

**EFFECTS OF BIPOLAR DISORDER ON INTRINSIC  
BRAIN NETWORKS**

by

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For the degree **Master of Medicine in Psychiatry**  
at  
**The University of Cape Town**

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# **Abstract - Effects of Bipolar Disorder on Intrinsic Brain Networks**

## **Introduction**

Bipolar disorder (BD) is a brain network disorder that affects cognitive and emotional functioning, and is associated with prefrontal and/or limbic dysfunction. Functional Magnetic Resonance Imaging (fMRI) allows identification of intrinsic brain networks (IBN), like the default mode network (DMN) and executive control network (ECN), which are consistent with previously established functional and anatomical relationships within the brain. Analysing the functional connectivity (integrity, extent and inter-relationships) of these networks, allows a deeper understanding of brain function in health and disease. In BD, there are functional connectivity changes in the DMN, ECN and cerebellar network (CERN). We evaluate IBN in BD, to explore changes in the functional connectivity between the cerebellum, fronto-cortical and paralimbic regions.

## **Methods**

Data from 14 BD subjects and 10 control subjects was analysed after fMRI. Changes were evaluated in 3 IBN (DMN, ECN and CERN) using an FMRIB Software Library (FSL) pipeline: MELODIC/ICA-AROMA, dual-regression, randomise and Local False Discovery Rate (FDR) to identify changes in functional connectivity bipolar subjects compared to controls.

## **Results**

Subjects with BD showed decreased connectivity between the CERN and a cluster in the right precuneus; and between the ECN and a cluster in the left OFC. There was also increased connectivity between the ECN and a cluster in the left temporal pole. No connectivity changes involving the DMN were identified. Voxels within the clusters were significant at  $p < 0.05$  with local FDR. Peaks within the clusters remained significant after further Bonferroni correction for multiple comparisons ( $p < 0.017$ ).

## **Conclusion**

The finding of altered functional connectivity in BD, in networks and regions involved in cognitive/emotional processes, highlights its complex neurobiology, and suggests that abnormal connectivity may help to explain the clinical picture. These findings should be replicated with larger samples, but may represent a further advance in understanding the role of functional connectivity in the pathology of BD, and contribute to laying the foundation for functional neuroimaging as a diagnostic tool in psychiatry

## **Acknowledgements**

Dr Neil Horn (University of Cape Town) was the primary supervisor and made the raw data (already collected prior to the involvement of the candidate) available for analysis. I completed the work described here after registration for the MMed degree. Dr Horn provided day-to-day support, particularly involving interpretation of results, as well as the university procedures relating to the completion of the dissertation. On any research article published from this research project, Dr Horn would be senior (last) author. Prof. Christian Beckmann (Donders Institute, Radboud University, the Netherlands) acted as a technical co-supervisor, and advised extensively at the outset on the software-based analysis approach used for the project. Prof. Beckmann would be a co-author (likely second author) on any published articles resulting from the project. Dr Victoria Ives-Deliperi (Co-PI on the original study from which the data was derived) would be listed as a co-author (likely third author). In addition, Dr Maarten Mennes (Donders Institute, Radboud University, the Netherlands) gave additional technical advice and some input regarding interpretation of results. Though he was not a co-supervisor, consideration would be given to including Dr Mennes as a co-author (likely fourth author) on any published articles resulting from the project, depending on the nature of his further involvement, and after discussion with the supervisor and co-supervisor. The candidate would be the first author on any research article published as a result of this project. I am thankful to my supervisors and Dr Mennes for their ongoing advice and support. I am also very thankful to my wife, Dr Ana Isabel Montoya Saldarriaga, for her kindness and support during the busy period of completing this project.

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## Abbreviations

<b>ACC</b>	Anterior Cingulate Cortex
<b>BD</b>	Bipolar Disorder
<b>BET</b>	Brain Extraction Tool
<b>BOLD</b>	Blood Oxygen Level Dependent
<b>CERN</b>	Cerebellar Network
<b>DLPFC</b>	Dorsolateral Prefrontal Cortex
<b>DMN</b>	Default Mode Network
<b>DSM-IV</b>	Diagnostic and Statistical Manual (4 <sup>th</sup> edition)
<b>DTI</b>	Diffusion Tensor Imaging
<b>ECN</b>	Executive Control Network
<b>FDR</b>	False Discovery Rate
<b>FHS</b>	Faculty of Health Sciences
<b>fMRI</b>	Functional Magnetic Resonance Imaging
<b>FMRIB</b>	(Oxford Centre for) Functional Magnetic Resonance Imaging of the Brain
<b>FSL</b>	FMRIB Software Library
<b>FWHM</b>	Full Width Half Maximum
<b>HADS</b>	Hospital Anxiety and Depression Score
<b>HREC</b>	Human Research Ethics Committee
<b>IBN</b>	Intrinsic Brain Network/s
<b>ICA</b>	Independent Components Analysis
<b>ICA-AROMA</b>	ICA-based Automatic Removal of Motion Artifacts
<b>MCFLIRT</b>	Motion Correction using FMRIB's Linear Image registration Tool
<b>MELODIC</b>	Multivariate Exploratory Linear Decomposition into Independent Components
<b>MNI</b>	Montreal Neurological Institute
<b>mPFC</b>	Medial Prefrontal Cortex
<b>MRI</b>	Magnetic Resonance Imaging
<b>NI</b>	Network Integrity
<b>OFC</b>	Orbitofrontal Cortex
<b>PET</b>	Positron Emission Tomography
<b>PFC</b>	Prefrontal Cortex
<b>SCID</b>	Structured Clinical Interview for DSM Disorders
<b>SGPFC</b>	Sub-genual Prefrontal cortex
<b>SPECT</b>	Single Photon Emission Computed Tomography
<b>TE</b>	Echo Time
<b>TP</b>	Temporal Pole
<b>TR</b>	Repetition Time
<b>VLPCF</b>	Ventrolateral Prefrontal Cortex
<b>YMRS</b>	Young Mania Rating Scale

## **Chapter 1: Introduction and Literature review**

### **1.1. Literature search strategy**

This was not a systematic review. Nevertheless, a structured approach was used in identifying and appraising the literature, as follows:

- Literature searches were performed on the PubMed, PsychINFO and ScienceDirect databases.
- Searches included combinations of the following terms: “bipolar disorder OR bipolar”, “mood disorder OR affective disorder”, “neurobiology OR neurobiological”, “neuroimaging OR brain imaging”, “functional magnetic resonance imaging OR fMRI”, “functional connectivity networks OR intrinsic connectivity networks OR resting state networks OR intrinsic brain networks”, “Default Mode Network OR DMN”, “Executive Control Network OR ECN”, “Cerebellar Network OR CER OR CERN”.
- Of the literature identified using this strategy, priority in selecting articles for further review was given to those which represented original research using fMRI to assess intrinsic brain networks in bipolar disorder. In addition, several review articles which summarized current knowledge on functional neuroimaging in mood disorders, especially bipolar, we selected, as well as review articles which summarized the clinical picture of bipolar, and/or the neurobiological models thought to explain the disorder. Apart from research identified directly through this search strategy, additional articles were identified from the reference lists of selected articles.

### **1.2. Bipolar disorder and its neurobiological underpinnings**

Bipolar disorder (BD) is a potentially disabling mood disorder occurring in about 2.5% of the adult population, which can involve episodes of both mania and depression, with interspersed periods of euthymia or subthreshold mood symptoms. In addition, BD is associated with neuropsychological abnormalities, including executive dysfunction, affective instability and dysregulation, neurovegetative abnormalities, impulsivity/disinhibition and psychosis (Delaloye et al., 2009; Green et

al., 2007; Strakowski et al., 2005). The clinical features of BD fall on a spectrum (Angst et al., 2003), ranging from severe illness with significant functional impairment, to cases in which there is a good recovery and high inter-episodic functioning (MacQueen et al., 2001) – this is important when considering how to apply research done on groups to the situation of any individual case. A number of clinical rating scales are used to evaluate symptoms in BD, including: the Young Mania Rating Scale (YMRS – Young et al., 1978<sup>1</sup>) – a multiple-choice questionnaire used to measure the presence and severity of manic symptoms; and the Hospital Anxiety and Depression Scale (HADS – Zigmond and Snaith, 1983<sup>2</sup>) – a fourteen-item scale measuring the presence and levels of anxiety and depression.

Importantly, neuropsychological impairments and difficulties with emotional regulation may persist even in the euthymic state, particularly involving ongoing problems with attention, working memory, processing speed and response inhibition/ impulse control (Goldberg and Chengappa, 2009; Kurtz and Gerraty, 2010; Robinson et al., 2006; Savitz et al., 2005; Torres et al., 2007). These abnormalities suggest that cognitive and anterior limbic brain networks are dysfunctional even in remitted patients, and structural, functional and spectroscopic imaging research is consistent in identifying abnormalities in prefrontal-striatal-thalamic regions and circuits which control complex socio-emotional behaviours, thus helping to explain the clinical picture (Blumberg et al., 2003; Cerullo et al. 2009; Kaladjian et al., 2009; Strakowski et al., 2005).

Neurobiological models of brain dysfunction in BD propose that either a relative loss of prefrontal modulation of subcortical and medial temporal limbic structures may underlie the symptoms, or limbic dysfunction may disrupt cortical inhibition, allowing affective symptoms to manifest (for example in the extreme clinical picture of mania). In support of this, the structural magnetic resonance imaging (MRI) literature (Drevets et al., 2008; Green et al., 2007; Hibar et al., 2014; Strakowski et al., 2005) has shown the following changes in BD: decreased grey matter volume in subregions of the prefrontal cortex, including the sub-genua prefrontal cortex (SGPFC), and anterior cingulate cortex, which have specific roles in

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<sup>1</sup> See appendix 11, page 49

<sup>2</sup> See appendix 10, page 48

linking the prefrontal cortex with anterior limbic system structures, and integrating cognitive and emotional information; enlargement of the striatum, which is significant given that prefrontal-striatal-thalamic loops regulate emotional, cognitive and social behaviour which is often seen to be abnormal in BD; changes in the size of the amygdala (both increases and decreases have been reported), a key structure in the limbic system with links to the prefrontal-striatal-thalamic loops, and has a prominent role in the regulation of emotion; and finally, decreased volume of the midline cerebellum which is now increasingly recognized as contributing towards emotion regulation through its strong connections to the limbic system (Schmahmann, 2004; Strakowski et al., 2005; Strick et al., 2009).

Functional imaging research (both task-based and task-free), including positron emission tomography (PET), single photon emission computed tomography (SPECT) and functional MRI (fMRI) (Chen et al., 2011; Drevets et al., 2008; Foland et al., 2008; Green et al., 2007; Strakowski et al., 2005) have, in summary, highlighted the following changes in BD: abnormal activation of the SGPF (decreased in bipolar depression and increased in mania suggesting a mood state-dependent effect); decreased activation of the orbito-frontal cortex (OFC), increased activation of the anterior cingulate, and both increased and decreased activation in sub regions of prefrontal cortices in the manic state (when cognitive control of mood may be impaired) compared with control subjects. Abnormalities on functional imaging persist even in the euthymic state, though there is some inconsistency in the literature with evidence for both increased and decreased activation of 'emotional' brain regions such as the ventrolateral prefrontal cortex (VLPFC). In addition to these cortical abnormalities, mood state-dependent and state-independent functional changes have been observed in the striatum, thalamus, amygdala and cerebellum, which highlight the possible role of these regions in the neurobiology of the condition, though there are once again some conflicting findings presented in the literature which remain to be clarified. Finally, functional studies have demonstrated a disturbed relationship between the PFC and the amygdala and other limbic structures, with decreased PFC control associated with increased limbic activation – a disinhibition which may lead to affective symptoms.

Overall, it is now recognised that while multiple cortical and subcortical brain areas are implicated (including amygdala, thalamus, anterior cingulate cortex - ACC, orbitofrontal cortex - OFC, medial prefrontal cortex - mPFC, dorsolateral prefrontal cortex - DLPFC, striatum, and cerebellum), BD is a problem of dysfunctional *brain networks* rather than one distinct brain region (Blumberg et al., 2003; Cerullo et al., 2009; Fountoulakis et al., 2008; Green et al., 2007; Strakowski et al., 2005). In attempting to clarify this, Diffusion Tensor Imaging (DTI) studies show decreased fractional anisotropy (a measure of white matter tract integrity) in multiple white matter tracts in BD, suggesting a possible mechanism for disordered *structural* connectivity across the brain (Nortje et al., 2013). Research exploring *functional* connections across the brain has provided further important insights.

### **1.3. fMRI and intrinsic brain networks in BD<sup>3</sup>**

Functional magnetic resonance imaging (fMRI) uses the different magnetic properties of oxy- and deoxyhaemoglobin to record changes in blood flow as a marker of neural activity – the Blood Oxygen Level Dependent or ‘BOLD’ signal – and is a useful tool for inferring neural activity (Malhi and Lagopoulos, 2008).

While the majority of fMRI studies continue to be task/activity based (Gusnard et al., 2001), there is growing interest in what the brain is doing in the absence of a task. This was originally studied with the brain in a “resting” state (awake but task-free), and the activity recorded by fMRI in this way is now recognised as being consistent, and structurally organised (Fox and Raichle, 2007). Subsequently, a consistent set of brain networks have been identified comprising areas that correspond temporally in their background fMRI activity, and mirror previously established functional and anatomical relationships within the brain (Beckmann et al., 2005; Damoiseaux et al., 2006). These networks are referred to as *resting-state networks*, *intrinsic brain networks* or *functional connectivity networks*, depending on the experimental approach used. Analysing the fluctuating integrity

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<sup>3</sup> PLEASE NOTE: The generic theoretical overview of fMRI and intrinsic brain networks presented here is equivalent to that described in the MSc thesis titled “Modulation of Resting State Networks by Fasting and Ghrelin: An fMRI study” submitted by Dr Jonathan Starke (myself) to Roehampton University in 2011 (unpublished but available in university library).

and spatial extent of these networks and the dynamic inter-relationships between them, allows a deeper understanding of brain function. A number of methods are used for identifying intrinsic brain networks, with Independent Components Analysis (ICA) – a computationally intensive and data-driven technique – now increasingly recognized as a robust, objective approach because it does not rely on a priori assumptions about the anatomical components of or relationships with and between networks, in contrast to alternatives such as seed-based approaches which do require these assumptions (Beckmann et al., 2005; Cole et al., 2010).

Whilst initially identified only at rest, activity within these networks has now been revealed to persist during and be modulated by a task or stimulus (Smith et al., 2009) – hence the terms *functional connectivity or intrinsic brain networks* (IBN). Taking this one step further, it is recognised that activity within the networks is predictive for activity in those same brain areas in response to tasks or stimuli which are presented *subsequently* (De Luca et al., 2005; Sadaghiani et al., 2010; Vincent et al., 2006). This suggests that the integrity of the networks in a particular group of participants or under a particular set of conditions (e.g. depressed vs. euthymic vs. manic BD subjects), is likely a key source of information for understanding the neurobiological underpinnings of behaviour (Cole et al., 2010), and the clinical picture of psychiatric illness. As a result, these networks have clarified aspects of brain function and provided diagnostic and prognostic insights into a number of diseases including Alzheimer’s disease, autism, depression and schizophrenia, and are now increasingly used in the study of BD (Fox and Raichle, 2007; Mamah et al., 2013; Meda et al., 2012).

Of particular interest in the context of BD, are the ‘Executive Control network’ (ECN) incorporating anterior cingulate cortex (ACC) and dorsolateral prefrontal cortex (DLPFC) which subserve executive functioning, and the ‘Default Mode Network’ (DMN) incorporating the precuneus, posterior cingulate and medial prefrontal cortex (mPFC) which mediate random episodic silent thought, reflections on self and aspects of consciousness) (Damoiseaux et al., 2006). These networks incorporate several of the anatomical areas already identified as abnormal or dysfunctional in BD, suggesting that studying them using both task-based and task-free approaches may be of value in understanding the disorder. In addition, the

cerebellar network (CERN) is of increasing interest in BD given the role the cerebellum is now thought to play in cognitive and emotional regulation (Bellebaum and Daum, 2007; Koziol et al., 2012; Schmahmann, 2004; Strick et al., 2009) and research identifying abnormalities in the cerebellum in BD (Strakowski et al., 2005; Phillips et al., 2015).

So far, network connectivity research in BD subjects using an oddball task has revealed changes within the ECN, with evidence of increased connectivity in the area of the DLPFC and ACC compared to control subjects (Calhoun et al., 2012). Regarding the DMN, the same researchers reported decreased connectivity in the precuneus and posterior cingulate, as well as areas of reduced connectivity between the DMN and limbic regions in the temporal lobes (Calhoun et al., 2012).

When the DMN was analysed in the resting state, additional differences emerged between depressed BD subjects and control subjects, specifically increased regional homogeneity (a measure of connectivity) in the left medial frontal gyrus and left inferior parietal lobe, which the authors interpreted as representing neuronal hyperactivity in those regions in BD, which they thought may represent a neuroimaging biomarker specific to bipolar depression (Liu et al., 2012). In contrast, in a study which examined changes in the DMN in BD subjects in the manic state, there was reduced DMN connectivity in the ventral mPFC in BD (Öngür et al., 2010). Chai et al. (2011) also demonstrated altered functional connectivity in manic BD subjects, finding a positive correlation in activity between the mPFC and ventrolateral PFC (VLPFC) as opposed to the normal anticorrelation between these areas seen in control subjects. Given the crucial role of these prefrontal regions in cognitive and executive functioning, this abnormal relationship may help to explain the neuropsychological impairments characteristic of BD.

Apart from these cortical changes, Mamah et al. (2013) also identified changes in functional connectivity involving the cerebellum in BD, showing decreased connectivity between the cerebellum and a network incorporating the cingulate, as well as between the cerebellum and a 'salience' network incorporating the anterior insula and anterior prefrontal cortex. These connectivity changes were significant in that they predicted the presence of psychotic symptoms within the bipolar group, highlighting the contribution of this imaging approach to developing

our understanding of the relationship between clinical features and the underlying neurobiological abnormalities in BD. Furthermore, given the evidence of cerebellar contributions to a number of functional connectivity networks, including those involved with executive functioning and salience (Habas et al., 2009), the research by Mamah et al. (2013) emphasizes the importance of including analysis of the cerebellum in neuroimaging studies in BD.

Importantly, given persisting neuropsychological abnormalities even when BD subjects are not depressed or manic (Torres et al., 2007), abnormal functional connectivity has also been documented in the euthymic state, with evidence of resting-state functional hyperconnectivity between the right amygdala and right VLPFC which was partially mediated by activity in the ACC (Torrissi et al., 2013), increased connectivity within the DMN in the right hippocampus and posterior cingulate (Vargas et al., 2014), and increased connectivity between a meso/paralimbic network and the right frontoparietal network (Lois et al., 2014). These findings are valuable in that they may represent trait-related biomarkers of BD and point to the possibility of functional neuroimaging as a diagnostic tool within psychiatry (Phillips and Vieta, 2007; Torrissi et al., 2013). Additional functional connectivity research in a heterogeneous group of BD subjects identified what appears to be a similar pathophysiological feature of BD, specifically increased connectivity between fronto-temporal and paralimbic networks involved in cognitive and emotional processing (Meda et al., 2012). This connectivity pattern was only present in BD subjects, which allowed them to be distinguished from both control subjects and subjects with schizophrenia, again highlighting the potential value of this imaging approach for diagnosis.

Taking into account the clinical abnormalities present in BD, the brain regions implicated and the usefulness of resting state and functional connectivity analysis in exploring the dynamics in networks incorporating these regions, it is valuable to further investigate network change in BD so as to deepen understanding of the underlying neurobiology of the disorder, and help to clarify current gaps and inconsistencies in the existing research. This will contribute towards a solid foundation for new diagnostic and treatment approaches for this common, potentially disabling condition.

## 1.4. Aims and Objectives

This project seeks to address the questions: what differences exist in the IBN of subjects with BD 1 compared with control subjects? And aims to clarify the following hypotheses: that functional connectivity between IBN incorporating prefrontal cortical regions (such as the ECN and DMN) and paralimbic brain regions will be decreased in BD subjects compared with healthy subjects, and that there will be decreased functional connectivity between the CERN and regions involved in cognitive and emotional processing.

As uncertainty remains surrounding the underlying neurobiology of BD and IBN analysis has proven valuable in understanding other psychiatric disorders, this project has the potential to uncover valuable insights. Particularly, as this is a secondary analysis, a chief benefit will be the use of the results for hypothesis generation to assist with future, larger studies specifically designed to address the issues of interest. In addition, IBN research is a relatively new field in South Africa, so this project has the additional objective of expanding knowledge, experience and interest in this exciting area.

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## Chapter 2: Publication-ready manuscript<sup>4</sup>

### Effects of Bipolar Disorder on Intrinsic Brain Networks

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### 2.1. INTRODUCTION

#### *Bipolar disorder and its neurobiological underpinnings*

Bipolar disorder (BD) is a common mood disorder marked by episodes of mania and depression, with interspersed euthymia. It is diagnosed clinically, with the defining psychopathological feature being mania. Apart from disturbances in mood, neuropsychological abnormalities occur, including impaired attention, executive dysfunction, affective dysregulation, and impulsivity/disinhibition which may persist during euthymia (Delaloye et al., 2009; Green et al., 2007; Goldberg and Chengappa, 2009; Kurtz and Gerraty, 2010; Robinson et al., 2006; Savitz et al., 2005; Strakowski et al., 2005; Torres et al., 2007).

Functional Magnetic Resonance Imaging (fMRI), has been particularly valuable for investigating the neurobiology of BD, revealing changes in brain activity in areas responsible for cognitive and emotional regulation, including the prefrontal cortex (PFC), orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), precuneus and cerebellum, including in the euthymic state (Chen et al., 2011; Drevets et al., 2008; Foland et al., 2008; Green et al., 2007; Houenou et al., 2011; Strakowski et al., 2005). In addition, functional imaging has highlighted reduced PFC control over the amygdala and other limbic structures (Chen et al., 2011; Foland et al. 2008). Overall, the literature indicates that while multiple cortical and subcortical areas are involved, BD is a problem of dysfunctional connections and interactions between these areas (Blumberg et al., 2003; Cerullo et al., 2009; Fountoulakis et al., 2008;

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<sup>4</sup> Format as required for submission to Journal of Affective Disorders, see appendix 12, page 51

Green et al., 2007; Strakowski et al., 2005). As a means to explain this, Diffusion Tensor Imaging (DTI) studies show reduced fractional anisotropy (a measure of white matter tract integrity) in multiple tracts in BD, suggesting a plausible mechanism for structural dysconnectivity across the brain (Nortje et al., 2013). More relevant in the context of our study, however, are the insights emerging from intrinsic brain network research.

### *fMRI and intrinsic brain networks*<sup>5</sup>

Understanding functional *relationships* in the brain is particularly relevant for psychiatry. Using fMRI, intrinsic brain networks have been identified comprising spatially distributed areas that correspond temporally in their activity, and mirror established functional and anatomical relationships (Beckmann et al., 2005; Biswal et al., 1995; Damoiseaux et al., 2006; Fox and Raichle, 2007). These networks are identified using various methods, with Independent Components Analysis (ICA) recognized as a robust, objective approach (Beckmann et al. 2005; Cole et al., 2010). Though initially identified in the “resting state” (awake but task-free), it is clear that intrinsic network activity persists during tasks or stimuli (Smith et al. 2009). We use the term *intrinsic brain networks* (IBN) to refer to established networks described in the literature, and *functional connectivity* to refer in a general way to functional relationships across the brain, even when not within a known network. We also use *network integrity* (NI) to indicate how cohesive a network is, or to what extent a voxel or cluster coheres to the activity of a network. As such, NI is a variable *measure of functional connectivity*.

The ‘Executive Control network’ (ECN – incorporating ACC and dorsolateral PFC and sub serving executive functioning) and the ‘Default Mode Network’ (DMN – incorporating the precuneus, posterior cingulate and medial PFC and mediating episodic silent thought, self-awareness and aspects of consciousness) (Damoiseaux et al., 2006), are frequently studied, and include anatomical areas that are dysfunctional in BD. A cerebellar network (CERN) is also consistently identified, and

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<sup>5</sup> PLEASE NOTE: The generic theoretical overview of fMRI and intrinsic brain networks presented here is similar to that described in the MSc thesis titled “Modulation of Resting State Networks by Fasting and Ghrelin: An fMRI study” submitted by Dr Jonathan Starke (myself) to Roehampton University in 2011 (unpublished but available in university library).

is significant given the cerebellum's role in cognitive and emotional regulation (Bellebaum and Daum, 2007; Koziol et al., 2012; Schmahmann, 2004; Strick et al., 2009), and research identifying cerebellar abnormalities in BD (Strakowski et al., 2005; Phillips et al., 2015). Analysing the fluctuating integrity, spatial extent and activation time-courses of IBN, and their dynamic inter-relationships gives key insights into the neurobiology of behaviour and psychiatric illness, including BD (Cole et al., 2010, Fox and Raichle, 2007; Mamah et al., 2013; Meda et al., 2012).

#### *Functional connectivity in BD*

IBN research in BD reveals increased connectivity within the ECN in the dorsolateral PFC (DLPFC) and ACC, and decreased connectivity within the DMN in the precuneus and posterior cingulate (Calhoun et al., 2012). There is also neuronal hyperactivity within the DMN in the left medial frontal gyrus and left inferior parietal lobe in BD depression, which could be a neuroimaging biomarker of bipolar depression (Liu et al., 2012). Conversely, in manic subjects, DMN connectivity is reduced in the ventromedial PFC (Öngür et al., 2010).

There are also functional connectivity changes *between* different areas and networks, including reduced connectivity between the DMN and temporal limbic structures (Calhoun et al., 2012), reduced connectivity between the CERN and networks incorporating the cingulate, anterior insula and anterior PFC (Mamah et al., 2013), and a positive correlation in activity between the medial PFC (mPFC) and ventrolateral PFC (VLPFC), as opposed to their usual antecorrelation (Chai et al. (2011). These changes appear to have clinical relevance (Mamah et al., 2013), which is understandable given the role of these regions in cognitive-emotional functioning. Further evidence of the value of this imaging approach comes from research in a heterogeneous BD group (including manic, depressed and euthymic subjects) showing increased connectivity between fronto-temporal and paralimbic networks involved in cognitive-emotional processing, a pattern allowing BD subjects to be distinguished from control subjects and those with schizophrenia (Meda et al., 2012).

Importantly, given persisting neuropsychological abnormalities when BD subjects are euthymic (Torres et al., 2007), abnormal functional connectivity remains

evident in euthymia. Vargas et al. (2014) found increased connectivity within the DMN in the right hippocampus and posterior cingulate, while (Lois et al., 2014) report increased connectivity between a meso/paralimbic network and the right frontoparietal network, and Torrisi et al. (2013) found resting-state hyperconnectivity between the right amygdala and right VLPFC, which was mediated by ACC activity. These findings may represent trait-related biomarkers of BD and point to the possibility of functional neuroimaging as a diagnostic tool (Phillips and Vieta, 2007; Torrisi et al., 2013).

Nevertheless, in their systematic review of the resting-state fMRI literature in BD, which summarised research using a variety of methodologies including ICA-based IBN analysis, Vargas et al. (2013) noted that while there was evidence for theories of BD that implicate dysconnectivity between frontal cortical areas (such as the PFC and ACC) and limbic areas (such as the amygdala), there was considerable heterogeneity of findings, making it difficult to come to any final conclusions. We therefore compared euthymic BD 1 subjects with controls to clarify IBN differences, specifically looking for functional connectivity changes between IBN incorporating prefrontal/cortical regions (such as the ECN and DMN) and paralimbic brain regions, and between the CERN and regions involved in cognitive and emotional processing, with the goal of confirming the presence and nature of trait-related functional neuroimaging biomarkers of BD 1.

## **2.2. METHODS<sup>6</sup>**

*Study sample:* 15 euthymic subjects with Bipolar 1 Disorder (13 females, age range 18 – 49yrs) and 10 control subjects (6 Females, age range 18 – 50yrs) were recruited as part of a larger study (Ives-Deliperi et al., 2013). For BD subjects, the diagnosis of BD 1 was confirmed using the Structured Clinical Interview for DSM-IV Disorders (First et al., 1996), and current euthymia (mild or sub-threshold symptoms only) was determined by Young Mania Rating Scale (YMRS – Young et al., 1978) and Hospital Anxiety and Depression Scale (HADS – Zigmond and Snaith 1983) scores of < 14 at the time of scanning.

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<sup>6</sup> See appendix 1, page 33 for a more detailed explanation of the theory of this methodology.

*Scanning procedure:* As part of a larger scan protocol which included structural MRI, task-related fMRI, and diffusion tensor imaging, all 25 subjects underwent a 6-minute ‘task-free’ period of fMRI scanning in which they were instructed to “*open awareness to the breath and present-moment bodily sensations, thoughts and emotions without judging or reacting to these mental and physical events*”. The functional scanning parameters were as follows: using a Siemens 3T Allegra MRI scanner, T2-weighted gradient echo, echo planar imaging sequence (TR = 2000 ms, TE = 30 ms, 34 interleaved slices, 3 mm thick, gap 0.9 mm, matrix size 64 x 64, resolution 3.125 x 3.125 x 3 mm<sup>3</sup>).

*Image pre-processing* (FMRIB, 2012; Jenkinson et al., 2012): Initial pre-processing was done within the Multivariate Exploratory Linear Decomposition into Independent Components (MELODIC) platform from the Functional Magnetic Imaging of the Brain Software Library (FSL) (Beckmann and Smith, 2004). Steps comprised: motion correction with MCFLIRT; brain extraction with BET; spatial smoothing at 6mm FWHM; intensity normalisation; and registration from functional space directly to a standard space brain (MNI152\_T1\_2mm\_brain) – as appropriate high resolution individual structural scans were not available for all subjects; resampling resolution 4mm. Further identification and removal of motion related artefacts was performed using an ICA-based Automatic Removal of Motion Artefacts (ICA-AROMA) approach (Pruim et al. 2015), which included high-pass temporal filtering (100s cut-off). At this point, one BD subject was removed due to excessive head motion (mean absolute displacement = 2.06mm), leaving 14 subjects for further analysis.

*Image analysis procedure* (FMRIB, 2012; Jenkinson et al., 2012): We evaluated differences between BD subjects and controls in 3 IBN (DMN, ECN, and CERN) using an FSL pipeline: Dual-regression (using standard ICA-derived IBN templates from the literature – Beckmann et al., 2009; Smith et al., 2009) to identify subject-specific activation time courses and spatial maps containing network integrity (NI) values for each voxel, for each of the 3 networks; followed by Randomise (Hayasaka and Nichols, 2003; Nichols and Holmes, 2002; Winkler et al., 2014) non-parametric

permutation testing to identify brain areas with different network integrity in BD subjects compared with controls for each of the 3 networks (5000 permutations with de-meaning, MNI152\_T1\_2mm\_brain.nii.gz used as mask, output in the form of tstat images). Post-hoc local False Discovery Rate (FDR – Genovese et al., 2002) correction for multiple comparisons was then performed within MELODIC on the tstat images derived from Randomise (mmthresh=0.05). Significant difference clusters were initially identified as those containing voxels with  $p < 0.05$  in the probability map output from FDR. Subsequently, further Bonferroni correction was performed to account for comparisons having been done for 3 networks, with  $p = 0.017$  regarded as the final threshold for significance. Anatomical areas were evaluated in accordance with the Harvard-Oxford Cortical and Subcortical Structural Atlases in fsview.

*Ethics*<sup>7</sup>: All subjects signed informed consent for the original study, and all data was handled confidentially. Approval for the original study was granted by the Human Research Ethics Committee at the University of Cape Town (HREC Ref 078/2009). Additional approval was given for this post-hoc data analysis (HREC Ref 219/2014). No further patient contact or additional data collection was necessary as part of the new study.

### **2.3. RESULTS**

For brain regions *within* the spatial extent of the 3 IBN analysed, there were no differences in NI. However, for the ECN and CERN, 3 significant clusters, which lay *outside* the spatial extent of the original networks, did show differences in functional connectivity in BD subjects compared to controls (Figures 1 – 3). For the ECN, there was decreased connectivity between the network as a whole and a cluster in the left OFC (Figure 1), and increased connectivity between the network and a cluster in the left temporal pole (Figure 2). For the CERN, there was decreased connectivity between the network as a whole and a cluster in the right precuneus (Figure 3). See Table 1 for further details of these clusters. There were no significant findings

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<sup>7</sup> Please appendices 2, 3, and 9 for details of information and consent forms and ethics approval

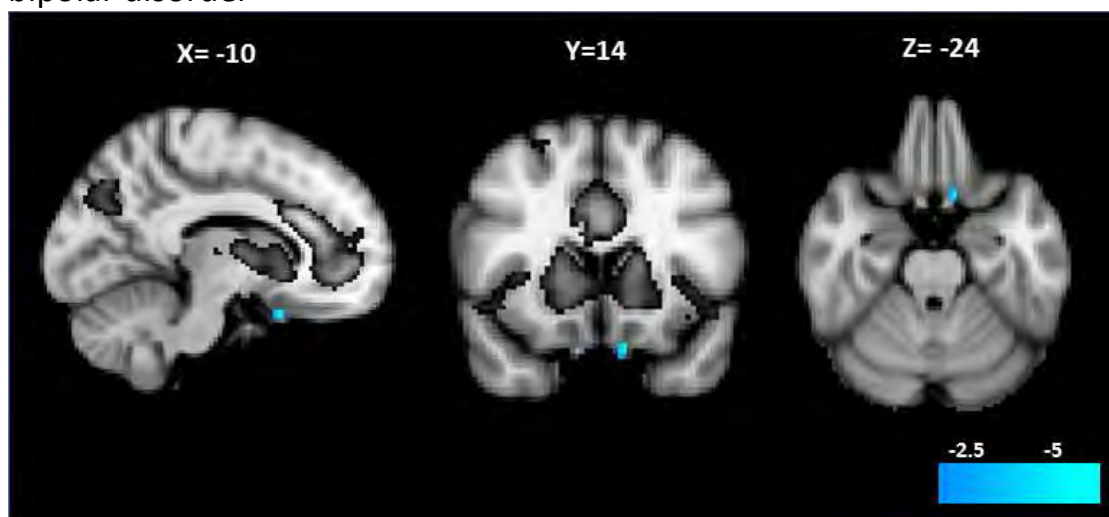
involving the DMN. The voxels showing significant change survived FDR correction for multiple comparisons across the brain ( $p < 0.05$ ). After additional Bonferroni correction of the p-value to account for comparisons involving 3 networks, the peaks within each of the clusters remained significant ( $p < 0.017$ ).

**Table 1:** Clusters showing functional connectivity differences in BD

Details of cluster	Peak Voxel Co- ordinates X, Y, Z (mm MNI Space)	Mean zstat of voxels within clusters	Size of cluster (No. of 2x2mm voxels)	
			FDR correction only ( $p < 0.05$ )	FDR and Bonferroni correction ( $p < 0.017$ )
<b>Decreased</b> connectivity btw. <b>ECN and left OFC</b>	-10, 14, -24	- 5.62	14	6
<b>Increased</b> connectivity btw. <b>ECN and left temporal Pole</b>	-26, 4, -32	+ 4.95	14	6
<b>Decreased</b> connectivity btw. <b>CERN and right precuneus</b>	12, -42, 46	- 5.13	45	18

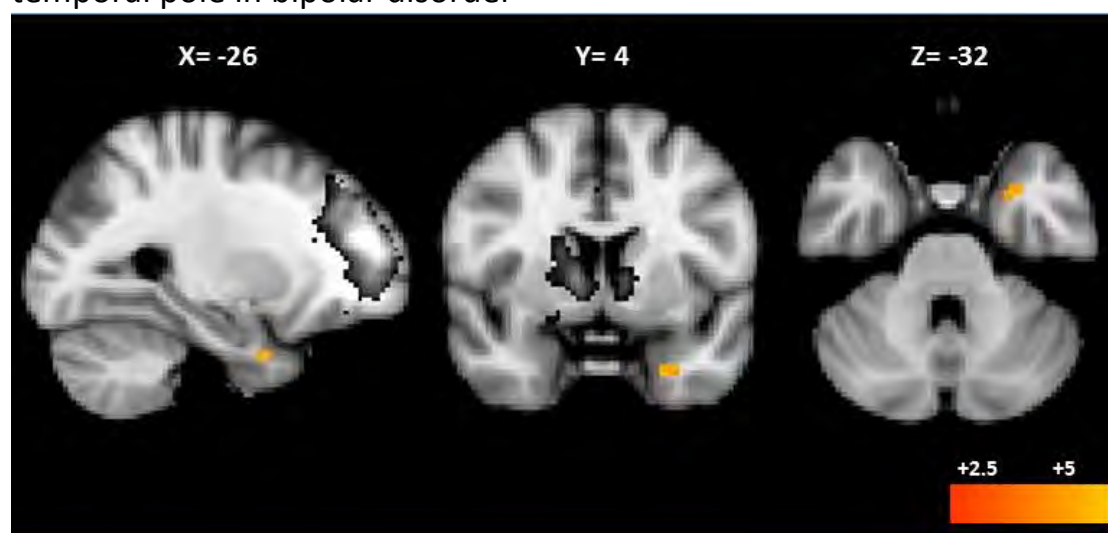
BD = bipolar disorder; CERN = Cerebellar network; ECN = executive control network, FDR = false discovery rate; MNI = Montreal Neurological Institute

**Figure 1:** Decreased functional connectivity between ECN and left OFC in bipolar disorder



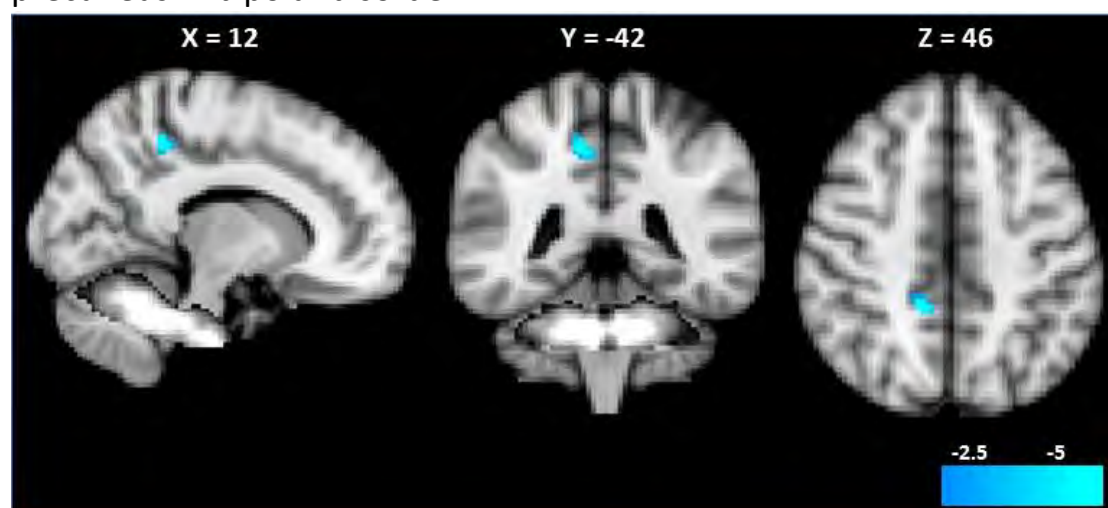
The ECN template (zstat image) shown in greyscale. The area of **decreased connectivity between the ECN and left OFC is in blue**. The cluster is a zstat image derived from FDR correction of randomised-based comparison. Colour bar shows zstat range. All voxels in the cluster are significant at the  $p < .05$  level using FDR. Images in radiological orientation. Background is MNI152 2mm brain, co-ordinates in mm of MNI space.

**Figure 2:** Increased functional connectivity between ECN and left temporal pole in bipolar disorder



The ECN template (zstat image) shown in greyscale. The area of **increased connectivity between the ECN and left temporal pole is in orange-yellow**. The cluster is a zstat image derived from FDR correction of randomise-based comparison. Colour bar shows zstat range. All voxels in the cluster are significant at the  $p < .05$  level using FDR. Images in radiological orientation. Background is MNI152 2mm brain, co-ordinates in mm of MNI space.

**Figure 3:** Decreased functional connectivity between CERN and right precuneus in bipolar disorder



The CERN template (zstat image) is shown in greyscale. The area of **decreased connectivity between the CERN and right precuneus is in blue**. The cluster is a zstat image derived from FDR correction of randomise-based comparison. Colourbar shows zstat range. All voxels in the cluster are significant at the  $p < .05$  level using FDR. Images in radiological orientation. Background is MNI152 2mm brain, co-ordinates in mm of MNI space.

## 2.4. DISCUSSION

The analysis of functional connectivity has already provided valuable insights into the neurobiology of BD (Mamah et al., 2013; Meda et al., 2012; Vargas et al., 2014). Our exploration of network changes in a group of euthymic BD 1 subjects adds novel findings to this existing research. Using a whole brain analysis, we show significantly decreased connectivity between the ECN and the left OFC, increased connectivity between the ECN and left temporal pole (TP), and decreased connectivity between the CERN and the right precuneus.

The ECN incorporates brain regions responsible for executive functioning (including planning, problem solving and response inhibition – Damoiseaux et al., 2006; Smith et al., 2009), while the OFC – which is often considered part of the ‘extended’ limbic system because of its extensive connections with the amygdala and cingulate – is responsible for integrating and regulating emotional information and responses, as well as salience processing (Altshuler et al., 2008; Torrisi et al., 2013). Given that emotional dysregulation is a frequent clinical feature of BD (Green et al., 2007), our finding of significantly decreased functional connectivity between the ECN and left OFC may help to explain the clinical picture by demonstrating a possible mechanism for impaired executive control of the limbic system. There is existing evidence for decreased OFC activity in BD (Altshuler et al. 2008; Strakowski et al., 2005), as well disruption in connectivity between the OFC and amygdala (Kanske et al., 2015), though as this research was task-based, it is not directly comparable with our own.

Though other reports of dysconnectivity between the ECN and OFC in euthymic BD subjects could not be found, increased functional connectivity between fronto-temporal and other paralimbic regions has been reported (Lois et al. 2014), as well hyperconnectivity between the right amygdala and right VLPFC (Torrisi et al., 2013 ). The fact that our findings differ from these other researchers is likely the result of differences in methodological approach, with Lois et al. 2014 focusing on inter-network changes as opposed to the whole-brain IBN-based approach that we used. Torrisi et al. (2013) on the other hand, based their analysis on predefined anatomical nodes, rather than ICA-derived IBN. It is also important to note that the

area of the OFC in which we found significant change is recognised as difficult to image accurately with fMRI due to 2 important confounding factors, firstly its proximity to the sphenoid sinus which can result in magnetic field inhomogeneities and signal dropout (Weiskopf et al., 2007), and secondly its proximity to the edge of the brain which increases the likelihood of motion-related artefact. While our established pre-processing approach, in particular our use of ICA-AROMA to address issues related to motion (Pruim et al., 2015), was an attempt to control for these issues, this finding should nevertheless be treated as preliminary, pending more extensive confirmation.

Though its precise functions are still the subject of some debate (Pascual et al., 2013), the temporal pole may have a role in emotional processing, specifically in social-emotional cognition, as a result of its dense connections to structures such as the amygdala, hippocampus, PFC, and fusiform gyrus (Wong and Gallate, 2012). There is also evidence that damage to the TP can manifest with mood dysregulation in a pattern equivalent to rapid cycling bipolar disorder, suggesting it may have a role in the underlying neurobiology of the condition (Murai and Fujimoto, 2003). Functional connectivity changes specifically involving the temporal pole in euthymic BD subjects have not previously been described in the literature, though in their meta-analysis of fMRI changes in BD, Chen et al., (2011) did find increased activation in the superior temporal gyrus. In addition, Mikawa et al., (2015), using a near-infrared spectroscopy approach in a task-based paradigm, reported significantly smaller increases in oxy-haemoglobin in bilateral temporal areas in BD. These studies are not directly comparable with our own due to significant differences in methodology, but they do indicate the likelihood of temporal abnormalities in BD. Furthermore, in their detailed resting-state functional connectivity analysis of the temporal pole in normal subjects, Pascual et al., (2013) used an anatomical seed closely approximating our own significant cluster in MNI space to show extensive connectivity between the TP and limbic structures including the amygdala, OFC, nucleus accumbens and cingulate cortex. This highlights the potential relevance of our findings, as increased connectivity between the ECN and TP, and thus the wider limbic system, may help to explain altered socioaffective expression in BD, such as the 'pro-social' behaviour which may occur, particularly in the manic state.

Due to the relative lack of functional connectivity research in euthymic bipolar 1 subjects, differences in study samples and methodological approaches used, and discrepancies amongst results that have been reported, it is difficult to come to any final conclusions. Nevertheless, our findings of altered connectivity between the ECN, left OFC and left TP appear consistent with models of BD which propose that a disruption in the relationship between executive areas (such as the prefrontal cortex) and limbic areas (including the temporal lobes and OFC) may underlie the clinical picture of BD (Chen et al., 2011; Foland et al., 2008; Green et al., 2007; Strakowski et al., 2005) .

Our finding of decreased connectivity between the CERN and the right precuneus was our most robust result in terms of the size of the significant cluster. The cerebellum has a key role in prediction and error detection (Bellebaum and Daum, 2007), and while this has usually been appreciated in the context of motor movement, increasing evidence suggests it plays a similar role in modulating executive functioning and emotional processes (Bellebaum and Daum, 2007; Koziol et al., 2012; Schmahmann, 2004). The precuneus, on the other hand, is densely connected to the rest of the cortex and functions as a hub for cognitive integration (Cavanna, 2006). In their meta-analysis of functional imaging research related to emotional processing in BD, Houenou et al., (2011) identified reduced activation in both the right cerebellum and right precuneus in euthymic BD subjects, while in a similar meta-analysis, Chen et al., (2011) found areas of both increased and decreased cerebellar activation in BD, though this was not specific to euthymic subjects. While these other findings were not based on functional connectivity analysis, they do indicate that the cerebellum and precuneus are likely important in the neurobiology of BD. Using a functional connectivity approach in normal subjects, others have demonstrated the contribution of the cerebellum to widely distributed IBN, including those encompassing the precuneus (Habas et al., 2009). Given the wide connectivity of both the cerebellum and precuneus with the rest of the brain (including multiple areas involved in cognitive and emotional processing), decreased connectivity between the CERN and the precuneus in BD may be important in explaining the diverse clinical picture. However, as this is a novel finding, additional research is needed to confirm and clarify its significance.

The fact that we did not find changes involving the DMN differs from what has been reported by others (Calhoun et al., 2012; Vargas et al., 2014). While Calhoun et al. (2012) used a task-based approach in a group of subjects who were not uniformly euthymic – making direct comparison with our results problematic – Vargas et al. (2014), used a very similar methodological approach to our own, though not the same ICBN templates, which may in part explain the discrepancy. More importantly though, differing findings between similar studies may indicate that the true picture is not yet certain, and that further work is needed for clarity.

Our study had limitations. As with similar studies, our sample size was small, which increases the likelihood for false positive findings. Nevertheless, our ICA-based analysis approach is well established for robustly identifying functional connectivity changes (Beckmann and Smith, 2004), and our use of ICA-AROMA to further account for motion artefact, represents an advancement over prior methodologies (Pruim et al., 2015). In addition our significant clusters, while small, survived comprehensive correction for multiple comparisons both across the brain (Genovese et al., 2002), and the 3 networks we explored. Finally, our study sample comprised a clearly defined group of euthymic bipolar 1 subjects, which adds a specificity to our findings that is absent from research on heterogeneous samples.

Taken together, our findings point to altered functional connectivity between networks and regions involved in cognitive/emotional processes, specifically dysconnectivity between cortical control and extended limbic system structures, and between centres responsible for prediction/error-detection and cognitive integration. This is valuable in shedding further light on the neurobiology of BD, as well as helping to explain the clinical picture. Our findings confirm other research, as well as supporting current theoretical models of BD, thus re-enforcing the value of brain network analysis as a tool in psychiatric research. It is important to note that similar functional connectivity changes in cortical and subcortical areas and networks have been reported in other psychiatric disorders, particularly schizophrenia (Mamah et al., 2013; Meda et al., 2012). This is consistent with the more generalised overlap in genetic and neurobiological features of BD and schizophrenia, which is currently the focus of intensive research (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013; Redpath et al., 2013). While there do

appear to be functional connectivity differences between the 2 disorders (Mamah et al., 2013; Meda et al., 2012), the extent to which our findings represent changes unique to BD remains to be confirmed, as we have not made comparisons with a schizophrenia sample. As such, our findings should be treated as preliminary, but as data accumulates and this approach is refined and standardised, reliable functional neuroimaging biomarkers specific to BD will be increasingly established, and the possibility of functional connectivity analysis as an adjunct to clinical assessment in psychiatric diagnosis will be brought ever closer (Phillips and Vieta, 2007). Future research with larger datasets will clarify the nature and extent of network changes within frontal executive networks, limbic networks, and cerebellar networks, as well as any changes in the dynamic relationships that exist between them, and how these findings relate to clinical measures.

## 2.5. REFERENCES

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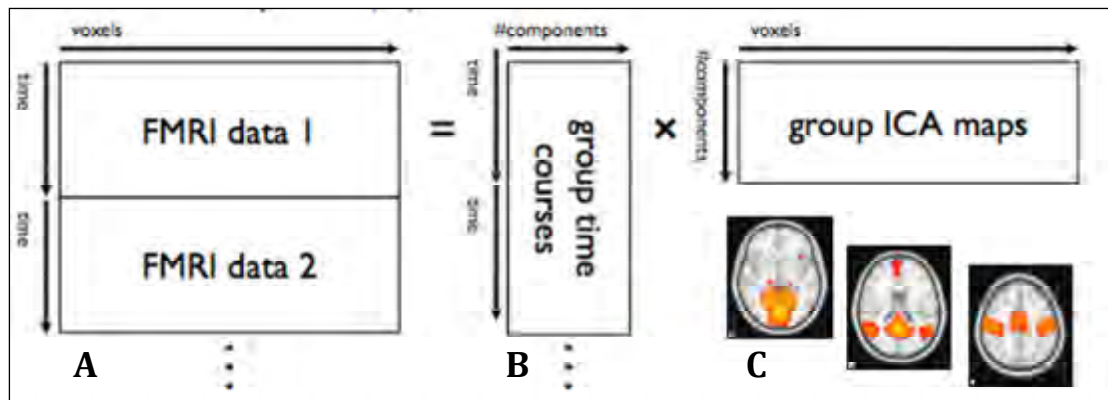
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## Technical appendix: Theoretical aspects of Methodology

### 1. Independent Components Analysis (ICA) (Beckmann and Smith 2004; Beckmann et al. 2009; Cole, Smith and Beckmann 2010; FMRIB: Brainmaps and RSNs; Smith et al. 2009)

ICA is a computational process whereby a signal (data) can be broken up and separated out into the multiple subcomponents which have combined to produce it. ICA identifies maximally independent components of the total signal, so they can then be used for further analysis. In addition, components which represent 'noise' within the data can be accounted for to minimize the confounding effect of noise on later analysis – this is of particular value in fMRI which is vulnerable to several sources of noise, especially physiological events (like the breath cycle and pulse beat) and head motion. ICA has proven especially useful in task-free fMRI as a means to identify and characterize the intrinsic brain networks (IBN) which exist amongst the components identified by ICA. The IBN templates we used as the starting point for our analysis were derived using ICA using the following process.



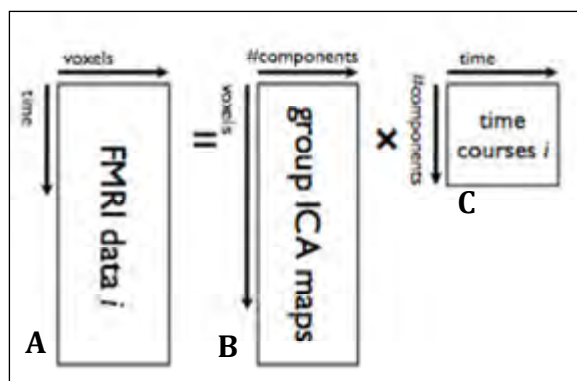
**1st:** Individual data for each participant - comprising the BOLD signal (activity) measured in each **voxel** at each point in **time** - is concatenated (joined into one large data set) = **A**

**2nd:** ICA splits the total data for the group into a number of maximally **independent** sub-components (including IBN), presenting these in two ways for the **group as a whole**:

- As a list of **time courses [B]** for each component (i.e. what the *group-level* activity is within each component at each point in time) - used directly in later analysis.
- As a collection of group-level **spatial maps [C]** showing the voxels comprising each component. **IBN templates are then identified from amongst these and fed into Dual Regression.** (Image from Beckmann et al. 2009)

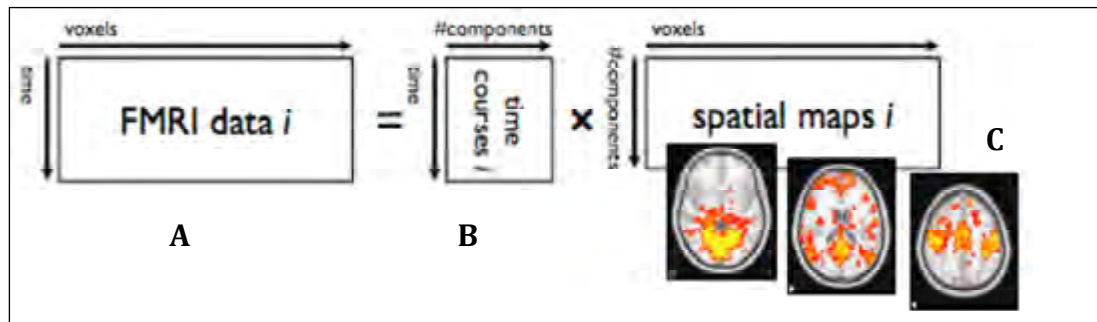
### 2. Dual Regression (Beckmann et al. 2009; Cole, Smith and Beckmann 2010; FMRIB 2012; Zuo et al 2010)

The group spatial maps for the IBN identified using ICA are then individualized for each subject in a two-stage process call **Dual Regression**. The data derived in this way forms the basis for comparisons between different scanning sessions in the same subject, or between different groups of subjects.



**1st regression:** each participant's data [A] is "probed" with the **group-level spatial maps [B]** derived from ICA (in our case standard network templates) to extract **individual time courses [C]** for each component (i.e. what the activity level is at each point in time in each individual **within each of the network templates**)

(Image from Beckmann et al. 2009)



**2nd regression:** each participant's data [A] is again “probed”, this time using the **time courses** [B] generated in the 1st regression to produce **individualised spatial maps** [C] for each of the network templates. As a result, each voxel is assigned a value which reflects the strength of its association with the network as a whole (i.e. how closely the activity in each voxel mirrors the activity within the individualised spatial map as a whole). This value is the **Network integrity**, which is then used for comparisons between subjects and groups in **Randomise**. (Image from Beckmann et al. 2009)

### 3. Randomise (Hayasaka and Nichols 2003; Nichols and Holmes 2001; Smith 2004; Winkler et al. 2014)

The images generated by fMRI, which are used for comparison between different conditions, groups and subjects are prepared for further analysis by converting them into statistical maps of the brain. Each voxel within these maps is assigned a value which quantifies the experimental effect at that location. In **task-based fMRI**, the timing of repeated task/stimulus ‘blocks’ is precisely controlled. BOLD activity during the periods *in-between* tasks represents the ‘null’ condition, and this can be compared with the BOLD response during task blocks. The degree of statistical significance of the association between the task/stimulus and the BOLD response in comparison to what is happening during the null condition can then be determined. If there is statistical evidence of a sufficient relationship, it can be concluded that the task is causing this response, and the null hypothesis (that there is no difference or effect as a result of the task) can be rejected.

**In task-free/ ‘resting-state’ analysis, this approach cannot be used** as there is no task-related timing sequence and no null condition against which the BOLD response can be compared. The ICA and Dual Regression steps already described are the first part of handling this difficulty – by separating valuable signal from noise within the BOLD data, and generating subject-specific data for comparison. Randomise multiple permutation testing is the final step. The basis of testing statistical significance with randomisation is the generation of a hypothetical null distribution for the data (i.e. how the data would look if the null hypothesis was true and there was no effect of the experimental conditions). **Randomise achieves this by first assuming that the null hypothesis is true, and that there is no significant effect.** If this were the case, then the arrangement or separation of data points between groups would be arbitrary, as whatever the distribution, the result would be the same, i.e. no significant effect. It then runs multiple permutations of the data along these lines by randomly switching the arrangement of data points, and by repeating this switching multiple times, a **null distribution of the data emerges**. The **actual distribution** of the data is then compared with this null distribution using a **General Linear Model**, which specifies the comparisons of interest, and if there is a significant difference, then the null hypothesis can be rejected and it can be concluded that there is an effect of the experimental conditions. **This effect is quantified as a statistical value (tstat)** assigned to each voxel in the output images.

In addition, **Randomise has a built-in method for controlling for multiple comparisons** – an important problem which can result in false positive results in fMRI research given the large number of voxels and hence the large number of comparisons involved in any analysis. The final output of Randomise therefore includes **family-wise error (FWE) corrected statistical images** containing p-values at each voxel, which can then be used to determine the final significance of the findings.

### 4. False Discovery Rate (Genovese, Lazar and Nichols 2002)

While the FWE correction performed by Randomise as just described is robust and widely used, it has been argued that this approach may in fact be *too* stringent, and may result in false negative findings. The False Discovery Rate (FDR) method is increasingly used as an alternative, especially when the

effect size is likely to be small. Instead of controlling for the chance of error in every comparison (whether initially significant or not), FDR only controls the rate of false 'discoveries' (i.e. sets an adjustable limit on the likelihood of false findings) for the much smaller number of seemingly significant findings (discoveries) from the initial comparison. As a result, **FDR has increased power to identify smaller effects while still controlling for multiple comparisons across the brain.** In this project, FDR was performed within the MELODIC platform (second usage option) as a post-hoc analysis step on the uncorrected tstat images produced by randomize. It outputs zstat images and probability map images, which can then be used to determine the final significance of the results, depending on the pre-determined significance threshold.

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24 March 2009

REC REF: 078/2009

Dr N Horn  
Psychiatry

Dear Dr Horn

**PROJECT TITLE: NEUROIMAGING THE EFFECTS OF MINDFULNESS TRAINING IN BIPOLAR DISORDER.**

Thank you for submitting your study to the Research Ethics Committee for review.

It is a pleasure to inform you that the Ethics Committee has **formally approved** the above-mentioned study.


**Approval is granted for one year till the 30<sup>th</sup> March 2010.**

Please submit an annual progress report if the research continues beyond the expiry date. Please submit a brief summary of findings if you complete the study within the approval period so that we can close our file.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

**Please quote the REC. REF in all your correspondence.**

Yours sincerely

  
**PROFESSOR M BLOCKMAN**  
**CHAIRPERSON, HSE HUMAN ETHICS**

Federal Wide Assurance Number: FWA00001637.  
Institutional Review Board (IRB) number: IRB00001938



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Website: [www.health.uct.ac.za/research/humanethics/forms](http://www.health.uct.ac.za/research/humanethics/forms)

14 April 2014

**HREC REF: 219/2014**

**Dr N Horn**  
Psychiatry  
Education Centre  
Valkenberg Hospital

Dear Dr Horn

**PROJECT TITLE: EFFECTS OF MBCT ON INTRINSIC BRAIN NETWORKS IN BIPOLAR DISORDER PATIENTS (MMED - Dr J Starke)**

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

**Approval is granted for one year until the 30<sup>th</sup> April 2015**

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

(Forms can be found on our website: [www.health.uct.ac.za/research/humanethics/forms](http://www.health.uct.ac.za/research/humanethics/forms))

***We acknowledge that the MMED student, Dr Jonathan Starke is also involved in this study.***

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC reference no in all your correspondence.

Yours sincerely

**PROFESSOR M BLOCKMAN**  
**CHAIRPERSON, FHS HUMAN ETHICS**

Federal Wide Assurance Number: FWA00001637.

Institutional Review Board (IRB) number: IRB00001938

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

HREC 219/2014

The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Gmail

**Starke : Confirmation of Approval of Study Proposal**

Jackie Cogill &lt;jackie.cogill@uct.ac.za&gt;

16 July 2014 at 14:18

To: "joestärke@gmail.com" &lt;joestärke@gmail.com&gt;

Cc: Neil Horn &lt;neil.horn@uct.ac.za&gt;, Lorraine McDonald &lt;lorraine.mcdonald@uct.ac.za&gt;

Dear Dr Starke

**Candidature Approval (STRJON008)**

Degree	MMed in Psychiatry
Title	Effects of MBCT on intrinsic brain networks in bipolar disorder patients
Department	Psychiatry & Mental Health
Supervisor	Dr N Horn
Ethics Approval	219/2014

I am pleased to advise that the Chair of the Dissertations/Doctoral & Masters Committee has approved your candidature for the above degree on behalf of the Committee. Formal approval was obtained by publication in the Dean's Circular, PG-Med May2014.

Yours sincerely

Jackie Cogill

**UNIVERSITY OF CAPE TOWN**

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HUMAN RESEARCH  
ETHICS COMMITTEE



UNIVERSITY OF CAPE TOWN - 7 SEP 2015

FACULTY OF HEALTH SCIENCES  
Human Research Ethics Committee



HEALTH SCIENCES FACULTY

**FHS016: Annual Progress Report / Renewal**

<b>HREC office use only (FWA00001837; IRB00001938)</b>			
This serves as notification of annual approval, including any documentation described below.			
<input checked="" type="checkbox"/> Approved	Annual progress report	Approved until/next renewal date	30.4.2016
<input type="checkbox"/> Not approved	See attached comments		
Signature Chairperson of the HREC		Date Signed	8/9/2015

Comments to PI from the HREC
See attached letter advising of change of title and scope of study as it related to Dr Starkes MMed mini-thesis

Principal Investigator to complete the following:

**1. Protocol Information**

Date (when submitting this form)	7.09.2015		
HREC REF Number	219/2014	Current Ethics Approval was granted until	30.04.2015
Protocol title	Effects of MBCT on intrinsic brain networks in Bipolar Disorder		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	NO		
If yes, could you please provide the HREC Ref's for all sub-studies? Note: A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Dr Neil Horn		
Department / Office Internal Mail Address	Psychiatry, Education Centre, Valkenberg Hospital doctor.neil.horn@gmail.com		

1.1 Does this protocol receive US Federal funding?	NO	
1.2 If the study receives US Federal Funding, does the annual report require full committee approval?	NA	
1.3 Has sponsorship of this study changed? If yes, please attach a revised summary of the budget.	NA	



## Department of Psychiatry and Mental Health

Professor **Dan J Stein, FRCPC, PhD, DPhil**

Head, Department of Psychiatry and Mental Health, UCT & GSH

Email: [dgs.stein@uct.ac.za](mailto:dgs.stein@uct.ac.za)

UCT Dept of Psychiatry and Mental Health, Groote Schuur Hospital (J-2), Anzio Rd.

Observatory 7925, Cape Town, South Africa

Phone: +27 21 4042137 Fax: +27 21 4488158

URL: <http://www.health.uct.ac.za/departments/psychiatry/about/>

05.09.2015

**The Chair, Human Research Ethics Committee  
Faculty of Health Sciences  
University of Cape Town**

Re: Update and Minor changes (HREC: 219/2014)

Thank you for considering the following with regards to our this study which specifically relates to an MMed:

- The project consists of a post-hoc analysis of an existing fMRI database. It does not involve any new data collection and involves no patient contact.
- The original research protocol included two components to data analysis. Firstly, a comparison of differences in intrinsic brain networks between patients with bipolar disorder and control subjects was to be made (baseline comparison). Secondly, the effects of a Mindfulness-Based Cognitive Therapy (MBCT) intervention on intrinsic brain networks in the bipolar patients was going to be performed (pre- vs. post comparison). The scope of this original protocol was significantly greater than that usually required for a minor MMed dissertation, and so a decision has been made to limit the scope to allow Dr Starke to complete the project in the available time, while ensuring that the project is still sufficiently complex to meet the requirements of a minor dissertation, which it still exceeds. As such, the project will now consist **only of the first comparison**.
- In light of the above, the original title for the project: "Effects of MBCT on intrinsic brain networks in bipolar disorder patients" will be changed to **"Effects of bipolar disorder on intrinsic brain networks"**.

Please see attached our amended protocol incorporating these changes. Please advise if the proposed changes to our project are acceptable, so that we can continue with our research.

A D9 form will be submitted to the Faculty of Health Sciences.

Sincerely

Dr Neil Horn (PI)



UNIVERSITY OF CAPE TOWN  
UNIBESITHI YASEKAPA - UNIVERSITEIT VAN KAAPSTAD

HUMAN RESEARCH  
ETHICS COMMITTEE

20 OCT 2015

FACULTY OF HEALTH  
SCIENCES



Form FHS006: Protocol Amendment  
FACULTY OF HEALTH SCIENCES FACULTY  
UNIVERSITY OF CAPE TOWN

HREC office use only (FWA00001637; IRB00001938)		
<input checked="" type="checkbox"/> Approved	<input checked="" type="checkbox"/> Type of review: Expedited	<input type="checkbox"/> Full committee
This serves as notification that all changes and documentation described below are approved.		
Signature Chairperson of the HREC		Date 21/10/2015
Note: All major amendments should include a PI Synopsis justifying the changes for the amendment (please see notice dated 23 April 2012)		
Comments to PI from the HREC		

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	20.10.2015	
HREC REF. Number	219/2014	
Protocol title	ORIGINAL TITLE: "Effects of MBCT on intrinsic brain networks in bipolar disorder patients"	
Protocol number (if applicable)		
Principal Investigator	Dr Neil Horn	
Department / Office Internal Mail Address	Psychiatry and Mental Health and Valkneberg Hospital doctor.neil.horn@gmail.com	
1.1 Is this a major or a minor amendment? (see FHS006hip) Major (tick box) Minor (tick box)	<input type="checkbox"/> Major	<input checked="" type="checkbox"/> Minor
1.2 Does this protocol receive US Federal funding?	<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No
1.3 If the amendment is a major amendment and receives US Federal Funding, does the amendment require full committee approval?	<input type="checkbox"/> Yes	<input type="checkbox"/> No



Jonathan Starke <joestärke@gmail.com>

**Starke : Confirmation of Approval of Study Proposal**

Jackie Cogill <jackie.cogill@uct.ac.za>  
To: jonathan starke <joestärke@gmail.com>

16 October 2015 at 14:52

Dear Joe

Yes, your change of title – Form D9, was approved and it was published in the August 2015 Dean's Circular:

Kind regards

Jackie

	<p>JACQUE COGILL   PG Administration   Faculty of Health Sciences   University of Cape Town    Room N2.19.1, Werner &amp; Beit Building North, Health Sciences Campus, Anso Road, Observatory, 7825    (+27 21 406 6750) fax +27 98 556 5778</p>
--	--

From: jonathan starke [mailto:joestärke@gmail.com]  
Sent: 15 October 2015 06:08 AM  
To: Jackie Cogill

[Quoted text hidden]

[Quoted text hidden]

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# University of Cape Town



Department of Psychiatry and Mental Health

## **PATIENT CONSENT FORM UCT REC/REF 078/2009 FUNCTIONAL MRI STUDY ON THE EFFECTS OF MINDFULNESS-BASED COGNITIVE THERAPY IN PEOPLE WITH BIPOLAR**

### **What is this study about?**

Bipolar Affective Disorder (or Manic Depression), is a condition which affects about 1% of the population. The Departments of Psychiatry, and Psychology, at the University of Cape Town, are investigating the biology, psychology and best treatments for Bipolar Disorder, and looking for the possible genetic basis of this illness. We would also like to find out whether, as has been suggested in other research, Mindfulness Based Cognitive Therapy assists people with Bipolar Disorder to cope with stress and depression.

Mindfulness can be defined as a state of heightened present-moment awareness. Acquiring mindfulness skills through training has shown to improve mental and physical health, and Mindfulness Based Cognitive Therapy (MBCT) has been shown to improve symptoms of depression and anxiety.

### **Who can participate?**

People aged 18 to 50 affected by Bipolar Disorder are invited to participate. If you have a treating doctor or psychiatrist, they should agree to your participation. As part of this research program we are also recruiting individuals who do not have Bipolar Disorder for comparative studies.

You are invited to take part in this study, which will investigate how emotions are regulated in people with Bipolar Disorder and investigate the effects of Mindfulness Based Cognitive Therapy (MBCT). If you agree to participate you will be interviewed by a psychiatrist about your condition and undergo

neuroimaging/brain scans and complete questionnaires. These will be repeated after MBCT training. You will initially be screened by a radiologist to ensure the MRI scan is safe for you and the total scanning time will be 60 minutes. During the scanning you will take part in four computerized tasks, which will be explained to you on the day of testing.

As a participant, you will be offered an 8-week MBCT intervention (weekly two-hour group sessions) which the Institute for the study of Affective Neuroscience at the University of Haifa, Israel, has kindly agreed to fund. This involves mindfulness training designed to cultivate non-judgmental observation of thoughts, emotions and bodily sensations.

Participation in this study is completely voluntary, and you are free to opt out any part of the study at any stage. If you are not participating in the UCT Bipolar Genetics study you will be invited to do so. Your data and individual identity will be kept strictly confidential. Please read the attached MRI Information Sheet before signing this consent.

## **MAGNETIC RESONANCE IMAGING INFORMATION SHEET**

Before taking part in this study you will be required to have an interview with a radiologist on the day of your scan to make sure that the MRI procedure is safe for you. Please feel free to raise any questions/ concerns you may have at this time, and prior to signing the consent form.

### **SAFETY AND METAL OBJECTS**

The MRI scanner is a powerful magnet. Because metal objects are strongly attracted to the magnet, any metal objects you are carrying or wearing must be removed prior to the MRI scan to avoid potentially severe injury.

### **UNEXPECTED FINDINGS**

In rare cases, researchers discover unexpected findings related to a participant's MRI scan in which case the scan is referred to a radiologist for further analysis and further tests may be recommended in order to determine the nature and significance of the unexpected finding, in which case the participant will be referred to a General Practitioner of their choosing.

### **ONGOING DISCLOSURE OF POTENTIAL HARMS**

If new findings about the potential harms of the MRI technique become available during the time of the study, the researcher will inform you.

### **CONTACT DETAILS**

Should you have any further queries you may contact:

Victoria Ives-Deliperi: 082 837 4017 [vives@mweb.co.za](mailto:vives@mweb.co.za)

Neil Horn: [neil.horn@uct.ac.za](mailto:neil.horn@uct.ac.za) Fleur Howells

The Faculty of Health Sciences Research Ethics Committee: 021 406 6492

## Consent Form

As a representative of this study, I have explained to the participant the purpose, the procedures, the possible benefits, and the risks of this research study; and how the participant's performance and other data will be collected, used and shared by others:

\_\_\_\_\_  
Signature of person obtaining consent

\_\_\_\_\_  
Date

You have been informed about this study's purpose, procedures, possible benefits, and risks; and how your performance and other data will be collected, used and shared with others. You have received a copy of this the information sheets. You have been given the opportunity to ask questions before you sign, and that you have been told that you can ask questions at any other time. You voluntarily agree to participate in this study. You hereby authorize the collection, use and sharing of your performance and other data. By signing this form, you are not waiving any of your legal rights.

I understand that I may opt out of the  
Questionnaires  
Memory assessment  
Brain Scan  
EEG  
Photography  
Mindfulness Based Cognitive Therapy

\_\_\_\_\_  
Signature of person consenting

\_\_\_\_\_  
Date

Home number: \_\_\_\_\_

Cell Phone: \_\_\_\_\_

E-mail address: \_\_\_\_\_

Mailing Address: \_\_\_\_\_

\_\_\_\_\_  
\_\_\_\_\_

### HOSPITAL ANXIETY AND DEPRESSION SCALE

In the last week: Choose option by bolding the text

1	<b>I feel tense or "wound up"</b>	Most of the time	A lot of the time	Occasionally	Not at all
2	<b>I still enjoy the things I used to</b>	Definitely as much	Not quite so much	Only a little	Hardly at all
3	<b>I get a sort of frightened feeling as if something awful is going to happen</b>	Very definitely and quite badly	Yes, but not too badly	A little but it doesn't worry me	Not at all
4	<b>I can laugh and see the funny side of things</b>	As much as I always could	Not quite so much now	Definitely not so much	Not at all
5	<b>Worrying thoughts go through my mind</b>	A great deal of the time	A lot of the time	From time to time but not too often	Only occasionally
6	<b>I feel cheerful</b>	Not at all	Not often	Sometimes	Most of the time
7	<b>I can sit at ease and feel relaxed</b>	Definitely	Usually	Not Often	Not at all
8	<b>I feel as if I am slowed down</b>	Nearly all the time	Very often	Sometimes	Not at all
9	<b>I get a sort of frightened feeling like 'butterflies' in the stomach</b>	Not at all	Occasionally	Quite often	Very often
10	<b>I have lost interest in my appearance</b>	Definitely	I don't take so much care as I should	I may not take quite as much care	I take just as much care as ever
11	<b>I feel restless as if I have to be on the move</b>	Very much indeed	Quite a lot	Not very much	Not at all
12	<b>I look forward with enjoyment to things</b>	As much as I ever did	Rather less than I used to	Definitely less than I used to	Hardly at all
13	<b>I get sudden feelings of panic</b>	Very often indeed	Quite often	not very often	Not at all
14	<b>I can enjoy a good book, radio or TV programme</b>	Often	Sometimes	Not often	Very seldom

## Young Mania Rating Scale (YMRS)

### GUIDE FOR SCORING ITEMS:

The purpose of each item is to rate the severity of that abnormality in the patient. When several keys are given for a particular grade of severity, the presence of only one is required to qualify for that rating.

The keys provided are guides. One can ignore the keys if that is necessary to indicate severity, although this should be the exception rather than the rule.

Scoring between the points given (whole or half points) is possible and encouraged after experience with the scale is acquired. This is particularly useful when severity of a particular item in a patient does not follow the progression indicated by the keys.

#### 1. Elevated Mood

- 0 Absent
- 1 Mildly or possibly increased on questioning
- 2 Definite subjective elevation; optimistic, self-confident; cheerful; appropriate to content
- 3 Elevated; inappropriate to content; humorous
- 4 Euphoric; inappropriate laughter; singing

#### 2. Increased Motor Activity-Energy

- 0 Absent
- 1 Subjectively increased
- 2 Animated; gestures increased
- 3 Excessive energy; hyperactive at times; restless (can be calmed)
- 4 Motor excitement; continuous hyperactivity (cannot be calmed)

#### 3. Sexual Interest

- 0 Normal; not increased
- 1 Mildly or possibly increased
- 2 Definite subjective increase on questioning
- 3 Spontaneous sexual content; elaborates on sexual matters; hypersexual by self-report
- 4 Overt sexual acts (toward patients, staff, or interviewer)

#### 4. Sleep

- 0 Reports no decrease in sleep
- 1 Sleeping less than normal amount by up to one hour
- 2 Sleeping less than normal by more than one hour
- 3 Reports decreased need for sleep
- 4 Denies need for sleep

#### 5. Irritability

- 0 Absent
- 2 Subjectively increased
- 4 Irritable at times during interview; recent episodes of anger or annoyance on ward
- 6 Frequently irritable during interview; short, curt throughout
- 8 Hostile, uncooperative; interview impossible



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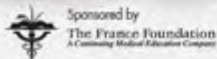
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## Young Mania Rating Scale (YMRS)

6. **Speech (Rate and Amount)**
  - 0 No increase
  - 2 Feels talkative
  - 4 Increased rate or amount at times, verbose at times
  - 6 Push; consistently increased rate and amount; difficult to interrupt
  - 8 Pressured; uninterruptible, continuous speech
7. **Language-Thought Disorder**
  - 0 Absent
  - 1 Circumstantial; mild distractibility; quick thoughts
  - 2 Distractible, loses goal of thought; changes topics frequently; racing thoughts
  - 3 Flight of ideas; tangentiality; difficult to follow; rhyming, echolalia
  - 4 Incoherent; communication impossible
8. **Content**
  - 0 Normal
  - 2 Questionable plans, new interests
  - 4 Special project(s); hyper-religious
  - 6 Grandiose or paranoid ideas; ideas of reference
  - 8 Delusions; hallucinations
9. **Disruptive-Aggressive Behavior**
  - 0 Absent, cooperative
  - 2 Sarcastic; loud at times, guarded
  - 4 Demanding; threats on ward
  - 6 Threatens interviewer; shouting; interview difficult
  - 8 Assaultive; destructive; interview impossible
10. **Appearance**
  - 0 Appropriate dress and grooming
  - 1 Minimally unkempt
  - 2 Poorly groomed; moderately disheveled; overdressed
  - 3 Disheveled; partly clothed; garish make-up
  - 4 Completely unkempt; decorated; bizarre garb
11. **Insight**
  - 0 Present; admits illness; agrees with need for treatment
  - 1 Possibly ill
  - 2 Admits behavior change, but denies illness
  - 3 Admits possible change in behavior, but denies illness
  - 4 Denies any behavior change

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ISSN: 0165-0327

### DESCRIPTION

The *Journal of Affective Disorders* publishes papers concerned with **affective disorders** in the widest sense: **depression, mania, anxiety and panic**. It is interdisciplinary and aims to bring together different approaches for a diverse readership. High quality papers will be accepted dealing with any aspect of affective disorders, including biochemistry, pharmacology, endocrinology, genetics, statistics, epidemiology, psychodynamics, classification, clinical studies and studies of all types of treatment.

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### IMPACT FACTOR

2014: 3.383 © Thomson Reuters Journal Citation Reports 2015

## ABSTRACTING AND INDEXING

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## GUIDE FOR AUTHORS

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*The Journal of Affective Disorders* publishes papers concerned with **affective disorders** in the widest sense: **depression, mania, anxiety and panic**. It is interdisciplinary and aims to bring together different approaches for a diverse readership. High quality papers will be accepted dealing with any aspect of affective disorders, including biochemistry, pharmacology, endocrinology, genetics, statistics, epidemiology, psychodynamics, classification, clinical studies and studies of all types of treatment.

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