INTRACORTICAL MYELIN IN BIPOLAR DISORDER

INTRACORTICAL MYELIN IN BIPOLAR DISORDER AND ITS RELATION TO COGNITIVE FUNCTION AND PERIPHERAL BLOOD MARKERS

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A Thesis Submitted to the School of Graduate Studies in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy

McMaster University DOCTOR OF PHILOSOPHY (2017) Hamilton, Ontario (Neuroscience)

TITLE: Intracortical myelin content in Bipolar Disorder and its relation to cognitive function and peripheral blood markers

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PAGES: xvii, 181

Abstract

Introduction

White matter abnormalities are routinely implicated in the pathophysiology of Bipolar Disorder (BD). Although the cerebral cortex is primarily composed of gray matter, myelinated axons are present throughout the cortical layers, most abundantly in the deeper layers. This is known as intracortical myelin (ICM). Many prior studies have shown that there are decreased counts and compromised integrity of oligodendrocytes in the prefrontal cortex of individuals with BD, in addition to altered expression of myelin protein genes. We have previously shown loss of ICM in the medial frontal gyrus in BD, using both MRI and histology. We, along with other groups, have also shown that in healthy humans, the development of ICM follows an "inverted-U" trajectory. To our knowledge, no prior studies have investigated whole brain ICM in BD *in vivo*.

The first aim of this thesis was to explore whole-brain age-related ICM maturation over young adulthood in individuals with BD-I in comparison to controls, using T_1-weighted MRI, and determine whether this was associated with any clinical variables such as the age of onset, duration of illness, number of mood episodes, lifetime psychosis, and medication use.

Due to ICM's essential function of maintaining neural synchrony and the integrity of local neural connections within the cerebral cortex, its role in preserving cognitive function is becoming increasingly recognized. Although poor cognitive function persists through euthymic states in BD, its neurobiological correlates remain undetermined. The

second aim of this thesis is to examine whole-brain ICM content and cognitive function in individuals with BD-I and controls.

Lastly, we explore whether the association between ICM and cognition is mediated by peripheral blood markers of the inflammatory response- C-Reactive Protein (CRP) and anti-myelin basic protein (anti-MBP).

Results

ICM follows an "inverted –U" pattern over age in healthy controls ($p<0.05$). In BD, this association with age is lost throughout the cortex, and appears to plateau. These effects are seen throughout cortex, but have the greatest magnitude in motor and premotor regions, followed by prefrontal and parietal regions. Exploratory analyses show that ICM is also associated with duration of illness, age of onset, the number of manic, hypomanic and mixed episodes, and mood stabilizer and antipsychotic use, although this does not survive correction for multiple comparisons.

In BD, verbal memory (VM) performance was strongly predicted by ICM throughout a cortical network identified *a priori* in association with performance. Executive function, processing speed, and reaction time were also predicted by ICM, although this does not survive Bonferroni correction.

Serum analysis showed that CRP was only associated with ICM in the right anterior cingulate cortex in BD, but not controls (Chapter 4). Further, CRP was associated with performance on verbal memory and Stoop tasks. anti-MBP levels were associated with ICM in regions of the bilateral visual, parietal, and somatosensory cortices, and left temporal, cingulate, and orbitofrontal cortices in healthy controls. In BD subjects, anti-

MBP levels were only associated with ICM in the left caudal medial visual cortex and the right cuneus. Anti-MBP was not associated with cognitive performance in either BD or control subjects.

Conclusion

The results from this thesis suggests a deficit in ICM maturation in young adults with BD. Whether this is developmental in origin is unclear. Further, ICM predicts cognitive performance on region-related tasks in BD, particularly verbal memory. Our work provides a novel potential structural correlate of cognitive performance in BD, and has significant implications for the underlying pathophysiology of cognitive dysfunction in the illness. We also identify anti-MBP as a potential marker of myelin maintenance and regenerative potential.

Acknowledgements

I would like to sincerely thank my supervisors Dr. Benicio Frey and Dr. Meir Steiner for their unwavering support and guidance throughout my years as their student. I have wholeheartedly appreciated your ability to provide guidance while allowing for independent study and discovery through my graduate school experience. Your mentorship and encouragement have been truly invaluable, both inside and outside of the lab, and your fervour for research and scholarship has been infectious. It has been both an honour and a privilege to have worked with you.

To my committee members: Dr. Roberto Sassi and Dr. Jacek Kwiecien. Thank you for all your helpful insight through the entire study process.

Sincerest thanks to Dr. Nicholas Bock and Christopher Rowley- the technical backbones of this work. Thank you, Nick, for allowing us to implement your technique within our study population. I will always be very thankful for this collaboration and for your neuroimaging expertise. Chris, without your image processing skills, this thesis would not have been possible.

A special thank you to Marg Coote for introducing me to research through bench work and for always being so kind and generous with your words of advice. Thank you to Helen Begin for all her help with the pilot for this study, and to Piumi Rajasekera for being my right hand.

Thank you to my peers at the Frey lab: Lauren Cudney (for all the delicious dinners and lovely chats), Jay Patel, Olivia Allega, Anastasiya Slyepchenko, and to Sabrina Syan for being a wonderful travel partner and for the check-ins during the writing

process. Further thanks to Roohie Sharma (an honorary Frey lab member) for all the great times spent by the Bayfront. Thank you to all for the laughs and times we shared.

To my parents, thank you for always letting me choose my own path and for always encouraging me to believe that you can accomplish anything you set your mind to. Your love and support have always meant the world. Thank you to my siblings Ishdeep, Harjot, Jagdeep, Jagjot, and Bhavraj for always putting the biggest smile on my face, and for truly making me believe that home is where the heart is. Thank you, Simba for being the most wonderful fur-ball. Thank you to Shreya Patel for being family, more so than a friend. I truly appreciate all your support and encouragement through this process.

Finally, thank you to all the participants in this study who gave their time and of themselves to help make this work a reality.

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List of Abbreviations

ACC	Anterior Cingulate Cortex
ANCOVA	Analysis of Covariance
Anti-MBP	Anti-myelin basic protein
ARC	Autocalibrating Reconstruction for Cartesian imaging
ATP	Adenosine triphosphate
BA	Brodmann Area
BD	Bipolar Disorder
BD-I	Bipolar Disorder – Type I
BD-II	Bipolar Disorder – Type II
BMI	Body mass index
BOLD	Blood-oxygen level dependent
CBS	Center for brain science
CNS	Central nervous system
CNSVS	CNS-Vital Signs
CPZ	Chlorpromazine
CRP	C-Reactive Protein
CRUISE	Cortical Reconstruction Using Implicit Surface Evolution
CU	Cuneus
DLPFC	Dorsolateral prefrontal cortex
DTI	Diffusion tensor imaging
DSM-IV	Diagnostic & statistical manual of mental disorders, 4th edition
DX	Diagnosis
EDTA	Ethylenediamine tetraacetic acid
EEG	Electroencephalography
ELISA	Enzyme-linked immunosorbent assay
FA	Fractional anisotropy
FANTASM	Fuzzy and Noise Tolerant Adaptive Segmentation Method
fMRI	Functional magnetic resonance imaging
FOV	Field of view
GABA	Gamma-amino Butyric Acid
GAD	Generalized anxiety disorder
GE	General electric
GLM	General linear model
GM	Grey matter
ICM	Intracortical myelin
IL-2	Interleukin-2
IL-6	Interleukin-6
IQ	Intelligence quotient
JIST	Java imaging science toolkit
MADRS	Montgomery Asberg Depression Rating Scale

MAG	Myelin associated glycoprotein
MATRICS	Measurement and Treatment Research to Improve Cognition in Schizophrenia
MBP	Myelin basic protein
MGDM	Multiple Object Geometric Deformable Model
MIPAV	Medical image processing, analysis & visualization
MNI	Montreal Neurological Institute
MOG	Myelin oligodendrocyte glycoprotein
MOSP	Myelin oligodendrocyte specific protein
mPFC	Medial prefrontal cortex
MP-RAGE	Magnetization-prepared rapidly-acquired gradient echo sequence
MRI	Magnetic resonance imaging
MS	Multiple Sclerosis
MTR	Magnetization transfer ratio
OCD	Obsessive compulsive disorder
OFC	Orbital Frontal Cortex
OPC	Oligodendrocyte precursor/progenitor cells
PFC	Prefrontal cortex
PFRDLS	Rostral Dorsolateral Superior Prefrontal Cortex
PLP	Proteolipid protein
PTSD	Post-traumatic stress disorder
RAVLT	Rey Auditory Verbal Learning Test
RBANS	Repeatable Battery for the Assessment of Neuropsychological Status
ROI	Region of interest
RF	Radiofrequency
SCID-I	Structured clinical interview for DSM-IV Axis I Disorders
SZ	Schizophrenia
TE	Echo time
TNF-α	Tumor necrosis factor-α
TR	Repetition time
VM	Verbal memory
WASI	Wechsler Abbreviated Scale of Intelligence
WM	White matter
WMH	White matter hyperintensity
YMRS	Young Mania Rating Scale

Declaration of Academic Achievement

Chapter 2

M. Sehmbi completed all data collection, demographic and clinical variable data analysis, and composed the manuscript. C. Rowley performed image processing and linear model analysis. L. Minuzzi provided statistical consultation and aided in manuscript composition. J.M. Kwiecien and F. Kapczinski aided in interpretation of results and manuscript composition. N.A. Bock and B.N. Frey contributed to project design, data analysis plan, formation of the initial research questions, and aided with composition of the manuscript.

This chapter, in its entirety, has been ***submitted*** to the **Journal of Psychiatry and Neuroscience**.

Chapter 3

M. Sehmbi completed all data collection and analysis, and composed the manuscript. C. Rowley performed image processing. L. Minuzzi provided statistical consultation and aided in manuscript composition. M. Steiner, F. Kapczinski, and R.B. Sassi aided in interpretation of results and manuscript composition. N.A. Bock and B.N. Frey contributed to project design, data analysis plan, formation of the initial research questions, and aided with composition of the manuscript.

This chapter, in its entirety, has been ***submitted*** to the journal **Cerebral Cortex**.

Chapter 4

All data presented in this chapter was collected and analyzed by M. Sehmbi. C. Rowley performed image processing. CRP assays were conducted by M. Coote and J. Gilchrist. N.A. Bock and B.N. Frey contributed to project design, data analysis plan, and formation of the initial research questions.

Chapter 5

All data presented in this chapter was collected and analyzed by M. Sehmbi. C. Rowley performed image processing. Anti-MBP assays were conducted by J. Gilchrist. N.A. Bock and B.N. Frey contributed to project design, data analysis plan, and formation of the initial research questions.

Chapter 1: Introduction

1.1 Bipolar Disorder

Bipolar Disorder (BD) is a highly disabling and chronic psychiatric condition. Initially dubbed "manic-depressive illness", BD is a mood disorder that is characterized by cycling periods of depression and mania, the cardinal symptom of BD. BD is a leading cause of disability worldwide, and has been associated with significant cognitive and functional impairment (1). Of note, BD patients experience a high rate of mortality, particularly an increased risk of suicide, and increased rates of both medical and psychiatric comorbidity.

1.1.1 Classification and Diagnosis

Currently, diagnosis is purely based upon symptom presentation, and is established using the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSM). In order to be diagnosed with BD, individuals must meet criteria for at least one past/current manic/hypomanic episode. Symptoms of mania include an inflated self-esteem and grandeur, a diminished need for sleep, racing thoughts, inability to concentrate, increased goal-directed activity or psychomotor agitation, and/or excessive involvement in enjoyable activities with potential for dire consequences. At least four of these above symptoms must be present, and must cause functional impairment for the individual, lasting at least a week or requiring

hospitalization. Hypomania is characterized by a similar symptomatic profile to mania, but is not severe enough to significantly impair the individual's daily functioning or to require hospitalization, with a minimum of 4 days of duration of symptoms (2).

Symptoms of depression include low mood that persists for most of the day, nearly every day, a severe decrease of interest in pleasurable activities, drastic changes in appetite and/or sleeping patterns, psychomotor agitation, difficulties with concentration, persistent fatigue, and/or suicidal ideation or attempts. Individuals must experience at least five of these symptoms over a period of at least 2 weeks, lasting most of the day, nearly every day in order to meet criteria for a depressive episode.

In the DSM-V, the specifier "with mixed features" is added to a diagnosis of BD if full criteria are met for a manic, hypomanic, or depressive episodes, and at least three symptoms of the contrasting mood profile are present for most of the days of the current or most recent episode (2). Periods of remission of mood symptoms are known as euthymia.

A diagnosis of BD-Type I require the individual to have experienced at least one manic episode. A diagnosis of BD-Type II requires at least one lifetime major depressive episode, accompanied by at least one hypomanic episode (2). BD-II is often mistakenly classified as a "milder" form of BD-I, however, individuals with both subtypes experience the same cumulative effect of illness. Although mania is more symptomatically intense than hypomania, individuals with BD-II experience an increased frequency of episodes, comorbidity, and suicidal behavior, and spend greater amounts of time in depressed states (3, 4). Due to the chronic and almost progressive nature of the

illness, the duration between episodes often shortens over time. This is especially true in cases where there is lack of treatment or low treatment adherence (5).

A diagnosis of BD can be further delineated using course specifiers such as anxious distress, mixed features, rapid cycling, melancholic features, atypical features, mood-congruent psychotic features, mood-incongruent psychotic features, catatonia, peri-partum onset, and/or seasonal pattern (2).

Up to 69% of individuals with BD are reported to have been initially and repeatedly misdiagnosed with an incorrect diagnosis- 60% with Major Depression, 26% with an anxiety disorder, 18% with Schizophrenia and 17%with antisocial personality disorders. This was shown to occur at least three to four times on average prior to correct diagnosis (6). One third of individuals with BD are only correctly diagnosed a decade after seeking help for their symptoms (7). When BD patients are misdiagnosed with Unipolar Depression, they are only treated with anti-depressants, and do not receive mood stabilizers. Antidepressants can trigger manic episodes in individuals who have BD, who are not taking mood stabilizers. This simply exacerbates the illness and furthers its progression (8).

Diagnostic criteria for BD have evolved through the history of psychiatry, and reflect our current understanding of the illness. This process is important both academically and clinically, as diagnosis guides both clinical treatment decisions and research sample composition (9). Solely using behavioral symptoms for diagnosis has many shortcomings, unfortunately. Firstly, clinical presentation in some patients may not exactly align with diagnostic criteria, and some patients may present with ambiguous

symptoms. During the clinical interview processes, patients may not be entirely forthcoming with their experience of symptoms, or they may lack insight to illness due to their symptoms or other secondary illness effects (9). Therefore, the exploration of biological correlates in psychiatric illness is of utmost importance.

1.1.2 Epidemiology

According to the World Health Organization World Mental Health Survey Initiative, the lifetime prevalence of BD is estimated to be between 1-4%. Lifetime prevalence is estimated at 0.6% for BD-I, 0.4% for BD-II, 1.4% for subthreshold BD, and 2.4% for the bipolar spectrum (10). Rates of BD are often suspected to be underreported, as bipolar Type II is thought to be underdiagnosed due to underreport of hypomania (11).

The peak of mean age of onset for BD is identified at 18 years for BD-I, and in the mid-20s for BD-II (2). A systematic review of the global distribution of BD revealed that there are no significant differences in prevalence according to sex, sample coverage, economic status and bipolar subtype (12).

1.1.3 Sex Differences

Although there are no sex differences in the prevalence of BD-I, BD-II is more prevalent in women (2). Sex differences in BD are exceedingly clinically significant, as males and females present with differing clinical features and illness courses. Women are at increased risk to develop mood episodes at times of intense hormone fluctuation, such as premenstrually, during pregnancy, and postpartum (13-15) Women experience rapid

cycling, mixed mania, and depressive episodes more often than men, and are twice as likely as men to develop bipolar type-II (14). Depressive episodes in females often last longer than in males, and are more likely to be treatment resistant (14). As a result, the treatment of BD is especially complex, and each case requires individualized consideration. Women with BD, especially bipolar type-II, are misdiagnosed with unipolar depression for up to two years longer than men, thus delaying the administration of appropriate treatment and potentially exacerbating their symptoms with improper therapies (14).

1.1.4 Risk Factors and Comorbidity

Both genetic and environmental risk factors have been identified in association with a diagnosis of BD. The estimated heritability of BD ranges between 32-85% (16-19), making family history the strongest risk factor for the onset of BD. Relatives of individuals with BD are at 10-fold greater risk than the general population. BD-I is more prevalent in separated, divorced, or widowed individuals than in individuals who have never been married or are currently married (2).

Individuals with BD have increased rates of psychiatric and medical comorbidities. The most common psychiatric comorbidities include anxiety disorders, occurring in 75% of individuals with BD, impulse-control/conduct/disruptive disorders, and substance use disorder, which affects over 50% of individuals with BD (2). Metabolic syndrome, migraines, and diabetes are amongst the most frequently observed physical comorbidities in individuals with BD (2, 20-22).

1.1.5 Current Treatment

Currently, there is no cure for BD, although symptoms can be managed through various avenues. Current treatment options include psychotropic medications and various forms of psychotherapy. Medications include lithium, anticonvulsants, anti-depressants, and antipsychotics. Available psychotherapies include Cognitive Behavioural therapy, Family-focused therapy, Interpersonal and social rhythm therapy, and psychoeducation. Some individuals respond well to pharmacotherapy, whereas others are extremely resistant to medication. Some patients benefit from combination therapy, including both medications and psychotherapy. Results vary between individuals, and each case requires personalized attention (7).

1.2 Neurobiology of BD

Magnetic resonance imaging studies (MRI) have tried to elucidate the structural and functional neurobiology of BD over the last few decades.

1.2.1 Structural Neural Correlates

Structural brain differences have been found in both cortical and subcortical regions in BD subjects, in comparison to healthy controls. Among the cortical regions, the prefrontal cortex is the most widely implicated in BD due to its roles in both cognitive and emotional processing. Subcortical structures implicated in the neurobiology of BD

include limbic structures, thalamus, basal ganglia, corpus callosum, and the cerebellum and vermis (9).

Three main regions of the prefrontal cortex have been associated with cognitive and emotional processing in BD: the anterior cingulate (ACC), dorsolateral prefrontal cortex (DLPFC), and the orbitofrontal cortex (OFC). The anterior cingulate cortex is widely connected with the amygdala, insula, thalamus and OFC. In BD, the ACC has been shown to have reduced volume and gray matter density (23, 24). First-episode mania subjects only showing volumetric reduction in the ACC (25), and those who show episode recurrence within one year of their first-episode also show greater reductions of ACC volume in comparison to those who do well within one year of follow-up (26). Post-mortem studies have shown both decreased neuronal (27) and glial density (28) in the ACC in BD. The DLPFC is a higher-order processing area that plays major roles in both working memory and executive function, and is connected with other areas of similar functionality in the temporal and parietal lobes. It has been found to be compromised in neuronal and glial density (29, 30), likely further reflected in the reduced GM volume in this region (31, 32). The OFC has been shown to be involved in tasks of attention, inhibition, and emotional processing in BD (9, 33-35).

Enlarged lateral ventricles are one of the most replicated findings in BD (36, 37), and have been associated with an increased number of episodes (38)

The hippocampus and amygdala are two limbic regions of interest in BD. The hippocampus is involved in tasks of declarative memory and memory consolidation (39). Gray matter atrophy and decreased size of the hippocampus is reported in adults with BD,

is more prominent during the first illness episode, and can be rescued by Lithium use (9, 40, 41).

The amygdala receives input from the fronto-temporal lobes, and relays information to the hippocampus, entorhinal cortex, thalamus, and cerebral cortex (42). It is consistently shown to be enlarged in BD. Studies have shown that in age-matched controls, amygdala volume decreases over age, whereas in BD subjects there is a volume increase over age (43).

The thalamus and basal ganglia are subcortical structures that have been investigated in BD. The thalamus serves as a relay station between cortical and subcortical structures, and is important for both motor and cognitive function (44). Although there is a general lack of consensus, many structural findings in the thalamus indicate reductions in thalamic volume in BD (45). The basal ganglia are composed of the caudate, putamen, and the globus pallidus, and are involved in motor and emotional functions, and are connected to both cortical and limbic structures. Enlarged volumes of the striatum, consisting of the caudate and putamen, are seen in BD (46, 47), in addition to altered shape of the structure (48).

The corpus callosum is a large bundle of white matter fibers that connects the two cerebral hemispheres, and play roles in attention, language and memory (49). In BD, it has been shown to be compromised both structurally, and functionally, with decreased area, decreased length of fibers, and altered signal intensity (50-53). This is often regarded as a reliable indicator of white matter damage in the illness.

Further, the cerebellum and the vermis play significant roles in regulating cognition, language and mood (54). Although there are conflicting reports, these structures are shown to have decreased volumes in BD (9, 55, 56).

1.2.2 Functional Neural Correlates

Oftentimes, BD is described as a disorder of brain connectivity, rather than a disorder that is associated with the structure or function of certain individual brain areas (57). Functional magnetic resonance imaging (fMRI) using blood-oxygen-level-dependent contrast has been the most popular technique used to assess *in vivo* neural activity in BD.

Two inter-related prefrontal-limbic networks have routinely been implicated in the pathophysiology of BD, and specifically, in the regulation of emotional behaviour. Highly similar to the Salience network, The Automatic/Internal emotional regulatory network comprises the ventromedial prefrontal cortex, subgenual ACC, nucleus accumbens, globus pallidus, and thalamus. This network is described to regulate amygdala responses to self-generated feeling states. The Volitional/External regulatory network comprises the ventrolateral PFC, mid and dorsal cingulate cortices, ventromedial striatum, globus pallidus, and thalamus. Both these networks are thought to exert prefrontal control over limbic regions, such as the amygdala, through feedback loops (9, 58).

1.2.3 White Matter Abnormalities

White matter hyperintensities (WMHs) are amongst the most replicated findings in BD. Additionally, the severity of these WMHs has been associated with increased hospitalizations and treatment resistance (59, 60). WMHs are more consistently found in deep WM, mostly in the deep frontal and prefrontal areas, than in periventricular WM. This suggests impaired connectivity between the fronto-cortical and subcortical regions (47, 61-63).

The corpus callosum is amongst the most widely studied WM subcortical regions in BD. The general consensus amongst volumetric studies is that the corpus callosum is smaller in individuals with BD than healthy controls (53). Patients who have received previous treatment with lithium display greater corpus callosum area than those who are lithium naïve, more closely resembling healthy controls. This suggests a possible therapeutic role for lithium, in ameliorating corpus callosum integrity(64).

Diffusion tensor imaging (DTI) studies have revealed significant WM abnormalities in BD. WM hyperintensities seen using MRI are the most widely replicated neurobiological findings in BD (65). They likely disrupt WM fibre function, and are thought to be associated with dilated perivascular spaces, localized demyelination, astrocytic gliosis, and atherosclerosis (66, 67). Widely used indices of measurement in DTI are anisotropy and diffusivity. At an anisotropy value of 0, otherwise known as total anisotropy, water moves in the *x, y*, and *z* planes with no preference for directionality. As we approach an anisotropy value of 1, the diffusivity of water is limited by the structures surrounding it (i.e.: axons and myelin) (68). This measure is known as fractional

anisotropy (FA), and provides a measure of directionality of axons in the brain. Diffusivity can be measured both radially and axially (68). Reduced axial diffusivity and increased radial diffusivity may be indicative of compromised axonal and myelin integrity (69).

Many DTI studies have focused on frontal WM tracts in BD. Using region-of interest approaches, reduced FA was initially reported in the anterior frontal WM, which connects the prefrontal cortex with other cortical and subcortical regions (70). Altered WM integrity has also been reported in the fronto-occipital fasciculus, which connects the frontal lobes with the temporal and occipital lobes (71). These WM changes were accompanied by reduced GM volume of the frontal cortex. The cingulum bundle, which connects the prefrontal and temporal lobes, also shows reduced FA in BD (72). Using voxel-based approaches, alterations in FA in the inferior longitudinal fascisulus, unicate fasciculus, optic radiation, and both anterior and superior thalamic radiations have also been reported in BD (69, 72, 73).

1.3 Cognition in BD

Cognitive dysfunction has been widely documented in BD in periods of acute depression and mania, and has also been found to persist in periods of symptom remission, known as euthymia (74, 75). Previous meta-analyses reveal deficits in executive functioning, verbal learning and memory, attention and processing speed in euthymic individuals with BD in comparison to healthy controls (76, 77). These studies support the notion that cognitive dysfunction is not transient and dependent on mood

state, but is rather a persistent feature of the disorder. Cognitive dysfunction has been associated with clinical variables such as number of hospitalizations, number of manic episodes, duration of illness (78, 79), history of psychosis (80), age of symptom onset and duration of treatment delay (75, 81). These studies suggest that individuals with BD experience deteriorating cognitive function with illness progression.

Only a handful of longitudinal studies on cognitive function in BD have been completed to date. Surprisingly, reviews have shown that the results of longitudinal studies of cognition in BD are not congruent with cross-sectional studies (82). Despite evidence from cross-sectional studies that certain clinical variables are associated with neurocognitive function, BD patients do not show a progressive neurocognitive decline over time with illness progression. The slight decline that is observed is comparable to that seen in healthy controls in most cases, and may be attributable to aging (82). A meta-analysis of studies on the longitudinal course of neurocognitive deficits in BD reports that individuals with BD do not display progressive cognitive decline after a follow-up period between 2 to 4 years in length (83).

Despite the lack of an accelerated decline in performance over time, cognitive dysfunction has been associated with reduced functionality in both cross-sectional and longitudinal studies. Results from a meta-analysis of 22 studies show that cognitive deficits contribute to a significant proportion of functional disability in individuals with BD (84). Cognitive dysfunction, therefore, severely impairs daily functioning in affected individuals and significantly contributes to the burden of the illness.

Interestingly, in a 6-year follow-up study of euthymic individuals with BD, although there was a lack of overall cognitive decline in the BD group, BD subjects who did display neuropsychological decline also reported worse psychosocial functioning (85).

In a staging model of BD, a progression from early- to late-stage BD was associated with both poorer functionality and poorer cognitive performance (86). Individuals in late-stages experienced far greater deficits in functionality, particularly in autonomy, and greater impairments in cognitive function than healthy controls and early-stage subjects (i.e. those with less severe courses of illness). Notably, unemployment status BD subjects has been related to deficits in verbal memory and attention (87, 88).

Despite an asymptomatic status, BD patients still experience ongoing neurocognitive and functional impairment. Only 43% of 166 individuals hospitalized for a first manic or mixed episode achieved functional recovery in 2 years of follow-up, despite being asymptomatic. These subjects were 2.6 times less likely to achieve functional recovery than symptomatic recovery (89). A second study found that only 24% of patients with BD-Type I achieved functional recovery following a midcourse hospitalization for a manic or mixed episode within one year (90).

Most individuals with BD are therefore unable to regain their level of premorbid occupational and residential status. It is essential to understand the cognitive and neurobiological correlates of functional impairment in order to develop treatment strategies and interventions for better patient care and to facilitate better societal integration for patients following severe mood episodes.

1.4 Myelin

Nervous tissue is divided into two categories: white matter (WM) and gray matter. The components of WM include myelinated axons, glia, and blood vessels (91). Myelin is a structure that wraps in a tight spiral around nerve axons, acting as an electrical insulator (91), and allowing for more efficient transmission of nerve impulses (92). In the CNS, myelin is comprised of cells called oligodendrocytes that wrap their processes around axons; in the PNS, Schwann cells perform this same function around peripheral nerves (91). By dry mass, myelin is composed of 70-85% lipids and 15-30% proteins (91). There are no myelin-specific lipids, although there is a collection of proteins that are unique to myelin. These include myelin basic protein (MBP), proteolipid protein (PLP), myelin-oligodendrocyte glycoprotein (MOG), myelin-associated glycoprotein (MAG), and myelin oligodendrocyte specific protein (MOSP) among others. The onset of psychiatric disorders in late adolescence or early childhood coincides temporally with increasing myelination of the prefrontal cortex (93, 94).

1.4.1 Neurobiology of Oligodendrocytes

The development of myelin is a highly regulated and complex process. Oligodendrocytes arise from oligodendrocyte progenitor cells (OPCs). These cells can differentiate into oligodendrocytes, glia or neurons. Neural progenitor cell domains include the floor plate and roof plate of the neural tube. Patterning molecules are released from these cell domains, instructing progenitor cells to assume identities unique to their

positioning along the embryonic axes. These include Sonic hedgehog, the ventralizing signal, and bone morphogenetic proteins, the dorsally secreted signals (95-97).

Some OPCs differentiate into myelinating cells, whereas others remain as immature cells. This is thought to play a role in the slow turnover of oligodendrocytes or for myelin regeneration (97, 98). The differentiation of oligodendrocytes is mediated mainly by the *Olig* genes, which are basic helix-loop-helix transcriptional factors (97, 99, 100). At birth, myelin is only present in the cerebellum and the brainstem, as seen in histological samples. From 0-3 months, myelination continues to occur in the cerebellum and begins in the internal capsule (101). The development of myelin follows a specific trajectory. Primary sensory and motor areas are the first to begin heavy myelination (102), whereas the frontal and temporal lobes continue to myelinate into the fifth decade of life as OPCs continue to differentiate into myelin producing cells (103, 104). In general, the pattern of cortical myelination follows the same trajectory, however, we have shown that although motor and premotor cortices begin to myelinate first, their peak of cortical myelination extends beyond other lobes (105). Is also follows the spatial development pattern of cortico-cortical associations (106). In subcortical structures, postnatal myelination at 3 months begins in the cerebellum, pons and internal capsule. It then proceeds caudo-cranially from the splenium of the corpus callosum and optic radiations between 3-4 months of age. The occipital and parietal lobes are myelinated between 4-6 months. The genu of the corpus callosum and frontal and temporal lobes are myelinated from 6-8 months (101).

During myelination, there is a strict association between axon diameter and myelin thickness- axons with larger diameters have proportionally thicker myelin and longer internodes. During remyelination, however, this relationship disintegrates, whereby the length and thickness of myelin is independent of axon diameter. Usually, remyelinated axons possess thinner myelin than that seen during development (97). It is important to note that the potential for remyelination decreases with age (107, 108). It seems that there is a delay with increasing age in both the recruitment and differentiation of OPCs (108).

1.4.2 Intracortical myelin

The cortex is the outermost layer of the brain that is responsible for many of higher-order functions. The main purpose of myelin is to increase conduction speed of electrical signals between neurons. It has been argued, however, that another reason for myelination in cortical gray matter (Intracortical myelin, ICM) is to prevent formation of incorrect synapses and potentially harmful connections (109). More recently, studies have shown that myelination factors inhibit growth of new axons in addition to synapse formation. This may reduce the plasticity of heavily myelinated regions (110, 111).

Heavily myelinated regions of the cortex include primary sensory/motor areas, and certain association areas including visual cortex, the intraparietal sulcus, and the ventral prefrontal cortex. These areas tend to myelinate early. Lightly myelinated cortical regions are largely association areas (112). Additionally, heavily myelinated regions do not expand as much postnatally as do lightly myelinated areas. Heavily myelinated

networks include the sensorimotor, auditory and visual networks. Lightly myelinated networks include association networks, namely the default mode network, cingulo-opercular network, ventral attention network and the language network (113, 114). It is hypothesized that heavily myelinated areas may need to be more stable and resistant to change to perform basal sensory and motor functions. Lightly myelinated areas, responsible for many higher-order functions, may need to be more plastic and dynamic (115).

1.5 Main Aims

Previously, the measurement of WM abnormalities *in vivo* was restricted to subcortical structures, with the use of DTI, and studies of ICM were limited to post-mortem tissue analysis, involving staining and microscopy techniques. We have recently developed a novel T_1- weighted imaging technique validated for *in vivo* measurement of ICM (105, 116-118). We aim to measure whole-cortex ICM in individuals with BD in comparison to healthy controls, and determine whether it is associated with cognitive performance and peripheral markers of inflammation and CNS-damage in BD.

1.6 Main Objectives

The specific objectives of this thesis are as follows:

1. Investigate whether whole-cortex ICM is altered in BD subjects in comparison to controls.

2. Examine if ICM is associated with cognitive performance in both controls and BD subjects.
3. To determine whether markers of inflammation or immune activation influence the relationship between ICM and cognition. We will investigate serum C-Reactive Protein (CRP) and anti-myelin basic protein (MBP).

1.7 Hypotheses

Our hypotheses for each previously outlined aim are as follows:

1. In light of previous studies showing compromised integrity of WM tracts in BD, and altered density and count of oligodendrocyte in the cerebral cortex, we hypothesize that individuals with BD will show decreased ICM levels in comparison to controls.
2. ICM has been shown to be important for the integrity of neural connections. Further, the cortex has been associated with many higher-order functions. We postulate ICM will be associated with cognitive performance in BD subjects.
3. Brain structure and cognitive performance have both been shown to be associated with both central and peripheral measures of inflammation and immune activation. Serum CRP and anti-MBP will be assessed in association with both ICM and cognition in BD.

1.8 References

1. Alonso J, Petukhova M, Vilagut G, Chatterji S, Heeringa S, Üstün TB, et al. Days out of role due to common physical and mental conditions: results from the WHO World Mental Health surveys. Molecular psychiatry. 2011;16(12):1234.

2. Association AP. Diagnostic and statistical manual of mental disorders (DSM-5®): American Psychiatric Pub; 2013.

3. Vieta E, Suppes T. Bipolar II disorder: arguments for and against a distinct diagnostic entity. Bipolar disorders. 2008;10(1p2):163-78.

4. Suppes T, Dennehy EB. Evidence-based long-term treatment of bipolar II disorder. Journal of Clinical Psychiatry. 2002;63:29-33.

5. Suppes T, Baldessarini RJ, Faedda GL, Tohen M. Risk of recurrence following discontinuation of lithium treatment in bipolar disorder. Archives of General Psychiatry. 1991;48(12):1082-8.

6. Economics A. for SANE Australia. Bipolar disorder: costs An analysis of the burden of bipolar disorder and related suicide in Australia Melbourne: Access Economics, for SANE Australia. 2003.

7. Hirschfeld R, Lewis L, Vornik LA. Perceptions and impact of bipolar disorder: how far have we really come? Results of the national depressive and manic-depressive association 2000 survey of individuals with bipolar disorder. The Journal of clinical psychiatry. 2003.

8. Severus E, Bauer M. Diagnosing bipolar disorders in DSM-5. International journal of bipolar disorders. 2013;1(1):14.

9. Bipolar Disorder: Clinical and Neurobiological Foundations. 1 ed. Singapore: Wiley-Blackwell; 2010.

10. Merikangas KR, Jin R, He J-P, Kessler RC, Lee S, Sampson NA, et al. Prevalence and correlates of bipolar spectrum disorder in the world mental health survey initiative. Archives of general psychiatry. 2011;68(3):241-51.

11. Akiskal HS, Bourgeois ML, Angst J, Post R, Möller H-J, Hirschfeld R. Re-evaluating the prevalence of and diagnostic composition within the broad clinical spectrum of bipolar disorders. Journal of affective disorders. 2000;59:S5-S30.

12. Ferrari AJ, Baxter AJ, Whiteford HA. A systematic review of the global distribution and availability of prevalence data for bipolar disorder. Journal of affective disorders. 2011;134(1):1-13.

13. Freeman MP, Smith KW, Freeman SA, McElroy SL, Kmetz G, Wright R, et al. The impact of reproductive events on the course of bipolar disorder in women. The Journal of clinical psychiatry. 2002;63(4):284-7.

14. Arnold LM. Gender differences in bipolar disorder. psychiatric clinics of North America. 2003;26(3):595-620.

15. Nivoli AM, Pacchiarotti I, Rosa AR, Popovic D, Murru A, Valenti M, et al. Gender differences in a cohort study of 604 bipolar patients: the role of predominant polarity. Journal of affective disorders. 2011;133(3):443-9.

16. Lichtenstein P, Yip BH, Björk C, Pawitan Y, Cannon TD, Sullivan PF, et al. Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. The Lancet. 2009;373(9659):234-9.

17. Wray NR, Gottesman II. Using summary data from the danish national registers to estimate heritabilities for schizophrenia, bipolar disorder, and major depressive disorder. Frontiers in genetics. 2012;3.

18. McGuffin P, Rijsdijk F, Andrew M, Sham P, Katz R, Cardno A. The heritability of bipolar affective disorder and the genetic relationship to unipolar depression. Archives of general psychiatry. 2003;60(5):497-502.

19. Smoller JW, Finn CT, editors. Family, twin, and adoption studies of bipolar disorder. American Journal of Medical Genetics Part C: Seminars in Medical Genetics; 2003: Wiley Online Library.

20. McIntyre RS, Konarski JZ, Soczynska JK, Wilkins K, Panjwani G, Bouffard B, et al. Medical comorbidity in bipolar disorder: implications for functional outcomes and health service utilization. Psychiatric Services. 2006;57(8):1140-4.

21. Goldstein BI, Kemp DE, Soczynska JK, McIntyre RS. Inflammation and the phenomenology, pathophysiology, comorbidity, and treatment of bipolar disorder: a systematic review of the literature. The Journal of clinical psychiatry. 2009;70(8):1078-90.

22. Kilbourne AM, Cornelius JR, Han X, Pincus HA, Shad M, Salloum I, et al. Burden of general medical conditions among individuals with bipolar disorder. Bipolar disorders. 2004;6(5):368-73.

23. Lochhead RA, Parsey RV, Oquendo MA, Mann JJ. Regional brain gray matter volume differences in patients with bipolar disorder as assessed by optimized voxel-based morphometry. Biological psychiatry. 2004;55(12):1154-62.

24. Sassi RB, Brambilla P, Hatch JP, Nicoletti MA, Mallinger AG, Frank E, et al. Reduced left anterior cingulate volumes in untreated bipolar patients. Biological psychiatry. 2004;56(7):467-75.

25. Bora E, Fornito A, Yücel M, Pantelis C. Voxelwise meta-analysis of gray matter abnormalities in bipolar disorder. Biological psychiatry. 2010;67(11):1097-105.

26. Kozicky JM, McGirr A, Bond DJ, Gonzalez M, Silveira LE, Keramatian K, et al. Neuroprogression and episode recurrence in bipolar I disorder: A study of gray matter volume changes in first-episode mania and association with clinical outcome. Bipolar disorders. 2016;18(6):511-9.

27. Bouras C, Kövari E, Hof PR, Riederer BM, Giannakopoulos P. Anterior cingulate cortex pathology in schizophrenia and bipolar disorder. Acta neuropathologica. 2001;102(4):373-9.

28. Öngür D, Drevets WC, Price JL. Glial reduction in the subgenual prefrontal cortex in mood disorders. Proceedings of the National Academy of Sciences. 1998;95(22):13290-5.

29. Rajkowska G, Halaris A, Selemon LD. Reductions in neuronal and glial density characterize the dorsolateral prefrontal cortex in bipolar disorder. Biological psychiatry. 2001;49(9):741-52.

30. Selemon L, Rajkowska G. Cellular pathology in the dorsolateral prefrontal cortex distinguishes schizophrenia from bipolar disorder. Current molecular medicine. 2003;3(5):427-36.

31. Abé C, Ekman C-J, Sellgren C, Petrovic P, Ingvar M, Landén M. Cortical thickness, volume and surface area in patients with bipolar disorder types I and II. Journal of psychiatry & neuroscience: JPN. 2016;41(4):240.

32. López-Larson MP, DelBello MP, Zimmerman ME, Schwiers ML, Strakowski SM. Regional prefrontal gray and white matter abnormalities in bipolar disorder. Biological psychiatry. 2002;52(2):93-100.

33. Altshuler LL, Bookheimer SY, Townsend J, Proenza MA, Eisenberger N, Sabb F, et al. Blunted activation in orbitofrontal cortex during mania: a functional magnetic resonance imaging study. Biological psychiatry. 2005;58(10):763-9.

34. Kronhaus DM, Lawrence NS, Williams AM, Frangou S, Brammer MJ, Williams SC, et al. Stroop performance in bipolar disorder: further evidence for abnormalities in the ventral prefrontal cortex. Bipolar disorders. 2006;8(1):28-39.

35. Fahim C, Stip E, Mancini-Marïe A, Potvin S, Malaspina D. Orbitofrontal dysfunction in a monozygotic twin discordant for postpartum affective psychosis: a functional magnetic resonance imaging study. Bipolar disorders. 2007;9(5):541-5.

36. Hibar D, Westlye LT, van Erp TG, Rasmussen J, Leonardo CD, Faskowitz J, et al. Subcortical volumetric abnormalities in bipolar disorder. Molecular psychiatry. 2016;21(12):1710-6.

37. Kempton MJ, Salvador Z, Munafò MR, Geddes JR, Simmons A, Frangou S, et al. Structural neuroimaging studies in major depressive disorder: meta-analysis and comparison with bipolar disorder. Archives of general psychiatry. 2011;68(7):675-90.

38. Strakowski SM, DelBello MP, Zimmerman ME, Getz GE, Mills NP, Ret J, et al. Ventricular and periventricular structural volumes in first-versus multiple-episode bipolar disorder. American Journal of Psychiatry. 2002;159(11):1841-7.

39. Wittenberg GM, Tsien JZ. An emerging molecular and cellular framework for memory processing by the hippocampus. Trends in neurosciences. 2002;25(10):501-5.

40. Otten M, Meeter M. Hippocampal structure and function in individuals with bipolar disorder: a systematic review. Journal of affective disorders. 2015;174:113-25.

41. Moorhead TWJ, McKirdy J, Sussmann JE, Hall J, Lawrie SM, Johnstone EC, et al. Progressive gray matter loss in patients with bipolar disorder. Biological psychiatry. 2007;62(8):894-900.

42. McGaugh JL, Cahill L, Roozendaal B. Involvement of the amygdala in memory storage: interaction with other brain systems. Proceedings of the National Academy of Sciences. 1996;93(24):13508-14.

43. Chen BK, Sassi R, Axelson D, Hatch JP, Sanches M, Nicoletti M, et al. Cross-sectional study of abnormal amygdala development in adolescents and young adults with bipolar disorder. Biological Psychiatry. 2004;56(6):399-405.

44. Mitchell AS, Sherman SM, Sommer MA, Mair RG, Vertes RP, Chudasama Y. Advances in understanding mechanisms of thalamic relays in cognition and behavior. Journal of Neuroscience. 2014;34(46):15340-6.

45. Ng WXD, Lau IY, Graham S, Sim K. Neurobiological evidence for thalamic, hippocampal and related glutamatergic abnormalities in bipolar disorder: a review and synthesis. Neuroscience & Biobehavioral Reviews. 2009;33(3):336-54.

46. Strakowski SM, DelBello MP, Sax KW, Zimmerman ME, Shear PK, Hawkins JM, et al. Brain magnetic resonance imaging of structural abnormalities in bipolar disorder. Archives of general psychiatry. 1999;56(3):254-60.

47. Aylward EH, Roberts-Twillie JV, Barta PE, Kumar AJ, Harris GJ, Geer M, et al. Basal ganglia volumes and white matter hyperintensities in patients with bipolar disorder. American Journal of Psychiatry. 1994;151(5):687-93.

48. Hwang J, Lyoo IK, Dager SR, Friedman SD, Oh JS, Lee JY, et al. Basal ganglia shape alterations in bipolar disorder. American Journal of Psychiatry. 2006;163(2):276-85.

49. Gazzaniga MS. Cerebral specialization and interhemispheric communication: Does the corpus callosum enable the human condition? Brain. 2000;123(7):1293-326.

50. Brambilla P, Nicoletti MA, Sassi RB, Mallinger AG, Frank E, Kupfer DJ, et al. Magnetic resonance imaging study of corpus callosum abnormalities in patients with bipolar disorder. Biological psychiatry. 2003;54(11):1294-7.

51. Hauser P, Dauphinais ID, Berrettini W, DeLisi LE, Gelernter J, Post RM. Corpus callosum dimensions measured by magnetic resonance imaging in bipolar affective disorder and schizophrenia. Biological Psychiatry. 1989;26(7):659-68.

52. Brambilla P, Nicoletti M, Sassi R, Mallinger A, Frank E, Keshavan M, et al. Corpus callosum signal intensity in patients with bipolar and unipolar disorder. Journal of Neurology, Neurosurgery & Psychiatry. 2004;75(2):221-5.

53. Arnone D, McIntosh A, Chandra P, Ebmeier K. Meta-analysis of magnetic resonance imaging studies of the corpus callosum in bipolar disorder. Acta Psychiatrica Scandinavica. 2008;118(5):357-62.

54. Tavano A, Borgatti R. Evidence for a link among cognition, language and emotion in cerebellar malformations. Cortex. 2010;46(7):907-18.

55. Mills NP, DelBello MP, Adler CM, Strakowski SM. MRI analysis of cerebellar vermal abnormalities in bipolar disorder. American Journal of Psychiatry. 2005;162(8):1530-3.

56. Baldaçara L, Nery-Fernandes F, Rocha M, Quarantini LdC, Rocha G, Guimarães J, et al. Is cerebellar volume related to bipolar disorder? Journal of affective disorders. 2011;135(1-3):305-9.

57. Maletic V, Raison C. Integrated neurobiology of bipolar disorder. Frontiers in psychiatry. 2014;5.

58. Strakowski SM, Adler CM, Almeida J, Altshuler LL, Blumberg HP, Chang KD, et al. The functional neuroanatomy of bipolar disorder: a consensus model. Bipolar disorders. 2012;14(4):313-25.

59. Breeze JL, Hesdorffer DC, Hong X, Frazier JA, Renshaw PF. Clinical significance of brain white matter hyperintensities in young adults with psychiatric illness. Harvard review of psychiatry. 2003;11(5):269-83.

60. Moore PB, Shepherd DJ, Eccleston D, Macmillan IC, Goswami U, McALLISTER VL, et al. Cerebral white matter lesions in bipolar affective disorder: relationship to outcome. The British Journal of Psychiatry. 2001;178(2):172-6.

61. Lyoo IK, Kim MJ, Stoll AL, Demopulos CM, Parow AM, Dager SR, et al. Frontal lobe gray matter density decreases in bipolar I disorder. Biological psychiatry. 2004;55(6):648-51.

62. Jose M, Greenwald BS, Alexopoulos GS, Kiosses DN, Ashtari M, Heo M, et al. Frontal signal hyperintensities in mania in old age. The American journal of geriatric psychiatry. 2006;14(7):598-604.

63. Bellani M, Boschello F, Delvecchio G, Dusi N, Altamura CA, Ruggeri M, et al. DTI and myelin plasticity in bipolar disorder: integrating neuroimaging and neuropathological findings. Frontiers in psychiatry. 2016;7.

64. Walterfang M, Wood AG, Barton S, Velakoulis D, Chen J, Reutens DC, et al. Corpus callosum size and shape alterations in individuals with bipolar disorder and their first-degree relatives. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2009;33(6):1050-7.

65. Kempton MJ, Geddes JR, Ettinger U, Williams SC, Grasby PM. Meta-analysis, database, and meta-regression of 98 structural imaging studies in bipolar disorder. Archives of general psychiatry. 2008;65(9):1017-32.

66. Soares JC, Mann JJ. The anatomy of mood disorders—review of structural neuroimaging studies. Biological psychiatry. 1997;41(1):86-106.

67. Taylor WD, Payne ME, Krishnan KRR, Wagner HR, Provenzale JM, Steffens DC, et al. Evidence of white matter tract disruption in MRI hyperintensities. Biological psychiatry. 2001;50(3):179-83.

68. Heng S, Song AW, Sim K. White matter abnormalities in bipolar disorder: insights from diffusion tensor imaging studies. Journal of neural transmission. 2010;117(5):639-54.

69. Versace A, Almeida JR, Hassel S, Walsh ND, Novelli M, Klein CR, et al. Elevated left and reduced right orbitomedial prefrontal fractional anisotropy in adults with bipolar disorder revealed by tract-based spatial statistics. Archives of general psychiatry. 2008;65(9):1041-52.

70. Adler CM, Holland SK, Schmithorst V, Wilke M, Weiss KL, Pan H, et al. Abnormal frontal white matter tracts in bipolar disorder: a diffusion tensor imaging study. Bipolar disorders. 2004;6(3):197-203.

71. Haznedar MM, Roversi F, Pallanti S, Baldini-Rossi N, Schnur DB, LiCalzi EM, et al. Fronto-thalamo-striatal gray and white matter volumes and anisotropy of their connections in bipolar spectrum illnesses. Biological psychiatry. 2005;57(7):733-42.

72. Wang F, Jackowski M, Kalmar JH, Chepenik LG, Tie K, Qiu M, et al. Abnormal anterior cingulum integrity in bipolar disorder determined through diffusion tensor imaging. The British Journal of Psychiatry. 2008;193(2):126-9.

73. Bruno S, Cercignani M, Ron MA. White matter abnormalities in bipolar disorder: a voxel-based diffusion tensor imaging study. Bipolar disorders. 2008;10(4):460-8.

74. Martínez-Arán A, Vieta E, Reinares M, Colom F, Torrent C, Sánchez-Moreno J, et al. Cognitive function across manic or hypomanic, depressed, and euthymic states in bipolar disorder. American Journal of Psychiatry. 2004;161(2):262-70.

75. Martínez-Arán A, Vieta E, Colom F, Torrent C, Sánchez-Moreno J, Reinares M, et al. Cognitive impairment in euthymic bipolar patients: implications for clinical and functional outcome. Bipolar disorders. 2004;6(3):224-32.

76. Bora E, Yucel M, Pantelis C. Cognitive endophenotypes of bipolar disorder: a meta-analysis of neuropsychological deficits in euthymic patients and their first-degree relatives. Journal of affective disorders. 2009;113(1):1-20.

77. Robinson LJ, Thompson JM, Gallagher P, Goswami U, Young AH, Ferrier IN, et al. A meta-analysis of cognitive deficits in euthymic patients with bipolar disorder. Journal of affective disorders. 2006;93(1):105-15.

78. Burdick K, Goldberg J, Harrow M. Neurocognitive dysfunction and psychosocial outcome in patients with bipolar I disorder at 15-year follow-up. Acta Psychiatrica Scandinavica. 2010;122(6):499-506.

79. Robinson LJ, Nicol Ferrier I. Evolution of cognitive impairment in bipolar disorder: a systematic review of cross-sectional evidence. Bipolar disorders. 2006;8(2):103-16.

80. Simonsen C, Sundet K, Vaskinn A, Birkenaes AB, Engh JA, Færden A, et al. Neurocognitive dysfunction in bipolar and schizophrenia spectrum disorders depends on history of psychosis rather than diagnostic group. Schizophrenia Bulletin. 2009:sbp034.

81. Post RM, Leverich GS, Kupka RW, Keck Jr PE, McElroy SL, Altshuler LL, et al. Early-onset bipolar disorder and treatment delay are risk factors for poor outcome in adulthood. The Journal of clinical psychiatry. 2010;71(7):864-72.

82. Budde M, Schulze TG. Neurocognitive correlates of the course of bipolar disorder. Harvard review of psychiatry. 2014;22(6):342.

83. Samamé C, Martino DJ, Strejilevich SA. Longitudinal course of cognitive deficits in bipolar disorder: a meta-analytic study. Journal of affective disorders. 2014;164:130-8.

84. Depp CA, Mausbach BT, Harmell AL, Savla GN, Bowie CR, Harvey PD, et al. Meta-analysis of the association between cognitive abilities and everyday functioning in bipolar disorder. Bipolar disorders. 2012;14(3):217-26.

85. Mora E, Portella M, Forcada I, Vieta E, Mur M. Persistence of cognitive impairment and its negative impact on psychosocial functioning in lithium-treated, euthymic bipolar patients: a 6-year follow-up study. Psychological medicine. 2013;43(6):1187-96.

86. Rosa AR, Magalhaes P, Czepielewski L, Sulzbach MV, Goi PD, Vieta E, et al. Clinical staging in bipolar disorder: focus on cognition and functioning. J Clin Psychiatry. 2014;75(5):e450-6.

87. O'Shea R, Poz R, Michael A, Berrios G, Evans J, Rubinsztein J. Ecologically valid cognitive tests and everyday functioning in euthymic bipolar disorder patients. Journal of affective disorders. 2010;125(1):336-40.

88. Dickerson FB, Boronow JJ, Stallings CR, Origoni AE, Cole S, Yolken RH. Association between cognitive functioning and employment status of persons with bipolar disorder. Psychiatric Services. 2004;55(1):54-8.

89. Tohen M, Zarate Jr CA, Hennen J, Khalsa H-MK, Strakowski SM, Gebre-Medhin P, et al. The McLean-Harvard first-episode mania study: prediction of recovery and first recurrence. American Journal of Psychiatry. 2003;160(12):2099-107.

90. Keck Jr PE, McElroy SL, Strakowski SM, West SA, Sax KW, Hawkins JM, et al. 12-month outcome of patients with bipolar disorder following hospitalization for a manic or mixed episode. Focus. 2003;1(1):44-52.

91. Quarles RH, Macklin WB, Morell P. Myelin formation, structure and biochemistry. Basic neurochemistry: molecular, cellular and medical aspects. 2006;7:51-71.

92. Lazzarini R, Griffin J, Lassmann H, Nave K, Trapp B. Myelin Biology and Disorders: Elsevier Academic Press. 2004.

93. Benes FM, Davidson J, Bird ED. Quantitative cytoarchitectural studies of the cerebral cortex of schizophrenics. Archives of General Psychiatry. 1986;43(1):31-5.

94. Tkachev D, Mimmack ML, Ryan MM, Wayland M, Freeman T, Jones PB, et al. Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. The Lancet. 2003;362(9386):798-805.

95. Jessell TM. Neuronal specification in the spinal cord: inductive signals and transcriptional codes. Nature reviews Genetics. 2000;1(1):20.

96. Marquardt T, Pfaff SL. Cracking the transcriptional code for cell specification in the neural tube. Cell. 2001;106(6):651-4.

97. Fancy SP, Chan JR, Baranzini SE, Franklin RJ, Rowitch DH. Myelin regeneration: a recapitulation of development? Annual review of neuroscience. 2011;34:21-43.

98. Tripathi RB, Rivers LE, Young KM, Jamen F, Richardson WD. NG2 glia generate new oligodendrocytes but few astrocytes in a murine experimental autoimmune encephalomyelitis model of demyelinating disease. Journal of Neuroscience. 2010;30(48):16383-90.

99. Li H, He Y, Richardson WD, Casaccia P. Two-tier transcriptional control of oligodendrocyte differentiation. Current opinion in neurobiology. 2009;19(5):479-85.

100. Lu QR, Sun T, Zhu Z, Ma N, Garcia M, Stiles CD, et al. Common developmental requirement for Olig function indicates a motor neuron/oligodendrocyte connection. Cell. 2002;109(1):75-86.

101. Deoni SC, Dean DC, O'muircheartaigh J, Dirks H, Jerskey BA. Investigating white matter development in infancy and early childhood using myelin water faction and relaxation time mapping. Neuroimage. 2012;63(3):1038-53.

102. Yakovlev P, Lecours A. a. L., A.(1967) The myelinogenetic cycles of regional maturation of the brain. Retional Development of the Brain Early in Life Blackwell Scientific Publications.3-70.

103. Ge Y, Grossman RI, Babb JS, Rabin ML, Mannon LJ, Kolson DL. Age-related total gray matter and white matter changes in normal adult brain. Part I: volumetric MR imaging analysis. American journal of neuroradiology. 2002;23(8):1327-33.

104. Bartzokis G. Age-related myelin breakdown: a developmental model of cognitive decline and Alzheimer's disease. Neurobiology of aging. 2004;25(1):5-18.

105. Rowley CD, Sehmbi M, Bazin PL, Tardif CL, Minuzzi L, Frey BN, et al. Age-related mapping of intracortical myelin from late adolescence to middle adulthood using T1-weighted MRI. Human Brain Mapping. 2017.

106. Fuster JM. Frontal lobe and cognitive development. Journal of neurocytology. 2002;31(3-5):373-85.

107. Shen S, Sandoval J, Swiss VA, Li J, Dupree J, Franklin RJ, et al. Age-dependent epigenetic control of differentiation inhibitors is critical for remyelination efficiency. Nature neuroscience. 2008;11(9):1024-34.

108. Sim FJ, Zhao C, Penderis J, Franklin RJ. The age-related decrease in CNS remyelination efficiency is attributable to an impairment of both oligodendrocyte progenitor recruitment and differentiation. Journal of Neuroscience. 2002;22(7):2451-9.

109. Braitenberg V. A note on myeloarchitectonics. Journal of comparative Neurology. 1962;118(2):141-56.

110. Chen MS, Huber AB, van der Haar ME, Marcus F. Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. Nature. 2000;403(6768):434.

111. McKerracher Ld, David S, Jackson D, Kottis V, Dunn R, Braun P. Identification of myelin-associated glycoprotein as a major myelin-derived inhibitor of neurite growth. Neuron. 1994;13(4):805-11.

112. Glasser MF, Van Essen DC. Mapping human cortical areas in vivo based on myelin content as revealed by T1-and T2-weighted MRI. Journal of Neuroscience. 2011;31(32):11597-616.

113. Yeo BT, Krienen FM, Sepulcre J, Sabuncu MR, Lashkari D, Hollinshead M, et al. The organization of the human cerebral cortex estimated by intrinsic functional connectivity. Journal of neurophysiology. 2011;106(3):1125-65.

114. Power JD, Cohen AL, Nelson SM, Wig GS, Barnes KA, Church JA, et al. Functional network organization of the human brain. Neuron. 2011;72(4):665-78.

115. Glasser MF, Goyal MS, Preuss TM, Raichle ME, Van Essen DC. Trends and properties of human cerebral cortex: correlations with cortical myelin content. Neuroimage. 2014;93:165-75.

116. Bock NA, Hashim E, Janik R, Konyer NB, Weiss M, Stanisz GJ, et al. Optimizing T 1-weighted imaging of cortical myelin content at 3.0 T. Neuroimage. 2013;65:1-12.

117. Bock NA, Kocharyan A, Liu JV, Silva AC. Visualizing the entire cortical myelination pattern in marmosets with magnetic resonance imaging. Journal of neuroscience methods. 2009;185(1):15-22.

118. Rowley CD, Bazin P-L, Tardif CL, Sehmbi M, Hashim E, Zaharieva N, et al. Assessing intracortical myelin in the living human brain using myelinated cortical thickness. Frontiers in neuroscience. 2015;9.

Chapter 2: Deficits in intracortical myelination in young adults with Bipolar I Disorder

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2.1 Abstract

Objectives

Prior studies have implicated white matter-related changes in the pathophysiology of Bipolar Disorder (BD). However, most of what is known is derived from *in-vivo* subcortical white matter imaging, or post-mortem studies. Here, whole-brain intracortical myelin (ICM) content is investigated in individuals with BD-I and controls.

Methods

T_1-weighted images were collected on a 3T GE scanner in 45 subjects with BD-Type I (BD-I) and 60 controls, ages 17-45, using an optimized sequence previously shown to be sensitive to ICM content. Images were analyzed using a surface-based approach. General linear models with quadratic age terms were used to examine the signal trajectory of ICM across this age range.

Results

T_1-weighted signal in healthy controls followed an "inverted –U" pattern over age, whereas in BD-I subjects the association between ICM and age followed a flat line pattern ($p<0.05$, Bonferroni corrected). Exploratory analyses showed that ICM signal intensity was associated with duration of illness, age of onset, anticonvulsant and antipsychotic use in BD-I subjects ($p<0.05$, uncorrected).

Conclusion

This study is the first to show global deficits in ICM maturation throughout the cortex in BD. Considering the impact of myelination on the maintenance of neural synchrony and

integrity of neural connections, this work may have significant implications for understanding cognitive and behavioural deficits seen in BD.

Key words: Age; Bipolar disorder; Intracortical myelin; MRI; myelin maturation

2.2 Introduction

White matter (WM) abnormalities are amongst the most consistently replicated neurobiological findings in individuals with Bipolar Disorder (BD). Neuroimaging studies focusing on subcortical tracts have linked WM abnormalities with a greater number of hospitalizations (1), increased treatment resistance (2), suicide attempts (3), and a history of psychosis (4).

WM is primarily composed of myelinated axons, where a myelin sheath formed by oligodendrocytes acts as an electrical insulator, allowing for increased speed of axonal signal propagation via saltatory conduction and increased signal integrity. Myelin also provides trophic support essential for neuronal survival (5). Additionally, oligodendrocyte precursor cells can receive presynaptic input from neurons and respond to neurotransmitters, allowing them to regulate neural circuits (6, 7). Myelin is therefore essential for establishing and maintaining neuronal circuitry, and myelinated fibres are widely distributed throughout the brain, including within the cerebral cortex. In healthy humans, the development of intracortical myelin (ICM) follows an "inverted-U" trajectory, and occurs in a heterochronous pattern, with association areas (frontal, temporal and parietal lobes) commencing myelination after primary process areas (motor and sensory cortices) (8, 9). ICM comprises myelinated axons within the cortical gray matter (GM), with deeper layers of the cortex containing greater amounts of myelin (10,

11). Importantly, animal studies have begun to link ICM with changes in behaviour, and show that neuronal activity stimulates oligodendrocyte precursor cell proliferation in the cortex and promotes oligodendrogenesis. This thickening of the myelin sheath enhances motor learning in mice (12). Furthermore, mice subjected to social isolation early in life displayed long-standing thinning of the myelin sheath of cortical axons and worse cognitive performance in adulthood (13).

While extensive research has shown both GM and WM abnormalities in BD, most knowledge of WM anomalies stems from *subcortical* anatomical or diffusion tensor imaging (DTI) studies of WM tracts. *Post-mortem* human studies report fewer overall oligodendrocytes, a decreased ratio of oligodendrocytes per neuron, and lower expression of myelin-related genes in the prefrontal cortex of individuals with BD (14-18), all suggesting possible deficits in ICM in BD. Here, we studied ICM in young adults with BD using an optimized MRI technique, previously validated for ICM measurement in humans and non-human primates (19, 20). We hypothesized that individuals with BD would show deficits in ICM relative to matched controls. In exploratory analyses, we investigated the association between ICM and clinical variables such as age of onset, number of manic, depressive, mixed and hypomanic episodes, duration of illness, lifetime psychosis, and medication use.

2.3 Methods

2.3.1: Study Participants

This study was approved by the Hamilton Integrated Research Ethics Board and signed

informed consent was obtained from each participant. 49 individuals with a diagnosis of BD type-I (28 F, 21 M) and 67 matched healthy individuals (37 F, 30 M) were imaged. All were right-handed, aged-17-45, and all female subjects were premenopausal. All participants completed the Structured Clinical Interview for DSM-IV (SCID-I) for diagnostic confirmation. Controls did not meet criteria for any current/lifetime Axis I psychiatric conditions. Subjects with BD-I did not meet criteria for any *current* Axis I psychiatric comorbidities. Other exclusion criteria were unstable medical/inflammatory conditions, alcohol/substance abuse within the last year (excluding caffeine or nicotine), past or current history of neurological disorders (including head trauma and migraines), and any MRI contraindications.

2.3.2 Imaging Acquisition

Images were acquired on a 3T General Electric scanner (Software Version 22.0), using a 32-channel receive-only radiofrequency (RF) coil for the head (MR Instruments) and a transmit RF body coil (GE) to produce a T_1-weighted image with optimized intracortical contrast for ICM analysis (20, 21). All images were acquired with 1mm isotropic resolution and the total time for the protocol was ~35 min. We have recently reported age-related ICM mapping in healthy controls (9). The imaging protocol is summarized below, with further details and parameter specifications available in our previous work and within the Supplementary Methods section (9).

2.3.2.1 Anatomical Reference Image

First, a typical 3D T_1-weighted whole-head anatomical image was acquired using a 3D inversion-recovery gradient echo sequence (GE 3D BRAVO). That was used for image registration in processing.

2.3.2.2: High Intracortical Contrast T1-weighted Image

Then, another 3D T_1-weighted whole-head image with strong intracortical contrast was acquired with an inversion-recovery gradient echo sequence (GE 3D BRAVO) to form the basis of our ICM maps.

2.3.3 Ratio Image

A final 3D proton density-weighted whole-head image was collected to correct intensity inhomogeneity and remove T_2*-weighting in the high intracortical contrast T_1-weighted image. This image was made with a 3D gradient-echo sequence (GE 3D SPGR).

The proton-density weighted image was registered to the high intracortical contrast T_1-weighted image using a 6-parameter rigid transform (FSL) and filtered with a 3D median filter with a 5 x 5 x 5 mm kernel size. The T_1-weighted image was then divided by the filtered proton-density weighted image to create the ratio image, which is a strongly T_1-weighted image with B_1- and some B_1+ inhomogeneities removed.

2.3.4 ICM Processing

Image processing was performed to map ICM content in the cortex of each subject and to register the subjects' maps to a common space for group analysis. To map ICM in a subject, the pial surface and a surface at the GM/WM boundary of the cortex were

found. A new surface at the middle depth of the cortex was calculated and the signal intensity of the ratio image was mapped onto this surface to depict ICM. This process is further described in the Supplementary Methods section.

2.3.5 Statistical Analysis

2.3.5.1 Demographics

All statistical analysis was completed using R version 3.3.2 (https://www.r-project.org). Shapiro-Wilk test and Bartlett test were used to determine the normality and homogeneity of variances of continuous variables, respectively. Between group differences in age, sex, BMI, years of education, and smoking status were tested using two-tailed independent t tests, Mann-Whitney test, or chi-square tests, as appropriate (Table 1).

2.3.5.2 Age and ICM

The MarsAtlas (22) was used to parcellate the cortex into 82 regions-of-interest (ROIs) for analysis. ICM maps were generated using Surfstat (http://www.math.mcgill.ca/keith/surfstat/) in Matlab (v R2015a). Signal intensity in the ratio image as a function of age was investigated for the ROIs using a general linear model (GLM): $Age + Age^2 + BMI + DX + DX*Age^2$, where *DX* was the subject diagnosis. The final interaction term tested whether there were differential trajectories in T_1-weighted signal over age in the cortex in BD-I. By investigating the Age^2 interaction term, we could determine if a diagnosis of BD affected the "inverted-U" shape of the ICM trajectory seen in healthy subjects. BMI was included as a covariate as it has been

shown to influence WM integrity (23). We have recently shown that there were no sex differences in ICM trajectory over age in healthy individuals (9). Six ROIs per hemisphere (twelve total) could not be analyzed due to topological errors in identification of the pial or GM/WM boundary surfaces in the cortex. These corresponded to the isthmus of the cingulate, the insula, rostral medial visual cortex, medial/rostral inferior temporal cortex, and rostral superior temporal cortex. The remaining 70 regions were used to compare age trajectories in T_1-weighted signal between controls and BD-I subjects. Signal was averaged across each individual ROI and analyzed in R. *p*-values for the linear model and the interaction term were extracted from the GLMs. Partial η^2 was calculated to determine the effect size of the interaction term, and to visualize the amount of variance in the data that could be attributed to the interaction term. Coefficients for the Age^2 term were used to determine the shape of the ICM signal trajectory over age in each population.

2.3.5.3 History of Psychosis

To determine whether history of psychosis affects ICM, we ran GLMs in each ROI in the BD-I sample only: $Age + Age^2 + BMI + Psychosis + Psychosis*Age^2$, where *Psychosis* was a binary variable (1 if the BD-I individual had experienced lifetime delusions/hallucinations, 0 otherwise). We aimed to investigate the final interaction term in order to determine whether a history of psychosis had any effect on ICM over age, after controlling for BMI.

2.3.5.4 Clinical Correlates

As per Foland-Ross et al. (2011), partial correlation analysis was used to determine the potential effects of age of onset, duration of illness, and number of episodes individually (depressive, manic, hypomanic, mixed) and as a total sum, on ICM throughout the cortex in BD-I subjects (24). Age, sex, and BMI were included as covariates in all analyses, except in cases where the dependent variable was expected to be highly collinear with age, such as duration of illness. In the latter case, only BMI and sex were included as covariates.

2.3.5.5 Medication

Medications were categorized as lithium, antidepressants, antipsychotics, anticonvulsants, and anxiolytics for each participant, and the dose of each class was coded as 0=absent, 1=low, or 2=high(25). Anticonvulsants and antidepressants were converted to high/low dose groupings according to Sackeim (2001)(26). Levels 1 and 2 were grouped as low dose, and levels 3 and 4 were grouped as high dose. Antipsychotics were converted to chlorpromazine (CPZ) dose equivalents. Individuals taking a dose equivalent below the mean effective daily dose of CPZ were defined as low dose, and vice-versa for high dose (27). Anxiolytics were converted to Lorazepam dose equivalents, and were determined as low or high dose using the Physician's Desk Reference recommended daily dose median. All individual medication low/high dose classifications were summed to determine the total medication load. Partial correlation analysis was used to determine the effects of individual medication classes and the composite medication score on ICM in BD-I, using age, sex, and BMI as covariates.

2.4 Results

Five control and two BD-I subjects were not included in the analysis due to missing BMI values. Additionally, two of each control and BD-I were removed due to poor quality ICM maps. Thus, the final age trajectory analysis was conducted with 60 controls and 45 BD-I subjects.

2.4.1 Demographics

BD-I subjects and controls were well matched on age, sex, BMI, years of education, and smoking status (all $p>0.05$, Table 1).

2.4.2 Age Trajectories of ICM Signal in Bipolar Disorder and Controls

Consistent with previous literature (9), ICM signal in controls followed an "inverted-U" trajectory over age, whereas the ICM signal over age was significantly flattened in individuals with BD-I, which suggests deficits in myelin maturation in young adults with BD (Figure 1).

GLMs met the conditions required for analysis (linearity, normality, homoscedasticity, and independence) and were Bonferroni corrected, such that only models with a *p*-value <0.0007 ($\alpha = 0.05/ 70$ tests) were considered significant. The $DX*Age^2$ interaction showed that ICM signal trajectory over age was significantly different in BD subjects relative to controls in several regions of the frontal, parietal and temporal cortices, most predominantly in the left hemisphere (Figure 2). This suggests that ICM deficits in young adults with BD are widespread and not localized. Partial η^2 was calculated for the $DX*Age^2$ term and mapped onto the cortex (**Figure 3)**. In most ROIs, we observed an effect between 0.01 - 0.1, with the largest effects seen in the

motor and premotor regions, mirroring regions of greatest difference between BD and controls from **Figure 2**. To analyze the trajectories, each diagnostic group was fitted with predictors: $BMI + Age + Age^2$, and coefficients for the Age^2 term were mapped on the cortex (Figure 4). Consistent with the $DX*Age^2$ analyses, the coefficient is largely negative in controls, corresponding to an "inverted-U" trajectory of ICM; the coefficient values in BD subjects were much closer to zero.

2.4.3 Intracortical Myelin and Clinical Correlates

Age of onset of BD was positively correlated with ICM signal in regions of the bilateral parietal, premotor, and prefrontal cortices, and left somatosensory cortex (R= 0.24-0.36, p= 0.002-0.046, supplementary Table S1), suggesting that earlier age of onset of BD was associated with less ICM.

Duration of illness was negatively correlated with ICM signal in the regions of the bilateral visual, temporal, parietal, cingulate, premotor cortices, bilateral cuneus, the left somatosensory and motor cortices, and right prefrontal cortex (R=-0.24—0.37, p=0.005-0.049, supplementary Table S1), suggesting that a longer duration of illness was associated with less ICM.

The number of depressive episodes was not significantly associated with ICM signal in any cortical region. The number of manic episodes was negatively correlated with ICM signal in the rostral middle temporal cortex (R=-0.24, p=0.047). The number of hypomanic episodes was negatively correlated with ICM signal in the bilateral posterior cingulate cortex (left R=-0.27, p=0.03, right R=-0.31, p=0.009, supplementary Table S2), and the right anterior cingulate cortex (R=-0.27, p=0.02, supplementary Table S2). The

number of mixed episodes was positively correlated with ICM signal in regions of the bilateral orbitofrontal, temporal, and prefrontal cortices, and right visual, parietal, and somatosensory cortices (R=0.24-0.42, p=0.0003-0.04, supplementary Table S2). In these exploratory analyses, only the correlation between number of mixed episodes and ICM in the left ventromedial prefrontal cortex survived Bonferroni correction (R=0.42, $p_{corrected}$=0.04, supplementary Table S2).

The total number of episodes was not significantly associated with ICM signal in any cortical region (p>0.05, supplementary Table S2). Similarly, the *Psychosis*Age*^{2} interaction was not significant in any model throughout the cortex (p>0.05), suggesting no differences in ICM between BD subjects with versus without a history of psychosis.

2.4.4 Medication

Lithium, antidepressant, and anxiolytic use were not significantly associated with ICM in any cortical region. Anticonvulsant use was negatively correlated with ICM signal in regions of the bilateral prefrontal and right orbitofrontal cortices (R=-0.23- -0.25, p=0.03-0.04, supplementary Table S3). Antipsychotic use was negatively correlated with ICM signal in regions of the bilateral parietal, cingulate, and motor cortices, right cuneus, and left visual cortex (R= -0.25- -0.34, p=0.003-0.04, supplementary Table S3). However, none of these medication analyses survived Bonferroni correction.

Total medication load was negatively associated with ICM signal in regions of the bilateral visual and motor cortices, the right cuneus, parietal and somatosensory cortices, and left posterior cingulate cortex (R=-0.25 - -0.38, p= 0.001- 0.04, supplementary Table S3). Total medication count was negatively associated with ICM signal in regions of the

bilateral motor, premotor, and prefrontal cortices, left visual and orbitofrontal cortices, and right temporal, parietal, and somatosensory cortices (R=-0.24- -0.33, p=0.005-0.049, supplementary Table S3). Similarly, none of these analyses survived Bonferroni correction.

2.5 Discussion

The main finding of our study is that the age-related trajectory of ICM signal in BD does not follow the same "inverted-U" pattern seen in controls over young adulthood, which suggests that BD is associated with deficits in ICM development and/or maturation. Deficits in ICM are widespread and primarily affect the frontal, parietal, and temporal cortices, with the strongest effects observed in motor and premotor regions, followed by prefrontal and parietal regions.

Using a 1.5 Tesla scanner, Jørgensen et al. (2016) recently reported increased T_1-weighted GM/WM contrast in BD in the motor cortex only (28). The motor cortex is one of the most heavily myelinated cortical regions, and therefore differences in this area are more readily identified using a variety of techniques, including increased T_1-weighted GM/WM contrast (29). Using a novel technique developed and optimized for enhanced sensitivity to detect ICM signal (9, 20, 21), we found that age-related deficits in ICM occur in several areas of the cerebral cortex in BD. Previously employed techniques likely did not have enough sensitivity to detect differences in ICM in more lightly myelinated cortical regions (30). Our imaging methodology seemingly endorses the increased sensitivity required for global analysis of ICM, considering the high variability in ICM content throughout the cortex. Our results are consistent with a large study (N=6503)

showing widespread patterns of reduced cortical thickness in frontal, temporal and parietal regions in individuals with BD (31), as well as a recent systematic review showing decreased cortical thickness in the left anterior cingulate/paracingulate and the left superior temporal gyrus, as well as several prefrontal regions in BD (32). In the ENIGMA study, the strongest effects were observed in the left cortex, specifically in the left pars opercularis, left fusiform gyrus, and left rostral middle frontal cortex. Our findings are consistent, with a more prominent left lateralization of ICM deficits (31, 32). It is conceivable that abnormal cortical thickness in BD may be, in part, due to changes in ICM since ICM content can affect overall thickness measures (20, 33). Our results also support many *post-mortem* studies showing deficits in cortical oligodendrocyte count and density in BD (14-17).

Recent animal studies have demonstrated the dynamic plasticity of ICM and its relation with behaviour. For instance, an optogenetic study in mice showed that neuronal activity stimulates oligodendrocyte precursor cell proliferation in the cortex, thereby promoting oligodendrogenesis, and leading to an increased thickness of the myelin sheath. Ultimately, these microstructural changes were associated with behavioral modulation in these mice (12). A reduction of myelin sheath thickness in the medial prefrontal cortex caused by early-life social isolation was linked with long-standing deficits in cognitive performance in adult mice (13). Therefore, it is conceivable that age-related ICM deficits in BD seen in the current study may be associated with emotional dysregulation and cognitive dysfunction, commonly observed in the course of BD. Future

studies should investigate the relation between ICM and cognitive/emotional processing in BD.

Interestingly, the strongest effect sizes of ICM deficits in our study were in the premotor and motor areas. Motor cortex is one of the most heavily myelinated regions, among other myelin-rich areas such as primary visual, auditory and somatosensory cortices. It is unique in comparison, however, as motor regions continue to myelinate for up to four years past other regions, peaking around 38 years of age (9). Notably, motor speed dysfunction has been shown in BD subjects and their first-degree relatives, and has recently been identified as an endophenotype of BD (34). Motor speed deficits are also seen in children of women with BD as young as one year of age (35). Deficits in ICM in these brain regions may, at least in part, account for these behavioural observations.

Our exploratory analyses suggest that illness characteristics such as earlier age of onset, longer duration of illness, and higher number of manic/hypomanic/mixed episodes may be associated with decreased ICM. However, these results should be considered preliminary and must be confirmed in future studies, ideally with a longitudinal design. The adolescent age of illness onset in our BD sample is commonly reported in the literature, and several lines of evidence have implicated abnormal myelination in the onset of psychiatric disorders, as the peak of onset of many psychiatric disorders coincides with a period of intense myelin development (36). Additionally, abnormalities in WM have routinely been identified during the first-episode of BD and in offspring and first-degree relatives of individuals with BD (37, 38). Our results are consistent with studies showing that longer duration of illness and number of mood episodes were

associated with declining GM and WM integrity (31, 39). Another exploratory/preliminary finding was that total medication load and, specifically, use of antipsychotics and anticonvulsants, may affect ICM. Unfortunately, we were unable to disentangle whether this was a direct effect of medication use, or an effect of illness severity, because individuals who are more ill tend to take more medications. Longitudinal studies are essential to examine potential effects of psychotropics on myelination *in vivo*.

Limited by the cross-sectional design, we were also precluded from examining the *progressive* impact of illness burden on ICM. Longitudinal studies are essential for the confirmation of our results, and will help further determine whether the effects seen here are due to dysmyelination or demyelination in BD, as well as understand the relationship between ICM and risk of mood relapse, and medication use. Other clinical factors that may affect ICM were not assessed in our study such as childhood trauma, medical and psychiatric comorbidities, and lifetime alcohol and substance use. Further, our study sample comprised young adults between ages 17-45. Pediatric and older adult samples may display distinct patterns of ICM trajectory over age. For instance, in a recent study investigating cortical thickness between childhood and the mid-30s in Autism Spectrum Disorder, there is an initial period of delayed cortical thinning, followed by a period of rapid cortical thinning that seemingly overcompensates for the initial delay (40). As previously mentioned, cortical thickness and ICM are likely intertwined, and therefore the trajectory of ICM over age may vary depending on the age range in question. It also remains to be determined whether ICM changes are accompanied by changes in axonal

signal propagation integrity and strength. The investigation of ICM in relation to cognitive performance will be important in determining whether there are functional consequences of aberrant ICM maturation over age in BD.

In conclusion, our study is the first evidence of widespread deficits in ICM maturation throughout the cerebral cortex in young adults with BD. Considering the potential role of ICM in establishing and maintaining neuronal circuitry and its ability to impact behavioural performance, future work should investigate the role of ICM in behavioural, cognitive, and emotional processing in BD.

2.6 References

1. Dupont RM, Jernigan TL, Butters N, Delis D, Hesselink JR, Heindel W, et al. Subcortical abnormalities detected in bipolar affective disorder using magnetic resonance imaging: clinical and neuropsychological significance. Archives of general psychiatry. 1990;47(1):55-9.

2. Regenold WT, Hisley KC, Phatak P, Marano CM, Obuchowski A, Lefkowitz DM, et al. Relationship of cerebrospinal fluid glucose metabolites to MRI deep white matter hyperintensities and treatment resistance in bipolar disorder patients. Bipolar disorders. 2008;10(7):753-64.

3. Grangeon MC, Seixas C, Quarantini LC, Miranda-Scippa A, Pompili M, Steffens DC, et al. White Matter Hyperintensities and Their Association With Suicidally in Major Affective Disorders: A Meta-Analysis of Magnetic Resonance Imaging Studies. CNS spectrums. 2010;15(06):375-81.

4. Sarrazin S, Poupon C, Linke J, Wessa M, Phillips M, Delavest M, et al. A multicenter tractography study of deep white matter tracts in bipolar I disorder: psychotic features and interhemispheric disconnectivity. JAMA psychiatry. 2014;71(4):388-96.

5. Nave K-A. Myelination and the trophic support of long axons. Nature reviews Neuroscience. 2010;11(4):275-83.

6. Bergles DE, Roberts JDB, Somogyi P, Jahr CE. Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. Nature. 2000;405(6783):187-91.

7. Wigley R, Hamilton N, Nishiyama A, Kirchhoff F, Butt AM. Morphological and physiological interactions of NG2-glia with astrocytes and neurons. Journal of Anatomy. 2007;210(6):661-70.

8. Huttenlocher PR, Dabholkar AS. Regional differences in synaptogenesis in human cerebral cortex. Journal of comparative Neurology. 1997;387(2):167-78.

9. Rowley CD, Sehmbi M, Bazin PL, Tardif CL, Minuzzi L, Frey BN, et al. Age-related mapping of intracortical myelin from late adolescence to middle adulthood using T1-weighted MRI. Human Brain Mapping. 2017.

10. Lake EM, Steffler EA, Rowley CD, Sehmbi M, Minuzzi L, Frey BN, et al. Altered intracortical myelin staining in the dorsolateral prefrontal cortex in severe mental illness. European Archives of Psychiatry and Clinical Neuroscience. 2016:1-8.

11. Geyer S, Weiss M, Reimann K, Lohmann G, Turner R. Microstructural parcellation of the human cerebral cortex–from Brodmann's post-mortem map to in vivo

mapping with high-field magnetic resonance imaging. Frontiers in human neuroscience. 2011;5:19.

12. Gibson EM, Purger D, Mount CW, Goldstein AK, Lin GL, Wood LS, et al. Neuronal activity promotes oligodendrogenesis and adaptive myelination in the mammalian brain. Science. 2014;344(6183):1252304.

13. Makinodan M, Rosen KM, Ito S, Corfas G. A critical period for social experience–dependent oligodendrocyte maturation and myelination. science. 2012;337(6100):1357-60.

14. Lewandowski KE, Ongür D, Sperry SH, Cohen BM, Sehovic S, Goldbach JR, et al. Myelin vs axon abnormalities in white matter in bipolar disorder. Neuropsychopharmacology. 2015;40(5):1243-9.

15. Uranova N, Orlovskaya D, Vikhreva O, Zimina I, Kolomeets N, Vostrikov V, et al. Electron microscopy of oligodendroglia in severe mental illness. Brain research bulletin. 2001;55(5):597-610.

16. Uranova NA, Vostrikov VM, Orlovskaya DD, Rachmanova VI. Oligodendroglial density in the prefrontal cortex in schizophrenia and mood disorders: a study from the Stanley Neuropathology Consortium. Schizophrenia research. 2004;67(2):269-75.

17. Öngür D, Drevets WC, Price JL. Glial reduction in the subgenual prefrontal cortex in mood disorders. Proceedings of the National Academy of Sciences. 1998;95(22):13290-5.

18. Tkachev D, Mimmack ML, Ryan MM, Wayland M, Freeman T, Jones PB, et al. Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. The Lancet. 2003;362(9386):798-805.

19. Bock NA, Kocharyan A, Liu JV, Silva AC. Visualizing the entire cortical myelination pattern in marmosets with magnetic resonance imaging. Journal of neuroscience methods. 2009;185(1):15-22.

20. Rowley CD, Bazin P-L, Tardif CL, Sehmbi M, Hashim E, Zaharieva N, et al. Assessing intracortical myelin in the living human brain using myelinated cortical thickness. Frontiers in neuroscience. 2015;9.

21. Bock NA, Hashim E, Janik R, Konyer NB, Weiss M, Stanisz GJ, et al. Optimizing T 1-weighted imaging of cortical myelin content at 3.0 T. Neuroimage. 2013;65:1-12.

22. Auzias G, Coulon O, Brovelli A. MarsAtlas: A cortical parcellation atlas for functional mapping. Human brain mapping. 2016.

23. Kullmann S, Schweizer F, Veit R, Fritsche A, Preissl H. Compromised white matter integrity in obesity. Obesity reviews. 2015;16(4):273-81.

24. Foland-Ross LC, Thompson PM, Sugar CA, Madsen SK, Shen JK, Penfold C, et al. Investigation of cortical thickness abnormalities in lithium-free adults with bipolar I disorder using cortical pattern matching. American Journal of Psychiatry. 2011;168(5):530-9.

25. Hassel S, Almeida JR, Kerr N, Nau S, Ladouceur CD, Fissell K, et al. Elevated striatal and decreased dorsolateral prefrontal cortical activity in response to emotional stimuli in euthymic bipolar disorder: no associations with psychotropic medication load. Bipolar disorders. 2008;10(8):916-27.

26. Sackeim HA. The definition and meaning of treatment-resistant depression. Journal of Clinical Psychiatry. 2001.

27. Davis JM, Chen N. Dose response and dose equivalence of antipsychotics. Journal of clinical psychopharmacology. 2004;24(2):192-208.

28. Jørgensen K, Nerland S, Norbom L, Doan N, Nesvåg R, Mørch-Johnsen L, et al. Increased MRI-based cortical grey/white-matter contrast in sensory and motor regions in schizophrenia and bipolar disorder. Psychological medicine. 2016;46(09):1971-85.

29. Miller DJ, Duka T, Stimpson CD, Schapiro SJ, Baze WB, McArthur MJ, et al. Prolonged myelination in human neocortical evolution. Proceedings of the National Academy of Sciences. 2012;109(41):16480-5.

30. Jefferson AL, Gifford KA, Damon S, Chapman GW, Liu D, Sparling J, et al. Gray & white matter tissue contrast differentiates Mild Cognitive Impairment converters from non-converters. Brain imaging and behavior. 2015;9(2):141-8.

31. Hibar D, Westlye L, Doan N, Jahanshad N, Cheung J, Ching C, et al. Cortical abnormalities in bipolar disorder: an MRI analysis of 6503 individuals from the ENIGMA Bipolar Disorder Working Group. Molecular Psychiatry. 2017.

32. Hanford LC, Nazarov A, Hall GB, Sassi RB. Cortical thickness in bipolar disorder: a systematic review. Bipolar disorders. 2016;18(1):4-18.

33. Nieuwenhuys R. The myeloarchitectonic studies on the human cerebral cortex of the Vogt–Vogt school, and their significance for the interpretation of functional neuroimaging data. Brain Structure and Function. 2013;218(2):303-52.

34. Correa-Ghisays P, Balanzá-Martínez V, Selva-Vera G, Vila-Francés J, Soria-Olivas E, Vivas-Lalinde J, et al. Manual motor speed dysfunction as a neurocognitive

endophenotype in euthymic bipolar disorder patients and their healthy relatives. Evidence from a 5-year follow-up study. Journal of Affective Disorders. 2017;215:156-62.

35. Santucci A, Singer L, Wisniewski S, Luther J, Eng H, Sit D, et al. One-Year Developmental Outcomes for Infants of Mothers With Bipolar Disorder. The Journal of clinical psychiatry. 2017.

36. Paus T, Keshavan M, Giedd JN. Why do many psychiatric disorders emerge during adolescence? Nature Reviews Neuroscience. 2008;9(12):947-57.

37. Vita A, De Peri L, Sacchetti E. Gray matter, white matter, brain, and intracranial volumes in first-episode bipolar disorder: a meta-analysis of magnetic resonance imaging studies. Bipolar disorders. 2009;11(8):807-14.

38. Versace A, Ladouceur CD, Romero S, Birmaher B, Axelson DA, Kupfer DJ, et al. Altered development of white matter in youth at high familial risk for bipolar disorder: a diffusion tensor imaging study. Journal of the American Academy of Child & Adolescent Psychiatry. 2010;49(12):1249-59. e1.

39. Moorhead TWJ, McKirdy J, Sussmann JE, Hall J, Lawrie SM, Johnstone EC, et al. Progressive gray matter loss in patients with bipolar disorder. Biological psychiatry. 2007;62(8):894-900.

40. Khundrakpam BS, Lewis JD, Kostopoulos P, Carbonell F, Evans AC. Cortical Thickness Abnormalities in Autism Spectrum Disorders Through Late Childhood, Adolescence, and Adulthood: A Large-Scale MRI Study. Cerebral Cortex. 2017;27(3):1721-31.

Table 1: Sociodemographic and clinical characteristics of the study population (n=105), listed as mean (SD), unless otherwise specified.

	Controls (n=60)	BD-I (n=45)	Test statistic	P value
Age	30.7 (8.3)	31.9 (8.1)	t=0.73	0.47
Female (%)	32.0 (53.3)	25.0 (55.6)	χ= 0.82	0.84
BMI	25.6 (5.2)	26.6 (4.4)	t=1.00	0.32
Education (years)	15.9 (2.8)	15.9 (3.3)*	t= 0.09	0.92
Smoking N (%) Yes No Past	 16 (26.7) 42 (70.0) 2 (3.3)	 13 (28.9) 25 (55.6) 7 (15.6)	χ= 5.37	0.07
Age of Onset	NA	16.7 (6.2)	NA	NA
Duration of Illness	NA	14.9 (8.0)	NA	NA
Current state Euthymic Depressed Manic Hypomanic Mixed	NA	 37 4 0 3 1	NA	NA
MADRS Euthymic Depressed Manic Hypomanic Mixed	1.7 (2.8)	 4.9 (5.6) 25.3 (7.9) NA 9.3 (2.1) 26	NA	NA
YMRS Euthymic Depressed Manic Hypomanic Mixed	0.4 (1.0)	 2.3 (3.9) 2.3 (2.6) NA 16.0 (2.6) 17	NA	NA
Lifetime Psychosis, N (%)	NA	27 (60.0)	NA	NA
Medication use (N) [µ] Unmedicated Lithium Anticonvulsants Antidepressants Antipsychotics Anxiolytics	NA	 5 14 20 16 27 12	NA	NA

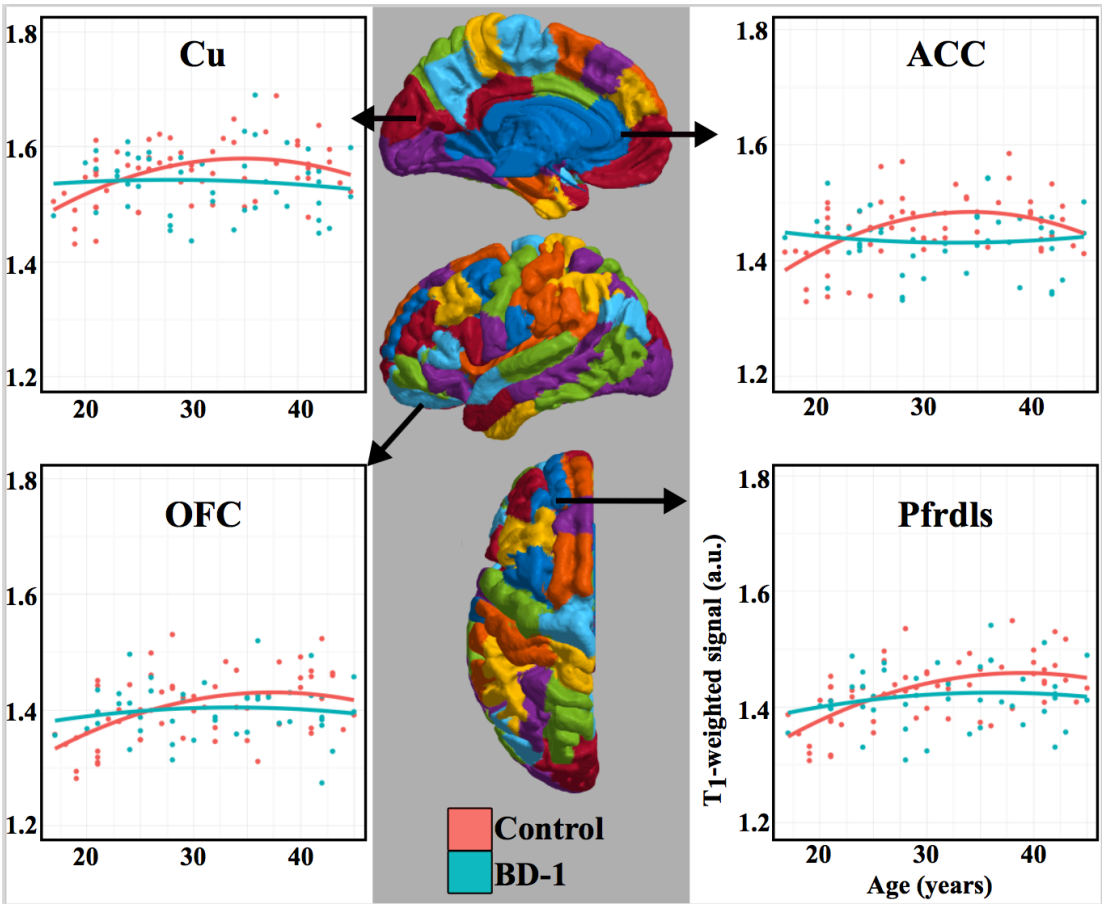
Number of previous mood episodes	NA		NA	NA
Depressed		16.4 (21.8)		
Manic		7.5 (15.9)		
Hypomanic		13.2 (20.1)		
Mixed		3.4 (8.7)		
Total		40.0 (46.9)		
Lifetime psychiatric diagnoses (N)	NA		NA	NA
Panic disorder		14		
Agoraphobia		10		
Social phobia		14		
OCD		1		
PTSD		8		
GAD		3		
Anorexia nervosa		2		
Binge eating disorder		2		
Alcohol dependence		5		
Alcohol abuse		14		
Substance dependence		7		
Substance abuse		13		

*Missing data (n=1)

μ Most participants were on polypharmacy, therefore totals of medication counts are greater than sample size (n=45)

MADRS- Montgomery Asberg Depression Rating Scale, YMRS- Young Mania Rating Scale, OCD- Obsessive Compulsive Disorder, PTSD- Post Traumatic Stress Disorder, GAD- Generalized Anxiety Disorder.

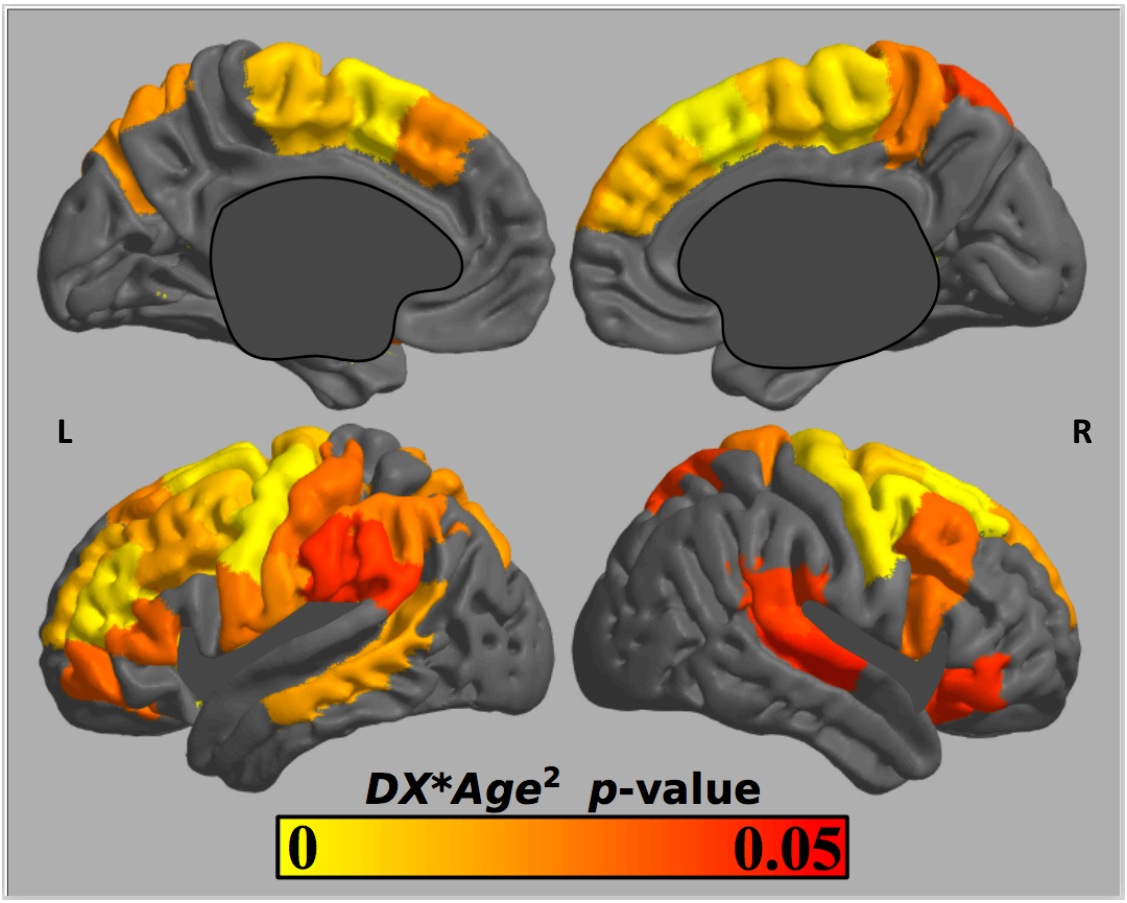
Figure 1. Intracortical myelin signal over age in bipolar disorder subjects (N=45) and healthy controls (N=60).



Half depth T_1- weighted ICM signal trajectory with age plotted in healthy controls (red) and BD-I subjects (blue). The four chosen ROI plots are areas typically associated with BD. In all regions, healthy controls show an "inverted-U" quadratic trajectory of ICM signal over age. This association is lost in BD subjects, where the association of ICM signal over age is severely blunted.

ROI: region of interest, Cu: Cuneus, ACC: Anterior Cingulate Cortex, OFC: Orbital Frontal Cortex, Pfrdls: Rostral Dorsolateral Superior Prefrontal Cortex.

Figure 2. *p*-values for the interaction term from the linear model of bipolar diagnosis against age^2.

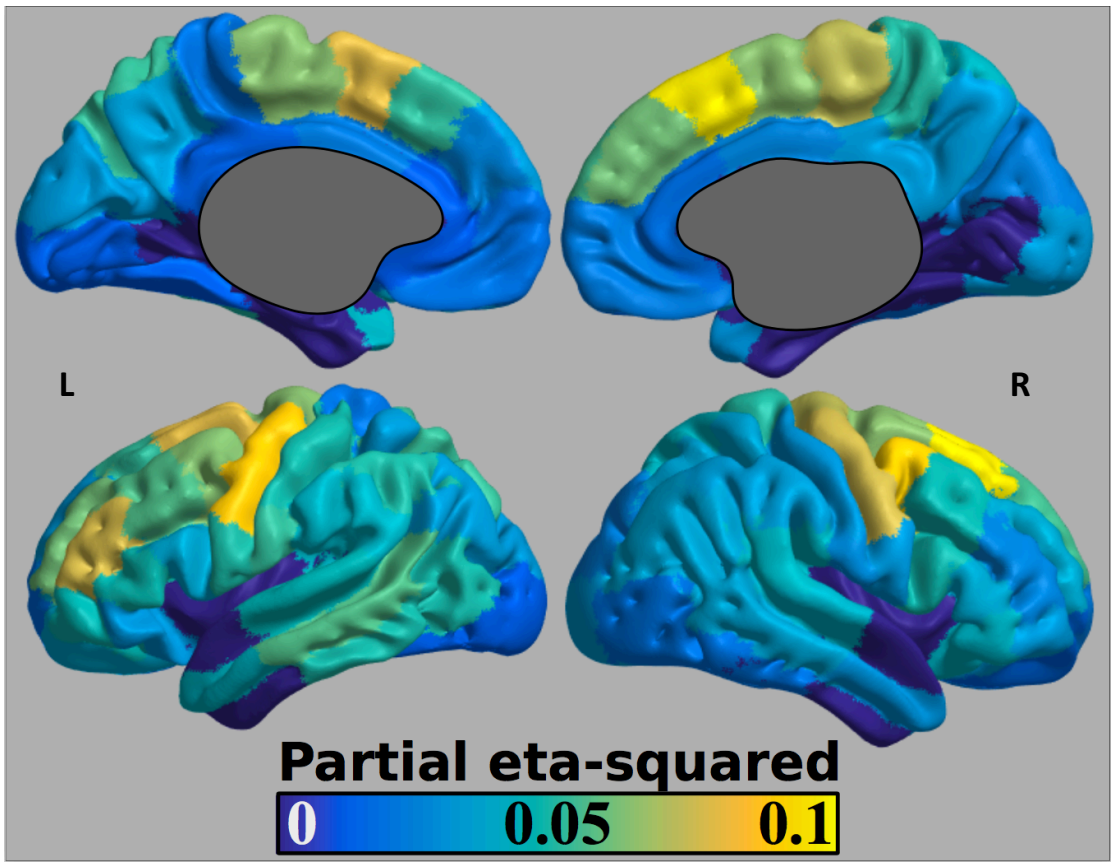


Yellow, orange, and red shaded regions show significantly different trajectories of ICM signal over age between BD (N=45) vs. control subjects (N=60) ($p_{Age2 \times Diagnosis} < 0.05$, where $p_{model} < 0.0007$ following Bonferroni correction for 70 regions).

Significant interactions between Age^2 and Diagnosis (BD vs control) are seen throughout the medial and lateral cortices, with a potential left lateralization in the posterior lateral cortex. The effect is largely bilateral in frontal cortices.

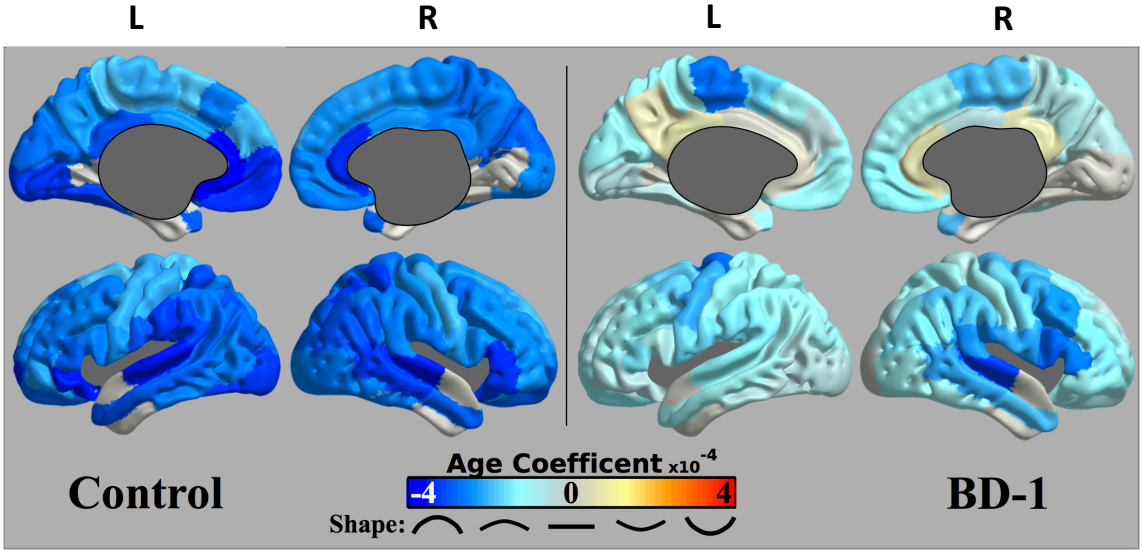
L: left hemisphere, R: right hemisphere, BD: Bipolar Disorder

Figure 3. Partial η^2 as an estimate of effect size for the Age^2 x Diagnosis interaction term, in the prediction of ICM signal.



Larger partial η^2 values suggest that the Age^2 x Diagnosis interaction term explains a greater degree of variance in ICM-related T_1-weighted signal. Thus, increased partial η^2 could be highlighting areas of strongest differences between control (N=60) and BD (N=45) subjects in this age range. The largest effect sizes are observed in motor and premotor regions. Left prefrontal regions also show notable effect sizes, although small to medium effect sizes are observed throughout the medial and lateral cortices. Regions with greatest effect size correspond with regions of greatest difference between BD and controls, seen in **Figure 2**.
L: left hemisphere, R: right hemisphere, BD; Bipolar Disorder.

Figure 4. The shape of the quadratic trajectory in controls (N=60) and BD-I (N-45).



Each population was fitted individually to find the coefficient for the Age^2 term. On the left of the figure, the Control population shows a global negative shape across cortex, representing an "inverted-U" trajectory. On the right, the BD population displays values closer to zero in widespread regions across the cortex, suggesting a flat-lined and severely blunted pattern in T_1-weighted signal over young adulthood in BD, (ages17-45).

L: left hemisphere, R: right hemisphere, BD: Bipolar Disorder.

SUPPLEMENTARY MATERIAL

Supplementary Methods

Image processing was performed predominantly in MIPAV v7.0.1 software (mipav.cit.nih.gov) using the JIST v3.0 (www.nitrc.org/projects/jist, TOADS-CRUISE vR3c (www.nitrc.org/projects/toads-cruise), and CBS High-Res Brain Processing Tools Version v3.0 (www.nitrc.org/projects/cbs-tools) plug-ins, and Amira v5.2 software (Visage Imaging). All processing was performed on the ratio image.

First, a mask of the cerebrum created from the SPECTRE 2010 algorithm (1) in MIPAV, was used to skull strip the ratio image. The skull-stripped ratio image was used as input to the Multiple Object Geometric Deformable Model (MGDM) Multi-contrast Brain Segmentation algorithm (2, 3) in MIPAV to generate initial probabilistic labels for tissue classes for each hemisphere of the brain. The probability labels generated for cerebral gray matter (GM) and white matter (WM) were used as the input to the CRUISE algorithm (4) to generate smoothed, topologically correct labels for the cerebrum. The CRUISE algorithm was performed in each hemisphere separately.

Subcortical structures and ventricles (as identified by the MGDM algorithm) were then removed from the labels for the left and right cerebrums and the labels remaining were combined. At this point, all GM segmentations were inspected and manual edits were made to replace missing cortex and to remove remaining dura mater arising from potential errors in the MGDM and CRUISE algorithms to ensure an accurate pial surface. This label for the entire cerebrum without the subcortical structures was then used to

mask the Ratio image such that it only contained the cerebral cortex and underlying major white matter tracts.

The manually corrected, cerebrum-masked Ratio image was next segmented into two main tissue classes: WM and GM, taking into account lightly and heavily myelinated components of GM (5) using the FANTASM algorithm (6) in MIPAV, as we found that this yields a more accurate WM label for our ICM analysis than the output from CRUISE. The hemispheres were segmented together to avoid potential hemispheric bias, such that the algorithm used the entire cerebrum for its classification. Following segmentations, labels for each tissue class were split back into left and right hemispheres for subsequent processing. The labels near the GM/WM-boundary were morphologically processed to remove all WM tissue not connected to the largest WM mass.

The corrected GM labels from CRUISE representing the pial surface, and WM labels from FANTASM representing the GM/WM boundary surface were used as inputs to a volume-preserving cortical depth model to generate an intracortical surface at the ½ cortical depth to sample the T_1-weighted image signal (7, 8) (**Supplementary Figure S1)**. Each subject's ½ depth surface was registered to the ½ depth surface generated from the MNI-152 atlas using a multi-contrast multi-scale surface registration approach (9). In the analysis, the signal was first sampled onto the individual subject's surface, which was then deformed to be in register with the MNI-152 equivalent surface.

References

1. Carass A, Cuzzocreo J, Wheeler MB, Bazin P-L, Resnick SM, Prince JL. Simple paradigm for extra-cerebral tissue removal: algorithm and analysis. NeuroImage. 2011;56(4):1982-92.

2. Bazin P-L, Weiss M, Dinse J, Schäfer A, Trampel R, Turner R. A computational framework for ultra-high resolution cortical segmentation at 7Tesla. Neuroimage. 2014;93:201-9.

3. Bogovic JA, Prince JL, Bazin P-L. A multiple object geometric deformable model for image segmentation. Computer Vision and Image Understanding. 2013;117(2):145-57.

4. Han X, Pham DL, Tosun D, Rettmann ME, Xu C, Prince JL. CRUISE: cortical reconstruction using implicit surface evolution. NeuroImage. 2004;23(3):997-1012.

5. Rowley CD, Bazin P-L, Tardif CL, Sehmbi M, Hashim E, Zaharieva N, et al. Assessing intracortical myelin in the living human brain using myelinated cortical thickness. Frontiers in neuroscience. 2015;9.

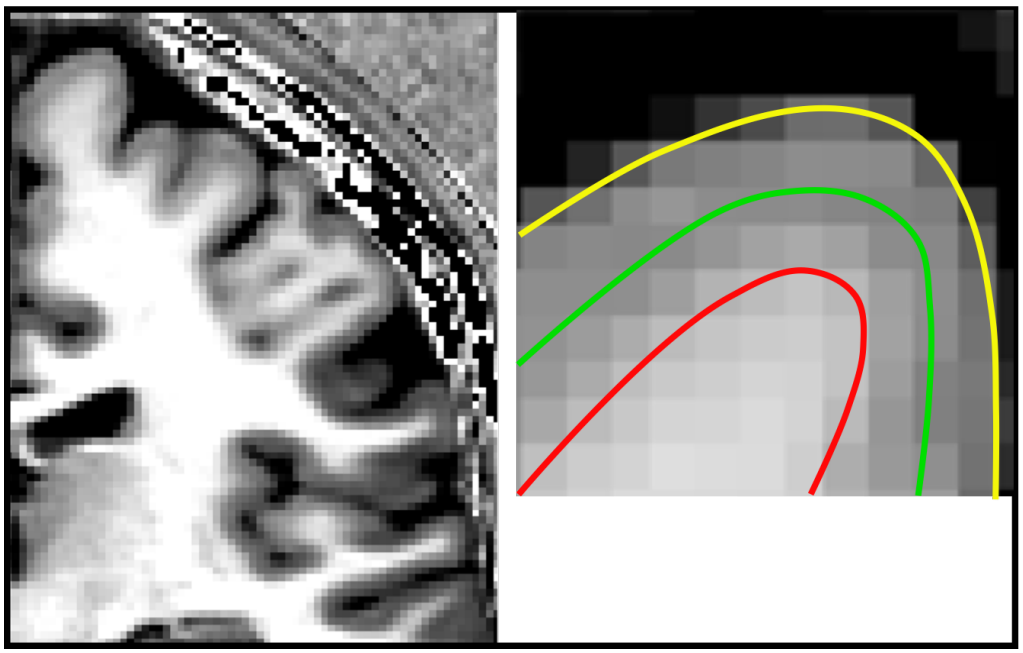
6. Pham DL, editor Robust fuzzy segmentation of magnetic resonance images. Computer-Based Medical Systems, 2001 CBMS 2001 Proceedings 14th IEEE Symposium on; 2001: IEEE.

7. Waehnert M, Dinse J, Weiss M, Streicher MN, Waehnert P, Geyer S, et al. Anatomically motivated modeling of cortical laminae. Neuroimage. 2014;93:210-20.

8. Rowley CD, Sehmbi M, Bazin PL, Tardif CL, Minuzzi L, Frey BN, et al. Age-related mapping of intracortical myelin from late adolescence to middle adulthood using T1-weighted MRI. Human Brain Mapping. 2017.

9. Tardif CL, Schäfer A, Waehnert M, Dinse J, Turner R, Bazin P-L. Multi-contrast multi-scale surface registration for improved alignment of cortical areas. Neuroimage. 2015;111:107-22.

Supplementary Figure S1. Half depth metric visualization.



Left: T_1-weighted image with high intracortical contrast.
Right: Illustration of the half depth signal surface, which is calculated from the pial and white matter surfaces.

Supplementary Table S1. Partial correlations with age of onset (years) and duration of illness (years) in BD subjects (N=45).

Cortical region	Left hemisphere			Right hemisphere		
	Partial R	P, uncorrected	P, corrected$^{\mu}$	Partial R	P, uncorrected	P, corrected$^{\mu}$
Age of onset						
Superior parietal cortex	0.17	0.16	1	0.25	0.04*	1
Medial parietal cortex	0.27	0.02*	1	0.34	0.006**	0.38
Dorsomedial somatosensory cortex	0.26	0.03*	1	0.20	0.10	1
Dorsolateral motor cortex	0.23	0.06	1	0.25	0.04*	1
Dorsomedial motor cortex	0.22	0.07	1	0.26	0.03*	1
Rostral ventral premotor cortex	0.15	0.21	1	0.26	0.03*	1
Dorsolateral premotor cortex	0.26	0.03*	1	0.22	0.07	1
Dorsomedial premotor cortex	0.36	0.002**	0.17	0.28	0.02*	1
Caudal dorsolateral prefrontal cortex	0.29	0.02*	1	0.25	0.04*	1
Caudal dorsomedial prefrontal cortex	0.30	0.01	0.72	0.28	0.02*	1
Rostral ventrolateral prefrontal cortex	0.28	0.02*	1	0.24	0.049*	1
Rostral dorsolateral superior prefrontal	0.25	0.04*	1	0.21	0.08	1

cortex						
Rostral dorsal prefrontal cortex	0.27	0.03*	1	0.24	0.046*	1
Rostral medial prefrontal cortex	0.36	0.002**	0.14	0.32	0.007**	0.48
Duration of illness (years)						
Caudal medial visual cortex	-0.33	0.005**	0.33	-0.31	0.009**	0.56
Lateral visual cortex	-0.26	0.03	1	-0.32	0.007**	0.43
Superior visual cortex	-0.26	0.03	1	-0.19	0.11	1
Cuneus	-0.33	0.006**	0.38	-0.26	0.03*	1
Caudal superior temporal cortex	-0.31	0.01*	0.60	-0.30	0.01	0.63
Dorsal inferior parietal cortex	-0.16	0.19	1	-0.25	0.03*	1
Superior parietal cortex	-0.21	0..09	1	-0.29	0.02*	0.84
Medial superior parietal cortex	-0.27	0.02*	1	-0.28	002*	0.85
Medial parietal cortex	-0.33	0.005**	0.36	-0.37	0.002**	0.12
Posterior cingulate cortex	-0.33	0.005*	0.37	-0.30	0.01*	0.71
Dorsomedial somatosensory cortex	-0.30	0.01*	0.70	-0.18	0.13	1
Ventral motor cortex	-0.23	0.05	1	-0.25	0.04*	1
Dorsolateral motor cortex	-0.23	0.06	1	-030	0.01*	0.73
Dorsomedial motor cortex	-0.28	0.02*	0.87	-0.23	0.06	1
Rostral	-0.24	0.049*	1	-0.23	0.06	1

ventral premotor cortex						
Dorsolateral premotor cortex	-0.27	0.02*	1	-0.22	0.07	1
Dorsomedial premotor cortex	-0.29	0.01*	0.75	-0.27	0.03*	1
Caudal dorsomedial prefrontal cortex	-0.29	0.01*	0.75	-0.20	0.09	1
Midcingulate cortex	-0.33	0.005**	0.37	-0.27	0.02*	1
Rostral ventrolateral prefrontal cortex	-0.24	0.04	1	-0.18	0.13	1
Rostral dorsolateral inferior prefrontal cortex	-0.25	0.04*	1	-0.29	0.02*	0.84
Rostral medial prefrontal cortex	-0.29	0.02*	0.85	-0.22	0.06	1
Ventrolateral orbitofrontal cortex	-0.24	0.048*	1	-0.20	0.09	1
Anterior cingulate cortex	-0.33	0.005**	0.33	-0.31	0.008**	0.53

[μ] p values corrected using Bonferroni correction.

Supplementary Table S2. Partial correlations with number of mood episodes in BD subjects (N=45).

Cortical Region	**Left hemisphere**			**Right hemisphere**		
	Partial R	P, uncorrected	P, corrected$^{\mu}$	Partial R	P, uncorrected	P, corrected$^{\mu}$
Number of manic episodes						
Rostral middle temporal cortex	-0.17	0.15	1	-0.24	0.047*	1
Number of hypomanic episodes						
Posterior cingulate cortex	-0.27	0.03*	1	-0.31	0.009**	0.67
Anterior cingulate cortex	-0.16	0.17	1	-0.27	0.02*	1
Number of mixed episodes						
Lateral visual cortex	0.08	0.51	1	0.26	0.03*	1
Superior visual cortex	0.09	0.46	1	0.27	0.03*	1
Caudal middle temporal cortex	0.20	0.09	1	0.31	0.009**	1
Rostral middle temporal cortex	0.29	0.01*	1	0.19	0.10	1
Ventral inferior parietal cortex	0.12	0.31	1	0.27	0.02*	1
Dorsal inferior parietal cortex	0.10	0.40	1	0.26	0.03*	1
Superior parietal cortex	0.04	0.77	1	0.24	0.045*	1
Ventral somatosensory	0.0009	0.99	1	0.29	0.02*	1

cortex						
Dorsolateral somatosensory cortex	0.18	0.14	1	0.26	0.03*	1
Rostral dorsolateral inferior parietal cortex	0.22	0.06	1	0.26	0.03*	1
Ventral orbitofrontal cortex	0.33	0.005**	1	0.29	0.01*	1
Ventromedial orbitofrontal cortex	0.37	0.001**	1	0.15	0.21	1
Ventromedial prefrontal cortex	0.42	0.0003***	0.04*	0.18	0.13	1

$^{\mu}$ p values corrected using Bonferroni correction.

Supplementary Table S3. Partial correlations with medication use in BD subjects (N=45).

Cortical Region	Left hemisphere			Right hemisphere		
	Partial R	P, uncorrected	P, corrected$^{\mu}$	Partial R	P, uncorrected	P, corrected$^{\mu}$
Anticonvulsant use						
Rostral dorsolateral inferior prefrontal cortex	-0.24	0.04*	1	-0.09	0.46	1
Rostral dorsal prefrontal cortex	-0.25	0.04*	1	-0.25	0.03*	1
Ventromedial orbitofrontal cortex	-0.12	0.34	1	-0.25	0.04*	1
Antipsychotic use						
Caudal medial visual cortex	-0.33	0.005**	1	-0.16	0.18	1
Cuneus	-0.23	0.06	1	-0.30	0.01*	1
Medial superior parietal cortex	-0.25	0.04*	1	-0.27	0.02*	1
Medial parietal cortex	-0.14	0.24	1	-0.25	0.04*	1
Posterior cingulate cortex	-0.29	0.02*	1	-0.26	0.03*	1
Dorsolateral motor cortex	-0.27	0.02*	1	-0.25	0.04*	1
Midcingulate cortex	-0.26	0.03*	1	-0.32	0.008**	1
Anterior cingulate cortex	-0.10	0.39	1	-0.35	0.003**	1
Total Hassel load						
Caudal medial visual cortex	-0.38	0.001**	0.70	-0.27	0.02*	1
Cuneus	-0.17	0.16	1	-0.27	0.03*	1
Medial	-0.13	0.25	1	-0.28	0.02*	1

parietal cortex						
Posterior cingulate cortex	-0.25	0.04*	1	-0.22	0.07	1
Dorsolateral somatosensory cortex	-0.13	0.28	1	-0.24	0.04*	1
Dorsolateral motor cortex	-0.30	0.01*	1	-0.30	0.01*	1
Dorsomedial motor cortex	-0.28	0.02*	1	-0.28	0.02*	1
Total medication count						
Caudal medial visual cortex	-0.33	0.006**	1	-0.22	0.06	1
Caudal middle temporal cortex	-0.06	0.62	1	-0.26	0.03*	1
Ventral inferior parietal cortex	-0.14	0.26	1	-0.27	0.03*	1
Dorsal inferior parietal cortex	-0.16	0.19	1	-0.26	0.03*	1
Superior parietal cortex	-0.13	0.28	1	-0.24	0.049*	1
Medial parietal cortex	-0.08	0.50	1	-0.25	0.03*	1
Dorsolateral somatosensory cortex	-0.18	0.12	1	-0.33	0.005**	1
Dorsomedial somatosensory cortex	-0.17	0.17	1	-0.25	0.03*	1
Dorsolateral motor cortex	-0.23	0.05	1	-0.29	0.02*	1
Dorsomedial motor cortex	-0.26	0.03*	1	-0.32	0.008**	1
Dorsolateral premotor cortex	-0.20	0.10	1	-0.25	0.04*	1
Dorsomedial premotor	-0.25	0.03*	1	-0.31	0.009**	1

cortex						
Caudal dorsomedial prefrontal cortex	-0.22	0.06	1	-0.25	0.04*	1
Rostral dorsolateral inferior prefrontal cortex	-0.29	0.02*	1	-0.15	0.23	1
Ventral orbitofrontal cortex	-0.31	0.01*	1	-0.22	0.07	1

[μ] p values corrected using Bonferroni correction

Chapter 3: Association of Intracortical Myelin and Cognitive Function in Bipolar I Disorder

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Abstract

Importance

Understanding the neural correlates of cognitive dysfunction can aid in the development of targeted therapeutics to improve functional outcomes in Bipolar Disorder (BD).

Objective

To investigate the association between intracortical myelin (ICM) and cognitive performance in BD-type I subjects.

Design, Setting, and Participants

This study took place at St. Joseph's Healthcare Hamilton between September 1, 2014-January 31, 2017. 40 subjects with BD-I and 60 age-, sex-, BMI-, education-, and IQ-matched controls (ages 17-45) were enrolled. We investigated ICM in domain-specific cortical networks, identified *a priori,* in relation to seven cognitive domains previously associated with BD: Psychomotor speed, reaction time, processing speed, verbal memory, simple attention, motor speed, and executive function.

Main Outcome and Measure(s)

T_1-weighted images (3T) optimized for measurement of ICM were analyzed using a surface-based approach, where the MRI signal was sampled at the middle depth of the cortex. Cognitive performance was measured using a standardized computerized testing battery, and paper and pencil Trails B.

Results

Mean (SD) ages of the BD-I and control subjects were 31.5 (8.2) and 30.6 (8.3), respectively, and 19 (48.7%) and 30 (51.7%) were female, respectively.

ICM was associated with verbal memory (VM) in BD throughout a cortical network identified in relation to VM function, with the strongest effect observed in the left caudal middle temporal cortex and the left dorsolateral prefrontal cortex ($p_{corrected}<0.05$).

Processing speed, executive function, and reaction time were also predicted by ICM signal in BD subjects, but not controls, although this did not survive Bonferroni correction. Subcomponent analyses revealed that the association between VM and ICM was specific to initial correct hits (correct word recognition, without delay).

Conclusions and Relevance

This is the first study to show that VM performance is associated with ICM in BD. ICM has previously shown to play a role in neural synchrony and integrity of neural connections. VM dysfunction is one of the most replicated cognitive abnormalities observed in BD. Therefore, these results provide a novel mechanism for understanding the neural correlates of cognitive dysfunction in BD.

Key Points

Question

Is intracortical myelin (ICM) associated with cognitive performance in Bipolar Disorder (BD)?

Findings

In this case-control study, verbal memory performance in BD was significantly associated with ICM in regions of the temporal, parietal, and prefrontal cortices, identified *a priori* in relation to verbal memory performance. This association is not seen in controls.

Meaning

Cognitive processes may have greater reliance on ICM in BD, than in controls. This finding may be particularly relevant to the development of therapeutics targeting cognitive remediation in BD.

3.1 Introduction

Cognitive dysfunction in Bipolar Disorder (BD) notably persists into periods of clinical remission, or euthymia, with deficits in executive functioning, verbal learning and memory, attention, and processing speed being the most replicated findings [1-3]. Cognitive impairment in BD has been associated with numerous clinical variables, including number of hospitalizations, number of manic episodes, duration of illness [4], history of psychosis [5], age of symptom onset, and duration of treatment delay [6]. Therefore, cognitive dysfunction in BD is not solely dependent on illness state, but is rather a persistent and disabling feature of the disorder, although its neurobiological correlates remain to be determined.

Certain brain regions have routinely been identified across species in association with certain cognitive tasks, notwithstanding the influence of differential experience amongst individuals. Specifically, associations between subcortical white matter (WM) abnormalities and cognitive dysfunction transcend diagnostic criteria and are seen across a myriad of conditions, in healthy individuals, and in unaffected first-degree relatives of individuals with BD, implying an inherent and potentially etiological relationship between WM integrity and cognitive processing [7-9]. For instance, subcortical WM tract integrity has been linked to both functional cortical activity and cognitive performance in BD and Schizophrenia [10,11].

Myelin content within the cerebral cortex (intracortical myelin, ICM) remains largely unexplored, however, especially in relation to cognitive processing despite being

highly relevant. Recent animal studies have begun to shown that ICM is essential for cognitive function. Rat pups exposed to neonatal maternal separation show decreased levels of myelination within the medial prefrontal cortex (mPFC) and display increased levels of anxiety and deficits in working memory and social interaction, all of which are mPFC-associated behaviors [12]. These findings were consistent with a social isolation paradigm, where deficits in rodent working memory performance were associated with decreased myelin maturation and myelination in the mPFC [13]. Even subtle aberrations in ICM in the prefrontal cortex have been associated with reduced prepulse inhibition in mice [14]. In healthy humans, Grydeland et al. (2013) have shown that ICM in the posterior cingulate cortex was associated with performance on the Eriksen Flanker task, a test of response inhibition, and that levels of ICM were directly associated with neural activity during this task [15]. We recently found that individuals with BD have extensive deficits in ICM development (Sehmbi et al, under review). Because of the emerging association between ICM and cognitive function in animals and humans [12-15], we aimed to determine whether ICM content is associated with cognitive performance in individuals with BD. For that, we employed an imaging technique optimized for ICM measurement in humans *in vivo* [16-19].

3. 2 Methods

3.2.1. Study participants

This study was approved by the Hamilton Integrated Research Ethics Board and signed informed consent was obtained from each study participant. Images were collected

in 40 individuals with a diagnosis of BD type-I (20 F, 20 M) and 60 matched controls (30 F, 30 M). All participants were right-handed, aged-17-45, and all female subjects were premenopausal. Study participants completed the Structured Clinical Interview for DSM-IV (SCID-I) for diagnostic assessment [20]. Controls did not meet criteria for any current or lifetime Axis I psychiatric conditions. Subjects with BD-I did not meet criteria for any *current* Axis I psychiatric comorbidities. We excluded individuals with unstable medical/inflammatory conditions, alcohol/substance abuse within the last year (excluding caffeine or nicotine), past/current history of neurological disorders (including head trauma and migraines), and any MRI contraindications. All participants were fluent in English, and groups were matched based on intelligence level using the Wechsler Abbreviated Scale of Intelligence (WASI) 2-subtest Intelligence Quotient (IQ) test.

3. 2.2 Imaging Methods

3. 2.2.1: Imaging Acquisition

Images were acquired on a 3T General Electric scanner (Software Version 22.0), using a 32-channel receive-only radiofrequency (RF) coil for the head (MR Instruments) and a transmit RF body coil (GE) to produce a T_1-weighted image with optimized intracortical contrast for ICM analysis [16,18]. All images were acquired with 1mm isotropic resolution and the total time for the protocol was ~35 min. We have recently reported age-related ICM mapping in healthy individuals (14). The imaging protocol is summarized below, with complete details and parameter specifications available in our previous work, and in the **Supplementary Methods** [19].

3. 2.2.2 Anatomical Reference Image

A typical 3D T_1-weighted whole-head anatomical image was acquired using a 3D inversion-recovery gradient echo sequence (GE 3D BRAVO). This was used for image registration in processing.

3.2.2.3: High Intracortical Contrast T1-weighted Image

Another 3D T_1-weighted whole-head image with strong intracortical contrast was acquired with an inversion-recovery gradient echo sequence (GE 3D BRAVO) to form the basis of our ICM maps.

3.2.2.4: Ratio Image

A final 3D proton density-weighted whole-head image was collected to correct intensity inhomogeneity and remove T_2*-weighting in the high intracortical contrast T_1-weighted image. This image was made with a 3D gradient-echo sequence (GE 3D SPGR).

The proton-density weighted image was registered to the high intracortical contrast T_1-weighted image using a 6-parameter rigid transform (FSL) and filtered with a 3D median filter with a 5 x 5 x 5 mm kernel size. The T_1-weighted image was then divided by the filtered proton-density weighted image to create the ratio image, which is a strongly T_1-weighted image with B_1- and some B_1+ inhomogeneities removed [21-23].

3.2.2.5: ICM Processing

Image processing was performed to map ICM content in the cortex of each subject and to register the subjects' maps to a common space for group analysis. To map ICM in a subject, the pial surface and a surface at GM/WM boundary of the cortex were found.

A new surface at the middle depth of the cortex was calculated and the signal intensity of the ratio image was mapped onto this surface to depict ICM. Henceforth, ICM signal intensity is referred to as ICM.

3.2.3 Cognition- CNS Vital Signs (CNSVS)

Each participant completed cognitive testing within the same week as, but not immediately following, the MRI session. This helped avoiding any stress or anxiety related to the MRI procedure. We administered a 25-minute computerized testing battery (CNS Vital Signs, CNSVS). This battery has previously been validated across a broad age range, with established reliability in patients with mild cognitive impairment and patients with depression [24-26].

We administered the Verbal Memory, Finger Tapping, Symbol Digit Coding, Stroop, and Continuous Performance tests. Test scores were recombined by the CNSVS program to obtain overall scores for the following cognitive domains: Psychomotor speed, reaction time, processing speed, verbal memory, simple attention and motor speed (**Supplementary Table 1**). Individual domain component scores were also obtained, and varied by test.

All scores used in the following analysis were deemed "valid" by the CNSVS internal assessment. Scores are considered valid when a suitable level of effort has been detected from the participant, in order to avoid potentially dubious results. This is based on a validity algorithm, outlined in **Supplementary Table 2.**

The paper and pencil Trails B was administered as a measure of executive function. Scores where the participant committed 3 or fewer errors were considered valid. Time of completion, in seconds (s), was used as the outcome of interest.

3.2.4 Statistical analysis

3.2.4.1 Demographics

All statistical analyses were completed using R version 3.3.2 (https://www.r-project.org). Shapiro-Wilk and Bartlett tests determined normality and homogeneity of variances of continuous variables, respectively. Between group differences in age, sex, BMI, years of education, WASI 2 subtest IQ, and smoking status were tested using two-tailed independent t-test, Mann-Whitney test, or chi-squared test, as appropriate.

3.2.4.2 Cognitive Performance

Shapiro-Wilk and Bartlett tests assessed the normality and homogeneity of variances, respectively, of cognitive domain scores to determine whether data met the assumptions for parametric testing. Between group differences in psychomotor speed, reaction time, processing speed, verbal memory, simple attention, motor speed, and executive function were subsequently assessed using two-tailed independent t-test or Mann-Whitney test, as appropriate.

Pearson's R was used to determine whether any continuous demographic or clinical variables were correlated with cognitive performance in our sample, by group. Unpaired two-tailed t-test, Mann-Whitney U test, or ANOVA was used for categorical

variables, as appropriate. All results were corrected for multiple comparisons using Bonferroni correction.

3.2.4.3 Cognition and ICM

MarsAtlas [27] was used to parcellate the cortex into 82 regions-of-interest (ROIs) for analysis. Cortical maps were generated using Surfstat (http://www.math.mcgill.ca/keith/surfstat/) in Matlab (v R2015a). We identified cortical networks pertaining to each cognitive domain *a priori,* from previous functional neuroimaging literature and meta-analyses within the field. Performance on cognitive tasks were investigated as a function of ICM by ROIs within each cognitive network, using a general linear model (GLM): $ICM + DX + DX*ICM$, where *DX* was the subject diagnosis. Our study aimed to investigate the *DX*ICM* interaction term, which would suggest whether there are differential relationships between ICM and cognitive performance in BD-I in comparison to controls.

Six ROIs per hemisphere (twelve total) could not be analyzed due to poor signal intensity profiles arising from topological errors in the identification of the GM/WM boundary surfaces: the isthmus of the cingulate, insula, rostral medial visual cortex, medial/rostral inferior temporal cortex, and rostral superior temporal cortex. These regions were excluded from all cortical networks. The signal was averaged across each individual ROI and then analyzed in R. *p*-values for the linear model and the interaction term were extracted from the GLMs. Partial η^2 was calculated to determine the amount of variance in the data that can be attributed to the interaction term. Coefficient values for

the ICM term were extracted by running the model in each population separately, in order to determine the slope of the association between ICM-related T_1-weighted signal intensity and cognitive performance within each study population.

For cognitive domains that did not meet the assumptions of linear regression (linearity, normality, homoscedasticity, and independence), each diagnostic group was assessed independently. Performance on cognitive tasks as a function of ICM signal intensity in the ratio image were investigated by ROI within each cognitive network using a second GLM. In this model, cognitive performance was assessed as the dependent variable and ICM signal as the independent variable. All results were corrected for multiple comparisons using the Bonferroni correction.

3.2.4.4 Secondary analyses - Verbal memory subcomponents

As exploratory analyses, we ran Pearson's R correlations to determine which specific subcomponents of Verbal Memory were associated with ICM in the network identified *a priori*. This recognition-based verbal memory test is administered twice: once at the start of the battery (initial test), and once following a 20-minute delay (delay test). During each test point, we collected the number of correct hits, correct passes, and target reaction time. We subsequently assessed whether ICM was associated with the following verbal memory subcomponents: initial/delayed correct hits, initial/delayed correct passes, and initial/delayed target reaction time. Bonferroni correction for multiple comparisons was applied.

3.2.4.5 Medication Effects

Medications were categorized as lithium, antidepressants, antipsychotics, anticonvulsants, and anxiolytics for each participant, and the dose of each medication class was coded as 0=absent, 1=low, or 2=high [28]. Anticonvulsants and antidepressants were converted to high or low dose groupings according to Sackeim [29]. Levels 1 and 2 were grouped as a low dose, and levels 3 and 4 were grouped as high dose. Antipsychotics were converted to chlorpromazine (CPZ) dose equivalents. Individuals taking a dose equivalent below the mean effective daily dose of CPZ were defined as low dose, and vice-versa for high dose [30]. Anxiolytics were converted to Lorazepam dose equivalents, and were determined as low or high dose using the Physician's Desk Reference recommended daily dose median. Individual medication classifications were summed to determine a total medication load. Pearson's R correlations were used to determine the effects of individual medication classes and the composite medication score on cognitive performance in BD-I subjects.

3.3 Results

3.3.1 Demographics

One female BD subject and two male controls were excluded from the analyses due to poor quality ICM maps. BD subjects (N=39) and controls (N=58) were matched on age, sex, BMI, years of education, WASI 2-subtest IQ and smoking status (all $p>0.05$, **Table 1)**. A majority (79%) of the BD-I sample was euthymic during testing.

3.3.2 Cognitive performance

There were no between group differences in psychomotor speed, reaction time, processing speed, verbal memory, simple attention, motor speed, or the Trails B (all $p>0.05$, **Supplementary Table 3).**

As expected, age, sex, education and IQ were associated with performance in some cognitive domains in both controls and BD-I, in addition to antipsychotic and anxiolytic use in BD (**Supplementary Table 4**). Notably, there was no association of current mood state or medication use with cognitive performance in our BD sample (**Supplementary Table 4**).

3.3.3 ICM and Cognition

GLMs were tested on whether they met the assumptions required for analysis within each cortical network (linearity, normality, homoscedasticity, and independence). Processing speed, executive function and verbal memory met the assumptions of the following regression model: *Cognitive performance ~ ICM + DX + DX* ICM*. Psychomotor speed, motor speed, and reaction time met the assumptions of the following regression model, excluding the interaction term: *Cognitive performance ~ ICM*. Simple attention scores did not meet the assumptions of linear regression, despite transformation, and therefore could not be explored here. Only regions with significant regression models are reported below. The complete network results are outlined in **Table 2, and Supplementary Tables 5-9.**

Verbal memory was predicted by a significant interaction between ICM and diagnosis in regions of the bilateral temporal, parietal, and prefrontal cortices. (R^2= 0.13-0.18, $p_{corrected}$= 0.004-0.04). The largest differences between BD-I and control subjects were seen in the left caudal middle temporal cortex and the left dorsolateral prefrontal cortex. These correspond to Brodmann areas 21/22/39, and 10/46, respectively [27].

Processing speed was predicted by a significant interaction between ICM and diagnosis in the left posterior cingulate (p=0.01), midcingulate (p=0.007), and anterior cingulate cortices (p=0.03) (**Supplementary Table 5**). Executive function was predicted by a significant interaction between ICM and diagnosis in the bilateral midcingulate cortex (left p=0.01, right p=0.008), and right anterior cingulate cortex (p=0.005) **(Supplementary Table 6)**. However, none of these survived Bonferroni correction.

Psychomotor and motor speed were not predicted by ICM in any ROI in either controls or BD-I subjects (all p>0.05, **Supplementary Tables 7 and 8**). Reaction time was predicted by ICM in the left posterior cingulate cortex, bilateral dorsomedial motor cortex, and left midcingulate cortex in individuals with BD-I, although these do not survive Bonferroni correction. ICM did not predict reaction time in any regions in controls (p>0.05, **Supplementary Table 9**).

To further explore the verbal memory findings, partial η^2 was calculated for the *DX*ICM* interaction term in ROIs where the regression model was significant, and was mapped onto the cortex in **Figure 1**. Most regions showed a small to medium effect size (0.01-0.08), with the largest effects seen in the left caudal middle temporal cortex (0.08), and the left dorsolateral prefrontal cortex (0.08).

The coefficient values for the *ICM* term was calculated in each population separately to determine the slope of the association between ICM and verbal memory performance. These values were mapped onto the cortex in **Figure 2**. Control subjects shows a blunted slope of verbal memory performance over ICM signal, with values ranging between 3.8-13.9. BD subjects display much steeper coefficient values, between 35.2 – 64.4, suggesting a much greater influence of ICM on verbal memory performance in BD, in comparison to controls.

3.3.4 Secondary analysis - Verbal memory subcomponents

Initial correct hits (correct recognition of a word from the presented list during immediate testing, without delay), were correlated with ICM in BD subjects in regions of the left temporal and bilateral prefrontal cortices (R= 0.19-0.24, $p_{corrected}$=0.001-0.003, **Table 3**). There were no significant correlations between initial correct hits and ICM in controls (p>0.05, **Table 3**).

Delayed correct hits were correlated with ICM in BD subjects in regions of the left temporal cortex, and bilateral parietal and prefrontal cortices (R=0.32-0.42, p=0.008-0.04, **Supplementary Table 12)**, but none of the above survived Bonferroni correction. There were no significant correlations between delayed correct hits and ICM in controls (p>0.05, **Supplementary Table 12**).

All significant correlations between delayed reaction time and ICM in BD subjects were lateralized to the right hemisphere, in regions of the temporal and prefrontal cortices (R=0.34-0.40, p=0.01-0.04, **Supplementary Table14**), but these did not survive

Bonferroni correction. There were no significant correlations between delayed reaction time and ICM in controls ($p>0.05$, **Supplementary Table 14).**

Initial correct passes **(Supplementary Table 10),** initial reaction time **(Supplementary Table 11)**, and delayed correct passes (**Supplementary Table 13)** were not correlated with ICM in either BD subjects or controls ($p>0.05$).

3.4 Discussion

The main finding of our study is that ICM was significantly associated with verbal memory performance in BD-I subjects but not in controls. The most robust effects were seen in the left caudal middle temporal and dorsolateral prefrontal cortices, although other temporal, parietal, and prefrontal areas also displayed significant effects. Sub-analyses revealed that this association was specific to initial correct hits performance (correct word recognition without delay), in the temporal, parietal, and prefrontal cortices. Similarly, BD subjects displayed significantly higher coefficient values for the ICM term than controls.

Verbal memory is one of the most prominently impaired cognitive domains in individuals with BD, alongside executive function and sustained attention [1-3]. Deficits in ICM maturation may therefore be associated with verbal memory dysfunction in individuals with BD. Our results parallel previous studies that routinely implicate the prefrontal cortex and temporo-limbic structures in verbal memory. Lateral temporal regions are essential for speech processing at both the phonemic and syllabic levels [31], with the left superior temporal plane involved in phonemic processing due a relatively

higher rate of information processing [32-34]. In addition to its role in processing, activity in the lateral temporal regions predicts whether sentential information is successfully recalled [35], and repeated exposure to auditory sentences shows suppressed activation [34,36]. Left frontal lobes lesions have been associated with poor verbal memory performance [37]. The frontal cortices show lateralized functionality in verbal memory tasks: the left prefrontal cortex is involved during acquisition, whereas the right prefrontal cortex is recruited during retrieval, in addition to other cortical areas such as the precuneus and the temporal lobes [38,39]. Taken together, these studies show significant contributions of temporal and frontal regions to optimal verbal memory. Our study suggests that ICM within temporal, parietal, and prefrontal cortical areas may be a biological correlate of verbal memory performance in individuals with BD-I. Consistent with this hypothesis, we found significantly higher coefficient values for the ICM term in BD subjects than in controls.

Myelination of cortical GABA-ergic parvalbumin interneurons has been recently implicated in cognitive dysfunction in Schizophrenia [40]. Parvalbumin interneurons comprise 40% of all GABA-ergic interneurons within the cortex, and are likely the most widely myelinated cortical interneurons [40]. Their optimal functioning has been associated with the synchronized inhibition of pyramidal networks in the cortex, essential for the production of cortical gamma frequency oscillations, thereby regulating cognitive control [41-43]. Although our current MRI protocol precludes comment on parvalbumin interneuron myelination, it is conceivable that the association between cognitive performance and ICM may be mediated by the integrity of myelinated cortical interneurons. Notably,

altered cortical gamma oscillations have been identified in BD in the right frontal, prefrontal, and posterior temporal regions [44].

We did not see any overall group differences between BD and controls on cognitive performance. The CNSVS may lack the sensitivity to detect between-group differences in cognitive performance in BD individuals who are highly educated and highly functioning (see **Table 1**). It has previously been well established, however, that cognitive dysfunction may be an endophenotype of BD. Therefore, future studies should assess ICM in BD using more sensitive cognitive batteries previously validated in BD, such as the MATRICS Consensus Cognitive Battery [45]. It is possible, however, that more sensitive cognitive tasks may be even more strongly associated with ICM. Processing speed, executive function, and reaction time performance were also associated with ICM, although they did not survive Bonferroni correction. These domains have previously been shown to be impaired in BD, and have been associated with cortical abnormalities [46].

We are limited by the cross-sectional nature of our study, and cannot comment on the sequential directionality between cognitive performance and ICM integrity. A sample with various mood states (although largely euthymic) could have influenced our results. Additionally, signal intensity was averaged across each ROI to obtain an average measure per region. Spatial acuity that may be pertinent to detecting subtle intra-regional differences potentially associated with cognitive function is therefore decreased. Cognitive function is also highly prone to cortical remodeling and compensation [47]. Therefore, future studies should include individuals at various stages of functionality, and should use a battery with increased sensitivity to further investigate the association

between ICM and cognitive performance. Strengths of our study include use of an imaging technique that has been well validated for ICM measurement *in vivo,* including histology [16-19]. Understanding the neurological basis of cognitive dysfunction in BD will aid in the development of new therapeutics targeting functional outcomes in patients with BD. Verbal memory dysfunction has been shown to be the strongest predictor of poor psychosocial functioning amongst BD subjects, in comparison to other cognitive domains such as attention and concentration deficits [48].

In conclusion, this is the first evidence that ICM is associated with verbal memory performance in individuals with BD. Longitudinal studies are required to determine the directionality of this relationship.

4.4 References

1. Bora E, Yucel M, Pantelis C. Cognitive endophenotypes of bipolar disorder: a meta-analysis of neuropsychological deficits in euthymic patients and their first-degree relatives. *Journal of affective disorders.* 2009;113(1):1-20.

2. Martínez-Arán A, Vieta E, Reinares M, et al. Cognitive function across manic or hypomanic, depressed, and euthymic states in bipolar disorder. *American Journal of Psychiatry.* 2004;161(2):262-270.

3. Robinson LJ, Thompson JM, Gallagher P, et al. A meta-analysis of cognitive deficits in euthymic patients with bipolar disorder. *Journal of affective disorders.* 2006;93(1):105-115.

4. Burdick K, Goldberg J, Harrow M. Neurocognitive dysfunction and psychosocial outcome in patients with bipolar I disorder at 15-year follow-up. *Acta Psychiatrica Scandinavica.* 2010;122(6):499-506.

5. Simonsen C, Sundet K, Vaskinn A, et al. Neurocognitive dysfunction in bipolar and schizophrenia spectrum disorders depends on history of psychosis rather than diagnostic group. *Schizophrenia Bulletin.* 2009:sbp034.

6. Post RM, Leverich GS, Kupka RW, et al. Early-onset bipolar disorder and treatment delay are risk factors for poor outcome in adulthood. *The Journal of clinical psychiatry.* 2010;71(7):864-872.

7. Filley CM, Fields RD. White matter and cognition: making the connection. *Journal of neurophysiology.* 2016;116(5):2093-2104.

8. Skudlarski P, Schretlen DJ, Thaker GK, et al. Diffusion tensor imaging white matter endophenotypes in patients with schizophrenia or psychotic bipolar disorder and their relatives. *American Journal of Psychiatry.* 2013;170(8):886-898.

9. Miskowiak KW, Kjærstad HL, Meluken I, et al. The search for neuroimaging and cognitive endophenotypes: A critical systematic review of studies involving unaffected first-degree relatives of individuals with bipolar disorder. *Neuroscience & Biobehavioral Reviews.* 2016.

10. McKenna BS, Theilmann RJ, Sutherland AN, Eyler LT. Fusing functional MRI and diffusion tensor imaging measures of brain function and structure to predict working memory and processing speed performance among inter-episode bipolar patients. *Journal of the International Neuropsychological Society.* 2015;21(5):330-341.

11. Kochunov P, Coyle TR, Rowland LM, et al. Association of White Matter With Core Cognitive Deficits in Patients With Schizophrenia. *JAMA psychiatry.* 2017.

12. Yang Y, Cheng Z, Tang H, et al. Neonatal Maternal Separation Impairs Prefrontal Cortical Myelination and Cognitive Functions in Rats Through Activation of Wnt Signaling. *Cerebral Cortex.* 2017;27(5):2871-2884.

13. Makinodan M, Rosen KM, Ito S, Corfas G. A critical period for social experience–dependent oligodendrocyte maturation and myelination. *science.* 2012;337(6100):1357-1360.

14. Poggi G, Boretius S, Möbius W, et al. Cortical network dysfunction caused by a subtle defect of myelination. *Glia.* 2016;64(11):2025-2040.

15. Grydeland H, Westlye LT, Walhovd KB, Fjell AM. Intracortical posterior cingulate myelin content relates to error processing: results from T1-and T2-weighted MRI myelin mapping and electrophysiology in healthy adults. *Cerebral Cortex.* 2016;26(6):2402-2410.

16. Bock NA, Hashim E, Janik R, et al. Optimizing T 1-weighted imaging of cortical myelin content at 3.0 T. *Neuroimage.* 2013;65:1-12.

17. Bock NA, Kocharyan A, Liu JV, Silva AC. Visualizing the entire cortical myelination pattern in marmosets with magnetic resonance imaging. *Journal of neuroscience methods.* 2009;185(1):15-22.

18. Rowley CD, Bazin P-L, Tardif CL, et al. Assessing intracortical myelin in the living human brain using myelinated cortical thickness. *Frontiers in neuroscience.* 2015;9.

19. Rowley CD, Sehmbi M, Bazin PL, et al. Age-related mapping of intracortical myelin from late adolescence to middle adulthood using T1-weighted MRI. *Human Brain Mapping.* 2017.

20. First M, Spitzer R, Gibbon M, Williams J. Research Version, Patient Edition With Psychotic Screen (SCID-I/PW/PSY SCREEN) New York: Biometrics Research, New York State Psychiatric Institute; 2002. *Structured Clinical Interview for DSM-IV-TR Axis I Disorders.*

21. Marques JP, Gruetter R. New Developments and Applications of the MP2RAGE Sequence-Focusing the Contrast and High Spatial Resolution R 1 Mapping. *PloS one.* 2013;8(7):e69294.

22. Van de Moortele P-F, Auerbach EJ, Olman C, Yacoub E, Uğurbil K, Moeller S. T 1 weighted brain images at 7 Tesla unbiased for Proton Density, T 2□ contrast and RF coil receive B 1 sensitivity with simultaneous vessel visualization. *Neuroimage.* 2009;46(2):432-446.

23. Wang J, Qiu M, Constable RT. In vivo method for correcting transmit/receive nonuniformities with phased array coils. *Magnetic resonance in medicine.* 2005;53(3):666-674.

24. Gualtieri CT, Johnson LG. Reliability and validity of a computerized neurocognitive test battery, CNS Vital Signs. *Archives of Clinical Neuropsychology.* 2006;21(7):623-643.

25. Gualtieri CT, Johnson LG. Neurocognitive testing supports a broader concept of mild cognitive impairment. *American Journal of Alzheimer's Disease & Other Dementias®.* 2005;20(6):359-366.

26. Gualtieri CT, Johnson LG, Benedict KB. Neurocognition in depression: patients on and off medication versus healthy comparison subjects. *The Journal of neuropsychiatry and clinical neurosciences.* 2006;18(2):217-225.

27. Auzias G, Coulon O, Brovelli A. MarsAtlas: A cortical parcellation atlas for functional mapping. *Human brain mapping.* 2016.

28. Hassel S, Almeida JR, Kerr N, et al. Elevated striatal and decreased dorsolateral prefrontal cortical activity in response to emotional stimuli in euthymic bipolar disorder: no associations with psychotropic medication load. *Bipolar disorders.* 2008;10(8):916-927.

29. Sackeim HA. The definition and meaning of treatment-resistant depression. *Journal of Clinical Psychiatry.* 2001.

30. Davis JM, Chen N. Dose response and dose equivalence of antipsychotics. *Journal of clinical psychopharmacology.* 2004;24(2):192-208.

31. Zhuang J, Tyler LK, Randall B, Stamatakis EA, Marslen-Wilson WD. Optimally efficient neural systems for processing spoken language. *Cerebral Cortex.* 2012;24(4):908-918.

32. Giraud A-L, Poeppel D. Cortical oscillations and speech processing: emerging computational principles and operations. *Nature neuroscience.* 2012;15(4):511-517.

33. Morillon B, Liégeois-Chauvel C, Arnal LH, Bénar C-G, Giraud A-L. Asymmetric function of theta and gamma activity in syllable processing: an intra-cortical study. *Frontiers in psychology.* 2012;3.

34. Hartzell JF, Davis B, Melcher D, et al. Brains of verbal memory specialists show anatomical differences in language, memory and visual systems. *Neuroimage.* 2016;131:181-192.

35. Hasson U, Nusbaum HC, Small SL. Brain networks subserving the extraction of sentence information and its encoding to memory. *Cerebral cortex.* 2007;17(12):2899-2913.

36. Dehaene-Lambertz G, Dehaene S, Anton JL, et al. Functional segregation of cortical language areas by sentence repetition. *Human brain mapping.* 2006;27(5):360-371.

37. Petrides M, Milner B. Deficits on subject-ordered tasks after frontal-and temporal-lobe lesions in man. *Neuropsychologia.* 1982;20(3):249-262.

38. Shallice T, Fletcher P, Frith C, Grasby P, Frackowiak R, Dolan R. Brain regions associated with acquisition and retrieval of verbal episodic memory. *Nature.* 1994;368(6472):633-635.

39. Kelley WM, Miezin FM, McDermott KB, et al. Hemispheric specialization in human dorsal frontal cortex and medial temporal lobe for verbal and nonverbal memory encoding. *Neuron.* 1998;20(5):927-936.

40. Stedehouder J, Kushner S. Myelination of parvalbumin interneurons: a parsimonious locus of pathophysiological convergence in schizophrenia. *Molecular psychiatry.* 2017;22(1):4.

41. Sohal VS, Zhang F, Yizhar O, Deisseroth K. Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature.* 2009;459(7247):698.

42. Lewis DA, Curley AA, Glausier JR, Volk DW. Cortical parvalbumin interneurons and cognitive dysfunction in schizophrenia. *Trends in neurosciences.* 2012;35(1):57-67.

43. Gonzalez-Burgos G, Lewis DA. GABA neurons and the mechanisms of network oscillations: implications for understanding cortical dysfunction in schizophrenia. *Schizophrenia bulletin.* 2008;34(5):944-961.

44. Özerdem A, Güntekin B, Atagün İ, Turp B, Başar E. Reduced long distance gamma (28–48Hz) coherence in euthymic patients with bipolar disorder. *Journal of affective disorders.* 2011;132(3):325-332.

45. Burdick KE, Goldberg TE, Cornblatt BA, et al. The MATRICS consensus cognitive battery in patients with bipolar I disorder. *Neuropsychopharmacology.* 2011;36(8):1587.

46. Clark L, Sahakian BJ. Cognitive neuroscience and brain imaging in bipolar disorder. *Dialogues in clinical neuroscience.* 2008;10(2):153.

47. Tan H-Y, Sust S, Buckholtz JW, et al. Dysfunctional prefrontal regional specialization and compensation in schizophrenia. *American Journal of Psychiatry.* 2006;163(11):1969-1977.

48. Martinez-Aran A, Vieta E, Torrent C, et al. Functional outcome in bipolar disorder: the role of clinical and cognitive factors. *Bipolar disorders.* 2007;9(1-2):103-113.

Table 1. Demographic and clinical characteristics.

	Control (n=58)	BD (n=39)	Test statistic	P value
Age	30.6 (8.3)	31.5 (8.2)	t=0.87	0.39
Female (%)	30 (51.7%)	19 (48.7%)	χ= 3.55	0.31
BMI	25.8 (5.2)	26.5 (4.7)	t=1.19	0.24
Education (years)	15.8 (2.9)	15.8 (3.3)	t= 0.44	0.66
WASI 2-subtest IQ	110.7 (11.0)	111.8 (13.9)	t=0.56	0.72
Smoking N (%) Yes No Past	 16 (27.6) 40 (69.0) 2 (3.4)	 11 (28.2) 21 (53.8) 7 (17.9)	χ= 8.85	0.12
Age of Onset	NA	17.1 (6.6)	NA	NA
Duration of Illness	NA	10.0 (11.4)	NA	NA
Current state Euthymic Depressed Manic Hypomanic Mixed	NA	 31 4 0 3 1	NA	NA
MADRS Euthymic Depressed Manic Hypomanic Mixed	1.8 (2.8)	 4.4 (3.6) 25.5 (7.9) - 10.3 (3.1) 25	NA	NA
YMRS Euthymic Depressed Manic Hypomanic Mixed	0.4 (1.1)	 1.9 (2.7) 2.2 (2.6) - 16.3 (2.3) 17	NA	NA
Psychosis (% Yes)	NA	26 (66.7%)	NA	NA
Medication use (N)* Unmedicated Lithium Anticonvulsants Antidepressants Antipsychotics Anxiolytics	NA	 5 11 18 15 23 9	NA	NA

Number of episodes Depressed Manic Hypomanic Mixed Total	NA	 16.3 (23.2) 5.2 (7.9) 3.6 (9.1) 13.1 (21.1) 37.8 (47.9)	NA	NA
Lifetime psychiatric diagnoses (N) Panic Disorder Agoraphobia Social Phobia OCD PTSD GAD Anorexia nervosa Binge eating disorder Alcohol dependence Alcohol abuse Substance dependence Substance abuse	NA	 11 10 11 1 7 4 1 2 5 11 7 11	NA	NA

* Most participants were on polypharmacy, therefore totals of medication counts are greater than sample size

BMI- Body mass index; MADRS- Montgomery Asberg Depression Rating Scale; YMRS- Young Mania Rating Scale; OCD- Obsessive Compulsive Disorder; PTSD- Post Traumatic Stress Disorder; GAD- Generalized Anxiety Disorder.

Table 2. Verbal memory, as predicted by ICM content and Diagnostic group (Control n=58 vs. BD n=39).

Cortical Region	*Left hemisphere*					*Right Hemisphere*				
	Model Adjusted R^2	Model p	ICM p	DX p	ICM*DX p	Model Adjusted R^2	Model p	ICM p	DX p	ICM*DX p
Caudal Middle Temporal Cortex	0.158	0.0003***	0.0002***	0.003**	0.004**	0.094	0.007**	0.008**	0.03*	0.04*
Rostral Middle Temporal Cortex	0.128	0.001**	0.001**	0.03*	0.04*	0.126	0.001**	0.002**	0.06	0.07
Ventral Inferior Parietal Cortex	0.128	0.001**	0.002**	0.04*	0.049*	0.137	0.0008***	0.0009***	0.02*	0.02*
Dorsal Inferior Parietal Cortex	0.166	0.0002***	0.0002***	0.01*	0.02*	0.104	0.004**	0.005**	0.04*	0.04*
Superior Parietal Cortex	0.160	0.0002*	0.0002***	0.02*	0.02*	0.131	0.001**	0.001**	0.02*	0.02*
Medial Superior Parietal Cortex	0.149	0.0004***	0.0005***	0.03*	0.03*	0.133	0.001**	0.001**	0.02*	0.03*
Caudal Dorsolateral Prefrontal Cortex	0.126	0.001**	0.002**	0.02*	0.03*	0.150	0.0004**	0.0004***	0.009**	0.01*
Caudal Dorsomedial Prefrontal Cortex	0.137	0.0008***	0.001**	0.02*	0.03*	0.128	0.001**	0.002**	0.03*	0.03*
Rostral Ventrolateral Prefrontal Cortex	0.140	0.0007***	0.001**	0.05	0.06	0.088	0.009**	0.02*	0.17	0.20
Rostral Dorsolateral Inferior Prefrontal Cortex	0.182	0.00007***	0.00006***	0.004**	0.006**	0.130	0.001**	0.001**	0.02*	0.02*
Rostral Dorsolateral Superior Prefrontal Cortex	0.106	0.004**	0.007**	0.10	0.12	0.075	0.02*	0.03*	0.16	0.18

Rostral Dorsal Prefrontal Cortex	0.102	0.005**	0.009**	0.12	0.15	0.105	0.004**	0.006**	0.05	0.06
Rostral Medial Prefrontal Cortex	0.104	0.004**	0.005**	0.04*	0.049*	0.085	0.01*	0.03*	0.23	0.27

ICM and Diagnosis predicted Verbal Memory performance throughout the cortical network identified *a priori.*
Predictions in the left caudal middle temporal cortex, left dorsal inferior parietal cortex, and bilateral ventral inferior parietal, superior parietal, medial superior parietal, caudal dorsolateral prefrontal, caudal dorsomedial prefrontal, and dorsolateral prefrontal cortices all survived Bonferroni correction.
The interaction term (ICM*DX) is the main outcome of interest and a significant p value for this interaction term denotes a differential relationship between ICM and Verbal Memory, by diagnostic group.

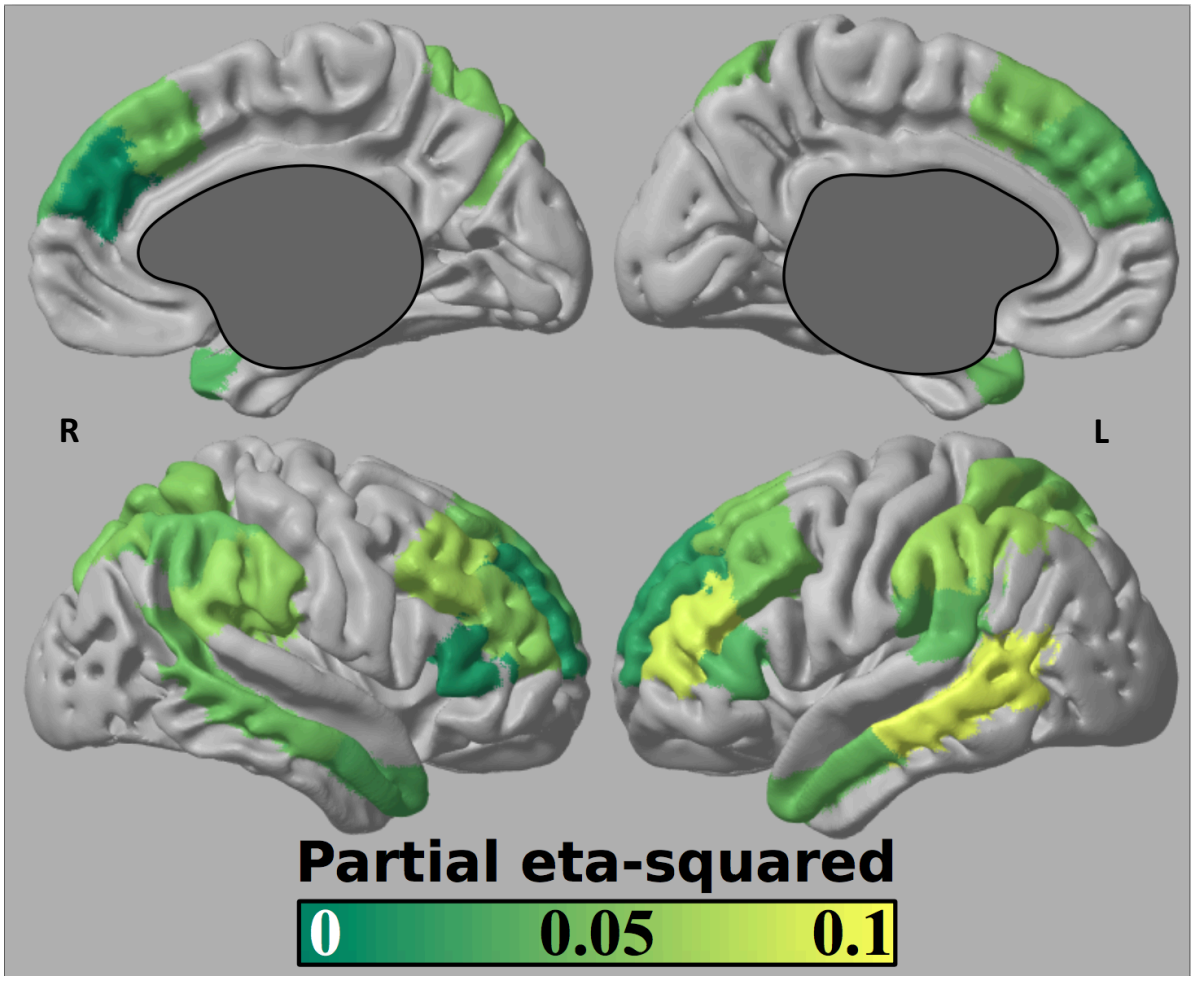
BD- Bipolar Disorder, DX- Diagnosis (Control vs. Bipolar Disorder)

Table 3. Pearson's R correlation between Verbal memory- initial correct hits and ICM in controls and BD subjects.

Cortical Region	*Healthy Control (N=58)*				*Bipolar Disorder (N=39)*			
	Left Hemisphere		Right Hemisphere		Left Hemisphere		Right Hemisphere	
	R	P	R	P	R	P	R	P
Caudal Middle Temporal Cortex	0.02	0.87	0	0.99	0.21	0.002**	0.12	0.02*
Rostral Middle Temporal Cortex	0.05	0.69	0.1	0.43	0.12	0.02*	0.17	0.006**
Ventral Inferior Parietal Cortex	0.08	0.56	0.05	0.72	0.14	0.01*	0.19	0.004**
Dorsal Inferior Parietal Cortex	0.08	0.53	0.04	0.78	0.17	0.006**	0.15	0.01*
Superior Parietal Cortex	0.08	0.57	0.05	0.73	0.15	0.01*	0.20	0.003**
Medial Superior Parietal Cortex	0.11	0.41	0.06	0.64	0.15	0.01*	0.18	0.004**
Caudal Dorsolateral Prefrontal Cortex	0.05	0.73	0.060	0.675	0.154	0.008**	0.243	0.001**
Caudal Dorsomedial Prefrontal Cortex	0.13	0.34	0.07	0.58	0.17	0.006**	0.19	0.003**
Rostral Ventrolateral Prefrontal Cortex	0.12	0.37	0.08	0.57	0.16	0.008**	0.13	0.01*
Rostral Dorsolateral Inferior Prefrontal Cortex	0.06	0.66	0.05	0.70	0.20	0.003**	0.17	0.006**
Rostral Dorsolateral Superior Prefrontal Cortex	0.10	0.47	0.05	0.69	0.11	0.03*	0.10	0.03*
Rostral Dorsal Prefrontal Cortex	0.12	0.37	0.06	0.65	0.10	0.03*	0.14	0.01*
Rostral Medial Prefrontal Cortex	0.05	0.72	0.13	0.32	0.17	0.006**	0.07	0.05

ICM is correlated with verbal memory initial correct hits in BD subjects in the left caudal middle temporal, right superior parietal, right caudal dorsolateral prefrontal, right caudal dorsomedial prefrontal, and left dorsolateral prefrontal cortices, following Bonferroni correction (Pearson's R, $p<0.004$, highlighted in red above). It is notable that prior to Bonferroni correction, significant correlations are seen bilaterally throughout this network, defined *a priori*. ICM is not associated with verbal memory initial correct hits in healthy controls in any cortical region across the network.

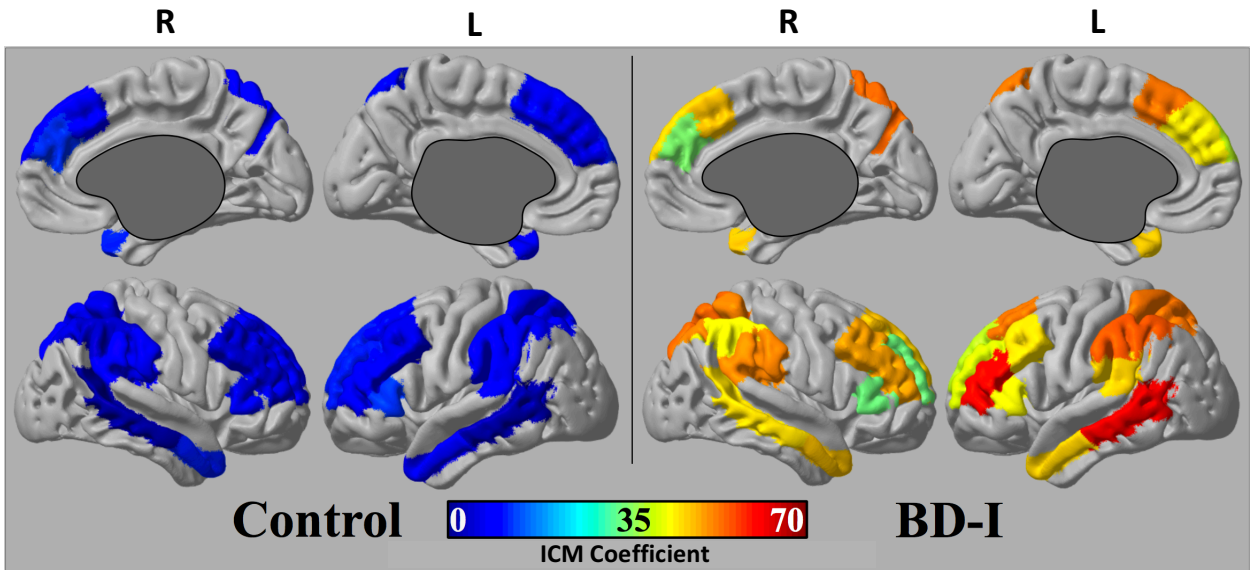
Figure 1. Partial η^2 as an estimate of effect size for the ICM signal x Diagnosis interaction term, in the prediction of verbal memory domain score.



Larger partial η^2 values suggest that the ICM signal x Diagnosis interaction term explains a greater degree of variance in verbal memory score. Thus, increased partial η^2 could be highlighting areas of strongest differences between control and BD subjects. The largest effect sizes are observed in the left caudal middle temporal cortex and the left dorsolateral prefrontal cortex. Small to medium effect sizes are observed throughout the verbal memory-related cortical network, defined *a priori* from the literature.

L: left hemisphere, R: right hemisphere, BD; Bipolar Disorder, ICM: intracortical myelin

Figure 2. The slope of the linear relationship between ICM and verbal memory performance in controls and BD subjects.



Each population was fitted individually to find the coefficient for the ICM term. On the left of the figure, the Control population shows a globally blunted slope of verbal memory performance over ICM-related T_1-weighted signal, with values ranging between 3.8-13.9. On the right, the BD population displays values between 35.2 – 64.4, suggesting a much greater influence of ICM signal on verbal memory performance in BD, in comparison to controls.

L: left hemisphere, R: right hemisphere, BD: Bipolar Disorder, ICM: intracortical myelin

SUPPLEMENTARY MATERIAL

Supplementary Methods

Intracortical Myelin Image Processing Methods

Image processing was performed predominantly in MIPAV v7.0.1 software (mipav.cit.nih.gov) using the JIST v3.0 (www.nitrc.org/projects/jist, TOADS-CRUISE vR3c (www.nitrc.org/projects/toads-cruise), and CBS High-Res Brain Processing Tools Version v3.0 (www.nitrc.org/projects/cbs-tools) plug-ins, and Amira v5.2 software (Visage Imaging). All processing was performed on the ratio image.

First, a mask of the cerebrum, created from the SPECTRE 2010 algorithm [1] in MIPAV, was used to skull strip the ratio image. The skull-stripped ratio image was used as input to the Multiple Object Geometric Deformable Model (MGDM) Multi-contrast Brain Segmentation algorithm [2,3] in MIPAV to generate initial probabilistic labels for tissue classes for each hemisphere of the brain. The probability labels generated for cerebral GM and white matter (WM) were used as the input to the CRUISE algorithm [4] to generate smoothed, topologically correct labels for the cerebrum. The CRUISE algorithm was performed in each hemisphere separately.

Subcortical structures and ventricles (as identified by the MGDM algorithm) were then removed from the labels for the left and right cerebrums and the labels remaining were combined. At this point, all GM segmentations were inspected and manual edits were made to replace missing cortex and to remove remaining dura mater arising from potential errors in the MGDM and CRUISE algorithms to ensure an accurate pial surface. This label for the entire cerebrum without the subcortical structures was then used to mask the Ratio image such that it only contained the cerebral cortex and underlying major white matter tracts.

The manually corrected, cerebrum-masked Ratio image was next segmented into two main tissue classes: WM and GM, taking into account lightly and heavily myelinated components of GM [5] using the FANTASM algorithm [6] in MIPAV, as we found that this yields a more accurate WM label for our ICM analysis than the output from CRUISE. The hemispheres were segmented together to avoid potential

hemispheric bias, such that the algorithm used the entire cerebrum for its classification. Following segmentations, labels for each tissue class were split back into left and right hemispheres for subsequent processing. The labels near the GM/WM-boundary were morphologically processed to remove all WM tissue not connected to the largest WM mass.

The corrected GM labels from CRUISE representing the pial surface, and WM labels from FANTASM representing the GM/WM boundary surface were used as inputs to a volume-preserving cortical depth model to generate an intracortical surface at the ½ cortical depth to sample the T_1-weighted image signal [7,8] (**Supplementary Figure 1)**. Each subject's ½ depth surface was registered to the ½ depth surface generated from the MNI-152 atlas using a multi-contrast multi-scale surface registration approach [9]. In the analysis, the signal was first sampled onto the individual subject's surface, which was then deformed to be in register with the MNI-152 equivalent surface.

References

1. Carass A, Cuzzocreo J, Wheeler MB, Bazin P-L, Resnick SM, Prince JL. Simple paradigm for extra-cerebral tissue removal: algorithm and analysis. *NeuroImage.* 2011;56(4):1982-1992.
2. Bazin P-L, Weiss M, Dinse J, Schäfer A, Trampel R, Turner R. A computational framework for ultra-high resolution cortical segmentation at 7Tesla. *Neuroimage.* 2014;93:201-209.
3. Bogovic JA, Prince JL, Bazin P-L. A multiple object geometric deformable model for image segmentation. *Computer Vision and Image Understanding.* 2013;117(2):145-157.
4. Han X, Pham DL, Tosun D, Rettmann ME, Xu C, Prince JL. CRUISE: cortical reconstruction using implicit surface evolution. *NeuroImage.* 2004;23(3):997-1012.
5. Rowley CD, Bazin P-L, Tardif CL, et al. Assessing intracortical myelin in the living human brain using myelinated cortical thickness. *Frontiers in neuroscience.* 2015;9.
6. Pham DL. Robust fuzzy segmentation of magnetic resonance images. Paper presented at: Computer-Based Medical Systems, 2001. CBMS 2001. Proceedings. 14th IEEE Symposium on2001.
7. Waehnert M, Dinse J, Weiss M, et al. Anatomically motivated modeling of cortical laminae. *Neuroimage.* 2014;93:210-220.
8. Rowley CD, Sehmbi M, Bazin PL, et al. Age-related mapping of intracortical myelin from late adolescence to middle adulthood using T1-weighted MRI. *Human Brain Mapping.* 2017.
9. Tardif CL, Schäfer A, Waehnert M, Dinse J, Turner R, Bazin P-L. Multi-contrast multi-scale surface registration for improved alignment of cortical areas. *Neuroimage.* 2015;111:107-122.

Supplementary Table 1. CNS-Vital Signs domain score calculation.

Cognitive Domain	Domain Score Calculations
Psychomotor Speed	FTT Right Taps Average + FTT Left Taps Average + SDC Correct Responses
Reaction Time	(ST Complex Reaction Time Correct + ST Reaction Time Correct) / 2
Processing Speed	SDC Correct Responses - SDC Errors
Verbal Memory	VBM Correct Hits Immediate + VBM Correct Passes Immediate + VBM Correct Hits Delay + VBM Correct Passes Delay
Simple Attention	CPT Correct Responses - CPT Commission Errors
Motor Speed	FTT Right Taps Average + FTT Left Taps Average

Domain score calculations per cognitive domain. Obtained from the CNSVS Brief Interpretation Guide.

Supplementary Table 2. CNS-Vital Signs validity indicator algorithm.

Cognitive Domain	Validity Criteria
Verbal Memory	VBM Correct Hits Immediate + VBM Correct Passes Immediate + VBM Correct Hits Delay + VBM Correct Passes Delay >30
Finger Tapping Test	FTT Right Taps Average + FTT Left Taps Average >=40
Symbol Digit Coding	SDC Correct Responses >=20 AND Correct Responses > Errors*
Stroop Test	[Simple RT < (Complex RT Correct *0.1) + Complex RT Correct] AND [Complex RT Correct < (Stroop RT Correct *0.1) +Stroop RT Correct] AND (Complex Correct > Complex Errors) AND (Stroop Correct > Stroop Errors)
Continuous Performance Test	CPT Correct Responses >= 30 AND CPT Correct Responses > CPT Commission Errors*
Executive Function	Number of errors <= 3

Domain score calculations per cognitive domain. Obtained from the CNSVS Brief Interpretation Guide.
*Errors are also denoted as incorrect responses.

Supplementary Table 3. Cognitive domain score by group.

Cognitive Domain	CONTROL Mean (SD) Range	BIPOLAR DISORDER Mean (SD) Range	Test statistic	P value
Psychomotor Speed	186.4 (30.5) 63-237	181.9 (22.5) 134-227	t = -1.06	0.29
Reaction Time	623.1 (77.9) 502-912	666.1 (113.9) 481-945	t = -1.72	0.09
Processing Speed	66.4 (12.5) 38-101	63.9 (13.3) 39-89	t = -0.93	0.36
Verbal Memory	53.7 (4.2) 40-60	51.1 (6.7) 33-60	W = 900.5	0.09
Simple Attention	38.2 (5.2) 7-40	38.3 (5.6) 8-40	W = 1320.5	0.11
Motor Speed	122.3 (7.8) 89-167	117.1 (16.1) 83-152	t = -1.46	0.15
Executive Function *	62.2 (22.2) 27.3 -117.3	78.9 (32.1) 40.5-182.1	t = 1.57	0.12

Cognitive performance (raw scores) descriptive by group and cognitive domain (N=58 healthy controls, N=39 Bipolar-type I subjects).
* Trails B, time of completion in seconds.

Supplementary Table 4. Association between demographic and clinical variables and cognition performance in control and BD subjects.

CONTROL (n=58)								
		Verbal memory	**Psychomotor speed**	**Motor speed**	**Reaction time**	**Processing speed**	**Simple attention**	**Executive Function**
Age	Statistic	R=0.1	R=-0.2	R=-0.01	R=0.15	R=-0.29	R=0.13	R=-0.10
	p	0.47	0.14	0.94	0.27	0.03*	0.32	0.43
Sex	Statistic	W=379.5	t=0.15	t=-1.1	t=-0.38	t=1.21	W=491	t=-2.14
	p	0.53	0.88	0.27	0.70	0.23	0.23	0.04*
BMI	Statistic	R=0.05	R=-0.12	R=-0.14	R=0.16	R=-0.24	R=0.07	R=0.008
	P	0.72	0.38	0.30	0.22	0.07	0.60	0.95
Education (years)	Statistic	R=-0.12	R=-0.09	R=-0.15	R=0.03	R=0.08	R=0.06	R=-0.11
	P	0.37	0.52	0.27	0.80	0.53	0.66	0.40
WASI 2-subtest IQ	Statistic	R=0.18	R=0.24	R=0.21	R=-0.23	R=0.29	R=0.03	R=-0.26
	P	0.18	0.07	0.11	0.09	0.03*	0.81	0.05
BIPOLAR DISORDER (n=39)								
		Verbal memory	**Psychomotor speed**	**Motor speed**	**Reaction time**	**Processing speed**	**Simple attention**	**Executive Function**
Age	Statistic	R=-0.01	R=-0.15	R=0.09	R=0.01	R=-0.39	R=0.12	R=-0.19
	p	0.56	0.37	0.58	0.94	0.01*	0.46	0.24
Sex	Statistic	W=270.5	t=1.71	t=0.94	t=-3.5	t=1.8	W=215.5	t=2.1
	p	0.02*	0.09	0.35	0.001**	0.08	0.37	0.05
BMI	Statistic	R=0.11	R=-0.01	0.11	R=-0.12	R=-0.16	R=-0.15	R=-0.14
	p	0.49	0.95	0.49	0.46	0.34	0.37	0.39
Education (years)	Statistic	R=0.34	R=-0.14	R=-0.17	R=-0.15	R=-0.04	R=0.06	R=-0.32
	p	0.04*	0.39	0.31	0.35	0.83	0.72	0.05
WASI 2 subtest IQ	Statistic	0.43	R=0.4	R=0.13	R=-0.42	R=0.53	R=0.25	R=-0.37
	p	0.007**	0.01*	0.42	0.008**	0.0005***	0.13	0.02*
Mood state	Statistic	Kruskal-Wallis χ^2=1.9	F=0.93	F=0.42	F=1.1	F=1.8	Kruskal-Wallis χ^2=3.2	F=0.23

	p	0.59	0.44	0.74	0.38	0.17	0.36	0.88
Lithium	Statistic	R= -0.1	R=-0.02	R=0.13	R=0.15	R=0.20	R=-0.06	R=-0.07
	p	0.53	0.91	0.43	0.35	0.23	0.70	0.65
Anticonvulsants	Statistic	R= 0.06	R=0.21	R=0.17	R=-0.14	R=0.16	R=0.18	R=-0.01
	p	0.72	0.20	0.30	0.39	0.31	0.26	0.94
Antidepressants	Statistic	R=0.15	R=0.06	R=-0.02	R=-0.13	R=0.11	R=0.04	R=-0.008
	p	0.38	0.71	0.92	0.45	0.49	0.79	0.96
Antipsychotics	Statistic	R=-0.12	R=-0.43	R=-0.36	R=0.26	R=-0.30	R=0.09	R=0.19
	p	0.48	0.006**	0.02*	0.11	0.07	0.58	0.24
Anxiolytics	Statistic	R=-0.20	R=-0.09	R=0.13	R=0.33	R=-0.30	R=0.01	R=0.27
	p	0.23	0.59	0.41	0.04*	0.06	0.93	0.10
Total medication load	Statistic	R=-0.06	R=-0.11	R=-0.005	R=0.16	R=-0.18	R=0.10	R=0.12
	p	0.71	0.50	0.97	0.33	0.26	0.51	0.46
Total medication count	Statistic	R=-0.20	R=-0.16	R=-0.05	R=0.20	R=-0.23	R=0.03	R=0.19
	p	0.23	0.32	0.77	0.22	0.16	0.83	0.25

Pearson's R was used for continuous variables. Unpaired t test/Mann-Whitney test used for categorical variables. Cognitive performance was associated with age, sex, years of education, and WASI-2 subtest IQ in both BD and control subjects, and antipsychotic and anxiolytic use in BD subjects. None of these associations survived Bonferroni correction.

Supplementary Table 5. Processing Speed domain score, as predicted by ICM and Diagnostic group (Control, n=58, vs. BD, n=39).

Cortical Region	Left hemisphere					Right Hemisphere				
	Model Adjusted R^2	Model p	ICM p	DX p	ICM*DX p	Model Adjusted R^2	Model p	ICM p	DX p	ICM*DX p
Ventral Inferior Parietal Cortex	0.004	0.34	0.37	0.13	0.13	0.009	0.28	0.34	0.10	0.11
Posterior Cingulate Cortex	**0.051**	**0.04***	**0.01***	**0.01***	**0.01 ***	0.048	0.06	0.01*	0.08	0.08
Mid Cingulate Cortex	**0.059**	**0.03***	**0.01***	**0.006****	**0.007****	0.044	0.07	0.02*	0.02*	0.02*
Anterior Cingulate Cortex	**0.042**	**0.07**	**0.02***	**0.03***	**0.03***	0.050	0.05	0.009 **	0.03*	0.03*
Dorsolateral Premotor Cortex	0.002	0.37	0.52	0.18	0.19	0.016	0.21	0.31	0.07	0.08
Dorsomedial Premotor Cortex	0.012	0.25	0.76	0.20	0.21	0.011	0.26	0.67	0.18	0.19
Caudal Dorsolateral Prefrontal Cortex	0.011	0.26	0.87	0.22	0.24	0.009	0.28	0.40	0.11	0.12
Caudal Dorsomedial Prefrontal Cortex	0.005	0.33	0.72	0.24	0.26	0.012	0.26	0.53	0.13	0.14
Rostral Ventrolateral Prefrontal Cortex	-0.020	0.78	0.88	0.65	0.68	-0.010	0.56	0.87	0.41	0.43
Rostral Dorsolateral Inferior Prefrontal Cortex	-0.011	0.58	0.52	0.29	0.31	-0.003	0.44	0.30	0.16	0.18
Rostral Dorsolateral Superior Prefrontal Cortex	-0.018	0.72	0.83	0.54	0.56	-0.002	0.43	0.46	0.19	0.20
Rostral Dorsal Prefrontal Cortex	-0.019	0.75	0.99	0.69	0.72	-0.018	0.72	0.95	0.71	0.73

ICM and Diagnosis predicted Processing Speed domain score in the left posterior, mid, and anterior cingulate cortices, although this does not survive correction for multiple comparisons. The interaction term (ICM*DX) is the main outcome of interest and a significant *p*-value for this term denotes a differential relationship between ICM and processing speed by diagnostic group.
BD- Bipolar Disorder, DX- Diagnosis (Control vs. BD)

Supplementary Table 6. Executive function, as predicted by ICM and Diagnostic group (Control, n=58, vs. BD, n=39

Cortical Region	Left hemisphere					Right Hemisphere				
	Model Adjusted R^2	Model p	ICM p	DX p	ICM*DX p	Model Adjusted R^2	Model p	ICM p	DX p	ICM*DX p
Caudal Middle Temporal Cortex	0.038	0.09	0.08.	0.04*	0.04*	0.030	0.12	0.18	0.06	0.07
Posterior Cingulate Cortex	0.037	0.09	0.15	0.04*	0.04*	0.025	0.15	0.38	0.09	0.10
Mid Cingulate Cortex	**0.059**	**0.03***	**0.09**	**0.01***	**0.01***	**0.077**	**0.02***	**0.16**	**0.006****	**0.008****
Anterior Cingulate Cortex	0.031	0.12	0.13	0.06	0.06	**0.078**	**0.01***	**0.05**	**0.004****	**0.005****
Dorsolateral Premotor Cortex	0.023	0.16	0.21	0.09	0.10	0.038	0.09	0.10	0.04*	0.04*
Dorsomedial Premotor Cortex	0.032	0.11	0.15	0.05	0.06	0.038	0.09	0.12	0.04*	0.04*
Caudal Dorsolateral Prefrontal Cortex	0.012	0.25	0.34	0.18	0.20	0.045	0.06	0.07	0.02*	0.03*
Caudal Dorsomedial Prefrontal Cortex	0.021	0.18	0.29	0.11	0.13	0.041	0.07	0.09	0.03*	0.03*
Rostral Ventrolateral Prefrontal Cortex	Assumptions not met					0.025	0.15	0.18	0.08	0.09
Rostral Dorsolateral Inferior Prefrontal Cortex	0.028	0.13	0.23	0.07	0.08	0.026	0.14	0.23	0.08	0.09
Rostral Dorsolateral Superior Prefrontal Cortex	0.018	0.19	0.28	0.12	0.13	0.021	0.18	0.33	0.11	0.13
Rostral Dorsal Prefrontal Cortex	0.005	0.33	0.45	0.28	0.31	0.002	0.36	0.50	0.34	0.37
Rostral Medial Prefrontal Cortex	0.023	0.16	0.16	0.09	0.10	0.033	0.10	0.21	0.05	0.06
Ventromedial Orbito Frontal Cortex	0.028	0.13	0.50	0.11	0.12	0.032	0.11	0.18	0.06	0.06

ICM and Diagnosis predicted Executive function (Trails B, time of completion) performance in the bilateral midcingulate cortex and right anterior cingulate cortex, although this does not survive Bonferroni correction.
The interaction term (ICM*DX) is the main outcome of interest and a significant *p*-value for this interaction term denotes a differential relationship between ICM and Verbal Memory by diagnostic group.
The data did not meet the assumptions of linear regression in the left rostral ventrolateral prefrontal cortex.
BD- Bipolar Disorder, DX- Diagnosis (Control vs. Bipolar Disorder)

Supplementary Table 7. Psychomotor speed domain score, as predicted by ICM in controls and BD.

Cortical Region	Healthy Control (N=58)				Bipolar Disorder (N=39)			
	Left Hemisphere		Right Hemisphere		Left Hemisphere		Right Hemisphere	
	Adjusted R^2	P	Adjusted R^2	P	Adjusted R^2	P	Adjusted R^2	P
Rostral middle temporal cortex	0.002	0.30	-0.002	0.36	-0.015	0.52	-0.016	0.54
Posterior cingulate cortex	-0.016	0.75	-0.017	0.81	0.024	0.17	0.041	0.11
Midcingulate cortex	-0.018	1	-0.017	0.83	0.031	0.15	0.034	0.14
Anterior cingulate cortex	-0.016	0.75	-0.011	0.56	-0.0001	0.33	0.041	0.11
Ventral motor cortex	-0.017	0.85	-0.01	0.53	-0.025	0.77	-0.025	0.77
Dorsolateral motor cortex	-0.011	0.54	-0.014	0.64	-0.027	0.91	-0.021	0.63
Dorsomedial motor cortex	-0.016	0.75	-0.01	0.50	-0.02	0.63	-0.019	0.59
Caudal dorsolateral prefrontal cortex	-0.006	0.41	-0.009	0.49	-0.022	0.67	-0.022	0.70
Rostral dorsolateral superior prefrontal cortex	-0.018	0.96	-0.015	0.69	-0.02	0.61	-0.027	0.99
Rostral dorsal prefrontal cortex	-0.018	0.96	-0.018	0.99	0.012	0.23	-0.003	0.36
Rostral medial prefrontal cortex	0.003	0.28	-0.012	0.59	-0.027	1	-0.026	0.82

ICM did not predict Psychomotor Speed domain score in controls or BD subjects in networks identified *a priori*.

Supplementary Table 8. Motor speed, as predicted by ICM in controls and BD.

Cortical Region	Healthy Control (N=58)				Bipolar Disorder (N=39)			
	Left Hemisphere		Right Hemisphere		Left Hemisphere		Right Hemisphere	
	Adjusted R^2	P	Adjusted R^2	P	Adjusted R^2	P	Adjusted R^2	P
Ventral Motor Cortex	-0.003	0.36	-0.008	0.47	-0.022	0.66	-0.025	0.77
Dorsolateral Motor Cortex	-0.010	0.51	0.002	0.30	-0.022	0.68	-0.027	0.99
Dorsomedial Motor Cortex	-0.005	0.41	-0.007	0.45	-0.024	0.75	-0.026	0.88
Ventrolateral Orbito Frontal Cortex	-0.017	0.85	-0.016	0.76	-0.009	0.43	-0.025	0.79
Ventral Orbito Frontal Cortex	-0.010	0.52	-0.006	0.41	0.003	0.30	-0.015	0.52
Ventromedial Orbito Frontal Cortex	-0.014	0.65	0.010	0.21	-0.025	0.77	-0.027	0.91
Ventromedial Prefrontal Cortex	-0.013	0.60	-0.017	0.81	-0.010	0.44	-0.020	0.61

ICM did not predict motor speed in either controls or BD subjects within the networks identified *a priori*.

Supplementary Table 9. Reaction Time domain score, as predicted by ICM in controls and BD.

Cortical Region	Healthy Control (N=58)				Bipolar Disorder (N=39)			
	Left Hemisphere		Right Hemisphere		Left Hemisphere		Right Hemisphere	
	Adjusted R^2	P	Adjusted R^2	P	Adjusted R^2	P	Adjusted R^2	P
Posterior cingulate cortex	-0.013	0.60	-0.017	0.84	**0.106**	**0.02***	0.069	0.06
Anterior cingulate cortex	-0.018	0.92	0.009	0.23	0.075	0.05	0.017	0.21
Ventral motor cortex	-0.016	0.75	-0.005	0.41	0.072	0.05	-0.009	0.42
Dorsolateral motor cortex	-0.010	0.50	-0.009	0.48	0.053	0.09	0.057	0.08
Dorsomedial motor cortex	0.003	0.29	0.003	0.29	**0.122**	**0.02***	**0.098**	**0.03***
Dorsolateral premotor cortex	-0.013	0.60	0.006	0.26	0.045	0.10	0.053	0.08
Dorsomedial premotor cortex	0.000	0.32	-0.010	0.51	0.036	0.13	0.027	0.16
Caudal Dorsolateral Prefrontal Cortex	-0.015	0.71	-0.002	0.34	0.030	0.15	0.041	0.11
Caudal Dorsomedial Prefrontal Cortex	0.003	0.28	0.001	0.31	0.014	0.22	0.050	0.09
Mid Cingulate Cortex	-0.008	0.46	-0.006	0.42	**0.127**	**0.01***	0.055	0.08
Rostral Ventrolateral Prefrontal Cortex	-0.016	0.77	-0.010	0.50	0.038	0.12	-0.020	0.63
Rostral Dorsolateral Inferior Prefrontal Cortex	-0.013	0.60	-0.009	0.50	0.020	0.19	-0.010	0.43
Rostral Dorsal Prefrontal Cortex	-0.012	0.56	-0.017	0.86	-0.026	0.82	-0.020	0.61
Rostral Medial Prefrontal Cortex	-0.001	0.34	-0.003	0.36	0.039	0.12	0.011	0.24
Ventrolateral Orbito Frontal Cortex	-0.016	0.76	-0.015	0.68	0.056	0.08	-0.002	0.34
Ventral Orbito Frontal Cortex	-0.015	0.71	-0.014	0.65	0.028	0.16	-0.026	0.82

ICM predicted Reaction Time domain score in BD subjects in the left posterior and midcingulate cortices, and the bilateral dorsomedial motor cortex, although this does not survive Bonferroni correction. ICM does not predict reaction time in controls in any cortical region across the *a priori* identified network.

Supplementary Table 10. Verbal memory- initial correct passes, correlations with ICM in controls and BD.

Cortical Region	Healthy Control (N=58)				Bipolar Disorder (N=39)			
	Left Hemisphere		Right Hemisphere		Left Hemisphere		Right Hemisphere	
	R	P	R	P	R	P	R	P
Caudal Middle Temporal Cortex	-0.03	0.84	0.07	0.62	0.11	0.50	0.03	0.84
Rostral Middle Temporal Cortex	0.02	0.86	0.04	0.74	0.11	0.51	0.03	0.86
Ventral Inferior Parietal Cortex	-0.03	0.81	0.07	0.60	0.13	0.43	0.04	0.82
Dorsal Inferior Parietal Cortex	0.05	0.69	0.02	0.87	0.19	0.24	0.04	0.83
Superior Parietal Cortex	-0.01	0.96	0.03	0.82	0.22	0.18	0.12	0.47
Medial Superior Parietal Cortex	0.00	0.97	0.04	0.79	0.19	0.26	0.09	0.57
Caudal Dorsolateral Prefrontal Cortex	0.05	0.71	0.05	0.70	0.20	0.23	0.07	0.69
Caudal Dorsomedial Prefrontal Cortex	0.02	0.88	0.08	0.57	0.22	0.19	0.09	0.60
Rostral Ventrolateral Prefrontal Cortex	0.09	0.50	0.10	0.48	0.15	0.36	0.02	0.91
Rostral Dorsolateral Inferior Prefrontal Cortex	0.04	0.74	0.07	0.58	0.26	0.12	0.10	0.55
Rostral Dorsolateral Superior Prefrontal Cortex	0.10	0.44	0.04	0.78	0.20	0.23	0.00	0.99
Rostral Dorsal Prefrontal Cortex	0.07	0.61	0.10	0.44	0.21	0.21	0.20	0.23
Rostral Medial Prefrontal Cortex	0.00	0.99	0.06	0.66	0.13	0.43	0.04	0.83

ICM was not associated with verbal memory initial correct passes in controls or BD subjects in any cortical region across the *a priori* identified network (Pearson's R, $p>0.05$).
R- Pearson's R.

Supplementary Table 11. Verbal memory- initial reaction time, correlations with ICM in controls and BD.

Cortical Region	Healthy Control (N=58)				Bipolar Disorder (N=39)			
	Left Hemisphere		Right Hemisphere		Left Hemisphere		Right Hemisphere	
	R	P	R	P	R	P	R	P
Caudal Middle Temporal Cortex	-0.17	0.19	-0.02	0.87	-0.23	0.17	-0.15	0.37
Rostral Middle Temporal Cortex	-0.05	0.72	0.05	0.70	-0.13	0.43	-0.15	0.37
Ventral Inferior Parietal Cortex	-0.08	0.55	-0.06	0.65	-0.25	0.13	-0.15	0.37
Dorsal Inferior Parietal Cortex	-0.01	0.94	-0.02	0.87	-0.17	0.32	-0.16	0.33
Superior Parietal Cortex	-0.04	0.79	0.03	0.81	-0.20	0.22	-0.17	0.29
Medial Superior Parietal Cortex	-0.04	0.78	-0.10	0.44	-0.13	0.43	-0.24	0.15
Caudal Dorsolateral Prefrontal Cortex	-0.05	0.72	-0.03	0.83	-0.08	0.65	-0.08	0.63
Caudal Dorsomedial Prefrontal Cortex	-0.06	0.68	-0.07	0.61	-0.15	0.38	-0.14	0.41
Rostral Ventrolateral Prefrontal Cortex	0.04	0.76	-0.05	0.73	-0.14	0.40	0.03	0.86
Rostral Dorsolateral Inferior Prefrontal Cortex	0.01	0.93	-0.02	0.87	-0.09	0.57	-0.03	0.88
Rostral Dorsolateral Superior Prefrontal Cortex	-0.04	0.76	-0.07	0.60	-0.03	0.84	-0.02	0.91
Rostral Dorsal Prefrontal Cortex	-0.05	0.70	-0.07	0.63	0.01	0.97	-0.01	0.96
Rostral Medial Prefrontal Cortex	-0.06	0.64	-0.08	0.56	-0.16	0.34	-0.07	0.66

ICM was not associated with verbal memory initial reaction time in controls or BD subjects in any cortical region across the *a priori* identified network (Pearson's R, $p>0.05$).
R- Pearson's R.

Supplementary Table 12. Verbal memory- delayed correct hits, correlations with ICM in controls and BD.

Cortical Region	Healthy Control (N=58)				Bipolar Disorder (N=39)			
	Left Hemisphere		Right Hemisphere		Left Hemisphere		Right Hemisphere	
	R	P	R	P	R	P	R	P
Caudal Middle Temporal Cortex	0.09	0.51	0.05	0.72	0.35	0.03*	0.26	0.12
Rostral Middle Temporal Cortex	0.15	0.26	0.15	0.27	0.25	0.13	0.29	0.07
Ventral Inferior Parietal Cortex	0.18	0.18	0.13	0.33	0.33	0.04*	0.37	0.02*
Dorsal Inferior Parietal Cortex	0.13	0.32	0.09	0.49	0.35	0.03*	0.32	0.047*
Superior Parietal Cortex	0.15	0.27	0.10	0.44	0.35	0.03*	0.37	0.02*
Medial Superior Parietal Cortex	0.17	0.20	0.14	0.28	0.31	0.06	0.34	0.04*
Caudal Dorsolateral Prefrontal Cortex	0.15	0.28	0.14	0.30	0.32	0.048*	0.42	0.008**
Caudal Dorsomedial Prefrontal Cortex	0.18	0.17	0.13	0.32	0.33	0.04*	0.37	0.02*
Rostral Ventrolateral Prefrontal Cortex	0.23	0.09	0.17	0.20	0.35	0.03*	0.24	0.15
Rostral Dorsolateral Inferior Prefrontal Cortex	0.12	0.36	0.13	0.35	0.38	0.02*	0.33	0.04*
Rostral Dorsolateral Superior Prefrontal Cortex	0.18	0.18	0.15	0.25	0.20	0.22	0.24	0.14
Rostral Dorsal Prefrontal Cortex	0.19	0.15	0.15	0.27	0.20	0.23	0.35	0.03*
Rostral Medial Prefrontal Cortex	0.12	0.37	0.20	0.13	0.34	0.04*	0.25	0.13

ICM was associated with verbal memory delayed correct hits in the left caudal middle temporal, bilateral ventral/dorsal inferior parietal, bilateral superior parietal, right medial superior parietal, bilateral caudal dorsolateral/dorsomedial prefrontal, left rostral ventrolateral prefrontal, bilateral dorsolateral prefrontal, right rostral dorsal prefrontal, and left rostral medial prefrontal cortices. These associations do not survive Bonferroni correction, *(*p>0.004*)*.
R- Pearson's R.

Supplementary Table 13. Verbal memory- delayed correct passes, correlations with ICM in controls and BD.

Cortical Region	Healthy Control (N=58)				Bipolar Disorder (N=39)			
	Left Hemisphere		Right Hemisphere		Left Hemisphere		Right Hemisphere	
	R	P	R	P	R	P	R	P
Caudal Middle Temporal Cortex	-0.05	0.72	-0.01	0.97	0.14	0.40	0.17	0.31
Rostral Middle Temporal Cortex	-0.04	0.76	0.02	0.86	0.22	0.19	0.15	0.36
Ventral Inferior Parietal Cortex	0.00	0.98	-0.01	0.95	0.10	0.55	0.14	0.41
Dorsal Inferior Parietal Cortex	-0.01	0.95	-0.06	0.67	0.26	0.12	0.18	0.29
Superior Parietal Cortex	-0.01	0.96	0.00	1.00	0.28	0.08	0.22	0.19
Medial Superior Parietal Cortex	-0.05	0.73	-0.02	0.89	0.28	0.08	0.20	0.22
Caudal Dorsolateral Prefrontal Cortex	-0.06	0.67	-0.07	0.62	0.26	0.12	0.19	0.26
Caudal Dorsomedial Prefrontal Cortex	-0.02	0.86	0.01	0.95	0.21	0.20	0.17	0.32
Rostral Ventrolateral Prefrontal Cortex	0.02	0.90	0.00	1.00	0.15	0.36	0.17	0.30
Rostral Dorsolateral Inferior Prefrontal Cortex	0.00	1.00	-0.03	0.80	0.31	0.05	0.20	0.23
Rostral Dorsolateral Superior Prefrontal Cortex	-0.06	0.66	-0.07	0.58	0.29	0.08	0.20	0.23
Rostral Dorsal Prefrontal Cortex	-0.08	0.57	-0.10	0.45	0.25	0.12	0.26	0.12
Rostral Medial Prefrontal Cortex	0.01	0.94	-0.01	0.95	0.12	0.46	0.20	0.23

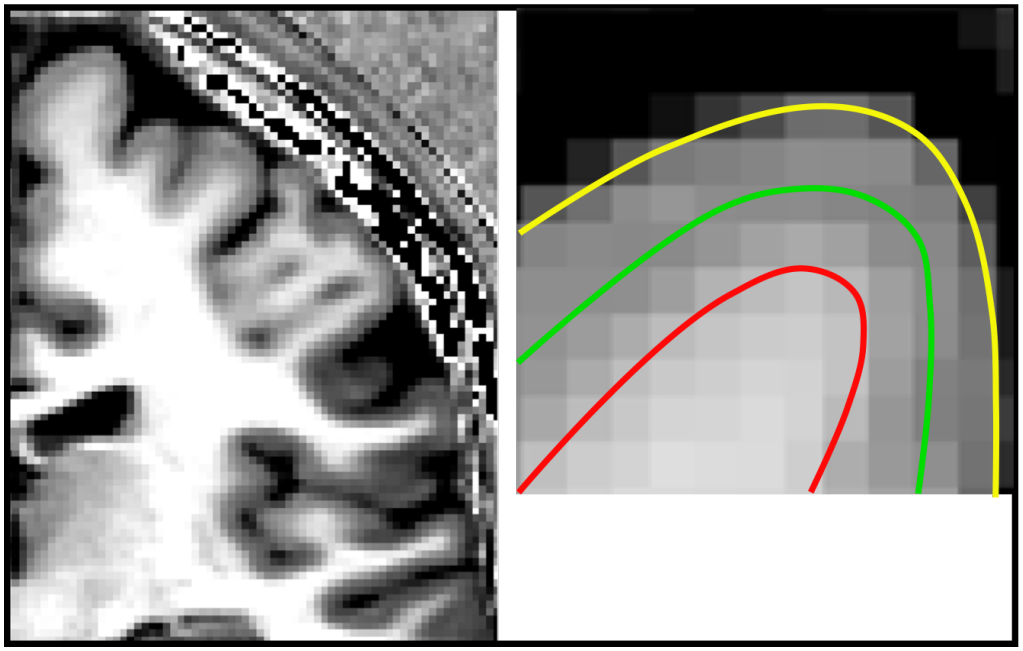
ICM was not associated with verbal memory delayed correct passes in controls or BD subjects in any cortical region across the *a priori* identified network (Pearson's R, $p>0.05$).
R- Pearson's R.

Supplementary Table 14. Verbal memory- delayed reaction time, correlations with ICM in controls and BD.

Cortical Region	Healthy Control (N=58)				Bipolar Disorder (N=39)			
	Left Hemisphere		Right Hemisphere		Left Hemisphere		Right Hemisphere	
	R	P	R	P	R	P	R	P
Caudal Middle Temporal Cortex	-0.11	0.43	-0.09	0.49	0.30	0.07	0.37	0.02*
Rostral Middle Temporal Cortex	0.00	0.98	-0.04	0.79	0.39	0.02	0.36	0.03*
Ventral Inferior Parietal Cortex	-0.08	0.56	-0.14	0.29	0.26	0.11	0.37	0.02*
Dorsal Inferior Parietal Cortex	-0.04	0.75	-0.07	0.61	0.22	0.18	0.33	0.046*
Superior Parietal Cortex	0	0.97	-0.08	0.54	0.19	0.25	0.28	0.09
Medial Superior Parietal Cortex	0	0.98	-0.10	0.43	0.24	0.15	0.20	0.23
Caudal Dorsolateral Prefrontal Cortex	-0.09	0.51	-0.12	0.35	0.29	0.08	0.37	0.02*
Caudal Dorsomedial Prefrontal Cortex	-0.13	0.32	-0.14	0.30	0.31	0.06	0.34	0.03*
Rostral Ventrolateral Prefrontal Cortex	-0.08	0.54	-0.13	0.32	0.30	0.07	0.38	0.02*
Rostral Dorsolateral Inferior Prefrontal Cortex	-0.04	0.74	-0.05	0.68	0.27	0.10	0.40	0.01*
Rostral Dorsolateral Superior Prefrontal Cortex	-0.06	0.68	-0.09	0.49	0.18	0.27	0.35	0.03*
Rostral Dorsal Prefrontal Cortex	-0.11	0.41	-0.15	0.27	0.28	0.09	0.35	0.03*
Rostral Medial Prefrontal Cortex	-0.06	0.63	-0.10	0.47	0.32	0.05	0.31	0.06

Following correction for multiple comparisons, ICM signal was associated with verbal memory delayed reaction time in right caudal middle temporal, rostral middle temporal, right ventral/dorsal inferior parietal, right caudal dorsolateral/dorsomedial prefrontal, right rostral ventrolateral prefrontal, bilateral dorsolateral inferior/superior prefrontal, and right rostral dorsal prefrontal cortices. These associations do not survive Bonferroni correction, *(*$p>0.004$).
R- Pearson's R.

Supplementary Figure 1. Half depth metric visualization.



Left: T1-weighted image with high intracortical contrast.
Right: Illustration of the half depth signal surface, which is calculated from the pial and white matter surfaces.

Chapter 4: The Association of Serum C-Reactive Protein and Intracortical Myelin and Cognitive Function

This chapter contains a compilation of work that is currently in progress and will be included in future manuscripts.

4.1 Introduction

High levels of peripheral and central (CNS) inflammation are consistently reported in Bipolar Disorder (BD) (1, 2). C-reactive protein (CRP) is a commonly studied marker. It is an acute inflammatory response protein that is produced in the liver and released into the bloodstream, and is part of a non-specific systemic inflammatory response (3). CRP has been shown to be elevated in the periphery across all states of BD, although highest levels are observed during manic states (3-8) .

This elevated inflammatory profile has further been associated with compromised structural and functional brain integrity in BD. Higher interleukin-6 (IL-6) and CRP have been associated with decreased white matter integrity (WM) throughout the brain, as measured using fractional anisotropy (FA)- an index of WM integrity in studies of diffusion tensor imaging (DTI) (9, 10). In the CNS, proinflammatory cytokines can activate microglia thereby contributing to brain atrophy, and reduced gray matter (GM) volume in both cortical and subcortical structures (11-13). They can also cause increases in tissue water that lead to increased mean diffusivity and reduced FA in DTI studies (14). Furthermore, declining levels of inflammation have been associated with preserved

integrity of WM tracts (15), which suggests a potentially etiological relationship between inflammation and WM integrity.

The energetic demands of oligodendrocytes are comparable to that of neurons, and are markedly higher during active myelination as they consume large amounts of oxygen and ATP, therefore rendering them just as vulnerable to neural damage and metabolic dysfunction (16). Alongside a high metabolic rate, oligodendrocytes also have very low concentrations of glutathione, a major CNS antioxidant, thus further compromising their ability to cope with oxidative stress on par with neurons (17, 18). They are exceptionally susceptible to the effects of neuroinflammation and oxidative stress during both oligodendrogenesis and myelination.

In moderate quantities, neuroinflammation is necessary for optimal rates of remyelination. Slightly elevated inflammation fosters a regenerative environment, as activated microglia actually promote oligodendrogenesis and oligodendrocyte differentiation (19, 20). However, during states of elevated or persistent neuroinflammation, these responses can be damaging and lead to cell death (21). The remyelination process is prone to impairment due to a shift towards anti-oligodendrogenic signals from immune cells that hinder oligodendrocyte maturation and myelination (22, 23). Notably, microglia are activated in the brain following trauma, infection, or illness, leaving oligodendrocytes particularly exposed to potential damage following these CNS insults.

Further, elevated inflammatory cytokines and oxidative stress markers in the periphery have been associated with poor cognitive performance in BD. Serum CRP

levels have been associated with poor performance on the immediate memory, language, and attention scores of the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) in BD (3). Longitudinally, baseline CRP levels were negatively associated with performance on Word Learning test over a 6-year follow-up period (24). Further, elevated expression of TNF-α, a proinflammatory cytokine, in the serum of BD subjects was associated with poorer delayed memory on the Rey Auditory Verbal Learning Test (RAVLT). Higher expression of two soluble TNF receptors (sTNFr1 and sTNFr2) was higher in euthymic BD subjects in comparison to healthy controls (25). This has been postulated as part of the reason why cognitive deficits persist into euthymia in BD (26). A recent systematic review in BD shows that CRP, IL-1 receptor antagonist, IL-6, and TNF-α and its receptors are involved in the development of cognitive dysfunction in BD. Many mechanisms of action have been put forth, such as neurogenesis, hippocampal-dependent synaptic plasticity, altered glutamatergic transmission systems, and the hypothalamic-pituitary-adrenal axis response (27-34).

The effects of inflammation on myelinated axons within the cerebral cortex (intracortical myelin, ICM) remain largely understudied, however, as most prior studies have focused on subcortical WM and GM structures. We have previously shown that the development of intracortical myelin (ICM) is compromised in BD (Sehmbi et al, under review), and that ICM is associated with performance on tasks of verbal memory, executive function, processing speed, and reaction time in BD (Sehmbi et al, under review).

4.2 Aims

In light of the breath of literature that implicates inflammation as a potential aggressor of WM-based pathology and cognitive dysfunction in BD, we aimed to measure serum CRP, and investigate its relation to ICM and cognitive performance in BD.

4.3 Methods

4.3.1 Study population

This study was approved by the Hamilton Integrated Research Ethics Board and signed informed consent was obtained from each study participant prior to commencement of the study. Images were collected in 47 individuals with a diagnosis of BD type-I and 64 matched controls. All participants were right-handed, aged-17-45, and all female subjects were premenopausal. Study participants completed the Structured Clinical Interview for DSM-IV (SCID-I) for diagnostic confirmation (35). Controls did not meet criteria for any current or lifetime Axis I psychiatric conditions. Subjects with BD-I did not meet criteria for *current* Axis I psychiatric comorbidities. We excluded individuals with unstable medical/inflammatory conditions, alcohol/substance abuse within the last year (excluding caffeine or nicotine), past/current history of neurological disorders (including head trauma and migraines), and any MRI contraindications. All participants were fluent in English, and groups were matched based on intelligence level using the Wechsler Scale of Intelligence (WASI) 2-subtest Intelligence Quotient (IQ) test.

Thirty individuals with a diagnosis of Multiple Sclerosis were recruited from the Hamilton Health Sciences Multiple Sclerosis clinic, as a positive control group for CRP analysis, considering the elevated levels of inflammatory-mediated WM damage that are characteristic of the illness. These participants did not complete any cognitive or MRI testing.

4.3.2 Imaging

The imaging methods are identical to those described previously in chapters 2 and 3.

4.3.3 Serum CRP Analysis

Blood samples were collected through venipuncture in non-EDTA collection tubes on the same day as the MRI scanning procedure. Once collected, whole-blood samples were allowed to clot at room temperature for 45 minutes, and subsequently spun at 3000rpm for 15 minutes. Serum was then aliquoted and immediately frozen at -80° C until assayed.

CRP levels were detected using ELISA (Enzyme Linked Immunosorbent Assay; R&D Systems Inc., Catalogue No: DCRP00, Range of Detection: 0.8 ng/mL – 50 ng/mL, Sensitivity: 0.022 ng/mL).

4.3.4 Cognition- CNS Vital Signs (CNSVS)

Each participant completed cognitive testing within the same week as, but not immediately following, the MRI session. This helped avoiding any stress or anxiety

related to the MRI procedure. We administered a 25-minute computerized testing battery (CNS Vital Signs, CNSVS). This battery has previously been validated across a broad age range, with established reliability in patients with mild cognitive impairment and patients with depression (36-38).

We administered the Verbal Memory, Finger Tapping, Symbol Digit Coding, Stroop, and Continuous Performance tests. Test scores were recombined by the CNSVS program to obtain overall scores for the following cognitive domains: Psychomotor speed, reaction time, processing speed, verbal memory, simple attention and motor speed (**Supplementary Table 1, Chapter 3**). Individual domain component scores were also obtained, and varied by test.

All scores used in the following analysis were deemed "valid" by the CNSVS internal assessment. Scores are considered valid when a suitable level of effort has been detected from the participant, in order to avoid potentially dubious results. This is based on a validity algorithm, outlined in **Supplementary Table 2, Chapter 3.**

The paper and pencil Trails B was administered as a measure of executive function. Scores where the participant committed 3 or fewer errors were considered valid. Time of completion, in seconds (s), was used as the outcome of interest.

4.3.5 Statistical Analysis

All statistical analyses were completed using R version 3.3.2 (https://www.r-project.org). Shapiro-Wilk and Bartlett tests determined normality and homogeneity of variances of continuous variables, respectively. Between group differences in age, sex,

BMI, years of education, WASI 2 subtest IQ, and smoking status were tested using two-tailed independent t-test, Mann-Whitney test, or chi-squared test, as appropriate.

Between group differences in CRP were tested using one-way ANOVA. The associations between CRP and ICM, and CRP and cognitive performance were testing using Pearson's R correlation.

4.4 Preliminary Results

4.4.1 Demographics

One female BD subject and two male controls were excluded from the analyses due to poor quality ICM maps. Two MS subjects and 1 female BD subject had CRP levels below the detectable limit for the ELISA, and were therefore also excluded. BD subjects (N=62) and controls (N=45) were matched on age, sex, BMI, years of education, WASI 2-subtest IQ and smoking status (all $p>0.05$, **Table 1)**. A majority (82%) of the BD-I sample was euthymic during testing.

4.4.2 CRP between groups

Between group means and standard deviations of serum CRP levels are outlined in **Table 2**. One-way ANCOVA using age, BMI, and smoking status as covariates revealed no between group differences in serum CRP levels ($F(2, 112)=2.6$, $p=0.08$). BMI was a significantly related to CRP levels, $F(1, 112)=21.8$, $p<0.0001$). Age and smoking status were not significantly associated with CRP levels ($p>0.05$).

Table 3 lists levels of CRP by MS-subtype: primary progressive, secondary progressive, or relapsing-remitting.

4.4.3 CRP and ICM

CRP was associated with ICM in the right anterior cingulate cortex (R= 0.29, $p_{uncorrected}$= 0.049) in BD subjects. There were no significant associations between CRP and ICM in controls ($p<0.05$) **(Table 4).**

4.4.4 CRP and Cognition

CRP was associated with initial correct passes on the verbal memory task in BD subjects (R=-0.47, $p_{uncorrected}$ =0.004), and correct response reaction time on the Stroop task (R=0.33, $p_{uncorrected}$ =0.048). There were no significant associations between CRP and cognition in controls ($p<0.05$) **(Table 5).**

4.5 Future Directions

We only saw an association between CRP and ICM within the right anterior cingulate cortex in BD subjects. We also observed as association between CRP and cognitive performance on subcomponents of the verbal memory and Stroop tests in BD subjects, but not in controls. Indeed, prior studies have shown that CRP is associated with cognitive performance in BD (3).

We will further investigate whether CRP has differential effects on ICM depending on cortical depth. We will assess ICM at both the ¼ and ¾ cortical depths in

relation to serum CRP using hierarchical linear models. We will investigate whether the relationships between CRP and ICM and CRP and cognition are better described when assessing CRP as a categorical variable, using previously established clinical cutoff ranges. We will further assess whether CRP is a potential mediating/moderating variable of the association between ICM and cognitive performance in BD at other cortical depths.

4.6 References

1. Berk M, Kapczinski F, Andreazza A, Dean O, Giorlando F, Maes M, et al. Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. Neuroscience & biobehavioral reviews. 2011;35(3):804-17.

2. Modabbernia A, Taslimi S, Brietzke E, Ashrafi M. Cytokine alterations in bipolar disorder: a meta-analysis of 30 studies. Biological psychiatry. 2013;74(1):15-25.

3. Dickerson F, Stallings C, Origoni A, Vaughan C, Khushalani S, Yolken R. Elevated C-reactive protein and cognitive deficits in individuals with bipolar disorder. Journal of affective disorders. 2013;150(2):456-9.

4. Cunha AB, Andreazza AC, Gomes FA, Frey BN, Da Silveira LE, Gonçalves CA, et al. Investigation of serum high-sensitive C-reactive protein levels across all mood states in bipolar disorder. European archives of psychiatry and clinical neuroscience. 2008;258(5):300-4.

5. Dickerson F, Stallings C, Origoni A, Boronow J, Yolken R. Elevated serum levels of C-reactive protein are associated with mania symptoms in outpatients with bipolar disorder. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2007;31(4):952-5.

6. Fernandes BS, Steiner J, Molendijk ML, Dodd S, Nardin P, Gonçalves C-A, et al. C-reactive protein concentrations across the mood spectrum in bipolar disorder: a systematic review and meta-analysis. The Lancet Psychiatry. 2016;3(12):1147-56.

7. De Berardis D, Conti C, Campanella D, Carano A, Scali M, Valchera A, et al. Evaluation of C-reactive protein and total serum cholesterol in adult patients with bipolar disorder. International journal of immunopathology and pharmacology. 2008;21(2):319-24.

8. Wium-Andersen MK, Ørsted DD, Nordestgaard BG. Elevated C-reactive protein and late-onset bipolar disorder in 78 809 individuals from the general population. The British Journal of Psychiatry. 2015:bjp. bp. 114.150870.

9. Verstynen TD, Weinstein A, Erickson KI, Sheu LK, Marsland AL, Gianaros PJ. Competing physiological pathways link individual differences in weight and abdominal adiposity to white matter microstructure. Neuroimage. 2013;79:129-37.

10. Gianaros PJ, Marsland AL, Sheu LK, Erickson KI, Verstynen TD. Inflammatory pathways link socioeconomic inequalities to white matter architecture. Cerebral Cortex. 2012;23(9):2058-71.

11. Cazettes F, Cohen JI, Yau PL, Talbot H, Convit A. Obesity-mediated inflammation may damage the brain circuit that regulates food intake. Brain research. 2011;1373:101-9.

12. Marsland AL, Gianaros PJ, Abramowitch SM, Manuck SB, Hariri AR. Interleukin-6 covaries inversely with hippocampal grey matter volume in middle-aged adults. Biological psychiatry. 2008;64(6):484-90.

13. Rosano C, Marsland AL, Gianaros PJ. Maintaining brain health by monitoring inflammatory processes: a mechanism to promote successful aging. Aging and disease. 2012;3(1):16.

14. Kullmann S, Schweizer F, Veit R, Fritsche A, Preissl H. Compromised white matter integrity in obesity. Obesity reviews. 2015;16(4):273-81.

15. Bettcher BM, Yaffe K, Boudreau RM, Neuhaus J, Aizenstein H, Ding J, et al. Declines in inflammation predict greater white matter microstructure in older adults. Neurobiology of aging. 2015;36(2):948-54.

16. McTigue DM, Tripathi RB. The life, death, and replacement of oligodendrocytes in the adult CNS. Journal of neurochemistry. 2008;107(1):1-19.

17. JUURLINK BH. Response of glial cells to ischemia: roles of reactive oxygen species and glutathione. Neuroscience & Biobehavioral Reviews. 1997;21(2):151-66.

18. Thorburne SK, Juurlink BH. Low glutathione and high iron govern the susceptibility of oligodendroglial precursors to oxidative stress. Journal of neurochemistry. 1996;67(3):1014-22.

19. Zhao C, Fancy SP, Kotter MR, Li W-W, Franklin RJ. Mechanisms of CNS remyelination—the key to therapeutic advances. Journal of the neurological sciences. 2005;233(1):87-91.

20. Olah M, Amor S, Brouwer N, Vinet J, Eggen B, Biber K, et al. Identification of a microglia phenotype supportive of remyelination. Glia. 2012;60(2):306-21.

21. Streit WJ, Mrak RE, Griffin WST. Microglia and neuroinflammation: a pathological perspective. Journal of neuroinflammation. 2004;1(1):14.

22. Kotter MR, Stadelmann C, Hartung H-P. Enhancing remyelination in disease—can we wrap it up? Brain. 2011:awr014.

23. Armada-Moreira A, Ribeiro FF, Sebastião AM, Xapelli S. Neuroinflammatory modulators of oligodendrogenesis. Neuroimmunology and Neuroinflammation. 2015;2(4):263-.

24. Teunissen C, Van Boxtel M, Bosma H, Bosmans E, Delanghe J, De Bruijn C, et al. Inflammation markers in relation to cognition in a healthy aging population. Journal of neuroimmunology. 2003;134(1):142-50.

25. Doganavsargil-Baysal O, Cinemre B, Aksoy UM, Akbas H, Metin O, Fettahoglu C, et al. Levels of TNF-α, soluble TNF receptors (sTNFR1, sTNFR2), and cognition in bipolar disorder. Human Psychopharmacology: Clinical and Experimental. 2013;28(2):160-7.

26. Bauer IE, Pascoe MC, Wollenhaupt-Aguiar B, Kapczinski F, Soares JC. Inflammatory mediators of cognitive impairment in bipolar disorder. Journal of psychiatric research. 2014;56:18-27.

27. Misiak B, Stańczykiewicz B, Kotowicz K, Rybakowski JK, Samochowiec J, Frydecka D. Cytokines and C-reactive protein alterations with respect to cognitive impairment in schizophrenia and bipolar disorder: A systematic review. Schizophrenia Research. 2017.

28. Borsini A, Zunszain PA, Thuret S, Pariante CM. The role of inflammatory cytokines as key modulators of neurogenesis. Trends in neurosciences. 2015;38(3):145-57.

29. McAfoose J, Baune B. Evidence for a cytokine model of cognitive function. Neuroscience & Biobehavioral Reviews. 2009;33(3):355-66.

30. Alboni S, Montanari C, Benatti C, Sanchez-Alavez M, Rigillo G, Blom JM, et al. Interleukin 18 activates MAPKs and STAT3 but not NF-κB in hippocampal HT-22 cells. Brain, behavior, and immunity. 2014;40:85-94.

31. Zalcman S, Green-Johnson JM, Murray L, Nance DM, Dyck D, Anisman H, et al. Cytokine-specific central monoamine alterations induced by interleukin-1,-2 and-6. Brain research. 1994;643(1):40-9.

32. Wang J, Dunn AJ. Mouse interleukin-6 stimulates the HPA axis and increases brain tryptophan and serotonin metabolism. Neurochemistry international. 1998;33(2):143-54.

33. Goshen I, Kreisel T, Licht T, Weidenfeld J, Ben-Hur T, Yirmiya R. Brain interleukin-1 mediates chronic stress-induced depression in mice via adrenocortical activation and hippocampal neurogenesis suppression. Molecular psychiatry. 2008;13(7):717.

34. Bortolato B, Miskowiak KW, Köhler CA, Vieta E, Carvalho AF. Cognitive dysfunction in bipolar disorder and schizophrenia: a systematic review of meta-analyses. Neuropsychiatric disease and treatment. 2015;11:3111.

35. First M, Spitzer R, Gibbon M, Williams J. Research Version, Patient Edition With Psychotic Screen (SCID-I/PW/PSY SCREEN) New York: Biometrics Research, New York State Psychiatric Institute; 2002. Structured Clinical Interview for DSM-IV-TR Axis I Disorders.

36. Gualtieri CT, Johnson LG. Reliability and validity of a computerized neurocognitive test battery, CNS Vital Signs. Archives of Clinical Neuropsychology. 2006;21(7):623-43.

37. Gualtieri CT, Johnson LG. Neurocognitive testing supports a broader concept of mild cognitive impairment. American Journal of Alzheimer's Disease & Other Dementias®. 2005;20(6):359-66.

38. Gualtieri CT, Johnson LG, Benedict KB. Neurocognition in depression: patients on and off medication versus healthy comparison subjects. The Journal of neuropsychiatry and clinical neurosciences. 2006;18(2):217-25.

Table 1. Clinical and Demographic characteristics.

	Control (n=62)	BD (n=45)	Test statistic	P value
Age	30.5 (8.2)	32.1 (7.9)	t=1.0	0.31
Female (%)	34 (54.8%)	26 (57.7%)	χ^2=0.09	0.76
BMI	25.3 (4.7)	26.6 (4.5)	t=1.44	0.15
Education (years)	15.9 (2.8)	15.8 (3.3)	t=0.19	0.85
WASI 2-subtest IQ	110.5 (11.0)	112.2 (13.7)	t=0.71	0.48
Smoking N (%)			χ^2=4.94	0.08
Yes	17	15		
No	43	24		
Past	2	6		
Age of Onset	NA	16.5 (5.5)	NA	NA
Duration of Illness	NA	15.3 (8.0)	NA	NA
Current state	NA		NA	NA
Euthymic		37		
Depressed		4		
Manic		0		
Hypomanic		3		
Mixed		1		
MADRS	1.7 (2.7)		NA	NA
Euthymic		4.7 (5.7)		
Depressed		25.3 (7.9)		
Manic		NA		
Hypomanic		9.3 (2.1)		
Mixed		26		
YMRS	04 (1.0)		NA	NA
Euthymic		2.2 (3.9)		
Depressed		2.2 (2.6)		
Manic		NA		
Hypomanic		16 (2.6)		
Mixed		17		
Psychosis (% Yes)	NA	29 (64.4%)	NA	NA
Medication use (N)*	NA		NA	NA
Unmedicated		7		
Lithium		13		
Anticonvulsants		19		
Antidepressants		15		

Antipsychotics		27		
Anxiolytics		12		
Number of episodes	NA		NA	NA
Depressed		16.9 (21.6)		
Manic		7.8 (15.8)		
Hypomanic		14.0 (20.1)		
Mixed		3.5 (8.8)		
Total		40.7 (46.1)		
Lifetime psychiatric diagnoses (N)	NA		NA	NA
Panic Disorder		12		
Agoraphobia		9		
Social Phobia		12		
OCD		1		
PTSD		9		
GAD		2		
Anorexia nervosa		2		
Binge eating disorder		1		
Alcohol dependence		5		
Alcohol abuse		13		
Substance dependence		7		
Substance abuse		14		

* Most participants were on polypharmacy, therefore totals of medication counts are greater than sample size

BMI- Body mass index; MADRS- Montgomery Asberg Depression Rating Scale; YMRS- Young Mania Rating Scale; OCD- Obsessive Compulsive Disorder; PTSD- Post Traumatic Stress Disorder; GAD- Generalized Anxiety Disorder.

HC- healthy control; BD- Bipolar Disorder; ICM- intracortical myelin

Table 2: CRP levels (mg/L) by Group.

CRP	HC (N= 62)	BD (N= 45)	MS (+ve control) (N=28)
Mean (SD), mg/L	7.2 (11.5)	4.0 (5.3)	3.9 (5.1)
Range, mg/L	0.5 -23.9	0.1 – 30.6	0.1 – 20.7

HC- healthy control; BD- Bipolar Disorder; MS- Multiple Sclerosis

Table 3: CRP (mg/L) by Multiple Sclerosis subgroup

MS Subtype	N	CRP Mean (SD), (mg/L)	Range (mg/L)
Primary progressive	1	3.4	NA
Secondary progressive	7	3.7 (4.5)	0.34 – 19.5
Relapsing-remitting	20	4.8 (7.3)	0.09 – 20.1

MS- Multiple Sclerosis

Table 4. Pearson's Correlation between CRP levels and ICM in HC (N=62) vs. BD (N=45).

Cortical Region	BA	HC				BD			
		Left Hemisphere		Right Hemisphere		Left Hemisphere		Right Hemisphere	
		R	p	R	p	R	p	R	p
Caudal Medial Visual Cortex	17/18	-0.04	0.76	-0.19	0.14	0.07	0.66	0.28	0.06
Lateral Visual Cortex	37/19/18	-0.08	0.54	-0.12	0.36	0.17	0.25	0.15	0.32
Superior Visual Cortex	19/39	-0.05	0.69	-0.09	0.49	-0.05	0.75	0.09	0.57
Cuneus	18/19	-0.19	0.13	-0.18	0.15	0.00	1.00	0.16	0.31
Caudal Middle Temporal Cortex	21/22/39	-0.08	0.54	-0.02	0.87	0.11	0.47	0.12	0.43
Caudal Superior Temporal Cortex	22/41/42	-0.02	0.85	-0.09	0.47	-0.02	0.91	0.10	0.53
Rostral Middle Temporal Cortex	21/22/38	0.00	1.00	0.08	0.51	0.18	0.25	0.13	0.38
Ventral Inferior Parietal Cortex	40	-0.02	0.88	-0.09	0.47	0.02	0.91	0.05	0.73
Dorsal Inferior Parietal Cortex	39/40/7	-0.06	0.63	-0.07	0.56	-0.08	0.61	0.08	0.61
Superior Parietal Cortex	7	-0.13	0.33	-0.09	0.50	-0.12	0.43	0.00	1.00
Medial Superior Parietal Cortex	7	-0.10	0.42	-0.15	0.25	-0.07	0.64	0.04	0.81
Medial Parietal Cortex	31	-0.10	0.45	-0.13	0.32	0.05	0.75	0.02	0.88
Posterior Cingulate Cortex	23	-0.05	0.72	-0.11	0.39	0.05	0.75	0.09	0.55
Ventral Somatosensory Cortex	24	-0.07	0.58	-0.12	0.35	0.08	0.61	0.08	0.62
Dorsolateral Somatosensory Cortex	24/32	-0.08	0.55	-0.12	0.36	-0.05	0.76	0.03	0.82
Dorsomedial Somatosensory Cortex	1/2/3	-0.07	0.57	-0.10	0.45	-0.08	0.62	0.04	0.81
Ventral Motor Cortex	1/2/3	-0.02	0.87	-0.09	0.50	0.06	0.67	0.16	0.31
Dorsolateral Motor Cortex	1/2/3/5/31	-0.05	0.70	-0.07	0.60	-0.01	0.97	0.03	0.86
Dorsomedial Motor Cortex	4/6	-0.05	0.69	-0.02	0.88	-0.07	0.66	0.06	0.68
Rostral Ventral Premotor Cortex	4	0.09	0.48	-0.03	0.81	0.05	0.72	0.04	0.82
Dorsolateral Premotor Cortex	4	0.02	0.89	0.03	0.79	-0.08	0.60	0.03	0.82

Dorsomedial Premotor Cortex	**44/45**	0.02	0.89	0.01	0.92	-0.05	0.77	0.03	0.83
Caudal Dorsolateral Prefrontal Cortex	**6/8**	0.03	0.83	-0.04	0.78	0.02	0.90	0.01	0.93
Caudal Dorsomedial Prefrontal Cortex	**6**	0.00	1.00	0.05	0.72	-0.04	0.79	0.01	0.95
Mid Cingulate Cortex	**45/46/9**	-0.03	0.79	-0.01	0.94	0.00	0.99	0.18	0.25
Rostral Ventrolateral Prefrontal Cortex	**6/8**	0.00	0.98	0.00	0.98	0.04	0.79	0.02	0.91
Rostral Dorsolateral Inferior Prefrontal Cortex	**47/45**	-0.04	0.78	-0.05	0.70	0.06	0.70	0.12	0.42
Rostral Dorsolateral Superior Prefrontal Cortex	**10/46**	-0.02	0.90	-0.06	0.66	0.03	0.87	0.07	0.63
Rostral Dorsal Prefrontal Cortex	**10/9**	-0.03	0.83	-0.08	0.54	-0.06	0.68	0.02	0.91
Rostral Medial Prefrontal Cortex	**10/9/8**	0.02	0.89	0.00	0.97	-0.03	0.83	0.03	0.83
Ventrolateral Orbito Frontal Cortex	**9/8**	-0.11	0.40	-0.01	0.94	-0.08	0.59	0.21	0.16
Ventral Orbito Frontal Cortex	**11/47**	-0.04	0.78	-0.03	0.79	-0.02	0.91	0.09	0.54
Ventromedial Orbito Frontal Cortex	**11/47**	-0.05	0.69	0.05	0.69	-0.06	0.70	0.11	0.46
Ventromedial Prefrontal Cortex	**10/11**	-0.08	0.52	0.09	0.48	-0.01	0.95	0.13	0.41
Anterior Cingulate Cortex	**32/10/11**	0.02	0.86	0.04	0.78	0.05	0.74	0.29	0.049*

CRP levels are correlated with ICM in the right anterior cingulate cortex in BD subjects ($p_{uncorrected}<0.05$). This is association is not seen in HC.

BD- Brodmann Area; BD- Bipolar Disorder; HC- Healthy control; ICM- intracortical myelin

Table 5. Pearson's Correlation between CRP levels and Cognitive function in HC (N=57) vs. BD (N=37).

	Control	Control	BD	BD
Domain Scores	**R**	**p**	**R**	**p**
Psychomotor speed	-0.13	0.32	-0.11	0.52
Reaction time	0.07	0.62	0.32	0.05
Processing Speed	-0.15	0.27	-0.17	0.31
Verbal memory	-0.02	0.87	-0.23	0.16
Simple attention	0.13	0.35	0.02	0.88
Motor speed	-0.24	0.07	0.00	0.99
Executive function*	0.12	0.37	0.08	0.63
Verbal Memory subcomponents				
Initial correct hits	-0.09	0.50	-0.13	0.44
Initial correct passes	0.08	0.56	-0.47	0.004**
Initial target reaction time	0.26	0.05	0.07	0.69
Delayed correct hits	-0.03	0.81	-0.12	0.48
Delayed correct passes	0.10	0.44	-0.15	0.37
Delayed target reaction time	0.15	0.26	0.21	0.20
Finger Tapping Test subcomponents				
Right taps	-0.21	0.13	0.06	0.71
Left taps	-0.26	0.05	-0.06	0.73
Symbol Digit Coding subcomponents				
Correct	-0.17	0.21	-0.18	0.28
Errors	-0.05	0.70	-0.13	0.45
Stroop Test subcomponents				
Simple reaction time	0.19	0.17	0.21	0.21
Complex- correct	0.08	0.57	0.07	0.69
Complex- reaction time, correct	0.08	0.56	0.26	0.12
Complex- commission errors	-0.06	0.68	0.17	0.33
Correct hits	-0.01	0.95	0.00	1.00
Reaction time-correct	0.05	0.72	0.33	0.048*
Commission errors	-0.05	0.71	-0.13	0.46
Continuous Performance Test subcomponents				
Correct responses	0.02	0.89	-0.09	0.58
Omission errors	-0.02	0.89	0.09	0.58
Commission errors	-0.14	0.31	-0.06	0.74
Correct choice reaction time	0.05	0.69	0.15	0.39

*Trails B, time of completion (s)
HC- healthy control; BD- Bipolar Disorder; ICM- intracortical myelin

Chapter 5: The Association of Serum Anti-Myelin Basic Protein and Intracortical Myelin and Cognitive Function

This chapter contains a compilation of work that is currently in progress and will be included in future manuscripts.

5.1 Introduction

Myelin basic protein (MBP) is the most abundant, extrinsic protein in myelin sheaths and possesses myelin specific antigens with distinctive sites for MBP specific T cells and antibodies (1). Traditionally, antibodies against myelin basic protein (anti-MBP) have been readily investigated in demyelinating diseases, in particular multiple sclerosis (MS) (1). The presence of anti-MBP is increased in MS in both serum (2) and cerebrospinal fluid (3), but its specific roles in pathogenicity and illness progression remain undetermined.

MS subjects with increased anti-MBP and anti-myelin oligodendrocyte glycoprotein (MOG) showed significantly more T_2- lesions on MRI. This increased lesion load indicated a higher likelihood of conversion from clinically isolated syndrome (CIS) to clinically definite MS. Individuals with anti-MBP also showed a trend towards more diffuse lesions in space (4), indicating a potential exacerbating role of anti-MBP presence in white matter damage. Further, anti-MBP has been shown to be a marker predictive of the conversion from clinically isolated syndrome to clinically definite syndrome (5), although there are conflicting results (4). The frequency of elevated anti-MBP also seems

to increase with degree of symptom severity (6). Whatsoever, it is not clear, whether anti-MBP plays a pathological role in MS or rather a defense mechanism limiting further damage to the myelin sheaths (7).

Notably, antibodies to myelin proteins do not seem to be a specific marker for MS, but rather a biomarker related to CNS damage (7-9). Elevated levels of anti-MBP have been found in other neurological diseases, including degenerative dementia, primary CNS lymphoma, Behcet disease, acute meningitis, motor neuron disease, multisystem atrophy, extrapyramidal syndromes, acute encephalitis, intracerebral haemorrhage, acute cerebral infarct, Wilson disease, Neimann-Pick disease, focal epilepsy and Friedrich ataxia. As such, anti-MBP is not a definite indicator of autoimmunity or of myelin damage, but is rather likely an index of CNS insult (10).

Counterintuitively, several studies in mice and rodents support the protective role of autoimmune reactions in relation to CNS damage. In a mouse model of virus-induced MS, monoclonal antibodies against surface antigens in oligodendrocytes increased remyelination (11). Likewise, in rodents with induced spinal cord injury and optic nerve lesions, autoimmune T cells specific for MBP have been observed to promote long lasting recovery of injured nervous tissue (12-14). Thus, there is robust evidence that autoimmune reactions involving antibodies seem to play neuroprotective roles in CNS injury, underscoring caution in the interpretation of autoimmunity as a pathological finding (11, 15).

Prior studies have shown that myelin-related gene expression is in fact altered in the prefrontal cortex in BD (16). Notably, the expression of MBP is increased during

both first-episode mania and first-episode psychosis on the Schizophrenia spectrum in antipsychotic-free subjects (17). Meta-analysis has further confirmed subcortical white matter volume reductions in first-episode BD (18), although the interplay of WM damage and MBP levels remains undetermined. Myelinated axons within the cortex (Intracortical myelin, ICM) may be especially susceptible to CNS insult. Prefrontal gray matter displays the steepest decline in volume over age, indicating a unique vulnerability in comparison to the rest of the cerebral cortex (19). Increased inflammation, microglial activation and inflammation-related gene expression have all found to be altered in the cortex of individuals with BD (20, 21).

5.2 Aims

We aim to determine whether serum anti-MBP levels are related to intracortical myelin (ICM) in individuals with BD, in comparison to healthy controls. Further, we explore whether anti-MBP levels are associated with cognitive performance in BD.

5.3 Methods

5.3.1 Study population

This study was approved by the Hamilton Integrated Research Ethics Board and signed informed consent was obtained from each study participant prior to commencement of the study. Images were collected in 46 individuals with a diagnosis of BD type-I and 52 matched controls. All participants were right-handed, aged-17-45, and all female subjects were premenopausal. Study participants completed the Structured

Clinical Interview for DSM-IV (SCID-I) for diagnostic confirmation (22). Controls did not meet criteria for any current or lifetime Axis I psychiatric conditions. Subjects with BD-I did not meet criteria for *current* Axis I psychiatric comorbidities. We excluded individuals with unstable medical/inflammatory conditions, alcohol/substance abuse within the last year (excluding caffeine or nicotine), past/current history of neurological disorders (including head trauma and migraines), and any MRI contraindications. All participants were fluent in English, and groups were matched based on intelligence level using the Wechsler Scale of Intelligence (WASI) 2-subtest Intelligence Quotient (IQ) test.

Thirty individuals with a diagnosis of Multiple Sclerosis were recruited from the Hamilton Health Sciences Multiple Sclerosis clinic, as positive control group for CRP analysis, considering the elevated levels of inflammatory-mediated WM damage that are characteristic of the illness. These participants did not complete any cognitive or MRI testing.

5.3.2 Imaging

The imaging methods are identical to those described previously in chapters 2 and 3.

5.3.3 Serum Anti-MBP Analysis

Blood samples were collected through venipuncture in non-EDTA collection tubes on the same day as the MRI scanning procedure. Once collected, whole-blood samples were allowed to clot at room temperature for 45 minutes, and subsequently spun

at 3000rpm for 15 minutes. Serum was then aliquoted and immediately frozen at -80° C until assayed.

Anti-MBP levels were detected using ELISA (Enzyme Linked Immunosorbent Assay; MyBioSource, Catalogue No: MBS733392, Range of Detection: 0 ng/ml – 250 ng/ml, Sensitivity: 1 ng/ml).

5.3.4 Cognition- CNS Vital Signs (CNSVS)

A subset of participants (N=50 controls, N=37 BD-I) completed cognitive testing within the same week as, but not immediately following, the MRI session. This helped avoiding any stress or anxiety related to the MRI procedure. We administered a 25-minute computerized testing battery (CNS Vital Signs, CNSVS). This battery has previously been validated across a broad age range, with established reliability in patients with mild cognitive impairment and patients with depression (23-25).

We administered the Verbal Memory, Finger Tapping, Symbol Digit Coding, Stroop, and Continuous Performance tests. Test scores were recombined by the CNSVS program to obtain overall scores for the following cognitive domains: Psychomotor speed, reaction time, processing speed, verbal memory, simple attention and motor speed (**Supplementary Table 1, Chapter 3**). Individual domain component scores were also obtained, and varied by test.

All scores used in the following analysis were deemed "valid" by the CNSVS internal assessment. Scores are considered valid when a suitable level of effort has been

detected from the participant, in order to avoid potentially dubious results. This is based on a validity algorithm, outlined in **Supplementary Table 2, Chapter 3.**

The paper and pencil Trails B was administered as a measure of executive function. Scores where the participant committed 3 or fewer errors were considered valid. Time of completion, in seconds (s), was used as the outcome of interest.

5.3.4 Statistical Analysis

All statistical analyses were completed using R version 3.3.2 (https://www.r-project.org). Shapiro-Wilk and Bartlett tests determined normality and homogeneity of variances of continuous variables, respectively. Between group differences in age, sex, BMI, years of education, WASI 2 subtest IQ, and smoking status were tested using two-tailed independent t-test, Mann-Whitney test, or chi-squared test, as appropriate.

Between group differences in anti-MBP levels were tested using one-way ANOVA. The associations between CRP and ICM, and CRP and cognitive performance were testing using Pearson's R correlation.

5.4 Preliminary Results

5.4.1 Demographics

One female BD subject and two male controls were excluded from the analyses due to poor quality ICM maps. BD subjects (N=45) and controls (N=50) were matched on age, sex, BMI, years of education, WASI 2-subtest IQ and smoking status (all $p>0.05$, **Table 1)**. A majority (82%) of the BD-I sample was euthymic during testing.

5.4.2 Anti-MBP between groups

All serum anti-MBP levels were within detection range of the ELISA kit. Between group means and standard deviations of serum anti-MBP levels are outlined in **Table 2**. One-way ANOVA ($F(2, 124)=0.66$, $p=0.52$) revealed no between group differences.

Table 3 lists levels of anti-MBP by MS-subtype: primary progressive, secondary progressive, or relapsing-remitting.

5.4.3 Anti-MBP and ICM

Anti-MBP was associated with ICM in regions of the bilateral visual, parietal, and somatosensory cortices, and left temporal, cingulate, and orbitofrontal cortices in healthy controls ($R= -0.28- -0.39$, $p_{uncorrected}= 0.006-0.049$). In BD subjects, significant associations were only seen in the left caudal medial visual cortex ($R=-0.31$, $p=0.04$), and the right cuneus ($R=-0.32$, $p_{uncorrected}=0.04$)

5.4.4 Anti-MBP and Cognition

Anti-MBP was not associated with cognitive performance across all the domains assessed. Trends are observed in relation to motor speed, right taps on the finger tapping test, correct responses on the complex Stroop task, and commission errors on the complex Stroop.

5.5 Future analyses

We observed a relationship between Anti-MBP and ICM in healthy controls, but not BD subjects. This observation may highlight the regenerative potential of anti-MBP, potentially indicating that individuals with BD lack the regenerative capacity that is seen healthy controls. In order to further assess this relationship, we will run regression models to determine whether anti-MBP levels can predict ICM in both populations. Due to exploratory nature of these analyses, with no *a priori* hypotheses regarding the potential differential effects of anti-MBP at various depths of the cortex, we will further investigate the potential differential effects of anti-MBP at the ¼ and ¾ cortical depths. We will examine whether anti-MBP is a potential mediating/moderating variable of the association between ICM and cognitive performance in control subjects through different cortical depths.

5.6 References

1. Deber CM, Reynolds SJ. Central nervous system myelin: structure, function, and pathology. Clinical biochemistry. 1991;24(2):113-34.

2. Egg R, Reindl M, Deisenhammer F, Linington C, Berger T. Anti-MOG and anti-MBP antibody subclasses in multiple sclerosis. Multiple Sclerosis Journal. 2001;7(5):285-9.

3. Warren K, Catz I. Relative frequency of autoantibodies to myelin basic protein and proteolipid protein in optic neuritis and multiple sclerosis cerebrospinal fluid. Journal of the neurological sciences. 1994;121(1):66-73.

4. Kuhle J, Lindberg RL, Regeniter A, Mehling M, Hoffmann F, Reindl M, et al. Antimyelin antibodies in clinically isolated syndromes correlate with inflammation in MRI and CSF. Journal of neurology. 2007;254(2):160-8.

5. Berger T, Rubner P, Schautzer F, Egg R, Ulmer H, Mayringer I, et al. Antimyelin antibodies as a predictor of clinically definite multiple sclerosis after a first demyelinating event. New England Journal of Medicine. 2003;349(2):139-45.

6. Reindl M, Linington C, Brehm U, Egg R, Dilitz E, Deisenhammer F, et al. Antibodies against the myelin oligodendrocyte glycoprotein and the myelin basic protein in multiple sclerosis and other neurological diseases: a comparative study. Brain : a journal of neurology. 1999;122 (Pt 11):2047-56.

7. Karni A, Bakimer-Kleiner R, Abramsky O, Ben-Nun A. Elevated levels of antibody to myelin oligodendrocyte glycoprotein is not specific for patients with multiple sclerosis. Arch Neurol. 1999;56(3):311-5.

8. Papuc E, Kurys-Denis E, Krupski W, Tatara M, Rejdak K. Can Antibodies Against Glial Derived Antigens be Early Biomarkers of Hippocampal Demyelination and Memory Loss in Alzheimer's Disease? J Alzheimers Dis. 2015;48(1):115-21.

9. Giovannoni G. Cerebrospinal fluid analysis. Handb Clin Neurol. 2014;122:681-702.

10. Karni A, Bakimer-Kleiner R, Abramsky O, Ben-Nun A. Elevated levels of antibody to myelin oligodendrocyte glycoprotein is not specific for patients with multiple sclerosis. Archives of neurology. 1999;56(3):311-5.

11. Warrington AE, Asakura K, Bieber AJ, Ciric B, Van Keulen V, Kaveri SV, et al. Human monoclonal antibodies reactive to oligodendrocytes promote remyelination in a model of multiple sclerosis. Proceedings of the National Academy of Sciences of the United States of America. 2000;97(12):6820-5.

12. Hauben E, Nevo U, Yoles E, Moalem G, Agranov E, Mor F, et al. Autoimmune T cells as potential neuroprotective therapy for spinal cord injury. Lancet. 2000;355(9200):286-7.

13. Moalem G, Leibowitz-Amit R, Yoles E, Mor F, Cohen IR, Schwartz M. Autoimmune T cells protect neurons from secondary degeneration after central nervous system axotomy. Nature medicine. 1999;5(1):49-55.

14. Moalem G, Yoles E, Leibowitz-Amit R, Muller-Gilor S, Mor F, Cohen IR, et al. Autoimmune T cells retard the loss of function in injured rat optic nerves. Journal of neuroimmunology. 2000;106(1-2):189-97.

15. Bieber AJ, Warrington A, Asakura K, Ciric B, Kaveri SV, Pease LR, et al. Human antibodies accelerate the rate of remyelination following lysolecithin-induced demyelination in mice. Glia. 2002;37(3):241-9.

16. Tkachev D, Mimmack ML, Ryan MM, Wayland M, Freeman T, Jones PB, et al. Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. The Lancet. 2003;362(9386):798-805.

17. Gouvea E, Ota V, Noto C, Santoro M, Spindola L, Moretti P, et al. Gene expression alterations related to mania and psychosis in peripheral blood of patients with a first episode of psychosis. Translational psychiatry. 2016;6(10):e908.

18. Vita A, De Peri L, Sacchetti E. Gray matter, white matter, brain, and intracranial volumes in first-episode bipolar disorder: a meta-analysis of magnetic resonance imaging studies. Bipolar disorders. 2009;11(8):807-14.

19. Raz N, Gunning FM, Head D, Dupuis JH, McQuain J, Briggs SD, et al. Selective aging of the human cerebral cortex observed in vivo: differential vulnerability of the prefrontal gray matter. Cerebral cortex (New York, NY: 1991). 1997;7(3):268-82.

20. Rao JS, Harry GJ, Rapoport SI, Kim H-W. Increased excitotoxicity and neuroinflammatory markers in postmortem frontal cortex from bipolar disorder patients. Molecular psychiatry. 2010;15(4):384.

21. Fillman S, Sinclair D, Fung S, Webster M, Weickert CS. Markers of inflammation and stress distinguish subsets of individuals with schizophrenia and bipolar disorder. Translational psychiatry. 2014;4(2):e365.

22. First M, Spitzer R, Gibbon M, Williams J. Research Version, Patient Edition With Psychotic Screen (SCID-I/PW/PSY SCREEN) New York: Biometrics Research, New York State Psychiatric Institute; 2002. Structured Clinical Interview for DSM-IV-TR Axis I Disorders.

23. Gualtieri CT, Johnson LG. Reliability and validity of a computerized neurocognitive test battery, CNS Vital Signs. Archives of Clinical Neuropsychology. 2006;21(7):623-43.

24. Gualtieri CT, Johnson LG. Neurocognitive testing supports a broader concept of mild cognitive impairment. American Journal of Alzheimer's Disease & Other Dementias®. 2005;20(6):359-66.

25. Gualtieri CT, Johnson LG, Benedict KB. Neurocognition in depression: patients on and off medication versus healthy comparison subjects. The Journal of neuropsychiatry and clinical neurosciences. 2006;18(2):217-25.

26. Auzias G, Coulon O, Brovelli A. MarsAtlas: A cortical parcellation atlas for functional mapping. Human brain mapping. 2016.

Table 1. Demographics and clinical characteristics.

	HC (n=50)	BD (n=45)	Test statistic	P value
Age	31.7 (8.3)	32.2 (8.2)	t=0.29	0.77
Female (%)	26 (52.0%)	26 (57.7%))	χ^2=0.32	0.57
BMI	25.9 (5.4)	26.7 (4.3)	t=0.78	0.44
Education (years)	15.7 (2.8)	15.9 (3.3)	t=0.36	0.72
WASI 2-subtest IQ	110.0 (11.2)	111.2 (13.9)	t=0.44	0.66
Smoking N (%) Yes No Past	 16 32 2	 14 24 7	χ^2=3.80	0.15
Age of Onset	NA	16.8 (6.2)	NA	NA
Duration of Illness	NA	15.1 (8.1)	NA	NA
Current state Euthymic Depressed Manic Hypomanic Mixed	NA	 37 4 0 3 1	NA	NA
MADRS Euthymic Depressed Manic Hypomanic Mixed	1.6 (2.6)	 4.6 (5.5) 25.3 (7.9) NA 9.3 (2.1) 26	NA	NA
YMRS Euthymic Depressed Manic Hypomanic Mixed	0.5 (1.1)	 2.2 (3.9) 2.3 (2.6) NA 16 (2.6) 17	NA	NA
Psychosis (% Yes)	NA	27 (60%)	NA	NA
Medication use (N)* Unmedicated Lithium Anticonvulsants Antidepressants	NA	 5 14 20 16 28	NA	NA

Antipsychotics Anxiolytics		12		
Number of episodes Depressed Manic Hypomanic Mixed Total	NA	 16.7 (21.8) 5.7 (7.7) 13.6 (20.0) 3.4 (8.7) 38.2 (44.8)	NA	NA
Lifetime psychiatric diagnoses (N) Panic Disorder Agoraphobia Social Phobia OCD PTSD GAD Anorexia nervosa Binge eating disorder Alcohol dependence Alcohol abuse Substance dependence Substance abuse	NA	 13 10 14 1 9 3 2 2 5 14 7 13	NA	NA

* Most participants were on polypharmacy, therefore totals of medication counts are greater than sample size

BMI- Body mass index; MADRS- Montgomery Asberg Depression Rating Scale; YMRS- Young Mania Rating Scale; OCD- Obsessive Compulsive Disorder; PTSD- Post Traumatic Stress Disorder; GAD- Generalized Anxiety Disorder.

HC- healthy control; BD- Bipolar Disorder; ICM- intracortical myelin

Table 2. Anti-MBP (ng/ml) between-group comparisons

Anti-MBP	HC (N=50)	BD (N= 45)	MS (+ve control) (N=30)
Mean (SD), ng/ml	9.5 (3.0)	7.9 (4.2)	7.6 (11.5)
Range, ng/ml	3.0 – 16.7	1.1 – 25.6	1.5 – 56

HC- healthy control; BD- Bipolar Disorder; MS- Multiple Sclerosis

Table 3: Anti-MBP (ng/ml) by Multiple Sclerosis subgroup

MS Subtype	N	Anti-MBP Mean (SD), (ng/ml)	Range (ng/ml)
Primary progressive	1	7.2	NA
Secondary progressive	8	4.1 (3.0)	1.5 – 10.4
Relapsing-remitting	21	8.9 (13.5)	1.7 - 56

MS- Multiple Sclerosis

Table 4. Pearson's Correlation between Anti-MBP levels and ICM in HC (N=50) vs. BD (N=45).

Cortical Region	BA	HC				BD			
		Left Hemisphere		Right Hemisphere		Left Hemisphere		Right Hemisphere	
		R	p	R	p	R	p	R	p
Caudal Medial Visual Cortex	17/18	-0.359	0.010*	-0.354	0.012*	-0.310	0.038*	-0.280	0.063
Lateral Visual Cortex	37/19/18	-0.369	0.008**	-0.240	0.093	-0.221	0.144	-0.140	0.358
Superior Visual Cortex	19/39	-0.318	0.025*	-0.214	0.135	-0.076	0.619	-0.096	0.531
Cuneus	18/19	-0.387	0.006**	-0.339	0.016*	-0.182	0.231	-0.315	0.035*
Caudal Middle Temporal Cortex	21/22/39	-0.333	0.018*	-0.198	0.167	-0.155	0.311	-0.104	0.497
Caudal Superior Temporal Cortex	22/41/42	-0.305	0.031*	-0.234	0.102	-0.112	0.463	-0.137	0.368
Rostral Middle Temporal Cortex	21/22/38	-0.297	0.037*	-0.263	0.065	-0.049	0.750	0.001	0.997
Ventral Inferior Parietal Cortex	40	-0.319	0.024*	-0.224	0.117	-0.051	0.738	-0.039	0.797
Dorsal Inferior Parietal Cortex	39/40/7	-0.273	0.055	-0.261	0.067	-0.011	0.942	-0.044	0.775
Superior Parietal Cortex	7	-0.256	0.073	-0.194	0.178	0.010	0.948	-0.019	0.901
Medial Superior Parietal Cortex	7	-0.231	0.106	-0.293	0.039*	-0.039	0.802	-0.085	0.577
Medial Parietal Cortex	31	-0.317	0.025*	-0.322	0.022*	-0.087	0.570	-0.100	0.514
Posterior Cingulate Cortex	23	-0.313	0.027*	-0.277	0.052	-0.155	0.309	-0.183	0.229
Mid Cingulate Cortex	24	-0.329	0.020*	-0.177	0.219	-0.048	0.753	-0.108	0.478
Anterior Cingulate Cortex	24/32	-0.368	0.009**	-0.256	0.073	-0.131	0.391	-0.099	0.519
Ventral Somatosensory Cortex	1/2/3	-0.281	0.048*	-0.213	0.137	0.003	0.987	-0.005	0.972
Dorsolateral Somatosensory Cortex	1/2/3	-0.227	0.113	-0.234	0.102	-0.043	0.780	-0.055	0.719
Dorsomedial Somatosensory Cortex	1/2/3/5/31	-0.244	0.088	-0.288	0.043*	-0.010	0.947	-0.042	0.784
Ventral Motor Cortex	4/6	-0.206	0.151	-0.224	0.117	-0.093	0.545	-0.025	0.868
Dorsolateral Motor Cortex	4	-0.191	0.183	-0.193	0.180	-0.222	0.142	-0.158	0.301

Dorsomedial Motor Cortex	**4**	-0.227	0.113	-0.216	0.132	-0.128	0.403	-0.064	0.676
Rostral Ventral Premotor Cortex	**44/45**	-0.225	0.117	-0.241	0.092	-0.066	0.668	0.042	0.785
Dorsolateral Premotor Cortex	**6/8**	-0.127	0.378	-0.137	0.342	-0.086	0.574	0.063	0.682
Dorsomedial Premotor Cortex	**6**	-0.182	0.207	-0.114	0.431	-0.105	0.493	0.029	0.849
Caudal Dorsolateral Prefrontal Cortex	**45/46/9**	-0.140	0.333	-0.181	0.209	-0.041	0.788	0.101	0.510
Caudal Dorsomedial Prefrontal Cortex	**6/8**	-0.172	0.232	-0.147	0.309	-0.023	0.883	0.026	0.866
Rostral Ventrolateral Prefrontal Cortex	**47/45**	-0.191	0.185	-0.209	0.145	0.018	0.907	0.045	0.771
Rostral Dorsolateral Inferior Prefrontal Cortex	**10/46**	-0.236	0.098	-0.196	0.174	0.100	0.514	0.015	0.922
Rostral Dorsolateral Superior Prefrontal Cortex	**10/9**	-0.240	0.093	-0.198	0.167	0.094	0.541	0.057	0.709
Rostral Dorsal Prefrontal Cortex	**10/9/8**	-0.139	0.335	-0.185	0.199	0.160	0.294	0.066	0.666
Rostral Medial Prefrontal Cortex	**9/8**	-0.204	0.155	-0.152	0.292	0.031	0.842	0.057	0.711
Ventrolateral Orbito Frontal Cortex	**11/47**	-0.335	0.017*	-0.356	0.011	-0.062	0.687	-0.052	0.732
Ventral Orbito Frontal Cortex	**11/47**	-0.246	0.084	-0.238	0.096	0.010	0.950	-0.048	0.755
Ventromedial Orbito Frontal Cortex	**10/11**	-0.325	0.021*	-0.165	0.253	0.043	0.779	-0.014	0.926
Ventromedial Prefrontal Cortex	**32/10/11**	-0.280	0.049*	-0.163	0.257	0.019	0.902	-0.002	0.989

Anti-MBP is much more widely associated with ICM in controls, than in BD subjects.
BA: Brodmann Area (26); HC- healthy control; BD- Bipolar Disorder; ICM- intracortical myelin

Table 5. Pearson's Correlation between CRP levels and Cognitive function in HC (N=50) vs. BD (N=37).

	HC		BD	
Domain Scores	**R**	**p**	**R**	**p**
Psychomotor speed	-0.20	0.16	-0.15	0.39
Reaction time	0.17	0.25	0.09	0.60
Processing Speed	-0.04	0.80	-0.13	0.44
Verbal memory	0.06	0.67	0.20	0.23
Simple attention	0.02	0.89	0.02	0.92
Motor speed	-0.25	0.09	-0.08	0.63
Executive function*	0.04	0.77	-0.22	0.18
Verbal Memory subcomponents				
Initial correct hits	0.03	0.83	0.16	0.33
Initial correct passes	0.15	0.31	0.20	0.23
Initial target reaction time	0.14	0.32	0.16	0.34
Delayed correct hits	0.04	0.81	0.14	0.41
Delayed correct passes	-0.01	0.96	0.10	0.56
Delayed target reaction time	0.04	0.78	0.17	0.32
Finger Tapping Test subcomponents				
Right taps	-0.28	0.05	-0.03	0.88
Left taps	-0.19	0.19	-0.13	0.46
Symbol Digit Coding subcomponents				
Correct	-0.09	0.53	-0.15	0.37
Errors	-0.23	0.10	-0.16	0.35
Stroop Test subcomponents				
Simple reaction time	0.23	0.10	0.25	0.13
Complex- correct	-0.26	0.07	0.02	0.88
Complex- reaction time, correct	0.16	0.27	0.07	0.67
Complex- commission errors	0.28	0.05	-0.11	0.51
Correct hits	-0.20	0.17	0.01	0.97
Reaction time-correct	0.15	0.31	0.12	0.47
Commission errors	0.13	0.37	0.09	0.60
Continuous Performance Test subcomponents				
Correct responses	0.01	0.97	0.04	0.84
Omission errors	-0.01	0.97	-0.04	0.84
Commission errors	-0.03	0.86	-0.01	0.97
Correct choice reaction time	0.03	0.86	0.13	0.44

*Trails B, time of completion (s)
HC- healthy control; BD- Bipolar Disorder; ICM- intracortical myelin

Chapter 6: General Discussion

6.1 Summary of Findings

Prior studies have shown widespread white matter-related changes in relation to the pathophysiology of Bipolar Disorder (BD). However, most of what is known is derived from *in-vivo* subcortical white matter imaging, or post-mortem studies (1-9). Myelinated axons within the cerebral cortex remain largely understudied and are often overlooked due to prior methodological constraints, despite their relevance to optimal brain functioning. Using a novel neuroimaging technique comprising an optimized sequence previously shown to be sensitive to ICM content (10-13), we investigated whole-cortex intracortical myelin (ICM) content in individuals with BD-I and controls.

In the first study (Chapter 2), we examined whether ICM follows the same "inverted-U" developmental trajectory seen in healthy controls across young adulthood (13), and whether this was associated with any illness characteristics in BD. T_1-weighted signal in healthy controls followed an "inverted –U" pattern over age, whereas in BD-I subjects the association between ICM and age followed a flat line pattern, and was severely blunted throughout the cortex, particularly in the left posterior lateral regions. Effects are seen throughout the frontal parietal and temporal cortices. Strongest effects between BD and controls were seen in motor and premotor regions, followed by prefrontal and parietal regions. Exploratory analyses showed that ICM signal intensity was associated with duration of illness, age of onset, anticonvulsant and antipsychotic use in BD-I subjects. This study is the first to show global deficits in ICM maturation

throughout the cortex in BD. In the only other imaging study of ICM measurement *in vivo* in BD, differences between BD and controls were reported solely in the motor cortex (14). In contrast, the results of our study underscore the increased sensitivity of our technique in the measurement of ICM, and highlight the widespread nature of ICM deficits, observed in comparison to healthy controls, as a potential neurobiological correlate of the disorder.

Due to the significant role of myelin in the maintenance of neuronal signal integrity and synchrony, we next investigated whether ICM was associated with cognitive performance in BD in Chapter 3. Cognitive dysfunction persists into asymptomatic states in BD, and is also associated with various illness variables, highlighting its disabling and chronic nature (15-20). We found that verbal memory was associated with ICM in BD, but not in controls. Strongest effects were seen in the left caudal middle temporal cortex and left dorsolateral prefrontal cortex. Processing speed, executive function, and reaction time were also associated with ICM, although this does not survive correction for multiple comparisons. This study highlights the behavioral impact of altered ICM content in BD.

In order to explore potential biological mechanisms underlying the relationship between ICM and cognitive function, we explored the relationship between two markers of inflammation and CNS damage and ICM and cognitive performance in BD in preliminary analyses. We found that CRP was only associated with ICM in the right anterior cingulate cortex in BD, but not controls (Chapter 4). Further, CRP was associated with performance on verbal memory and Stoop tasks. Prior studies have indeed shown

that CRP is associated with cognitive performance in BD (21, 22). This study illustrates that an acute inflammatory response protein may not be reflective of potential inflammation-mediated effects on ICM.

Counterintuitively, anti-MBP levels were associated with ICM in regions of the bilateral visual, parietal, and somatosensory cortices, and left temporal, cingulate, and orbitofrontal cortices in healthy controls. In contrast, anti-MBP levels were only associated with ICM in the left caudal medial visual cortex and the right cuneus in BD. Anti-MBP was not associated with cognitive performance in either BD or control subjects. Prior studies have shown that autoimmune markers harbor certain protective roles within the brain and can increase remyelination and recovery of injured tissue (23-27). Our preliminary findings allude to the regenerative potential of anti-MBP with regards to myelin maintenance, that is perhaps lost in BD.

6.2 Significance

The significance of each of our major findings are discussed in detail within each chapter. This work is the first series of analyses to show that ICM is compromised throughout the cerebral cortex *in vivo* in BD, and that furthermore, ICM is associated with cognitive function in BD.

Subcortical neuroimaging and *post-mortem* studies of WM have both alluded to potentially compromised ICM in BD, however, prior methodological constraints precluded the translation and confirmation of these findings in an *in vivo* human neuroimaging study. Our technique endorses the increased sensitivity required to assess

ICM throughout the cortex, including in lightly myelinated regions where nuances in ICM are more difficult to detect. Additionally, our imaging technique has previously been validated for ICM measurement using histology (10-12).

We have shown that ICM is compromised in BD throughout the cortex, across the range of young adulthood investigated (17-45 years). This finding highlights ICM as longstanding neural abnormality in BD. Myelin is imperative for maintaining neural synchrony and integrity, and other studies have postulated that the role for myelin within the cerebral cortex through a developmental perspective, is to prevent the formation of aberrant connections (28-30). As the peak of onset of many psychiatric disorders coincides with a period of intense myelination within the brain, the two may be tied in etiology (31). Due to the widespread and persistent deficits of ICM across young adulthood, our work highlights ICM as a microstructural property of the cerebral cortex with huge potential for further functional exploration pertaining to the behavioural correlates of BD.

WM hyperintensities (WMH) have largely been the most replicated neurobiological findings in BD (32). Prior works have suggested that at a pathophysiological level, they may reflect dilation of perivascular spaces, localized demyelination, astrocytic gliosis, and atherosclerosis Further, it has been suggested that their presence is likely to impede the function of WM tracts (33-36). We did not assess WMHs in addition to ICM in our study. It remains unknown whether deficits in ICM are seen in tandem with WMHs in individuals with BD or not, or whether one neurobiological marker is prodromal for the development/presence of the other.

Furthermore, WMHs have also been associated with deficits in cognitive performance, and have routinely been observed in tracts that are heavily related to cognitive processing (37). Interestingly, cognitive reserve has been shown to lessen the burden of WMH on executive functioning in BD (38, 39). Again, whether WM hyperintensities bear greater influence on cognitive processing in BD in comparison to ICM remains unknown, but we suspect that the two work together to influence both cognitive and emotional phenotypes of the illness.

Determining the neural correlates of cognitive dysfunction in BD in imperative to the development of targeted therapeutics that can improve functional outcomes in BD. We found that ICM is most strongly correlated with verbal memory- one of the most widely impaired cognitive domains in BD across the entire cortical network identified *a priori* in association with verbal memory function (15-17). Further, verbal memory dysfunction has been shown to have the greatest effect on functional outcome in comparison to other domains such as attention and concentration (40). Although cognitive dysfunction does not display progressive decline over time in longitudinal studies overall, it is important to note that individuals who do display a decline also report worse psychosocial functionality. Understanding the neurobiological basis of cognitive dysfunction will allow for the identification of potential makers of illness progression and/or daily functionality, which are imperative to the quality of life experienced by affected individuals (40).

Although we only explore cognitive function as a behavioural correlate of ICM in BD here, it is important to note that the cortical regions of difference between BD and

controls are also involved in other core features of the disorder, such as emotional regulation (36, 41-44). Our work provides a previously unexplored potential neural correlate of various behavioural deficits seen in BD. ICM deficits in the cerebral cortex may further explain previous literature showing deficits in cortical thickness and altered functional connectivity in BD (44). We have previously shown that changes in ICM can affect overall thickness measures of the cortex, and changes in WM have been shown to be associated with functional connectivity (12, 45, 46).

The relevance of WM abnormalities in relation to cognitive function has largely been explored in relation to subcortical WM tracts in the brain. In tandem, subtle differences in ICM may have the potential to influence WM tract functionality, as subcortical tracts are connected to the cortex through subcortical-cortical connections. Further, the effects of ICM are also important for the fidelity of corticocortical connections within the cerebral cortex (30). Therefore, the effects of ICM on brain functionality have the potential to be far-reaching both through direct and indirect means. Future studies combining imaging modalities will be able to further delineate this possibility, and are further outlined in the Future Directions section of this chapter.

Our study adds to the rapidly growing literature of WM deficits in BD. Studies using various imaging modalities such as T_2-weighted MRI, DTI, and Magnetization Transfer Ratio (MTR) Imaging all show myelin deficits in BD. T_2- weighted imaging has revealed hyperintensities in both deep and periventricular WM, as discussed previously. DTI studies show reduced fractional anisotropy (FA) and increased radial diffusivity (both considered markers of reduced WM integrity) in BD. Many ROI-based DTI studies

have focused on frontal WM, highlighting compromised connections between the prefrontal cortex and other cortical and subcortical regions, with these being the most replicated DTI-based findings (36). Tracts such as the anterior fronto-occipital fasciculus, which connects the orbitofrontal cortex with the temporal and occipital lobes, and the cingulum bundle, which connects the frontal and temporal lobes, also display reduced FA values in BD (47, 48). Although MTR imaging has been studied to a lesser extent in BD in comparison to conditions such as SZ, it is nonetheless still notable that the few studies that have been conducted in BD also show compromised myelin content. Further, using both MTR and diffusion tensor spectroscopy, Lewandowski et al. (2015) show that although there is compromised myelin integrity in BD, there are not accompanying changes in axonal integrity (7). In combination, these studies highlight the role of myelin-based deficits in the pathophysiology of BD, and the work contained in this thesis further illustrates that these deficits are also seen throughout the cerebral cortex, and indeed do carry bearing on cognitive performance in individuals with BD.

Other major strengths of our study include the study of a largely euthymic population, with no current psychiatric comorbidities. Therefore, we can be confident that the cross-sectional results observed in the study are not clouded by affective mood state or variable psychiatric diagnoses.

Our work further provides potential immunomodulatory mechanisms of ICM maintenance, seen through the effects of anti-MBP on ICM. Anti-MBP has been shown to have regenerative potential in previous animal studies, and may play a significant role in myelin turnover within the brain. It is important to note that there may be differences of

vulnerability between subcortical WM and ICM in this context. Because ICM is less "bundled" and dense than myelin in WM tracts, perhaps the relative physical sparsity renders it more vulnerable to damage from immune/ neurotoxic insult. Developmentally, perhaps ICM is more vulnerable to small alterations in the developmental blueprint. Subtle variations in ICM, as shown in animal studies, can have profound impacts well into adulthood, and can be modified by experience, including childhood adversity (49-51).

Although we provide a potential neuromodulatory pathway for the maintenance of ICM, the effects could not be due to inflammatory mediators at all, as seen through the lack of association between CRP and ICM. This could be accomplished through other pathways, where increased inflammation is a by-product such as the kynurenine pathway, oxidative stress, and neurotrophic factors.

6.3 Limitations

The implications of scientific studies should be considered within their limitations. Limitations specific to each study are outlined in the relevant chapters. Here, we discuss overall study limitations.

First and foremost, the cross-sectional nature of our study design does not tap into the prospective effects of ICM on illness progression. Cross-sectionally, we have shown that ICM is associated with duration of illness, age of onset, anticonvulsant and antipsychotic use in BD-I subjects. These findings must be confirmed using a longitudinal design in order to determine the true effects of ICM in association with clinical

characteristics and variates of BD, and whether the two change in tandem over time. Although the age of onset is associated with BD, we are unable to comment on whether deficits in ICM are present prior to illness onset, or whether they are a secondary result of the illness itself and another primary brain process. Along those lines, we are also unable to determine whether the results seen here are due dysmyelination or demyelination within the illness.

We were unable to detect any overall differences in cognitive performance in BD subjects in comparison to controls. Cognitive dysfunction has been identified as an endophenotype of the illness, and is present during all phases of the illness, and is seen in first-degree relatives of individuals with BD. Therefore, our results indicate the lack of sensitivity of the cognitive battery that we used- the CNS- Vital Signs, CNSVS- in a population of BD subjects. It has previously been validated in both subjects with depression and mild cognitive impairment. However, its functionality may not translate to a BD population. It is notable, however, that despite a lack of overall group differences in cognitive function between groups, we were able to detect an association with ICM in the BD group, but not in controls. This further highlights the potential lack of sensitivity of the CNSVS in this population. Although we have shown that ICM is correlated with verbal memory, executive function, processing speed, and reaction time, it remains unknown whether these findings are indicative of separate impairments within each cognitive domain, or whether there is a single fundamental abnormality in cognitive functioning, such as working memory, that is subsequently reflected across various domains.

A lack of group differences on the cognitive measures and serum marker levels (CRP and Anti-MBP) may also have stemmed from potential Type II error. Increased sample size in future studies would help better elucidate whether these differences between study populations exist, and were not detected here due to sampling error.

Our BD sample was composed of subjects in a variety of current mood states, although it was largely euthymic (79-82%). Indeed, prior studies have shown that WM abnormalities vary with illness state in BD. Although mood state was not a significant factor in our statistical analyses, it is an important limitation of our study.

Another limitation of our study is that we average the ICM signal across regions of interest in order to obtain a singular signal output for each region. In this process, we are losing spatial acuity that may be relevant to differential effects of ICM on behavioural aspects of BD within certain regions themselves.

In the analyses of peripheral blood markers using CRP and anti-MBP and their effects on ICM (Chapters 3 and 4), we lack *a priori* hypotheses regarding potential differential effects of inflammatory-mediated markers by cortical region in BD. This precludes us from conducting region specific analysis, and compromises power of our sample, as the analysis is exploratory in nature.

Although we assess the effects of medication on ICM in BD using a previously developed and widely used medication index (41, 52), it is important to note that we are only assessing the effects of the current psychotropic regimen of the individual. Past medication history is not assessed. Further, the potential effects of psychotherapy are not taken into account in our analyses.

6.4 Future Directions

Our work provides the foundation for many future studies regarding ICM and its role in the pathophysiology of BD. Firstly, the combination of imaging modalities will be useful to enhance the understanding of the ICM deficits seen in our current study in relation to other neurobiological hallmarks of BD. Adding DTI and fMRI protocols to ICM scans would allow 1) the identification of the connection between cortical and subcortical brain regions through the structural means of subcortical WM tracts and 2) the determination of whether the potentially altered connectivity, mediated through ICM, has consequences for the functional connectivity (BOLD signal) in BD. Using concurrent electroencephalography (EEG) would further help identify whether deficits in ICM are accompanied by concurrent changes in neuronal signal fidelity. A prior study showed that altered ICM in the posterior cingulate cortex is associated with altered EEG signaling and cognitive performance in controls (44). In addition, longitudinal study designs will help assess ICM in relation to illness progression in a prospective manner. This will help confirm the initial findings of our work, which indicate an association between ICM and illness characteristics of BD.

Our study focused on assessing the role of ICM in BD across young adulthood. Future studies should extend to both pediatric and older-adult populations. Studies in pediatric and at-risk samples (especially discordant twin pairs) will help determine whether ICM deficits occur before illness onset, and whether they are prodromal in nature Further, studies of at-risk individuals will help determine whether ICM deficits are an endophenotype of BD. Together, longitudinal design studies in pediatric populations will

be integral in the delineation between dysmyelination and demyelination in BD. Furthermore, it is unclear whether there is a threshold or range of ICM levels that can account for optimal brain functioning in BD (or controls), and whether illness onset or progression is associated with surpassing this threshold and/or range. Further, these studies will help identify potential markers of risk and resilience in relation to ICM and the onset and progression of BD.

Future studies should also examine the association between ICM and cognitive performance in BD using more sensitive testing batteries, previously validated in BD, such as the MATRICS Consensus Cognitive Battery (45, 46).

Other factors that may affect ICM that were not investigated in our studies should be considered. Childhood trauma, obesity, smoking status, lifetime psychiatric/medical comorbidity, and prior medication/therapy exposure have been shown to influence brain function and subcortical WM (43, 47-50). Understanding the effects of these variants and their influence will help understand the role of ICM in the pathophysiology of BD.

Although prior studies have investigated ICM in Schizophrenia, it has largely been limited to the effects of the antipsychotic risperidone. ICM data in both Schizophrenia (in more broad contexts) and Major Depressive Disorder would help disentangle whether ICM aberrations are characteristic of disorders of (psychotic) mania, depressive symptomatology, or general psychopathology.

Studies assessing potential inflammatory mechanisms of ICM dysregulation and, potential mediators of the relationship between ICM and cognitive function should explore indices of toxicity and inflammatory marker panels in BD (e.g. multiplex assays).

6.5 Conclusion

In conclusion, the work contained within this thesis shows that age-related ICM content is disrupted throughout the cerebral cortex in BD subjects. Further, we found that ICM may be a novel microstructural correlate of cognitive dysfunction in BD, particularly verbal memory performance. We also identify anti-MBP as a potential serum marker of ICM. This work has significant implications for the behavioural/cognitive dysfunction commonly observed through the course of BD, and provides a novel avenue of research into a potential marker of illness progression and symptom remediation in BD.

6.6 References

1. Uranova N, Orlovskaya D, Vikhreva O, Zimina I, Kolomeets N, Vostrikov V, et al. Electron microscopy of oligodendroglia in severe mental illness. Brain research bulletin. 2001;55(5):597-610.

2. Uranova NA, Vostrikov VM, Orlovskaya DD, Rachmanova VI. Oligodendroglial density in the prefrontal cortex in schizophrenia and mood disorders: a study from the Stanley Neuropathology Consortium. Schizophrenia research. 2004;67(2):269-75.

3. Adler CM, Holland SK, Schmithorst V, Wilke M, Weiss KL, Pan H, et al. Abnormal frontal white matter tracts in bipolar disorder: a diffusion tensor imaging study. Bipolar disorders. 2004;6(3):197-203.

4. Heng S, Song AW, Sim K. White matter abnormalities in bipolar disorder: insights from diffusion tensor imaging studies. Journal of neural transmission. 2010;117(5):639-54.

5. Ji A, Godwin D, Rutlin J, Kandala S, Shimony JS, Mamah D. Tract-based analysis of white matter integrity in psychotic and nonpsychotic bipolar disorder. Journal of Affective Disorders. 2017;209:124-34.

6. Mahon K, Burdick KE, Szeszko PR. A role for white matter abnormalities in the pathophysiology of bipolar disorder. Neuroscience & Biobehavioral Reviews. 2010;34(4):533-54.

7. Lewandowski KE, Ongür D, Sperry SH, Cohen BM, Sehovic S, Goldbach JR, et al. Myelin vs axon abnormalities in white matter in bipolar disorder. Neuropsychopharmacology. 2015;40(5):1243-9.

8. Öngür D, Drevets WC, Price JL. Glial reduction in the subgenual prefrontal cortex in mood disorders. Proceedings of the National Academy of Sciences. 1998;95(22):13290-5.

9. Lake EM, Steffler EA, Rowley CD, Sehmbi M, Minuzzi L, Frey BN, et al. Altered intracortical myelin staining in the dorsolateral prefrontal cortex in severe mental illness. European Archives of Psychiatry and Clinical Neuroscience. 2016:1-8.

10. Bock NA, Hashim E, Janik R, Konyer NB, Weiss M, Stanisz GJ, et al. Optimizing T 1-weighted imaging of cortical myelin content at 3.0 T. Neuroimage. 2013;65:1-12.

11. Bock NA, Kocharyan A, Liu JV, Silva AC. Visualizing the entire cortical myelination pattern in marmosets with magnetic resonance imaging. Journal of neuroscience methods. 2009;185(1):15-22.

12. Rowley CD, Bazin P-L, Tardif CL, Sehmbi M, Hashim E, Zaharieva N, et al. Assessing intracortical myelin in the living human brain using myelinated cortical thickness. Frontiers in neuroscience. 2015;9.

13. Rowley CD, Sehmbi M, Bazin PL, Tardif CL, Minuzzi L, Frey BN, et al. Age-related mapping of intracortical myelin from late adolescence to middle adulthood using T1-weighted MRI. Human Brain Mapping. 2017.

14. Jørgensen K, Nerland S, Norbom L, Doan N, Nesvåg R, Mørch-Johnsen L, et al. Increased MRI-based cortical grey/white-matter contrast in sensory and motor regions in schizophrenia and bipolar disorder. Psychological medicine. 2016;46(09):1971-85.

15. Bora E, Yucel M, Pantelis C. Cognitive endophenotypes of bipolar disorder: a meta-analysis of neuropsychological deficits in euthymic patients and their first-degree relatives. Journal of affective disorders. 2009;113(1):1-20.

16. Martínez-Arán A, Vieta E, Reinares M, Colom F, Torrent C, Sánchez-Moreno J, et al. Cognitive function across manic or hypomanic, depressed, and euthymic states in bipolar disorder. American Journal of Psychiatry. 2004;161(2):262-70.

17. Robinson LJ, Thompson JM, Gallagher P, Goswami U, Young AH, Ferrier IN, et al. A meta-analysis of cognitive deficits in euthymic patients with bipolar disorder. Journal of affective disorders. 2006;93(1):105-15.

18. Burdick K, Goldberg J, Harrow M. Neurocognitive dysfunction and psychosocial outcome in patients with bipolar I disorder at 15-year follow-up. Acta Psychiatrica Scandinavica. 2010;122(6):499-506.

19. Simonsen C, Sundet K, Vaskinn A, Birkenaes AB, Engh JA, Færden A, et al. Neurocognitive dysfunction in bipolar and schizophrenia spectrum disorders depends on history of psychosis rather than diagnostic group. Schizophrenia Bulletin. 2009:sbp034.

20. Post RM, Leverich GS, Kupka RW, Keck Jr PE, McElroy SL, Altshuler LL, et al. Early-onset bipolar disorder and treatment delay are risk factors for poor outcome in adulthood. The Journal of clinical psychiatry. 2010;71(7):864-72.

21. Teunissen C, Van Boxtel M, Bosma H, Bosmans E, Delanghe J, De Bruijn C, et al. Inflammation markers in relation to cognition in a healthy aging population. Journal of neuroimmunology. 2003;134(1):142-50.

22. Misiak B, Stańczykiewicz B, Kotowicz K, Rybakowski JK, Samochowiec J, Frydecka D. Cytokines and C-reactive protein alterations with respect to cognitive impairment in schizophrenia and bipolar disorder: A systematic review. Schizophrenia Research. 2017.

23. Warrington AE, Asakura K, Bieber AJ, Ciric B, Van Keulen V, Kaveri SV, et al. Human monoclonal antibodies reactive to oligodendrocytes promote remyelination in a model of multiple sclerosis. Proceedings of the National Academy of Sciences. 2000;97(12):6820-5.

24. Hauben E, Nevo U, Yoles E, Moalem G, Agranov E, Mor F, et al. Autoimmune T cells as potential neuroprotective therapy for spinal cord injury. The Lancet. 2000;355(9200):286-7.

25. Moalem G, Yoles E, Mor F, Cohen IR, Schwartz M. Autoimmune T cells protect neurons from secondary degeneration after central nervous system axotomy. Nature medicine. 1999;5(1).

26. Moalem G, Yoles E, Leibowitz-Amit R, Muller-Gilor S, Mor F, Cohen IR, et al. Autoimmune T cells retard the loss of function in injured rat optic nerves. Journal of neuroimmunology. 2000;106(1):189-97.

27. Bieber AJ, Warrington A, Asakura K, Ciric B, Kaveri SV, Pease LR, et al. Human antibodies accelerate the rate of remyelination following lysolecithin-induced demyelination in mice. Glia. 2002;37(3):241-9.

28. Braitenberg V. A note on myeloarchitectonics. Journal of comparative Neurology. 1962;118(2):141-56.

29. Chen MS, Huber AB, van der Haar ME, Marcus F. Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. Nature. 2000;403(6768):434.

30. Glasser MF, Goyal MS, Preuss TM, Raichle ME, Van Essen DC. Trends and properties of human cerebral cortex: correlations with cortical myelin content. Neuroimage. 2014;93:165-75.

31. Paus T, Keshavan M, Giedd JN. Why do many psychiatric disorders emerge during adolescence? Nature Reviews Neuroscience. 2008;9(12):947-57.

32. Kempton MJ, Geddes JR, Ettinger U, Williams SC, Grasby PM. Meta-analysis, database, and meta-regression of 98 structural imaging studies in bipolar disorder. Archives of general psychiatry. 2008;65(9):1017-32.

33. Soares JC, Mann JJ. The anatomy of mood disorders—review of structural neuroimaging studies. Biological psychiatry. 1997;41(1):86-106.

34. Taylor WD, Payne ME, Krishnan KRR, Wagner HR, Provenzale JM, Steffens DC, et al. Evidence of white matter tract disruption in MRI hyperintensities. Biological psychiatry. 2001;50(3):179-83.

35. Awada KA, Jackson DR, Baumann SB, Williams JT, Wilton DR, Fink PW, et al. Effect of conductivity uncertainties and modeling errors on EEG source localization using a 2-D model. IEEE Transactions on Biomedical Engineering. 1998;45(9):1135-45.

36. Bipolar Disorder: Clinical and Neurobiological Foundations. 1 ed. Singapore: Wiley-Blackwell; 2010.

37. Kieseppä T, Mäntylä R, Tuulio-Henriksson A, Luoma K, Mantere O, Ketokivi M, et al. White matter hyperintensities and cognitive performance in adult patients with bipolar I, bipolar II, and major depressive disorders. European Psychiatry. 2014;29(4):226-32.

38. Rolstad S, Abé C, Olsson E, Eckerström C, Landén M. Cognitive reserve lessens the burden of white matter lesions on executive functions in bipolar disorder. Psychological medicine. 2016;46(15):3095-104.

39. Bearden CE, Hoffman KM, Cannon TD. The neuropsychology and neuroanatomy of bipolar affective disorder: a critical review. Bipolar disorders. 2001;3(3):106-50.

40. Martinez-Aran A, Vieta E, Torrent C, Sanchez-Moreno J, Goikolea J, Salamero M, et al. Functional outcome in bipolar disorder: the role of clinical and cognitive factors. Bipolar disorders. 2007;9(1-2):103-13.

41. Hassel S, Almeida JR, Kerr N, Nau S, Ladouceur CD, Fissell K, et al. Elevated striatal and decreased dorsolateral prefrontal cortical activity in response to emotional stimuli in euthymic bipolar disorder: no associations with psychotropic medication load. Bipolar disorders. 2008;10(8):916-27.

42. Altshuler LL, Bookheimer SY, Townsend J, Proenza MA, Eisenberger N, Sabb F, et al. Blunted activation in orbitofrontal cortex during mania: a functional magnetic resonance imaging study. Biological psychiatry. 2005;58(10):763-9.

43. Abé C, Ekman C-J, Sellgren C, Petrovic P, Ingvar M, Landén M. Cortical thickness, volume and surface area in patients with bipolar disorder types I and II. Journal of psychiatry & neuroscience: JPN. 2016;41(4):240.

44. Hibar D, Westlye L, Doan N, Jahanshad N, Cheung J, Ching C, et al. Cortical abnormalities in bipolar disorder: an MRI analysis of 6503 individuals from the ENIGMA Bipolar Disorder Working Group. Molecular Psychiatry. 2017.

45. McKenna BS, Theilmann RJ, Sutherland AN, Eyler LT. Fusing functional MRI and diffusion tensor imaging measures of brain function and structure to predict working memory and processing speed performance among inter-episode bipolar patients. Journal of the International Neuropsychological Society. 2015;21(5):330-41.

46. Kochunov P, Coyle TR, Rowland LM, Jahanshad N, Thompson PM, Kelly S, et al. Association of White Matter With Core Cognitive Deficits in Patients With Schizophrenia. JAMA psychiatry. 2017.

47. Haznedar MM, Roversi F, Pallanti S, Baldini-Rossi N, Schnur DB, LiCalzi EM, et al. Fronto-thalamo-striatal gray and white matter volumes and anisotropy of their connections in bipolar spectrum illnesses. Biological psychiatry. 2005;57(7):733-42.

48. Wang F, Jackowski M, Kalmar JH, Chepenik LG, Tie K, Qiu M, et al. Abnormal anterior cingulum integrity in bipolar disorder determined through diffusion tensor imaging. The British Journal of Psychiatry. 2008;193(2):126-9.

49. Yang Y, Cheng Z, Tang H, Jiao H, Sun X, Cui Q, et al. Neonatal Maternal Separation Impairs Prefrontal Cortical Myelination and Cognitive Functions in Rats Through Activation of Wnt Signaling. Cerebral Cortex. 2017;27(5):2871-84.

50. Makinodan M, Rosen KM, Ito S, Corfas G. A critical period for social experience–dependent oligodendrocyte maturation and myelination. science. 2012;337(6100):1357-60.

51. Poggi G, Boretius S, Möbius W, Moschny N, Baudewig J, Ruhwedel T, et al. Cortical network dysfunction caused by a subtle defect of myelination. Glia. 2016;64(11):2025-40.

52. Phillips ML, Travis MJ, Fagiolini A, Kupfer DJ. Medication effects in neuroimaging studies of bipolar disorder. American Journal of Psychiatry. 2008;165(3):313-20.

53. Grydeland H, Westlye LT, Walhovd KB, Fjell AM. Intracortical posterior cingulate myelin content relates to error processing: results from T1-and T2-weighted MRI myelin mapping and electrophysiology in healthy adults. Cerebral Cortex. 2016;26(6):2402-10.

54. Burdick KE, Goldberg TE, Cornblatt BA, Keefe RS, Gopin CB, DeRosse P, et al. The MATRICS consensus cognitive battery in patients with bipolar I disorder. Neuropsychopharmacology. 2011;36(8):1587.

55. Yatham LN, Torres IJ, Malhi GS, Frangou S, Glahn DC, Bearden CE, et al. The International Society for Bipolar Disorders–Battery for Assessment of Neurocognition (ISBD-BANC). Bipolar disorders. 2010;12(4):351-63.

56. McCarthy-Jones S, Oestreich LK, Lyall AE, Kikinis Z, Newell DT, Savadjiev P, et al. Childhood adversity associated with white matter alteration in the corpus callosum, corona radiata, and uncinate fasciculus of psychiatrically healthy adults. Brain imaging and behavior. 2017:1-10.

57. Cazettes F, Cohen JI, Yau PL, Talbot H, Convit A. Obesity-mediated inflammation may damage the brain circuit that regulates food intake. Brain research. 2011;1373:101-9.

58. Kullmann S, Schweizer F, Veit R, Fritsche A, Preissl H. Compromised white matter integrity in obesity. Obesity reviews. 2015;16(4):273-81.

59. Rüsch N, Weber M, Il'yasov KA, Lieb K, Ebert D, Hennig J, et al. Inferior frontal white matter microstructure and patterns of psychopathology in women with borderline personality disorder and comorbid attention-deficit hyperactivity disorder. Neuroimage. 2007;35(2):738-47.