al., 2015a) who, using the same sample, also found similar group-by-age interactions in grey matter volume. Moreover, multiple other studies using both cross-sectional and longitudinal designs have indicated that structural deviations occur over time in adolescent MDD, and likely precede the onset of the illness (Foland-Ross et al., 2015; Hagan et al., 2015a; Vulser et al., 2015; Whittle et al., 2014). Given these relatively consistent findings, it does appear that adolescent MDD has a strong neurodevelopmental aspect and highlights a need to further approach the illness from a developmental perspective, utilising more longitudinal designs rather than only cross-sectional comparisons to healthy controls.

During typical adolescent neurodevelopment, the brain undergoes substantial structural changes that affect both grey and white matter. Generally, the rate of myelination accelerates within the brain during adolescence (Lu & Sowell, 2009; Weir et al., 2012), with the prefrontal cortex – including the anterior cingulate and orbitofrontal cortices – being a region that undergoes some of the most substantial structural changes. These processes of myelination lead to improved signal transmission and function within the prefrontal cortex, and is thought to bring about the improvements in cognition, executive function, and emotion regulation observed during the later stages of adolescence (Weir et al., 2012). Therefore, in typical adolescence, measures of grey matter,

such as grey matter volume and cortical thickness, appear to decrease with age as myelin encroaches into the grey matter boundary. Our findings suggests that these neurodevelopmental patterns may be perturbed in adolescent MDD patients. In healthy controls, cortical thickness showed a negative association with age within the pregenual anterior cingulate cortex, as would be expected during typical adolescent brain development. However, in the adolescent MDD group, cortical thickness increased with age – counter to what would be expected during typical adolescent brain development. Alongside the finding that adolescent MDD patients showed greater cortical thickness within the subgenual anterior cingulate and medial orbitofrontal cortices, the work in this thesis suggests that myelination trajectories may be markedly curtailed in adolescent MDD. Given that myelination improves signal transmission of brain regions, its curtailment in regions crucial for emotion processing and regulation may lead to disrupted brain function in brain networks involving the anterior cingulate and medial orbitofrontal cortices, and ultimately contribute to the deficits in emotion processing and regulation observed in adolescent MDD. Furthermore, the likely developmental aspect of the illness warrants further investigation at earlier ages, as if these developmental deviations do indeed occur before its onset, an understanding of how its neurobiological precursors manifest themselves in late childhood and early adolescence may provide a greater understanding of MDD's origins and could have implications for early clinical interventions.

Overall, whilst the work within this thesis does shed some light on the characteristics of cortical thickness and white matter volume in adolescent MDD, and their development throughout the illness, further studies utilising large sample sizes and longitudinal designs at earlier stages in development are required before explanations of the illness' genesis and progression can be proposed with any degree of confidence.

7.4. Pre-treatment brain function in adolescent major depressive disorder

Past work on resting-state functional connectivity in adolescent MDD has implicated four key networks that appear to be disrupted by the illness, being the fronto-limbic, default mode, central executive and salience networks. However, whilst these networks had repeatedly been implicated in MDD, giving rise to hypotheses explaining symptoms of MDD based on their disruption, the direction of their disrupted connectivity was disputed. When investigating pre-treatment functional disruption within our adolescent MDD sample, we found that, in all analyses, pre-treatment functional disruption was characterised by overconnectivity within and between each of the brain networks. This finding of overconnectivity in adolescent MDD has implications for past work that has attempted to explain the illness and its symptoms as being caused by underconnectivity within the brain, which have previously argued that this underconnectivity leads to poor communication within brain networks and to the under regulation of emotion that gives rise to symptoms of MDD (Anand et al., 2005; Carballedo et al., 2011; Pannekoek et al., 2014; Sacchet et al., 2016).

Additionally, the work from this thesis has also demonstrated the importance of the dynamic processes of adolescent MDD, which may lead to neurobiological differences between adolescent and adult forms of the illness. We found that some of the patterns of overconnectivity seen in our adolescent MDD patients differed from previous reports in adult MDD, particularly regarding the resting-state functional connectivity of the amygdala. These differences between adolescent and adult MDD patients highlight a need to view the illness as a progressive disorder that changes over time, rather than simply studying it as the same illness regardless of age. This view that the neurobiological characteristics of MDD change greatly over time further show why longitudinal research in MDD is required in order to gain an understanding of its development and progression.

Moreover, whilst work from this thesis may have partly discerned the direction of disruption to resting-state functional connectivity in adolescent MDD, the clinical meaning and implications of overconnectivity in the illness are still unknown. We were unable to find any links between the

clusters of overconnectivity found in our total adolescent MDD sample and general symptoms of depression or anxiety. Furthermore, much of the past literature has also failed to correlate disruption in functional connectivity in adolescent MDD to general symptoms of the illness (Cullen et al., 2009; Kaiser et al., 2015; Pannekoek et al., 2014; Sacchet et al., 2016). This lack of a clear link between functional overconnectivity and symptoms of MDD makes it unclear as to what these findings mean with respect to the aetiology of the illness. For example, the overconnectivity found within the fronto-limbic network may indicate that the functional inputs from limbic regions overwhelm the regulatory effects of frontal brain regions, leading to overactive emotional responses in adolescent MDD patients. On the other hand, this overconnectivity may conversely suggest an overregulation of emotion from the frontal regions of the network, giving rise to blunted emotional responses to pleasant events and stimuli. This fundamental lack of understanding as to what functional connectivity actually means with respect to emotion and behaviour makes it almost impossible to understand the aetiology of the illness and how it manifests itself in its symptoms. This highlights the need for a far greater understanding of what this commonly studied measure actually signifies in adolescent MDD, as until it is fully understood, the field will struggle to make any headway in comprehending the neurobiological causes of the illness.

7.5. The effects of cognitive behavioural therapy on brain function in adolescent major depressive disorder

Previous work investigating the effects of CBT on brain function in MDD have consistently suggested that CBT has a normalising effect on disrupted brain function (Franklin, Carson, & Welch, 2016; Jacobs et al., 2016; Ritchey et al., 2011; Shou et al., 2017; Straub et al., 2017; Yoshimura et al., 2017). However, much of the literature has suffered from multiple methodological limitations by either not directly investigating pre-treatment functional disruption, not longitudinally studying a healthy control group alongside a patient group, or by not keeping any pre-treatment analyses statistically

independent of post-treatment analyses. These issues have led the field to view the effects of CBT somewhat in the same light as those of antidepressant medications, whereby it normalises MDD-related functional disruption. Conversely, the work in this thesis studying the potential effects of CBT on resting-state functional connectivity did investigate pre-treatment functional disruption, did longitudinally track healthy controls, and most importantly, did maintain statistical independence between pre- and post-treatment analyses. In doing so, we found the exact opposite of the past literature, in that CBT does not appear to have a normalising effect on resting-state functional connectivity in adolescent MDD and instead its effects appear greatest in regions that show the weakest levels of pre-treatment functional disruption. Our findings regarding the potential effects of CBT on brain function demonstrate why both rigorous methodological and statistical designs are required when attempting to study longitudinal effects of treatment in adolescent MDD, as vastly different results with conflicting implications can be generated if these strict designs are not adhered to. This is particularly pertinent to the literature investigating psychotherapy, as psychological therapies are the first line treatment for mild adolescent MDD in the UK (National Institute for Health and Care Excellence, 2019), making it of vital importance that we understand their effects upon the brain.

Moreover, this work suggesting that CBT has a rehabilitative effect on brain function, by targeting brain regions that are least affected by the illness, is partly in line with subjective reports from adolescent patients themselves. Adolescent MDD patients often report that successful treatment from CBT leads to a better understanding of their emotional states, viewing their experiences from different perspectives, and to improved coping strategies (Donnellan, Murray, & Harrison, 2013; Wilmots, Midgley, Thackeray, Reynolds, & Loades, 2019). Importantly, whilst many adolescent MDD patients do report CBT has a positive effect on their illness, they do not necessarily report that their subjective feelings of depression entirely dissipate in a trend that would be expected if CBT had a "normalising" effect on the brain's function. Instead, adolescent MDD patients often report that the therapy has helped them better manage their symptoms in a rehabilitative trend as may be

expected from the work within this thesis. Indeed, even reports from the patients and their parents, used in our sample, further evidence the rehabilitative effects that CBT may have. For example, described in (Hagan et al., 2015b), one patient reported experiencing a lack of control over their emotions when they were depressed:

"It's not really easy to make sense of [because] when you're in that mood, you don't think of anything like you don't think logically, but then like once you've sort of calmed down and everything, I sort of sit and think 'Why was I like that?'" (Hagan et al., 2015b)

Whereas the patient's mother's description of the effects of CBT suggested that whilst her son's emotional symptoms were not necessarily completely alleviated, their ability to manage them and address their own behavior had been rehabilitated by the treatment.

"He's certainly not in the dark place where he was…he can now say 'this is upsetting me' or 'that makes me angry'—he's now able to analyse some of his feelings" (Hagan et al., 2015b)

With this perspective in mind, there appears to be a discrepancy between conclusions from past literature claiming that CBT normalises disrupted brain function in adolescent MDD and the subjective reports from the patients themselves – which this work may have partly revealed. However, it should be noted that different changes in brain function could lead to the same subjective experience, and given that we found no relationship between the changes in brain function and improvements in symptoms of depression, it could equally be possible that a normalising effect on disrupted brain function could still produce similar changes in subjective symptoms of MDD. In line with this, as we were unable to identify which specific aspects of changes in resting-state functional connectivity led to the improvements in symptoms of MDD, multiple different functional changes, including both rehabilitative and normalising effects, could be responsible for symptom improvements.

This discrepancy demonstrates a need for the neuroimaging field to take greater heed from qualitative reports from patients, and potentially use them to help guide the direction and interpretations of its findings – for if this does not happen, the disconnect between neurobiological and patient-centered approaches will hinder the progression of our understanding of adolescent MDD.

7.6. Subtypes of major depressive disorder based on familial loading

A common occurring theme when examining and reviewing the past literature focusing on the neurobiology of adolescent MDD is the inconsistency in its findings. Clearly, as demonstrated earlier in this chapter, the small sample sizes of past studies have greatly contributed to this inconsistency but another potential contributor is the grouping of potentially heterogenous subtypes of MDD patients. In this work, we found that adolescent MDD patients with differing familial loadings for the illness varied in both the brain structure and function of the default mode network, the severity of their rumination symptoms, and how those symptoms related to brain structure and function. This finding that these subtypes of adolescent MDD patients differ both at the neurobiological and symptom level, based on their familial loading for the illness, demonstrates the heterogeneity of adolescent MDD and how it may have contributed to the inconsistencies of past neurobiological case-control comparisons.

Moreover, this subtyping of adolescent MDD patients, based on familial loading, demonstrates the heterogeneity of the illness and why far more emphasis is needed on identifying its subtypes if the field's understanding of the illness is to progress. For example, according to both DSM-5 and the older DSM-IV criteria, there are nearly 1000 possible combinations of symptoms that can lead to a diagnosis of MDD (Fried & Nesse, 2015; Østergaard et al., 2011), with it being possible for two MDD patients to have no symptoms in common. This multitude of ways to experience MDD is unlikely to have the exact same neurobiological characteristics and underpinnings, which leads to the

conclusion that subtypes of MDD will exist in order to account for the sheer number of its possible manifestations. Therefore, using methods to help identify these potential subtypes is an avenue that should be thoroughly studied as it is an issue that has likely hindered our understanding of the illness.

Furthermore, aside from explaining the inconsistencies within the past literature, an emphasis on identifying subtypes of MDD could lead to greater leaps in our understanding of which patients may respond best to certain treatments and interventions. In this thesis, we found that adolescent MDD patients differed in rumination symptoms, and it is therefore conceivable that they may respond differently to treatments that specifically target rumination in MDD. Subtyping MDD patients has a potential to change the way the illness is viewed and treated, and a dimensional approach may eventually be required to fully understand the illness, rather than a simple categorisation of either having the illness or not.

7.7 Overall limitations

Whilst the work in this thesis has strengths, its many limitations should be discussed. Firstly, while the sample size used for investigations of brain structure and pre-treatment functional disruption are large in comparison to past neuroimaging studies investigating adolescent MDD, it is still relatively small when attempting to obtain a complete understanding of the neurobiology of an illness as heterogenous as adolescent MDD. In particular, while the size of the MDD group was large, a sample size of 36 healthy controls is small and will likely have hindered the statistical power of analyses investigating group differences between the MDD and healthy control groups. Substantially larger sample sizes, numbering in the thousands, are required to provide a truly conclusive understanding of brain structure and function in adolescent MDD. Similarly, the sample size used for analyses investigating the potential effects of CBT is again small, though unfortunately this is common for the field. Therefore, it is possible that while only changes to the fronto-limbic and

default mode networks were associated with receiving CBT, functional changes to both the central executive and salience networks may have been present but undetected due to the study's lack of power. Moreover, when investigating differences between adolescent MDD patients with differing familial loadings for the illness, the neuroimaging and clinical samples were again small, and these findings should be viewed with caution and be seen as exploratory.

Moreover, adolescent MDD patients with co-morbid anxiety were included in these studies. Co-morbid anxiety is extremely common in adolescent MDD, and the exclusion of patients with co-morbid anxiety would have made the sample unrepresentative of the adolescent MDD population. However, it is therefore unclear to what extent the findings in this thesis can be attributed to depression itself or anxiety. For example, in the study investigating potential effects of CBT on brain function, both symptoms of depression and anxiety were ameliorated after adolescent MDD patients received CBT, and as there were no associations between changes in resting-state functional connectivity and improvements in symptoms of depression or anxiety, it is unclear whether the changes observed in functional brain networks occurred in line with the improvement to symptoms of either depression or anxiety.

Additionally, methodological issues of the work in this thesis should be discussed. The neuroimaging studies relied on the SMFQ (Angold et al., 1995) as a measure of current symptoms of depression. While this questionnaire is short and easy to administer, by only having 13 items to assess symptoms of depression it likely failed to capture the full range of symptoms that participants with MDD were experiencing. This lack of precision in such a key measure for these studies likely hindered their ability to detect an association between aspects of brain structure and function, and may explain why no associations were found between the SMFQ and any neuroimaging measures. This explanation is further supported when viewing the results regarding rumination symptoms in **Chapter 6**. Here a much more precise measure was used to find an association between symptoms and neuroimaging measures, focusing on one specific symptom of MDD. In these analyses,

associations were indeed found between both the structure and function of the default mode network and rumination symptoms, suggesting that more precise and specific measures of depressive symptoms are required in order to understand how they relate to the brain.

Another methodological limitation of the neuroimaging studies conducted in this thesis is their reliance on a priori brain regions. In **Chapter 2**, analyses relied on the subgenual anterior cingulate and medial orbitofrontal cortex as key regions of interest. In **Chapters 3, 4 and 5,** seed-based approaches using predetermined regions as seeds were applied, primarily focusing on masks of predetermined functional brain networks. While all a priori brain regions used in these studies were well-justified and were often complimented by whole brain analyses, this reliance on predetermined brain regions as the focus of neuroimaging investigations may have hindered their ability to identify other brain regions that might be important in the neurobiology of MDD, as they were not as thoroughly investigated.

Finally, the studies in this thesis investigating the possibility of subtyping adolescent MDD patients based on their familial loading should be viewed with caution, due to their exploratory nature. Both the IMPACT and MR-IMPACT studies were not designed to investigate potential effects of familial loading on symptoms of MDD or its neurobiological characteristics. Due to this, the measure used to assess familial loading for MDD may not have been adequate to fully capture the extent of an individual's family history of MDD. The results regarding familial loading therefore require replication using substantially larger samples sizes and more precise measures specifically designed to assess familial loading for MDD.

7.8 Conclusion

This thesis has identified some of the neural characteristics of adolescent MDD, potential mechanisms of CBT's effects upon the depressed adolescent brain, as well as identifying potential

subtypes of the illness. However, whilst some light has been shed on the neurobiological underpinnings of the illness, this work is a mere flicker of what is required if we are to understand the illness in a way that provides meaningful improvements to the lives of adolescents suffering from MDD, and far more work is needed before the workings of the illness can be fully illuminated.

8. References

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9. Appendix

Table 9.1. Shows details of the significant clusters in cortical thickness, comparing medication-free MDD patients to controls.

Cortical thickness cluster	Number of voxels	*Peak pcorr*-value	Peak *t*-score	Peak MNI Coordinates	Cohen's *d*	Regions involved (peak region in bold)
A) Case-control – Cluster 1	41	0.024	3.71901	49, 82, 35		**Left subgenual anterior cingulate**, left medial orbitofrontal cortex
A) Case-control – Cluster 2	68	0.026	3.43852	41, 81, 31		Right anterior cingulate, **right medial orbitofrontal cortex**
B) Case-control – Cluster 1	18	0.04	4.33793	60, 49, 72		Left precentral gyrus, **left postcentral gyrus**
B) Case-control – Cluster 2	427	0.016	4.91213	46, 49, 71		**Right precentral gyrus**, left supplementary motor area, right supplementary motor area, right postcentral gyrus, left paracentral lobule, right paracentral lobule,
C) Group-by-age interaction	627	0.006	4.07601	47, 81, 41		Left pregenual anterior cingulate, **right pregenual anterior cingulate**
D) Group-by-age interaction	3	0.048	4.02823	46, 53, 50		**Left midcingulate**

Table 9.2. Shows details of the significant clusters in white matter volume, comparing medication-free MDD patients to controls.

White matter volume cluster	Number of voxels	*Peak pcorr*-value	Peak *t*-score	Peak MNI Coordinates	Regions involved (peak region in bold)
A) Case-control	544	0.015	3.72219	39, 90, 51	Left superior frontal gyrus, right superior frontal gyrus, left medial frontal gyrus, **right medial frontal gyrus**
B) Case-control – cluster 1	399	0.014	4.71327	31, 56, 31	Right insula, **right hippocampus**, right amygdala, right putamen, right pallidum
B) Case-control – cluster 2	435	0.028	3.86595	59, 57, 31	**Left hippocampus, left** parahippocampus, left amygdala, left caudate, right caudate, left putamen, left pallidum, left insula
C) Group-by-age interaction	83	0.30	3.34585	51, 81, 42	**Right pregenual anterior cingulate**

Table 9.3. Shows the significant clusters in resting-state functional connectivity, when using whole-brain analyses to compare adolescent MDD patients to healthy controls.

Seed Region	Number of voxels	*Peak pcorr*-value	Peak *t*-score	Peak MNI Coordinates	Regions showing greater connectivity (peak region in bold)
Right Amygdala -Cluster 1	6	0.048	3.91	62, 77, 27	**Right orbital part of the inferior frontal gyrus**
Right Amygdala -Cluster 2	6	0.049	3.27	71, 62, 40	**Right Rolandic operculum**
Right Amygdala -Cluster 3	9	0.048	3.78	68, 55, 39	**Right Heschl's gyrus**
Right Amygdala -Cluster 4	796	0.024	4.95	66, 70, 31	Right orbital part of the inferior frontal gyrus, right Rolandic operculum, right gyrus rectus, **right insula**, right putamen, right pallidum
Right Subgenual Anterior Cingulate - Cluster 1	2	0.049	3.16	27, 65, 40	**Right medial frontal gyrus**
Right Subgenual Anterior Cingulate - Cluster 2	2	0.049	3.38	28, 66, 54	**Left inferior frontal gyrus**
Right Subgenual Anterior Cingulate - Cluster 3	4	0.049	3.42	28, 69, 32	**Left caudate**
Right Subgenual Anterior Cingulate - Cluster 4	4	0.049	3.37	23, 68, 37	**Right insula**
Right Subgenual Anterior Cingulate - Cluster 5	5	0.049	3.62	29, 57, 32	**Left precentral gyrus**
Right Subgenual Anterior Cingulate - Cluster 6	9	0.049	3.39	34, 89, 39	**Right gyrus rectus**
Right Subgenual Anterior Cingulate - Cluster 7	17	0.044	4.15	41, 79, 33	**Left gyrus rectus**
Right Subgenual Anterior Cingulate - Cluster 8	21	0.047	3.50	59, 55, 38	**Left caudate**
Right Subgenual Anterior Cingulate - Cluster 9	22	0.047	3.50	64, 81, 45	**Left pallidum**
Right Subgenual Anterior Cingulate - Cluster 10	26	0.047	3.28	56, 95, 35	**Left insula**
Right Subgenual Anterior Cingulate - Cluster 11	34	0.044	3.97	36, 91, 44	Left precentral gyrus, **left middle frontal gyrus**
Right Subgenual Anterior Cingulate - Cluster 12	42	0.033	5.52	65, 42, 19	**Right caudate**
Right Subgenual Anterior Cingulate - Cluster 13	55	0.042	3.67	53, 96, 40	**Left middle frontal gyrus**

Table 9.4. Shows the different groupings of the seeds and in order of effect size strength, when correlating case-control *z*-scores to group-by-time interaction *z*-scores.

	Seed Grouping				
Order of effect size strength, for averaged target regions	**Fronto-limbic Network**	**Default Mode Network**	**Central Executive Network**	**Salience Network**	**Average of all 118 Seed Regions**
1st	Medial orbitofrontal Gyrus Right	Medial orbitofrontal Gyrus Right	Medial orbitofrontal Gyrus Right	Lobules I and II of Vermis	Medial orbitofrontal Gyrus Right
2nd	Lobule X of Cerebellum Right	Lobule IX of Vermis	Posterior Cingulate Right	Lobule X of Vermis	Lobule X of Cerebellum Left
3rd	Paracentral Leftobule Left	Lobule VIII of Vermis	Lobule X of Cerebellum Left	Superior Parietal Lobule Right	Lobule III of Cerebellum Left
4th	Pallidum Left	Lobules I and II of Vermis	Heschls Gyrus Left	Pars Opercularis Right	Lobule VIII of Vermis
5th	Paracentral Leftobule Right	Lobule X of Cerebellum Left	Putamen Left	Lobule X of Cerebellum Left	Posterior Cingulate Right
6th	Pars Opercularis Left	Lobule X of Cerebellum Right	Lobule VIII of Vermis	Middle Temporal Pole Right	Lobule X of Cerebellum Right
7th	Superior Frontal Gyrus Right	Paracentral Leftobule Right	Heschls Gyrus Right	Superior Frontal Gyrus Right	Lobules I and II of Vermis
8th	Posterior Cingulate Left	Rolandic Oper Right	Orbital Part of Middle Frontal Right	Pars Opercularis Left	Superior Parietal Lobule Left
9th	Lobules I and II of Vermis	Amygdala Left	Orbital part of Superior Frontal Gyrus Left	Insula Left	Paracentral Leftobule Left
10th	Lobule X of Cerebellum Left	Paracentral Leftobule Left	Postcentral Gyrus Left	Rectus Right	Lobule IX of Vermis
11th	Lobule VIII of Vermis	Pars Opercularis Left	Putamen Right	Medial orbitofrontal Gyrus Left	Putamen Left
12th	Hippocampus Left	Superior Frontal Gyrus Right	Rectus Right	Orbital Part of Middle Frontal Right	Paracentral Leftobule Right
13th	Lobules IV and V of Cerebellum Right	Lobule VIIb of Cerebellum Right	Superior Parietal Lobule Left	Medial Frontal Gyrus Right	Middle Temporal Pole Right
14th	Heschls Gyrus Left	Parahippocampus Left	Superior Temporal Pole Right	Posterior Cingulate Right	Lobule X of Vermis
15th	Supramarginal Gyrus Left	Lobule III of Cerebellum Left	Angular Gyrus Left	Subgenual Anterior Cingulate Left	Supplementary Motor Area Right
16th	Parahippocampus Left	Lobule III of Vermis	Amygdala Left	Medial Frontal Gyrus Left	Pars Opercularis Left
17th	Lobule III of Cerebellum Left	Lobule VIII of Cerebellum Right	Pallidum Right	Lobule X of Cerebellum Right	Insula Left
18th	Lobule III of Cerebellum Right	Hippocampus Left	Lobule X of Cerebellum Right	Angular Gyrus Left	Postcentral Gyrus Left
19th	Orbital part of Superior Frontal Gyrus Left	Superior Occipital Gyrus Left	Paracentral Leftobule Left	Inferior Temporal Gyrus Right	Superior Frontal Gyrus Right
20th	Orbital Part of Middle Frontal Right	Supramarginal Gyrus Left	Superior Frontal Gyrus Left	Medial orbitofrontal Gyrus Right	Middle Temporal Pole Left
21st	Lobule VIIb of Cerebellum Right	Lobules IV and V of Cerebellum Right	Orbital part of Middle Frontal Gyrus Left	Middle Temporal Pole Left	Hippocampus Left
22nd	Superior Occipital Gyrus Left	Lobule X of Vermis	Superior Frontal Gyrus Right	Lobule III of Cerebellum Right	Amygdala Right

23rd	Lobule VIII of Cerebellum Right	Pallidum Left	Pallidum Left	Heschls Gyrus Right	Rectus Right
24th	Lobule IX of Vermis	Lobule VI of Cerebellum Left	Lobule III of Cerebellum Left	Putamen Left	Orbital part of Superior Frontal Gyrus Left
25th	Lobule III of Vermis	Putamen Left	Amygdala Right	Lobule VIIb of Cerebellum Right	Lobule III of Cerebellum Right
26th	Amygdala Left	Heschls Gyrus Right	Rectus Left	Hippocampus Left	Subgenual Anterior Cingulate Right
27th	Precentral Gyrus Right	Lobule III of Cerebellum Right	Posterior Cingulate Left	Middle Temporal Gyrus Left	Pallidum Right
28th	Precentral Gyrus Left	Middle Temporal Pole Left	Medial orbitofrontal Gyrus Left	Lobule III of Cerebellum Left	Orbital Part of Middle Frontal Right
29th	Putamen Left	Lobules IV and V of Vermis	Inferior Parietal Lobule Left	Paracentral Leftobule Left	Pallidum Left
30th	Rolandic Oper Right	Precentral Gyrus Left	Inferior Occipital Cortex Left	Pars Triangularis Right	Rectus Left
31st	Superior Frontal Gyrus Left	Lobule IX of Cerebellum Right	Middle Temporal Pole Left	Pregenual Anterior Cingulate Left	Superior Temporal Pole Right
32nd	Lobule IX of Cerebellum Right	Superior Frontal Gyrus Left	Hippocampus Left	Paracentral Leftobule Right	Subgenual Anterior Cingulate Left
33rd	Posterior Cingulate Right	Superior Parietal Lobule Right	Lobule VI of Vermis	Middle Frontal Gyrus Left	Inferior Parietal Lobule Left
34th	Middle Temporal Pole Right	Middle Temporal Pole Right	Paracentral Leftobule Right	Supplementary Motor Area Right	Posterior Cingulate Left
35th	Supramarginal Gyrus Right	Pars Triangularis Right	Hippocampus Right	Amygdala Right	Lobule III of Vermis
36th	Angular Gyrus Left	Heschls Gyrus Left	Medial Frontal Gyrus Left	Superior Parietal Lobule Left	Superior Frontal Gyrus Left
37th	Rectus Right	Postcentral Gyrus Left	Lobules IV and V of Vermis	Middle Frontal Gyrus Right	Lobule VIIb of Cerebellum Right
38th	Superior Temporal Pole Right	Insula Left	Insula Left	Lobule VI of Cerebellum Right	Amygdala Left
39th	Insula Left	Orbital part of Superior Frontal Gyrus Left	Pars Orbitalis Left	Inferior Parietal Lobule Right	Pars Opercularis Right
40th	Pars Triangularis Left	Orbital Part of Middle Frontal Right	Lobules I and II of Vermis	Orbital part of Superior Frontal Gyrus Left	Medial Frontal Gyrus Left
41st	Inferior Occipital Cortex Left	Pars Opercularis Right	Lobule IX of Vermis	Rectus Left	Heschls Gyrus Left
42nd	Lobule VI of Cerebellum Right	Inferior Parietal Lobule Left	Pregenual Anterior Cingulate Right	Superior Frontal Gyrus Left	Heschls Gyrus Right
43rd	Pregenual Anterior Cingulate Left	Middle Occipital Gyrus Left	Precuneus Right	Putamen Right	Angular Gyrus Left
44th	Pars Opercularis Right	Superior Parietal Lobule Left	Lobules IV and V of Cerebellum Left	Rolandic Oper Right	Medial Frontal Gyrus Right
45th	Supplementary Motor Area Right	Supplementary Motor Area Right	Subgenual Anterior Cingulate Right	Lobule III of Vermis	Supramarginal Gyrus Right
46th	Pregenual Anterior Cingulate Right	Putamen Right	Supramarginal Gyrus Left	Pregenual Anterior Cingulate Right	Putamen Right
47th	Pars Triangularis Right	Lobule IX of Cerebellum Left	Lobule VIIb of Cerebellum Right	Pallidum Left	Orbital part of Middle Frontal Gyrus Left
48th	Middle Temporal Pole Left	Posterior Cingulate Left	Pars Opercularis Right	Lobule IX of Vermis	Olfactory Cortex Left
49th	Calcarine Sulcus Left	Precentral Gyrus Right	Middle Temporal Gyrus Left	Pars Triangularis Left	Lobule VII of Vermis
50th	Inferior Parietal Lobule Left	Rolandic Operculum Left	Insula Right	Precuneus Right	Lobule VI of Vermis

51st	Olfactory Cortex Left	Postcentral Gyrus Right	Supplementary Motor Area Right	Angular Gyrus Right	Rolandic Oper Right
52nd	Medial orbitofrontal Gyrus Left	Pregenual Anterior Cingulate Left	Olfactory Cortex Right	Crus I of Cerebellum Right	Lobules IV and V of Vermis
53rd	Pallidum Right	Fusiform Gyrus Left	Caudate Left	Lobules IV and V of Cerebellum Right	Supramarginal Gyrus Left
54th	Fusiform Gyrus Left	Posterior Cingulate Right	Precentral Gyrus Right	Pallidum Right	Medial orbitofrontal Gyrus Left
55th	Putamen Right	Pars Orbitalis Right	Superior Temporal Pole Left	Orbital part of Middle Frontal Gyrus Left	Fusiform Gyrus Left
56th	Medial Frontal Gyrus Left	Cuneus Left	Superior Parietal Lobule Right	Lobule IX of Cerebellum Right	Middle Frontal Gyrus Right
57th	Amygdala Right	Lobule VIIb of Cerebellum Left	Supplementary Motor Area Left	Supplementary Motor Area Left	Superior Parietal Lobule Right
58th	Postcentral Gyrus Left	Pars Orbitalis Left	Supramarginal Gyrus Right	Fusiform Gyrus Left	Supplementary Motor Area Left
59th	Lobules IV and V of Cerebellum Left	Rectus Left	Fusiform Gyrus Left	Insula Right	Hippocampus Right
60th	Heschls Gyrus Right	Medial Frontal Gyrus Left	Middle Temporal Pole Right	Pars Orbitalis Right	Parahippocampus Right
61st	Lobule IX of Cerebellum Left	Lobule VI of Vermis	Precuneus Left	Lobule VIII of Vermis	Pars Triangularis Right
62nd	Medial Frontal Gyrus Right	Cuneus Right	Rolandic Operculum Left	Lobule IX of Cerebellum Left	Lobule VI of Cerebellum Right
63rd	Superior Parietal Lobule Right	Pars Triangularis Left	Subgenual Anterior Cingulate Left	Supramarginal Gyrus Left	Pregenual Anterior Cingulate Left
64th	Precuneus Left	Lobule VI of Cerebellum Right	Middle Frontal Gyrus Right	Supramarginal Gyrus Right	Inferior Occipital Cortex Left
65th	Rectus Left	Orbital part of Superior Frontal Gyurus Right	Pars Triangularis Right	Precentral Gyrus Right	Parahippocampus Left
66th	Parahippocampus Right	Rectus Right	Rolandic Oper Right	Crus II of Cerebellum Right	Lobule VIII of Cerebellum Right
67th	Lobule VII of Vermis	Lobules IV and V of Cerebellum Left	Lobule IX of Cerebellum Left	Inferior Parietal Lobule Left	Lobule VIIb of Cerebellum Left
68th	Lobule VI of Vermis	Superior Temporal Pole Right	Medial Frontal Gyrus Right	Lobules IV and V of Vermis	Precentral Gyrus Right
69th	Orbital part of Middle Frontal Gyrus Left	Amygdala Right	Lobule III of Vermis	Calcarine Sulcus Right	Precuneus Right
70th	Cuneus Left	Medial orbitofrontal Gyrus Left	Inferior Parietal Lobule Right	Calcarine Sulcus Left	Middle Temporal Gyrus Left
71st	Superior Parietal Lobule Left	Orbital part of Middle Frontal Gyrus Left	Lobule VIII of Cerebellum Right	Lobule VI of Vermis	Lobules IV and V of Cerebellum Right
72nd	Middle Temporal Gyrus Left	Pallidum Right	Pregenual Anterior Cingulate Left	Olfactory Cortex Left	Rolandic Operculum Left
73rd	Supplementary Motor Area Left	Medial Frontal Gyrus Right	Olfactory Cortex Left	Middle Temporal Gyrus Right	Pars Orbitalis Left
74th	Pars Orbitalis Right	Crus II of Cerebellum Right	Postcentral Gyrus Right	Lobule VIII of Cerebellum Left	Pars Orbitalis Right
75th	Insula Right	Inferior Occipital Cortex Left	Lingual Gyrus Right	Inferior Occipital Cortex Left	Midcingulate Left
76th	Lobules IV and V of Vermis	Supplementary Motor Area Left	Pars Orbitalis Right	Hippocampus Right	Cuneus Left
77th	Hippocampus Right	Insula Right	Parahippocampus Left	Lobules IV and V of Cerebellum Left	Lobule IX of Cerebellum Left

78th	Middle Occipital Gyrus Left	Inferior Temporal Gyrus Right	Fusiform Gyrus Right	Lobule VIIb of Cerebellum Left	Lobule IX of Cerebellum Right
79th	Postcentral Gyrus Right	Fusiform Gyrus Right	Lobule VIIb of Cerebellum Left	Fusiform Gyrus Right	Lobules IV and V of Cerebellum Left
80th	Precuneus Right	Precuneus Left	Caudate Right	Heschls Gyrus Left	Inferior Temporal Gyrus Right
81st	Lobule VI of Cerebellum Left	Parahippocampus Right	Angular Gyrus Right	Cuneus Right	Pregenual Anterior Cingulate Right
82nd	Lobule X of Vermis	Hippocampus Right	Middle Occipital Gyrus Right	Posterior Cingulate Left	Midcingulate Right
83rd	Middle Frontal Gyrus Left	Supramarginal Gyrus Right	Precentral Gyrus Left	Rolandic Operculum Left	Middle Temporal Gyrus Right
84th	Middle Frontal Gyrus Right	Caudate Left	Superior Temporal Right	Superior Temporal Gyurs Left	Olfactory Cortex Right
85th	Rolandic Operculum Left	Angular Gyrus Left	Lobules IV and V of Cerebellum Right	Subgenual Anterior Cingulate Right	Pars Triangularis Left
86th	Midcingulate Left	Pregenual Anterior Cingulate Right	Lobule VI of Cerebellum Right	Caudate Left	Superior Occipital Gyrus Left
87th	Pars Orbitalis Left	Subgenual Anterior Cingulate Left	Orbital part of Superior Frontal Gyurus Right	Lobule VIII of Cerebellum Right	Angular Gyrus Right
88th	Lobule VIIb of Cerebellum Left	Olfactory Cortex Right	Pars Opercularis Left	Inferior Temporal Gyrus Left	Caudate Left
89th	Crus II of Cerebellum Right	Precuneus Right	Lobule VII of Vermis	Inferior Occipital Cortex Right	Insula Right
90th	Crus II of Cerebellum Left	Inferior Temporal Gyrus Left	Crus II of Cerebellum Left	Pars Orbitalis Left	Precentral Gyrus Left
91st	Superior Temporal Pole Left	Middle Frontal Gyrus Left	Midcingulate Left	Superior Temporal Pole Left	Lobule VI of Cerebellum Left
92nd	Orbital part of Superior Frontal Gyurus Right	Subgenual Anterior Cingulate Right	Middle Temporal Gyrus Right	Precuneus Left	Calcarine Sulcus Left
93rd	Subgenual Anterior Cingulate Left	Thalamus Right	Calcarine Sulcus Left	Parahippocampus Right	Fusiform Gyrus Right
94th	Inferior Temporal Gyrus Left	Middle Temporal Gyrus Left	Inferior Temporal Gyrus Left	Postcentral Gyrus Right	Inferior Temporal Gyrus Left
95th	Middle Temporal Gyrus Right	Midcingulate Left	Lobule III of Cerebellum Right	Middle Occipital Gyrus Left	Superior Temporal Pole Left
96th	Cuneus Right	Middle Frontal Gyrus Right	Middle Frontal Gyrus Left	Superior Temporal Right	Inferior Parietal Lobule Right
97th	Midcingulate Right	Lobule VII of Vermis	Pars Triangularis Left	Amygdala Left	Thalamus Right
98th	Fusiform Gyrus Right	Thalamus Left	Middle Occipital Gyrus Left	Superior Temporal Pole Right	Crus II of Cerebellum Left
99th	Superior Occipital Gyrus Right	Superior Occipital Gyrus Right	Crus II of Cerebellum Right	Precentral Gyrus Left	Postcentral Gyrus Right
100th	Crus I of Cerebellum Left	Calcarine Sulcus Left	Thalamus Right	Crus II of Cerebellum Left	Middle Frontal Gyrus Left
101st	Lingual Gyrus Left	Crus I of Cerebellum Right	Superior Occipital Gyrus Left	Postcentral Gyrus Left	Precuneus Left
102nd	Thalamus Right	Lobule VIII of Cerebellum Left	Crus I of Cerebellum Right	Olfactory Cortex Right	Cuneus Right
103rd	Olfactory Cortex Right	Midcingulate Right	Lingual Gyrus Left	Orbital part of Superior Frontal Gyurus Right	Middle Occipital Gyrus Left
104th	Inferior Temporal Gyrus Right	Lingual Gyrus Left	Midcingulate Right	Superior Occipital Gyrus Right	Crus I of Cerebellum Right
105th	Caudate Left	Calcarine Sulcus Right	Cuneus Left	Middle Occipital Gyrus Right	Crus II of Cerebellum Right

106th	Thalamus Left	Lingual Gyrus Right	Crus I of Cerebellum Left	Midcingulate Right	Caudate Right
107th	Angular Gyrus Right	Crus II of Cerebellum Left	Thalamus Left	Lingual Gyrus Right	Calcarine Sulcus Right
108th	Inferior Parietal Lobule Right	Middle Occipital Gyrus Right	Lobule IX of Cerebellum Right	Thalamus Left	Superior Temporal Right
109th	Lingual Gyrus Right	Superior Temporal Gyurs Left	Cuneus Right	Cuneus Left	Orbital part of Superior Frontal Gyurus Right
110th	Superior Temporal Gyurs Left	Angular Gyrus Right	Parahippocampus Right	Superior Occipital Gyrus Left	Lobule VIII of Cerebellum Left
111th	Subgenual Anterior Cingulate Right	Olfactory Cortex Left	Lobule VIII of Cerebellum Left	Midcingulate Left	Superior Occipital Gyrus Right
112th	Calcarine Sulcus Right	Inferior Parietal Lobule Right	Calcarine Sulcus Right	Lobule VI of Cerebellum Left	Middle Occipital Gyrus Right
113th	Lobule VIII of Cerebellum Left	Inferior Occipital Cortex Right	Inferior Temporal Gyrus Right	Parahippocampus Left	Lingual Gyrus Right
114th	Superior Temporal Right	Superior Temporal Pole Left	Inferior Occipital Cortex Right	Thalamus Right	Inferior Occipital Cortex Right
115th	Crus I of Cerebellum Right	Middle Temporal Gyrus Right	Superior Occipital Gyrus Right	Crus I of Cerebellum Left	Lingual Gyrus Left
116th	Caudate Right	Superior Temporal Right	Superior Temporal Gyurs Left	Caudate Right	Thalamus Left
117th	Inferior Occipital Cortex Right	Crus I of Cerebellum Left	Lobule VI of Cerebellum Left	Lingual Gyrus Left	Crus I of Cerebellum Left
118th	Middle Occipital Gyrus Right	Caudate Right	Lobule X of Vermis	Lobule VII of Vermis	Superior Temporal Gyurs Left