EXAMINING THE IMPACT OF CHILDHOOD TRAUMA AND GENETIC RISK FACTORS ON MYELIN INTEGRITY IN MAJOR DEPRESSIVE DISORDER; CLINICAL AND THERAPEUTIC IMPLICATIONS

EXAMINING THE IMPACT OF CHILDHOOD TRAUMA AND GENETIC RISK FACTORS ON MYELIN INTEGRITY IN MAJOR DEPRESSIVE DISORDER; CLINICAL AND THERAPEUTIC IMPLICATIONS

By

ERICA L. TATHAM
B.Sc (Honours)

A Thesis submitted to
The School of Graduate Studies in Partial
Fulfillment of the Requirements for the Degree
Master of Science

McMaster University

EXAMINING THE IMPACT OF CHILDHOOD TRAUMA AND GENETIC RISK FACTORS ON MYELIN INTEGRITY IN MAJOR DEPRESSIVE DISORDER; CLINICAL AND THERAPEUTIC IMPLICATIONS

TABLE OF CONTENTS

Table of Content- Figures and Tables

Prelude

This project was carried out as a result of collaboration between Dr. Geoffrey Hall, a neuroimaging expert at McMaster University, and Dr. Rajamannar Ramasubbu, an academic psychiatrist and clinical investigator at the University of Calgary. This study assessed depression severity, history of childhood adversity, genetic polymorphisms in BDNF and 5-HTTLPR, and collected diffusion tensor imaging scans in medication-free individuals with major depressive disorder and in controls. Following baseline assessment, patients underwent an 8-week treatment regime and were randomly assigned to either citalopram or quetiapine XR (extended release) anti-depressant treatment. Depression severity was monitored throughout the treatment regime and at endpoint.

The data for this study was collected at the Hotchkiss Brain Institute at the University of Calgary. Clinical assessments of depression diagnosis and severity, collection of blood samples for genotyping and diffusion tensor imaging scan data were collected under the supervision of Dr. Ramasubbu. Blood samples were genotyped for polymorphisms in the brain derived neurotrophic factor (BDNF)(val66met) and serotonin transporter linked polymorphic region (5-HTTLPR) under the supervision of Dr. Jane Foster, a neuroscientist at McMaster University. Tract-based spatial statistic and probabilistic tractography analyses were completed on the diffusion tensor imaging data, under the supervision of Dr. Geoffrey Hall.

Previous research has determined that genetic risk factors and early childhood adversity influence the development of depression (Caspi, 2003).

Furthermore, previous research has determined that depression significantly influences myelin microstructure (M. L. Murphy & Frodl, 2011). Extending these findings, the present investigation aimed to investigate whether depression severity, severity of childhood maltreatment and genetic risk factors influence measures of myelin integrity (i.e. fractional anisotropy). Furthermore, this investigation will delineate the different types of childhood adversity to determine their differential impact on neuroconnectivity.

Diffusion tensor imaging may also have diagnosis and prognostic potential for major depressive disorder. The present investigation attempts to extend original studies in geriatric populations (Alexopoulos, Kiosses, Choi, Murphy, & Lim, 2002) to further understand the implications diffusion tensor imaging has in predicting depression prognosis following treatment. Furthermore, this study is advantageous as it assesses the influence genetic risk factors have on treatment response, and how these genetic factors moderate the influence neuroconnectivity has on treatment response.

These studies strongly contribute to the major depression literature, and provide insights on whether gene and environment factors alter neuroconnectivity in major depression, and how these neuroconnectivity outcomes influence response to antidepressant medication.

References:

Alexopoulos, G. S., Kiosses, D. N., Choi, S. J., Murphy, C. F., & Lim, K. O. (2002). Frontal white matter microstructure and treatment response of late-life depression: a preliminary study. *American Journal of Psychiatry*, *159*(11), 1929–1932.

Caspi, A. (2003). Influence of Life Stress on Depression: Moderation by a Polymorphism in the 5-HTT Gene. *Science*, *301*(5631), 386–389. doi:10.1126/science.1083968

Murphy, M. L., & Frodl, T. (2011). Meta-analysis of diffusion tensor imaging studiesshows altered fractional anisotropy occurring indistinct brain areas in association with depression. *Biology of Mood & Anxiety Disorders*, *1*(1), 3. doi:10.1186/2045-5380-1-3

CHAPTER 1: **White Matter Integrity Changes in Major Depressive Disorder: Implications of 5-HTTLPR and BDNF Polymorphisms, and Childhood Trauma**

Abstract

Early life stress has been found to be a strong predictor of depression severity, with genetic risk factors such as the serotonin transporter promotor (5-HTTLPR) and brain derived neurotrophic factor (BDNF) polymorphisms moderating depression development. Our investigation aims to extend these findings to examine the impact of depression severity, genetic risk factors, and childhood maltreatment on neuronal connectivity changes using tract based spatial statistics (TBSS) and probabilistic tractography. Fifty-five medication-free patients with major depressive disorder (MDD) [$\bar{x}$ age: 36.4, M/F: 22/33] and 18 controls [$\bar{x}$ age: 33.2, M/F: 8/10] underwent diffusion tensor imaging scanning, genotyping and completed the Childhood Trauma Questionairre. Corrected TBSS findings revealed trends toward significantly reduced FA in the right anterior internal capsule [p=0.051], fornix [p=0.085] and right anterior corona radiata [p=0.084] in the MDD group. Probabilistic tractography analysis examined fractional anisotropy (FA) in the cingulum bundle, uncinate fasciculus and superior longitudinal fasciculus. Individuals scoring high in depression severity and who experienced severe childhood physical neglect (PN) and emotional neglect (EN) had higher FA in the uncinate [PN: p=0.003, EN: p=0.029] and superior longitudinal fasciculus [PN: p=0.0748], with BDNF and 5-HTTLPR moderating these associations. BDNF polymorphisms also exhibited a stronger impact on uncinate FA in individuals with high depression severity, with val-BDNF exhibiting higher FA than met carriers [p=0.021]. In conclusion, MDD patients exhibit widespread decreases in FA across

many neural regions. Furthermore, the impact that depression severity has on FA is considerably influenced by early life neglect.

1.0 Introduction

1.1 Depression

1.1.1 What is Depression?

Major Depressive Disorder (MDD) is characterized by changes in mood that result in anhedonia, sadness, changes in appetite, fatigue, insomnia or oversleeping, irritability, feelings of worthlessness, difficulty completing executive function based tasks and thoughts of suicide. Women are particularly vulnerable to develop depression, with approximately twice as many women experiencing a depressive episode each year [women: 12%, men: 7%] (Nolen-Hoeksema, 1987) and within a lifetime [women 21%, men: 12%] (Kessler et al., 2005). Depression is a heritable disorder with estimated heritability coefficients of 0.36-0.66 (Sullivan, Neale, & Kendler, 2000).

1.1.2 Treatment for Depression

Depression can be treated with a variety of antidepressants including selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAO-Is), tricyclic antidepressants (TCAs), serotonin-norepinephrine reuptake inhibitors (SNRIs). SSRI's are the most prescribed type of antidepressant as they have minimal side effects. The pronounced effectiveness of SSRIs has been taken as evidence to support the Serotonin Theory of depression, which hypothesizes that disruption in the serotonin (5-HT) system is the basis for depression development.

SSRI's exert their effects by acting at the junction between two communicating neurons, called the synapse. Neurons communicate by releasing neurotransmitters from the presynaptic terminal of one neuron and binding to post-synaptic terminal receptor sites on another neuron. Following neurotransmitter (e.g. serotonin) release, excess neurotransmitter is reabsorbed from the synapse to the presynaptic terminal. SSRI's function by decreasing the amount of presynaptic re-uptake, resulting in a net increase in synapse serotonin and potentially serotonin signaling. Knowledge of this mechanism provides the theoretical rationale for depression research examining the serotonin transporter system.

1.1.3 Depression and the HPA-axis

Another system studied in depression is the hypothalamic-pituitary-adrenal axis, which is postulated to be hyperactive in a significant proportion of depressed individuals, making them hypervigilant and vulnerable to stressful life experiences. Previous research has found an interaction between the 5-HT system and the HPA axis. Mouse research indicates that maternal separation at p.n.d. 2-14 resulted in a reduction of 5-HT cell firing in the raphe nucleus and hippocampal glucocorticoid receptor density, reflecting poor HPA feedback inhibition. Additionally, Meaney et al. (2000) found that hippocampal glucocorticoid receptor changes following early life stress are dependent on serotonin (Heim et al., 2000). Experiences of early life stress are predictive of depression later in life and changes in cortisol profiles (Kang et al., 2011; Heim, Plotsky & Nemeroff, 2004). Morning cortisol levels have delineated experience of childhood abuse and neglect, with experiences of abuse and neglect associated with higher and blunted levels of cortisol, respectively(Bruce,

Fisher, Pears & Levine, 2009). Exposure to early life stress can suppress glial cell division and influence neuronal hormones like cortisol and catecholamines. This can critically impact myelination processes, neurogenesis, and selective pruning; ultimately reducing FA in myelinated tracts (Lauder, 1983; Teicher et al., 2003).

1.2 Diffusion Tensor Imaging

1.2.1 Tract Based Spatial Statistics

Neuroimaging is a tool that can be used to better understand the neuroanatomical and physiological changes associated with a disorder and aid in the diagnosis and treatment of depression (Mayberg, 2003). One relatively new neuroimaging method is diffusion tensor imaging (DTI). This technique allows non-invasive tracking of water perfusion in the brain, and is indicative of myelin integrity and thus neuronal connectivity between associated brain regions (See Diffusion Tensor Imaging below for more information). Myelination refers to the natural production of the myelin sheath from glial cells. Myelin sheath is a fatty tissue that insulates neurons and allows for fast and efficient transmission of information between brain regions. When neurons are heavily myelinated, water perfusion is highly restricted to a specific axis. Fractional anisotropy (FA) is a DTI parameter that reflects a ratio of directional to non-directional water movement in a single imaging voxel and provides information on axon size, myelination, axon connections and orientation. In regions with reduced white matter structure integrity and axon connectivity, FA has lower values. The potential use of diffusion tensor imaging as a tool to guide treatment has promoted investigations on the

predictive nature of FA on depression severity, illness course/duration and treatment outcomes.

Fractional anisotropy has been shown to be reduced in frontal regions in individuals with depression (Li et al., 2007). White matter abnormalities are also found in tracts and regions including the superior longitudinal fasciculus (Dalby et al., 2010; Wu et al., 2011; Zuo et al., 2012; meta-analysis by Frodl & Murphy, 2011), the fornix (K. Zou et al., 2008), internal capsule (Guo et al., 2012; Zhu et al., 2011; K. Zou et al., 2008)
(Zuo et al., 2012), external capsule (Guo et al., 2012), corpus callosum (Guo et al., 2012; Kieseppä et al., 2010), sagittal stratum (Kieseppä et al., 2010), hippocampus (Zhou et al., 2011; Zhu et al., 2011), cingulum (Keedwell et al., 2012), uncinate fasciculus (Cullen et al., 2010; Dalby et al., 2010) and projection fibers associated with the thalamus (Korgaonkar, Cooper, Williams, & Grieve, 2012).

Based on the above findings, it is evident that there are a multitude of regions and tracts within overlapping neural circuits that are associated with depression. More specifically, dysregulation and abnormal connectivity in depression has repeatedly been reported in the limbic-dorsolateral prefrontal cortex (dlPFC)-thalamic circuit that encompasses many of the above mentioned regions (Korgaonkar et al., 2012). Another region in the limbic-dlPFC-thalamus circuit, whose neuronal integrity correlates with depression severity is the anterior internal capsule (K. Zou et al., 2008; Zuo et al., 2012). This structure integrates frontal cortical structures with subcortical regions such as the thalamus(Mori et al., 2008). This information provides further evidence for the pivotal role of the internal

capsule in the development of depression and potential use of DTI as a diagnostic tool for depression.

When investigating the regions most substantially affected by depression, Korgaonkar and colleagues (2010), found that FA changes in the fornix, a structure that connects the septohippocampal system, to be most significant. This region provides serotonin transmission from the midbrain raphe to the hippocampus and plays a major role in the mediation of anxiety (Degroot & Treit, 2004). The 13.5% decrease in FA was also accompanied by increased radial diffusivity and axial diffusivity, which are additional DTI parameters that indicate disruption in myelin integrity {Korgaonkar:2010iu}. Although not part of the limbic-dlPFC-thalamus circuit, changes in this region could greatly impact serotonin transmission and mood regulation.

1.2.2 Probabilistic Tractography

Although the majority of diffusion tensor imaging investigations on depression have used voxel-wise comparisons, a relatively more recent analytical method called tractography is gaining in popularity. Tractography allows for the remodeling of neural tracts and the ability to assess average FA across an entire tract. This method also allows the specification of a seed region to assess fractional anisotropy in any tract emerging from that particular seed. With a seed in the subgenual anterior cingulate cortex (sgACC), Cullen et al. (2010) found substantial reductions in FA in the right uncinate fasciculus, a tract that connects the sgACC and amygdala, in adolescents with depression (Cullen et al., 2010). The depression literature also indicates activation abnormilties in the sgACC through fMRI studies

(Mayberg, 2003). Furthermore, Keedwell et al. (2012) demonstrated an 8% reduction in FA in this region in individuals with a family history of depression. This same group also found a 3 and 5% reduction in FA in the right and left cingulum bundles, which correlated with reductions in hedonic tone (an assessment of positive affect)(Keedwell et al., 2012).

1.3 Depression Theories

1.3.1 Environmental Adversities

Depression is thought to be a result of environmental and genetic risk factors. Although a specific risk factor has yet to be determined to cause depression, there are many candidate genes and specific adversities that have been highly correlated with the onset, chronicity and outcome of depression. One environmental risk factor associated with depression onset is stressful life events, particularly early life stress. The prevalence of childhood maltreatment in the general population is quite substantial with approximately 41% of men, and 30% of women experiencing at least one form of child maltreatment (Scher, Forde, McQuaid, & Stein, 2004). Epidemiological studies have revealed that stress or emotional trauma are associated with increased risk for depression, particularly when experiencing stress early in life(Agid, Kohn, & Lerer, 2000). Experiencing a stressful experience in childhood has also been associated with an earlier onset, more chronic depression (Heim & Nemeroff, 2001) and a higher number of co-morbidities (Bernet & Stein, 1999). Additionally, women who were abused as children are four times more likely to develop depression symptoms relative to women who were not abused. The

extent of the abuse also correlates with severity of depression symptomology in adulthood (Mullen, Martin, Anderson, Romans, & Herbison, 1996).

Childhood maltreatment has been associated with both physical and psychological effects. Furthermore, physical types of abuse can lead to psychological outcomes, which are associated with more negative long-term effects (Claussen & Crittenden, 1991). These types of experiences can have lasting effects on an individual's physiological wellbeing. Animal studies have found that early adversities result in alterations to the serotonin system that persists into adulthood (Barr et al., 2004). This manuscript will focus on 5 distinct types of childhood maltreatment as outlined by Bernstein's (2003) Childhood Trauma Questionairre, and include: physical abuse, emotional abuse, sexual abuse, physical neglect and emotional neglect (Bernstein et al., 2003). Abuse consists of frightening, hurtful, and/or threatening actions by the caregiver directed at the child. Neglect, on the other hand, constitutes a lack of the required and expected basic necessities by the caregiver, and can take several forms. Following experiences of physical abuse, children exhibit enhanced attention to negative emotional cues, while neglected children demonstrate deficits in discriminating emotional expressions (Pollak & Sinha, 2002; Pollak & Tolley-Schell, 2003; Pollak, Cicchetti, Hornung, & Reed, 2000). Experiences of self-report childhood abuse are also more strongly associated with experiences of other forms of maltreatment relative to neglect, which seems to be more isolated. In addition, subgroups of adversity have been delineated on depression severity (Anguilera et al., 2009), emotion processing (Pollak, Cicchetti, Hornung & Reed, 2000), morning cortisol levels (Bruce, Fisher, Pears & Levine,

2009) and grey matter volumes changes (Edmiston et al. 2011). These findings exemplify the kinds of processing, physiological and neuroanatomical changes that have origins during childhood and may contribute to long-term changes in mental health and wellbeing.

1.3.2 Early Environmental Adversities – Neuroanatomical/Function changes

The childhood maltreatment literature indicates that early exposure to adversity is a consistent, reliable and pervasive risk factor for depression during adulthood. Childhood maltreatment is also associated with a more chronic courses of depression in adulthood (G. W. Brown & Harris, 2008). This close association between maltreatment and depression, further explains the overlap of brain regions associated with depression, stress and childhood maltreatment. Specifically, reductions in volume and function of the hippocampus, prefrontal cortical and fronto-limbic circuitry are seen in adults exposed to maltreatment experiences as children as well as in depressed individuals (Teicher, Andersen, Polcari, Anderson, & Navalta, 2002; Treadway et al., 2009; van Harmelen et al., 2010). It has been suggested that since childhood adversity has such a pivotal role in depression development, depressed individuals who have experienced early life stress may be a subgroup of depression based on their distinct physiological response to stress and neuroanatomical differences (Heim et al., 2000; Vythilingam et al., 2002). Neural volumetric changes following early life stress have found reductions in the anterior cingulate cortex, hippocampus, insula, orbitofrontal cortex and caudate nuclei, (Carballedo, Lisiecka, et al., 2012b; Cohen et al., 2006; Dannlowski et al., 2012; Hart, 2012; Pollak et al., 2010), and increases in amygdala volume in previously

institutionalized children (Tottenham et al., 2010). However, in an examination of the structural and functional neuronal changes associated with early life stress, regression analysis has revealed no major impact of childhood trauma on fronto-striatal or limbic circuits (Bren and Walitza, 2012 for review).

Only a few studies have examined connectivity and integrity of white matter tracts in subjects who have experienced childhood adversity. Alterations have been detected in the uncinate fasciculus (connecting the orbitofrontal cortex to the amygdala) (Eluvathingal, 2006), arcinate fasciculus, fornix and cingulum (J. Choi, Jeong, Rohan, Polcari, & Teicher, 2009) and consistent volume reductions in the corpus callosum have been reported (De Bellis & Thomas, 2003; De Bellis et al., 2002; Teicher et al., 2004), see Brem and Walitza, 2012 for review). In fact, it is suggested that the regions to which these tracts project from have different windows of sensitivity to early experience (Teicher et al., 2004). Accordingly, Andersen et al. (2008) suggests that the effects of adversity are most prominent during these sensitive periods or do not manifest until specific critical periods. A cross-sectional study examining grey matter volume suggested that the frontal cortex is especially vulnerable between the ages of 14-16yrs, the hippocampus between 3-5 and 11-13 yrs and corpus callosum between ages 9-10 years (Andersen et al., 2008).

1.3.3 5-HTTLPR

Serotonin system irregularities have been a promising candidate risk factor for the development of depression as disruptions in this system are exhibited in currently depressed individuals and are reversed following anti-depressant

treatment. With the serotonin transporter being the primary target of anti-depressant treatment, this system has been a major focus in depression risk research. The human 5-HT transporter (5-HTT) is encoded by the SLC6A4 gene on chromosome 17p12, and encodes a transmembrane protein that is pivotal in presynaptic re-uptake of serotonin following neurotransmitter release (Lesch et al., 1995). Two 5-HTT polymorphisms are known to regulate the level of 5-HTT gene expression, and therefore expected to influence transporter concentrations. The first, STin2, is a variable number of tandem repeats (VNTR) polymorphism, consisting of 10 and 12 repeats within intron 3 (Lesch et al., 1995). The second is a VNTR in the promoter regions of the 5-HTT gene and is referred to as the serotonin transporter linked polymorphic region (5-HTTLPR)(Heils et al., 1996). The major alleles of the 5-HTTLPR are the short ('S') and long ('L') alleles, consisting of 14 and 16 repeats, respectively. Lesch and colleagues found that these two variants have different transcription efficiencies, with the short allele having significantly reduced transcription efficiency relative to the long allele (Lesch et al., 1996). Further research has determined that the long allele actually consists of two distinct variants, L_A and L_G, with the L_G variants having transcription efficiency significantly less than the L_A, and similar to the S_A variant {Hu:2005eu}. As a result, genetic risk associated within the 5-HTT gene has been examined from both biallelic (S and L) and triallelic (L_A, L_A, and S_A) frameworks.

Although the 5-HTT gene locus has many polymorphic regions (Nakamura, Ueno, Sano, & Tanabe, 2000), the 5-HTTLPR, and more specifically the S allele is most strongly associated with depression and anxiety symptomology(Bellivier et al.,

1998; Collier et al., 1996; Furlong et al., 1998; Lesch et al., 1995; 1996), although conflicting evidence exists (Seretti et al., 1999).

Early positron emission tomography (PET) studies revealed that S allele carriers exhibit reduced binding potentials across all brain regions relative to the LL genotypes. Additionally, genetic variants within the 5-HTTLPR gene, but not single nucleotide polymorphisms in the 5-HT1$_A$ gene, affect 5-HT1$_A$ receptor binding in humans (David, 2005). These original studies had very little power and required very large samples to identify significant results.

1.3.4 5-HTTLPR X Environment

Although there is still a search for candidate risk genes for depression, research has determined that depression is most accurately conferred by a combination of factors, both genetic and environmental. In a substantially powered study of 2,164 individuals, Kendler and colleagues investigated whether genetic and environmental components interact in the development of depression. Genetics seemed to control sensitivity to the depression-inducing effects of stressful life events. Twins with high genetic risk for depression and exposure to stressful life events had an increased risk of developing depression relative to those unexposed to a severe event and/or at low genetic risk (Kendler et al., 1995).

Genetic risk was specifically linked to the 5-HTTLPR in a study by Caspi et al. (2003) indicating increased depression symptoms, diagnosis and suicidality in individuals with 1 or 2 copies of the risk S allele following stressful life events (Caspi, 2003). Supportive results were reported in a subsequent meta-analysis, with stronger effect sizes in individuals with exposure to childhood maltreatment

relative to stressful life events (Karg, 2011). Childhood adversity was associated with higher depression symptomology, which was moderated by the risk S allele (Aguilera et al., 2009). Although research findings and meta-analyses have documented supportive results (Zalsman et al., 2006), (Bennett et al., 2002; Karg, 2011; Taylor et al., 2006) conflicting reports are also evident (Chipman et al., 2007; Risch et al., 2009) (Wilhelm, 2006). Reasons for these discrepancies have been discussed by Uher and McGuffin (2007) {Uher:2007gv}.

Others have suggested that the S allele results in an increased sensitivity to the impact of mild stressors (Kendler, Kuhn, Vittum, Prescott, & Riley, 2005) or environment in general, whether positive or negative (Owens et al., 2012). A cognitive and emotional profile in individuals with the 5-HTTLPR risk allele seemed to make them susceptible to depression and anxiety following experiences of childhood adversity (Owens et al., 2012).

Sibille and Lewis's (2006) review of Parsey's et al. (2006) study, suggests that polymorphisms in the 5-HTTLPR might disrupt serotonin transporter levels in cortical and subcortical brain areas during development, rather than impacting on current levels. Indicating that risk for depression is a result of disruption in early neuron maturation rather than current serotonin state(Sibille & Lewis, 2006). This may explain why Karg et al. (2011) found that childhood adversities conferred stronger depression risk relative to stressful life event experiences 6 months prior to a depressive episode. Although this finding is reflected across the literature, the impact of stressful life events should not be disregarded as interactions have been

found between stressful life events and genetic risk factors in first episode depression (Bukh et al., 2009; Wilhelm, 2006).

1.3.5 5-HTTLPR and Neuroimaging Findings

From the earliest stages of neuroimaging research, depression has been a focus of investigation. PET studies found reductions in activity in the left dorsal anterolateral prefrontal cortex activity, and found that changes in the anterior cingulate cortex (ACC) correlated with improvement in depression scores following medication treatment (Baxter et al., 1989). Due to the high heritability of the disorder, these changes in neural structure and activity may be instantiated by genetic factors.

Neuroanatomical and functional changes associated with depression are also seen in healthy controls whom have depression risk-alleles. For example, abnormalities in the ACC are not only observed in depressed individuals, but also in healthy individuals who are carriers of risk genes for depression. Smaller grey matter volumes in the perigenual cingulate and amygdala were found in healthy s-allele carriers, a previously reported finding in depressed populations. These regions encompass a circuit that is of specific importance for emotional feedback regulation when viewing negative affective stimuli. Altered white matter structure and an uncoupling of this circuit was found in s-allele carriers, and accounted for 30% of the variance in temperamental anxiety (Pacheco et al., 2009; Pezawas et al., 2005).

It is speculated that uncoupling the ACC and amygdala results in reduced top-down regulation, which is further validated by findings of increased amygdala

activity to aversive stimuli in s-allele carriers (Hariri et al., 2005; Heinz et al., 2004). Furthermore, imaging-genetic investigations have shown 5-HTTLPR to moderate the influence early life experience has on amygdala reactivity in adolescents viewing emotionally salient face stimuli (Walsh et al., 2012). These findings were further supported by a meta-analysis and a gene-environment interaction study, but suggest effect sizes for genetic risk may be much smaller and only account for 10% of the variance in amygdala activation(Alexander et al., 2012; Munafò, Brown, & Hariri, 2008). These findings were in healthy subjects, and therefore it should be noted that associations between neuroanatomical changes and genetics (Frodl et al., 2008) or childhood adversity (Frodl et al., 2012) may be delineated in depressed populations.

Thus far it has been suggested the genetic variants are associated with neural connectivity changes. Current research indicates that the serotonin system and connectivity within the human brain seem to be overlapped by common genes. A genome-wide association study on myelin integrity (as indicated by FA), found an association between FA and a SNP in HTR7 intron, a g-protein coupled receptor within the serotonin system. Although the function of this gene has yet to be determined, it is thought to mediate antidepressant response and be relevant in functions of neuroplasticity and circadian rhythms (Sprooten, 2013).

1.3.6 BDNF

The serotonin system and brain-derived neurotrophic factor (BDNF) seem to interact and ultimately affect the regulation of neural circuits in affective disorders (Lu & Martinowich, 2008). For example, BDNF promotes the survival and

differentiation of 5-HT neurons (Mattson, Maudsley, & Martin, 2004). In the brain, BDNF is secreted by glial cells, which have a well-established reciprocal relationship with neurons for their mutual development, growth and functioning. Glial cells are also involved in the myelination and synaptic plasticity of neurons through the release of myelin, BDNF, nerve growth factors and glial-derived growth factors, by oligodendrocytes, microglia and astrocytes. The role of glia in neuron survival and white matter microstructure may indicate that it has a role in volume and activation abnormalties (when viewing emotional stimuli) that are detected in depression (Cannon, 2010).

Based on its role in synaptic plasticity and development, polymorphisms within the BDNF gene are thought to confer risk for depression. The BDNF gene is located on chromosome 11p14, and has several polymorphic markers including a single nucleotide polymorphism (SNP) at nucleotide 196 (G to A), resulting in a valine (val) to methionine (met) amino acid substitution at codon 66 (Val66Met). This SNP is located in the pro region of the BDNF gene and therefore does not affect the functioning of the mature form, but affects intracellular processing and secretion of BDNF. Reduced secretion observed in met allele carriers of the BDNF gene (met-BDNF) seems to be a failure of sorting and packaging of vesicles, due to a lack of vesicle-synapse associations relative to val-BDNF (Egan et al., 2003).

Meta-analyses have made the BDNF gene an attractive candidate gene for depression, as blood BDNF levels revert to normal following antidepressant treatment, and improvements in depression correlate with serum BDNF levels (Brunoni, Lopes, & Fregni, 2008). Furthermore, suicide victims (who typically

express strong depression-like symptomology) exhibit reduced expression of BDNF in the hippocampus and prefrontal cortex (Dwivedi et al., 2003). Chen and colleagues (2006), also evidenced that defective BDNF secretion in the met-BDNF mouse was accompanied by increased anxiety-like behaviours, that could not be reversed with fluoxetine, an antidepressant (Chen et al., 2006). However, results have not consistently associated a specific variant of the Val66Met polymorphism with depression symtomology and/or antidepressant response (Kato & Serretti, 2008; Verhagen et al., 2010; Y.-F. Zou et al., 2010).

BDNF is secreted in the hippocampus in response to neuronal activity (Farhadi et al., 2000). Polymorphisms within this gene have a critical role in activity-dependent secretion of BDNF in hippocampus-based synaptic plasticity and learning. Met-BDNF individuals exhibit significantly lower scores on hippocampus based cognitive tasks including the Wechsler Memory Scale (a test of verbal episodic memory)(Egan et al., 2003), episodic memory (Hariri et al., 2003) and spatial working memory tasks. Reduced scores on such tasks in met carriers was accompanied by reduced hippocampus volumes, relative to val-BDNF individuals (Bath & Lee, 2006).

Infusion of BDNF into the hippocampus exerts antidepressant-like effects (Yu & Chen, 2011). However, BDNF may have different or opposite effects in other regions of the brain. For example, in contrast to the hippocampus, the nucleus accumbens (NAc) increases BDNF expression in the response to chronic stress. Infusion of BDNF into the nucleus accumbens (NAc) also exerts depression inducing effects in a social defeat task, in comparison to blockage of BDNF which exerts an

antidepressant-like effect by opposing the development of social avoidance behaviours associated with social defeat (Berton et al., 2006). Thus, the location of BDNF in the neural circuit may be pivotal to the differential role BDNF has in depression.

1.3.7 Rumination

Another aspect of depression is rumination, which is the tendency to repeatedly rehash and brood on past events (Nolen-Hoeksema, Wisco, & Lyubomirsky, 2008). Hilt and colleagues (2007) suggest that rumination may be an endophenotype in the pathway between BDNF polymorphic genetic risk and development of major depression, as it is a relatively stable trait regardless of present depression levels. This study also found differing risk factors for early and late depression development. Although the onset of depression was associated with increased rumination in all cases, early and adult depression onset were linked with the val-BDNF and met-BDNF genotypes, respectively (Hilt, Sander, Nolen-Hoeksema, & Simen, 2007).

Dysregulations in attention networks involving the dlPFC and ACC have also been observed in depressed individuals during inhibitory control processes, involved in the suppression of ruminative-related thoughts. Activity dependent dysregulation in these regions was significantly higher for depressed individuals, followed by at-risk and then healthy controls (Carew, Milne, Tatham, MacQueen, & Hall, 2013). These regions are connected by the superior longitudinal fasciculus, which has a major role in attention and cognitive control. This tract has reduced FA in depressed populations, which negatively correlated with ruminative state (Zuo et

al., 2012). Furthermore, stable FA reductions in the superior longitudinal fasciculus has been identified in a meta-analysis of 7 DTI papers on depression (M. L. Murphy & Frodl, 2011). This tract and other tracts involved in the attention network will be further examined in the present study, and will provide a more thorough examination of attention control deficits seen in individuals who ruminate or experience depression.

1.3.8 BDNF X Environment

Genetic and environmental interactions have also been observed in studies investigating the role of BDNF and adversity on depression development. BDNF's distribution profile spans cerebral regions involved in emotional and behavioural regulation (Gratacos et al., 2007), making it a strong candidate risk gene for depression. Chen and colleagues (2006), have tied the more rare met-BDNF variant of the val66met polymorphism with diminished BDNF secretion, smaller hippocampal volumes, less dendritic arbors and 30% reductions in activity dependent secretion of BDNF (Chen et al., 2006). A largely powered gene by environment association study found that the impact of childhood adversity (specifically sexual abuse) on depression symtomology was moderated by the met-allele (Aguilera et al., 2009). Furthermore, met carriers exhibit dose dependent reductions in serum BDNF levels following exposure to childhood adversities (Elzinga et al., 2010).

Similar studies in post mortem suicide victims have been inconsistent, with both met-BDNF (Pregelj et al., 2011) and val-BDNF (Perroud et al., 2008) genotypes

exhibiting greater genetic moderation on the association between childhood adversity and violent suicide attempts.

1.3.9 BDNF and Neuroimaging Findings

Early morphometric imaging studies found that BDNF polymorphisms were associated with reduced hippocampal and amygdala volumes (Montag:2009jj}(Frodl, Meisenzahl, Zetzsche, Born, et al., 2002a; Frodl, Meisenzahl, Zetzsche, Bottlender, et al., 2002b) and activity during navigation tasks in healthy humans (Banner, Bhat, Etchamendy, Joober, & Bohbot, 2011). More recently, advanced techniques such as DTI have been used to investigate the role of genetic risk factors on myelin integrity in depressed individuals. Reduced FA in the uncinate fasciculus (which connects the amygdala and hippocampal regions with orbital frontal regions) was associated with met-BDNF carriers with depression, relative to depressed val-BDNF homozygotes or healthy controls. This group also found a three-way interaction between FA in the cingulum regions (rostral, dorsal and parahippocampal regions), hemisphere and BDNF genotype. Higher FA was detected in the left rostral regions of the cingulum for met carriers relative to val-BDNF homozygotes (Carballedo, Lisiecka, et al., 2012b). In contrast, Tost and colleagues reported widespread reductions in FA in the corpus callosum and posterior corona radiata for val-homozygotes relative to met carriers (Tost et al., 2013).

Other original investigations examined the effect of both BDNF and 5-HTTLPR on depression. Three way interactions between severe childhood adversity, met-BDNF and 2 S alleles of 5-HTTLPR was found in children (Kaufman et al., 2006)

or L'S' genotype in adult females (Wichers et al., 2008). Extending these findings to neuroconnectivity changes, it has been suggested that the met-BDNF allele may be protective against the negative effects the 5-HTTLPR S allele has on neural circuitry encompassing the ACC and amygdala. Individuals with the S allele did not show the associated volume reductions of the anterior cingulate when they were met-BDNF carriers (Canli & Lesch, 2007).

1.3.10 BDNF X 5-HTTLPR on cortisol activity

For many years, increased hypothalamic-pituitary-adrenal (HPA) axis reactivity, and in turn cortisol response, have been a predominant hypothesis regarding the development of depression (Heim et al., 2000; Heim & Nemeroff, 2001). In the context of depression, HPA axis reactivity has been suggested as a possible endophenotype, as it appears to be party heritable, temporally stable and observed in depressed patients (Miller, Wankerl, Stalder, Kirschbaum, & Alexander, 2012).

This is in accordance with findings of increased morning waking salivary cortisol levels in at-risk adolescents in the presence of the 5-HTTLPR S allele (Goodyer, Bacon, Ban, Croudace, & Herbert, 2009) and met-BDNF allele (Goodyer, Croudace, Dudbridge, Ban, & Herbert, 2010). A recent meta-analysis of 11 data sets found a small, but significant effect of 5-HTTLPR genotype ('s' allele carriers) and increase HPA-axis reactivity (indicated by increased cortisol reactivity) with acute psychosocial stress (Miller et al., 2012). Similarly, animal research suggests that the effects of 5-HTTLPR likely involves persistent alterations in the neural development of cortico-limbic circuits known to heighten HPA reactivity (D. L. Murphy & Lesch,

2008). And in reverse, corticoids in the hippocampus are known to inhibit the effects of neurotrophins such as BDNF, leading to neuronal loss (Kumamaru et al., 2008; Tessner, Walker, Dhruv, Hochman, & Hamann, 2007). These findings suggest that individuals with genetic risk are less resistant and/or more sensitive to corticoid-mediated neurotoxicity in the presence of stress.

The objective of the present study is to draw together a number of the above findings, by assessing the influence of childhood maltreatment, 5-HTTLPR and BDNF polymorphisms on connectivity and myelin integrity in the brain. Furthermore, we stratified our study groups by type of abuse/neglect to delineate the impact of specific types of maltreatment on FA. The 5-HTTLPR and BDNF genes were chosen as candidate risk factors for depression as these two genes confer the largest risk for depression and have been shown to moderate the effect of childhood adversity on depression development (Bukh et al., 2009). Connectivity was assessed by two analysis methods: tract based spatial statistics and probabilistic tractography. Myelin integrity within white matter fiber tracts within the fronto-limbic circuit including the cingulum, uncinate and superior longitudinal fasciculus were examined, as these tracts have been implicated in depression. We anticipate that childhood maltreatment and genetic risk factors will moderate the effect of depression on neuronal connectivity. This manuscript is consistent with the objectives laid out in the National Institute of Mental Health's 2009 Strategic Plan (www.nimh.nih.gov) to identify sensitive physiological indicators of mental health.

2.0 Methods

2.1 Study Participants

The study included fifty-five major depressive disorder patients and eighteen controls, whom were of either sex, caucasian and within an age range of 19-58 years. Depressed individuals met DSM-IV's (American Psychiatric Association, 2000) criteria for major depressive disorder according to the Structured Clinical Interview (First, Spitzer, Williams, Gibbon, First, 1997) for DSM-IV Axis 1 disorder. Participants were recruited from the Calgary Health Region Outpatient Mental Health Clinics, tertiary care hospitals, University of Calgary through flyers, and from the community through local newspaper advertisements. Exclusion criteria included bipolar disorder, psychosis, a history of substance abuse within 6 months of study participation, anxiety disorders, severe medical and neurological disorders, left handedness and previous history of treatment failure with citalopram or quetiapine XR mono/add-on therapy. Edinburgh Inventory was used to determine participant handedness (Oldfield, 1971). All MDD participants scored 18 or higher on the 17-item Hamilton Depression Rating Scale (HDRS)(Hamilton, 1960) and were free of antidepressant and psychotropic medications for at least 3-4 weeks prior to baseline assessments, MRI scan, genotyping and medication randomization. The local review board approved this study, and informed consent was obtained from all subjects prior to participating in the study.

2.2 Assessments

MRI scan and Childhood Trauma Questionnaire- Short Form (CTQ-SF) were completed at baseline. The CTQ-SF is a 28-item questionnaire shortened from the

original 70-item Childhood Trauma Questionnaire (Bernstein et al., 2003). Both questionnaires are well-validated assessments of childhood abuse and neglect. The CTQ-SF consists of 25 clinical items and 3 validation items. Items on the CTQ consists of questions about experiences of neglect and/or abuse in childhood, which are to be rated on a 5-point scale, with response options ranging from Never True to Very Often True. The questionnaire contains 5 questions for each factor of abuse/neglect assessed [i.e. physical abuse, emotional abuse, sexual abuse, physical neglect and emotional neglect]. Scores for each factor are calculated based on the mean value of the five individual items for each subscale and range from 5 to 25. The CTQ has good reliability and validity, as well as convergent and discriminate validity within structured interviews by therapists and clinical-rated interviews, and has been further corroborated by independent data (Fink, Bernstein, handelsman, Foote, & Lovejoy, 1995).

2.3 Genotyping and Blood Collection

2.3.1 Blood Collection

Whole blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. The collected blood was stored at -20°C and then thawed to room temperature when DNA extraction processes were to proceed. Of the sample, 200μL was set aside for extraction and the remaining blood was separated into 500μL aliquots. This reduces how frequent the blood is thawed, which reduces chances of degradation of the blood leading to low DNA yields. DNA was isolated using QIAmp DNA Blood Mini Kit from Qiagen. The blood cells were lysed and the DNA was bound to a filter in the spin column. The DNA was washed in

the column and then eluted into a new tube to produce the final DNA. The DNA was then stored at 4°C until needed.

2.3.2 Genotyping

Genotyping for the 5-HTTLPR and BDNF val66met was completed by first amplifying the genes using polymerase chain reaction (PCR), and then a portion of the PCR product underwent restriction fragment length polymorphism (RFLP) analysis. This process uses a restriction enzyme to digest the DNA into fragments that can be translated to genotype. The genotyping was performed without knowledge of the subject's clinical status.

2.3.3 5-HTTLPR

Either a 484 or a 528 base pair fragment was generated using forward primer 5'-GGCGTTGCCGCTCTGAATGC and reverse primer 5'-GAGGGACTGAGCTGGACAACCAC (Lesch et al., 1996). The genes were amplified using AccuPrime GC-Rich DNA polymerase (Invitrogen 12337-016). The 25 μL amplification mixture contained 100 ng of genomic DNA, 0.2 μM of each primer, 1U of AccuPrime GC-Rich DNA polymerase and 1X AccuPrime GC-Rich Buffer A. The conditions for cycling were: (1) initial denaturation at 95°C for 3 m followed by 7 cycles of 95°C for 30 s, 68°C for 30 s and 72°C for 1 m, (2) 7 cycles of 95°C for 30 s, 67°C for 30 s and 72°C for 1 m (3) 7 cycles of 95°C for 30 s, 66°C for 30 s and 72°C for 1 m. Final extension took place at 72°C for 10 minutes. The final product (uncut) was run on 1.5% agarose gel at 50V for 90 m. 12 μL of the PCR product was then cut using the restriction enzyme MSPI (New England Biolabs) for 3 hours at 37°C (Prachak-Rieder et al., 2007). To separate the bands, this cut product was run on a

4-20% Tris/Borate/EDTA (TBE) gel at 14 mA for 100 m to separate the bands (invitrogen EC6225BOX). The 5-HTTLPR is a 44 base pair deletion in the promoter region at base pair 1212 to 1255 (on chromosome 17), and results in a short (S) or long (L) allele (Heils et al., 1996). Within the extra 44 basepairs associated with the long allele, there is also a guanine to adenine single nucleotide polymorphism, resulting in L_G and L_A (Hu et al., 1996). The band pattern for L_A was 340 base pairs, L_G was 166+174 base pairs, and S_A was 297 base pairs (Prachak-Rieder et al., 2007).

2.3.4 BDNF

A 113 base pair fragment was amplified using forward primer 5'-GAGGCTTGACATCATTGGCT and reverse primer 5'-CGTGTACAAGTCTGCGTCCT (Neves-Pereora et al., 2002). The 25 μL amplification mixture contained 50 ng of genomic DNA, 0.2 μM of each primer, and 1X AccuStart Taq DNA polymerase (Quanta Biosciences). The conditions for cycling were: (1) initial denaturation at 95°C for 2 m, (2) 35 cycles of 94°C for 30s (3) 60°C for 30s (4) 72°C for 30s, (5) final extension following the completion of the cycles of 72°C for 5 m (Rybakoski et al., 2003). The 7.5 μL PCR product was cut with the restriction enzyme Eco 721 (Fermentas #ER0361) for 3 h at 37°C. The bands were separated using a 4-20% TBE gel (invitrogen EC6225BOX) at 14 mA for 60 m. The BDNF val66met polymorphism we are examining is a guanine to adenine single nucleotide polymorphism at nucleotide 196, that results in a valine to methionine switch at codon 66 (Egan et al., 2003). The resulting banding pattern for the methionine and valine allele was 113 base pairs and two bands at 78 and 35 base pairs, respectively.

2.4 Diffusion Tensor Imaging

Magnetic resonance imaging (MRI) is one of the most powerful and flexible imaging tools used today. This technique allows non-invasive in-vivo investigation of soft tissue with anatomical structure resolution. A relatively new MRI method called diffusion tensor imaging (DTI) allows for the detection of microstructure and cellular changes by assessing the magnitude and direction of water diffusion. White matter neurons surrounded by myelin have restricted diffusion profiles, with water diffusing along the neuron (axon) rather than perpendicular to the neuron. Changes in the degree of restricted diffusion indicate a lack of structural integrity and connectivity within a region. Water diffusion that is restricted to a specific direction is referred to as anisotropic movement. Free diffusion or isotropic diffusion is found in areas of the brain where diffusion is unrestricted, such as in cerebral spinal fluid, and is observed in neurons with reduced myelin integrity. DTI is a powerful tool that utilizes measures of diffusion as an indicator of cellular integrity, although neurons are much smaller than the measureable MRI 3D voxel.

The diffusion of water is mapped as an ellipsoid diffusion tensor. A tensor is a mathematical construct of water movement indicating both integrity of microstructure but also coherence of fibre tracts in a voxel. The tensor is a construct of 3 eigenvectors (corresponding to the 3 major diffusion axes) that have individual radii eigenvalues.

Based on the ellipsoid diffusion tensor, several parameters of diffusion can be mapped which provide an estimation of microstructure integrity. The most commonly studied and most indicative of myelin integrity is fractional anisotropy

(FA). Fractional anisotropy is a value between zero (unrestricted) and one (restricted) describing the degree of anisotropy within a diffusion process. High FA is generally found in regions of white matter with high neuron coherence, as parallel myelinated neurons restrict water movement such that it aligns with the direction of the fiber tracts. This restricted movement is affected if coherence of axons or myelin integrity in an area is disrupted.

Another parameter used in DTI is mean diffusivity (MD). This parameter averages the diffusion along the 3 principal diffusion axes and indicates the average restriction of water movement in all directions. Although FA is more sensitive to diffusion along the primary diffusion axis, generally MD and FA have an inverse relationship. Other measures of microstructure are axial (parallel) and radial (perpendicular) diffusion. Axial diffusion is the diffusion along the principal eigenvalue [$\lambda 1$], the axis with the greatest perfusion. Radial diffusion is the average diffusion along the second and third eigenvalues [$(\lambda 2 + \lambda 3)/2$], which have lower perfusion relative to $\lambda 1$.

When acquiring a DTI scan, specific gradient directions are applied during the scan to obtain a probability distribution of water diffusion. Magnetic gradients are applied to determine the probability of water moving along a specific gradient direction, with typical scans having 6 to 60 gradient directions. The more gradient directions used the more precisely one can determine the direction of water diffusion. The minimum required directions are 6, which align to the x, y, z axes of a sphere.

Although DTI parameters are good indicators of white matter integrity at the subject level, only recently was a solution developed to adequately align FA images for multiple subjects, and determine the required spatial smoothing for voxel-wise statistical analysis. This relatively new method is called tract based spatial statistics (TBSS) and resolves many of these issues. Through sensitive non-linear alignments and projections onto average tract representations, researchers have been able to get a more sensitive and objective analysis of diffusion. TBSS allows for a voxel-by-voxel comparison of diffusion parameters between two groups.

2.4.1 Tract Based Spatial Statistical Analyses

To complete TBSS analyses, images were exported into a format that was readable by Oxford Center for FMRIB Diffusion Toolbar and TBSS software tools, part of FSL 5.0. (Smith et al., 2006). Raw DTI data was converted from analyze format to nifti format and then corrected for head movement and eddy current distortions (Jenkinson & Smith, 2001). Following this, a brain extraction tool (BET) was applied to each image using a binary mask to differentiate between brain and skull structures. A tensor model was then fitted to each voxel within the brain mask. Using FLIRT and FNIRT (FMRIB linear and non-linear registation tools), FA images were aligned to the FMRIB58_FA template and transformed into Montreal Neurological Institute (MNI)-152 $1mm^3$ standard space (Rueckert et al., 1999). A mean FA skeleton image was created to represent all major white matter tracts, and was threshold at FA ≥ 0.2 to avoid retaining skeleton tracts within distal extremes where cross subject variability is high and registration is poor. The FA skeleton images were then processed through a permutation testing procedure called

Randomise that performs voxel-wise cross-group statistics with 5000 permutations (Nichols & Holmes, 2002). This application allows for group comparisons using two-sample t-tests. To avoid setting an inappropriate predetermined threshold, the threshold-free cluster enhancement (TFCE) method was used for all TBSS statistical analysis (Smith & Nichols, 2009). TFCE allows the user to minimize error due to predetermined arbitary threshold setting while maintaining the benefits of cluster-based thresholding. This produces a voxel-wise comparison image representing the amount of local spatial support for significant clusters. Images were then statistically thresholded to $p<0.05$ and family wise error (FWE) corrected for multiple comparisons. Significant clusters (in corrected and uncorrected FA maps) were identified following between group comparisons, and were labeled using the John Hopkins University International Consortium for Brain Mapping (JHU ICBM)-DTI-81 white matter labels atlas (Hua et al., 2009; Wakana, Jiang, Nagae-Poetscher, van Zijl, & Mori, 2004).This provided a relatively good indication of the region and tract where the significant differences were present.

2.4.2 Probabilistic Tractography Analyses

Probabilistic tractography, another commonly used DTI method, models white matter tracts between two neural regions and uses parameters of water diffusion to assess connectivity. Similar to TBSS, changes in FA indicate fiber coherence and possible pathology. Probabilistic tractography requires a build up of probability distributions for water movement along the principal diffusion vector at each voxel. This distribution indicates the most probable path of water movement through each voxel, while accounting for uncertainty. Factors such as motion and

crossing or splitting fibres may contribute to this uncertainty. This is the main advantage of probabilistic tractography relative to other methods such as deterministic tractography, as it indicates the 'most likely' path of water diffusion leaving a particular seed region (starting region or voxel) and ending at a target region (termination region or voxel).

By specifying particular seed and target regions, one can model known tracts that bridge two regions. Accordingly, by specifying regions that are known to interact and connect via white matter projections, this method allows the modeling and averaging of diffusion parameters (such as FA) along real white matter tracts. This method is limited, as directionality of water movement from the seed to target, or vice versa, cannot be determined.

In order to run probabilistic tractography analyses, preprocessed data must run through a Bayesian Estimation of Diffusion Parameters (BEDPOST), which uses Markov Chain Monte Carlo sampling distributions. This technique builds up distributions by sampling and re-sampling clusters of voxels to get the most accurate indication of diffusion parameters at a specific voxel (Behrens et al., 2003). Following this, the identification of seed and target regions, can be completed by manually tracing the region, or through the use of an automatic pipeline from Freesurfer. This pipeline creates cortical parcelations, surface maps, and cortical thickness measurements. Automatic processing pipelines allow for an increased level of reliability and certainty in region specification across individuals.

Following cortical parcelation, a multi-fiber diffusion probabilistic model was applied to the diffusion data. Probabilistic tractography allows the exact orientation

and trajectory of white matter fiber tracts to be assessed in each subject. The association white matter tracts investigated in the present study were the cingulum bundle, uncinate fasciculus and superior longitudinal fasciculus. These tracts have exhibited significant microstructure alterations in previous DTI studies on depression. To isolate these tracts a previously used multiple region seed and target approach was implemented. Tracts were defined in standard space using anatomically defined regions of interest seed and targets from the cortical parcelation (Fischl, 2004). The cingulum bundle is a medial tract that arches superior to the corpus callosum, encompassing much of the limbic system and connects the anterior cingulate to the occipital cortex. We defined the seed and target regions as the rostral anterior cingulated and the isthmus cingulated, respectively. The uncinate fasiculus connects the frontal cortex with the hippocampus and the amygdala. We defined our seed regions as the lateral orbital frontal, medial orbital frontal and parsorbitalis, and our target regions as the entorhinal cortex and temporal pole. Finally, the superior longitudinal fasciculus connects the inferior parietal with inferior frontal lobe. The seed regions used to define this tract include the parsopercularis and caudal middle frontal cortex, and our targets were defined as superior temporal and inferir parietal lobe (see figure 1).

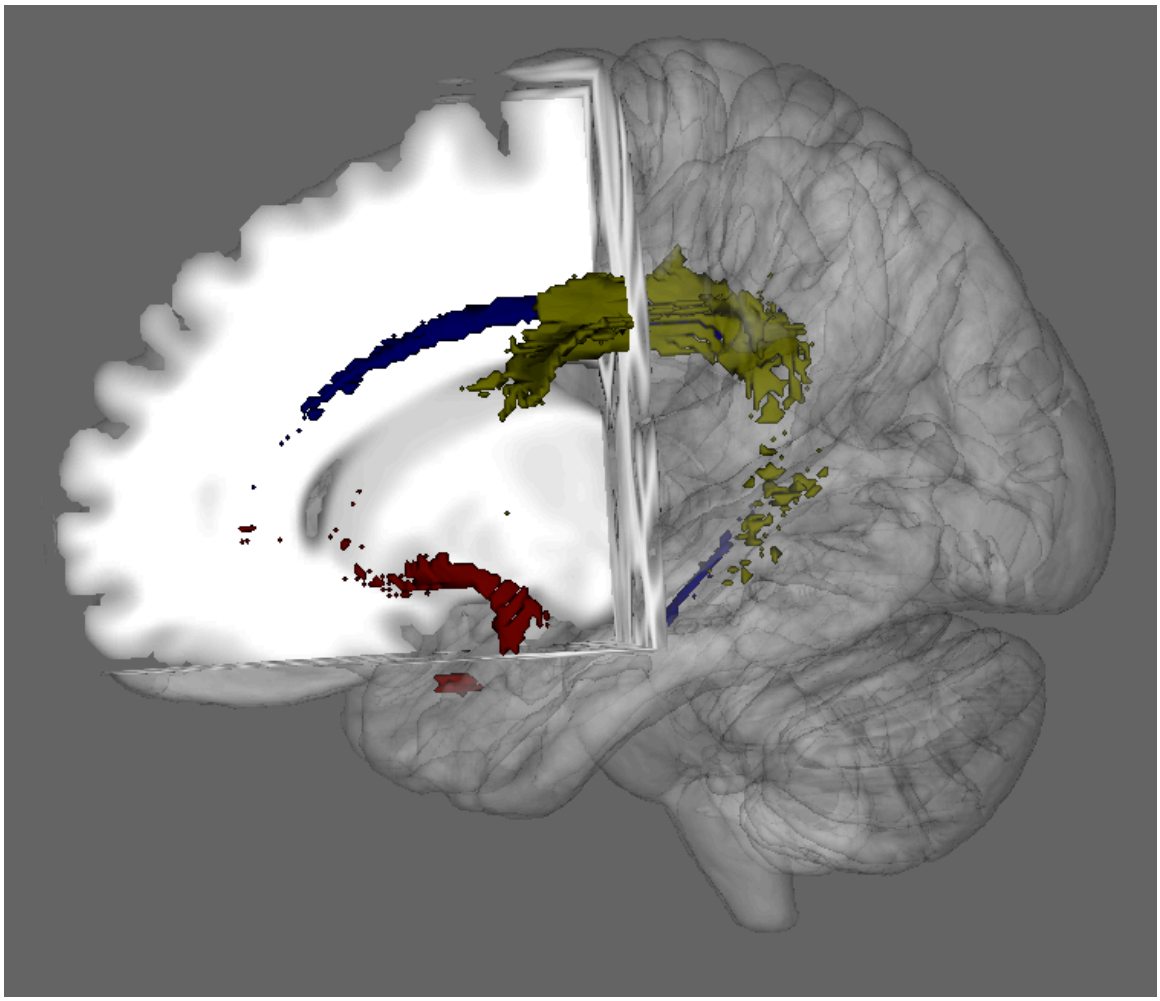


Figure 1: Trace of cingulum bundle, unincate fasciculus and superior longitudinal fasciculus. A 3-D depiction of the cingulum bundle (blue), uncinate fasciculus (red) and superior longitudinal fasciculus (green) from John Hopkins tractography atlas.

A limitation of the present investigation is that only 12 collinear directions were used to assess water perfusion rather than 60 directions, which are used in more recent studies. Tracts derived using fewer directions are consistently smaller then those derived using 60 direction data. For this reason, we did not assess tract volume changes in our sample. Studies using fewer diffusion directions are also less sensitive to changes in perfusion and crossing fibers within a single voxel. However,

the number of diffusion directions does not have a significant effect on reproducibility, and therefore we believe the findings from the present study are valid. (Heiervang, Behrens, Mackay, Robson, & Johansen-Berg, 2006).

2.5 Diffusion Imaging Parameters

Diffusion-weighted MRI scans were acquired using a single-shot echo planar imaging sequence. Scan parameters were as follows: TR: 10 000ms, slice thickness: 3 mm , FOV (field of view): 24 cm, acquisition matrix: 96 x 96, spacing: 0 mm, b-value: 850 sec/mm^2, and 12 collinear directions with 1 non-weighted image. DTI images were monitored for common artifacts such as ring artifacts and slice wise intensity related artifacts (Liu, Klomp, & Heynderickx, 2010).

2.6 Grouping of variables

Multiple linear regression models were used to assess the influence of genetic risk factors and experiences of childhood trauma on myelin integrity. Within the probabilistic tractography analysis, myelin integrity was assessed by measures of fractional anisotropy in association fiber tracts including, the cingulum bundle, uncinate fasciculus, and superior longitudinal fasciculus.

Brain derived neurotropic factor (BDNF) genotypes were collapsed across met/val and met/met, in accord with much of the literature, as homozygous met/met genotype is more rare in Caucasians (~4%){Pregelj:2011hz}. Tri-allelic serotonin transporter polymorphisms were collapsed into categories based on transcription efficiency {Hu:2005eu}. Previous research has indicated that L_A has high transcription efficiency, while the L_G and S_A genotype have relatively equal and lower transcription efficiency. We stratified our 5-HTTLPR genotype groups into L'L'

representing two transcriptionally efficient alleles (L_A/L_A) , L'S' representing 1 high and 1 low transcriptionally efficient alleles (L_A/L_g or L_A/S_A) and S'S' representing 2 low transcriptionally efficient alleles (S_A/S_A, L_G/S_A or L_G/L_G).

Allele Character	Description
S'	Allele that is transcribed more efficiently (L_A)
L'	Allele that is transcribed less efficiently (L_G and S_A)

2.7 Diffusion Tensor Imaging Statistical Analysis

2.7.1 Tract-Based Spatial Statistics

Tract based spatial statistics allowed t-test voxel-wise comparisons between controls and MDD patients, and the assessment of whether depression severity, genetic and environmental predictors impact FA. Fractional anisotropy was compared between depressed patients and controls using TBSS analyses. FA within regions of interest were extracted for further t-test and regression analyses. Between group differences were investigated in the genu, body and splenium of the corpus callosum, fornix, right and left anterior internal capsule, right and left anterior corona radiata, hippocampus and sagittal stratum.

Within the depressed group TBSS analyses were used to determine differences between each 5-HTTLPR tri-allelic grouping in depressed individuals alone [(1)L'L' vs. L'S' (2) L'L' vs. S'S' (3) L'S' vs. S'S']. Within group comparisons were also completed in the depressed group for BDNF (met carriers vs. val/val) and CTQ (High vs. Low).

Both corrected and uncorrected TBSS findings have been reported in the literature (Cullen et al., 2010; Huang, Fan, Williamson, & Rao, 2010; Montag, Schoene-Bake, Faber, Reuter, & Weber, 2010). Voxel-wise uncorrected p-values from TBSS analyses have been shown to be useful for apriori selected regions (Randomise: FSL 5.0). If corrected results were not found, uncorrected TBSS findings were reported .

2.7.2 Probabilistic Tractography

Multiple linear regression analysis was used to examine to impact 5-HTTLPR, BDNF val66met, CTQ scores and depression severity (HDRS scores) had on FA in association tracts including the cingulum bundle, uncinate fasciculus, superior longitudinal fasciculus, and significant regions from TBSS analyses. Fractional anisotropy was averaged across left and right hemispheres for each association tract studied in this investigation.

Two-way interactions were fitted to a linear regression model to predict changes in FA. Interactions between HDRS scores and severity of each type of childhood trauma were regressed against FA. Significant interactions were further investigated using t-tests and graphical representations that segmented types of abuse into high and low based on standardized cut-offs (Scher et al., 2004; E. A. Walker et al., 1999).

In regions and tracts that exhibited a sensitivity to depression severity that was moderated by experiences of trauma, exploratory three-way interaction models were regressed with FA to determine the moderating impact genetic risk factors have on connectivity changes in these regions/tracts [i.e. (1) HDRS score, type of

childhood trauma & BDNF polymorphisms regressed against FA, (2) HDRS scores, type of childhood trauma & 5-HTTLPR polymorphisms were regressed against FA].

Each model was tested for normality, heteroscedasticity, multi-collinearity, autocorrelation, outliers, high leverage and influential points. Specific cases were only evaluated for exclusion if the case was a corrected outlier, had a leverage value 3 times the average leverage value (Stevens, 2002), and had a Cook's distance (effect of a single case on a model) value >0.7 (Cook & Weisberg, 1982)(Field, Miles, Field, 2012).

3.0 Results

3.1 Demographics

3.1.1 Participant Selection

Fifty-five patients and 18 healthy controls were recruited for the study. Two MDD patients did not complete the MRI portion of the study, and 11 MDD patients and 1 control did not complete the Childhood Trauma Questionnaire. 5-HTTLPR genotype data was not extracted for two MDD patients and two controls, and BDNF genotype data was not extracted for the same two controls and two different MDD patients. Groups did not significantly differ in age (t=1.13, p>0.05), but had significantly different baseline HDRS scores [t=17.7, p<0.00, MDD: 21.4 (±4.0), controls :3.5(± 3.0)] and childhood trauma scores [t=10.94, p<0.00, MDD: 56.4(±8.1), controls: 32.2(±6.7)] (See Table 1). The S'S' genotype of the 5-HTTLPR was not associated with reporting significantly more severe childhood trauma, relative to L'L' genotype [p>0.05, S'S': 50.294 (±12.27), L'L': 44.42 (±16.63)]. Additionally, met-BDNF allele carriers did not report more severe childhood trauma relative to val-

BDNF homozygotes [p>0.05, G/G: 50.66 (±12.13), G/A: 44.77 (±16.00)]. Individuals who reported high CTQ scores did not self-report higher depression symptoms relative to individuals reporting low CTQ scores [p>0.05, High CTQ: 23.04167 (3.972), Low CTQ: 13 (9.0431)]

Table 1 : Demographics of MDD patients and controls

	MDD Patients (n=55) mean (sd)	Controls (n=18) mean (sd)	t-test	p
Age	36.4 (10.5)	33.2 (10.2)	1.13	0.262
Age of onset	25.2 (10.6)			
HAM-D score	21.5 (3.95)	3.5 (3.0)	17.70	<0.00
CTQ	56.4 (8.1)	32.2 (6.7)	10.94	<0.00
Physical Abuse	7.5 (3.7)	5.4 (0.7)		0.02
Emotional Abuse	11.2 (6.1)	7.1 (2.5)		0.01
Sexual Abuse	7.4 (4.9)	5.8 (2.0)		0.184
Physical Neglect	12.8 (1.5)	5.7 (1.4)		<0.00
Emotional Neglect	17.4 (5.2)	8.2 (3.4)		<0.00
	n (% of group)	n (% of group)		
5-HTTLPR				
S'/S'	17 (32%)	4 (25%)		0.574[A]
S'/L'	26 (49%)	7 (44%)		
L'/L'	10 (19%)	5 (31%)		
BDNF (Val66Met)				
Met/Met & Met/Val	11 (20%)	6 (37%)		0.197[A]
Val/Val	42 (80%)	10 (63%)		

[A] Fisher's Exact Test

Table 1: Demographic Data

3.1.2 Genetics

Hardy weinberg allele frequency distribution was maintained for the 5-HTTLPR genotype [MDD patients: x2=0, controls: x2=0.25, p>0.05] and BDNF val66met genotype [MDD patients: x2=2.36, controls: x2=0.12, p>0.05]. Allele frequency proportions were similar for controls and MDD patients for the 5-HTTLPR [p=0.574] and BDNF [p=0.197].

3.2 Tract Based Spatial Statistics

3.2.1 FA in Controls vs. Participants

Whole brain voxel-wise statistics contrasting MDD patients and controls revealed significant reductions in FA in the MDD group, that spanned the brain and including many white matter regions. Post-hoc analyses allowed extraction of FA values in regions of interest (ROI) and comparisons across group. John Hopkins White Matter Labels Atlas was used to extract FA values for regions including the genu, body and splenium of the corpus callosum, fornix, left and right anterior internal capsule, left and right hippocampus, and left and right anterior corona radiata. Uncorrected MDD patients showed a trend towards significant reduction in FA in the right anterior internal capsule (W=648, p=0.05097), fornix ($t(71)=1.7421$, p=0.08582), and right anterior corona radiata ($t(62.43)=1.7581$, p=0.0836) relative to controls (see figure 2, 3, 4 and table 2). Trends, rather than significant differences were likely found as ROI extraction averages FA values across the whole region-of-interest, rather than determining significant peak differences in FA. No significant differences were found for contrasts examining other regions ($p>0.05$).

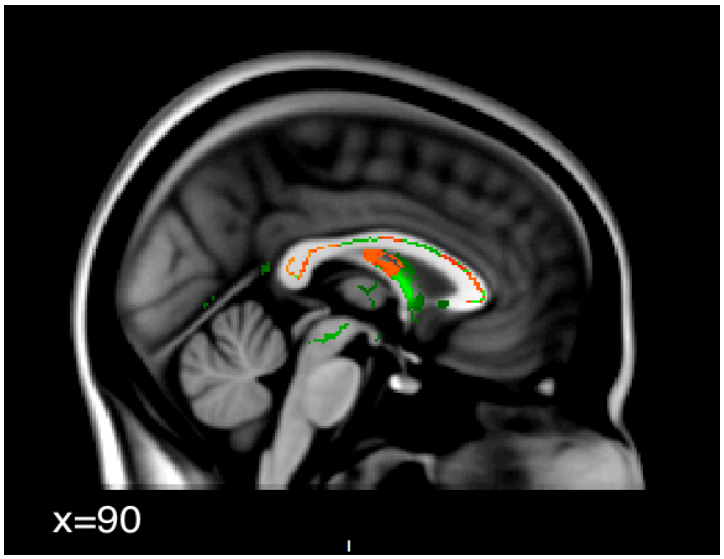


Figure 2: Tract Based Spatial Statistics: MDD vs. Controls – Fornix. Sagittal view of corrected tract based spatial statistics analysis between major depressive disorder patients and controls, indicating significant differences in FA in the fornix.

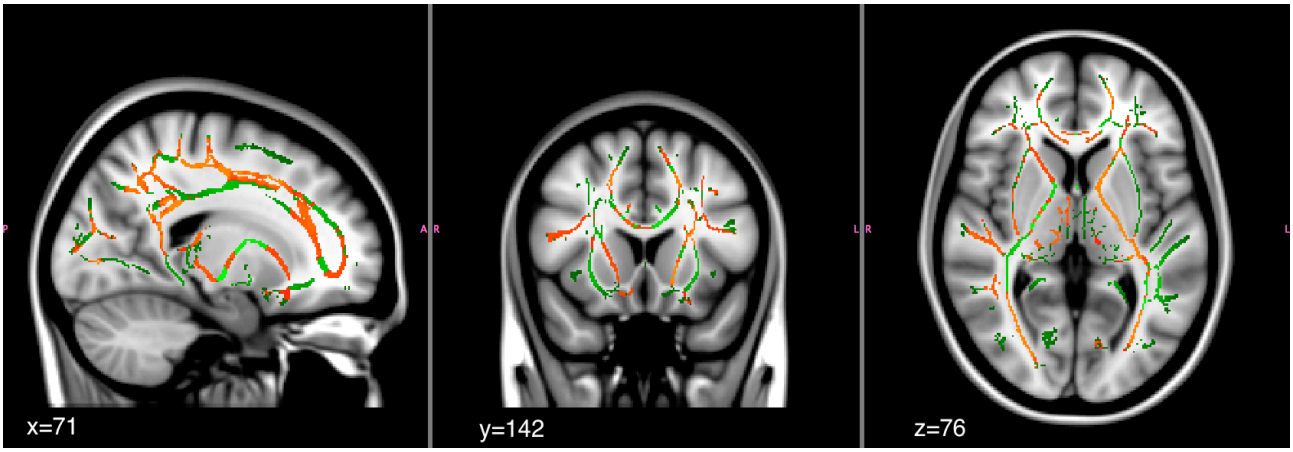


Figure 3: Tract Based Spatial Statistics: MDD vs. Controls – Right Anterior Internal Capsule. Corrected tract based spatial statistics analysis between major depressive disorder patients versus. controls, indicating significant differences in FA in the right anterior internal capsule.

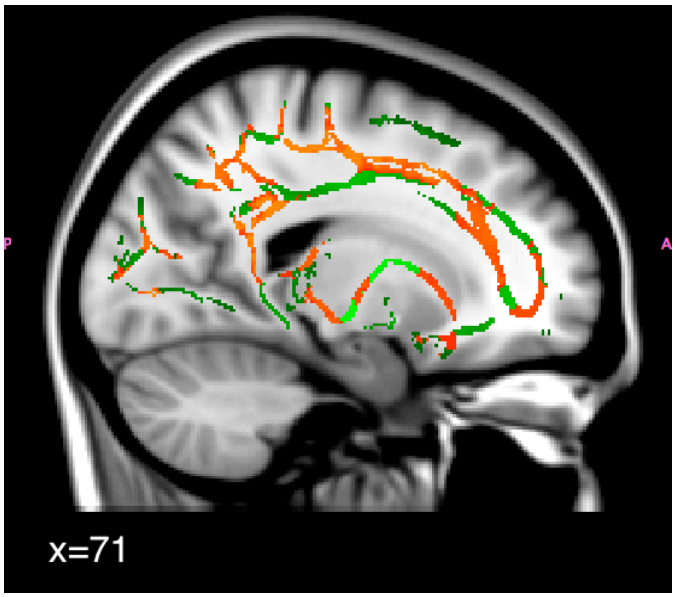


Figure 4: Tract Based Spatial Statistics: MDD vs. Controls – Right Anterior Corona Radiata.

Sagittal view of corrected tract based spatial statistics analysis between major depressive disorder patients and controls, indicating significant differences in FA in the right anterior corona radiata.

Tract Based Spatial Statistics: MDD vs. Controls ROI analysis			
White Matter Region	MDD patients	Controls	p-value
Corpus Callosum			
Genu	0.628	0.671	0.1896
Body	0.574	0.614	0.3151
Splenium	0.72	0.777	0.4238
Fornix	**0.422**	**0.459**	**0.0858**
Right Anterior Internal Capsule	**0.545**	**0.583**	**0.0509**
Left Anterior Internal Capsule	0.51	0.57	0.2314
Right Hippocampus	0.43	0.46	0.1853
Left Hippocampus	0.43	0.46	0.3029
Right Anterior Corona Radiata	**0.45**	**0.477**	**0.0836**
Left Anterior Corona Radiata	0.469	0.463	0.725

Mean (SD)

Table 2: Tract Based Spatial Statistics: MDD vs. Controls – ROI Analysis

3.2.2 5-HTTLPR Comparisons

Corrected for multiple comparisons

The S'S' depressed subgroup exhibited significantly higher FA in the splenium of the corpus callosum [x=105, y=84, z=80, voxels=2245, p=0.046] and anterior and posterior limb of internal capsule [x=102, y=104, z=54, voxels=2809, p=0.046]

relative to L'S' depressed subgroup (See figure 5 and table 3). No other corrected comparisons showed significant results.

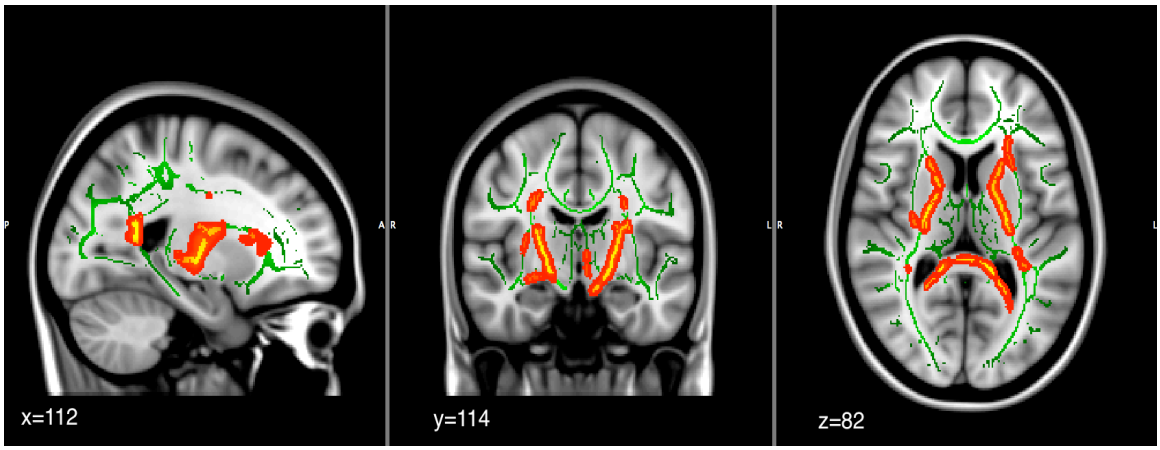


Figure 5: Tract Based Spatial Statistics: 5-HTTLPR: Depressed S'L' vs. S'S'. Filled tract based spatial statistics analysis between major depressive disorder patients with the S'S' and S'L' genotype of the 5-HTTLPR. Results indicate significant differences in the splenium of the corpus callosum and left anterior and posterior limb of internal capsule

Tract Based Spatial Statistics: 5-HTTLPR (S'S'>S'L')							
	MNI co-ordinates (vox)						
Voxels	X	Y	Z	Hemi-sphere	p-value	JHU White Matter Label	Harvard Oxford Cortical Region
2809	102	104	54	L	0.046	Anterior and Posterior Limb of Internal Capsule, Cerebral Peduncle	
2393	73	109	61	R	0.046	Anterior and Posterior Limb of Internal Capsule, Cerebral Peduncle	
2245	105	84	80	L	0.046	Splenium and Body of Corpus Callosum	Cingulate Gyrus
92	122	103	68	L	0.05	Sagittal Stratum	
57	116	118	98	L	0.05	Superior Corona Radiata	

Tract Based Spatial Statistics: 5-HTTLPR (L'L'>S'S')*							
36	130	55	98	L	0.01		Lateral Occipital Cortex
10	99	116	126	L	0.01		Juxtapositional Lobule Cortex
9	126	140	67	L	0.032		Insular Cortex
8	49	137	65	R	0.008		Insular Cortex

Threshold= 5 voxels

Tract Based Spatial Statistics: 5-HTTLPR (L'L'>S'L') *							
1273	124	166	84	L	0.01		Frontal Pole
1041	70	179	61	R	0.012		Frontal Pole
704	130	92	95	L	0.012		Parietal Operculum Cortex
666	142	99	76	L	0.018		Planum Temporale
544	100	42	90	L	0.008		Cuneal Cortex
490	127	46	72	L	0.008		Lateral Occipital Cortex
479	62	155	103	R	0.008		Middle Frontal Gyrus
434	71	32	72	R	0.022		Occipital Pole
320	67	98	56	R	0.004	Cingulum (Hippocampus)	Parahippocampal Gyrus
299	50	101	121	R	0.006		Postcentral Gyrus
278	47	66	78	R	0.01		Lateral Occipital Cortex
251	109	72	129	L	0.012		Superior Parietal Lobule
238	106	93	75	L	0.014		
236	64	100	92	R	0.014	Posterior Corona Radiata	
234	120	56	80	L	0.032	Posterior Thalamic Radiation (include optic radiation)	
199	72	126	82	R	0.028	Anterior Limb of Internal Capsule	
191	54	137	116	R	0.008		Middle Frontal Gyrus
182	135	127	50	L	0.014		Planum Pole
177	107	138	57	L	0.02		Frontal Orbital Cortex
176	73	146	51	R	0.018		Frontal Orbital Cortex
175	51	69	59	R	0.024		Temporal Occipital Fusiform Cortex

Threshold = 175 voxels
* uncorrected

Table 3: Tract Based Spatial Statistics: 5-HTTLPR

Uncorrected for multiple comparisons

TBSS contrasts between L'L' vs. S'L' depressed individuals revealed that the L'L' depressed group has higher FA in the right anterior corona radiata [x=70, y=179, z=61, voxels=1041, p=0.012] or [x=63, y=155, z=84, voxels=1041, p=0.012] relative to S'L'. Comparisons between L'L' and S'S' in the depressed group revealed no significant differences in FA between groups.

3.2.3 BDNF Comparisons

FA maps were constructed for contrasts between met-BDNF carriers versus homozygous val-BDNF in depression individuals. No between group differences were found in the ROIs in this contrasts.

3.2.4 CTQ Comparisons

Depressed participants were median split (median =55) based on self report CTQ scores (Wichers et al., 2008). FA maps were constructed for contrasts between individuals with depression who self-reported high vs. low total childhood trauma questionnaire scores based on a median split. No corrected or uncorrected between group differences were found within the ROIs in this contrasts.

3.3 Probabilistic Tractography

One control participant was removed from probabilistic tractography analysis due to image artifacts affecting the processing pipeline. Analyses included all participants with tract specific fractional anisotropy output.

3.3.1 Cingulum Bundle

Fractional anisotropy did not significantly regress with a multiple linear regression model including depression severity and total CTQ ($F(2,39)=1.071$, $p=0.3525$). There was a significant main effect of BDNF polymorphisms on FA in the cingulum bundle ($p=0.0279$), with met-BDNF carriers having higher FA than val-BDNF homozygotes [G/A: 0.439, G/G: 0.386](see figure 6). There was no main effect of depression severity, any type of childhood trauma (p's>0.05) or 5-HTTLPR polymorphisms ($p=0.9061$) on FA in the cingulum bundle.

Fractional anisotropy did not significantly regress with interactions between depression severity and (1) types of childhood trauma (p's>0.05) or (2) genetic risk factors [BDNF ($p=0.1765$) or 5-HTTLPR polymorphisms ($p=0.959$)].

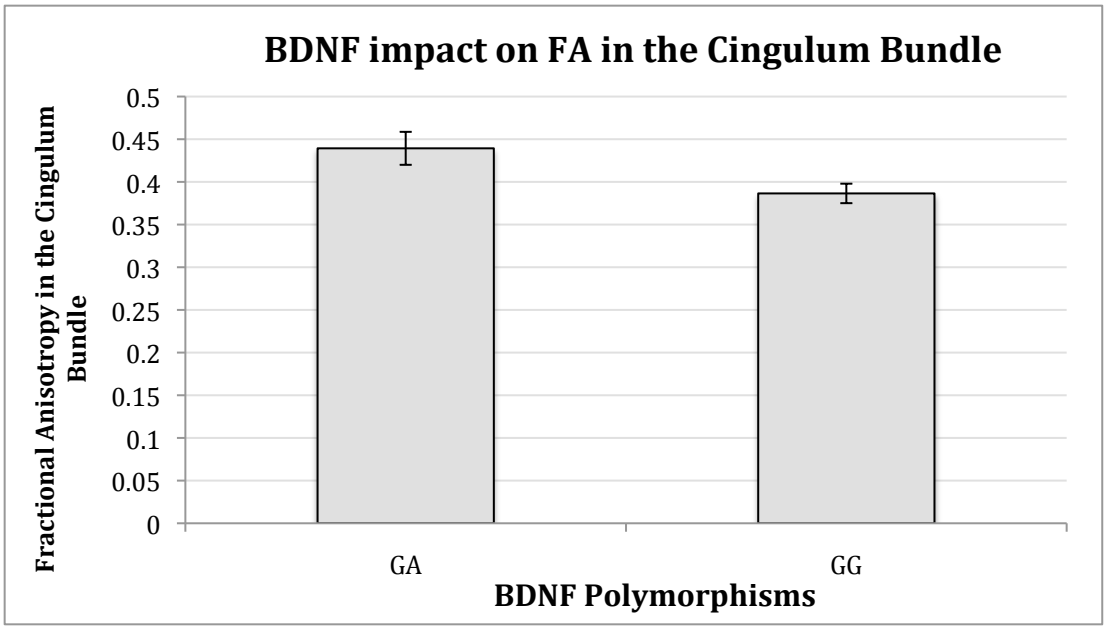


Figure 6: Influence of BDNF polymorphisms on FA in the cingulum bundle

3.3.2 Uncinate Fasciculus

There was a significant main effect of BDNF polymorphisms (p=0.0045) on FA in the uncinate fasciculus, with homozygous val-BDNF individuals having higher FA then met-BDNF carriers [G/G: 0.28, G/A: 0.24] (see figure 7). There were no significant effects of 5-HTTLPR polymorphisms (p=0.364), types of childhood trauma (p's>0.05) or depression severity (p=0.385) on FA in the uncinate fasciculus.

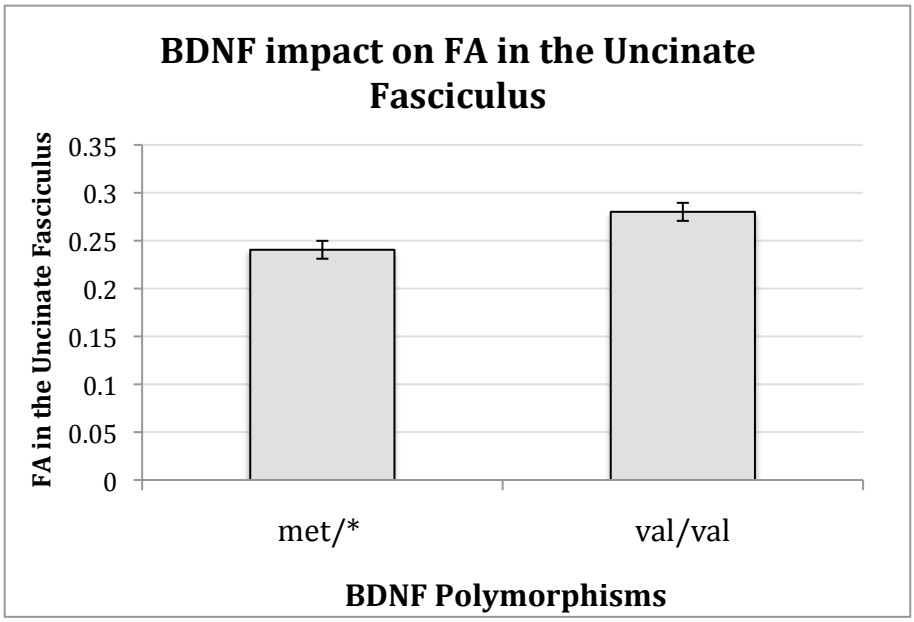


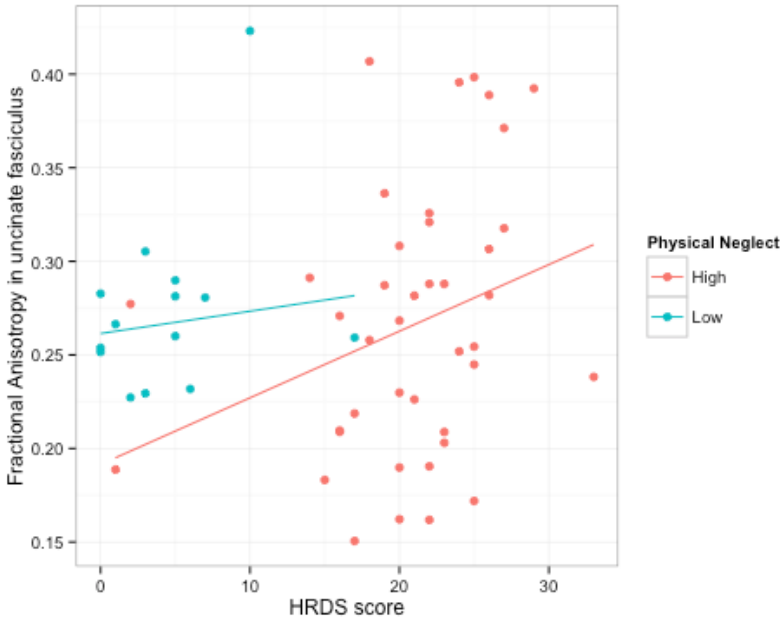
Figure 7: Influence of BDNF polymorphisms on FA in the uncinate fasciculus.

Fractional anisotropy exhibited a trend towards significantly regressing with a multiple linear regression model including depression severity and total CTQ ($F(2,51)=4.62$, $p=0.0143$). Fractional anisotropy significantly regressed with an interaction between pretreatment depression severity and (1) physical neglect ($F(4,49)=4.618$, $p=0.00304$, $r^2=0.2743$) and showed trends with (2) emotional neglect ($F(4,49)=2.937$, $p=0.0297$, $r^2=0.1934$) beyond the main effect of the associated variables and total CTQ (figure 8a,b). Individuals with high levels of depression with exposure to severe childhood neglect exhibited higher FA values in the uncinate fasciculus, relative to individuals with low levels of depression [physical neglect: $p=0.03$, emotional neglect: $p=0.06$].

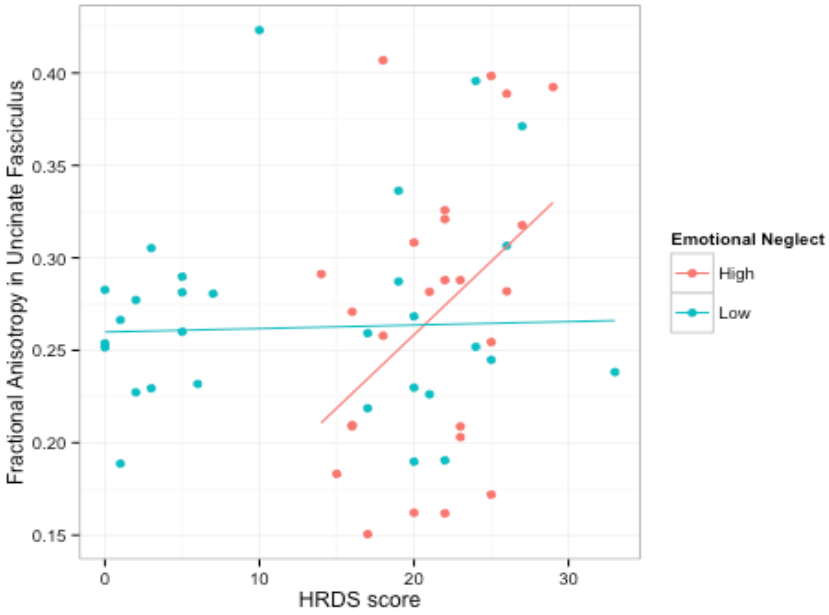
Fractional anisotropy significantly regressed with an interaction between pretreatment depression severity and BDNF polymorphisms ($F(3,56)=2.674$, $p=0.055$) beyond the main effect of the associated variables and total CTQ (figure

8c). Indicating that as depression severity increased BDNF polymorphisms had a more substantial effect on FA in the uncinate, with val-BDNF individuals having higher FA relative to met-BDNF carriers [$t(35)$=-2.3979, p=0.02]. Interactions between depression severity and other types of trauma or 5-HTTLPR polymorphisms did not significantly regress with FA in the uncinate (p>0.05's).

a)



b)


Fractional Anisotropy in Uncinate Fasciculus
0.40
0.35
0.30
0.25
0.20
0.15
0
10
20
30
HRDS score
Emotional Neglect
High
Low

c)

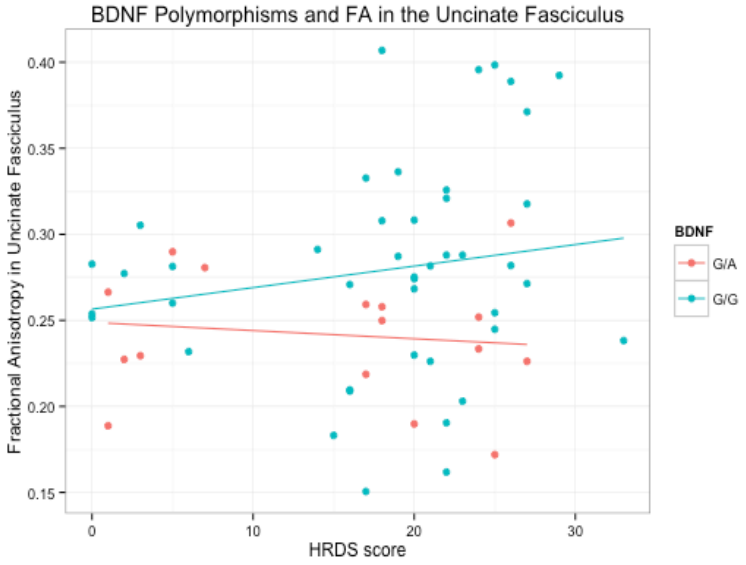

BDNF Polymorphisms and FA in the Uncinate Fasciculus
Fractional Anisotropy in Uncinate Fasciculus
0.40
0.35
0.30
0.25
0.20
0.15
0
10
20
30
HRDS score
BDNF
G/A
G/G

Figure 8: Tractography – Depression Severity on FA in the uncinate fasciculus, with a moderator of early neglect [(a) Physical Neglect (b) Emotional Neglect] and (c) BDNF polymorphism. To graphically represent trends within the data, categories of childhood maltreatment were categorized. Physical and emotional neglect were split into high and low based on cuts offs (Scher et al., 2004; E. A. Walker et al., 1999). For physical neglect, a score of 8 or under was considered low, and for emotional neglect, a score of 15 or lower was considered low. [G/G: val/val, G/A: met/*]

To determine whether these effects are lateralized, analysis was repeated on both the left and right uncinate fasciculus for types of trauma exhibiting significant interactions in the above results. Results were lateralized as interactions between depression severity and physical neglect significantly regressed with FA in the left ($F(4,45)=6.187$, $p=0.000469$), but not right ($F(4,40)=0.916$, $p=0.464$) uncinate fasciculus, beyond the main effect of the associated variables and total CTQ . With respect to emotional neglect, interactions between depression severity and emotional neglect significantly regressed with FA in the left ($F(4,45)=5.314$, $p=0.0014$), but not right right ($F(4,40)=0.592$, $p=0.671$) uncinate fasciculus, beyond the main effect of the associated variables and total CTQ .

Exploratory, three-way interactions examined the moderating effect of specific risk genes on the significant interactions in the above analyses. A three-way interaction between depression severity, genetic risk factors (BDNF or 5-HTTLPR polymorphisms) and severity of physical or emotional neglect were regressed against FA in the uncinate fasciculus. Significant three-way interactions were found between depression severity, BDNF and physical neglect ($F(7,44)=2.202$, $p=0.05237$) or emotional neglect ($F(7,44)=2.468$, $p=0.03162$). In contrast, no

significant interactions were found between depression severity, 5-HTTLPR polymorphisms and physical neglect ($F(11,40)=1.491$, $p=0.1713$) or emotional neglect ($F(11,40)=1.279$, $p=0.2712$). These results suggest that although depression severity and childhood trauma have a stronger impact on fractional anisotropy, genetic variants seem to moderate their influence. Overall, the data indicates that as depression severity increases, individuals who experienced severe neglect show increased FA values in the uncinate fasciculus.

3.3.3 Superior Longitudinal Fasciculus

Fractional anisotropy did not significantly regress with a multiple linear regression model with depression severity and total CTQ ($F(2,50)=0.999$, $p=0.3752$). There were no significant effects of depression severity, type of childhood trauma or genetic risk factors (BDNF or 5-HTTLPR polymorphisms) on FA in the superior longitudinal fasciculus ($p's>0.05$).

Fractional anisotropy exhibited a trend towards significantly regressing with an interaction between pretreatment HDRS scores and physical neglect ($F(4,47)=2.278$, $p=0.0748$), but not with emotional neglect ($F(4,47)=0.9299$, $p=0.4548$), beyond the main effect of the associated variables and total CTQ (see figure 9a,b). Individuals with high levels of depression and experienced severe physical neglect exhibited a trend towards higher FA values in the superior longitudinal fasciculus [physical neglect: $p=0.055$], relative to low depression severity. There were no significant interactions between pretreatment HDRS scores and other types of trauma or genetic risk factors (BDNF or 5-HTTLPR polymorphisms) on FA in the superior longitudinal fasciculus ($p>0.05's$).

a)

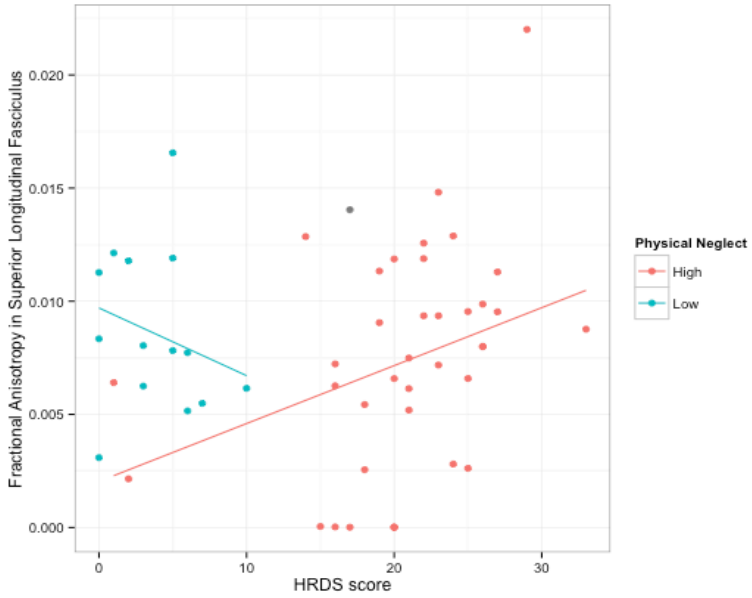


Figure 9: Tractography – Depression Severity on FA in the superior longitudinal fasciculus, with a moderator of early physical neglect . To graphically represent trends within the data, categories of childhood maltreatment were categorized. Physical neglect was split into high and low based on cuts offs (Scher et al., 2004; E. A. Walker et al., 1999). For physical neglect, a score of 8 or under was considered low. Data has been transformed with an exponential transform to fit normality [transformed ^6].

To determine whether these effects are lateralized, analysis was repeated on both the left and right superior longitudinal fasciculus for types of trauma exhibiting significant interactions in the above results. Two-way interactions of depression severity and physical neglect significantly regressed with FA in the left superior longitudinal fasciculus ($F(4,41)=3.821$, $p=0.0099$) and exhibited a trend with the

right (F(4,48)=2.356, p=0.067) superior longitudinal fasciculus, beyond the main effect of the associated variables and total CTQ.

Exploratory, three-way interactions examined the moderating effect of specific risk genes on the significant interactions in the above analyses. A three-way interaction between depression severity, genetic risk factors (BDNF gene polymorphisms and 5-HTTLPR) and severity of a physical neglect were regressed against FA in the superior longitudinal fasciculus. Significant three-way interactions were found between depression severity, physical neglect, and BDNF polymorphisms (F(7,42)=3.195, p=0.00838) and a trend for 5-HTTLPR polymorphisms (F(11,38)=1.931, p=0.06577). These results suggest that although depression severity and childhood trauma have a stronger impact on fractional anisotropy, genetic variants seem to moderate their influence. Overall, the data indicates that as depression severity increases, individuals who experienced severe neglect show increased FA values in the superior longitudinal fasciculus.

3.4 Regression Analyses with TBSS Findings

3.4.1 Right Anterior Internal Capsule

There was a significant main effect of physical neglect (p=0.018) on FA in the right anterior internal capsule (R-AntIntCap), with FA decreasing as scores of physical neglect increased. There was no significant effect of depression severity (p=0.1399), other types of childhood trauma (p's>0.05), or genetic risk factors (BDNF or 5-HTTLPR polymorphisms) on FA in the R-AntIntCap.

FA in the right internal capsule exhibited a trend towards significantly regressing with an interaction between pretreatment HDRS scores and physical neglect

(F(3,57)=2.198, p=0.0982)(see figure 10), but not with genetic risk factors (BDNF or 5-HTTLPR polymorphisms) or other types of trauma (p>0.05's). Individuals with low levels of depression and low childhood exposure to physical neglect exhibited higher FA in the R-AntIntCap [p=0.03], relative to high childhood exposure to physical neglect.

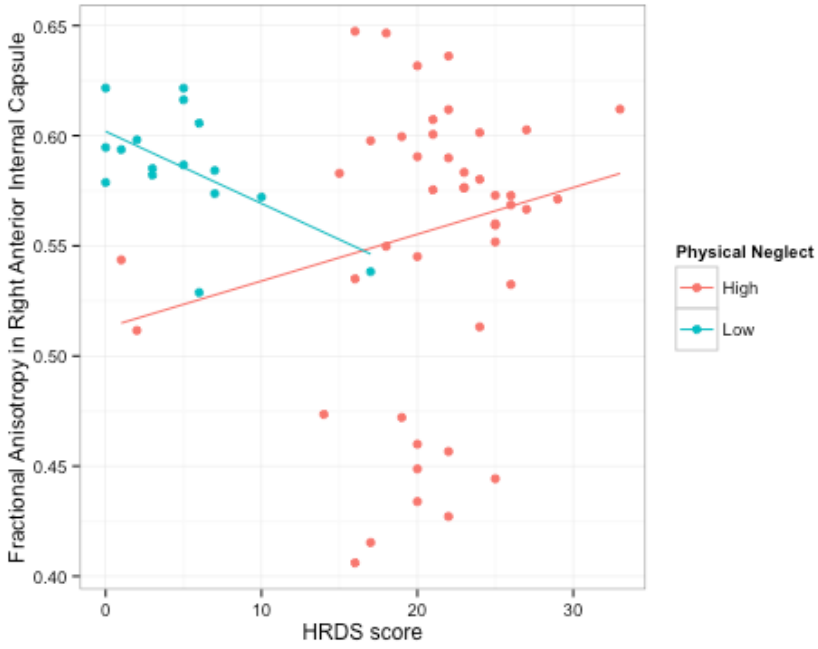


Figure 10: Tractography – Depression Severity on FA in the Right Anterior Internal Capsule, with a moderator of early neglect [Physical Neglect]. To graphically represent trends within the data, categories of childhood maltreatment were categorized. Physical neglect were split into high and low based on cuts offs (Scher et al., 2004; E. A. Walker et al., 1999). For physical neglect, a score of 8 or under was considered low.

Exploratory, three-way interactions examined the moderating effect of specific risk genes on the significant interactions in the above analyses. A three-way interaction between depression severity, genetic risk factors (BDNF gene

polymorphisms and 5-HTTLPR) and severity of physical neglect were regressed against FA in the R-AntIntCap. No significant interactions within FA in the R-AntIntCap were found between depression severity, physical neglect, and BDNF or 5-HTTLPR polymorphisms (p's>0.05).

3.4.2 Fornix

There were no significant main effects of depression severity (p=0.222), any type of childhood trauma (p's>0.05) or genetic risk factors [5-HTTLPR (p=0.364) or BDNF polymorphisms (p=0.792)] on FA in the fornix .

FA in the fornix did not significantly regress with interactions between pretreatment HDRS scores and (1) severity of any type of trauma or (2) genetic risk factors (BDNF or 5-HTTLPR polymorphisms) (p>0.05's).

3.4.2 Right Anterior Corona Radiata

There were no significant main effects of depression severity (p=0.6217), any type of childhood trauma (p's>0.05) or genetic risk factors [5-HTTLPR (p=0.906) or BDNF polymorphisms (p=0.9426)] on FA in the right anterior corona radiata.

FA in the right anterior corona radiata did not significantly regress with interactions between pretreatment HDRS scores and (1) severity of any type of trauma or (2) genetic risk factors (BDNF or 5-HTTLPR polymorphisms) (p>0.05's).

Eleven participants were scanned on a new magnetic imaging scanner implemented into the Calgary Imaging Center. Scanner parameters were consistent across scanners and controlled for variability by the on-site physicist. Analyses for

all significant findings were repeated, and were significant even in the smaller sample that excluded new scanner participants ($p<0.05$).

Due to the limited number of diffusion directions used in the present study, probabilistic tractography analysis of specific tracts was unable to extract diffusion parameters for specific remodeled association tracts in a portion of our sample. Participant data was excluded in cases where diffusion data could not be extracted.

4.0 Discussion

Our analysis used tract-based spatial statistics and probablistic tractography to investigate fractional anisotropy differences across MDD and control groups. Regression modeling was used to examine how genetic factors, environmental experiences and depression severity influence FA changes in specific white matter association tracts. The present study suggests widespread differences in FA between MDD patients and controls, concentrated in the right anterior internal capsule, fornix and right anterior corona radiata. The data also suggest that the effects of depression on neuronal connectivity are moderated by experiences of childhood adversity. Individuals who experience high levels of depression severity following childhood neglect exhibited increased fractional anisotropy in the uncinate and superior longitudinal fasciculus. Additionally, at low levels of emotional and physical neglect there were no differences in fraction anisotropy in the uncinate and superior longitudinal fasciculus between individuals with high and low depression severity. As rated experiences of early life neglect increased, more prominent differences were found between groups.

This work has replicated many original and previous findings of decreased FA across many neural regions in MDD patients relative to controls (M. L. Murphy & Frodl, 2011). Differences in FA represent a lack of myelination, cohesion, organization or connectivity between communicating neural regions. Trends towards significant between group differences were found in the anterior internal capsule, fornix, and anterior corona radiata. The anterior internal capsule contains thalamocortical projection fibers that connect the medial dorsal thalamic nuclei and the frontal cortex (Gutman, Holtzheimer, Behrens, Johansen-Berg, & Mayberg, 2009; Zhu et al., 2011). The fornix is related to the limbic system; extends to the hippocampus and is involved in learning and memory (Frodl et al., 2012). The anterior corona radiata consists of projection fibers anterior to the genu of the corpus callosum and supports thalamic cortical connections (Mori et al., 2008; Mueller et al., 2010). These findings provide support for the role thalamic cortical connections have on the etiology of MDD (Carballedo, Amico, et al., 2012a; Jonassen, 2012; Lafer, Renshaw, & Sachs, 1997; Pacheco et al., 2009; Tost et al., 2013). Compared to other regions affected in depression, the fornix exhibits the most substantial (13.5%) decrease in FA (Korgaonkar et al., 2011; Tost et al., 2013). Low FA levels in this region are also associated with remission failure (Hoogenboom et al., 2014; Pacheco et al., 2009).

Our data demonstrate consistent trends in FA across many neural regions. Early neglect moderates the influence depression has on FA. Non-significant differences in FA were observed between individuals high and low depression severity who experienced low levels of neglect. In contrast, individuals who

experienced high levels of childhood adversity, specifically neglect, showed increases in FA as depression severity increased.

Consistent with our findings, Frodl and colleagues (2012) found similar results in a DTI investigation on the neuronal connectivity changes associated with childhood adversity in depression at-risk, and healthy controls (Frodl, 2012; Jonassen, 2012). The authors reported similar between group differences in FA in many overlapping regions. Healthy relatives of depressed individuals who retrospectively reported childhood maltreatment exhibited increased FA across regions such as the superior longitudinal fasciculus and the fornix, but also the body and splenium of the corpus callosum and inferior fronto-occipital fasciculus. Additionally, the healthy controls showed a decrease in FA in these regions following experiences of childhood maltreatment. Since the literature has primarily associated childhood maltreatment with reduced FA (Hart, 2012; Lebel et al., 2012), the authors speculated that the increase in FA in the unaffected healthy relatives was associated with their resilience to depression development. The present findings suggest that rather than resilience, an increase in FA following childhood maltreatment may confer risk for the development of depression. It is possible that the reported affected regions exhibit a sensitivity to depression risk factors, which confers risk for depression following exposure to early life stress. Early life stress has been associated with abnormalities within the HPA axis, and thus alterations to cortisol levels in response to stress (Heim & Binder, 2011). It can be speculated that the reduced cortisol profiles exhibited in individuals who have experienced childhood neglect may provide an environment for unhindered myelin production.

Without the limiting effects of cortisol, oligodendrocytes may have the capability to overproduce myelin. In the present study, tracts involved in emotion regulation and cognitive control were most affected, as these tracts connect regions that may have been overactive following experiences of neglect. In accord, early consolidation of limbic circuits has also been reported following early deprivation experiences (Gee et al., 2013). Increased connections within affective circuits may also be association with reduced top-down regulation, and thus reduced ability to regulate emotional response and an increased sensitivity to environmental cues. These types of changes have been associated with the development of depression and a decreased ability to regulate emotion (Beauregard, Paquette & Levesque, 2006).

Interestingly, the present findings indicate a specific neuroconnectivity profile in individuals who self-report childhood experiences of neglect, that was not found in other subtypes of adversity. More heterogeneous experiences of adversity, consisting of individuals whom experienced more severe abuse, rather than neglect, may explain the discrepancies in myelin integrity findings between present and previous findings (Bernstein et al., 2003). Thus, future investigations should investigate the neuroanatomical and functional effects of neglect and the implications these changes have on depression development relative to other subtypes of adversity,

In agreement with our findings, increased FA has also been found in the superior temporal gyrus in healthy subjects exposed to parental verbal abuse. This finding, in conjunction with our findings, may suggest that the neural region affected by childhood adversity depends on the type of adversity experienced and the

regions involved in processing those experiences (J. Choi, Jeong, Polcari, Rohan, & Teicher, 2012; Tomoda et al., 2011; Wichers et al., 2008). Additionally, childhood adversity, specifically neglect, seems to strengthen connections between neural regions, suggesting a possible compensatory mechanism to cope with the adversity or temporal changes in the developmental processes of circuit consolidation and myelination.

Frodl and colleagues also found moderating affects of early life adversity on grey matter volume differences in individuals at risk for depression. At-risk depressed individuals with a history of emotional abuse had smaller grey matter volumes in regions associated with tracts affected in the present study, namingly the dorsolateral prefrontal cortices, medial prefrontal cortices and anterior cingulate cortex (Carballedo, Lisiecka, et al., 2012b; Hu et al., 2005).

Building on previous literature our group has identified that experiences of neglect in childhood have a strong impact on neural connections that are dysregulated in depression. These results indicate, that childhood trauma is a strong moderating factor affecting fractional anisotropy, and that the lack of conclusive associations between depression and neuroconnectivity (K. S. Choi et al., 2014; Dougherty & Rauch, 2007; Fournier et al., 2010; Huang et al., 2010) may be attributed to studies not including factors of early life stress, particularly neglect.

Neglect involves the lack of stimulation or interaction required for the brain's proper maturation (De Bellis, 2005). General deprivation of experiences may also reduce neuronal activity, survival, synaptic connectivity and neurotrophic production, which would result in neural network organization abnormalities and

lasting negative consequences (Anda et al., 2005). Research by Anguilera et al. (2009) determined that childhood adversity, specifically emotional neglect, sexual abuse and emotional abuse had the strongest impact on depression symptoms in healthy adults (Aguilera et al., 2009). It is possible that neglect is different then the other forms of childhood trauma as it has a greater impact on neurobiological structures involved in emotional, cognitive and stress control. Abnormal neural organization resulting in FA aberrations, were observed in adolescents who experienced early neglect (Hanson et al., 2013). The white matter regions affected in these individuals are overlapped by the superior longitudinal fasciculus, a white matter tract that has exhibited neural structure alterations that correlate with declines in neurocognitive performance (Hanson et al., 2013). In addition to the more broadly studied forms of childhood adversity including witnessing a domestic violence, verbal abuse, physical abuse and sexual abuse, the present findings provide support for investigating the impact neglect has on depression development.

Overlapping neuroanatomical changes are documented in individuals with depression and exposed to childhood maltreatment, including alterations to regions such as the corpus callosum (Teicher et al., 2004), superior longitudinal fasciculus, uncinate (Govindan, Behen, Helder, Makki, & Chugani, 2010), cingulum (Keedwell et al., 2012) and fornix (Frodl et al., 2012). The superior longitudinal fasciculus connects lateral parts of the inferior parietal lobule with the lateral inferior prefrontal lobe, and plays a role in the fronto-parietal circuit involved in working memory (Preuss & Goldman Rakic, 1989). Stable reductions in FA in the superior

longitudinal fasciculus have been found in a meta-analysis of DTI studies on depression (M. L. Murphy & Frodl, 2011).

The largest fronto-limbic tract is the uncinate fasciculus, which is involved in cognitive emotion regulation, and connects orbitomedial and ventrolateral aspects of the prefrontal cortex with the ipsilateral hippocampal gyrus and amygdala (Catani, Howard, Pajevic, & Jones, 2002). Healthy individuals who were exposed to early deprivation have decreased FA in the uncinate (Eluvathingal, 2006) and superior longitudinal fasciculus (Govindan et al., 2010). These decreases have been associated with anxiety traits, and neurocognitive/behavioural functioning (Eluvathingal, 2006). A number of studies on the fronto-limbic system in maltreated individuals have also found abnormal activity in the amygdala and hippocampus during fear conditioning (Bremner et al., 2005; Maheu et al., 2010).

The cingulum is a medial tract that arches superiorly to the corpus callosum from the anterior cingulate to the occipital lobe. It connects the orbitofrontal cortex to the ipsilateral temporal gyrus, as well as the cingulate cortex to the ipsilateral medial frontal, parietal, occipital, and temporal lobes (Catani et al., 2002). The cingulum is involved in attention, memory and emotion regulation. Adults following exposure to childhood adversity have shown reduced FA in the cingulum (by the posterior tail of the left hippocampus) and left fornix (J. Choi et al., 2009).

The anterior internal capsule contains thalamocortical projection fibers that connect the medial dorsal thalamic nuclei and the frontal cortex (Gutman et al., 2009; Zhu et al., 2011). Some of the original DTI studies on depression found abnormalities in this region (Zhu et al., 2011; K. Zou et al., 2008), which may reflect

a loss of white-matter integrity and dysregulation in fronto-striatal and limbic-DLPFC-thalamic systems known to be affected in depression (Drevets, Price, & Furey, 2008; Guo et al., 2012; Korgaonkar et al., 2011). This region has also been targeted by deep brain stimulation in the treatment of depression (Gutman et al., 2009; Malone et al., 2009). Increased fronto-striatal connectivity may predict treatment outcomes, as stronger connections within this circuit has also been associated with increased positive affect and stronger treatment response (Heller et al., 2013; Danuta Lisiecka et al., 2010). This same fronto-striatal network is affected in maltreated subjects. A study on response inhibition found increased activation in the inferior frontal cortex and striatum in previously maltreated subjects relative to controls (Mueller et al., 2010). These findings may explain deficits in inhibitory control seen in individuals exposed to early life stress.

The majority of previous literature on this topic has focused on exploring how genetic risk factors for depression influence FA in healthy controls(Carballedo, Amico, et al., 2012a; Jonassen, 2012; Pacheco et al., 2009; Tost et al., 2013). Main effects of BDNF polymorphisms in the present study revealed differing effects across white matter tracts. Within the cingulum bundle and uncinate fasciculus met-carriers exhibited higher and lower FA, respectively. This may suggest that the regions overlapped by these white matter tracts use BDNF differently, similarly to how BDNF infusion into the hippocampus and nucleus accumbens produce opposing effects in a social defeat paradigm (Berton et al., 2006). Alternatively, the availability of BDNF may have differential effects on myelin production, ultimately influencing measures of fractional anisotropy.

The present study also found that the association between depression severity and FA in the uncinate fasciculus is moderated by polymorphisms in the BDNF gene. With no significant differences in uncinate FA in met-carriers and val-homozygotes at low depression severity, but significant differences between groups at high depression severity. Therefore, as depression severity increased genetic risk factors had a more substantial impact on fractional anisotropy. Carballedo and colleagues (2012) reported similar findings, as patients who had high depression severity and were met-BDNF carriers had lower FA in the UF relative to patients with the val-BDNF allele. This is in contrast to findings in healthy individuals, who exhibited lower FA in the uncinate for val-BDNF homozygotes relative to met-BDNF carriers (Tost et al., 2013). Based on these findings, it is possible that BDNF polymorphisms have differing effects in depressed individuals and healthy controls. Furthermore, the effects of BDNF polymorphisms seem to differ across tracts, and may indicate a sensitivity to changes in BDNF in regions connected by specific tracts. Future studies are required to tease out these differences, and to further understand the role BDNF has in pathology and myelin integrity within different regions of interest.

Although not assessed in this study, age significantly influences BDNF's effect on FA in the UF (Pacheco et al., 2009), or completely eliminates genetic effects (Jonassen, 2012). The present study is advantageous as the mean age of participants is similar to the age at which peak FA values are identified in the uncinate fasciculus (Lebel et al., 2012), and therefore may provide a more accurate estimation of the influence of genetics on neuroconnectivity. Further studies are required to

understand how age influences the effect depression, genetic risk and adversity have on neural connectivity, and how these factors interact in the development of depression.

TBSS results in the present study revealed that highly depressed individuals with the S'S' alleles of the 5-HTTLPR had higher FA in the right uncinate relative to patients with the S'L' genotype. Previous literature has indicated that the effects of gene and environment on depression severity are strongest in individuals with the SL allele (Wichers et al., 2008), which may be reflected in the low uncinate FA found in this group. Future large powered investigations are required to understand the possible connections between polymorphisms within the 5HTTLPR and neuroconnectivity changes.

Results were analyzed based on the tri-allelic theory of 5-HTTLPR (L_A, L_G & S_A) and were categorized based on transcription efficiency. We grouped alleles to increase power in our analyses. We believe that collapsing L_G and S_A did not impact our results as this is a common practice in the literature. Previous findings also indicate that the L_G and S_A allele are not significantly different in transcription efficiency, with the S_A allele having lower efficiency in absolute terms (Hu et al., 2005).

4.1 Conclusion

It has been shown that early life neglect has a strong influence on whether depression severity influences neural connectivity within the brain. Regions most affected by early life stress were the superior longitudinal fasciculus and uncinate fasciculus, which are involved in emotional cognitive regulation and cognitive

control. The data also suggest that connectivity within the uncinate is affected by polymorphisms within the BDNF gene. Furthermore, as depression severity increases polymorphisms within this gene have a stronger effect on FA in the uncinate fasciculus. These findings suggest that connectivity within frontal and limbic regions are affects in depression and are influenced by early life experiences and genetic risk factors.

References

Agid, O., Kohn, Y., & Lerer, B. (2000). Environmental stress and psychiatric illness. *Biomedicine & Pharmacotherapy*, *54*(3), 135–141.

Aguilera, M., Arias, B., Wichers, M., Barrantes-Vidal, N., Moya, J., Villa, H., et al. (2009). Early adversity and 5-HTT/BDNF genes: new evidence of gene–environment interactions on depressive symptoms in a general population. *Psychological Medicine*, *39*(09), 1425. doi:10.1017/S0033291709005248

Alexander, N., Klucken, T., Koppe, G., Osinsky, R., Walter, B., Vaitl, D., et al. (2012). Interaction of the Serotonin Transporter-Linked Polymorphic Region and Environmental Adversity: Increased Amygdala-Hypothalamus Connectivity as a Potential Mechanism Linking Neural and Endocrine Hyperreactivity. *Bps*, *72*(1), 49–56. doi:10.1016/j.biopsych.2012.01.030

Anda, R. F., Felitti, V. J., Bremner, J. D., Walker, J. D., Whitfield, C., Perry, B. D., et al. (2005). The enduring effects of abuse and related adverse experiences in childhood. *European Archives of Psychiatry and Clinical Neuroscience*, *256*(3), 174–186. doi:10.1007/s00406-005-0624-4

Andersen, S., Tomada, A., Vincow, E., Valente, E., Polcari, A., & Teicher, M. (2008). Preliminary evidence for sensitive periods in the effect of childhood sexual abuse on regional brain development. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *20*(3), 292–301.

Banner, H., Bhat, V., Etchamendy, N., Joober, R., & Bohbot, V. D. (2011). The brain-derived neurotrophic factor Val66Met polymorphism is associated with reduced functional magnetic resonance imaging activity in the hippocampus and increased use of caudate nucleus-dependent strategies in a human virtual navigation task. *European Journal of Neuroscience*, *33*(5), 968–977. doi:10.1111/j.1460-9568.2010.07550.x

Barr, C. S., Newman, T. K., Schwandt, M., Shannon, C., Dvoskin, R. L., Lindell, S. G., et al. (2004). Sexual dichotomy of an interaction between early adversity and the serotonin transporter gene promoter variant in rhesus macaques. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(33), 12358–12363.

Bath, K. G., & Lee, F. S. (2006). Variant BDNF (Val66Met) impact on brain structure and function. *Cognitive, Affective, & Behavioral Neuroscience*, *6*(1), 79–85.

Baxter, L. R., Jr, Schwartz, J. M., Phelps, M. E., Mazziotta, J. C., Guze, B. H., Selin, C. E., et al. (1989). Reduction of prefrontal cortex glucose metabolism common to three types of depression. *Archives of General Psychiatry*, *46*(3), 243.

Beauregard, M., Paquette, V., & Levesque, J. (2006). Dysfunction in the neural circuitry of emotional self-regulation in major depressive disorder. *NeuroReport*, *17*(8), 843–846.

Behrens, T. E. J., Woolrich, M. W., Jenkinson, M., Johansen-Berg, H., Nunes, R. G., Clare, S., et al. (2003). Characterization and propagation of uncertainty in diffusion-weighted MR imaging. *Magnetic Resonance in Medicine*, *50*(5), 1077–1088. doi:10.1002/mrm.10609

Bellivier, F., Henry, C., Szöke, A., Schürhoff, F., Nosten-Bertrand, M., Feingold, J., et al. (1998). Serotonin transporter gene polymorphisms in patients with unipolar or

bipolar depression. *Neuroscience Letters*, *255*(3), 143–146.
Bennett, A. J., Lesch, K. P., Heils, A., Long, J. C., Lorenz, J. G., Shoaf, S. E., et al. (2002). Early experience and serotonin transporter gene variation interact to influence primate CNS function. *Molecular Psychiatry*, *7*(1).
Bernet, C. Z., & Stein, M. B. (1999). Relationship of childhood maltreatment to the onset and course of major depression in adulthood. *Depression and Anxiety*, *9*(4), 169–174.
Bernstein, D. P., Stein, J. A., Newcomb, M. D., Walker, E., Pogge, D., Ahluvalia, T., et al. (2003). Development and validation of a brief screening version of the Childhood Trauma Questionnaire. *Child Abuse & Neglect*, *27*(2), 169–190. doi:10.1016/S0145-2134(02)00541-0
Berton, O., McClung, C. A., DiLeone, R. J., Krishnan, V., Renthal, W., Russo, S. J., et al. (2006). Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science*, *311*(5762), 864–868.
Brem, S., Walitza, S. (2002) Structural and functional alterations in the brain of patients exposed to childhood violence.
Bremner, J. D., Vermetten, E., Schmalh, C., Vaccarino, V., Vythilingam, M., AFZAL, N., et al. (2005). Positron emission tomographic imaging of neural correlates of a fear acquisition and extinction paradigm in women with childhood sexual-abuse-related post-traumatic stress disorder. *Psychological Medicine*, *35*(6), 791–806. doi:10.1017/S0033291704003290
Brown, G. W., & Harris, T. O. (2008). Depression and the serotonin transporter 5-HTTLPR polymorphism: A review and a hypothesis concerning gene–environment interaction. *Journal of Affective Disorders*, *111*(1), 1–12. doi:10.1016/j.jad.2008.04.009
Bruce, J., Fisher, P. A., Pears, K. C., & Levine, S. (2009). Morning cortisol Levels in preschool-aged foster children: Differential effects of maltreatment type. *Developmental Psychobiology*, *51*(1), 14–23. doi:10.1002/dev.20333
Brunoni, A. R., Lopes, M., & Fregni, F. (2008). A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. *The International Journal of Neuropsychopharmacology*, *11*(08), 1169. doi:10.1017/S1461145708009309
Bukh, J. D., Bock, C., Vinberg, M., Werge, T., Gether, U., & Kessing, L. V. (2009). Journal of Affective Disorders. *Journal of Affective Disorders*, *119*(1-3), 107–115. doi:10.1016/j.jad.2009.02.023
Canli, T., & Lesch, K.-P. (2007). Long story short: the serotonin transporter in emotion regulation and social cognition. *Nature Neuroscience*, *10*(9), 1103–1109. doi:10.1038/nn1964
Cannon, D. M. (2010). Neuroimaging and the pathophysiology and treatment of depression: recent advances and future needs.
Carballedo, A., Amico, F., Ugwu, I., Fagan, A. J., Fahey, C., Morris, D., et al. (2012a). Reduced fractional anisotropy in the uncinate fasciculus in patients with major depression carrying the met-allele of the Val66Met brain-derived neurotrophic factor genotype. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *159B*(5), 537–548. doi:10.1002/ajmg.b.32060
Carballedo, A., Lisiecka, D., Fagan, A., Saleh, K., Ferguson, Y., Connolly, G., et al.

(2012b). Early life adversity is associated with brain changes in subjects at family risk for depression. *World Journal of Biological Psychiatry*, *13*(8), 569–578. doi:10.3109/15622975.2012.661079

Carew, C. L., Milne, A. M., Tatham, E. L., MacQueen, G. M., & Hall, G. B. C. (2013). Neural systems underlying thought suppression in young women with, and at-risk, for depression. *Behavioural Brain Research*, *257*, 13–24. doi:10.1016/j.bbr.2013.09.016

Caspi, A. (2003). Influence of Life Stress on Depression: Moderation by a Polymorphism in the 5-HTT Gene. *Science*, *301*(5631), 386–389. doi:10.1126/science.1083968

Catani, M., Howard, R. J., Pajevic, S., & Jones, D. K. (2002). Virtual in Vivo Interactive Dissection of White Matter Fasciculi in the Human Brain. *NeuroImage*, *17*(1), 77–94. doi:10.1006/nimg.2002.1136

Chen, Z.-Y., Jing, D., Bath, K. G., Ieraci, A., Khan, T., Siao, C.-J., et al. (2006). Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science*, *314*(5796), 140–143.

Chipman, P., Jorm, A. F., Prior, M., Sanson, A., Smart, D., Tan, X., & Easteal, S. (2007). No interaction between the serotonin transporter polymorphism (5-HTTLPR) and childhood adversity or recent stressful life events on symptoms of depression: Results from two community surveys. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *144B*(4), 561–565. doi:10.1002/ajmg.b.30480

Choi, J., Jeong, B., Polcari, A., Rohan, M. L., & Teicher, M. H. (2012). Reduced fractional anisotropy in the visual limbic pathway of young adults witnessing domestic violence in childhood. *NeuroImage*, *59*(2), 1071–1079. doi:10.1016/j.neuroimage.2011.09.033

Choi, J., Jeong, B., Rohan, M. L., Polcari, A. M., & Teicher, M. H. (2009). Preliminary Evidence for White Matter TractAbnormalities in Young Adults Exposed toParental Verbal Abuse. *Bps*, *65*(3), 227–234. doi:10.1016/j.biopsych.2008.06.022

Choi, K. S., Holtzheimer, P. E., Franco, A. R., Kelley, M. E., Dunlop, B. W., Hu, X. P., & Mayberg, H. S. (2014). Reconciling Variable Findings of White Matter Integrity in Major Depressive Disorder, 1–8. doi:10.1038/npp.2013.345

Claussen, A. H., & Crittenden, P. M. (1991). Physical and psychological maltreatment: Relations among types of maltreatment. *Child Abuse & Neglect*, *15*(1), 5–18.

Cohen, R. A., Grieve, S., Hoth, K. F., Paul, R. H., Sweet, L., Tate, D., et al. (2006). Early Life Stress and Morphometry of the Adult Anterior Cingulate Cortex and Caudate Nuclei. *Biological Psychiatry*, *59*(10), 975–982. doi:10.1016/j.biopsych.2005.12.016

Collier, D. A., Arranz, M. J., Sham, P., Battersby, S., Vallada, H., Gill, P., et al. (1996). The serotonin transporter is a potential susceptibility factor for bipolar affective disorder. *NeuroReport*, *7*(10), 1675–1679.

Cook, R. D., & Weisberg, S. (1982). Residuals and influence in regression.

Cullen, K. R., Klimes-Dougan, B., Muetzel, R., Mueller, B. A., Camchong, J., Houri, A., et al. (2010). NEW RESEARCHAltered White Matter Microstructurein Adolescents With Major Depression:A Preliminary Study. *Jaac*, *49*(2), 173–183.e1.

doi:10.1016/j.jaac.2009.11.005
Dalby, R. B., Frandsen, J., Chakravarty, M. M., Ahdidan, J., Sørensen, L., Rosenberg, R., et al. (2010). Psychiatry Research: Neuroimaging. *Psychiatry Research: Neuroimaging*, *184*(1), 38–48. doi:10.1016/j.pscychresns.2010.06.008
Dannlowski, U., Stuhrmann, A., Beutelmann, V., Zwanzger, P., Lenzen, T., Grotegerd, D., et al. (2012). Limbic Scars: Long-Term Consequences of ChildhoodMaltreatment Revealed by Functional and StructuralMagnetic Resonance Imaging. *Bps*, *71*(4), 286–293. doi:10.1016/j.biopsych.2011.10.021
David, S. P. (2005). A Functional Genetic Variation of the Serotonin (5-HT) Transporter Affects 5-HT1A Receptor Binding in Humans. *Journal of Neuroscience*, *25*(10), 2586–2590. doi:10.1523/JNEUROSCI.3769-04.2005
De Bellis, M. D. (2005). The Psychobiology of Neglect. *Child Maltreatment*, *10*(2), 150–172. doi:10.1177/1077559505275116
De Bellis, M. D., & Thomas, L. A. (2003). Biologic findings of post-traumatic stress disorder and child maltreatment. *Current Psychiatry Reports*, *5*(2), 108–117.
De Bellis, M. D., Keshavan, M. S., Shifflett, H., Iyengar, S., Beers, S. R., Hall, J., & Moritz, G. (2002). Brain structures in pediatric maltreatment-related posttraumatic stress disorder: a sociodemographically matched study. *Bps*, *52*(11), 1066–1078.
Degroot, A., & Treit, D. (2004). Anxiety is functionally segregated within the septo-hippocampal system. *Brain Research*, *1001*(1-2), 60–71. doi:10.1016/j.brainres.2003.10.065
Dougherty, D. D., & Rauch, S. L. (2007). Brain Correlates of Antidepressant Treatment Outcome from Neuroimaging Studies in Depression. *Psychiatric Clinics of North America*, *30*(1), 91–103. doi:10.1016/j.psc.2006.12.007
Drevets, W. C., Price, J. L., & Furey, M. L. (2008). Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Structure and Function*, *213*(1-2), 93–118. doi:10.1007/s00429-008-0189-x
Dwivedi, Y., Rizavi, H. S., Conley, R. R., Roberts, R. C., Tamminga, C. A., & Pandey, G. N. (2003). Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects. *Archives of General Psychiatry*, *60*(8), 804–815.
Edmiston, E. E., Wang, F., Mazure, C. M., Guiney, J., Sinha, R., Mayes, L. C., & Blumberg, H. P. (2011). Corticostriatal-limbic gray matter morphology in adolescents with self-reported exposure to childhood maltreatment. *Archives of Pediatrics & Adolescent Medicine*, *165*(12), 1069–1077.
Egan, M. F., Kojima, M., Callicott, J. H., Goldberg, T. E., Kolachana, B. S., Bertolino, A., et al. (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell*, *112*(2), 257–269.
Eluvathingal, T. J. (2006). Abnormal Brain Connectivity in Children After Early Severe Socioemotional Deprivation: A Diffusion Tensor Imaging Study. *Pediatrics*, *117*(6), 2093–2100. doi:10.1542/peds.2005-1727
Elzinga, B. M., Molendijk, M. L., Oude Voshaar, R. C., Bus, B. A. A., Prickaerts, J., Spinhoven, P., & Penninx, B. J. W. H. (2010). The impact of childhood abuse and recent stress on serum brain-derived neurotrophic factor and the moderating

role of BDNF Val66Met. *Psychopharmacology*, *214*(1), 319–328. doi:10.1007/s00213-010-1961-1

Farhadi, H. F., Mowla, S. J., Petrecca, K., Morris, S. J., Seidah, N. G., & Murphy, R. A. (2000). Neurotrophin-3 sorts to the constitutive secretory pathway of hippocampal neurons and is diverted to the regulated secretory pathway by coexpression with brain-derived neurotrophic factor. *The Journal of Neuroscience*, *20*(11), 4059–4068.

Field, A., Miles, J., Field, Z. (2012) Discovering Statistics using R. SAGE Publications, London, England.

Fink, L., Bernstein, D. P., handelsman, L., Foote, J., & Lovejoy, M. (1995). Initial reliability and validity of the childhood trauma interview: a new multidimensional measure of childhood interpersonal trauma. *Am J Psychiatry*, *1*(52), 1329.

First, M. B., Spitzer, R. L., Williams, J. B., Gibbon, M., First. (1997). *Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I), Clinical Version*. American Psychiatric Publishing, Incorporated.

Fischl, B. (2004). Automatically Parcellating the Human Cerebral Cortex. *Cerebral Cortex*, *14*(1), 11–22. doi:10.1093/cercor/bhg087

Fournier, J. C., DeRubeis, R. J., Hollon, S. D., Dimidjian, S., Amsterdam, J. D., Shelton, R. C., & Fawcett, J. (2010). Antidepressant drug effects and depression severity. *JAMA: the Journal of the American Medical Association*, *303*(1), 47–53.

Frodl, T. (2012). Effects of early-life adversity on white matter diffusivity changes in patients at risk for major depression. *Journal of Psychiatry & Neuroscience*, *37*(1), 37–45. doi:10.1503/jpn.110028

Frodl, T., Carballedo, A., Fagan, A. J., Lisiecka, D., Ferguson, Y., & Meaney, J. F. (2012). Effects of early-life adversity on white matter diffusivity changes in patients at risk for major depression. *Journal of Psychiatry & Neuroscience: JPN*, *37*(1), 37.

Frodl, T., Koutsouleris, N., Bottlender, R., Born, C., Jäger, M., Mörgenthaler, M., et al. (2008). Reduced gray matter brain volumes are associated with variants of the serotonin transporter gene in major depression. *Molecular Psychiatry*, *13*(12), 1093–1101. doi:10.1038/mp.2008.62

Frodl, T., Meisenzahl, E. M., Zetzsche, T., Born, C., Groll, C., Jäger, M., et al. (2002a). Hippocampal changes in patients with a first episode of major depression. *American Journal of Psychiatry*, *159*(7), 1112–1118.

Frodl, T., Meisenzahl, E., Zetzsche, T., Bottlender, R., Born, C., Groll, C., et al. (2002b). Enlargement of the amygdala in patients with a first episode of major depression. *Bps*, *51*(9), 708–714.

Furlong, R. A., Ho, L., Walsh, C., Rubinsztein, J. S., Jain, S., Paykel, E. S., et al. (1998). Analysis and meta‐analysis of two serotonin transporter gene polymorphisms in bipolar and unipolar affective disorders. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *81*(1), 58–63.

Goodyer, I. M., Bacon, A., Ban, M., Croudace, T., & Herbert, J. (2009). Serotonin transporter genotype, morning cortisol and subsequent depression in adolescents. *The British Journal of Psychiatry*, *195*(1), 39–45. doi:10.1192/bjp.bp.108.054775

Goodyer, I. M., Croudace, T., Dudbridge, F., Ban, M., & Herbert, J. (2010). Polymorphisms in BDNF (Val66Met) and 5-HTTLPR, morning cortisol and subsequent depression in at-risk adolescents. *The British Journal of Psychiatry*, *197*(5), 365–371. doi:10.1192/bjp.bp.110.077750

Govindan, R. M., Behen, M. E., Helder, E., Makki, M. I., & Chugani, H. T. (2010). Altered Water Diffusivity in Cortical Association Tracts in Children with Early Deprivation Identified with Tract-Based Spatial Statistics (TBSS). *Cerebral Cortex*, *20*(3), 561–569. doi:10.1093/cercor/bhp122

Guo, W.-B., Liu, F., Chen, J.-D., Xu, X.-J., Wu, R.-R., Ma, C.-Q., et al. (2012). Progress in Neuro-Psychopharmacology & Biological Psychiatry. *Progress in Neuropsychopharmacology & Biological Psychiatry*, *38*(2), 201–206. doi:10.1016/j.pnpbp.2012.03.012

Gutman, D. A., Holtzheimer, P. E., Behrens, T. E. J., Johansen-Berg, H., & Mayberg, H. S. (2009). A Tractography Analysis of Two Deep Brain Stimulation White Matter Targets for Depression. *Bps*, *65*(4), 276–282. doi:10.1016/j.biopsych.2008.09.021

Hamilton, M. (1960). A rating scale for depression. *Journal of Neurology, Neurosurgery, and Psychiatry*, *23*(1), 56.

Hanson, J. L., Adluru, N., Chung, M. K., Alexander, A. L., Davidson, R. J., & Pollak, S. D. (2013). Early Neglect Is Associated With Alterations in White Matter Integrity and Cognitive Functioning. *Child Development*, *84*(5), 1566–1578. doi:10.1111/cdev.12069

Hariri, A. R., Drabant, E. M., Munoz, K. E., Kolachana, B. S., Mattay, V. S., Egan, M. F., & Weinberger, D. R. (2005). A susceptibility gene for affective disorders and the response of the human amygdala. *Archives of General Psychiatry*, *62*(2), 146–152.

Hariri, A. R., Goldberg, T. E., Mattay, V. S., Kolachana, B. S., Callicott, J. H., Egan, M. F., & Weinberger, D. R. (2003). Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *The Journal of Neuroscience*, *23*(17), 6690–6694.

Hart, H. (2012). Neuroimaging of child abuse: a critical review, 1–24. doi:10.3389/fnhum.2012.00052/abstract

Heiervang, E., Behrens, T. E. J., Mackay, C. E., Robson, M. D., & Johansen-Berg, H. (2006). Between session reproducibility and between subject variability of diffusion MR and tractography measures. *NeuroImage*, *33*(3), 867–877. doi:10.1016/j.neuroimage.2006.07.037

Heils, A., Teufel, A., Petri, S., Stöber, G., Riederer, P., Bengel, D., & Lesch, K. P. (1996). Allelic variation of human serotonin transporter gene expression. *Journal of Neurochemistry*, *66*(6), 2621–2624.

Heim, C., & Nemeroff, C. B. (2001). The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Bps*, *49*(12), 1023–1039.

Heim, C., Newport, D. J., Heit, S., Graham, Y. P., Wilcox, M., Bonsall, R., et al. (2000). Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. *JAMA: the Journal of the American Medical Association*, *284*(5), 592–597.

Heim, C., Plotsky, P. M., & Nemeroff, C. B. (2004). Importance of Studying the

Contributions of Early Adverse Experience to Neurobiological Findings in Depression. *Neuropsychopharmacology*, *29*(4), 641–648. doi:10.1038/sj.npp.1300397

Heinz, A., Braus, D. F., Smolka, M. N., Wrase, J., Puls, I., Hermann, D., et al. (2004). Amygdala-prefrontal coupling depends on a genetic variation of the serotonin transporter. *Nature Neuroscience*, *8*(1), 20–21. doi:10.1038/nn1366

Heller, A. S., Johnstone, T., Light, S. N., Peterson, M. J., Kolden, G. G., Kalin, N. H., & Davidson, R. J. (2013). Relationships between changes in sustained fronto-striatal connectivity and positive affect in major depression resulting from antidepressant treatment. *American Journal of Psychiatry*, *170*(2), 197–206.

Hilt, L. M., Sander, L. C., Nolen-Hoeksema, S., & Simen, A. A. (2007). The BDNF Val66Met polymorphism predicts rumination and depression differently in young adolescent girls and their mothers. *Neuroscience Letters*, *429*(1), 12–16. doi:10.1016/j.neulet.2007.09.053

Hoogenboom, W. S., Perlis, R. H., Smoller, J. W., Zeng-Treitler, Q., Gainer, V. S., Murphy, S. N., et al. (2014). Limbic system white matter microstructure and long-term treatment outcome in major depressive disorder: A diffusion tensor imaging study using legacy data. *World Journal of Biological Psychiatry*, *15*(2), 122–134. doi:10.3109/15622975.2012.669499

Hu, X., Oroszi, G., Chun, J., Smith, T. L., Goldman, D., & Schuckit, M. A. (2005). An Expanded Evaluation of the Relationship of Four Alleles to the Level of Response to Alcohol and the Alcoholism Risk. *Alcoholism: Clinical & Experimental Research*, *29*(1), 8–16. doi:10.1097/01.ALC.0000150008.68473.62

Hu X-Z., Lipsky R.H., Zhu G., Akhtar L.A., Taubman J., Greenberg B.D., Xu K., Arnold P.D., Richter M.A., Kennedy J.L., Murphy D.L., Goldman D. (2006). Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. *Am J Hum Genet* 78:815-26.

Hua, K., Oishi, K., Zhang, J., Wakana, S., Yoshioka, T., Zhang, W., et al. (2009). Mapping of Functional Areas in the Human Cortex Based on Connectivity through Association Fibers. *Cerebral Cortex*, *19*(8), 1889–1895. doi:10.1093/cercor/bhn215

Huang, H., Fan, X., Williamson, D. E., & Rao, U. (2010). White Matter Changes in Healthy Adolescents at Familial Risk for Unipolar Depression: A Diffusion Tensor Imaging Study. *Neuropsychopharmacology*, *36*(3), 684–691. doi:10.1038/npp.2010.199

Jenkinson, M., & Smith, S. (2001). A global optimisation method for robust affine registration of brain images. *Medical Image Analysis*, *5*(2), 143–156.

Jonassen, R. (2012). The effects of the serotonin transporter polymorphism and age on frontal white matter integrity in healthy adult women, 1–5. doi:10.3389/fnhum.2012.00019/abstract

Kalia, M. (2005). Neurobiological basis of depression: an update. *Metabolism*, *54*(5), 24–27. doi:10.1016/j.metabol.2005.01.009

Karg, K. (2011). The Serotonin Transporter Promoter Variant (5-HTTLPR), Stress, and Depression Meta-analysis RevisitedEvidence of Genetic Moderation5-HTTLPR, Stress, and Depression Meta-Analysis. *Archives of General Psychiatry*, *68*(5), 444. doi:10.1001/archgenpsychiatry.2010.189

Kato, M., & Serretti, A. (2008). Review and meta-analysis of antidepressant pharmacogenetic findings in major depressive disorder. *Molecular Psychiatry*, *15*(5), 473–500. doi:10.1038/mp.2008.116

Kaufman, J., Yang, B.-Z., Douglas-Palumberi, H., Grasso, D., Lipschitz, D., Houshyar, S., et al. (2006). Brain-Derived Neurotrophic Factor–5-HTTLPR Gene Interactions and Environmental Modifiers of Depression in Children. *Biological Psychiatry*, *59*(8), 673–680. doi:10.1016/j.biopsych.2005.10.026

Keedwell, P. A., Chapman, R., Christiansen, K., Richardson, H., Evans, J., & Jones, D. K. (2012). Cingulum White Matter in Young Women at Risk ofDepression: The Effect of Family History andAnhedonia. *Bps*, *72*(4), 296–302. doi:10.1016/j.biopsych.2012.01.022

Kendler, K. S., Kessler, R. C., Walters, E. E., MacLean, C., Neale, M. C., Heath, A. C., & Eaves, L. J. (1995). Stressful life events, genetic liability, and onset of an episode of major depression in women. *American Journal of Psychiatry*, *152*(6), 833–842.

Kendler, K. S., Kuhn, J. W., Vittum, J., Prescott, C. A., & Riley, B. (2005). The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: a replication. *Archives of General Psychiatry*, *62*(5), 529.

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey replication: Erratum.

Kieseppä, T., Eerola, M., Mäntylä, R., Neuvonen, T., Poutanen, V.-P., Luoma, K., et al. (2010). Journal of Affective Disorders. *Journal of Affective Disorders*, *120*(1-3), 240–244. doi:10.1016/j.jad.2009.04.023

Korgaonkar, M. S., Cooper, N. J., Williams, L. M., & Grieve, S. M. (2012). Mapping inter-regional connectivity of the entire cortex to characterize major depressive disorder. *NeuroReport*, *23*(9), 566–571. doi:10.1097/WNR.0b013e3283546264

Korgaonkar, M. S., Grieve, S. M., Koslow, S. H., Gabrieli, J. D. E., Gordon, E., & Williams, L. M. (2011). Loss of white matter integrity in major depressive disorder: Evidence using tract-based spatial statistical analysis of diffusion tensor imaging. *Human Brain Mapping*, *32*(12), 2161–2171. doi:10.1002/hbm.21178

Kumamaru, E., Numakawa, T., Adachi, N., Yagasaki, Y., Izumi, A., Niyaz, M., et al. (2008). Glucocorticoid Prevents Brain-Derived Neurotrophic Factor-Mediated Maturation of Synaptic Function in Developing Hippocampal Neurons through Reduction in the Activity of Mitogen-Activated Protein Kinase. *Molecular Endocrinology*, *22*(3), 546–558. doi:10.1210/me.2007-0264

Lafer, B., Renshaw, P. F., & Sachs, G. S. (1997). Major depression and the basal ganglia. *Psychiatric Clinics of North America*, *20*(4), 885–896.

Lauder, J. M. (1983). Hormonal and humoral influences on brain development. *Psychoneuroendocrinology*, *8*(2), 121–155.

Lebel, C., Gee, M., Camicioli, R., Wieler, M., Martin, W., & Beaulieu, C. (2012). Diffusion tensor imaging of white matter tract evolution over the lifespan. *NeuroImage*, *60*(1), 340–352. doi:10.1016/j.neuroimage.2011.11.094

Lesch, K. P., Gross, J., Franzek, E., Wolozin, B. L., Riederer, P., & Murphy, D. L. (1995). Primary structure of the serotonin transporter in unipolar depression and bipolar disorder. *Bps*, *37*(4), 215–223.

Lesch, K.-P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*, *274*(5292), 1527–1531.

Li, L., Ma, N., Li, Z., Tan, L., Liu, J., Gong, G., et al. (2007). Prefrontal white matter abnormalities in young adult with major depressive disorder: A diffusion tensor imaging study. *Brain Research*, *1168*, 124–128. doi:10.1016/j.brainres.2007.06.094

Lisiecka, Danuta, Meisenzahl, E., Scheuerecker, J., Schoepf, V., Whitty, P., Chaney, A., et al. (2010). Neural correlates of treatment outcome in major depression. *The International Journal of Neuropsychopharmacology*, *14*(04), 521–534. doi:10.1017/S1461145710001513

Liu, H., Klomp, N., & Heynderickx, I. (2010). A No-Reference Metric for Perceived Ringing Artifacts in Images. *IEEE Transactions on Circuits and Systems for Video Technology*, *20*(4), 529–539. doi:10.1109/TCSVT.2009.2035848

Lu, B., & Martinowich, K. (2008). Cell biology of BDNF and its relevance to schizophrenia, *289*, 119.

Maheu, F. S., Dozier, M., Guyer, A. E., Mandell, D., Peloso, E., Poeth, K., et al. (2010). A preliminary study of medial temporal lobe function in youths with a history of caregiver deprivation and emotional neglect. *Cognitive, Affective, & Behavioral Neuroscience*, *10*(1), 34–49. doi:10.3758/CABN.10.1.34

Malone, D. A., Dougherty, D. D., Rezai, A. R., Carpenter, L. L., Friehs, G. M., Eskandar, E. N., et al. (2009). Deep Brain Stimulation of the Ventral Capsule/Ventral Striatum for Treatment-Resistant Depression. *Bps*, *65*(4), 267–275. doi:10.1016/j.biopsych.2008.08.029

Mattson, M. P., Maudsley, S., & Martin, B. (2004). BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. *Trends in Neurosciences*, *27*(10), 589–594. doi:10.1016/j.tins.2004.08.001

Mayberg, H. S. (2003). Modulating dysfunctional limbic-cortical circuits in depression: towards development of brain-based algorithms for diagnosis and optimised treatment. *British Medical Bulletin*, *65*(1), 193–207. doi:10.1093/bmb/ldg65.193

Miller, R., Wankerl, M., Stalder, T., Kirschbaum, C., & Alexander, N. (2012). The serotonin transporter gene-linked polymorphic region (5-HTTLPR) and cortisol stress reactivity: a meta-analysis. *Molecular Psychiatry*. doi:10.1038/mp.2012.124

Montag, C., Schoene-Bake, J. C., Faber, J., Reuter, M., & Weber, B. (2010). Genetic variation on the BDNF gene is not associated with differences in white matter tracts in healthy humans measured by tract-based spatial statistics. *Genes, Brain and Behavior*, *9*(8), 886–891. doi:10.1111/j.1601-183X.2010.00626.x

Mori, S., Oishi, K., Jiang, H., Jiang, L., Li, X., Akhter, K., et al. (2008). Stereotaxic white matter atlas based on diffusion tensor imaging in an ICBM template. *NeuroImage*, *40*(2), 570–582.

Mueller, S. C., Maheu, F. S., Dozier, M., Peloso, E., Mandell, D., Leibenluft, E., et al. (2010). Neuropsychologia. *Neuropsychologia*, *48*(10), 3037–3044. doi:10.1016/j.neuropsychologia.2010.06.013

Mullen, P. E., Martin, J. L., Anderson, J. C., Romans, S. E., & Herbison, G. P. (1996). The

long-term impact of the physical, emotional, and sexual abuse of children: a community study. *Child Abuse & Neglect*, *20*(1), 7–21.

Munafò, M. R., Brown, S. M., & Hariri, A. R. (2008). Serotonin Transporter (5-HTTLPR) Genotype and Amygdala Activation: A Meta-Analysis. *Biological Psychiatry*, *63*(9), 852–857. doi:10.1016/j.biopsych.2007.08.016

Murphy, D. L., & Lesch, K.-P. (2008). Targeting the murine serotonin transporter: insights into human neurobiology. *Nature Reviews Neuroscience*, *9*(2), 85–96. doi:10.1038/nrn2284

Murphy, M. L., & Frodl, T. (2011). Meta-analysis of diffusion tensor imaging studiesshows altered fractional anisotropy occurring indistinct brain areas in association with depression. *Biology of Mood & Anxiety Disorders*, *1*(1), 3. doi:10.1186/2045-5380-1-3

Nakamura, M., Ueno, S., Sano, A., & Tanabe, H. (2000). The human serotonin transporter gene linked polymorphism (5-HTTLPR) shows ten novel allelic variants. *Molecular Psychiatry*, *5*(1), 32.

Neves-Pereira M., Mundo E., Muglia P., King N., Macciardi F., Kennedy J.L. (2002) The brain-derived neurotrophic factor gene confers susceptibility to bipolar disorder: evidence from a family-based association study. *American Journal of Human Genetics*, 71, 651-655.

Nichols, T. E., & Holmes, A. P. (2002). Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Human Brain Mapping*, *15*(1), 1–25.

Nolen-Hoeksema, S. (1987). Sex differences in unipolar depression: evidence and theory. *Psychological Bulletin*, *101*(2), 259.

Nolen-Hoeksema, S., Wisco, B. E., & Lyubomirsky, S. (2008). Rethinking Rumination. *Perspectives on Psychological Science*, *3*(5), 400–424. doi:10.1111/j.1745-6924.2008.00088.x

Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia*, *9*(1), 97–113.

Owens, M., Goodyer, I. M., Wilkinson, P., Bhardwaj, A., Abbott, R., Croudace, T., et al. (2012). 5-HTTLPR and Early Childhood Adversities Moderate Cognitive and Emotional Processing in Adolescence. *PLoS ONE*, *7*(11), e48482. doi:10.1371/journal.pone.0048482.s005

Pacheco, J., Beevers, C. G., Benavides, C., McGeary, J., Stice, E., & Schnyer, D. M. (2009). Frontal-Limbic White Matter Pathway Associations with the Serotonin Transporter Gene Promoter Region (5-HTTLPR) Polymorphism. *Journal of Neuroscience*, *29*(19), 6229–6233. doi:10.1523/JNEUROSCI.0896-09.2009

Perroud, N., Courtet, P., Vincze, I., Jaussent, I., Jollant, F., Bellivier, F., et al. (2008). Interaction between BDNF Val66Met and childhood trauma on adult's violent suicide attempt. *Genes, Brain and Behavior*, *7*(3), 314–322. doi:10.1111/j.1601-183X.2007.00354.x

Pezawas, L., Meyer-Lindenberg, A., Drabant, E. M., Verchinski, B. A., Munoz, K. E., Kolachana, B. S., et al. (2005). 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. *Nature Neuroscience*, *8*(6), 828–834. doi:10.1038/nn1463

Pollak, S. D., & Sinha, P. (2002). Effects of early experience on children's recognition

of facial displays of emotion. *Developmental Psychology*, *38*(5), 784–791. doi:10.1037//0012-1649.38.5.784

Pollak, S. D., & Tolley-Schell, S. A. (2003). Selective attention to facial emotion in physically abused children. *Journal of Abnormal Psychology*, *112*(3), 323–338. doi:10.1037/0021-843X.112.3.323

Pollak, S. D., Cicchetti, D., Hornung, K., & Reed, A. (2000). Recognizing emotion in faces: developmental effects of child abuse and neglect. *Developmental Psychology*, *36*(5), 679.

Pollak, S. D., Nelson, C. A., Schlaak, M. F., Roeber, B. J., Wewerka, S. S., Wiik, K. L., et al. (2010). Neurodevelopmental effects of early deprivation in postinstitutionalized children. *Child Development*, *81*(1), 224–236.

Prachak-Rieder N., Kennedy J., Wilson A.A., Hussey D., Boovariwala A., Willeit M., Ginovart N., Tharmalingam S., Maselllis M., Houle S., Meyer J.H. (2007). Novel 5-HTTLPR allele associates with higher serotonin transporter binding in putamen: a [(11)C] DASB positron emission tomography study. *Biol Psychiatry*. 62:327-331.

Pregelj, P., Nedic, G., Paska, A. V., Zupanc, T., Nikolac, M., Balažic, J., et al. (2011). Journal of Affective Disorders. *Journal of Affective Disorders*, *128*(3), 287–290. doi:10.1016/j.jad.2010.07.001

Preuss, T. M., & Goldman Rakic, P. S. (1989). Connections of the ventral granular frontal cortex of macaques with perisylvian premotor and somatosensory areas: anatomical evidence for somatic representation in primate frontal association cortex. *Journal of Comparative Neurology*, *282*(2), 293–316.

Risch, N., Herrell, R., Lehner, T., Liang, K.-Y., Eaves, L., Hoh, J., et al. (2009). Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression. *JAMA: the Journal of the American Medical Association*, *301*(23), 2462–2471.

Rueckert, D., Sonoda, L. I., Hayes, C., Hill, D. L., Leach, M. O., & Hawkes, D. J. (1999). Nonrigid registration using free-form deformations: application to breast MR images. *Medical Imaging, IEEE Transactions on*, *18*(8), 712–721.

Rybakowski J.K., Borkowska A., Czerski P.M., Skibimska M., Hauser J. (2003). Polymorphism of the brain-derived neurotrophic factor gene and performance on a cognitive prefrontal test in bipolar patients. *Bipolar Disorders*, 5, 468-472.

Scher, C. D., Forde, D. R., McQuaid, J. R., & Stein, M. B. (2004). Prevalence and demographic correlates of childhood maltreatment in an adult community sample. *Child Abuse & Neglect*, *28*(2), 167–180. doi:10.1016/j.chiabu.2003.09.012

Seretti, A., Cusin, C., Lattuada, E., Di Bella, D., Catalano, M., & Smeraldi, E. (1999). Serotonin transporter gene (5-HTTLPR) is not associated with depressive symptomatology in mood disorders. *Molecular Psychiatry*, *4*(3), 280–283.

Sibille, E., & Lewis, D. A. (2006). SERT-ainly involved in depression, but when? *American Journal of Psychiatry*, *163*(1), 8–11.

Smith, S. M., & Nichols, T. E. (2009). Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *NeuroImage*, *44*(1), 83–98.

Smith, S. M., Jenkinson, M., Johansen-Berg, H., Rueckert, D., Nichols, T. E., Mackay, C.

E., et al. (2006). Tract-based spatial statistics: Voxelwise analysis of multi-subject diffusion data. *NeuroImage*, *31*(4), 1487–1505. doi:10.1016/j.neuroimage.2006.02.024

Sprooten E. et al. (2014) Common genetic variants and gene expression associated with white matter microstructure in the human brain. *Neuroimage,*1053-8119

Sullivan, P. F., Neale, M. C., & Kendler, K. S. (2000). Genetic epidemiology of major depression: review and meta-analysis. *American Journal of Psychiatry*, *157*(10), 1552–1562.

Stevens, J. P. (2002) Applied multivariate statistics for the social sciences (4th Ed.) Hillsdale, NJ: Erlbaum.

Taylor, S. E., Way, B. M., Welch, W. T., Hilmert, C. J., Lehman, B. J., & Eisenberger, N. I. (2006). Early Family Environment, Current Adversity, the Serotonin Transporter Promoter Polymorphism, and Depressive Symptomatology. *Biological Psychiatry*, *60*(7), 671–676. doi:10.1016/j.biopsych.2006.04.019

Teicher, M. H., Andersen, S. L., Polcari, A., Anderson, C. M., & Navalta, C. P. (2002). Developmental neurobiology of childhood stress and trauma. *Psychiatric Clinics of North America*, *25*(2), 397–426.

Teicher, M. H., Andersen, S. L., Polcari, A., Anderson, C. M., Navalta, C. P., & Kim, D. M. (2003). The neurobiological consequences of early stress and childhood maltreatment. *Neuroscience and Biobehavioral Reviews*, *27*(1-2), 33–44. doi:10.1016/S0149-7634(03)00007-1

Teicher, M. H., Dumont, N. L., Ito, Y., Vaituzis, C., Giedd, J. N., & Andersen, S. L. (2004). Childhood neglect is associated with reduced corpus callosum area. *Biological Psychiatry*, *56*(2), 80–85. doi:10.1016/j.biopsych.2004.03.016

Tessner, K. D., Walker, E. F., Dhruv, S. H., Hochman, K., & Hamann, S. (2007). The relation of cortisol levels with hippocampus volumes under baseline and challenge conditions. *Brain Research*, *1179*, 70–78. doi:10.1016/j.brainres.2007.05.027

Tomoda, A., Sheu, Y.-S., Rabi, K., Suzuki, H., Navalta, C. P., Polcari, A., & Teicher, M. H. (2011). Exposure to parental verbal abuse is associated with increased gray matter volume in superior temporal gyrus. *NeuroImage*, *54*(S1), S280–S286. doi:10.1016/j.neuroimage.2010.05.027

Tost, H., Alam, T., Geramita, M., Rebsch, C., Kolachana, B., Dickinson, D., et al. (2013). Effects of the BDNF Val, *38*(3), 525–532. doi:10.1038/npp.2012.214

Tottenham, N., Hare, T. A., Quinn, B. T., McCarry, T. W., Nurse, M., Gilhooly, T., et al. (2010). Prolonged institutional rearing is associated with atypically large amygdala volume and difficulties in emotion regulation. *Developmental Science*, *13*(1), 46–61. doi:10.1111/j.1467-7687.2009.00852.x

Treadway, M. T., Grant, M. M., Ding, Z., Hollon, S. D., Gore, J. C., & Shelton, R. C. (2009). Early Adverse Events, HPA Activity and Rostral Anterior Cingulate Volume in MDD. *PLoS ONE*, *4*(3), e4887. doi:10.1371/journal.pone.0004887

van Harmelen, A. L., van Tol, M. J., van der Wee, N. J. A., Veltman, D. J., Aleman, A., Spinhoven, P., et al. (2010). Reduced Medial Prefrontal Cortex Volume in Adults Reporting Childhood Emotional Maltreatment. *Bps*, *68*(9), 832–838. doi:10.1016/j.biopsych.2010.06.011

Verhagen, M., van der Meij, A., van Deurzen, P. A. M., Janzing, J. G. E., squez, A. A.-V. A.,

Buitelaar, J. K., & Franke, B. (2010). Meta-analysis of the BDNF Val66Met polymorphism inmajor depressive disorder: effects of gender and ethnicity. *Molecular Psychiatry*, *15*(3), 260–271. doi:10.1038/mp.2008.109

Vythilingam, M., Heim, C., Newport, J., Miller, A. H., Anderson, E., Bronen, R., et al. (2002). Childhood trauma associated with smaller hippocampal volume in women with major depression. *The American Journal of Psychiatry*, *159*(12), 2072.

Wakana, S., Jiang, H., Nagae-Poetscher, L. M., van Zijl, P. C. M., & Mori, S. (2004). Fiber Tract-based Atlas of Human White Matter Anatomy. *Radiology*, *230*(1), 77–87. doi:10.1148/radiol.2301021640

Walker, E. A., Gelfand, A., Katon, W. J., Koss, M. P., Korff, Von, M., Bernstein, D., & Russo, J. (1999). Adult health status of women with histories of childhood abuse and neglect. *The American Journal of Medicine*, *107*(4), 332–339.

Walsh, N. D., Dalgleish, T., Dunn, V. J., Abbott, R., Clair, M. C. S., Owens, M., et al. (2012). 5-HTTLPR–environment interplay and its effects on neural reactivity in adolescents. *NeuroImage*, *63*(3), 1670–1680. doi:10.1016/j.neuroimage.2012.07.067

Wichers, M., Kenis, G., Jacobs, N., Mengelers, R., Derom, C., Vlietinck, R., & van Os, J. (2008). The BDNF Val66Met × 5-HTTLPR × child adversity interaction and depressive symptoms: An attempt at replication. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *147B*(1), 120–123. doi:10.1002/ajmg.b.30576

Wilhelm, K. (2006). Life events, first depression onset and the serotonin transporter gene. *The British Journal of Psychiatry*, *188*(3), 210–215. doi:10.1192/bjp.bp.105.009522

Wu, F., Tang, Y., Xu, K., Kong, L., Sun, W., Wang, F., et al. (2011). Psychiatry Research: Neuroimaging. *Psychiatry Research: Neuroimaging*, *191*(1), 80–83. doi:10.1016/j.pscychresns.2010.09.002

Zalsman, G., Huang, Y.-Y., Oquendo, M., Burke, A., Hu, X.-Z., Brent, D., et al. (2006). Association of a triallelic serotonin transporter gene promoter region (5-HTTLPR) polymorphism with stressful life events and severity of depression. *American Journal of Psychiatry*, *163*(9), 1588–1593.

Zhou, Y., Qin, L.-D., Chen, J., Qian, L.-J., Tao, J., Fang, Y.-R., & Xu, J.-R. (2011). Brain microstructural abnormalities revealed by diffusion tensor images in patients with treatment-resistant depression compared with major depressive disorder before treatment. *European Journal of Radiology*, *80*(2), 450–454. doi:10.1016/j.ejrad.2010.06.041

Zhu, X., Wang, X., Xiao, J., Zhong, M., Liao, J., & Yao, S. (2011). Altered white matter integrity in first-episode, treatment-naive young adults with major depressive disorder: A tract-based spatial statistics study. *Brain Research*, *1369*(C), 223–229. doi:10.1016/j.brainres.2010.10.104

Zou, K., Huang, X., Li, T., Gong, Q., Li, Z., Ou-yang, L., et al. (2008). Alterations of white matter integrity in adults with major depressive disorder: a magnetic resonance imaging study. *Journal of Psychiatry & Neuroscience: JPN*, *33*(6), 525.

Zou, Y.-F., Ye, D.-Q., Feng, X.-L., Su, H., Pan, F.-M., & Liao, F.-F. (2010). Meta-analysis of BDNF Val66Met polymorphism association with treatment response in patients

with major depressive disorder. *European Neuropsychopharmacology*, *20*(8), 535–544. doi:10.1016/j.euroneuro.2009.12.005

Zuo, N., Fang, J., Lv, X., Zhou, Y., Hong, Y., Li, T., et al. (2012). White Matter Abnormalities in Major Depression: A Tract-Based Spatial Statistics and Rumination Study. *PLoS ONE*, *7*(5), e37561. doi:10.1371/journal.pone.0037561.t004

CHAPTER 2: The Role of White Matter Integrity Changes, 5-HTTLPR and BDNF Polymorphisms in Predicting Depression Treatment Response

Abstract

Approximately 50% of patients with major depressive disorder do not respond optimally to antidepressant medication. Linking neuronal connectivity and genetic risk factors in predicting antidepressant response has clinical implications. Our investigation assessed whether indices of fractional anisotropy (FA) (examined using tract based spatial statistics (TBSS) and probabilistic tractography), and genetic polymorphisms (serotonin transporter promotor (5-HTTLPR) and brain derived neurotrophic factor (BDNF)), predicted magnitude of depression symptom change following anti-depressant treatment. Forty-six medication-free patients with major depressive disorder participated in a diffusion tensor imaging scan prior to completing an 8-week treatment regime with Citalopram ($\bar{x}$ age: 36.3, M/F:12/12) or Quetiapine XR ($\bar{x}$ age: 39.8, M/F:8/14). Indexed improvements in Hamilton Depression Rating Scale score from baseline to 8-week endpoint were used as an indicator of depression improvement. Uncorrected TBSS results revealed significantly higher FA in hippocampal portions of the cingulum bundle in responders and remitters, compared to non-responders [p=0.0116]. Probabilistic tractography identified that higher FA in the left uncinate fasciculus predicted percent change in depression severity [p=0.08], and BDNF moderated this association [p=0.0178]. Carriers of the BDNF met-allele also showed lower FA values in the left uncinate fasciculus [p=0.009]. An interaction between FA in the right uncinate fasciculus and 5-HTTLPR also predicted percent change in depression severity [p=0.00184]. Fractional anisotropy in the hippocampus and uncinate fasciculus may provide accurate predictors of antidepressant response across a

range of medications, with BDNF gene polymorphisms moderating these associations.

1.0 Introduction

1.1 Depression Treatment

1.1.1. Treatments of Depression

Although there are a variety of antidepressant treatments available, 30-40% of depressed individuals don't respond to antidepressant treatment. Furthermore, large improvements in depression following antidepressant treatment are only seen in individuals with severe baseline depression (Fournier et al., 2010). Some examples of antidepressant treatments include selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAO-I's), tricyclic antidepressants (TCAs), and serotonin-norepinephrine reuptake inhibitors (SNRIs). SSRI's such as Citalopram, are the most prescribed type of antidepressant, having minimal side effects. Quetiapine is an atypical antipsychotic that is often used as an antipsychotic in the treatment of schizophrenia and bipolar disorder, but can be used as an antidepressant agent for major depressive disorder.

1.1.2. Quetiapine and Citalopram

Although all types of antidepressant medications work to decrease depression symptomology, each function through different mechanisms. For example, quetiapine (branded as Seroquel) is typically a short acting atypical antipsychotic, although an extended release formula is used in the present study. Quetiapine, is metabolized to N-desalkyl quetiapine in humans, and is thought to exert its beneficial effects on mood through antagonistic activity on serotonin type 2

(5-HT2) receptors, dopaminergic receptors (D1 & D2), and noradrenergic receptors & transporters (Arango & Bernardo, 2005). Quetiapine is often used in adjunct with another antidepressants, but quetiapine has shown more robust antidepressant action as a monotherapy in controlled clinical trials (Baune, 2008; Weisler, 2009; El-Khalili, 2008; Montgomery, 2008) and is effective for short-term and maintainence treatment of MDD (Baune, 2008).

Citalopram (branded as Celexa, Cipramil) is an antidepressant of the selective serotonin reuptake inhibitor class, and significantly improves depression severity as indicated by placebo controlled trials (Stahl, 2000). The effectiveness of citalopram is attributed to the s-enantiomer, which has been recently isolated and patented for prescription (Henry et al., 2013). Citalopram is also the most selective SSRI (Hyttel, 1995) and has low risk for side effects and drug-drug interactions(Stahl, 2000). It is a slow release anti-depressant with a half life of 33hrs (Baune, 2008).

1.2 5-HTTLPR, BDNF and Treatment Response

1.2.1 5-HTTLPR

It has been demonstrated that information about genetic polymorphisms can help inform decisions on the diagnosis and treatment of depression. As SSRI treatments target the 5-HT transporter (5-HTT), and the 5-HTTLPR is associated with depression symptomology, polymorphisms in this allele are an attractive candidate risk factor for depression. The human 5-HTT is encoded by the SLC6A4 gene and contains three variants linked to depression; the more recently differentiated long alleles (L_G and L_A) and one short variant (S_A). The long alleles can

be differentiated according to transcription efficiency, as the L_G variants has transcription efficiency significantly lower than L_A, and similar to the S_A variant (Hu et al., 2005).

An original European investigation determined that depressed individuals with the l/l genotype of the 5-HTTLPR had better responses to serotonergic antidepressants (Smeraldi et al., 1998). Although this finding has been further replicated in European samples (Zanardi et al., 2000), the findings do not validate across ethnicities and demographic regions. Asian, Middle-East and Korean sample findings have been inconsistent, with supportive results (Y. W.-Y. Yu, Tsai, Chen, Lin, & Hong, 2002) in males (Sahraian et al., 2013) and opposing findings in Japanese patients (Yoshida et al., 2002). Additionally, in a large American sample there was no association found between 5-HTTLPR polymorphisms and treatment response (Kraft et al., 2007). Prior to this study, Serretti and colleagues conducted a meta-analysis of papers across ethnicities and replicated original findings of the l/l homozygotes having more favourable responses and remission rates (Serretti, Kato, De Ronchi, & Kinoshita, 2006).

1.2.2. Brain Derived Neurotrophic Factor

BDNF serum levels decrease during a depressive episode, and return to normal levels following antidepressant treatment (Aydemir et al., 2006), making it another attractive candidate risk factor for depression development and predictor of treatment response(Brunoni, Lopes, & Fregni, 2008). Research on BDNF has focused on discovering its role in hippocampus function and structure, a region highly affected by depression. It is postulated that reductions in volume and

abnormal activity within the hippocampus are due to reductions in BDNF levels (Frodl et al., 2007). This is further corroborated by findings in post-mortem studies of decreased BDNF levels in the hippocampus of depressed individuals being treated with antidepressants prior to, or at time of death relative to antidepressant-untreated depressed individuals (B. Chen, Dowlatshahi, MacQueen, Wang, & Young, 2001). One single nucleotide polymorphism (SNP) that has gained strong attention is val66met, which is a guanine to adenosine polymorphism in the pro-region of the BDNF gene. This SNP is associated with inhibited vesicle trafficking and secretion but not mature protein function (Egan et al., 2003). The role of this polymorphism in depression treatment outcomes has been inconclusive (M.-J. Choi, Kang, Lim, Oh, & Lee, 2006; Domschke et al., 2009; Tsai, Cheng, Yu, Chen, & Hong, 2003). Heterozygous genotypes (val/met) have exhibited a trend to improved antidepressant response after 4-weeks of fluoxetine treatment, relative to homozygous depressed patients (met/met or val/val) (Tsai et al., 2003; Zou et al., 2010). Others have found no association, or stronger antidepressant responses in met carriers relative to val/val genotypes (see review Kato & Serretti, 2008).

A more recent investigation on the association and interaction between 9 genetic polymorphisms and stressful life events (i.e. events within 6 months of interview) found no main effect or interaction on response to antidepressants (Bukh et al., 2009). This study examined the variants most strongly associated with depression, including the two discussed above; BDNF and 5-HTTLPR. These null findings may be a consequence of the types of measures used in this study, as

stressful life events do not provide as strong or reliable indictor of depression development, relative to early life stress (Karg, 2011).

1.3 Neuroimaging and Treatment Response

One of the fundamental aims of neuroimaging research has been to determine the validity of various techniques as diagnostic tools. The demand to critically assess quantitative neuroimaging techniques has recently become a topic of interest as a result of the National Institute of Mental Health's 2009 Strategic Plan (www.nimh.nih.gov) to identify sensitive physiological indicators of mental health status. Although there is a surge of interest on this topic, it is not a new area of research. Early electroencephalography (EGG) and positron emission tomography (PET) studies on depression found concordant neural activity and increased frontal activity, respectively, which predicted stronger responses to antidepressants (I. A. Cook et al., 1999). Increased volume in the left dlPFC following a 12-week treatment regime of sertraline support these findings. Volume changes in this region were also significantly correlated with reductions in self-report depression scores.

One of the leading theories on depression development suggests that it results from frontal-limbic dysregulation, which may have a role in illness course and remission. It also suggests that functional associations between regions within this network may provide a biomarker for treatment response, relapse risk and vulnerability to depression (Mayberg, 2003). Changes within cortico-limbic regions have been repeatedly reported in the depression literature, with higher pre-treatment amygdala reactivity associated with stronger treatment responses. Response and/or remission to antidepressant treatment is also associated with

attenuation and modulation of amygdala and frontal activity (Delaveau et al., 2011; Mayberg et al., 2000)(see Mayberg 2003 for review). These results extend to changes in network integrity, as alterations in fronto-limbic connections have distinguished treatment responders from non-responders across medication and psychotherapy treatments (Seminowicz et al., 2004).

White matter alterations have strong diagnostic and prognostic potential for depression, as white matter can predict clinical treatment outcomes with an accuracy of 65% (Gong et al., 2011). An in-vivo, non-invasive assessment of white matter integrity can be completed using MR imaging. This type of MR imaging is called diffusion tensor imaging (DTI), and uses water diffusivity in the brain as an indicator of myelin integrity. Fractional anisotropy (FA) is a DTI parameter with values ranging between 0 and 1 that reflect a ratio of directional to non-directional water movement in a single imaging voxel and provides information on axon size, myelination, axon connections and orientation.

Remission with antidepressant administration can be predicted by baseline assessments of myelin integrity, as lower FA in frontal white matter regions is associated with reduced remission rates in geriatric populations (Alexopoulos, Kiosses, Choi, Murphy, & Lim, 2002; Alexopoulos et al., 2008). These findings further substantiate theories of cortico-striato-limbic network dysfunction in depression, as white matter regions most affected include the rostral and dorsal anterior cingulate, dorsolateral prefrontal cortex (dlPFC), genu of the corpus callosum and white matter surrounding the hippocampus (Alexopoulos et al., 2002; 2008).

Although fewer studies have investigated whether pre-treatment neuronal status predicts depression remission, a number of studies have investigated neuronal changes pre to post treatment. For example, both increases in uncinate FA (Lai, Wu, Yu, & Yuan, 2013) and reductions in ACC FA (W. D. Taylor et al., 2011) have been associated with remission. Changes in FA are also seen across pathologies, as medication naive, first episode panic disorder individuals exhibited increases in FA following medication treatment, although post-treatment FA values did not normalize to levels of healthy controls (Lai et al., 2013). Based on observations of persistent changes in the uncinate fasciculus and associated regions such as the orbitofrontal cortex, it has been suggested that reductions in the functional integrity of this network persist following anti-depressant treatment relative to other regions such as the dlPFC (Lamar et al., 2013). Although these studies provide insights into the effects that therapeutics have on neural structure and functionality, the present study provides strong prognostic insights for therapeutic treatment.

A study by Delorenzo and colleagues (2012) has also taken this approach, investigating whether FA in tracts connecting the midbrain/raphe to the amygdala or hippocampus predict SSRI treatment outcomes. The midbrain/raphe nucleus is the principal source of serotonin in the brain, and connects regions affected by depression (i.e. amygdala and hippocampus). In the tract leading to the right amygdala, elevated baseline FA was associated with remitters, and positively correlated with depression severity improvements (DeLorenzo, 2013). Based on these findings we predict that we will find alterations in associations tracts that are connected to the amygdala and hippocampus.

The present study utilizes baseline predictors of neuronal connectivity to predict antidepressant response (following citalopram or quetiapine XR treatment). Tract based spatial statistics permit voxelwise comparisons of FA, and the assessment of connectivity changes associated with antidepressant response. Probabilistic tractography allows the assessment of FA in remodeled association tracts including the cingulum bundle, uncinate fasciculus and superior longitudinal fasciculus. Regression models will be used to assess the impact neuronal connectivity and polymorphisms in the 5-HTTLPR and BDNF gene have on the magnitude of improvement in depression severity following antidepressant treatment.

2.0 Methods

See Chapter 1: Methods for information on (i) Genotyping (ii) Grouping variables (iii) Diffusion Tensor Imaging (iv) Diffusion Imaging Parameters

2.1 Participants

2.1.1 Participant Selection

Forty-six major depressive disorder patients who were of either sex, Caucasian and within an age range of 19-58 were included in this study. Depressed individuals met DSM-IV's (American Psychiatric Association, 2000) criteria for major depressive disorder according to the Structured Clinical Interview (First, Spitzer, Williams, Gibbon, First, 1997) for DSM-IV Axis 1 disorders. Participants were recruited from the Calgary Health Region Outpatient Mental Health Clinics, tertiary care hospitals, University of Calgary through flyers, and from the community through local newspaper advertisements. Exclusion criteria included

bipolar disorder, psychosis, a history of substance abuse within 6 months of study participation, anxiety disorders, severe medical and neurological disorders, left handedness and previous history of treatment failure with citalopram or quetiapine XR mono/add-on therapy. Edinburgh Inventory was used to determine participant handedness (Oldfield, 1971). All MDD participants scored 18 or higher on the 17-item Hamilton Depression Rating Scale (HDRS)(Hamilton, 1960) and were free of antidepressant and psychotropic medications for at least 3-4 weeks prior to baseline assessments, MRI scan, genotyping and medication randomization. The local review board approved this study and informed consent was obtained from all subjects prior to participating in the study.

2.1.2 Treatment

MDD patients were randomly assigned to either citalopram or quetiapine XR treatment in a 1:1 ratio based on computer generated randomized numbers. After randomization but prior to treatment initiation, participants were MRI scanned. The present study was an 8-week, randomized, parallel-group, double-blind comparative trial. For the citalopram treatment group, the initial dose was 10 mg/day and titrated up to 20 mg/day until day 4. For the quetiapine XR treatment group, quetiapine XR was initiated at 50 mg/day for the first 2 days, 150 mg on the third day and titrated up to 300 mg/day by day 4. If patients could not tolerate the maximum dose of 300mg/day of quetiapine XR or 20mg/day of citalopram, the dose was reduced according to clinical judgment to 150 mg/day and 10mg/day, respectively. Patients who could not tolerate 10mg/day of citalopram or 150 mg/day of quetiapine XR were terminated from the study.

2.1.3 Assessment

Depression symptoms were assessed using the HDRS at baseline, at week one following treatment titration, and at week 8. The main clinical outcome was percent change in HDRS scores from baseline to endpoint at 8 weeks. A secondary clinical outcome included index of response and remission. Response was defined as an indexed change in HDRS scores [((pre-post)/pre)*100%] (DeLorenzo et al., 2013) over the 8-week treatment regime. Non-remitters were defined by ≥ 50% reduction in HDRS at endpoint, and remission was defined by HDRS endpoint scores of ≤ 7.

2.2 Diffusion Tensor Imaging Statistical Analysis

2.2.1 Tract Based Spatial Statistics

Impact of fractional anisotropy and genetic risk factors on treatment response was assessed through TBSS and probabilistic tractography. The FMRIB Software Tools (FSL) allows t-test comparisons between groups (refer to Diffusion Tensor Imaging Section for more information). Voxel-wise FA differences were assessed between antidepressant responders (non-remitters and remitters) vs. non-responders, non-remitters vs. non-responders, and remitters vs. non-responders. Between group differences were investigated in regions of interest including the hippocampus and anterior internal capsule.

2.2.2 Probabilistic Tractography

The utility of myelin integrity to predict treatment outcome was assessed by regressing fractional anisotropy in the left or right cingulum bundle, uncinate fasciculus and superior longitudinal fasciculus with antidepressant response. To

assess the predictive value of FA in combination with genetics on antidepressant response, linear regression models were used to regress two-way interactions between FA and genetic risk factors (5-HTTLPR or BDNF polymorphisms) against treatment response. To assess the impact of genetic risk factors on myelin integrity, FA in the left and right cingulum bundle, uncinate fasciculus and superior longitudinal fasciculus, were compared across allelic groupings.

More specifically, each model was tested for normality, heteroscedasticity, multi-collinearity, autocorrelation, outliers, high leverage and influential points. Specific cases were only evaluated for exclusion if the case was a corrected outlier, had a leverage value 3 times the average leverage value (Stevens, 2002), and had a Cook's distance (effect of a single case on a model) value >0.7 (R. D. Cook & Weisberg, 1982).

3.0 Results

3.1 Participants

Forty-six patients diagnosed with major depressive disorder completed the study, which excludes the nine patients who dropped-out or were excluded over the 8-week treatment regime (see table 1). Hardy Weinberg allele frequency distribution was maintained for the 5-HTTLPR genotype [$x^2=0$ $p>0.05$] and BDNF val66met genotype [$x^2=2.36$, $p>0.05$].

Table 1: Characteristics of patients with MDD receiving Quetiapine XR and citalopram

Characteristics	Citalopram (N=24)*	Quetiapine XR (N=22)*	p-value
Sex (F/M)	12/12	14/8	
Age (yrs)	36.3(9.7)	39.8(10.0)	0.23
Age of onset	25.5(10.0)	27.9 (10.6)	0.43
HDRS Baseline	21.3(4.5)	20.9(3.5)	0.74
HDRS Week 8	9.6(6.8)	7.8(4.1)	0.27
Current episode duration (wks)	249(293)	204(261)	0.59
Number of Episodes	2.4(4.5)	2.1(3.0)	0.79
Medication dosage	20 mg/day	300 mg/day	
Responders	8(37.3%)	6(27%)	
Remitters	8(33.3%)	11(50%)	
Responders + Remitters	17(71%)	17(77%)	
Non-responders	7(29%)	5(23%)	

HDRS=Hamilton Depression Rating Scale; Mean (S.D)s are presented

*Completers

Table 1: Demographic Data

3.2 Tract based spatial statistics

3.2.1 Responders (Non-remitters / Remitters) vs. Non-Responders

FA maps were constructed for contrasts between antidepressant responders (non-remitters and remitters) and non-responders. No between group differences were found in these contrasts at a corrected level of FWE $p<0.05$. Comparisons uncorrected for multiple comparisons revealed significantly higher FA in the hippocampal portion of the left cingulum [x=117, y=97, z=56, voxels=165, p=0.016]

for antidepressant responders relative to non-responders (see figure 1 & table 2a).

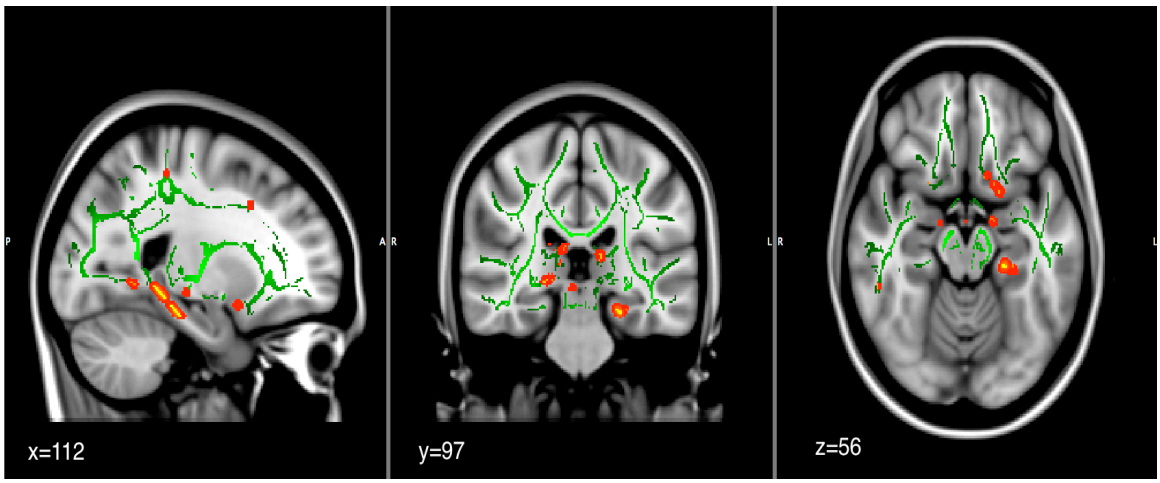


Figure 1: Tract Based Spatial Statistics – Responders vs. Non-Responders. Filled tract based spatial statistics analysis between major depressive disorder patients who responded to antidepressant treatment, relative to non-responders. Based on John Hopkins University tract labels significant differences were observed in the hippocampal regions of the cingulum bundle.

3.2.2 Non-remitters vs. Non-Responders

FA maps were constructed for contrasts between antidepressant non-remitters and non-responders. No corrected between group differences were found in these contrasts. Uncorrected comparisons found significantly higher FA in the hippocampal portion of the left cingulum [x=115, y=86, z=66, voxels=64, p=0.024] for antidepressant non-remitters relative to non-responders (see table 2b).

3.2.3 Remitters vs. Non-Responders

FA maps were constructed for contrasts between remitters and non-responders. No corrected between group results were found in these contrasts. Uncorrected comparisons revealed significantly higher FA in the hippocampal region of the left cingulum [x=117, y=97, z=56, voxels=165, p=0.016] in remitters, relative to non-responders (see table 2c).

(a) Tract Based Spatial Statistics: Remitters + Responders > Non-Responders *							
	MNI co-ordinates (vox)						
Voxels	X	Y	Z	Hemisphere	p-value	JHU White Matter Label	Harvard Oxford Cortical Region
165	117	97	56	L	0.016	Cingulum (Hippocampus)	Parahippocampal Gyrus
79	128	52	82	L	0.01		Lateral Occipital Cortex
55	41	72	89	R	0.026		Angular Gyrus
50	61	40	84	R	0.002		Lateral Occipital Cortex
40	85	131	68	R	0.03		Unclassified
34	112	99	53	L	0.02	Cingulum (Hippocampus)	Parahippocampal Gyrus
threshold = 30 voxels							
(b) Tract Based Spatial Statistics: Responders > Non-Responders *							
64	115	86	66	L	0.024	Cingulum (Hippocampus)	Parahippocampal
46	49	102	120	R	0.024		Postcentral Gyrus
34	129	55	96	L	0.008		Lateral Occipital Cortex
31	46	83	110	R	0.03		Supramarginal Gyrus
threshold = 30 voxels							
(c) Tract Based Spatial Statistics: Remitters > Non-Responders *							
12	111	96	56	L	0.046	Cingulum (Hippocampus)	Parahippocampal Gyrus
5	56	144	70	R	0.006	Unclassified	Insular Cortex

Threshold =5 voxels
* uncorrected

Table 2: Tract Based Spatial Statistics – Response [(a) Non-remitters/Remitters vs. Non-Responders (b) Non-remitters vs. Non-Responders, (c) Remitters vs. Non-Responders].

3.3 Genetic Risk Factors

There was no significant main effects of genetic risk factors [5-HTTLPR: $F(2,40)=0.1961$, $p=0.8227$; BDNF: $F(1,40)=0.07278$, $p=0.7887$] on percent change in depression severity.

3.4 Probabistic Tractography

3.4.1 Cingulum Bundle

Change in depression severity did not significantly regress with a multiple linear regression model with fractional anisotropy in the left cingulum bundle, 5-HTTLPR and BDNF polymorphisms ($F(4,16)=0.0986$, $p=0.9814$). In the left cingulum bundle, there was no significant association between fractional anisotropy and indexed change in depression severity ($F(1,20)=0.6131$, $p=0.4428$). There was no significant association in the regression between polymorphisms in BDNF or 5-HTTLPR with FA in the left cingulum bundle (BDNF: $p=0.963$, 5-HTTLPR: $p=0.248$).

No significant association was identified in the regression of percent change in depression severity and a two-way interactions between fractional anisotropy in the left cingulum bundle and BDNF polymorphisms ($F(3,18)=0.398$, $p=0.756$) or 5-HTTLPR polymorphisms ($F(5,15)=0.191$, $p=0.961$).

Change in depression severity did not significantly regress with a multiple linear regression model with fractional anisotropy in the right cingulum bundle, 5-HTTLPR and BDNF polymorphisms ($F(4,17)=0.705$, $p=0.5991$). In the right cingulum bundle, there was no significant association between fractional anisotropy and indexed change in depression severity ($F(1,21)=0.162$, $p=0.6914$). There was no significant association in the regression between polymorphisms in BDNF or 5-HTTLPR and FA in the right cingulum bundle (BDNF:$p=0.247$, 5-HTTLPR: $p=0.294$).

There was no significant association in the regression of percent change in depression severity and two-way interactions between fractional anisotropy in the right cingulum bundle and BDNF polymorphisms ($F(3,19)=0.1334$,$p=0.939$), or 5-HTTLPR polymorphisms ($F(5,16)=0.3374$, $p=0.883$).

3.4.2 Uncinate Fasciculus

Change in depression severity did not significantly regress with a multiple linear regression model with fractional anisotropy in the left uncinate fasciculus, 5-HTTLPR and BDNF polymorphisms ($F(4,28)=1.34$, $p=0.2808$). In the left uncinate fasciculus, there was a trend towards significant association between fractional anisotropy and change in depression severity ($F(1,33)=3.073$, $p=0.08891$) (see figure 2). As FA increased, patients experienced a larger improvement in depression severity following antidepressant treatment. There was a significant association

between polymorphisms in the BDNF gene and FA in the left uncinate fasiculus ($F(1,40)=7.314$, $p=0.009$), with val homozygotes exhibiting higher FA in the left uncinate relative to met carriers (see figure 3). No significant association was found in the regression of 5-HTTLPR polymorphisms and FA in the left uncinate fasciculus ($p=0.195$).

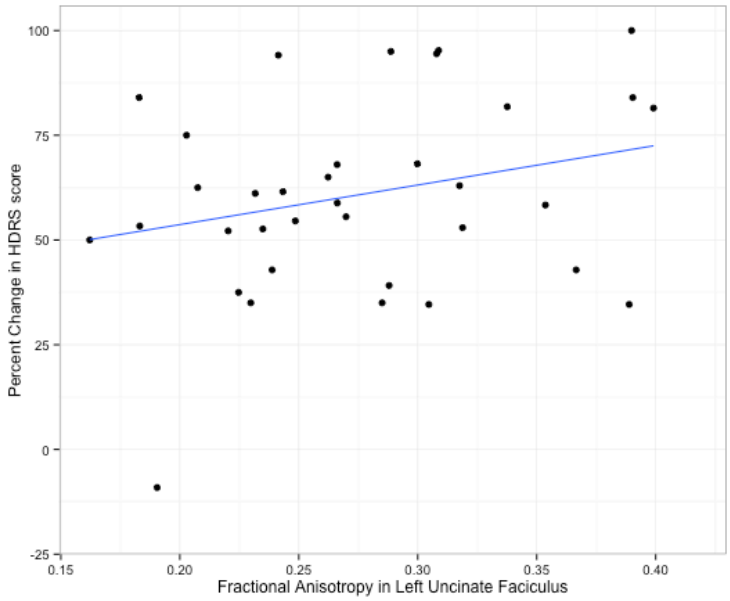


Figure 2: Influence of Fractional Anisotropy in the left uncinate fasciculus on improvement in HAMD score

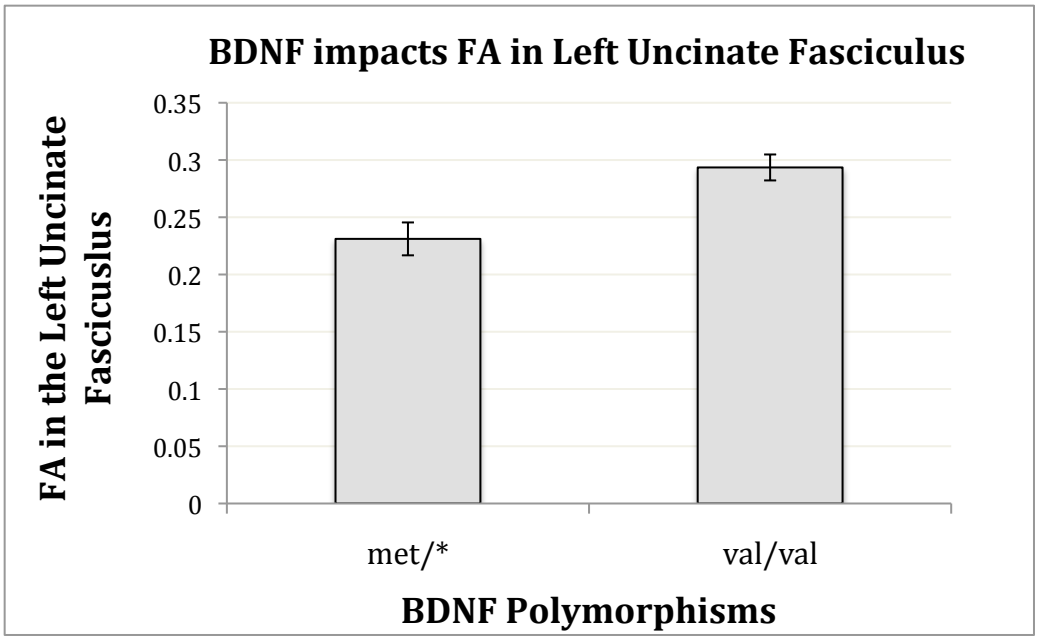


Figure 3: Influence of BDNF polymorphisms on FA in the uncinate fasciculus

There was a significant association in the regression of percent change in depression severity and a two-way interaction between FA in the left uncinate and BDNF polymorphisms ($F(3,30)=3.923$, $p=0.0178$), but not with 5-HTTLPR polymorphisms ($F(5,28)=0.737$, $p=0.602$). These results indicate that as fractional anisotropy increased homozygous val individuals (G/G) had a more substantial improvement in depression severity following antidepressant treatment relative to met carriers (G/A) (Figure 4).

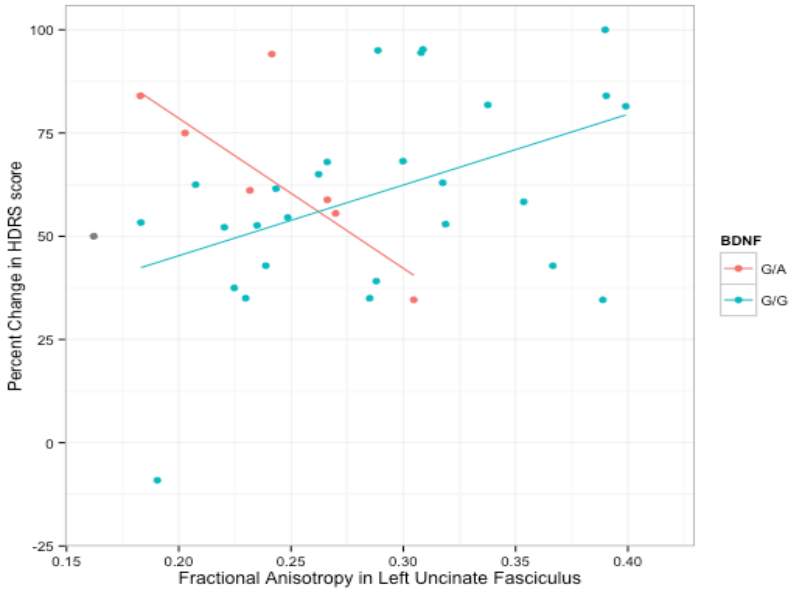


Figure 4:Influence of Fractional Anisotropy in the left uncinate fasciculus on improvement in HAMD score, with a moderator of BDNF polymorphisms. [BDNF legend: G/G: val/val, G/A: met/*]

Change in depression severity did not significantly regress with a multiple linear regression model with fractional anisotropy in the right uncinate fasciculus, 5-HTTLPR and BDNF polymorphisms ($F(4,26)=2.692$, $p=0.05314$). In the right uncinate fasciculus, there was no significant association between fractional anisotropy and indexed change in depression severity ($F(1,30)=0.8491$, $p=0.3642$). There was no significant association in the regression between polymorphisms in BDNF or 5-HTTLPR and FA in the right uncinate fasciculus (BDNF: $p=0.151$, 5-HTTLPR: $p=0.432$).

A significant association was identified in the regression of percent change in depression severity and a two-way interaction between FA in the right uncinate and 5-HTTLPR polymorphism ($F(5,25)=5.315$, $p=0.00184$), but not with val66met BDNF polymorphism ($F(3,28)=0.6825$, $p=0.57$). Indicating that as FA increased,

individuals who were homozygous for the S'S' or L'L' alleles had a more significant improvement in depression severity following treatment, in contrast to S'L' heterozygotes who exhibited more minute improvements in depression severity (Figure 5). These results indicate that as fractional anisotropy increased homozygous val individuals (G/G) had a more substantial improvement in depression severity following antidepressant treatment relative to met carriers (G/A) (figure 6).

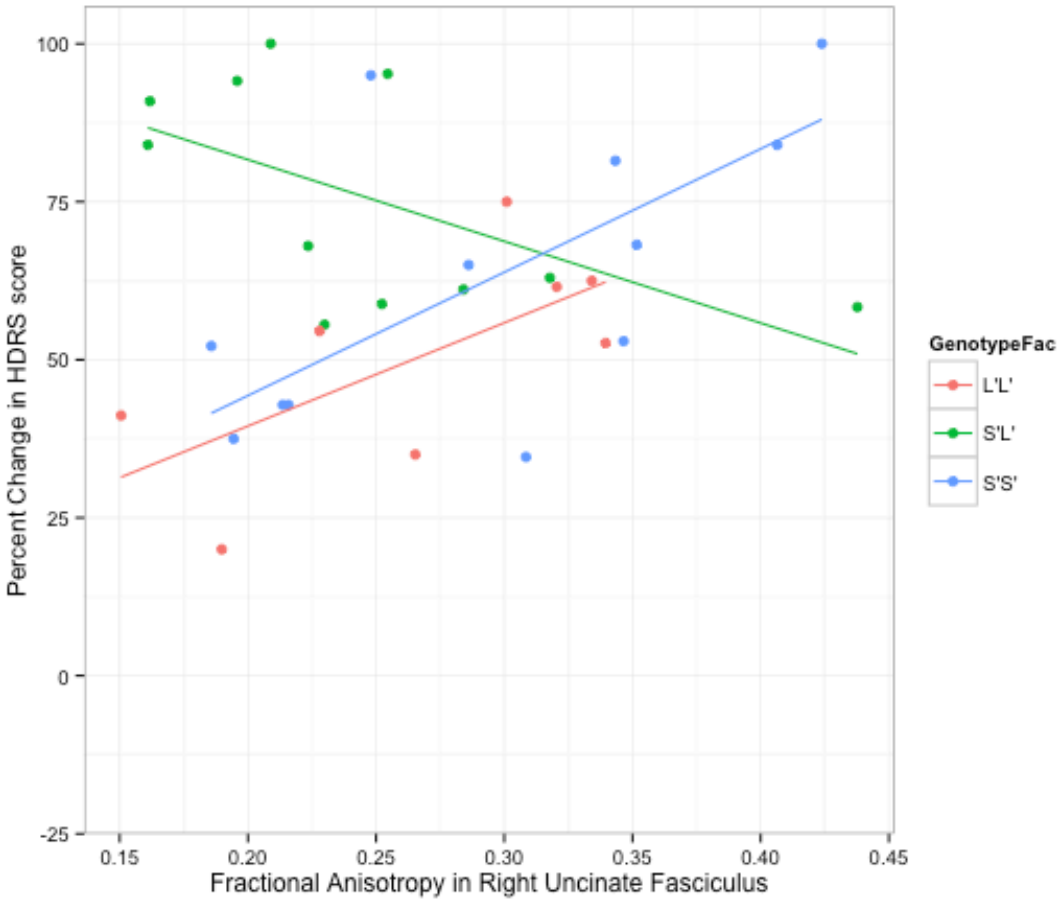


Figure 5: Influence of fractional anisotropy in the right uncinate fasciculus on improvement in HAMD score, with a moderator of 5-HTTLPR polymorphisms

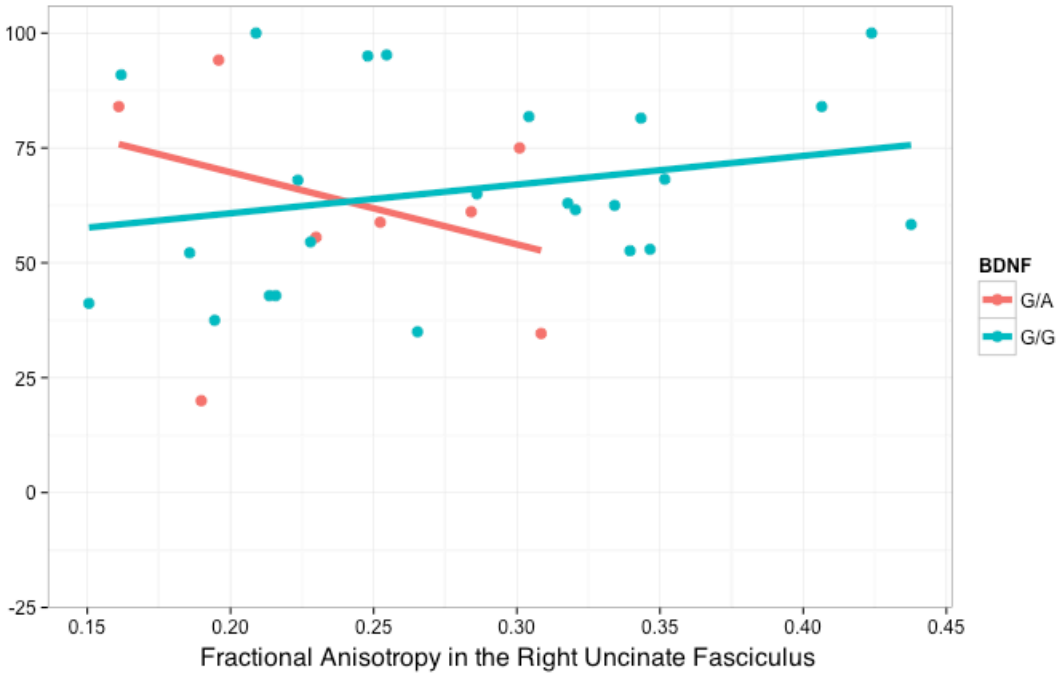


Figure 6: Influence of fractional anisotropy in the right uncinate fasciculus on improvement in HAMD score, with a moderator of BDNF polymorphism

3.4.3 Superior Longitudinal Fasciculus

Change in depression severity did not significantly regress with a multiple linear regression model with fractional anisotropy in the left superior longitudinal fasciculus, 5-HTTLPR and BDNF polymorphisms ($F(4,25)=0.328$, $p=0.856$). In the left superior longitudinal fasciculus, there was no significant association between fractional anisotropy and indexed change in depression severity ($F(1,29)=0.0605$, $p=0.807$). There was no significant association in the regression of polymorphisms in the BDNF gene or 5-HTTLPR and FA in the left superior longitudinal fasciculus (BDNF: $p=0.917$, 5-HTTLPR: $p=0.149$).

No significant association was identified in the regression of percent change in depression severity and two-way interactions between fractional anisotropy in the left superior longitudinal fasciculus and BDNF ($F(3,27)=0.05$, $p=0.985$) or 5-HTTLPR polymorphisms ($F(5,24)=0.569$, $p=0.723$).

Change in depression severity did not significantly regress with a multiple linear regression model with fractional anisotropy in the right superior longitudinal fasciculus, 5-HTTLPR and BDNF polymorphisms ($F(4,30)=0.147$, $p=0.856$). In the right superior longitudinal fasciculus, there was no significant association between fractional anisotropy and indexed change in depression severity ($F(1,35)=0.1944$, $p=0.662$). There was no significant association in the regression of polymorphisms in BDNF or 5-HTTLPR and FA in the right superior longitudinal fasciculus (BDNF: $p=0.406$, 5-HTTLPR: $p=0.457$).

No significant association was identified in the regression of percent change in depression severity and two-way interactions between fractional anisotropy in the right superior longitudinal fasciculus and BDNF ($F(3,32)=0.049$, $p=0.986$) or 5-HTTLPR polymorphisms ($F(5,30)=0.777$, $p=0.574$).

Due to the implementation of a new MR scanner, eleven participants were imaged on a new scanner. Although an onsite physicist controlled for consistency across both MR scanners, all significant regressions were computed in a smaller sample that excluded these participants to control for possible variability. Smaller sample analysis revealed similar results to the full group findings, with all interacting regressions remaining significant ($p<0.05$).

Due to the limited number of diffusion directions used in the present study, probabilistic tractography analysis of specific tracts was unable to extract diffusion parameters for specific remodeled association tracts in a portion of our sample. Participant data was excluded in cases where diffusion data could not be extracted.

4.0 Discussion

In the present study we investigated the predictive value of genetic risk factors and neuroimaging parameters on response to antidepressants. Tract based spatial statistics permitted voxel-wise comparisons of antidepressant responders and remitters with non-responders. Regression analyses were used to access whether FA in the superior longitudinal fasciculus, cingulum and uncinate fasciculus, alone or in conjunction with polymorphisms in the 5-HTTLPR and BDNF gene predicted a larger indexed change in depression severity. We found that antidepressant remitters and responders showed higher FA in the hippocampus, relative to non-responders. Probabilistic tractography analysis revealed that pretreatment FA values in the left uncinate alone and in combination with BDNF polymorphisms predicted larger improvements in depression severity. BDNF polymorphisms moderated the association between FA and change in depression severity, with depressed individuals who had higher FA in the left uncinate and were val/val homozygotes exhibiting larger improvements in depression severity following antidepressant treatment. The data also showed that the depressed individuals with the val/val BDNF genotype had higher left uncinate fasciculus FA values than those who were met carriers. Finally, we found that depressed individuals who were homozygotes (S'S' or L'L') for the 5-HTTLPR and have high FA

in the right uncinate, exhibited larger improvements in depression severity relative to S'L' genotypes.

The present findings are consistent with previous research that associated higher FA in frontal regions with remission following anti-depressant treatment (Alexopoulos et al., 2002; 2008). The regions exhibiting the strongest association with treatment response were the hippocampal portion of the cingulum and the uncinate fasciculus, both of which are involved in cognitive aspects of emotion regulation.

The cingulum bundle connects the cingulate gyrus to the hippocampus; arches superiorly to the corpus callosum, and is involved in planning, attention, memory and emotion regulation (Catani, Howard, Pajevic, & Jones, 2002; J. Choi, Jeong, Rohan, Polcari, & Teicher, 2009; Mori et al., 2008; Schermuly et al., 2010). Our TBSS findings indicated higher FA in the hippocampal region of the cingulum for responders/remitters, relative to non-responders, a finding that has been replicated in dorsal regions of the cingulum (Hoogenboom et al., 2014). Abnormal hippocampus volume and function are well documented findings in the depression literature (Campbell, Marriott, Nahmias, & MacQueen, 2004; Milne, MacQueen, & Hall, 2012). Smaller hippocampi volume has been associated with lower remission rates (MacQueen, Yucel, Taylor, Macdonald, & Joffe, 2008) and impairments in attention, memory and executive function (O'Brien, Lloyd, McKeith, Gholkar, & Ferrier, 2004). Based on previous and present findings, the size and integrity of the hippocampus seem to be pivotal in depression development and remission. High hippocampal integrity may indicate strong functionality and less severe

hippocampal-based depression symptoms, which are non-surprisingly associated with stronger response to anti-depressants.

The TBSS findings may not of been replicated in the tractography analysis, as FA differences were localized to the hippocampus. Tractography analysis averages FA across the entire remodeled tract, resulting in a dissipation of region specific significant results across large tracts such as the cingulum. Future studies should partition the cingulum bundle into subregions, to further investigate the role cingulum bundle subregions, specifically FA in the hippocampus, have on antidepressant response (Carballedo et al., 2012; Hoogenboom et al., 2014).

The uncinate fasciculus is the largest fronto-limbic tract, and connects both orbitomedial and ventrolateral aspects of the prefrontal cortex with the ipsilateral temporal pole, uncus, hippocampal gyrus, and amygdala (Catani et al., 2002). High FA in this tract indicates strong connections between the ventrolateral PFC and the amygdala and hippocampus (Steffens, Taylor, Denny, Bergman, & Wang, 2011). Reduced myelin integrity, and thus FA, within the uncinate has been repeatedly reported in the depression literature (Cullen et al., 2010; Dalby et al., 2010; Versace et al., 2008; Zhang et al., 2011), but higher FA within this regions has also been reported in depression(Aghajani et al., 2013) and bipolar disorder (Versace et al., 2008). Low FA in this region has also been associated with trait anxiety (Baur, Hänggi, & Jäncke, 2012) and early onset depression, relative to mid and late-onset (W. D. Taylor, MacFall, Gerig, & Krishnan, 2007).

Limbic-cortical connections, specifically fronto-limbic connections have distinguished treatment responders from non-responders across medication and

psychotherapy treatments (Seminowicz et al., 2004). The number of previous depressive episodes and severity of symptoms have also been reported to have a significant impact on FA in the ventromedial PFC, a region connected to the uncinate fasciculus (de Diego-Adeliño et al., 2013). These previous findings and the present results suggest that depressed individuals whom have strong white matter integrity, and thus high FA, in fronto-limbic regions exhibit larger improvements in depression severity following antidepressant treatments. Microstructure abnormalties within this circuit may result in interrupted or unmodulated regulation between regions, which has been associated with poor depression severity improvements (Alexopoulos et al., 2002; 2008).

Future studies should be cautious of null pre-post antidepressant treatment differences in uncinate fasciculus FA, as having high pretreatment FA may bias an individual to larger changes in depression severity. Furthermore, it can be speculated that this tract may not be substantially altered over the course of treatment, as there may be ceiling effects in FA changes. Increased pretreatment FA may suggest a predisposition to more favourable outcomes, as prior to treatment these individuals have the existing neuroconnectivity profiles necessary to support the functional changes associated with remission. For example, having strong fronto-limbic connectivity prior to treatment may assist in top-down regulation of emotion and the attenuation of amygdala reactivity. Future DTI studies using probabilistic tractography are necessary to address this hypothesis.

Functional connectivity deficits in fronto-limbic tracts have been demonstrated in depression and shown to normalize following antidepressant

treatment. Remission following antidepressant treatment is associated with changes in neural activity including increases in activity in frontal regions such as the dorsolateral prefrontal cortex and dorsal ACC, and decreases in ventral limbic and para-limbic structures (Alexopoulos et al., 2008; Mayberg, 2003; Mayberg et al., 1999; Sheline et al., 2001) . Additionally, elevated pre-treatment amygdala and frontal activity predicts stronger improvements in depression (Canli et al., 2005; Langenecker et al., 2007), but persistently higher amygdala reactivity following treatment is associated with lower remission rates and a risk for relapse (Drevets, 1999). It is possible that these functional deficits are secondary to structural and connectivity changes in conferring vulnerability (Alexopoulos et al., 2008). Consistent with this theory and vulnerability theories of depression, our results indicate dysfunction in networks that overlap many regions affected in depression, rather than pin-pointing dysfunction to specific regions (Mayberg, 2003).

The present association of higher pretreatment uncinate fasciculus FA values with greater change in HDRS scores is consistent with related research that examined tracts connecting the raphe nuclei/midbrain with the amygdala or hippocampus. DeLorenzo and colleagues (2013) found an association between FA values in tracts to the amygdala and depression improvement following SSRI treatment. This group also found fewer number of tracts to the right amygdala in non-remitters relative to remitters (DeLorenzo, 2013). Although our group did not investigate the same tract, the uncinate fasciculus is connected to the same two target regions, the hippocampus and amygdala. From these studies it can be suggested that reduced connectivity in the uncinate may impair the effectiveness of

antidepressant treatment (Pizzagalli, 2010). Thus, reduced pretreatment fronto-limbic connectivity may inhibit the normalization associated with a strong anti-depressant response, and in-turn decreases antidepressant effectiveness.

Recent DTI investigations have reported that carriers of the met BDNF allele who had high depression severity exhibit lower FA values in the uncinate relative to homozygous val depressed individuals or healthy controls (Carballedo et al., 2012). Our research extends these findings to antidepressant response, as FA increased BDNF met-carriers exhibited limited improvements in depression severity, relative to val homozygotes who exhibited more significant improvements following medication. These findings indicate that carriers of the met-allele exhibit reduced depression improvement even with the neuroconnectivity profile optimal for antidepressant response. The present findings support previous associations between BDNF profiles and antidepressant response as we found that higher pretreatment uncinate BDNF is associated with a stronger antidepressant response. Based on these results, it is clear that in addition to neuroimaging parameters, genetic risk factors can provide valuable insights regarding antidepressant response.

Analysis of genetic risk factors indicated that the 5-HTTLPR moderated the association of uncinate myelin integrity on depression improvement. As uncinate FA increased, S'S' and L'L genotypes exhibited larger improvements in depression severity, in contrast to S'L' heterozygotes who showed less marked improvements in depression. Such findings are in line with more recent literature indicating a lack of association between a specific variant of the 5-HTTLPR and depression or antidepressant response. While other studies have found no associations between a

number of depression risk genes including 5-HTTLPR and anti-depressant response (Bukh et al., 2010) it can also be postulated that the 5-HTTLPR alleles that confer risk for depression do not extend their influence to myelin integrity or treatment response, or rather there is a lack of sensitivity on myelin integrity since effect sizes between 5-HTTLPR and depression are small. Further analyses are required to investigate the relative value of neuroimaging and genetic risk factors in predicting antidepressant response.

Our group also investigated the impact that genetic polymorphisms had on FA specifically. BDNF polymorphisms were significantly associated with FA in the left uncinate, and had val homozygotes exhibit higher FA then met carriers. Associations between BDNF and FA has been observed in prior studies on healthy controls, but in contrast the val/val genotype was associated with lower FA (Chiang et al., 2011; Tost et al., 2013). Although the majority of previous literature on this topic has been in healthy controls, the present findings may indicate that BDNF has differing roles in depression; future studies are required to investigate this hypothesis. Contrary to our expectations there was no association between the 5-HTTLPR polymorphisms and neuronal connectivity, as direct impact on functional organization (Jonassen, 2012; Pacheco et al., 2009) and reactivity (Costafreda et al., 2013; Hariri, 2002) have been previously documented, though effect sizes were small (Munafò, Brown, & Hariri, 2008).

4.1 Conclusion

These findings provide support for the use of diffusion tensor imaging as a tool for determining antidepressant treatment prognosis. Our research has

demonstrated that myelin integrity can be used to predict treatment response. Measures of myelin integrity in regions and tracts affected by depression, including the hippocampus and fronto-limbic tracts, are shown here to delineate responders and remitters from non-responders. Furthermore, our findings suggest an association between strong fronto-limbic connectivity and depression severity improvement following antidepressant treatment. Although genetics did not seem to have a direct impact on antidepressant response, they seem to exacerbate effects of neuroconnectivity on improvement in depression severity.

References:

Aghajani, M., Veer, I. M., van Lang, N. D. J., Meens, P. H. F., van den Bulk, B. G., Rombouts, S. A. R. B., et al. (2013). Altered white-matter architecture in treatment-naive adolescents with clinical depression. *Psychological Medicine*, 1–12. doi:10.1017/S0033291713003000

Alexopoulos, G. S., Kiosses, D. N., Choi, S. J., Murphy, C. F., & Lim, K. O. (2002). Frontal white matter microstructure and treatment response of late-life depression: a preliminary study. *American Journal of Psychiatry*, *159*(11), 1929–1932.

Alexopoulos, G. S., Murphy, C. F., Gunning-Dixon, F. M., Latoussakis, V., Kanellopoulos, D., Klimstra, S., et al. (2008). Microstructural White Matter Abnormalities and Remission of Geriatric Depression. *American Journal of Psychiatry*, *165*(2), 238–244. doi:10.1176/appi.ajp.2007.07050744

Arango, C., & Bernardo, M. (2005). The effect of quetiapine on aggression and hostility in patients with schizophrenia. *Human Psychopharmacology: Clinical and Experimental*, *20*(4), 237–241. doi:10.1002/hup.686

Aydemir, C., Yalcin, E. S., Aksaray, S., Kisa, C., Yildirim, S. G., Uzbay, T., & Goka, E. (2006). Brain-derived neurotrophic factor (BDNF) changes in the serum of depressed women. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *30*(7), 1256–1260. doi:10.1016/j.pnpbp.2006.03.025

Baune, B. T. (2008). New developments in the management of major depressive disorder and generalized anxiety disorder: role of quetiapine. *Neuropsychiatric Disease and Treatment*, *4*(6), 1181.

Baur, V., Hänggi, J., & Jäncke, L. (2012). Volumetric associations between uncinate fasciculus, amygdala, and trait anxiety. *BMC Neuroscience*, *13*(1), 4. doi:10.1186/1471-2202-13-4

Brunoni, A. R., Lopes, M., & Fregni, F. (2008). A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. *The International Journal of Neuropsychopharmacology*, *11*(08), 1169. doi:10.1017/S1461145708009309

Bukh, J. D., Bock, C., Vinberg, M., Werge, T., Gether, U., & Kessing, L. V. (2009). Journal of Affective Disorders. *Journal of Affective Disorders*, *119*(1-3), 107–115. doi:10.1016/j.jad.2009.02.023

Bukh, J. D., Bock, C., Vinberg, M., Werge, T., Gether, U., & Kessing, L. V. (2010). No interactions between genetic polymorphisms and stressful life events on outcome ofantidepressant treatment. *European Neuropsychopharmacology*, *20*(5), 327–335. doi:10.1016/j.euroneuro.2009.11.004

Campbell, S., Marriott, M., Nahmias, C., & MacQueen, G. M. (2004). Lower hippocampal volume in patients suffering from depression: a meta-analysis. *American Journal of Psychiatry*, *161*(4), 598–607.

Canli, T., Cooney, R. E., Goldin, P., Shah, M., Sivers, H., Thomason, M. E., et al. (2005). Amygdala reactivity to emotional faces predicts improvement in major depression. *NeuroReport*, *16*(12), 1267–1270.

Carballedo, A., Amico, F., Ugwu, I., Fagan, A. J., Fahey, C., Morris, D., et al. (2012). Reduced fractional anisotropy in the uncinate fasciculus in patients with major depression carrying the met-allele of the Val66Met brain-derived neurotrophic

factor genotype. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *159B*(5), 537–548. doi:10.1002/ajmg.b.32060

Catani, M., Howard, R. J., Pajevic, S., & Jones, D. K. (2002). Virtual in Vivo Interactive Dissection of White Matter Fasciculi in the Human Brain. *NeuroImage*, *17*(1), 77–94. doi:10.1006/nimg.2002.1136

Chen, B., Dowlatshahi, D., MacQueen, G. M., Wang, J.-F., & Young, L. T. (2001). Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Bps*, *50*(4), 260–265.

Chiang, M.-C., Barysheva, M., Toga, A. W., Medland, S. E., Hansell, N. K., James, M. R., et al. (2011). BDNF gene effects on brain circuitry replicated in 455 twins. *NeuroImage*, *55*(2), 448–454. doi:10.1016/j.neuroimage.2010.12.053

Choi, J., Jeong, B., Rohan, M. L., Polcari, A. M., & Teicher, M. H. (2009). Preliminary Evidence for White Matter TractAbnormalities in Young Adults Exposed toParental Verbal Abuse. *Bps*, *65*(3), 227–234. doi:10.1016/j.biopsych.2008.06.022

Choi, M.-J., Kang, R.-H., Lim, S.-W., Oh, K.-S., & Lee, M.-S. (2006). Brain-derived neurotrophic factor gene polymorphism (Val66Met) and citalopram response in major depressive disorder. *Brain Research*, *1118*(1), 176–182. doi:10.1016/j.brainres.2006.08.012

Cook, I. A., Leuchter, A. F., Witte, E., Abrams, M., Uijtdehaage, S. H., Stubbeman, W., et al. (1999). Neurophysiologic predictors of treatment response to fluoxetine in major depression. *Psychiatry Research*, *85*(3), 263–273.

Cook, R. D., & Weisberg, S. (1982). Residuals and influence in regression.

Costafreda, S. G., McCann, P., Saker, P., Cole, J. H., Cohen-Woods, S., Farmer, A. E., et al. (2013). Journal of Affective Disorders. *Journal of Affective Disorders*, *150*(1), 96–103. doi:10.1016/j.jad.2013.02.028

Cullen, K. R., Klimes-Dougan, B., Muetzel, R., Mueller, B. A., Camchong, J., Houri, A., et al. (2010). Altered White Matter Microstructurein Adolescents With Major Depression:A Preliminary Study. *Jaac*, *49*(2), 173–183.e1. doi:10.1016/j.jaac.2009.11.005

Dalby, R. B., Frandsen, J., Chakravarty, M. M., Ahdidan, J., Sørensen, L., Rosenberg, R., et al. (2010). Psychiatry Research: Neuroimaging. *Psychiatry Research: Neuroimaging*, *184*(1), 38–48. doi:10.1016/j.pscychresns.2010.06.008

de Diego-Adeliño, J., Pires, P., Gómez-Ansón, B., Serra-Blasco, M., Vives-Gilabert, Y., Puigdemont, D., et al. (2013). Microstructural white-matter abnormalities associated with treatment resistance, severity and duration of illness in major depression. *Psychological Medicine*, 1–12. doi:10.1017/S003329171300158X

Delaveau, P., Jabourian, M., Lemogne, C., Guionnet, S., Bergouignan, L., & Fossati, P. (2011). Journal of Affective Disorders. *Journal of Affective Disorders*, *130*(1-2), 66–74. doi:10.1016/j.jad.2010.09.032

DeLorenzo, C. (2013). Prediction of selective serotonin reuptake inhibitor response using diffusion-weighted MRI, 1–8. doi:10.3389/fpsyt.2013.00005/abstract

Domschke, K., Lawford, B., Laje, G., Berger, K., Young, R., Morris, P., et al. (2009). Brain-derived neurotrophic factor (BDNF) gene: no major impact on antidepressant treatment response. *The International Journal of Neuropsychopharmacology*, *13*(01), 93. doi:10.1017/S1461145709000030

Drevets, W. C. (1999). Prefrontal Cortical ‐ Amygdalar Metabolism in Major Depression. *Annals of the New York Academy of Sciences*, *877*(1), 614–637.

Egan, M. F., Kojima, M., Callicott, J. H., Goldberg, T. E., Kolachana, B. S., Bertolino, A., et al. (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell*, *112*(2), 257–269.

First, M. B., Spitzer, R. L., Williams, J. B., Gibbon, M., First. (1997). *Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I), Clinical Version*. American Psychiatric Publishing, Incorporated.

Fournier, J. C., DeRubeis, R. J., Hollon, S. D., Dimidjian, S., Amsterdam, J. D., Shelton, R. C., & Fawcett, J. (2010). Antidepressant drug effects and depression severity. *JAMA: the Journal of the American Medical Association*, *303*(1), 47–53.

Frodl, T., Schüle, C., Schmitt, G., Born, C., Baghai, T., Zill, P., et al. (2007). Association of the brain-derived neurotrophic factor Val66Met polymorphism with reduced hippocampal volumes in major depression. *Archives of General Psychiatry*, *64*(4), 410–416.

Gong, Q., Wu, Q., Scarpazza, C., Lui, S., Jia, Z., Marquand, A., et al. (2011). Prognostic prediction of therapeutic response in depression using high-field MR imaging. *NeuroImage*, *55*(4), 1497–1503. doi:10.1016/j.neuroimage.2010.11.079

Hamilton, M. (1960). A rating scale for depression. *Journal of Neurology, Neurosurgery, and Psychiatry*, *23*(1), 56.

Hariri, A. R. (2002). Serotonin Transporter Genetic Variation and the Response of the Human Amygdala. *Science*, *297*(5580), 400–403. doi:10.1126/science.1071829

Henry, M. E., Lauriat, T. L., Lowen, S. B., Churchill, J. H., Hodgkinson, C. A., Goldman, D., & Renshaw, P. F. (2013). Psychiatry Research: Neuroimaging. *Psychiatry Research: Neuroimaging*, *213*(3), 217–224. doi:10.1016/j.pscychresns.2013.05.008

Hoogenboom, W. S., Perlis, R. H., Smoller, J. W., Zeng-Treitler, Q., Gainer, V. S., Murphy, S. N., et al. (2014). Limbic system white matter microstructure and long-term treatment outcome in major depressive disorder: A diffusion tensor imaging study using legacy data. *World Journal of Biological Psychiatry*, *15*(2), 122–134. doi:10.3109/15622975.2012.669499

Hu, X., Oroszi, G., Chun, J., Smith, T. L., Goldman, D., & Schuckit, M. A. (2005). An Expanded Evaluation of the Relationship of Four Alleles to the Level of Response to Alcohol and the Alcoholism Risk. *Alcoholism: Clinical & Experimental Research*, *29*(1), 8–16. doi:10.1097/01.ALC.0000150008.68473.62

Jonassen, R. (2012). The effects of the serotonin transporter polymorphism and age on frontal white matter integrity in healthy adult women, 1–5. doi:10.3389/fnhum.2012.00019/abstract

Karg, K. (2011). The Serotonin Transporter Promoter Variant (5-HTTLPR), Stress, and Depression Meta-analysis RevisitedEvidence of Genetic Moderation5-HTTLPR, Stress, and Depression Meta-Analysis. *Archives of General Psychiatry*, *68*(5), 444. doi:10.1001/archgenpsychiatry.2010.189

Kato, M., & Serretti, A. (2008). Review and meta-analysis of antidepressant

pharmacogenetic findings in major depressive disorder. *Molecular Psychiatry*, *15*(5), 473–500. doi:10.1038/mp.2008.116

Kim DK, Lim SW, Lee S, Sohn SE, Kim S, Hahn CG et al. Serotonin transporter gene polymorphism and antidepressant response. Neuroreport 2000; 11: 215–219.

Kraft, J. B., Peters, E. J., Slager, S. L., Jenkins, G. D., Reinalda, M. S., McGrath, P. J., & Hamilton, S. P. (2007). Analysis of Association Between the Serotonin Transporter and Antidepressant Response in a Large Clinical Sample. *Biological Psychiatry*, *61*(6), 734–742. doi:10.1016/j.biopsych.2006.07.017

Lai, C.-H., Wu, Y.-T., Yu, P.-L., & Yuan, W. (2013). Journal of Affective Disorders. *Journal of Affective Disorders*, *150*(2), 330–336. doi:10.1016/j.jad.2013.04.014

Lamar, M., Charlton, R. A., Ajilore, O., Zhang, A., Yang, S., Barrick, T. R., et al. (2013). Neuropsychologia. *Neuropsychologia*, *51*(8), 1463–1470. doi:10.1016/j.neuropsychologia.2013.05.004

Langenecker, S. A., Kennedy, S. E., Guidotti, L. M., Briceno, E. M., Own, L. S., Hooven, T., et al. (2007). Frontal and Limbic Activation During Inhibitory Control Predicts Treatment Response in Major Depressive Disorder. *Biological Psychiatry*, *62*(11), 1272–1280. doi:10.1016/j.biopsych.2007.02.019

MacQueen, G. M., Yucel, K., Taylor, V. H., Macdonald, K., & Joffe, R. (2008). Posterior Hippocampal Volumes Are Associated with Remission Rates in Patients with Major Depressive Disorder. *Biological Psychiatry*, *64*(10), 880–883. doi:10.1016/j.biopsych.2008.06.027

Mayberg, H. S. (2003). Modulating dysfunctional limbic-cortical circuits in depression: towards development of brain-based algorithms for diagnosis and optimised treatment. *British Medical Bulletin*, *65*(1), 193–207. doi:10.1093/bmb/ldg65.193

Mayberg, H. S., Brannan, S. K., Tekell, J. L., Silva, J. A., Mahurin, R. K., McGinnis, S., & Jerabek, P. A. (2000). Regional metabolic effects of fluoxetine in major depression: serial changes and relationship to clinical response. *Bps*, *48*(8), 830–843.

Mayberg, H. S., Liotti, M., Brannan, S. K., McGinnis, S., Mahurin, R. K., Jerabek, P. A., et al. (1999). Reciprocal limbic-cortical function and negative mood: converging PET findings in depression and normal sadness. *American Journal of Psychiatry*, *156*(5), 675–682.

Milne, A. M., MacQueen, G. M., & Hall, G. B. (2012). Abnormal hippocampal activation in patients with extensive history of major depression: an fMRI study. *Journal of Psychiatry & Neuroscience: JPN*, *37*(1), 28.

Mori, S., Oishi, K., Jiang, H., Jiang, L., Li, X., Akhter, K., et al. (2008). Stereotaxic white matter atlas based on diffusion tensor imaging in an ICBM template. *NeuroImage*, *40*(2), 570–582.

Munafò, M. R., Brown, S. M., & Hariri, A. R. (2008). Serotonin Transporter (5-HTTLPR) Genotype and Amygdala Activation: A Meta-Analysis. *Biological Psychiatry*, *63*(9), 852–857. doi:10.1016/j.biopsych.2007.08.016

O'Brien, J. T., Lloyd, A., McKeith, I., Gholkar, A., & Ferrier, N. (2004). A longitudinal study of hippocampal volume, cortisol levels, and cognition in older depressed subjects. *American Journal of Psychiatry*, *161*(11), 2081–2090.

Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia*, *9*(1), 97–113.

Pacheco, J., Beevers, C. G., Benavides, C., McGeary, J., Stice, E., & Schnyer, D. M. (2009). Frontal-Limbic White Matter Pathway Associations with the Serotonin Transporter Gene Promoter Region (5-HTTLPR) Polymorphism. *Journal of Neuroscience*, *29*(19), 6229–6233. doi:10.1523/JNEUROSCI.0896-09.2009

Pizzagalli, D. A. (2010). Frontocingulate Dysfunction in Depression: Toward Biomarkers of Treatment Response. *Neuropsychopharmacology*, *36*(1), 183–206. doi:10.1038/npp.2010.166

Sahraian, S., Babashams, M., Reza-Soltani, P., Najmabadi, H., Kahrizi, K., & Gorgani, S. H. (2013). Serotonin Transporter Polymorphism (5-HTTLPR) and Citalopram Effectiveness in Iranian Patients with Major Depressive Disorder. *Iranian Journal of Psychiatry*, *8*(2), 86–91.

Schermuly, I., Fellgiebel, A., Wagner, S., Yakushev, I., Stoeter, P., Schmitt, R., et al. (2010). Association between cingulum bundle structure and cognitive performance: An observational study in major depression. *European Psychiatry*, *25*(6), 355–360. doi:10.1016/j.eurpsy.2010.05.001

Seminowicz, D. A., Mayberg, H. S., McIntosh, A. R., Goldapple, K., Kennedy, S., Segal, Z., & Rafi-Tari, S. (2004). Limbic–frontal circuitry in major depression: a path modeling metanalysis. *NeuroImage*, *22*(1), 409–418. doi:10.1016/j.neuroimage.2004.01.015

Serretti, A., Kato, M., De Ronchi, D., & Kinoshita, T. (2006). Meta-analysis of serotonin transporter gene promoter polymorphism (5-HTTLPR) association with selective serotonin reuptake inhibitor efficacy in depressed patients. *Molecular Psychiatry*. doi:10.1038/sj.mp.4001926

Sheline, Y. I., Barch, D. M., Donnelly, J. M., Ollinger, J. M., Snyder, A. Z., & Mintun, M. A. (2001). Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: an fMRI study. *Bps*, *50*(9), 651–658.

Smeraldi, E., Zanardi, R., Benedetti, F., Di Bella, D., Perez, J., & Catalano, M. (1998). Polymorphism within the promoter of the serotonin transporter gene and antidepressant efficacy of fluvoxamine. *Molecular Psychiatry*, *3*(6), 508–511.

Stahl, S. M. (2000). Placebo-controlled comparison of the selective serotonin reuptake inhibitors citalopram and sertraline. *Bps*, *48*(9), 894–901.

Steffens, D. C., Taylor, W. D., Denny, K. L., Bergman, S. R., & Wang, L. (2011). Structural Integrity of the Uncinate Fasciculus and Resting State Functional Connectivity of the Ventral Prefrontal Cortex in Late Life Depression. *PLoS ONE*, *6*(7), e22697. doi:10.1371/journal.pone.0022697.s001

Stevens, J. P. (2002) Applied multivariate statistics for the social sciences (4th Ed.) Hillsdale, NJ: Erlbaum.

Taylor, W. D., MacFall, J. R., Boyd, B., Payne, M. E., Sheline, Y. I., Krishnan, R. R., & Murali Doraiswamy, P. (2011). One-Year Change in Anterior Cingulate Cortex White Matter Microstructure: Relationship With Late-Life Depression Outcomes. *The American Journal of Geriatric Psychiatry*, *19*(1), 43–52. doi:10.1097/JGP.0b013e3181e70cec

Taylor, W. D., MacFall, J. R., Gerig, G., & Krishnan, R. R. (2007). Structural integrity of

the uncinate fasciculus in geriatric depression: Relationship with age of onset. *Neuropsychiatric Disease and Treatment*, *3*(5), 669.

Tost, H., Alam, T., Geramita, M., Rebsch, C., Kolachana, B., Dickinson, D., et al. (2013). Effects of the BDNF Val, *38*(3), 525–532. doi:10.1038/npp.2012.214

Tsai, S.-J., Cheng, C.-Y., Yu, Y. W.-Y., Chen, T.-J., & Hong, C.-J. (2003). Association study of a brain-derived neurotrophic-factor genetic polymorphism and major depressive disorders, symptomatology, and antidepressant response. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *123B*(1), 19–22. doi:10.1002/ajmg.b.20026

Versace, A., Almeida, J. R., Hassel, S., Walsh, N. D., Novelli, M., Klein, C. R., et al. (2008). Elevated left and reduced right orbitomedial prefrontal fractional anisotropy in adults with bipolar disorder revealed by tract-based spatial statistics. *Archives of General Psychiatry*, *65*(9), 1041–1052.

Weisler R, Joyce M, McGill L, Lazarus A, Szamosi J, Eriksson H; (2009). Extended release quetiapine fumarate monotherapy for major depressive disorder: results of a double-blind, randomized, placebo-controlled study. *CNS Spectr*. 14(6), 299-313.

Yoshida, K., Ito, K., Sato, K., Takahashi, H., Kamata, M., Higuchi, H., et al. (2002). Influence of the serotonin transporter gene-linked polymorphic region on the antidepressant response to fluvoxamine in Japanese depressed patients. *Progress in Neuropsychopharmacology & Biological Psychiatry*, *26*(2), 383–386.

Yu, Y. W.-Y., Tsai, S.-J., Chen, T.-J., Lin, C.-H., & Hong, C.-J. (2002). Association study of the serotonin transporter promoter polymorphism and symptomatology and antidepressant response in major depressive disorders. *Molecular Psychiatry*, *7*(10), 1115–1119. doi:10.1038/sj.mp.4001141

Zanardi, R., Benedetti, F., Daniela, D., Catalano, M., Smeraldi, E. (2000) Efficacy of Paroxetine in Depression is influenced by a Functional Polymorphism Within the Promoter of the Serotonin Transporter Gene. *Journal of Clinical Psychology*. 20(1), 105-107.

Zhang, A., Leow, A., Ajilore, O., Lamar, M., Yang, S., Joseph, J., et al. (2011). Quantitative Tract-Specific Measures of Uncinate and Cingulum in Major Depression Using Diffusion Tensor Imaging. *Neuropsychopharmacology*, *37*(4), 959–967. doi:10.1038/npp.2011.279

Zou, Y.-F., Ye, D.-Q., Feng, X.-L., Su, H., Pan, F.-M., & Liao, F.-F. (2010). Meta-analysis of BDNF Val66Met polymorphism association with treatment response in patients with major depressive disorder. *European Neuropsychopharmacology*, *20*(8), 535–544. doi:10.1016/j.euroneuro.2009.12.005

Final Notes

The present research contributes to the understanding of depression development and treatment response. These findings provide insight into whether risk factors for depression influence neuroconnectivity in depressed individuals, and whether such risk factors and neuroconnectivity changes predict treatment response.

Our findings indicate that individuals with depression exhibit a reduction in fractional anisotropy in a number of white matter regions. Furthermore, we demonstrated that childhood adversity, specifically emotional and physical neglect, moderate the influence depression severity has on myelin integrity in the uncinate and superior longitudinal fasciculus. Importantly, these white matter association tracts are involved in cognitive control and cognitive-emotion regulation. These diffusion tensor imaging findings were further extended to antidepressant response, with myelin integrity in the hippocampus delineating antidepressant remitters/responders from non-responders. Additionally, higher myelin integrity in the uncinate fasciculus predicted greater improvements in depression severity.

Our findings also indicate that polymorphisms in the BDNF gene influence uncinate fasciculus myelin integrity, across all participants and within the depressed group alone. Furthermore, polymorphisms within the BDNF gene seem to moderate the association between depression severity and uncinate myelin integrity. These findings were also extended to treatment response; with BDNF and 5-HTTTLPR polymorphisms moderating the influence uncinate FA has on percent

change in depression severity. From these findings, it is evident that there is a relationship between BDNF, fronto-limbic connectivity and treatment response.

An advantage of the present study is that the depressed population consisted of medication naive patients, who were scanned and assessed prior to medication administration. Medications have been shown to influence neural structure and connectivity, and known to reverse some of the negative side effects of depression (Dougherty & Rauch, 2007; Fournier et al., 2010). This type of study design provides an informative understanding of neural connectivity changes in depression, without the confounding effects of medication.

Limitations

Although the present study has many advantages, certain limitations of the findings should be acknowledged. Sample size differences between controls and patients reduced power when comparing across groups. To attenuate this, depression severity was used as a continuous regressor in the probabilistic tractography analyses rather than categorical variable separating individuals into depressed or control groups. Additionally, the analysis program used for TBSS analysis controlled for differences in sample size, and thus mitigating some of the loss in power.

The assessment of water perfusion was also limited to 12 co-linear directions. Previous findings have determined that the number of diffusion directions does not have a significant effect on reproducibility, but tracts derived using 12 directions were smaller than tracts derived with 60 diffusion directions. Fewer diffusion

directions decreases can sensitivity to changes in perfusion and crossing fibers within a single voxel (Heiervang et al., 2006).

Although controlled for, it is also possible that there was bias in the scans collected on the new scanner. Since the new scanner was only used on 15% of the participants, and original findings were replicated after excluding these participants, our team is confident that the change in scanner did not significantly impact our findings.

With the use of the Childhood Trauma Questionnaire, participants were also required to retrospectively self-report childhood adversity. Retrospective reporting may have affected our findings as depressed individuals have more negatively loaded schemas of their traumas and are more likely to report more severe childhood trauma (Uher & McGuffin, 2010). The large range in current depressive episode length may have also affected our findings. Our population ranged from first episode depression, to persistent depressive episodes lasting months to years.

Conclusion

These present findings indicate that abnormalties in white matter within the cortico-striatal-limbic network confer risk/vulnerability to depression, which may be associated with more chronic, treatment resistant, and severe forms of depression. This network also seems to be vulnerable to early life adversity including neglect, and may provide a greater understanding of how such abnormalties predispose an individual to depression and predict their treatment outcome. Finally, genetic risk factors appear to influence fronto-limbic

neuroconnectivity, and influence the association between depression severity, myelin integrity and depression severity improvement.

References:

Dougherty, D. D., & Rauch, S. L. (2007). Brain Correlates of Antidepressant Treatment Outcome from Neuroimaging Studies in Depression. *Psychiatric Clinics of North America*, *30*(1), 91–103. doi:10.1016/j.psc.2006.12.007

Fournier, J. C., DeRubeis, R. J., Hollon, S. D., Dimidjian, S., Amsterdam, J. D., Shelton, R. C., & Fawcett, J. (2010). Antidepressant drug effects and depression severity. *JAMA: the Journal of the American Medical Association*, *303*(1), 47–53.

Heiervang, E., Behrens, T. E. J., Mackay, C. E., Robson, M. D., & Johansen-Berg, H. (2006). Between session reproducibility and between subject variability of diffusion MR and tractography measures. *NeuroImage*, *33*(3), 867–877. doi:10.1016/j.neuroimage.2006.07.037

Uher, R., & McGuffin, P. (2010). The moderation by the serotonin transporter gene of environmental adversity in the etiology of depression: 2009 update. *Molecular Psychiatry*, *15*(1), 18–22. doi:10.1038/mp.2009.123