2014-05-01

Measurement of the Cerebellar Vermis in Bipolar Disorder and the Effect of Lithium Treatment

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Measurement of the Cerebellar Vermis in Bipolar Disorder and the Effect of Lithium Treatment

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF NEUROSCIENCE

CALGARY, ALBERTA

April 2014

Abstract

Previous studies have suggested that the cerebellar vermis may be smaller in individuals with bipolar disorder, but these findings are inconsistent and have not considered the potential impact of medication. To address these knowledge gaps, the cerebellar vermis was measured using structural magnetic resonance imaging in both an adolescent and an adult sample. Analysis of variance was performed on the cross-sectional area of the three vermal lobes and on the total area. There were no significant differences between adolescent or adult bipolar subjects compared to healthy controls in any region, regardless of lithium treatment. In addition, a medication-naïve subset of the adult population underwent a two-year course of lithium treatment and was then reassessed. No changes in vermis area were found within subjects across the treatment. These results, combined with literature meta-analysis, indicate no clear effect of either bipolar disorder, or lithium treatment, on the size of the cerebellar vermis.

Acknowledgements

I would like to thank the following individuals: my supervisors, Dr. Glenda MacQueen and Dr. Frank MacMaster, as well as Dr. Natalia Jaworska, for their guidance, advice, and support throughout my course of study; committee members Dr. Scott Patten and Dr. Matt Hill for their sound guidance, helpful suggestions, and technical expertise; Anthony Nazarov for his help with data and analysis; Allegra Courtright and Jacqueline Bobyn for good company and occasional commiseration; and lastly my unshakably fast friend and staunch supporter, Charlie Edman.

Dedication

This work is dedicated to Charlie Edman, although what use she will have for it I can't imagine.

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List of Symbols, Abbreviations and Nomenclature

Symbol	Definition
2-AG	2-Arachidonoylglycerol
ACC	Anterior cingulate cortex
AEA	Anandamide
AFNI	Analysis of Functional Neuroimages (software)
AIMS	Abnormal Involuntary Movement Scale
APA	American Psychiatric Association
BD	Bipolar disorder
BDNF	Brain-derived neurotrophic factor
BD-I	Bipolar I disorder
BD-II	Bipolar II disorder
CT	Computed tomography
DLPFC	Dorsolateral prefrontal cortex
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders, 4th Edition Text Revision
fMRI	Functional magnetic resonance imaging
GABA	*gamma*-Aminobutyric acid
GSK-3	Glycogen synthase kinase-3
HamD-17	17-Item Hamilton Depression Rating Scale
HPA	Hypothalamic-pituitary-adrenal
ICC	Interclass correlation coefficient
Li	Lithium
LTP	Long-term potentiation
LTD	Long-term depression
MDD	Major depressive disorder
MDQ	Mood Disorder Questionnaire
MRI	Magnetic resonance imaging
NIMH	National Institutes of Mental Health
NMR	Nuclear magnetic resonance

OFC	Orbitofrontal cortex
SAS	Simpson-Angus Scale
SCID	Structured Clinical Interview for DSM-IV
SSRI	Selective serotonin reuptake inhibitor
TCV	Total cerebral volume
TMT	Trail-Making Test
TrkB	Tropomysin receptor kinase B
V1	Anterior lobe of cerebellar vermis
V2	Superior-posterior lobe of cerebellar vermis
V3	Inferior-posterior lobe of cerebellar vermis
VLPFC	Ventrolateral prefrontal cortex
YMRS	Young Mania Rating Scale

Epigraph

All living things contain a measure of madness that moves them in strange, sometimes inexplicable ways. This madness can be saving; it is part and parcel of the ability to adapt. Without it, no species would survive.

-Yann Martel

Chapter One: **Introduction**

1.1 Outline of the Research Problem

Interest in imaging of the cerebellum in bipolar disorder (BD) stems from findings of abnormalities in the limbic system and paralimbic cortex associated with BD (Blond, Fredericks, & Blumberg, 2012; Cerullo, Adler, Delbello, & Strakowski, 2009), coupled with the discovery that there is significant functional connectivity between the cerebellum and limbic structures such as the cingulate cortex and hypothalamus (Baldacara, Borgio, Lacerda, & Jackowski, 2008; Baumann & Mattingley, 2012; Mamah, Barch, & Repovs, 2013). Specifically, the mid-sagittal portion of the cerebellum, called the cerebellar vermis, appears to be the locus of cerebellar-limbic connectivity (Grimaldi & Manto, 2012). In contrast with imaging of limbic structures, the literature is not clear on what effects BD may have on the vermis, or how medication may mediate any such effects. A handful of imaging studies have addressed the possibility of cerebellar vermal abnormalities in BD (DelBello, Strakowski, Zimmerman, Hawkins, & Sax, 1999; Mills, Delbello, Adler, & Strakowski, 2005), but these have not controlled for medication use. This last point is particularly salient since BD is treated with a broad range of medications; some of which- notably lithium- are already known to affect the size and connectivity of the aforementioned structures of interest (Yucel et al., 2008). A recent review of imaging in BD stresses the need for more work in that area, as the literature is characterised by low power and heterogeneous methodology (Emsell & McDonald, 2009), and this message is echoed by researchers working to understand cerebellar deficits, who admit that "the debate on the exact function of the cerebellum remains" (Grimaldi & Manto, 2012).

1.2 Objectives

We aim to synthesize two previous findings: first that the cerebellar vermis displays structural abnormalities in subjects with BD (no previous studies have controlled for medication status) (Mills et al., 2005; Womer et al., 2009), and second that lithium directly exerts structural effects on the brain (Hajek et al., 2012; Yucel et al., 2007; Yucel et al., 2008). This study consists of several components, each of which is designed to address a key question: whether BD directly impacts the cerebellum, or whether past findings of abnormal cerebellar vermis volume in BD are a consequence of lithium treatment. It is possible that both the illness and the treatment have independent effects, which has already been reported to be the case in the hippocampus of patients with BD (Hajek et al., 2012).

Our dataset has detailed clinical, demographic and treatment information on patients, includes a longitudinal component of patients scanned both prior to and following initiation of lithium, and represents a substantially larger dataset to be analyzed than previous studies of the vermis in BD. The large size of our sample will enable us to examine for medication effects and other variables such as sex and illness duration that might also influence vermis volumes. An additional dataset consisting of adolescent, treatment-naive subjects with BD and healthy controls will allow us to examine the potential effects of the illness on the cerebellar vermis in the absence of long illness duration and medication effects. Our methods and analysis plan are structured by three primary hypotheses.

1.3 Primary hypotheses

1. Cerebellar vermis area will not differ between adolescent, untreated BD patients and age-matched controls.

2. Adult BD patients not taking lithium will have smaller vermis areas than healthy control subjects, however this effect will be normalized by lithium treatment, so that vermis area in patients taking lithium will not differ from healthy controls.

3. Vermis area will increase over two years of lithium treatment in BD patients with no previous exposure to lithium.

1.4 Structure of the thesis

This thesis is divided into five chapters. Chapter One provides a very brief introduction to the thesis, its aims and hypotheses, and a statement of the research problem. Chapter Two provides: a brief introduction to Magnetic Resonance Imaging, the primary tool used in this investigation; an overview of bipolar disorder, including incidence, treatment, and what is known about the bipolar brain; and a thorough description of the region of interest to this study, the cerebellar vermis. Chapter Two is intended to provide sufficient coverage of the relevant background that a non-expert in biological psychiatry can understand the thesis in full, and furthermore is intended to justify the relevance of the questions addressed therein. Chapter Three describes the methods employed in recruitment of subjects for this study, the acquisition of clinical and imaging data, and the methods employed in data analysis. Chapter Four presents the results of the study, addressing each of the three hypotheses given above specifically, as well as exploring several

other factors of potential interest, the selection of which is driven by suggestions from past literature. Chapter Five interprets and discusses the results from Chapter Four. Firstly, Chapter Five answers the hypotheses directly, leading into the conclusions of this thesis. From there Chapter Five discusses the degree to which the findings of this study agree with- or challenge-past work. Considerable attention is given to the confounding factors and limitations present in this study, and common to studies of this nature. In the author's opinion it is extremely important to undertake study of the brain in BD, or any mental illness, very carefully and with an eye to specific hypotheses, thus Chapter Five closes with the implications of this work, and how future studies may benefit from lessons learned.

1.5 Roles of collaborators

The adult imaging data and clinical and demographic information used in this work were collected by researchers at St. Joseph's Healthcare, Hamilton, ON; Anthony Nazarov and Dr. Kaan Yücel were particularly helpful in compiling these data. Adolescent data were collected previously by Dr. MacMaster at Dalhousie University. All cerebellar measurements, statistical analysis, literature review and meta-analysis were performed by Mr. Mahnke, with periodic guidance from Drs. MacMaster, MacQueen, Patten, and Hill. Fellow student Bernice Fonseka learned and performed a number of cerebellar measurements independently for the purpose of inter-rater validation.

Chapter Two: **Background and Literature Review**

2.1 Magnetic Resonance Imaging

2.1.1 The phenomenon of nuclear magnetic resonance

Nuclear magnetic resonance (NMR) was first described by Isidor Rabi in 1938 (Rabi, Zacharias, Millman, & Kusch, 1938). The discovery was of revolutionary importance in the field of physics, earning Rabi the 1944 Nobel prize in Physics. NMR researchers earned two more Nobel prizes in Physics around the same time, followed by two Nobel prizes in Chemistry around the turn of the 20^{th} century. The essential discovery consists of three parts:

1. The nuclei of many common elements (including ^{1}H) have an intrinsic magnetic moment (picture a bar magnet)
2. When such a nucleus is placed in an external magnetic field, two different energy states emerge; a low-energy state in which the nuclear moment is aligned with the external field, and a high-energy state in which the nuclear moment opposes the external field
3. A nucleus in an external magnetic field can absorb or emit electromagnetic radiation in transitioning between these two energy states

These observations led to the technique of NMR spectroscopy; by placing a substance within a strong magnetic field and recording the frequencies of electromagnetic radiation it will absorb (under common conditions NMR occurs at radio frequencies), one can obtain a lot of detail about molecular and electronic structure. As a result, NMR has become a mainstay in the field of chemistry for its ability to rapidly identify most compounds.

2.1.2 Structural imaging of the brain

Engineering developments extended the application of NMR from its beginnings in theoretical physics to chemistry, materials science, and ultimately to clinical medicine: the 2003 Nobel prize in Medicine was awarded for the development of Magnetic Resonance Imaging (MRI) (Paul Lauterbur and Peter Mansfield 2003). MRI is based on the fact that the body is composed largely of water (each molecule of which has two spin-active ^{1}H nuclei), and that different tissues have different amounts of water. The development of practical MRI required two major challenges to be overcome. The first was a strong superconducting magnet large enough to fit a human within it; the first of these, "The Indomitable", was successfully tested in 1977. The second challenge was a means of spatially resolving the NMR signal; a spatial average, though quite useful in chemistry, gives no useful information about the human body. Spatial resolution was accomplished by augmenting the strong primary magnet with gradient magnets, so that the field could be altered across three-dimensional space. These early MRI machines could image the human brain, but they were slower, more expensive, and produced lower-quality images than Computed Tomography (CT), which was readily available at the time. Through the tenacity of those developing new MRI technology, though, it has since become an invaluable tool across the whole of medicine.

2.1.3 Imaging in the context of this thesis

MRI is an ideal tool for a quantitative examination of brain structures, such as in this work, primarily due to its safety. Although CT is comparatively faster and cheaper to perform than MRI, it also exposes the subject to ionizing radiation and thus presents a non-negligible cancer risk. As such, it could be considered unfeasible to employ CT in a research study such as this

one, since doing so presents a risk without conferring any benefit to the subject. Conversely MRI exposes the subject to a strong magnetic field- which is harmless unless there is metal present- and to radio-frequency light, also harmless in the absence of metal, and thus raises no ethical issues. The largest concern of exposing subjects to MRI is that the bore of the instrument is very confining, and can induce panic in claustrophobic individuals; this possibility can be screened out during recruitment though. An additional advantage of MRI is that it provides excellent contrast between different soft tissues, and thus gives a highly detailed picture of the brain, including clear distinctions between gray matter and white matter.

The images analyzed in this thesis are T_1-weighted, where T_1 is the *spin-lattice relaxation time* or the average time it takes for excited protons to emit electromagnetic energy and return to ground state. T_1 differs considerably between grey matter and white matter, so this type of scan is ideal for resolving fine structure of the brain; in these images, grey matter appears grey, and white matter white. T_1-weighted images are frequently used to quantify both normal and abnormal brain structure and volume, either by manual tracing of specific structures in the brain, or by automated methods such as voxel-based morphometry (Whitwell, 2009). Also common are T_2-weighted images, where T_2 is the *spin-spin relaxation time*. T_2 images do not differentiate grey and white matter as well, and also require a long echo time and long repetition time, thus it takes considerably longer to acquire T_2-weighted images than T_1-weighted images of comparable resolution. T_2-weighted images do differentiate strongly between tissue and fluid, so they are useful in identifying abnormal fluid accumulation such as in white matter disease, edema, and cerebral infarction (Muftuler, 2013).

2.2 Bipolar Disorder

2.2.1 Clinical presentation, prevalence, course, and impact of bipolar disorder

Bipolar disorder (BD) is characterized by recurrent episodes of mania and depression (Schneider, DelBello, McNamara, Strakowski, & Adler, 2012). Mania commonly presents with symptoms of elevated mood and energy levels, reduced need for sleep, racing thoughts, disinhibition, and in the extreme, psychosis; a manic episode is defined by the DSM-5 (American Psychiatric Association, 2013) as a period of at least seven days in which some or all of the above symptoms are present, and cause obvious difficulty in occupational or social functioning. The DSM also recognizes hypomanic episodes, a milder form of the manic state. Conversely, symptoms of depression include feelings of hopelessness and sadness, fatigue, disrupted appetite and sleep, and anhedonia. In the DSM these symptoms constitute a major depressive episode if some or all symptoms persist for at least two weeks and cause significant social or occupational distress.

Based on these concepts of mania and depression, BD is divided into two categories: bipolar I disorder (BD-I) in which patients have experienced at least one manic episode, and- likely but not necessarily- episodes of hypomania and major depression as well; and bipolar II disorder (BD-II), in which patients have experienced at least one depressive and one hypomanic episode.

A recent study compiling longitudinal data from over 500 BD-I and BD-II patients found that, on average, the patients were euthymic (not manic or depressed) half the time, depressed approximately 36% of the time, and in a manic, hypomanic, or mixed state for the remaining 14% (Kupka et al., 2007).

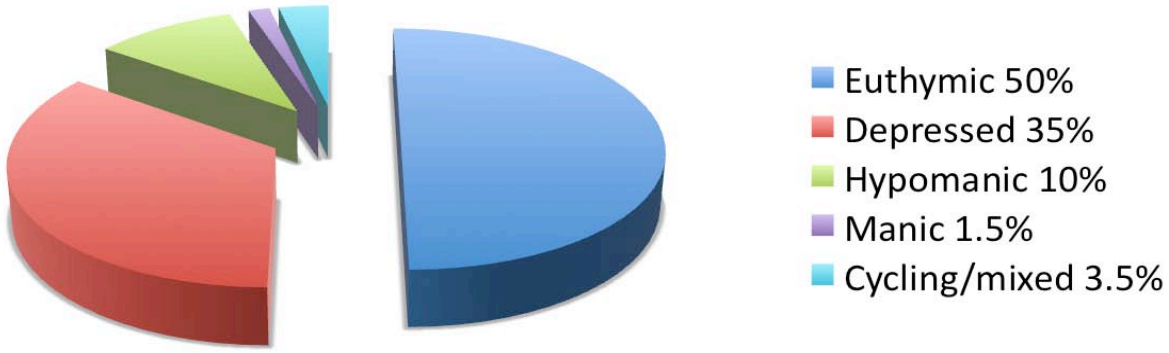

Figure 1: Time spent in different mood states in BD patients, adapted from (Kupka et al., 2007)

BD is a chronic, lifelong condition; clinically, symptom remission (attaining a euthymic state) is usually the goal for treatment of BD as the disorder is not thought to ever "go away". Furthermore, patients with BD continue to experience significant cognitive and functional impairment during remission (Fagiolini et al., 2005; Robinson et al., 2006). BD is associated with numerous psychosocial impacts, including increased suicidal thoughts and behaviours, poor social functioning, unemployment and low income, and decreased quality of life and lifespan (Judd et al., 2005).

BD has been estimated to affect about 1% of the population in North America, with prevalence rates roughly constant worldwide (Judd & Akiskal, 2003; Kleinman et al., 2003). Others argue that the disorder is under-diagnosed and the true lifetime prevalence is as high as 4-6%, taking into account 'subthreshold' symptoms, particularly the likelihood that diagnosticians fail to identify hypomania (MacQueen & Young, 2001; Merikangas et al., 2011). BD is a major contributor to morbidity and mortality in the North American population, and the direct and indirect costs of the disorder in the US have recently been estimated at $150 billion annually (Dilsaver, 2011).

2.2.2 Challenges to diagnosis

BD is regarded as a particularly difficult condition to diagnose (Cardoso de Almeida & Phillips, 2013; Correa et al., 2010; Keshavan et al., 2011). A prominent issue in the literature regarding BD diagnosis is the fact that depression is easier to diagnose than mania or hypomania, thus BD is often miscategorised as MDD (Fagiolini et al., 2013). As shown in Figure 1 above, bipolar individuals spend much more time in a depressed state than a manic or hypomanic state (Kupka

et al., 2007), and a bipolar individual's first episode is usually a depressive episode (Duffy, 2007). Additionally some symptoms of mania, e.g. high energy and decreased need for sleep, may be overlooked because individuals do not identify them as distressing (MacQueen & Young, 2001). As a result, patients can suffer with uncontrolled BD symptoms for up to 10 years before receiving a correct diagnosis (Fagiolini et al., 2013). During this time they may be treated with antidepressant medications such as SSRIs, which may in fact exacerbate BD symptoms by inducing mood state switching (Sidor & MacQueen, 2012). This risk has been identified as particularly salient in young people, and even extended to those at high risk for BD but who have not actually experienced a manic episode (Strawn et al., 2013). The question of antidepressant use in BD has been the subject of intensive study, and to date remains controversial (Pacchiarotti et al., 2013).

On the other end of the spectrum, there is uncertainty around the distinction between diagnoses of BD and schizophrenia as well, owing to similarities in the genetics and clinical presentation of these two disorders; this particular debate dates back to Kraepelin's work over 100 years ago (Keshavan et al., 2011; Potash & Bienvenu, 2009). For the above reasons, there is much interest in developing new means for diagnosis of BD that do not rely on symptomatology, and advancing a more biologically-based understanding of the nature and boundaries of BD.

One such initiative- spearheaded by the National Institute of Mental Health (NIMH)- is called the Research Domain Criteria (RDoC). In contrast to the DSM-5, RDoC is intended purely as a research tool, and it bears little resemblance to the classification system used in the DSM. RDoC focuses on five 'domains' of functioning: negative valence systems, positive valence systems, cognitive systems, systems for social processes, and arousal/regulatory systems (Cuthbert and Kozak, 2013). The utility and validity of the RDoC criteria are not yet known.

Regardless of the outcome though, it represents a radical departure, as research has largely been informed by the DSM alone since the release of DSM-III in 1980. RDoC will be entirely agnostic towards DSM categories, and NIMH director Thomas Insel has been unequivocally opposed to research use of the DSM in recent years (Insel et al., 2010).

2.2.3 Rating scales and tests for bipolar disorder

There exist a plethora of self-rated and clinician-rated scales for depression, for mania, and for bipolar disorder. These scales are generally unable to characterize many of the social and biological components of bipolar illness. In light of the difficulties discussed above, rating scales are no substitute for clinician judgment in diagnosis of BD- they are generally referred to as screening tools, not diagnostic tools- however in a research context they can help to identify and quantify the symptoms of BD, and rate illness severity. A recent report comparing self-rated and clinician-rated scores on the Mood Disorder Questionnaire (MDQ; Hirschfeld et al., 2000), one of the screening tools for BD, found that the self-rated MDQ was a poor predictor of BD-I or BD-II diagnoses, but if the MDQ was delivered as a semi-structured interview (clinician-rated), it was a reliable predictor of these diagnoses (Goldberg, Garakani, & Ackerman, 2012). A recent comparison of two common screening tools for depression, the self-rated Beck Depression Inventory (BDI; Beck, Ward, Mendelson, Mock, & Erbaugh, 1961) and the clinician-rated Montgomery-Åsberg Depression Rating Scale (MADRS; Montgomery & Asberg, 1979), found significant discrepancy between the measures, suggesting that age, sex, and degree of rumination can all contribute to patient under- or over-reporting of symptoms (J. D. Carter, Frampton, Mulder, Luty, & Joyce, 2010).

Two scales were used to evaluate symptoms within our study population, the 17-item Hamilton Depression Rating Scale (HamD-17; Hamilton, 1960) and the Young Mania Rating Scale (YMRS; Young, Biggs, Ziegler, & Meyer, 1978). Both scales are clinician-rated, and together they are used to evaluate the degree of patients' depressive and manic symptoms at the time of assessment.

2.2.3.1 The Trail-Making Test

The Trail-Making Test (TMT) is a popular neuropsychological tool used to assess several domains of cognitive functioning (Bowie & Harvey, 2006). The test consists of two parts, A and B: in part A, the subject uses a pencil to connect the numbers 1-25 in consecutive order; in part B, the series of 25 items alternates between letters and numbers (e.g. 1-A-2-B-3…). The primary metric of the test is the time taken to complete each part, and errors are not recorded. (Tombaugh, 2004)

2.2.3.2 Abnormal Involuntary Movement Scale and Simpson-Angus Scale

The Abnormal Involuntary Movement Scale (AIMS) and Simpson-Angus Scale (SAS) are both clinician-rated scales used to assess abnormal movements in patients. These tests are specifically designed to capture extrapyramidal symptoms, which are common side effects of antipsychotic medications, and potential side effects of lithium treatment as well, especially when combined with antipsychotics (Dols et al., 2013).

2.2.4 Bipolar disorder in children and adolescents

There is substantial controversy around diagnosis and treatment of BD in children (Parens & Johnston, 2010), and indeed there is controversy as to whether the disorder even exists in children (Duffy, 2007). Studies of high-risk children and adolescents find that BD often first

presents with a depressive episode, which may be preceded by nonspecific mood disturbances also. The evidence suggests, in youth and adolescents, BD progresses through stages before developing into the full-blown disorder (Duffy, Alda, Hajek, Sherry, & Grof, 2010). Treating BD in early stages may be more successful in the long run than treatment in adults (Duffy, Milin, & Grof, 2009), so sorting out the diagnosis of BD in adolescents is notably important, but also very challenging. Careful consideration of treatment is indicated also, as little is known about the potential detrimental effects of psychotropic medications on development (Singh & Chang, 2012). The best identifier of young people at high risk for BD at present seems to be not clinical presentation, but rather family history, owing to the disorder's high degree of heritability (Barnett & Smoller, 2009).

2.2.5 Anatomy and biology of bipolar disorder

In recent years advances in understanding of the cognitive and affective functions of the brain, largely driven by neuroimaging studies, have led to the proposal of a neural network model for bipolar disorder. There remains some debate about the specific regions of the brain that should be included in this model, but taken together the literature implicates dysfunction in a corticolimbic network of emotion perception and regulation as a hallmark of BD (Phillips, Ladouceur, & Drevets, 2008). This network includes regions of the prefrontal cortex, particularly the orbitofrontal cortex (OFC), dorsolateral prefrontal cortex (DLPFC), and anterior cingulate cortex (ACC), as well as limbic regions, particularly the hippocampus and amygdala. Some researchers argue for inclusion of the cerebellar vermis in this model (Schmahmann & Sherman, 1998), which will be discussed in greater detail later in this chapter.

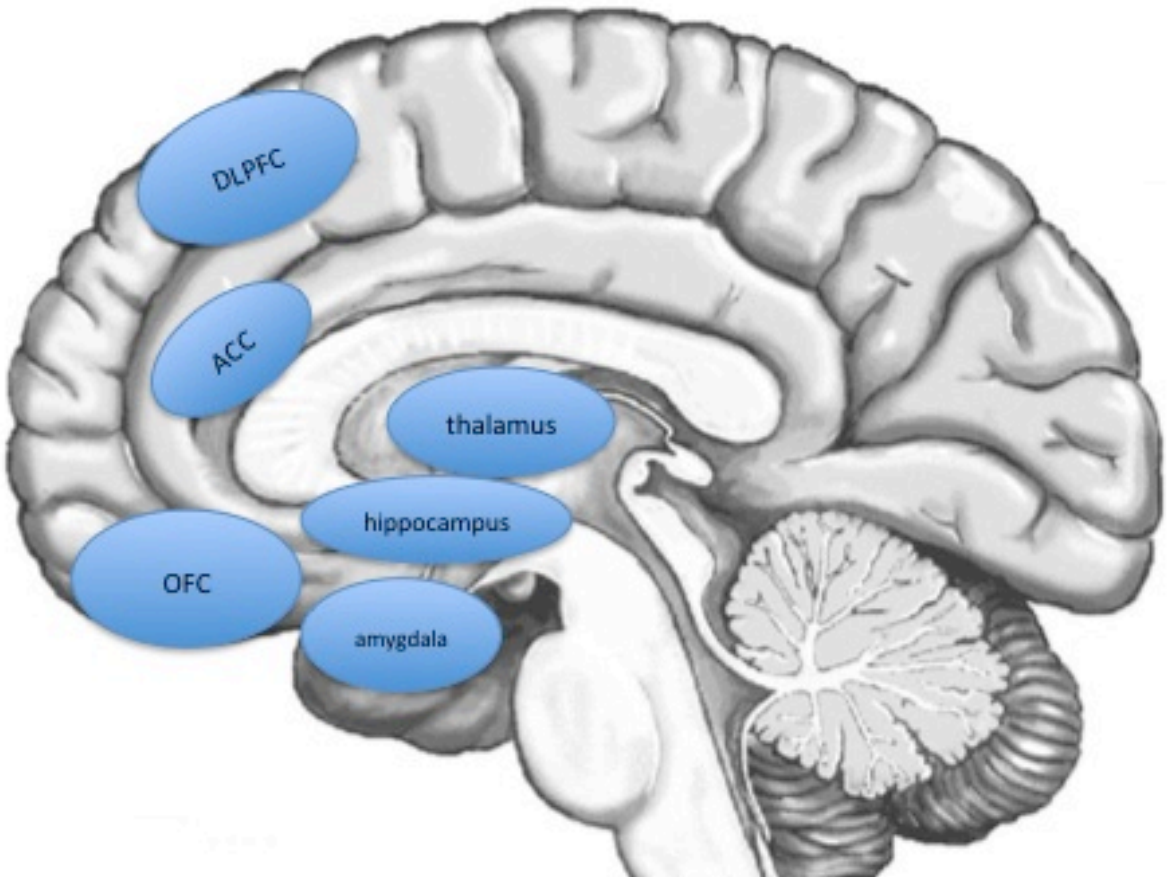

Figure 2: Emotion perception and regulation areas implicated in bipolar disorder, adapted from (Phillips et al., 2008). DLPFC = dorsolateral prefrontal cortex; ACC = anterior cingulate cortex; OFC = orbitofrontal cortex.

2.2.5.1 Neuroplasticity and bipolar disorder

Adult neuroplasticity- the ability of the adult brain to change via new cell growth (neurogenesis), programmed cell death (apoptosis), or changes in connectivity (synaptic plasticity)- has become a fundamentally important concept to mood disorders research. The first suggestions of the existence of plasticity in the adult brain date back 100 years and more to the works of William James, Karl Lashley, and Santiago Ramon y Cajal, among others; however, the idea has only gained real traction in the past couple of decades. Detrimental changes to neuroplasticity, especially in the hippocampus, are now considered by many fundamental to the nature of depression (Kempermann & Kronenberg, 2003; MacQueen et al., 2003), and these changes are proposed to be fundamental to BD as well (Berk et al., 2013; Elvsashagen et al., 2012; Kapczinski, Frey, Kauer-Sant'Anna, & Grassi-Oliveira, 2008; Schloesser, Huang, Klein, & Manji, 2008).

While this work focuses on the macroscopic properties of brain structure identifiable by magnetic resonance imaging (MRI), much has also been learned about cellular plasticity changes in BD which form the basis for understanding macroscopic structural changes. Abnormalities in the hippocampus of people with BD are one of the most robust findings in BD research, and post mortem studies have identified a reduction in the size of hippocampal pyramidal cells in BD patients (Liu, Schulz, Lee, Reutiman, & Fatemi, 2007), as well as a reduction in nonpyramidal cell density (Benes, Kwok, Vincent, & Todtenkopf, 1998), later narrowed specifically to hippocampal interneurons (Konradi et al., 2011). One proposed reason for these observations concerns the hypothalamic-pituitary-adrenal (HPA) axis. Patients with BD have elevated levels of cortisol (a glucocorticoid) associated with stress (Steen et al., 2011); hippocampal cells, rich

in glucocorticoid receptors, have been shown to respond to elevated cortisol with dendritic retraction, and possibly cell death (McEwen & Magarinos, 2001). Neuroplasticity changes in BD have also been linked to lower levels of a protein, brain-derived neurotrophic factor (BDNF) (Post, 2007), a factor important to neurogenesis, cell survival, and synaptic plasticity (Acheson et al., 1995). Low levels of BDNF are, in turn, linked to a variation in the *BDNF* gene known as *val66met* polymorphism. This single-nucleotide polymorphism is thought to affect secretion of BDNF protein, so that individuals with two copies of the *met*-allele have lower levels of secreted BDNF, smaller hippocampal volume, decreased prefrontal grey matter, and increased susceptibility to psychiatric disorders, most notably depression and BD (Chepenik et al., 2009; Pezawas et al., 2004).

Recent work has elaborated the role that the endocannabinoid system plays in neuroplasticity and response to stress as well. The endocannabinoids are two closely-related neurotransmitters, anandamide (AEA), and 2-arachidonoylglycerol (2-AG), now known to be ubiquitous throughout the brain. Primarily retrograde signals, AEA and 2-AG are released from the postsynaptic terminal after synaptic activity, and are important modulators of synaptic plasticity (Castillo, Younts, Chavez, & Hashimotodani, 2012). Impairments in AEA and 2-AG signalling have been identified in chronic stress (Hill & McEwen, 2010), and concomitantly in mood and anxiety disorders (Hill & Gorzalka, 2009; Tambaro & Bortolato, 2012).

2.2.6 Treatments for bipolar disorder

A wide variety of approved pharmacological treatments for BD exist, including the mood stabilizer lithium, anticonvulsants such as valproate and lamotrigine, and antipsychotics such as olanzapine and quetiapine (Sanches, Newberg, & Soares, 2010). Many BD patients are treated

with a combination of medications (Sachs, Peters, Sylvia, & Grunze, 2013), with the possible inclusion of antidepressant medications as well (Vazquez, Tondo, Undurraga, & Baldessarini, 2013). The chemical and physiological effects of these medications have been studied to varying degrees which will not be detailed here: the point is that in BD patients, the chemical environment is extremely complex, and may differ greatly from one patient to the next. This complexity presents a fundamental challenge to clinical studies of BD, and is a primary motivation for this work. We have chosen to look at the physiological effects of lithium specifically.

2.2.6.1 Lithium pharmacotherapy

Lithium (usually in the form of lithium carbonate or lithium citrate) has been the gold standard medication for BD since its discovery as a mood-stabilizing agent in 1948, and is still one of the most effective treatments available, particularly in reducing the risk of suicide (Cipriani, Hawton, Stockton, & Geddes, 2013). The study of lithium remains important for three reasons: first, clinical response to lithium may reflect important genetic factors underlying BD. Lithium is very effective at treating BD in a subset of patients, whereas for others it is ineffective (McCarthy, Leckband, & Kelsoe, 2010). This observation suggests that lithium responders and lithium non-responders may represent distinct BD subtypes. This position is strengthened by the fact that responders and non-responders differ significantly in their family history and clinical course, suggesting that genetic factors are important (Grof et al., 2002; Passmore et al., 2003). A recent large-scale study concluded that low-dose lithium is in fact an ineffective treatment for patients with BD, however the authors emphasize the importance of research into individual-level predictors of treatment response (Nierenberg et al., 2013), which could be genetic, structural, or chemical markers.

Second, although biochemical research has revealed a great deal about lithium's mechanisms of action, the picture is far from complete. Lithium's pharmacological effects are complex and are known to involve growth factors, excitotoxicity, neurogenesis, neuronal differentiation, and apoptosis (Pasquali, Busceti, Fulceri, Paparelli, & Fornai, 2010; Quiroz, Machado-Vieira, Zarate, & Manji, 2010). Several biochemical pathways have been identified that may be important, most notably that lithium directly inhibits glycogen synthase kinase-3 (GSK-3), which impacts a number of biochemical cascades and appears to have a net neuroprotective effect (Valvezan & Klein, 2012). Lithium also promotes brain-derived neurotrophic factor (BDNF), which acts to protect neurons from glutamate excitotoxicity, and is also dysregulated in patients with BD (Post, 2007).

Third, lithium is still a commonly prescribed medication with a number of potentially serious side effects including hypothyroidism, weight gain, and renal toxicity, as well as postural tremor (Gelenberg & Jefferson, 1995) and cerebellar atrophy and toxicity (Fischera, Anneken, Evers, Kloska, & Husstedt, 2009; Niethammer & Ford, 2007). Lithium-induced tremor is a well known side effect of lithium treatment (Tyrer, Lee, & Trotter, 1981), but beyond clinical phenomenology, its aetiology remains largely unknown (Gelenberg & Jefferson, 1995). The 8-12 Hz postural tremor associated with lithium use differs from the classic cerebellar tremor, which is a ~5 Hz intention tremor, however it has been clinically established that cerebellar damage can be accompanied by the higher-frequency postural tremor (Deuschl, Bain, & Brin, 1998). Thus, while no causal link has been illustrated, it is reasonable to hypothesize that the lithium-induced tremor- which occurs in up to 65% of patients (Gelenberg & Jefferson, 1995) - may be mediated by lithium acting on the cerebellum to affect motor control via known molecular mechanisms (Duman & Voleti, 2012; Gould, Quiroz, Singh, Zarate, & Manji, 2004; Pasquali et al., 2010).

2.2.7 Neuroimaging in bipolar disorder

The umbrella term *biomarker* is used to describe any physically measurable parameter that may be used to identify a particular state. Neuroimaging is a promising method for the development of biomarkers for BD (Fleck et al., 2008), an important goal due to the difficulties inherent in diagnosis and treatment of BD. The literature on BD reveals a number of effects of the illness on brain structure. Decreased hippocampal volume is a robust finding in studies of BD (Blumberg, Kaufman et al., 2003; Chepenik et al., 2012; Elvsashagen et al., 2013; Kempton et al., 2011), although not every study corroborates this finding (Foland-Ross et al., 2013). A consensus has not been reached, perhaps due in part to the confounding effects of medication (Schneider et al., 2012). A recent mega-analysis of data from several research groups identified two important sources of heterogeneity in BD patients are illness duration and lithium use (Hallahan et al., 2011).

There is also evidence of increased hippocampal volume associated with lithium treatment longitudinally in bipolar patients (Hallahan et al., 2011; van Erp et al., 2012; Yucel et al., 2007; Yucel et al., 2008), whereas reduction in hippocampal volume compared to controls has been reported in some studies of BD (Chepenik et al., 2012; Frey et al., 2007; Mamah et al., 2010). It is important to note that none of the latter studies controlled for psychotropic medication; trophic effects due to lithium could potentially explain why many studies of BD did not find volumetric differences in the hippocampus compared to controls (Frey et al., 2007). Another important finding is reduction in total grey matter volume in BD (Lisy et al., 2011; Savitz & Drevets, 2009); the subgenual, rostral, and dorsal anterior cingulate cortex (ACC), orbitofrontal cortex (OFC), and dorsolateral prefrontal cortex (DLPFC) have been identified as

some of the key areas of grey matter loss (Drevets, Savitz, & Trimble, 2008; Haznedar et al., 2005; Savitz & Drevets, 2009). Broadly, these cortical areas share connectivity with the hippocampus, amygdala, thalamus, and hypothalamus, and are some of the key brain regions responsible for emotion regulation, as shown in Figure 2.

Functional magnetic resonance imaging (fMRI) is an increasingly valuable tool in BD research. fMRI is a specific application of magnetic resonance that uses the magnetic properties of oxygen to detect regional changes in blood flow in the brain. Briefly, when a brain area is activated, blood flow increases (*haemodynamic response*); the resulting local increase in oxyhaemoglobin alters the local MR signal. This change in the signal is called the blood-oxygen-level dependent (BOLD) signal, and is used as a measure of what brain regions are activated under various tasks and conditions. fMRI findings in BD are broadly consistent with those of structural studies, implicating the DLPFC, ACC, amygdala, hippocampus, thalamus, and striatum primarily (Cerullo et al., 2009). The ACC and DLPFC are typically underactive in BD patients compared to controls during cognitive and attentional tasks (Adler, Holland, Schmithorst, Tuchfarber, & Strakowski, 2004), although some have found increased activity in these areas during memory tasks, and one study also found concomitant increased ventrolateral PFC (VLPFC) activity (Blumberg, Leung et al., 2003). Memory and affective tasks designed to activate the limbic system have fairly reliably identified increased activation in the amygdala, thalamus, hippocampus, and striatum (Cerullo et al., 2009).

An issue in the study of BD is the question of state- vs. trait-dependence. Most of the published neurostructural findings in BD mirror those of MDD, but few studies have directly compared BD to MDD patients (Cardoso de Almeida & Phillips, 2013), and imaging of patients with BD is often obtained during symptomatic periods. To further complicate matters, many

studies neglect to report the mood state of participants or consist of patients in different mood states (Strakowski et al., 2012). As such, it is not always clear if the differences seen may be the result of the trait of bipolar disorder or the state of a depressive episode, and little can be inferred regarding state-dependent abnormalities during mania. Structural neuroimaging studies of BD for the most part have not controlled for medications, and thus any effects owing directly to lithium treatment are obfuscated. Furthermore, since lithium is prescribed primarily for BD, and cannot be administered to a control group due to the heavy burden of side effects, there are no studies examining the effect of the medication on brain structure or function independent of the underlying illness state.

There is, however, patient-based information on the effect of lithium on brain structure and function. A recent study by Hajek *et al* indicates that BD patients without significant lithium treatment have smaller hippocampi than either healthy controls or lithium-treated BD patients, while they found no difference between controls and Li-treated patients (Hajek et al., 2012). Two recent longitudinal studies also concluded that lithium treatment in previously lithium-naïve BD patients resulted in an increase in hippocampal volume (Yucel et al., 2007; Yucel et al., 2008). Another longitudinal study reported that lithium treatment increases grey matter in the subgenual ACC in BD patients (Moore et al., 2009). These findings all support lithium's proposed neuroprotective/neurotrophic effects as described in the previous section. Furthermore, another recent longitudinal study found that the Li-mediated increase in hippocampal volume in BD patients is not associated with treatment response, i.e. the effect of Li on brain structure is present whether or not the treatment is effective in reducing symptoms (Hajek et al., 2013). Lending the issue some complexity, a recent study reported smaller grey matter volume in non-Li BD patients compared to both Li-treated patients and controls (consistent with above), but

also found that Li-treated patients have smaller total brain volumes than healthy controls with increasing age; the authors suggest that Li may only be neuroprotective in younger patients (Abramovic et al., 2012).

2.2.7.1 Neuroimaging of bipolar disorder in children and adolescents

Imaging data in pediatric BD are scarce. What results there are, though, are partially consistent with the adult literature: according to reports, the subgenual ACC is smaller (although not significant statistically) in pediatric BD compared to controls, and mood stabilizers may exert a hypertrophic effect in this region (Mitsunaga et al., 2011); subgenual PFC was also found to be smaller compared to controls, again with mood stabilizers normalizing this effect (Baloch et al., 2010). There is one study in the literature of the cerebellar vermis in pediatric BD, which found a trend towards smaller V2 lobe compared to controls (Monkul et al., 2008). fMRI findings are also consistent with adult studies, namely frontolimbic network dysfunction including amygdala hyperactivity in BD patients compared to controls (Wegbreit et al., 2011), and more specifically that cognitive tasks elicit less DLPFC activity in BD than controls, and more VLPFC activity (Pavuluri, Passarotti, Parnes, Fitzgerald, & Sweeney, 2010).

2.3 The Cerebellar Vermis

2.3.1 Vermal anatomy

The cerebellum (literally *little brain*) is the largest part of the hindbrain, located posterior to the brainstem and inferior to the cerebral hemispheres (Figure 3). The cerebellar cortex is very tightly folded compared to the cerebral cortex, and despite its small size contains a majority of all the neurons in the brain (Kandel, Schwartz, & Jessell, 2000). The specific area of interest to this study is the cerebellar vermis, which is the midsagittal portion of the cerebellum. The vermis

consists of ten lobules, most easily visible in a midsagittal section of the cerebellum. For convenience the vermis is often divided into three lobes: *V1* or the anterior lobe, consisting of lobules I-V; *V2* or the superior-posterior lobe, consisting of lobules VI and VII; and *V3* or the inferior-posterior lobe, consisting of lobules VIII-X (Figure 4; Bogovic et al., 2012; Yucel et al., 2012). The primary fissure clearly separates lobe V1 from V2, and the pre-pyramidal fissure likewise separates V2 from V3.

The vermis displays a highly organized and regular structure making up two circuits: a main excitatory loop, and a cortical inhibitory loop. In the excitatory loop, glutamatergic mossy fibres and climbing fibres project directly to deep cerebellar neurons, which then project to other brain structures. Both fibres also extend to the cerebellar cortex, forming an orthogonal matrix of glutamatergic projections onto Purkinje cells. The GABAergic Purkinje cells directly inhibit the main excitatory loop, closing the cortical inhibitory loop (Figure 5; Kandel et al., 2000).

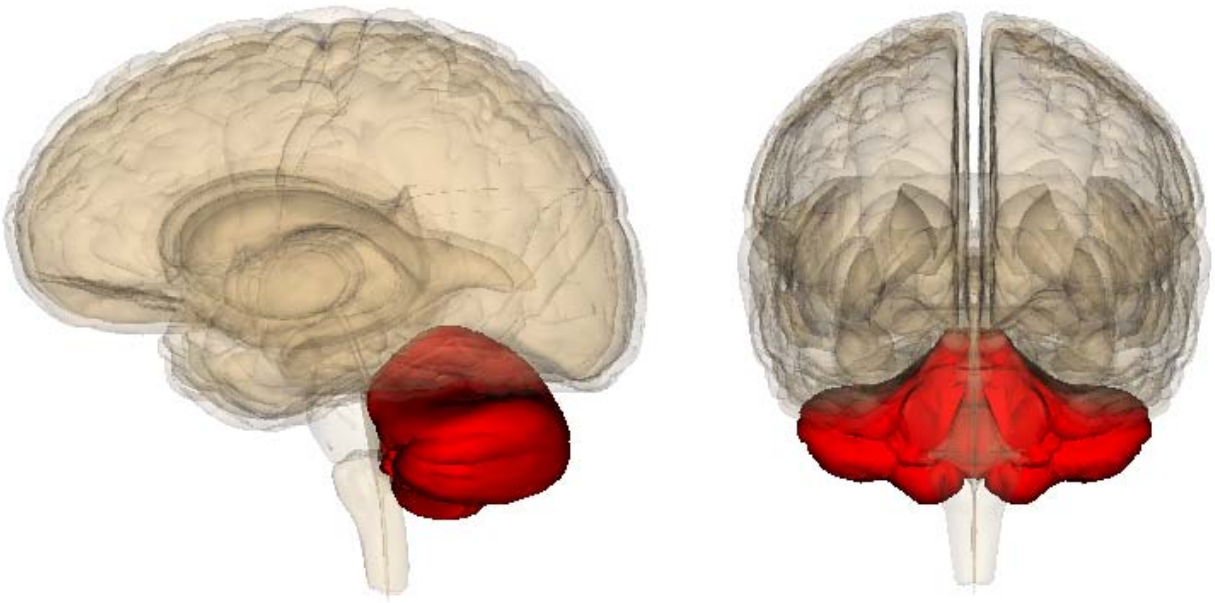

Figure 3: A graphical depiction of the human cerebellum (in red) from the side and from the back. The cerebellar vermis occupies the sagittal midline, with cerebellar lobes extending outward laterally.

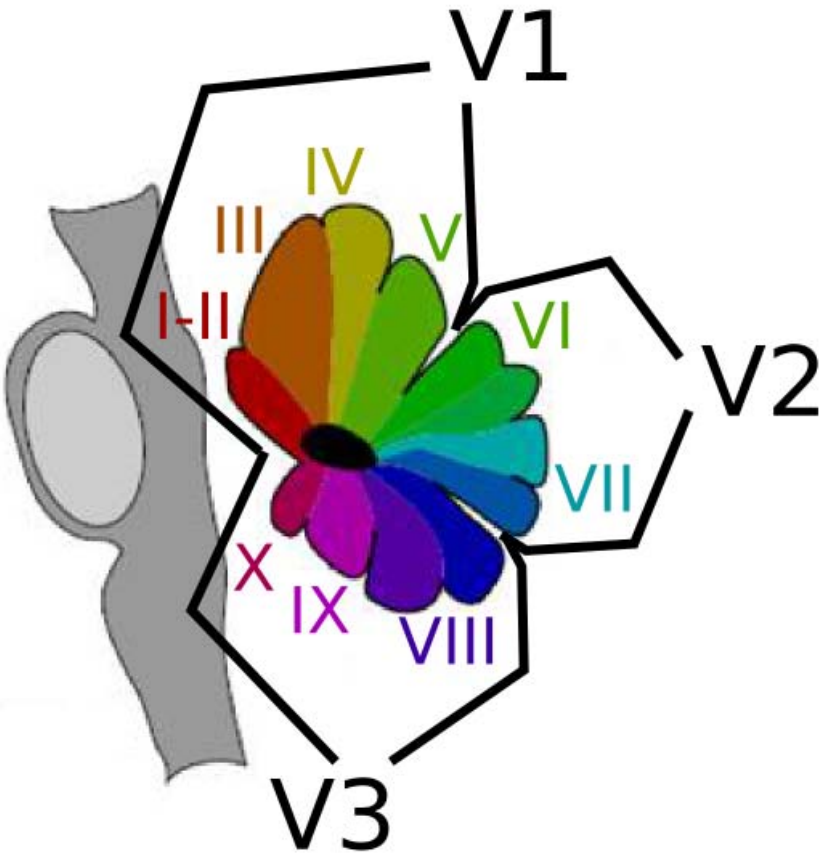

Figure 4: Subdivisions of the cerebellar vermis diagrammed as a midsagittal slice (adapted from Bogovic et al., 2012)

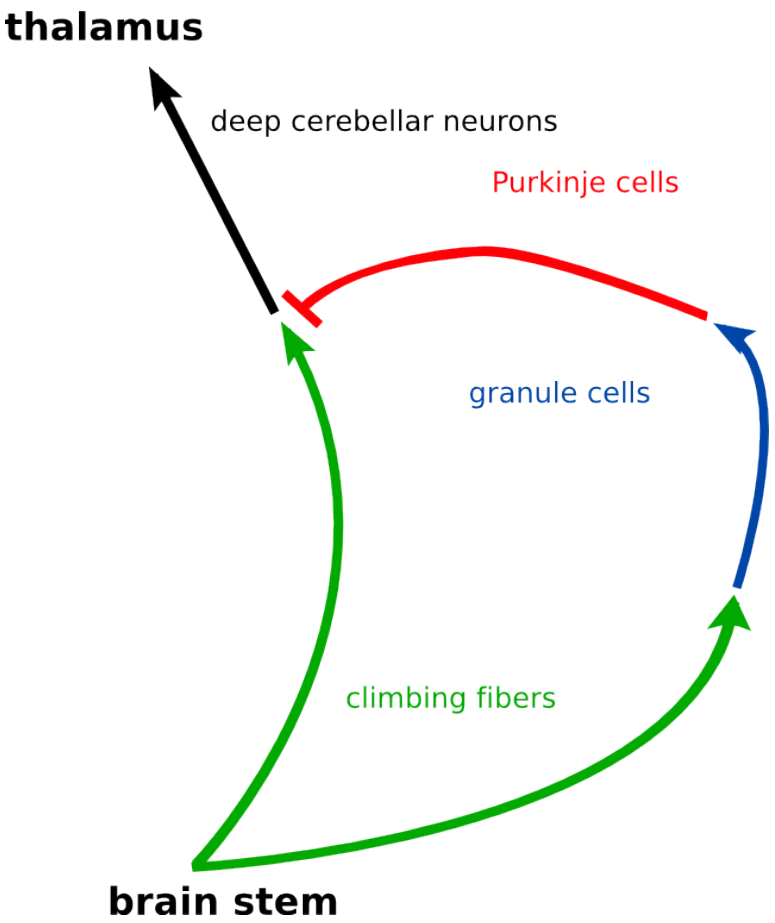

Figure 5: A simplified representation of the cerebellar excitatory and inhibitory loops.

2.3.2 The "limbic cerebellum"

Although the cerebellum is classically considered to be involved only in movement-related tasks such as balance, coordination, and precision, newer studies are implicating it, and particularly the vermis, in many cognitive and emotional functions as well (Baumann & Mattingley, 2012; Schmahmann & Caplan, 2006; Schmahmann, Weilburg, & Sherman, 2007; Stoodley, Valera, & Schmahmann, 2012). The role of the cerebellum in cognition has been a question for at least 20 years (Ivry & Baldo, 1992), and reports of cerebellar abnormalities related to psychiatric disorder date back even further (Nasrallah, Jacoby, & McCalley-Whitters, 1981), but this field has received heightened interest in the past several years. A seminal report from Schmahmann *et al* details the clinical phenomenology of patients with vermal lesions of various aetiologies. The array of symptoms displayed by these patients included anxious and obsessive behaviours, depressive-type symptoms including rumination, dysphoria, and apathy, as well as symptoms commonly seen in mania, such as irritability, disinhibition, and impulsiveness, which were collectively termed the "Cerebellar Cognitive Affective Syndrome" (Schmahmann et al., 2007). While not specifically indicative of BD, the aforementioned observations strongly suggest that the vermis is in some way important to mood regulation.

Recent MRI connectivity studies identify distinct connectivity between the cerebellum and corticolimbic areas (Buckner, Krienen, Castellanos, Diaz, & Yeo, 2011; Habas et al., 2009). Building the case even further, functional imaging work by Baumann *et al* shows that the cerebellum is active in response to a broad range of emotional stimuli, with much of the activity located in the posterior vermis (Baumann & Mattingley, 2012). The authors of this work go on to

describe the patterns of activation for different emotions, which were partially but not entirely overlapping with one another.

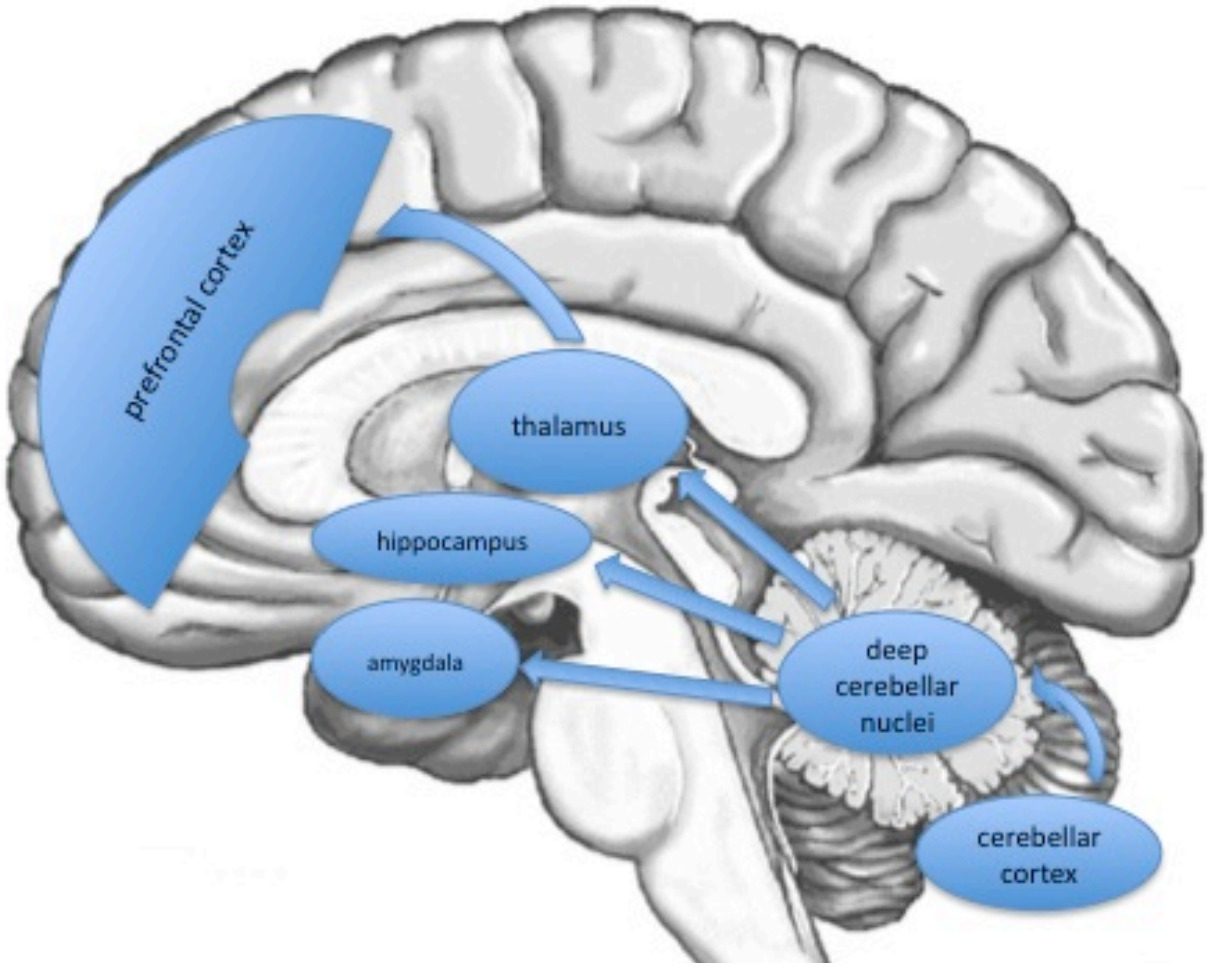

Figure 6: Proposed connectivity of the cerebellar vermis with the frontolimbic network

2.3.3 Vermal neurochemistry and plasticity

The cerebellum has long been known to have a profound capacity to recover from lesion-induced deficits (Ito, 2001) as well as to change substantially in response to motor learning (Park et al., 2009), indicating a high degree of neuroplasticity. This region is not nearly as well-studied as the hippocampus, however cerebellar plasticity shares several mechanisms with hippocampal plasticity. BDNF and its receptor, tropomyosin related kinase B (TrkB)- implicated in hippocampal changes associated with BD- have been identified as important to plasticity in cerebellar synapses as well (A. R. Carter, Chen, Schwartz, & Segal, 2002). Glucocorticoid receptors- thought to play an important role in the pathophysiology of BD due to HPA axis dysfunction observed in BD- are present in the cerebellum also (Daban, Vieta, Mackin, & Young, 2005; Metz, Schwab, & Welzl, 2001). In keeping with the model of plasticity and bipolar disorder presented in section 2.2.5.1, endocannabinoid signalling has also been described as an important modulator of long-term potentiation (LTP) and depression (LTD) at synapses in the cerebellar cortex (Safo, Cravatt, & Regehr, 2006; van Beugen, Nagaraja, & Hansel, 2006). There is modest evidence that neurogenesis occurs in the adult cerebellum, demonstrated in rabbits and in goldfish (Delgado & Schmachtenberg, 2011; Ponti, Peretto, & Bonfanti, 2008); however, there is no evidence for similar mechanisms in humans. It seems more likely that neuroplastic changes in the human adult cerebellum are mediated by synaptic plasticity and/or changes in neuron size.

2.3.4 The vermis and bipolar disorder

Owing to the connectivity of the cerebellum with the limbic system, and its apparent involvement in emotional processing (Baumann & Mattingley, 2012; Schmahmann & Caplan, 2006; Schmahmann et al., 2007), we are interested in investigating the potential role of the cerebellar vermis in BD, and whether it is affected by lithium pharmacotherapy. Previous studies of the cerebellum in BD have not controlled for medication (DelBello et al., 1999; Mills et al., 2005; Womer et al., 2009), and there are no extant reports describing the effect of lithium on the cerebellum. Given the apparent involvement of the cerebellum in mood regulation, one might expect to see structural cerebellar abnormalities in MR images of patients with mood disorders. Here the literature is scant, but what has been shown is consistent with this expectation. The only study to date of the cerebellar vermis in major depressive disorder found a larger anterior lobe (V1) in male MDD patients with a long history of antidepressant medication use compared to healthy controls, but found no other significant differences (Yucel et al., 2012). An early structural MRI analysis of the vermis and cerebellar hemispheres revealed a significantly smaller inferior-posterior vermis (V3) in bipolar patients with a long history of illness compared to healthy controls (DelBello et al., 1999). This report was later confirmed, with the addition of a smaller V2 in bipolar patients as well (Mills et al., 2005). Consistent with the above, a study of adolescent bipolar patients reported trends towards smaller V2 and V3, but with none of the comparisons meeting statistical significance criteria (Monkul et al., 2008). Conversely, a recent study found region V1 significantly *larger* in patients with BD than controls (Womer et al., 2009). Finally, though not necessarily related to BD, one study found a robust correlation between smaller cerebellar volume and increased trait neuroticism in healthy volunteers, citing the vermis as an area of particular interest (Schutter, Koolschijn, Peper, & Crone, 2012). This

finding raises the possibility that decreased cerebellar volume may be a non-specific effect of psychiatric illness.

One reason for the discrepancies in the BD findings may be that none controlled for medication use in patients with BD. BD has a wide range of pharmaceutical treatments, including several each of antipsychotics, mood stabilizers, and antidepressants. We are particularly interested in studying lithium; in light of Hajek *et al*'s finding that illness burden and lithium treatment have opposing effects on hippocampal volume, we are interested in investigating the possibility of a similar effect in the vermis (Hajek et al., 2012). In addition, the finding in MDD of larger V1 only in the case of long-term antidepressant use suggests the possibility that these drugs influence vermal volume as well, since they have established neurotrophic effects (Castren, 2004). To understand changes in the cerebellar vermis in BD, then, it seems necessary to control to the greatest extent possible the effects of pharmacotherapy.

The potential impact of lithium pharmacotherapy on the cerebellum has not been addressed to date, but this question is interesting in light of recent neuroimaging findings of Li-induced volume increase in the ACC and hippocampus, coupled with the fact that the vermis also displays experience-dependent plasticity, which may be mediated by similar molecular mechanisms.

Chapter Three: **Methods**

3.1 Subjects

3.1.1 Cross-sectional adolescent data

20 adolescent subjects were recruited: 10 diagnosed with BD according to DSM-IV criteria, mean age=16.6, SD=1.6, 7 female, 3 male; 10 healthy controls, mean age=17.0, SD=2.3, 5 female, 5 male). Patients were recruited after being referred to the Department of Psychiatry, Dalhousie University, Halifax, Nova Scotia. Healthy controls were recruited through local advertisement. All patients met DSM-IV criteria for BD as determined by the Kiddie Schedule for Affective Disorders and Schizophrenia- Present and Lifetime Version (Kaufman et al., 1997) by the attending psychiatrist. Exclusion criteria for participation in this study were (1) history of neurological illness; (2) history of head trauma; (3) any underlying medical disorder potentially related to endocrine dysfunction or resulting in central nervous system symptoms; (4) claustrophobia; (5) age less than 14 years or greater than 20 years; (5) MRI incompatibility. One patient had started medication within the last two weeks (risperidone); the remainder of clinical subjects were treatment-naïve. Control subjects had no current or lifetime psychiatric illness.

3.1.2 Cross-sectional adult data

Clinical and neuroimaging data were obtained from 75 subjects: 27 with a primary diagnosis of BD and no lithium treatment (mean age=30.6, SD=11.8, 16 female, 11 male), 20 with a primary diagnosis of BD with current lithium treatment (mean age=31.9, SD=12.5, 10 male, 10 female), and 29 healthy controls with no current or lifetime history of psychiatric illness (mean age=33.3, SD=10.0, 18 female, 11 male). Subjects were recruited by the Mood Disorders Program at St. Joseph's Healthcare in Hamilton, Ontario through inpatient and outpatient programs. All patients

were diagnosed by the Structured Clinical Interview for DSM-IV (SCID) (First, 2002). Patients were variously medicated; detailed medication history was be obtained from patient records for use in the analysis and interpretation of results. Exclusion criteria for all groups were (1) substance-use related disorder within the past six months as determined by the SCID; (2) lifetime history of substance dependence as measured by the SCID; (3) post-traumatic stress disorder as determined by the SCID; (4) use of alcohol or illicit psychoactive substance within 48 hours of testing; (5) untreated medical illness such as uncontrolled diabetes or other endocrine disorders; (6) history of head injury with loss of consciousness; (7) history of neurological disease; and (8) past treatment with electroconvulsive therapy (ECT), transcranial magnetic stimulation, or psychotherapy.

3.1.3 Longitudinal adult data

17 subjects (mean age=27.2, SD=8.7, 8 female, 9 male) with a primary diagnosis of BD were recruited by the Mood Disorders Program at St. Joseph's Healthcare in Hamilton, Ontario. All patients were diagnosed by the Structured Clinical Interview for DSM-IV (SCID) (First, 2002). Exclusion criteria were identical to those above, with the addition of: (9) past treatment with pharmacotherapy before entry into the study (greater than 5 days lifetime with any psychotropic medication including stimulants). The baseline scan and initiation of treatment with lithium occurred on average within one month of each other. All participants received follow-up scanning at the first interval, taken on average 2.0 years (range 1.1-2.8) after the baseline scan. 10 longitudinal control subjects were also recruited (mean age=28.5, SD=10.6, 7 female, 3 male); controls had the same exclusion criteria as BD patients, with the addition of: (10) any lifetime psychiatric diagnosis. Controls received follow-up scanning after an average of 2.4 years (range 1.2-3.4).

Table 1: Adolescent subject demographic information

	Healthy control	BD
Sample size	10	10
Age /years (SD)	17.0 (2.3)	16.6 (1.6)
female/male	5/5	7/3

Table 2: Adult subject demographic information

	Healthy control	Non-Lithium treated	Lithium-treated
Sample size	29	27	20
Age (SD)	33.3 (10.0)	30.6 (11.8)	31.9 (12.5)
female/male	18/11	16/11	10/10
Number returned for rescan	10	17	-
Interscan time /years (SD)	2.4 (0.9)	2.0 (0.4)	-

3.2 Data Acquisition

Adult cross-sectional and longitudinal scan data were obtained on a 1.5-Tesla Sigma GE Genesis-based Echo-Speed scanner (General Electric Medical Systems, Milwaukee, WI, USA) running version 5.7 software and using a standard 30-cm circularly polarized head coil. Sagittal anatomic images were acquired using a 3D/FSPGR/20 sequence [flip angle = 20; echo delay time in-phase (TE), minimum repetition time (TR) = 300 ms; inversion recovery = 300 ms; matrix = 512 9 256; field of view (FOV) = 24 cm; scan thickness = 1.2 mm]. The remaining 35 (33.4 % of the total) subjects were scanned on a 3-T MRI Sigma GE Genesis (General Electric Medical Systems, Milwaukee, WI, USA). Here, sagittal T-1 weighted images were acquired

using a 3D FSPGR-IR sequence, (TR/TE = 10.3/2.1 ms; flip angle = 20; inversion time = 300; matrix = 512 9 256; FOV = 24; and slice thickness = 1.2 mm).

Adolescent scan data using a 1.5-Tesla Siemens Vision system (Erglington, Germany) at the Queen Elizabeth II Health Sciences Centre in Halifax. Coronal fast low angle shot sequence parameters were: TE 1⁄4 25 ms, TE 1⁄4 5.40 ms, flip angle 1⁄4 40°, slice thickness 1⁄4 1.45 mm, 124 slices, and matrix 1⁄4 256 · 256 pixels.

The AFNI software package (National Institute of Mental Health, Bethesda, Maryland, MD, USA; http:// afni.nimh.nih.gov/afni/) was used to analyze the data. Due to the use of different instruments and scanning protocols, adolescent and adult data were not combined, but rather analyzed as separate datasets throughout.

3.3 Vermal Measurements

Vermal volumes were measured in AFNI (Cox, 1996) using primarily the sagittal plane, double-checking and trimming the measurements in the coronal and axial planes using a method similar to Yucel (Yucel et al., 2012). The vermis is divided into the anterior (V1), superior–posterior (V2), and inferior–posterior (V3) lobes (see Figure 4). The anterior lobe is composed of lobules I–V and is located between the anterior (superior) medullary velum and the primary fissure; the primary fissure lies between the V1 and V2 regions (Mackie et al., 2007). The V2 region consists of lobules VI and VII, and is separated from the V3 region by the prepyramidal fissure (Mackie et al., 2007). V3 includes the lobules VIII–X.

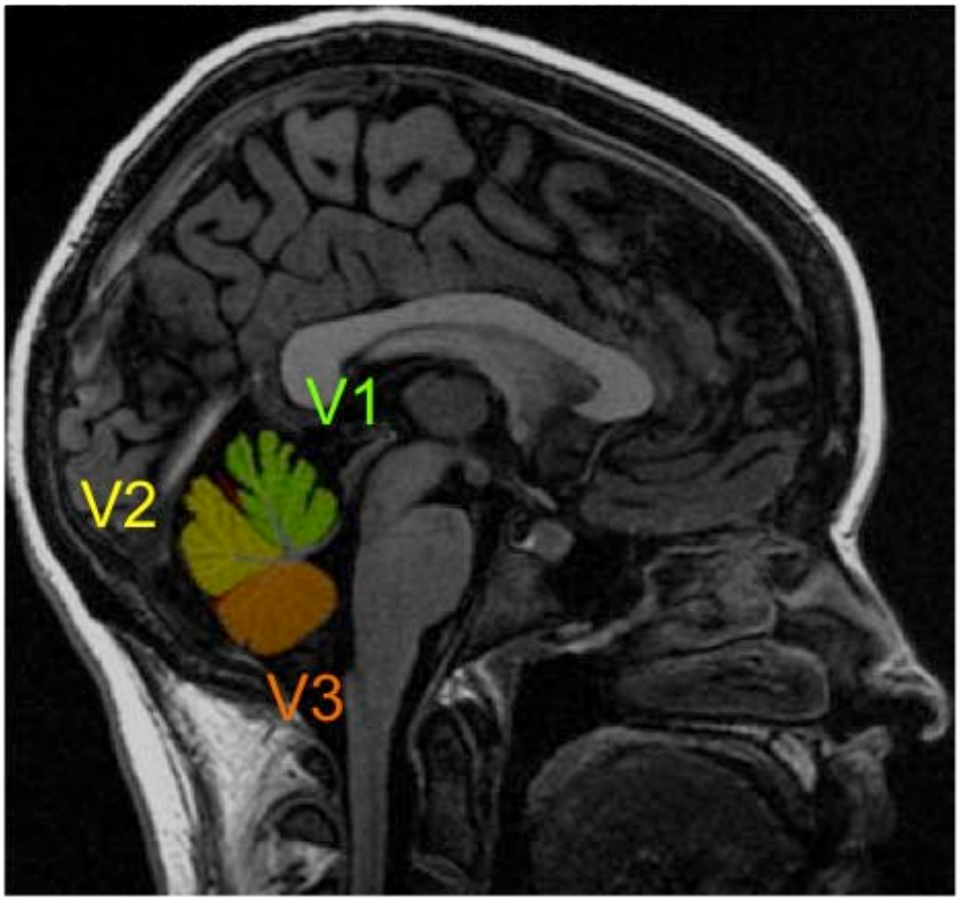

Figure 7: The cerebellar vermis on a typical mid-sagittal MR image

Measurements began with identification of the two fissures that demarcate the vermal lobes. The primary and prepyramidal fissures were clearly delineated on the mid-sagittal slice but became relatively less prominent laterally. The mid-sagittal slice was defined as that in which the fine, branching structure of the vermis was readily apparent, the aqueduct of Sylvius open, the cerebral fissures and corpus callosum visible, and the occipital gyri were ambiguous. We found it difficult to set the lateral borders of the vermis on both sides for V1 particularly, as the paravermian sulcus (which runs between vermis and cerebellar hemispheres) is not prominent or constant across slices throughout its course. The vermis abuts at an angle the anterior medullary velum and continues with the vermal white matter. We set the lateral border of the vermis on both sides as the slice preceding the last slice where anterior medullary velum is still seen on the sagittal plane. Moreover the process of lateral boundary definition is guided by the qualitative differences between fine branching vermis tissue and amorphous cerebellar hemisphere tissue.

After tracing all lobes in the sagittal plane, we trimmed the tracings in the coronal and axial planes, removing any included cerebellar hemisphere or cerebral cortex. This protocol is simple and reliable (inter-rater ICC consistently exceeding 0.90) but some ambiguity remains in the definition of lateral boundaries, as there is no discontinuity between the vermis and cerebellar hemispheres. The total vermis volume was the sum of the subregions V1, V2, and V3. Due to interscan variability in contrast parameters, the vermis was not parcellated into gray and white matter. Care was taken to exclude the cerebellar tonsils and *velum medullare* (white matter tracts connecting the vermis to the medulla/pons).

We also conducted measurements of average vermal cross-sectional area, in an effort to circumvent the issue of ambiguous lateral vermal boundaries. The accuracy and reproducibility of this approach was greater, with clearer boundaries and less latitude for rater judgment, while the assumption involved is minor, namely that the vermal cross-section at the midline is a good index of vermal volume. Given the highly regular cellular structure of the vermis, consisting all along the sagittal domain of arrays of Purkinje cells innervated by climbing fibers and granule cells, we find it likely that any evident changes in cell number, size, or connectivity are well represented in the midline cross-section. This procedure was essentially an abridged version of the volumetric method described above: the midline sagittal slice was identified and V1, V2, and V3 traced in this slice, and in the two adjacent slices to the left and right. The three areas for each of V1, V2, and V3 were then averaged to obtain mean areas, and summed to obtain the total vermal area.

3.3.1 Method validation

Area and volume measurements were performed by the same rater (DM) on a subset of 19 subjects for the purpose of method validation. The values were highly correlated (Pearson's $r > 0.85$, $p = 0.000$), except for region V3 which was less strongly correlated but still highly significant (Pearson's $r = 0.734$, $p = 0.000$) (Table 3, Figure 8).

Table 3: Comparison of vermal volume to area measurements

Measurement	Region V1	Region V2	Region V3	total vermis
Vermal area /mm^2	419.0	278.7	335.5	1033.2
Vermal volume /mm^3	3268.8	2336.3	2195.3	7800.4
Correlation (Pearson's *r*)	0.898	0.850	0.734	0.900
Significance	p = 0.000	p = 0.000	p = 0.000	p = 0.000

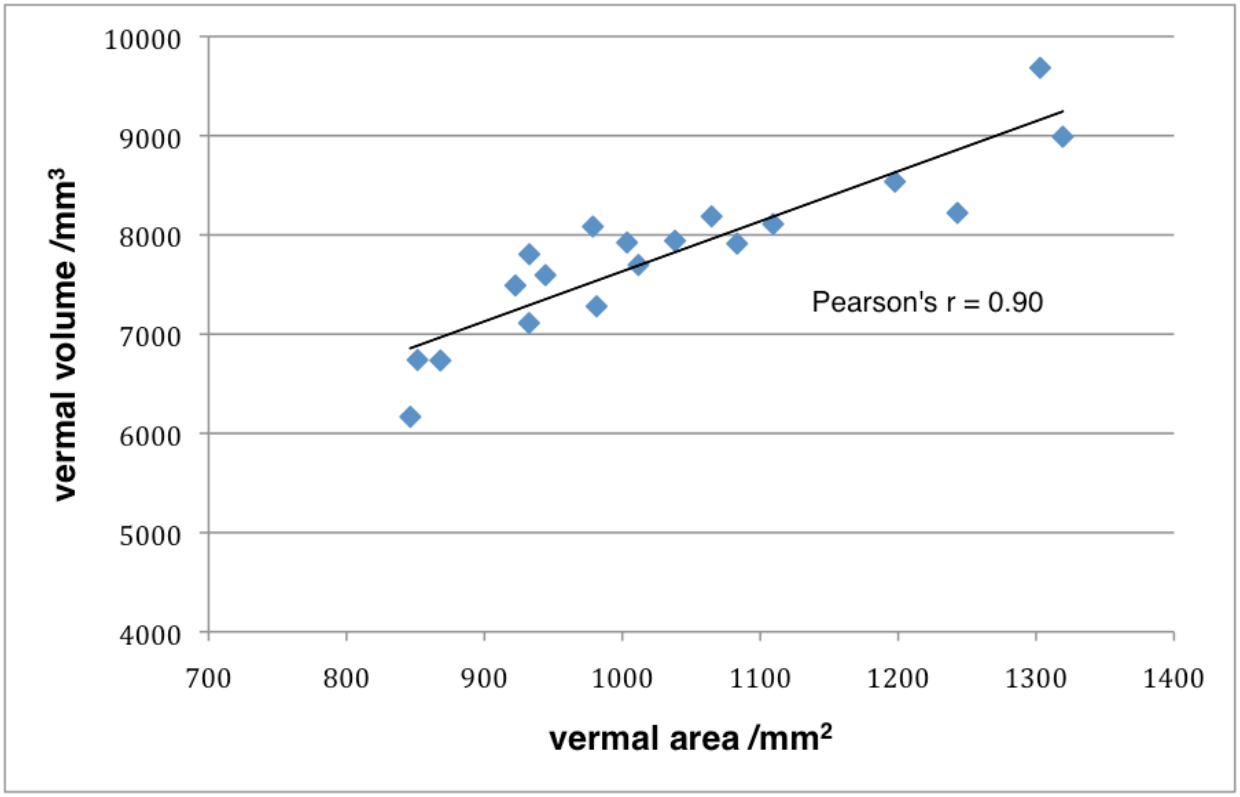

Figure 8: Correlation of total vermal area and volume measurements

All measurements were carried out by a single rater, DM, blinded to subject diagnosis and medication history. A subset of 12 scans, randomly selected, were measured again by DM, as well as by a second rater, BF. This sample revealed excellent intrarater reliability (ICCs for individual lobes and total vermis all >0.97), and very good interrater reliability (ICCs for individual lobes and total vermis all >0.89); these values were consistent with, and even exceeded, our expectations based on literature (Table 4) (Yucel et al., 2012).

Table 4: Intra- and interrater reliability

	average V1 area /mm^2	average V2 area /mm^2	average V3 area /mm^2	average total vermal area /mm^2
Rater DM	444.2	272.8	336.0	1052.8
second measurement	444.7	275.6	335.8	1055.7
Rater BF	422.9	264.2	322.8	1009.9
Intrarater reliability (ICC)	0.996	0.972	0.976	0.989
Interrater reliability (ICC)	0.975	0.930	0.889	0.975

3.4 Clinical measures

The following rating instruments were administered at the time of scan for all subjects: 17-item Hamilton Depression Rating Scale (Hamilton, 1960); Young Mania Rating Scale (Young et al., 1978); Simpson-Angus Scale (Simpson & Angus, 1970); Abnormal Involuntary Movement Scale (AIMS); and Trail-Making Tests A and B (Bowie & Harvey, 2006). The HamD and YMRS questionnaires were administered by a psychiatrist; the AIMS and SAS were also

completed by a psychiatrist, however these scales were based on the rater's observations. The Trail-Making Tests were administered by trained raters.

3.5 Statistical Analysis Plan

3.5.1 Cross-sectional adolescent data

1. Cerebellar vermis area will not differ between adolescent, untreated BD patients and age-matched controls.

To address our first hypothesis, we measured vermal areas for adolescent healthy controls (N = 10, 5 female, 5 male) and age-matched adolescent, medication-naïve BD patients (N = 10, 7 female, 3 male) as described above, and tested for statistical significance using a two-tailed between groups t-test. Because this test is designed to quantify evidence against the null hypothesis rather than to specifically confirm the null hypothesis, we also calculated a 95% confidence interval of the difference between the group means, and used this to estimate the maximum difference between groups at the 95% confidence level according to our results.

3.5.2 Cross-sectional adult data

2. Adult BD patients not taking lithium will have smaller vermis areas than healthy control subjects, however this effect will be normalized by lithium treatment, so that vermis area in patients taking lithium will not differ from healthy controls.

Pursuant to our second hypothesis we compared the cross-sectional area of the cerebellar vermis from magnetic resonance (MR) images of 20 lithium-treated BD patients, 27 non-lithium-treated BD patients, and 29 control subjects. To determine the impact of lithium use on vermal area, we performed a one-way analysis of variance (ANOVA), using the three groups defined above. While not part of our hypothesis, the literature suggests that cerebellar effects related to mood

disorders are more defined in- and perhaps limited to- males, therefore sex was an important variable to consider. We repeated the ANOVA as described above using male-only and female-only samples to investigate this possibility. We also anticipated that age- a very rough indicator of illness duration- may be negatively correlated with vermal area, thus we performed a Pearson correlation of age with vermal area in our total population, with the intention of including age as a covariate in our main analysis if it proved a significant factor. We followed up the main ANOVA test with Tukey's pairwise comparisons to explore statistically significant effects. Although we expected vermal areas to be smaller in non-lithium-treated patients, we were not confident of the direction of the difference, and would be interested to find any statistical differences between groups, therefore we employed the more conservative two-tailed parameters throughout the analysis. We also used descriptive statistics (mean area and associated 95% confidence intervals) prior to analysis to decide if any potentially interesting relationships were present at the level of individual lobes (V1, V2, and V3), and thereafter decided whether to analyze the lobes separately or combine them into a total area.

3.5.3 Longitudinal adult data

3. Vermis area will increase over two years of lithium treatment in BD patients with no previous exposure to lithium.

After analyzing vermal areas in our longitudinal sample of adult BD patients before and after lithium treatment (N = 17, 9 male, 8 female, mean interscan interval = 2.0±0.4 years), as well as longitudinal control subjects (N = 10, 7 female, 3 male, mean interscan interval = 2.4±0.9 years), we conducted a repeated measures (paired samples) t-test on the results to determine if a significant change in vermal area occurred in association with lithium treatment. Again, we would be interested to find either an increase or a decrease in area, so we employed two-tailed

parameters. This test more than any other component of our analysis directly addresses the effect of lithium on the cerebellar vermis within subjects and with limited confounds.

3.5.4 Analysis of literature results

Total vermal area or volume was reported in six prior studies identified herein. These results were synthesized in a Forest plot to test for an overall significant difference in vermal size between BD subjects and healthy controls. The Forest plot was prepared using Stata's *metan* function (StatCorp, 2007).

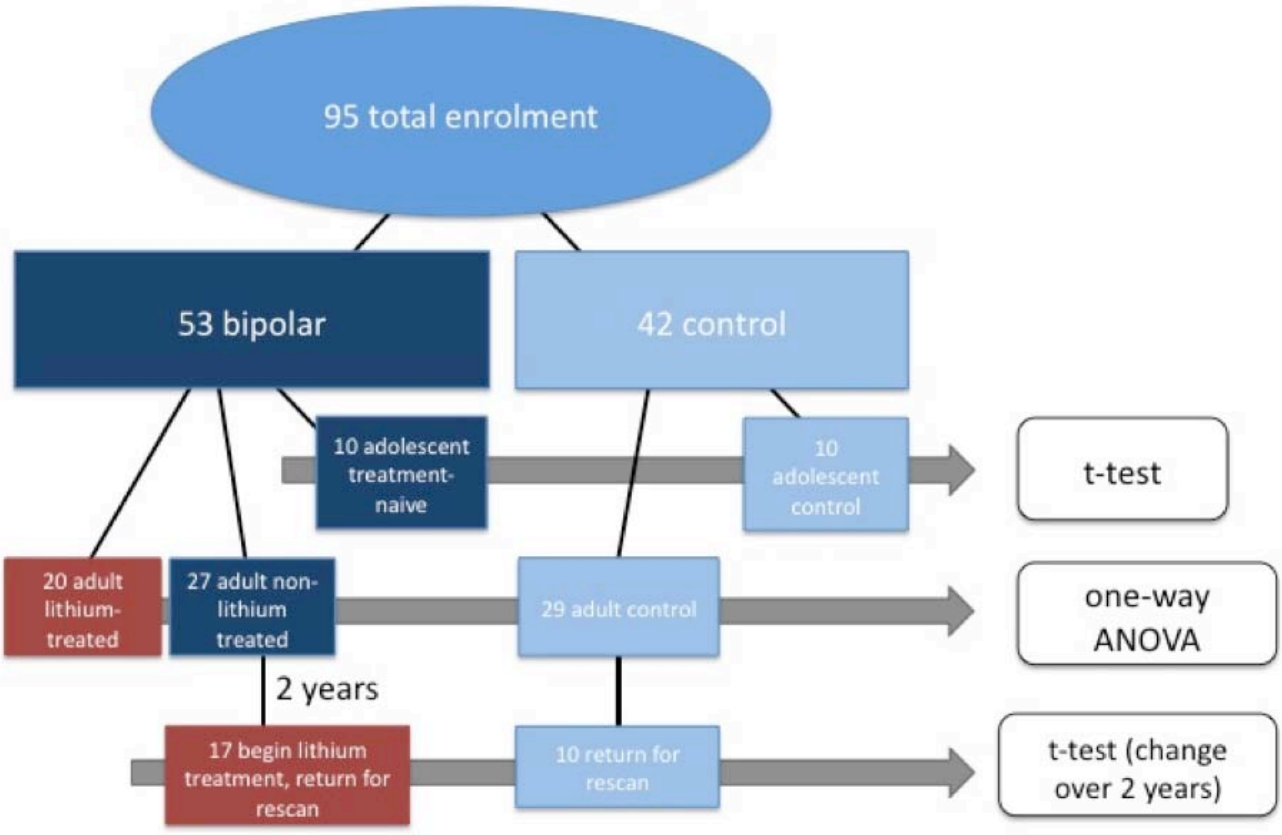

Figure 9: Flowchart of patient enrolment and statistical analysis

Chapter Four: **Results**

4.1 Primary findings

4.1.1 Cross-sectional adolescent data

1. Cerebellar vermis area will not differ between adolescent, untreated BD patients and age-matched controls.

Comparisons of the three vermis regions and total vermis area using Student's t-test did not yield any significant differences (Table 5). We also observed a very small effect size in the total vermis area (Cohen's $d = 0.02$) representing an overall difference of 2.1 mm^2 between groups, with 95% confidence interval spanning the range -52.5 to +56.7 mm^2.

Table 5: Vermal area in adolescent, unmedicated bipolar patients and healthy controls

Group	N	Mean V1 area /mm^2	Mean V2 area /mm^2	Mean V3 area /mm^2	Mean total vermal area /mm^2
Bipolar*	10	504.9 ± 16.7	321.8 ± 14.0	416.2 ± 11.7	1242.9 ± 24.6
Healthy control*	10	507.6 ± 18.4	319.7 ± 7.9	413.5 ± 11.9	1240.8 ±25.7
p-value (2-tailed t-test)	-	0.916	0.896	0.706	0.953
95% confidence interval of difference	-	-	-	-	-52.5 to +56.7

*Values are presented with ± standard error of the mean.

4.1.2 Cross-sectional adult data

2. Adult BD patients not taking lithium will have smaller vermis areas than healthy control subjects, however this effect will be normalized by lithium treatment, so that vermis area in patients taking lithium will not differ from healthy controls.

Comparisons of the three vermis regions and total vermis area using a one-way analysis of variance (ANOVA) did not yield any significant differences (Table 6). A weak trend was noted for larger V1 area in lithium-treated BD patients compared to both non-lithium-treated patients and healthy controls (p=0.13). The effect size (Cohen's *d*) for the difference between the lithium-treated and control groups was 0.58, which is moderately sized according to Cohen's original guidelines (Cohen, 1992).

Table 6: Vermal area in healthy controls and bipolar subjects with and without lithium treatment

Group	N	Mean V1 area /mm^2	Mean V2 area /mm^2	Mean V3 area /mm^2	Mean total vermal area /mm^2
Bipolar, non-lithium-treated*	27	419.2 ± 11.5	260.1 ± 7.8	341.4 ± 7.8	1020.7 ± 23.6
Bipolar, lithium-treated*	20	446.6 ± 13.3	262.6 ± 12.2	335.6 ± 8.2	1044.7 ± 28.2
Healthy control*	29	414.7 ± 9.5	257.7 ± 7.9	332.1 ± 5.5	1004.5 ± 19.3
p-value (ANOVA)	-	0.13	0.93	0.62	0.50

*Values are presented with ± standard error of the mean.

4.1.3 Longitudinal adult data

3. Vermis area will increase over two years of lithium treatment in BD patients with no previous exposure to lithium.

Comparisons of the three vermis regions and total vermis area using a paired-samples t-test yielded one marginally significant difference, a slight decrease in region V1 in the lithium-treatment group as compared to controls (p=0.048, Table 7). The statistical significance of this difference is due equally to an increase in V1 area in the control group, and a decrease in the lithium-treatment group. We also note that this change in V1 area is not reflected in the total vermis area. In Trail-making Test times, both the treatment and control groups showed a significant improvement in TMT-A performance over time (p=0.027), but no such difference in TMT-B performance. There was no difference between groups on either test (Table 8). No extrapyramidal symptoms were evident in the treatment group: all subjects scored 0 on the Abnormal Involuntary Movement Scale and Simpson-Angus Scale at both assessments.

Table 7: Vermal area before and after 2-year lithium treatment

Region	Group (BD n=17, HC n=10)	Pre-treatment area /mm^2	Post-treatment area /mm^2	Change /mm^2	p-value (t-test)
anterior vermis (V1)*	BD	420.4 ± 12.9	415.7 ± 12.2	-4.7	0.048
	HC	422.6 ± 12.0	427.1 ± 11.2	+4.5	
superior-posterior vermis (V2)*	BD	272.1 ± 10.1	266.4 ± 12.0	-5.7	0.54
	HC	261.6 ± 13.7	260.1 ± 12.0	-1.5	
Inferior-posterior vermis (V3)*	BD	339.2 ± 8.7	343.2 ± 8.5	+4.0	0.80
	HC	345.0 ± 11.0	351.0 ± 10.7	+6.0	
Total vermis*	BD	1031.7 ± 26.7	1025.3 ± 26.0	-6.4	0.24
	HC	1029.2 ± 28.5	1039.1 ± 25.4	+9.9	

*Values are presented with ± standard error of the mean.

Table 8: Average trail-making test times before and after 2-year lithium treatment

Group	Pre-treatment TMT /s	Post-treatment TMT /s	p-value (time)	p-value (time*group)
		Trail-making test 1		
Bipolar	26.4	23.0	0.027	0.16
Healthy control	40.2	25.9		
		Trail-making test 2		
Bipolar	55.5	49.4	0.40	0.84
Healthy control	53.8	50.0		

4.2 Age, sex, and other factors

We found no correlation between total vermal area and age, either in the total adult sample (Pearson's r = -0.055, *ns*, Figure 10) or in our sample of adult BD patients only (Pearson's r = -0.055, *ns*, Figure 11). Accordingly, age was not included as a factor or covariate in our analyses of adult subjects.

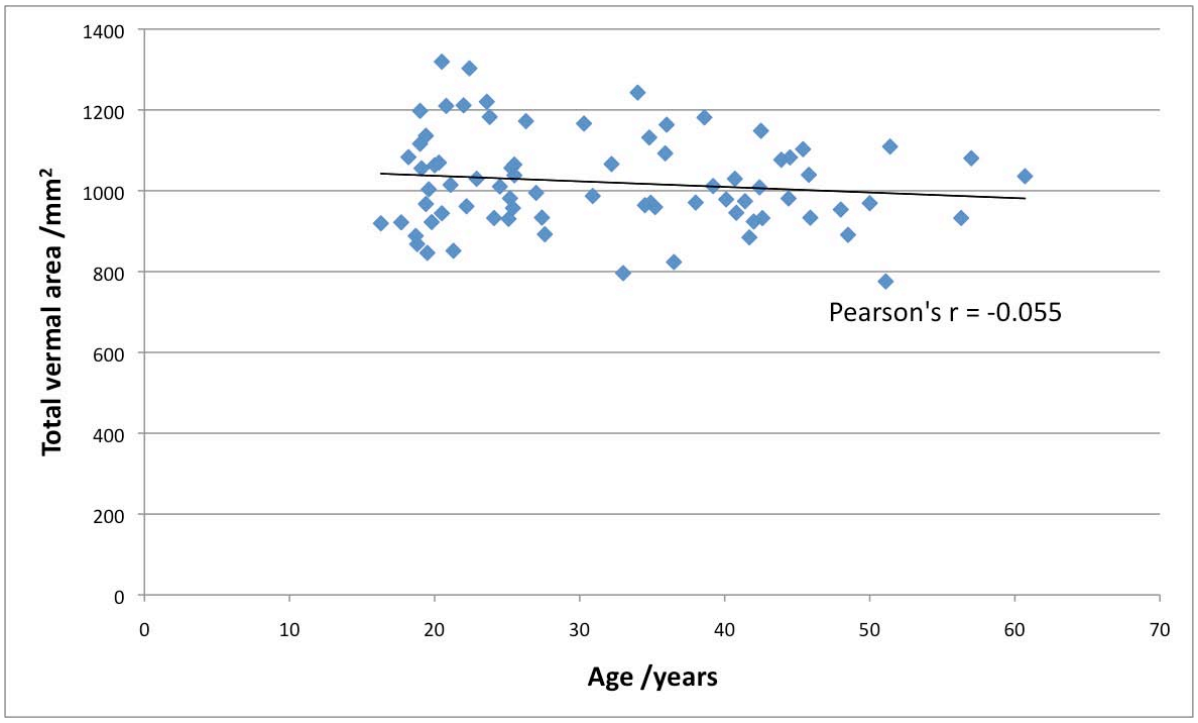

Figure 10: Vermal area as a function of age for all subjects

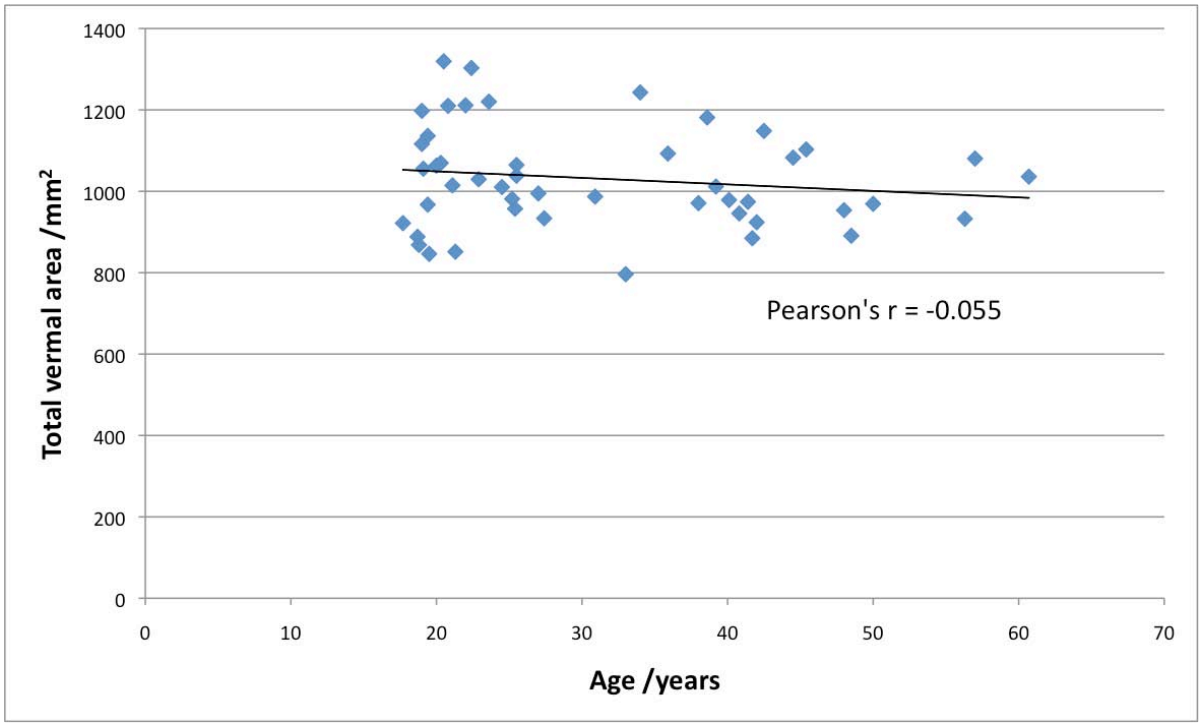

Figure 11: Vermal area as a function of age for bipolar subjects

Similar analyses to that presented in Section 4.1.2 were performed separately on male subjects and on female subjects (Tables 9 and 10). It appears that the trend towards larger region V1 in the lithium-treated group is driven by male subjects ($p = 0.10$ in males, $p = 0.85$ in females). The effect size (Cohen's *d*) for the difference between the male lithium-treated and control groups was 1.04, which is large according to Cohen's original guidelines (Cohen, 1992). There was also a more apparent trend within male subjects for larger total vermis in lithium-treated individuals compared to controls ($p=0.16$, Cohen's $d = 0.89$).

Table 9: Vermal area in healthy controls and bipolar subjects, male only

Group	N	Mean V1 area /mm^2	Mean V2 area /mm^2	Mean V3 area /mm^2	Mean total vermal area /mm^2
Bipolar, lithium-negative*	11	427.1 ± 20.3	254.5 ± 11.1	360.8 ± 11.6	1042.4 ± 37.1
Bipolar, lithium-positive*	10	467.0 ± 19.4	268.2 ± 14.0	348.7 ± 7.4	1083.8 ± 36.0
Healthy control*	11	411.7 ± 13.4	239.2 ± 11.4	333.8 ± 10.1	984.7 ± 32.5
p-value (ANOVA)	-	0.10	0.26	0.17	0.16

*Values are presented with ± standard error of the mean.

Table 10: Vermal area in healthy controls and bipolar subjects, female only

Group	N	Mean V1 area /mm^2	Mean V2 area /mm^2	Mean V3 area /mm^2	Mean total vermal area /mm^2
Bipolar, lithium-negative*	16	413.8 ± 13.8	262.9 ± 10.8	328.1 ± 9.3	1005.9 ± 30.9
Bipolar, lithium-positive*	10	426.1 ± 16.5	257.0 ± 20.6	322.5 ± 13.8	1005.6 ± 41.5
Healthy control*	18	416.6 ± 13.3	269.0 ± 10.0	331.1 ± 6.5	1016.7 ± 24.1
p-value (ANOVA)	-	0.85	0.82	0.83	0.96

*Values are presented with ± standard error of the mean.

Comparison of total vermal area with total cerebral volume (TCV) in our adult sample showed only a modest, marginally significant positive correlation (Pearson's $r = 0.27$, $p = 0.048$). As such, all of our analyses were carried out using raw vermal measurements *without* correction or covariation for TCV.

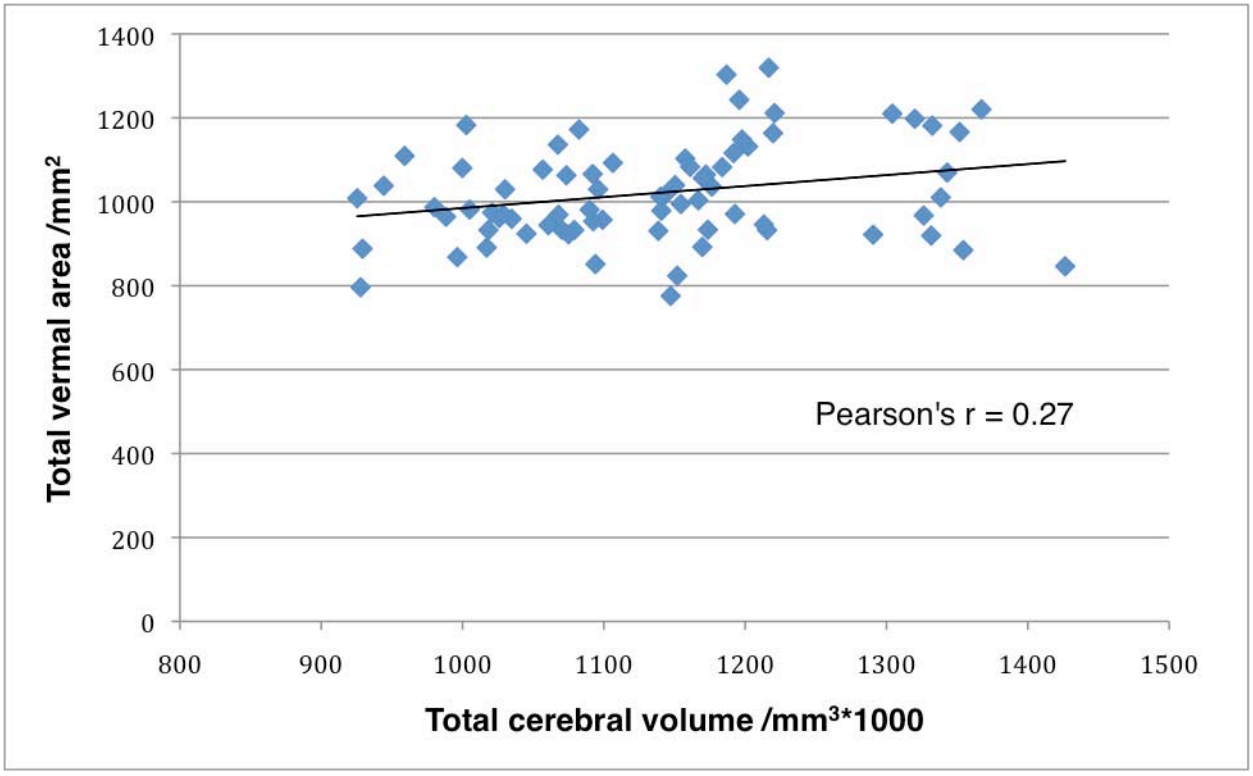

Figure 12: Vermal area and total cerebral volume for all subjects

Chapter Five: **Discussion**

5.1 Primary findings regarding hypotheses

1. Cerebellar vermis area will not differ between adolescent, untreated BD patients and age-matched controls.

We did not find a difference in cerebellar vermis area between adolescent BD patients and control subjects; however, this is not the same as confirming the hypothesis. Finding no statistical difference between these groups could mean that there is in fact no difference, or that the study was flawed or insufficient in some way (i.e. that there is in fact a real difference, but a type II error has been committed). In this case our sample consisted of 10 BD patients and 10 controls, and normally studies of this type indicate that increasing sample size will be necessary to confirm or reject the hypothesis. The very small effect size we observed though (Cohen's d = 0.02), suggests that no reasonable increase in sample size will yield different results. Our results are therefore consistent with the hypothesis, and a 95% confidence interval of between-groups difference indicates that the maximum amount by which the vermal area could differ between BD patients and controls is 8.5%.

2. Adult BD patients not taking lithium will have smaller vermis areas than healthy control subjects, however this effect will be normalized by lithium treatment, so that vermis area in patients taking lithium will not differ from healthy controls.

Comparing the three groups using vermal measurements uncorrected for total cerebral volume (TCV) by ANOVA revealed no significant between-groups differences. There was possibly a

trend towards larger V1 in lithium-treated BD subjects (p=0.13, Cohen's d = 0.58, see Table 6), and a breakdown of this analysis by sex reveals that the trend is driven by male subjects (Tables 9 and 10). The effect size in male subjects only was in fact larger (Cohen's d = 1.04), and was reflected in the total vermal area as well, both of which observations suggest that this finding could reach statistical significance in a larger sample using similar methodology.

3. Vermis area will increase over two years of lithium treatment in BD patients with no previous exposure to lithium.

Contrary to the hypothesis, we found no significant change in total vermis area in BD patients over a two-year course of lithium treatment. Performing repeated-measures analysis of variance (rmANOVA) on each of the three vermal regions independently yielded a marginally significant result in V1 only (p = 0.048). It is apparent from inspection of the data that this result comes about due to a decrease in V1 area of about 1% in BD patients, coupled with an increase in V1 area of about 1% in controls. We consider the statistical significance of this result more likely to be a type I error (false positive) than a real substantial difference between the two groups longitudinally.

5.2 Total cerebral volume

We noted that there was only a modest correlation between vermis size and total cerebral volume (TCV) (Pearson's r = 0.27). It is a common practice to normalize measurement of brain structures against a subject's TCV to account for differences in brain size- for example, one expects a person with a larger head to have a larger hippocampus- and several of the studies summarized in Table 11 performed this kind of normalization. Our data suggest, though, that the

same relationship does not exist between the cerebrum and the cerebellum, perhaps because they reside in different compartments separated by the *tentorium cerebelli*. For this reason we performed all analyses using uncorrected values.

5.3 Intra- and interrater reliability

Our vermal measurements were shown to be highly reproducible, with intrarater ICC (absolute agreement) values all greater than 0.97. The interrater ICCs were comparable for region V1, and for total vermal area, but substantially lower for regions V2 and V3. Since the total vermal areas remained consistent between raters, it is apparent that the discrepancy was in definition of the boundary between V2 and V3. This boundary, the prepyramidal fissure, is indeed the most ambiguous feature of the vermis, and since the ambiguity is apparent in measurements by different individuals we would be cautious of any significant findings limited to regions V2 or V3.

5.4 Secondary findings

5.4.1 The roles of age and sex

Across our subjects, total cerebral volume (TCV) was on average about 10% greater in males than in females, irrespective of other factors, which is consistent with the literature (Tiemeier et al., 2010). Interestingly though, no such sex difference was apparent in vermal area, which was comparable across female and male subjects. This finding further supports the weak relationship between TCV and vermis area discussed above.

We found no significant correlation between age and vermal area, although relevant literature suggests an age-related decline in vermal area beginning around age 50 (Luft et al.,

1999), corroborated by findings of age-related loss of cerebellar cortical cells in cat (Zhang, Hua, Zhu, & Luo, 2006). Our study included very few subjects over age 50, none of whom were part of the longitudinal lithium treatment component, and as such it is unlikely that effects of age on the cerebellar vermis could have affected our results.

5.4.2 Clinical measures

5.4.2.1 HAMD and YMRS rating scales

We found no relationships between vermal measurements and clinical ratings of depression and mania symptoms at the time of scan; this result was expected. Visible changes to the size of the cerebellar vermis such as we proposed to investigate would likely be long-term, cumulative effects, taking weeks or months to become apparent. Conversely, HAMD and YMRS scores will fluctuate widely on this time scale as subjects move in and out of symptomatic episodes.

5.4.2.2 Abnormal Involuntary Movement Scale and Simpson-Angus Scale

A part of our reasoning in looking at the effect of lithium on the cerebellar vermis was that some of lithium's side effects, notably tremor, might be representative of detrimental effects on the vermis. Any correlation between abnormal movements and vermal area over the course of the 2-year treatment would have been an interesting topic for future research; however, every BD patient included in this study rated 0 on both scales at both assessments.

5.4.2.3 Trail-Making Tests A and B

Performance on the Trail-Making Tests did not significantly differ between BD patients and healthy controls at intake or after 2 years of lithium treatment in the BD group. We did find a significant improvement in both groups on TMT-A over the 2 years, though ($F(1,19) = 5.76$, $p = 0.027$), which we ascribe to a practice effect. The TMT is identical each time it is administered, and within our population it was administered several times over the 2-year course, thus it is

expected that subjects would become progressively more proficient at it. This proposition is consistent with the fact that the improvement was seen in control subjects also, although we may have expected to see a similar improvement on TMT-B, which interestingly was absent.

5.5 Fit with literature

5.5.1 Vermal area measurement vs. volume measurement

A first point to discuss is our use in this work of the midsagittal cross-sectional area of the vermis as our primary metric. As covered in Chapter Three, the lateral boundaries of the vermis are poorly defined and a chief source of potential variability, whereas the midsagittal vermis is unambiguous. We further reasoned that any substantive changes to the vermis are likely to be apparent in the cross-section. Of the literature we surveyed, three studies reported vermal volumes only, one reported cross-sectional area, and two reported both measures. Our concern about the lateral boundaries seems substantiated, as all reports of vermal cross-section are within the range 970-1220 mm^2, whereas volume measurements from different studies range from 4490-10100 mm^3. It is clear that definitions of the vermal boundaries must have differed widely across studies, calling into question the reproducibility of these studies.

In this study, area and volume measurements performed by the same rater (DM) on a subset of 19 subjects were highly correlated (Pearson's $r > 0.85$, $p = 0.000$), except for region V3 which was less strongly correlated but still highly significant (Pearson's $r = 0.734$, $p = 0.000$). This is likely explained by the fact that V3 extends farther laterally than do V1 and V2.

5.5.2 Literature findings

We identified seven prior studies of the cerebellar vermis in bipolar disorder, salient details of which are summarized below in Table 11. Qualitatively, the most-repeated finding in the literature is a reduction in the size of the vermis in subjects with bipolar disorder, particularly in region V3. It should be noted that on the whole this finding is far from conclusive, considering that three of the seven studies failed to replicate it, and Baldacara *et al* did not parse the vermis into separate lobes. It is also important that all studies are cross-sectional in nature, and most are of modest size. Lastly, we note that Womer *et al* report a difference only in male subjects (no others identified a sex effect), and DelBello *et al* and Mills *et al* found a significant effect only in multiple-episode BD patients. We must consider the possibility that such findings are erroneous, the result of multiple comparisons.

Table 11: Literature findings on the cerebellar vermis in bipolar disorder

Study	Sample BD/HC	Primary finding reported	total vermal area /mm^2 BD/HC	total vermal volume /mm^3 BD/HC
Womer et al 2009	44 / 43	larger V1 in BD vs. HC; effect driven by male subjects	-	7110 / 6750
Mills et al 2005	39 / 32	smaller V2 and V3 in multi-episode BD vs. HC	-	4490 / 4650
Baldacara et al 2011	40 / 22	whole cerebellum and whole vermis smaller in BD than HC; no correlation with clinical variables	-	4760 / 5620
DelBello et al 1999	30 / 15	smaller V3 in multi-episode BD than either first-episode or HC	970 / 975	-
Brambilla et al 2001	22 / 22	no vermal abnormalities in BD vs. HC	1110 / 1040	9300 / 9300
Monkul et al 2008	16 / 21	no significant differences	1180 / 1220	10100 / 10100
Kim et al 2013	49 / 50	Voxel-based morphometry: less V3 grey matter in BD vs. HC	-	-

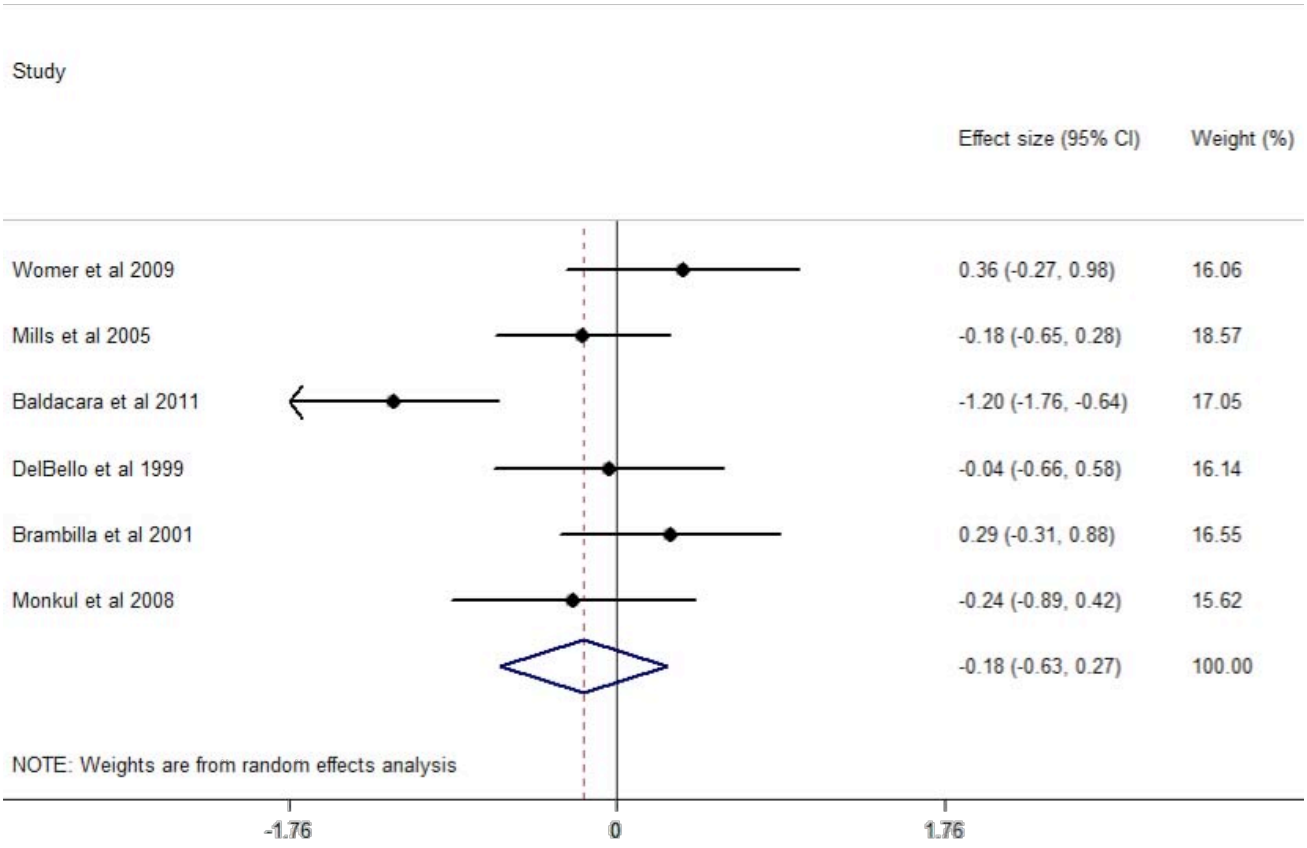

Figure 13: Forest plot of literature reports of total vermis size in BD patients compared to healthy controls

Figure 13 above is a Forest Plot, a meta-analytical tool for combining the results of several studies on a single metric. The analysis did not provide any evidence for heterogeneity across the six studies ($I2 = 0.0\%$, $p = 0.457$). Despite this finding, we employed a random-effects model to acknowledge the fact that these studies differed in their primary measurement (vermal area or volume), and that qualitatively they differed greatly in the magnitude of vermal measurements (Table 11) indicating that the manual tracing protocols differed considerably also. Where studies reported both area and volume, we used area values for the plot. This analysis indicates that overall, measurements of the vermis in BD patients do not differ significantly from healthy controls.

5.6 Limitations

This study has several strengths over those discussed in Section 5.4 above; we studied a large cohort of BD patients and controls, considered the possible effect that medication with lithium could have on our measurements, and we studied subjects longitudinally to assess the effects of lithium specifically. Still, there are a number of other factors that may have influenced our results, and we suggest these be considered carefully in future works.

5.6.1 Confounding factors

Literature reports indicate that illness duration and number of episodes are important factors to consider in investigation of the cerebellum in BD (DelBello et al., 1999; Mills et al., 2005). While not directly addressed, these factors were controlled in our longitudinal analysis by nature of the within-subjects comparisons, and they were also not likely a factor in our adolescent

population, since all subjects included were young and recently diagnosed. Illness duration and course may have had influence on our cross-sectional analysis of adult subjects.

Substance abuse comorbidities are very common in BD, especially alcohol abuse (Cassidy, Ahearn, & Carroll, 2001). Furthermore, it has been shown that alcohol in particular can have a profound detrimental effect on the cerebellum, inducing apoptosis and cellular abnormalities (Jaatinen & Rintala, 2008). Degree of alcohol consumption, even below levels that would be considered substance abuse, could have an impact on vermal size. It has also been reported that prenatal exposure to alcohol and cigarette smoke can be apparent in reduced cerebellar volume (de Zeeuw, Zwart, Schrama, van Engeland, & Durston, 2012; Lewandowska et al., 2012).

On the other hand, positive aspects of cerebellar plasticity have been reported. Most notably, that learning a complex motor skill such as playing basketball or badminton is associated with an increase in cerebellar gray matter, particularly in the vermis (Di et al., 2012; Park et al., 2009). The new acquisition- or discontinuation- of the practice of a sport or some other complex motor skill could have a measurable impact on vermal size.

Neurostructural effects of lithium have been reported in the hippocampus (Hajek et al., 2012; Hanson, Nemeroff, & Owens, 2011; Yucel et al., 2007), however these have recently been questioned on technical grounds. It was shown that lithium can alter *apparent* gray matter volume (GMV) on T_1-weighted MRI scans without affecting the actual GMV, by decreasing water's T_1 relaxation time and thus increasing the signal from gray matter (Cousins, Aribisala, Nicol Ferrier, & Blamire, 2013). If such a mechanism were indeed active in the present study, it would have masked a lithium-induced *decrease* in GMV in our longitudinal subjects, which would certainly have been interesting, but still counter to our hypothesis.

5.6.2 Cross-sectional versus longitudinal methodology

It is clear that many of the potential confounding factors in a study such as this one are obviated by the use of a longitudinal study design. Many factors differing between individuals with BD, including comorbidities such as substance abuse and anxiety disorders, genetics, and history could potentially impact physiological measures. The role of these factors will not be elucidated until they are properly controlled, which is most expediently achieved by evaluating subjects across different time points to look directly at the effects of BD itself, or of other medications or treatments, while minimizing other variables.

5.7 Conclusions

We found no differences in the area of the cerebellar vermis associated with bipolar disorder as compared to healthy controls in adolescents. We found a weak trend for a larger anterior vermis (V1) in BD patients who were treated with lithium as compared to both non-lithium-treated BD patients and healthy controls; however, this trend was evident only in a cross-sectional sample of individuals. In a longitudinal study of previously lithium-naïve BD patients over a 2-year course of lithium treatment, we found no significant changes to the cerebellar vermis as a result of lithium treatment. Since longitudinal design is less susceptible to various confounding factors than a cross-sectional design, we consider the latter results more authoritative. Our findings thus indicate that neither the disease process of bipolar disorder, nor treatment with lithium, have a measurable effect on the cerebellar vermis. This finding accords with our meta-analysis of literature findings related to the cerebellar vermis in BD, which showed no overall difference between BD patients and healthy controls. We were not able to probe the possibility of a

relationship between vermal area and lithium-induced tremor, for the simple reason that none of our study subjects presented this side effect. We were not certain beforehand if tremor would be apparent within our sample, as incidence estimates of tremor due to lithium treatment vary widely, from 4%-65% (Gelenberg & Jefferson, 1995). Our conclusion is thus as follows: either there is no direct effect of the course of BD, or its treatment with lithium, on the cerebellar vermis; or, such an effect is sufficiently small or sufficiently influenced by extraneous factors as to be functionally undetectable.

5.8 Implications and significance

Estimates of the proportion of the American population that will be diagnosed with Bipolar Disorder at some point in their lives vary from 1.3% to nearly 5% (Kleinman et al., 2003; Merikangas et al., 2011), and the diagnosis has been rising sharply in recent years especially in youth (Moreno et al., 2007). Whether the increase is due to an actual increase in sickness, or to changes in diagnostic practice and criteria, is an issue hotly contested; however, regardless of the reason why BD is gaining such prominence, the importance of developing a physiological understanding is paramount. Biomarkers for psychiatric illness, long the stated goal of organizations like the American Psychiatric Association (APA) and National Institute of Mental Health (NIMH), have proven maddeningly elusive, and BD has been no exception. The search continues, though, and just as it is important to know where to look, it is also important to know where *not* to look. Based on the findings herein, as well as the past findings we reviewed, there is little apparent value in continuing to look for structural abnormalities in the cerebellar vermis of bipolar patients.

References

Abramovic, L., van Haren, N. E. M., Boks, M. P. M., Bootsman, F., Schnack, H. G., Brouwer, R. (2012). The effect of lithium and age on brain volume in patients with bipolar disorder. *Bipolar Disorders, 14*, 38-38.

Acheson, A., Conover, J. C., Fandl, J. P., DeChiara, T. M., Russell, M., Thadani, A. (1995). A BDNF autocrine loop in adult sensory neurons prevents cell death. *Nature, 374*(6521), 450-453.

Adler, C. M., Holland, S. K., Schmithorst, V., Tuchfarber, M. J., & Strakowski, S. M. (2004). Changes in neuronal activation in patients with bipolar disorder during performance of a working memory task. *Bipolar Disord, 6*(6), 540-549.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th Edition). Arlington, VA: American Psychiatric Publishing.

Baldacara, L., Borgio, J. G., Lacerda, A. L., & Jackowski, A. P. (2008). Cerebellum and psychiatric disorders. *Rev Bras Psiquiatr, 30*(3), 281-289.

Baldacara, L., Nery-Fernandes, F., Rocha, M., Quarantini, L. C., Rocha, G. G., Guimaraes, J. L. (2011). Is cerebellar volume related to bipolar disorder? *J Affect Disord, 135*(1-3), 305-309.

Baloch, H. A., Hatch, J. P., Olvera, R. L., Nicoletti, M., Caetano, S. C., Zunta-Soares, G. B. (2010). Morphology of the subgenual prefrontal cortex in pediatric bipolar disorder. *J Psychiatr Res, 44*(15), 1106-1110.

Barnett, J. H., & Smoller, J. W. (2009). The genetics of bipolar disorder. *Neuroscience, 164*(1), 331-343.

Baumann, O., & Mattingley, J. B. (2012). Functional topography of primary emotion processing in the human cerebellum. *Neuroimage, 61*(4), 805-811.

Beck, A. T., Ward, C. H., Mendelson, M., Mock, J., & Erbaugh, J. (1961). An inventory for measuring depression. *Arch Gen Psychiatry, 4*, 561-571.

Benes, F. M., Kwok, E. W., Vincent, S. L., & Todtenkopf, M. S. (1998). A reduction of nonpyramidal cells in sector CA2 of schizophrenics and manic depressives. *Biol Psychiatry, 44*(2), 88-97.

Berk, M., Berk, L., Dodd, S., Cotton, S., Macneil, C., Daglas, R. (2013). Stage managing bipolar disorder. *Bipolar Disord*.

Blond, B. N., Fredericks, C. A., & Blumberg, H. P. (2012). Functional neuroanatomy of bipolar disorder: structure, function, and connectivity in an amygdala-anterior paralimbic neural system. *Bipolar Disord, 14*(4), 340-355.

Blumberg, H. P., Kaufman, J., Martin, A., Whiteman, R., Zhang, J. H., Gore, J. C. (2003). Amygdala and hippocampal volumes in adolescents and adults with bipolar disorder. *Arch Gen Psychiatry, 60*(12), 1201-1208.

Blumberg, H. P., Leung, H. C., Skudlarski, P., Lacadie, C. M., Fredericks, C. A., Harris, B. C. (2003). A functional magnetic resonance imaging study of bipolar disorder: state- and trait-related dysfunction in ventral prefrontal cortices. *Arch Gen Psychiatry, 60*(6), 601-609.

Bogovic, J. A., Jedynak, B., Rigg, R., Du, A., Landman, B. A., Prince, J. L. (2012). Approaching expert results using a hierarchical cerebellum parcellation protocol for multiple inexpert human raters. *Neuroimage, 64C*, 616-629.

Bowie, C. R., & Harvey, P. D. (2006). Administration and interpretation of the Trail Making Test. *Nat Protoc, 1*(5), 2277-2281.
Buckner, R. L., Krienen, F. M., Castellanos, A., Diaz, J. C., & Yeo, B. T. (2011). The organization of the human cerebellum estimated by intrinsic functional connectivity. *J Neurophysiol, 106*(5), 2322-2345.
Cardoso de Almeida, J. R., & Phillips, M. L. (2013). Distinguishing between unipolar depression and bipolar depression: current and future clinical and neuroimaging perspectives. *Biol Psychiatry, 73*(2), 111-118.
Carter, A. R., Chen, C., Schwartz, P. M., & Segal, R. A. (2002). Brain-derived neurotrophic factor modulates cerebellar plasticity and synaptic ultrastructure. *J Neurosci, 22*(4), 1316-1327.
Carter, J. D., Frampton, C. M., Mulder, R. T., Luty, S. E., & Joyce, P. R. (2010). The relationship of demographic, clinical, cognitive and personality variables to the discrepancy between self and clinician rated depression. *J Affect Disord, 124*(1-2), 202-206.
Cassidy, F., Ahearn, E. P., & Carroll, B. J. (2001). Substance abuse in bipolar disorder. *Bipolar Disord, 3*(4), 181-188.
Castillo, P. E., Younts, T. J., Chavez, A. E., & Hashimotodani, Y. (2012). Endocannabinoid signaling and synaptic function. *Neuron, 76*(1), 70-81.
Castren, E. (2004). Neurotrophic effects of antidepressant drugs. *Curr Opin Pharmacol, 4*(1), 58-64.
Cerullo, M. A., Adler, C. M., Delbello, M. P., & Strakowski, S. M. (2009). The functional neuroanatomy of bipolar disorder. *Int Rev Psychiatry, 21*(4), 314-322.
Chepenik, L. G., Fredericks, C., Papademetris, X., Spencer, L., Lacadie, C., Wang, F. (2009). Effects of the brain-derived neurotrophic growth factor val66met variation on hippocampus morphology in bipolar disorder. *Neuropsychopharmacology, 34*(4), 944-951.
Chepenik, L. G., Wang, F., Spencer, L., Spann, M., Kalmar, J. H., Womer, F. (2012). Structure-function associations in hippocampus in bipolar disorder. *Biol Psychol, 90*(1), 18-22.
Cipriani, A., Hawton, K., Stockton, S., & Geddes, J. R. (2013). Lithium in the prevention of suicide in mood disorders: updated systematic review and meta-analysis. *BMJ, 346*, f3646.
Cohen, J. (1992). A power primer. Psychol Bull, 112(1), 155-159.
Correa, R., Akiskal, H., Gilmer, W., Nierenberg, A. A., Trivedi, M., & Zisook, S. (2010). Is unrecognized bipolar disorder a frequent contributor to apparent treatment resistant depression? *Journal of Affective Disorders, 127*(1-3), 10-18.
Cousins, D. A., Aribisala, B., Nicol Ferrier, I., & Blamire, A. M. (2013). Lithium, gray matter, and magnetic resonance imaging signal. *Biol Psychiatry, 73*(7), 652-657.
Cox, R. W. (1996). AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Comput Biomed Res, 29*(3), 162-173.
Cuthbert, B. N., & Kozak, M. J. (2013). Constructing constructs for psychopathology: the NIMH research domain criteria. *J Abnorm Psychol, 122*(3), 928-937.
Daban, C., Vieta, E., Mackin, P., & Young, A. H. (2005). Hypothalamic-pituitary-adrenal axis and bipolar disorder. *Psychiatr Clin North Am, 28*(2), 469-480.

de Zeeuw, P., Zwart, F., Schrama, R., van Engeland, H., & Durston, S. (2012). Prenatal exposure to cigarette smoke or alcohol and cerebellum volume in attention-deficit/hyperactivity disorder and typical development. *Transl Psychiatry, 2*, e84.

DelBello, M. P., Strakowski, S. M., Zimmerman, M. E., Hawkins, J. M., & Sax, K. W. (1999). MRI analysis of the cerebellum in bipolar disorder: a pilot study. *Neuropsychopharmacology, 21*(1), 63-68.

Delgado, L. M., & Schmachtenberg, O. (2011). Neurogenesis in the adult goldfish cerebellum. *Anat Rec (Hoboken), 294*(1), 11-15.

Deuschl, G., Bain, P., & Brin, M. (1998). Consensus statement of the Movement Disorder Society on Tremor. Ad Hoc Scientific Committee. *Mov Disord, 13 Suppl 3*, 2-23.

Di, X., Zhu, S., Jin, H., Wang, P., Ye, Z., Zhou, K. (2012). Altered resting brain function and structure in professional badminton players. *Brain Connect, 2*(4), 225-233.

Dilsaver, S. C. (2011). An estimate of the minimum economic burden of bipolar I and II disorders in the United States: 2009. *J Affect Disord, 129*(1-3), 79-83.

Dols, A., Sienaert, P., van Gerven, H., Schouws, S., Stevens, A., Kupka, R. (2013). The prevalence and management of side effects of lithium and anticonvulsants as mood stabilizers in bipolar disorder from a clinical perspective: a review. *Int Clin Psychopharmacol, 28*(6), 287-296.

Drevets, W. C., Savitz, J., & Trimble, M. (2008). The subgenual anterior cingulate cortex in mood disorders. *CNS Spectr, 13*(8), 663-681.

Duffy, A. (2007). Does bipolar disorder exist in children? A selected review. *Canadian Journal of Psychiatry-Revue Canadienne De Psychiatrie, 52*(7), 409-417.

Duffy, A., Alda, M., Hajek, T., Sherry, S. B., & Grof, P. (2010). Early stages in the development of bipolar disorder. *J Affect Disord, 121*(1-2), 127-135.

Duffy, A., Milin, R., & Grof, P. (2009). Maintenance treatment of adolescent bipolar disorder: open study of the effectiveness and tolerability of quetiapine. *BMC Psychiatry, 9*, 4.

Duman, R. S., & Voleti, B. (2012). Signaling pathways underlying the pathophysiology and treatment of depression: novel mechanisms for rapid-acting agents. *Trends Neurosci, 35*(1), 47-56.

Elvsashagen, T., Moberget, T., Boen, E., Boye, B., Englin, N. O., Pedersen, P. O. (2012). Evidence for impaired neocortical synaptic plasticity in bipolar II disorder. *Biol Psychiatry, 71*(1), 68-74.

Elvsashagen, T., Westlye, L. T., Boen, E., Hol, P. K., Andersson, S., Andreassen, O. A. (2013). Evidence for reduced dentate gyrus and fimbria volume in bipolar II disorder. *Bipolar Disord, 15*(2), 167-176.

Emsell, L., & McDonald, C. (2009). The structural neuroimaging of bipolar disorder. *Int Rev Psychiatry, 21*(4), 297-313.

Fagiolini, A., Forgione, R., Maccari, M., Cuomo, A., Morana, B., Dell'Osso, M. C. (2013). Prevalence, chronicity, burden and borders of bipolar disorder. *J Affect Disord, 148*(2-3), 161-169.

Fagiolini, A., Kupfer, D. J., Masalehdan, A., Scott, J. A., Houck, P. R., & Frank, E. (2005). Functional impairment in the remission phase of bipolar disorder. *Bipolar Disord, 7*(3), 281-285.

First, M. B., Spitzer, R. L., Gibbon, M, Williams, J. B. W. (2002). *Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition*. New York: Biometrics Research; New York State Psychiatric Institute

Fischera, M., Anneken, K., Evers, S., Kloska, S., & Husstedt, I. W. (2009). Cerebellar atrophy after long-term treatment with low-dose lithium. *Pharmacopsychiatry, 42*(3), 125-126.

Fleck, D. E., Nandagopal, J., Cerullo, M. A., Eliassen, J. C., DelBello, M. P., Adler, C. M. (2008). Morphometric magnetic resonance imaging in psychiatry. *Top Magn Reson Imaging, 19*(2), 131-142.

Foland-Ross, L. C., Thompson, P. M., Sugar, C. A., Narr, K. L., Penfold, C., Vasquez, R. E. (2013). Three-dimensional mapping of hippocampal and amygdalar structure in euthymic adults with bipolar disorder not treated with lithium. *Psychiatry Res, 211*(3), 195-201.

Frey, B. N., Andreazza, A. C., Nery, F. G., Martins, M. R., Quevedo, J., Soares, J. C. (2007). The role of hippocampus in the pathophysiology of bipolar disorder. *Behav Pharmacol, 18*(5-6), 419-430.

Gelenberg, A. J., & Jefferson, J. W. (1995). Lithium tremor. *J Clin Psychiatry, 56*(7), 283-287.

Goldberg, J. F., Garakani, A., & Ackerman, S. H. (2012). Clinician-rated versus self-rated screening for bipolar disorder among inpatients with mood symptoms and substance misuse. *J Clin Psychiatry, 73*(12), 1525-1530.

Gould, T. D., Quiroz, J. A., Singh, J., Zarate, C. A., & Manji, H. K. (2004). Emerging experimental therapeutics for bipolar disorder: insights from the molecular and cellular actions of current mood stabilizers. *Mol Psychiatry, 9*(8), 734-755.

Grimaldi, G., & Manto, M. (2012). Topography of cerebellar deficits in humans. *Cerebellum, 11*(2), 336-351.

Grof, P., Duffy, A., Cavazzoni, P., Grof, E., Garnham, J., MacDougall, M. (2002). Is response to prophylactic lithium a familial trait? *J Clin Psychiatry, 63*(10), 942-947.

Habas, C., Kamdar, N., Nguyen, D., Prater, K., Beckmann, C. F., Menon, V. (2009). Distinct cerebellar contributions to intrinsic connectivity networks. *J Neurosci, 29*(26), 8586-8594.

Hajek, T., Bauer, M., Simhandl, C., Rybakowski, J., O'Donovan, C., Pfennig, A. (2013). Neuroprotective effect of lithium on hippocampal volumes in bipolar disorder independent of long-term treatment response. *Psychol Med*, 1-11.

Hajek, T., Cullis, J., Novak, T., Kopecek, M., Hoschl, C., Blagdon, R. (2012). Hippocampal volumes in bipolar disorders: opposing effects of illness burden and lithium treatment. *Bipolar Disord, 14*(3), 261-270.

Hallahan, B., Newell, J., Soares, J. C., Brambilla, P., Strakowski, S. M., Fleck, D. E. (2011). Structural magnetic resonance imaging in bipolar disorder: an international collaborative mega-analysis of individual adult patient data. *Biol Psychiatry, 69*(4), 326-335.

Hamilton, M. (1960). A rating scale for depression. *J Neurol Neurosurg Psychiatry, 23*, 56-62.

Hanson, N. D., Nemeroff, C. B., & Owens, M. J. (2011). Lithium, but not fluoxetine or the corticotropin-releasing factor receptor 1 receptor antagonist R121919, increases cell proliferation in the adult dentate gyrus. *J Pharmacol Exp Ther, 337*(1), 180-186.

Haznedar, M. M., Roversi, F., Pallanti, S., Baldini-Rossi, N., Schnur, D. B., Licalzi, E. M. (2005). Fronto-thalamo-striatal gray and white matter volumes and anisotropy of their connections in bipolar spectrum illnesses. *Biol Psychiatry, 57*(7), 733-742.

Hill, M. N., & Gorzalka, B. B. (2009). Impairments in endocannabinoid signaling and depressive illness. *JAMA, 301*(11), 1165-1166.
Hill, M. N., & McEwen, B. S. (2010). Involvement of the endocannabinoid system in the neurobehavioural effects of stress and glucocorticoids. *Prog Neuropsychopharmacol Biol Psychiatry, 34*(5), 791-797.
Hirschfeld, R. M., Williams, J. B., Spitzer, R. L., Calabrese, J. R., Flynn, L., Keck, P. E., Jr. (2000). Development and validation of a screening instrument for bipolar spectrum disorder: the Mood Disorder Questionnaire. *Am J Psychiatry, 157*(11), 1873-1875.
Insel, T., Cuthbert, B., Garvey, M., Heinssen, R., Pine, D. S., Quinn, K. (2010). Research domain criteria (RDoC): toward a new classification framework for research on mental disorders. *Am J Psychiatry, 167*(7), 748-751.
Ito, Masao. (2001). Cerebellar Plasticity *eLS*: John Wiley & Sons, Ltd.
Ivry, R. B., & Baldo, J. V. (1992). Is the cerebellum involved in learning and cognition? *Curr Opin Neurobiol, 2*(2), 212-216.
Jaatinen, P., & Rintala, J. (2008). Mechanisms of ethanol-induced degeneration in the developing, mature, and aging cerebellum. *Cerebellum, 7*(3), 332-347.
Judd, L. L., & Akiskal, H. S. (2003). The prevalence and disability of bipolar spectrum disorders in the US population: re-analysis of the ECA database taking into account subthreshold cases. *J Affect Disord, 73*(1-2), 123-131.
Judd, L. L., Akiskal, H. S., Schettler, P. J., Endicott, J., Leon, A. C., Solomon, D. A. (2005). Psychosocial disability in the course of bipolar I and II disorders: a prospective, comparative, longitudinal study. *Arch Gen Psychiatry, 62*(12), 1322-1330.
Kandel, Eric R., Schwartz, James H., & Jessell, Thomas M. (2000). *Principles of neural science* (4th ed.). New York: McGraw-Hill, Health Professions Division.
Kapczinski, F., Frey, B. N., Kauer-Sant'Anna, M., & Grassi-Oliveira, R. (2008). Brain-derived neurotrophic factor and neuroplasticity in bipolar disorder. *Expert Rev Neurother, 8*(7), 1101-1113.
Kaufman, J., Birmaher, B., Brent, D., Rao, U., Flynn, C., Moreci, P. (1997). Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): initial reliability and validity data. *J Am Acad Child Adolesc Psychiatry, 36*(7), 980-988.
Kempermann, G., & Kronenberg, G. (2003). Depressed new neurons--adult hippocampal neurogenesis and a cellular plasticity hypothesis of major depression. *Biol Psychiatry, 54*(5), 499-503.
Kempton, M. J., Salvador, Z., Munafo, M. R., Geddes, J. R., Simmons, A., Frangou, S. (2011). Structural neuroimaging studies in major depressive disorder. Meta-analysis and comparison with bipolar disorder. *Arch Gen Psychiatry, 68*(7), 675-690.
Keshavan, Matcheri S., Morris, David W., Sweeney, John A., Pearlson, Godfrey, Thaker, Gunvant, Seidman, Larry J. (2011). A dimensional approach to the psychosis spectrum between bipolar disorder and schizophrenia: The Schizo-Bipolar Scale. *Schizophrenia Research, 133*(1-3), 250-254.
Kleinman, L., Lowin, A., Flood, E., Gandhi, G., Edgell, E., & Revicki, D. (2003). Costs of bipolar disorder. *Pharmacoeconomics, 21*(9), 601-622.

Konradi, C., Zimmerman, E. I., Yang, C. K., Lohmann, K. M., Gresch, P., Pantazopoulos, H. (2011). Hippocampal interneurons in bipolar disorder. *Arch Gen Psychiatry, 68*(4), 340-350.

Kupka, R. W., Altshuler, L. L., Nolen, W. A., Suppes, T., Luckenbaugh, D. A., Leverich, G. S. (2007). Three times more days depressed than manic or hypomanic in both bipolar I and bipolar II disorder. *Bipolar Disord, 9*(5), 531-535.

Lewandowska, E., Stepien, T., Wierzba-Bobrowicz, T., Felczak, P., Szpak, G. M., & Pasennik, E. (2012). Alcohol-induced changes in the developing cerebellum. Ultrastructural and quantitative analysis of neurons in the cerebellar cortex. *Folia Neuropathol, 50*(4), 397-406.

Lisy, M. E., Jarvis, K. B., DelBello, M. P., Mills, N. P., Weber, W. A., Fleck, D. (2011). Progressive neurostructural changes in adolescent and adult patients with bipolar disorder. *Bipolar Disord, 13*(4), 396-405.

Liu, L., Schulz, S. C., Lee, S., Reutiman, T. J., & Fatemi, S. H. (2007). Hippocampal CA1 pyramidal cell size is reduced in bipolar disorder. *Cell Mol Neurobiol, 27*(3), 351-358.

Luft, A. R., Skalej, M., Schulz, J. B., Welte, D., Kolb, R., Burk, K. (1999). Patterns of age-related shrinkage in cerebellum and brainstem observed in vivo using three-dimensional MRI volumetry. *Cereb Cortex, 9*(7), 712-721.

Mackie, S., Shaw, P., Lenroot, R., Pierson, R., Greenstein, D. K., Nugent, T. F., 3rd. (2007). Cerebellar development and clinical outcome in attention deficit hyperactivity disorder. *Am J Psychiatry, 164*(4), 647-655.

MacQueen, G. M. (2009). Magnetic resonance imaging and prediction of outcome in patients with major depressive disorder. *J Psychiatry Neurosci, 34*(5), 343-349.

MacQueen, G. M., Campbell, S., McEwen, B. S., Macdonald, K., Amano, S., Joffe, R. T. (2003). Course of illness, hippocampal function, and hippocampal volume in major depression. *Proc Natl Acad Sci U S A, 100*(3), 1387-1392.

MacQueen, G. M., & Young, L. T. (2001). Bipolar II disorder: symptoms, course, and response to treatment. *Psychiatr Serv, 52*(3), 358-361.

Mamah, D., Barch, D. M., & Repovs, G. (2013). Resting state functional connectivity of five neural networks in bipolar disorder and schizophrenia. *J Affect Disord, 150*(2), 601-609.

Mamah, D., Wang, L., Csernansky, J. G., Rice, J. P., Smith, M., & Barch, D. M. (2010). Morphometry of the hippocampus and amygdala in bipolar disorder and schizophrenia. *Bipolar Disord, 12*(3), 341-343.

McCarthy, M. J., Leckband, S. G., & Kelsoe, J. R. (2010). Pharmacogenetics of lithium response in bipolar disorder. *Pharmacogenomics, 11*(10), 1439-1465.

McEwen, B. S., & Magarinos, A. M. (2001). Stress and hippocampal plasticity: implications for the pathophysiology of affective disorders. *Hum Psychopharmacol, 16*(S1), S7-S19.

Merikangas, K. R., Jin, R., He, J. P., Kessler, R. C., Lee, S., Sampson, N. A. (2011). Prevalence and correlates of bipolar spectrum disorder in the world mental health survey initiative. *Arch Gen Psychiatry, 68*(3), 241-251.

Metz, G. A., Schwab, M. E., & Welzl, H. (2001). The effects of acute and chronic stress on motor and sensory performance in male Lewis rats. *Physiol Behav, 72*(1-2), 29-35.

Mills, N. P., Delbello, M. P., Adler, C. M., & Strakowski, S. M. (2005). MRI analysis of cerebellar vermal abnormalities in bipolar disorder. *Am J Psychiatry, 162*(8), 1530-1532.

Mitsunaga, M. M., Garrett, A., Howe, M., Karchemskiy, A., Reiss, A., & Chang, K. (2011). Increased subgenual cingulate cortex volume in pediatric bipolar disorder associated with mood stabilizer exposure. *J Child Adolesc Psychopharmacol, 21*(2), 149-155.

Monkul, E. S., Hatch, J. P., Sassi, R. B., Axelson, D., Brambilla, P., Nicoletti, M. A. (2008). MRI study of the cerebellum in young bipolar patients. *Prog Neuropsychopharmacol Biol Psychiatry, 32*(3), 613-619.

Montgomery, S. A., & Asberg, M. (1979). A new depression scale designed to be sensitive to change. *Br J Psychiatry, 134*, 382-389.

Moore, G. J., Cortese, B. M., Glitz, D. A., Zajac-Benitez, C., Quiroz, J. A., Uhde, T. W. (2009). A longitudinal study of the effects of lithium treatment on prefrontal and subgenual prefrontal gray matter volume in treatment-responsive bipolar disorder patients. *J Clin Psychiatry, 70*(5), 699-705.

Moreno, C., Laje, G., Blanco, C., Jiang, H., Schmidt, A. B., & Olfson, M. (2007). National trends in the outpatient diagnosis and treatment of bipolar disorder in youth. *Arch Gen Psychiatry, 64*(9), 1032-1039.

Muftuler, L. Tugan. (2013). *Quantifying morphology and physiology of the human body using MRI*. Boca Raton, FL: CRC Press/Taylor & Francis Group.

Nasrallah, H. A., Jacoby, C. G., & McCalley-Whitters, M. (1981). Cerebellar atrophy in schizophrenia and mania. *Lancet, 1*(8229), 1102.

Nierenberg, A. A., Friedman, E. S., Bowden, C. L., Sylvia, L. G., Thase, M. E., Ketter, T. (2013). Lithium treatment moderate-dose use study (LiTMUS) for bipolar disorder: a randomized comparative effectiveness trial of optimized personalized treatment with and without lithium. *Am J Psychiatry, 170*(1), 102-110.

Niethammer, M., & Ford, B. (2007). Permanent lithium-induced cerebellar toxicity: three cases and review of literature. *Mov Disord, 22*(4), 570-573.

Pacchiarotti, I., Bond, D. J., Baldessarini, R. J., Nolen, W. A., Grunze, H., Licht, R. W. (2013). The International Society for Bipolar Disorders (ISBD) Task Force Report on Antidepressant Use in Bipolar Disorders. *Am J Psychiatry, 170*(11), 1249-1262.

Parens, E., & Johnston, J. (2010). Controversies concerning the diagnosis and treatment of bipolar disorder in children. *Child Adolesc Psychiatry Ment Health, 4*, 9.

Park, I. S., Lee, K. J., Han, J. W., Lee, N. J., Lee, W. T., Park, K. A. (2009). Experience-dependent plasticity of cerebellar vermis in basketball players. *Cerebellum, 8*(3), 334-339.

Pasquali, L., Busceti, C. L., Fulceri, F., Paparelli, A., & Fornai, F. (2010). Intracellular pathways underlying the effects of lithium. *Behav Pharmacol, 21*(5-6), 473-492.

Passmore, M. J., Garnham, J., Duffy, A., MacDougall, M., Munro, A., Slaney, C. (2003). Phenotypic spectra of bipolar disorder in responders to lithium versus lamotrigine. *Bipolar Disord, 5*(2), 110-114.

Pavuluri, M. N., Passarotti, A. M., Parnes, S. A., Fitzgerald, J. M., & Sweeney, J. A. (2010). A pharmacological functional magnetic resonance imaging study probing the interface of cognitive and emotional brain systems in pediatric bipolar disorder. *J Child Adolesc Psychopharmacol, 20*(5), 395-406.

Pezawas, L., Verchinski, B. A., Mattay, V. S., Callicott, J. H., Kolachana, B. S., Straub, R. E. (2004). The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. *J Neurosci, 24*(45), 10099-10102.

Phillips, M. L., Ladouceur, C. D., & Drevets, W. C. (2008). A neural model of voluntary and automatic emotion regulation: implications for understanding the pathophysiology and neurodevelopment of bipolar disorder. *Mol Psychiatry, 13*(9), 829, 833-857.
Ponti, G., Peretto, P., & Bonfanti, L. (2008). Genesis of neuronal and glial progenitors in the cerebellar cortex of peripuberal and adult rabbits. *PLoS One, 3*(6), e2366.
Post, R. M. (2007). Role of BDNF in bipolar and unipolar disorder: clinical and theoretical implications. *J Psychiatr Res, 41*(12), 979-990.
Potash, J. B., & Bienvenu, O. J. (2009). Neuropsychiatric disorders: Shared genetics of bipolar disorder and schizophrenia. *Nat Rev Neurol, 5*(6), 299-300.
Quiroz, J. A., Machado-Vieira, R., Zarate, C. A., Jr., & Manji, H. K. (2010). Novel insights into lithium's mechanism of action: neurotrophic and neuroprotective effects. *Neuropsychobiology, 62*(1), 50-60.
Rabi, I. I., Zacharias, J. R., Millman, S., & Kusch, P. (1938). A new method of measuring nuclear magnetic moment. *Physical Review, 53*(4), 318-318.
Robinson, L. J., Thompson, J. M., Gallagher, P., Goswami, U., Young, A. H., Ferrier, I. N. (2006). A meta-analysis of cognitive deficits in euthymic patients with bipolar disorder. *J Affect Disord, 93*(1-3), 105-115.
Sachs, G. S., Peters, A. T., Sylvia, L., & Grunze, H. (2013). Polypharmacy and bipolar disorder: what's personality got to do with it? *Int J Neuropsychopharmacol*, 1-9.
Safo, P. K., Cravatt, B. F., & Regehr, W. G. (2006). Retrograde endocannabinoid signaling in the cerebellar cortex. *Cerebellum, 5*(2), 134-145.
Sanches, M., Newberg, A. R., & Soares, J. C. (2010). Emerging drugs for bipolar disorder. *Expert Opin Emerg Drugs, 15*(3), 453-466.
Savitz, J., & Drevets, W. C. (2009). Bipolar and major depressive disorder: neuroimaging the developmental-degenerative divide. *Neurosci Biobehav Rev, 33*(5), 699-771.
Schloesser, R. J., Huang, J., Klein, P. S., & Manji, H. K. (2008). Cellular plasticity cascades in the pathophysiology and treatment of bipolar disorder. *Neuropsychopharmacology, 33*(1), 110-133.
Schmahmann, J. D., & Caplan, D. (2006). Cognition, emotion and the cerebellum. *Brain, 129*(Pt 2), 290-292.
Schmahmann, J. D., & Sherman, J. C. (1998). The cerebellar cognitive affective syndrome. *Brain, 121 (Pt 4)*, 561-579.
Schmahmann, J. D., Weilburg, J. B., & Sherman, J. C. (2007). The neuropsychiatry of the cerebellum - insights from the clinic. *Cerebellum, 6*(3), 254-267.
Schneider, M. R., DelBello, M. P., McNamara, R. K., Strakowski, S. M., & Adler, C. M. (2012). Neuroprogression in bipolar disorder. *Bipolar Disord, 14*(4), 356-374.
Schutter, D. J., Koolschijn, P. C., Peper, J. S., & Crone, E. A. (2012). The cerebellum link to neuroticism: a volumetric MRI association study in healthy volunteers. *PLoS One, 7*(5), e37252.
Sidor, M. M., & MacQueen, G. M. (2012). An update on antidepressant use in bipolar depression. *Curr Psychiatry Rep, 14*(6), 696-704.
Simpson, G. M., & Angus, J. W. (1970). A rating scale for extrapyramidal side effects. *Acta Psychiatr Scand Suppl, 212*, 11-19.
Singh, M. K., & Chang, K. D. (2012). The neural effects of psychotropic medications in children and adolescents. *Child Adolesc Psychiatr Clin N Am, 21*(4), 753-771.

StataCorp. (2007). Stata Statistical Software (Version Release 10). College Station, TX: StataCorp LP.
Steen, N. E., Methlie, P., Lorentzen, S., Hope, S., Barrett, E. A., Larsson, S. (2011). Increased systemic cortisol metabolism in patients with schizophrenia and bipolar disorder: a mechanism for increased stress vulnerability? *J Clin Psychiatry, 72*(11), 1515-1521.
Stoodley, C. J., Valera, E. M., & Schmahmann, J. D. (2012). Functional topography of the cerebellum for motor and cognitive tasks: an fMRI study. *Neuroimage, 59*(2), 1560-1570.
Strakowski, S. M., Adler, C. M., Almeida, J., Altshuler, L. L., Blumberg, H. P., Chang, K. D. (2012). The functional neuroanatomy of bipolar disorder: a consensus model. *Bipolar Disord, 14*(4), 313-325.
Strawn, J. R., Adler, C. M., McNamara, R. K., Welge, J. A., Bitter, S. M., Mills, N. P. (2013). Antidepressant tolerability in anxious and depressed youth at high risk for bipolar disorder: a prospective naturalistic treatment study. *Bipolar Disord*.
Tambaro, S., & Bortolato, M. (2012). Cannabinoid-related agents in the treatment of anxiety disorders: current knowledge and future perspectives. *Recent Pat CNS Drug Discov, 7*(1), 25-40.
Tiemeier, H., Lenroot, R. K., Greenstein, D. K., Tran, L., Pierson, R., & Giedd, J. N. (2010). Cerebellum development during childhood and adolescence: a longitudinal morphometric MRI study. *Neuroimage, 49*(1), 63-70.
Tombaugh, T. N. (2004). Trail Making Test A and B: normative data stratified by age and education. *Arch Clin Neuropsychol, 19*(2), 203-214.
Tyrer, P., Lee, I., & Trotter, C. (1981). Physiological characteristics of tremor after chronic lithium therapy. *Br J Psychiatry, 139*, 59-61.
Valvezan, A. J., & Klein, P. S. (2012). GSK-3 and Wnt Signaling in Neurogenesis and Bipolar Disorder. *Front Mol Neurosci, 5*, 1.
van Beugen, B. J., Nagaraja, R. Y., & Hansel, C. (2006). Climbing fiber-evoked endocannabinoid signaling heterosynaptically suppresses presynaptic cerebellar long-term potentiation. *J Neurosci, 26*(32), 8289-8294.
van Erp, T. G., Thompson, P. M., Kieseppa, T., Bearden, C. E., Marino, A. C., Hoftman, G. D. (2012). Hippocampal morphology in lithium and non-lithium-treated bipolar I disorder patients, non-bipolar co-twins, and control twins. *Hum Brain Mapp, 33*(3), 501-510.
Vazquez, G. H., Tondo, L., Undurraga, J., & Baldessarini, R. J. (2013). Overview of antidepressant treatment of bipolar depression. *Int J Neuropsychopharmacol, 16*(7), 1673-1685.
Wegbreit, E., Ellis, J. A., Nandam, A., Fitzgerald, J. M., Passarotti, A. M., Pavuluri, M. N. (2011). Amygdala functional connectivity predicts pharmacotherapy outcome in pediatric bipolar disorder. *Brain Connect, 1*(5), 411-422.
Whitwell, J. L. (2009). Voxel-based morphometry: an automated technique for assessing structural changes in the brain. *J Neurosci, 29*(31), 9661-9664.
Womer, F. Y., Wang, F., Chepenik, L. G., Kalmar, J. H., Spencer, L., Edmiston, E. (2009). Sexually dimorphic features of vermis morphology in bipolar disorder. *Bipolar Disord, 11*(7), 753-758.
Young, R. C., Biggs, J. T., Ziegler, V. E., & Meyer, D. A. (1978). A rating scale for mania: reliability, validity and sensitivity. *Br J Psychiatry, 133*, 429-435.

Yucel, K., McKinnon, M. C., Taylor, V. H., Macdonald, K., Alda, M., Young, L. T. (2007). Bilateral hippocampal volume increases after long-term lithium treatment in patients with bipolar disorder: a longitudinal MRI study. *Psychopharmacology (Berl), 195*(3), 357-367.

Yucel, K., Nazarov, A., Taylor, V. H., Macdonald, K., Hall, G. B., & Macqueen, G. M. (2012). Cerebellar vermis volume in major depressive disorder. *Brain Struct Funct*.

Yucel, K., Taylor, V. H., McKinnon, M. C., Macdonald, K., Alda, M., Young, L. T. (2008). Bilateral hippocampal volume increase in patients with bipolar disorder and short-term lithium treatment. *Neuropsychopharmacology, 33*(2), 361-367.

Zhang, C., Hua, T., Zhu, Z., & Luo, X. (2006). Age-related changes of structures in cerebellar cortex of cat. *J Biosci, 31*(1), 55-60.