NEURAL CORRELATES OF PREMENSTRUAL DYSPHORIC DISORDER IN WOMEN WITH BIPOLAR DISORDER

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Abstract

Introduction: Women with bipolar disorder (BD) have higher rates of premenstrual syndrome (PMS) and premenstrual dysphoric disorder (PMDD). The primary goal of this thesis was to examine the neural correlates of bipolar disorder and comorbid PMDD and identify changes in brain structure or function that may mediate emotional and cognitive dysregulation in the late luteal phase.

Results: In healthy women with no history of PMDD, absolute levels of estradiol, progesterone, allopregnanolone and dehydroepiandrosterone sulfate (DHEAS) were correlated with patterns of functional coupling in multiple regions associated with emotional and cognitive processes, in the mid-follicular and late luteal menstrual phases. A systematic review of the literature on resting state functional connectivity (Rs-FC) in BD during euthymia highlighted consistent patterns of resting state functional connectivity (Rs-FC) using ICA and SBA; including stability of the default mode network (DMN), salience network (SN) and fronto-parietal network (FPN) relative to controls. Available literature largely failed to control for sex, menstrual cycle phase or menstrual cycle disorders. Thus, we conducted the first fMRI studies to control for menstrual cycle phase in BD. During the mid-follicular phase, we found increased Rs-FC between critical nodes of the default mode and frontoparietal networks in BD compared to controls and increased functional connectivity between the somatosensory cortex and the insular cortex, inferior prefrontal gyrus and frontal orbital cortex in BD compared to controls. Voxel based morphometry analysis showed decreased gray matter in the somatosensory cortex in the same population compared to controls. Finally, women with BD and co-morbid PMDD displayed different patterns of Rs-FC using the right and left hippocampi as seed regions than women with BD without co-morbid PMDD and controls with PMDD. Differences in cortical thickness between controls with

and without PMDD and with and without BD were also found in regions central to emotional regulation and cognitive processing.

Conclusions: Results highlight the influence of sex hormones on Rs-FC and support the need to control for menstrual phase and PMDD diagnosis. Differences in structural and functional connectivity, and the clinical profile of women with BD and those with BD and co-morbid PMDD highlights the impact of PMDD on BD and the need for future research in this area.

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List of Abbreviations

BD – Bipolar Disorder
PMDD – Premenstrual Dysphoric Disorder
PMS – Premenstrual Syndrome
Rs-FC – Resting State Functional Connectivity
ICA – Independent Component Analysis
SBA – Seed Based Analysis
E2 – Beta 17 Estradiol
P4 - Progesterone
ALLO - Allopregnanolone
DHEAS - Dehydroepiandrosterone Sulphate
5HT – Serotonin
GABAa – Gamma-amino Butyric Acid Receptor Subtype a
DRSP – Daily Record of Severity of Problems
PSST – Premenstrual Symptom Screening Tool
SCID-IV-TR – Structured Clinical Interview for DSM-IV
SCID-PMDD - Structured Clinical Interview for Premenstrual Dysphoric Disorder
MADRS – Montgomery Asberg Depression Rating Scale
HAMD – Hamilton Depression Rating Scale
YMRS – Young Mania Rating Scale
STAI – State Trait Anxiety Inventory
BRIAN – Biological Rhythms Interview of Assessment in Neuropsychiatry
PSQI – Pittsburg Sleep Quality Index
FON - Fronto-occipital Network
CER - Cerebellum/Midbrain Network
DMN - Default Mode Network
nDMN - Posterior Default Mode Network
aDMN - Anterior Default Mode Network
PL - Paralimbic Network
MPN – Meso Paralimbic Network
BGN - Fronto-thalamic/Basal Ganglia Network
SN - Salience Network
SMN - Sensorimotor Network
FPN – Frontoparietal Network
rFPN - Right Fronto Parietal Network
lFPN - Left Fronto-Parietal Network
PN - Parietal Network
ARN - Auditory Related Network
VRN - Vision Related Network
VSN - Visospatial Network
AUD - Auditory Network
CC - Cognitive Control Network
TIN - Temporo-Insular Network
MN - Motor Network
ECN - Executive Control Network
SFG - Superior Frontal Gyrus

MFG - Middle Frontal Gyrus
OFC - Orbitofrontal Cortex
ACC - Anterior Cingulate Cortex
PFC - Prefrontal Cortex
vlPFC - Ventrolateral Prefrontal Cortex
BRM - German Version Bech Rafaelsen Mania
BDI – II - Beck Depression Inventory-II
PCC - Posterior Cingulate Cortex
ACC - Anterior Cingulate Cortex
vACC - Ventral Anterior Cingulate Cortex
mPFC - Medial Prefrontal Cortex
dlPFC – Dorsolateral Prefrontal Cortex
sgACC – Subgenual Anterior Cingulate Cortex
vmPFC – Ventromedial Prefrontal Cortex
LSTG - Left Superior Temporal Gyrus
LMTG - Left Middle Temporal Gyrus
LANG - Left Angular Gyrus
FusiformG – Fusiform Gyrus
A1 – Primary Auditory Cortex
pEC – Posterior Entrorhinal Cortex
S1 – Primary Somatosensory Cortex
M1 – Primary Motor Cortex
ITG – Inferior Temporal Gyrus
DFC – Dorsal Frontal Cortex
AMYG – Amygdala
V2, V3 – Associative Visual Cortex
V1 – Primary Visual Cortex
S2 – Somatosensory Association Cortex
BA – Brodmann Area
MNI – Montreal Neurological Institute

Declaration of Academic Achivement

Chapter 2: All data presented in this chapter was collected and analyzed by S.K. Syan. M. Smith and O. Allega aided with aspects of data collection. All hormonal assays were conducted by M. Coote. L Minuzzi and B.N. Frey contributed to the project design, composition of the manuscript, data analysis plan and aided with formation of the intial research questions. G. Hall aided with neuroimaging analysis and D. Costescu was involved with analysis of hormone levels.

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Chapter 5: S.K. Syan completed data collection, resting state functional connectivity and clinical analysis and contributed to the writing of the manuscript. L Minuzzi composed the manuscript and completed the structural analysis. B.N. Frey contributed to the project design, composition of the manuscript. G. Hall aided with neuroimaging analysis. A. Hall aided with subject recruitment.

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Chapter 1: Introduction

1.1 Bipolar Disorder

1.1.1 Clinical Definition

Bipolar Disorder (BD) is a psychiatric illness characterized by acute mood episodes of depression and mania (BD type-I) or hypomania (BD type-II) (1). A depressive episode is defined as depressed mood and a marked loss of interest or pleasure in most activities, which present for the majority of the day, nearly every day for a period of two weeks or longer (1). This period is accompanied by additional symptoms including: (i) significant changes in weight, defined as weight loss or gain of greater than a 5% of body weight in a month; (ii) changes in sleep – insomnia or hypersomnia; (iii) psychomotor agitation or retardation; (iv) pronounced fatigue and/or loss of energy; (v) difficulty concentrating and/or indecisiveness; (vi) feelings of worthlessness or guilt; (vii) suicidality (1). Further, these symptoms must result in clinically significant distress or impairment domains that are important to the patients' functioning (social and occupational) (1).

Mania is defined as a period of abnormally persistent, elevated, expansive or irritable mood, in conjunction with a marked increase in goal-directed activity and energy, taking place nearly all day, every day for at least a week, and in severe cases requiring hospitalization and including symptoms of psychosis (1). This period also includes symptoms such as: (i) an inflated self-esteem (grandiosity); (ii) decreased need for sleep; (iii) increased desire to remain talkative; (iv) a flight of ideas and/or racing thoughts; (v) greater tendency to partake in risky activities with a high potential for volatile consequences (unprotected sex, drug use, fiscal irresponsibility) (1). This change in an individual's behaviour is commonly noticeable by others and similar to a

depressive episode, causes impairment in social and occupational domains (1). A hypomanic episode shares the same features of a manic episode, however presents nearly all day, every day for four consecutive days and does not cause significant impairment (1).

Additionally, a diagnosis of BD can be followed by specifiers, which include information about episode or illness trajectory such as: (i) anxious distress; (ii) mixed features, (iii) rapid cycling; (iv) melancholic features; (v) atypical features; (vi) mood-congruent psychotic features; (vii) mood-incongruent psychotic features; (viii) catatonia; (ix) peripartum onset; (x) seasonal pattern (1).

1.1.2. Prevalence, Course and Comorbidities

Bipolar disorder is estimated to have a prevalence of 1-4% of the general population (1,2). In a pooled sample from eleven countries the 12-month prevalence of BD was estimated to be 0.4%, 0.3%, 0.8% and 1.5% of the population, for BD type-I, type-II, subthreshold BD and bipolar spectrum (2). Bipolar disorder is highly heritable and studies estimates a it carries a heritability of 40-70% (3-7). The mean age of onset is estimated to be 18 years of age for BD type-I and the mid-20s for BD type-II. Due to its chronicity, early age of onset and progressive nature, BD carries a high risk of suicidality. Severity of mood episodes and risk of suicidality increases along the bipolar spectrum from subthreshold BD to BD type-1 (2). Research estimates that individuals with a diagnosis of BD encompass 25% of completed suicides and carry a 15-fold greater risk of suicide than the general population; this risk is greatest in those with co-morbid alcohol use disorder (1).

BD is also highly co-morbid with other psychiatric illnesses. Individuals with BD type-1 report high rates of co-morbid anxiety disorders (75%), attention deficient hyperactivity disorder (ADHD) (over 50%) and substance use disorder (over 50%). Interestingly, approximately 60% of individuals with BD-type-2 report 3 or more co-morbid psychiatric conditions, including anxiety

disorders (75%) and substance use disorders (37%) (1). In this case, co-morbid conditions may show entrainment or exacerbation in episodic illness phases; for instance, a greater incidence of anxiety disorders associated with depressive episodes. Further BD carries a high rate of medical co-morbidities - specifically metabolic illnesses (8-11). Further contributing to its high burden of illness and disability-adjusted life years (DALYs) lost to illness, is the functional impairment that follows acute mood episodes and presents in remitted (euthymic) illness phases in 30% of individuals with BD type-1 and 15% in BD type-2 (1). In this subset of patients with BD, functional impairment persists after symptoms are alleviated, leading to impairment in occupational domains and may lead to inability to maintain a socioeconomic status similar to healthy controls with similar years of education.

1.1.3. Neurobiological Models of BD

Bipolar disorder can be regarded as a disorder of emotional regulation and biological and regulatory processes (12-16). Clinically relevant features of BD can be attributed to fluctuations through extreme mood states, cognitive dysregulation and presence of neurovegetative symptoms (12-16). Neuroimaging studies have helped to inform neurobiological models of BD by highlighting regions of neural pathways that may underlie its pathophysiology (12-16). Many neurobiological models suggest that the pathophysiology of BD is less likely due to differences in the structure and function of particular brain regions, however more likely responds to changes in large scale brain networks (14-16). Dysregulation in pathways involved in emotional processing and control are postulated to result from of a loss of top down prefrontal modulation of limbic circuitry; and aberrant functioning two interrelated networks responsible for mediating emotional regulation: (i) lateral prefrontal cortical system (originating in the ventrolateral PFC); and (ii) medial prefrontal cortical system (originating in the ventromedial PFC) (14,16-18). It is hypothesized that an imbalance between these two neural streams leads to the onset of affective

episodes and clinical symptoms experienced in BD (14,16-18). In complex emotional states, both networks function in synchrony to modulate the activity of the amygdala (14,16).

The lateral prefrontal cortical system has also been described as the external regulatory network and dorsal neural stream (14,16,18). This network is thought to regulate external emotional states, voluntary aspects of emotional regulation, and is central to the cognitive regulation of affect, especially suppression of negative affect (14,16,18). It is postulated to originate between the robust connections of the dorsolateral prefrontal cortex (dlPFC) and ventrolateral prefrontal cortex (vlPFC) (14,16,18). It includes the vlPFC, mid and dorsal anterior cingulate cortex (ACC), ventromedial striatum, globus paladus and thalamus (14,16,18). These regions show considerable overlap with the central executive network (14,16). The medial prefrontal cortical system is also referred to as the internal/automatic emotional regulatory network (14,16). This network is involved in the regulation of implicit emotional states and mediates the amygdala's response to endogenously generated emotional states (14,16). It originates in the ventromedial PFC and includes the subgenual ACC, nucleus accumbens, globus palladus and thalamus (14,16).

Differences in thickness and volume of brain regions have been reported across various primary research studies and meta-analyses in BD compared to controls. Consistent findings include increased lateral ventricle (19-21) and right putamen volume (19) and decreased volume in the cerebellar vermis (22), corpus callosum (23), prefrontal cortex (24,25), insula (26,27) and both the peri and subgenual anterior cingulate corticies (26,27). Findings regarding amygdala enlargement in adults with BD are discrepant(28), and more commonly seen by patients taking lithium (29,30). Decreases in cortical thickness have been found in the left anterior cingulate gyrus, superior temporal gyrus and bilateral superior frontal gyri in BD compared to controls (31).

Studies investigating Rs-FC have found aberrant connectivity of various resting state networks during episodic illness phases and less during periods of euthymia (32-35). Most commonly, literature highlights dysregulation of the default mode network (DMN) during episodic illness phases, with studies showing hypoconnectivity during mania and increased connectivity during depressive phases. The absence of differences in Rs-FC of the DMN during periods of remission highlights its potential for acting as a potential marker for periods of euthymia in BD. Specific to periods of euthymia, studies using a seed based analysis (SBA) have found increased coupling between the amygdala and medial PFC (mPFC) (36,37) between the amygdala and the vlPFC in BD type I (38) and between the amygdala and the right dlPFC (39). Studies using the same seed based approach also found decoupling between the dlPFC and the mPFC (36) and between the right dlPFC and the amygdala (37). Most studies using independent component analysis (ICA); a data driven approach used to investigate the functional connectivity functional brain networks, have consistently found no differences between individuals with euthymic BD and controls in the DMN, fronto-parietal network (FPN), mesoparalimbic network (MPN) and salience network (SN) (40-43). There are two exceptions to this; (i) a small study (n=15) of medication- naïve subjects, which found increased activity of the bilateral insula and putamen comprising the temporo- insular network in individuals with BD type II (43); (ii) two studies that found hypoconnectivity of the DMN in a group of patients with BD and history of psychosis compared to controls (44,45).

1.1.4 Bipolar Disorder in Women

Although men and women are equally represented in BD type-I, literature suggests that the presentation of BD in women is different than that within men. Clinically, women tend to report more depressive and mixed episodes than men and are more prone to develop the type-II and rapid-cycling subtypes of BD (46-53). Bipolar females also experience higher rates of

comorbid post-traumatic stress disorder, eating disorders and personality disorders (1,48,49,54). In women, a diagnosis of BD also carried a higher risk of alcoholism than men (53). Further, this study found that alcoholism in women with BD was associated with polysubstance use (53). Female reproductive life events such the perinatal period, menopause and the menstrual cycle may also serve as windows of vulnerability for illness relapse and mood worsening (55). Further, independent studies have shown higher rates of premenstrual worsening of mood in women with BD as described below (56-58).

Periods of hormonal fluctuation associated with the reproductive lifespan may predispose some women with BD to the onset of a mood episode (55). The hormonal changes associated with the peri and postpartum periods and menopausal transition may, in some women, create unique and periods of psychiatric vulnerability for mood changes (59). In recent years, independent studies have also highlighted higher rates of premenstrual worsening of mood in women with BD (54,56-58,60-62).

1.1.5 Bipolar Disorder and PMS

Mood instability during the premenstrual phase in women with BD has been reported across numerous studies (61). Literature shows that women with BD display high rates of premenstrual syndrome (PMS); with studies estimating that approximately 51%-68% of women with BD report mood symptoms during the premenstrual period (47,56,63,64). Choi et al. investigated premenstrual exacerbation among women with BD to find that 51.6% of women with BD type-II displayed moderate to severe premenstrual syndrome as compared to 23.3% of women with BD type-I and 19.7% of healthy controls (56). Symptoms of PMS were measured according to the Premenstrual Symptom Screening Tool (PSST) and not confirmed through use of prospective charting through the menstrual cycle; therefore, these studies may reflect an overestimation of the prevalence of PMS in BD. Further, Fornaro and Perugi investigated the

impact of premenstrual dysphoric disorder (PMDD) in a sample of 92 women with BD (57). In their sample, 27.2% of women met criteria for PMDD according to a clinical semi-structured interview. This subset of women with BD and co-morbid PMDD displayed a higher number of axis I co-morbidities than those without PMDD (57). Common comorbidities associated with PMDD relative to no PMDD in this population included: post-partum depression, obsessive-compulsive disorder and body dysmorphic disorders (57). Again, it is important to note that the studies discussed above did not confirm premenstrual mood changes with prospective charting of mood across the menstrual cycle; thus, the prevalence of PMDD in this sample may also reflect an overestimation of PMDD.

Dias and colleagues conducted a large prospective study, which found that women with a diagnosis of BD and history of premenstrual exacerbation of mood have a worse course of their bipolar illness. This was characterized by shorter time to relapse, and greater symptom severity - to a greater extent for depressive symptoms (58). Further studies with a primary objective of examining prevalence of PMDD in community-based samples have also highlighted its association with BD. Wittchen and colleagues reported that women with PMDD found that they are 8 times more likely to have a diagnosis of BD (65). It is important to note that smaller studies have failed to find an association with BD and PMS (66-69).

1.2. The Menstrual Cycle

The menstrual cycle is a hormonally mediated process by which the female reproductive tract produces and release of a follicle/oocyte that has the potential of being fertilized and implanted into the uterus to achieve pregnancy (70). In the event that the oocyte is not fertilized, the corpus luteum resorbs and the uterus sheds its lining. The normal menstrual cycle can range from 21-35 days, with an average cycle length of 28 days (70). The menstrual cycle is split into 4

key phases, (i) menstrual phase; (ii) follicular (proliferative) phase; (iii) ovulatory phase; (iv) luteal (secretory) phase(70).

The menstrual cycle is regulated by the hypothalamic-pituitary-ovarian (HPO) axis. The hypothalamus releases gonadotropin-releasing hormone (GnRH) in a pulsatile fashion, which acts on the gonadotrophic cells of the anterior pituitary gland causing the release of follicle stimulating hormone (FSH) and lutenizing hormone (LH) (70). Follicle stimulating hormone stimulates the granulosa cells surrounding the follicle to produce estradiol, which increases through the follicular phase and peaks just prior to ovulation (70). Lutenizing hormone is released after E2 peaks, signalling ovulation (70). Progesterone (P4) remains at low levels through the follicular phase and rises through the luteal phase with the development of the corpus luteum; the primary source of circulating P4 (70). In the absence of fertilization, luteolysis occurs and the corpus luteum degrades which results in a decline in E2 and P4 levels, which precipitates the onset of menstrual flow (70). This steep decline in E2 and P4 in the late luteal phase may mediate the development of premenstrual symptoms in certain women (71) . The average menstrual cycle is between 21-35 days (72).

1.2.1. Hormones and the Brain

17-β-Estradiol (E2) is a primary sex hormone, which profoundly affects numerous systems throughout the body including cardiovascular, reproductive, skeletal and central nervous system (CNS). Its effects on CNS suggest a role in the regulation of emotional and cognitive processes and the pathophysiology of mood disorders. Postmortem studies investigating estrogen receptor- α and estrogen receptor-β messenger RNA expression have found greatest localization in the hippocampal formation, claustrum, cerebral cortex, amygdala, hypothalamus, subthalamic nucleus, and the thalamus (73-76). E2 also affects neurotransmitter receptor function and availability (77). Specifically, E2 is known to (i) enhance 5HT synthesis through the increase of

tryptophan hydrolase; (ii) regulate 5HT transporters in the synaptic cleft; (iii) increase 5HT receptors in brain regions dense in E2 receptors; (iv) increase 5HT2a receptor subtypes while decreasing 5HT1a subtypes; (v) act as a monoamine oxidase inhibitor (MAO) by decreasing the activity of MAO-A and MAO-B (77,78). Further, E2 exerts affects on other neurotransmitter systems by (i) enhancing norepinephrine synthesis by increasing tyrosine hydroxylase; (ii) enhancing dopaminergic (DA) synthesis, release and turnover by modifying the firing rates of DA neurons via E2 membrane receptors; (iii) enhancing gene expression of dopamine B-hydroxylase; and (iv) upregulating CREB (77-79).

Progesterone is another important female sex hormone and with reproductive and non-reproductive central nervous system functions including mitochondrial function, neurogenesis, cognition and emotional regulation (80). Although there is no literature that investigates messenger RNA expression in the human brain, a postmortem study reported high concentrations of progesterone in the amygdala, hypothalamus, and cerebellum (80,81). In the brain, progesterone is metabolized into its neuroactive metabolite allopregnanolone (82,83).

Allopregnanolone (3α-hydroxy-5α-pregnane-20-one) is a γ-aminobutyric (GABA)-a receptor agonist and allosteric modulator (83,84). It is synthesized in the CNS and the corpus luteum (85). Concentrations vary across the menstrual cycle, and mirror that of progesterone. Plasma levels of allopregnanolone range from 0.2-0.5 nmol/L in the follicular phase up and may go up to 4nmol/L in the luteal phase (86). As a GABA-a agonist, allopregnanolone exerts effects on the brain's major inhibitory system; therefore creating a foundation for its role in mood disorders and aetiology for premenstrual syndrome (82-84). Further, it is highly lipophilic and passes through the blood-brain-barrier. In high concentrations, allopregnanolone's effects can be sedative and anxiolytic in nature. In a certain subset of individuals, it may induce negative mood

changes and provoke anxiogenic effects (82,84,87). The prevalence of adverse emotional reactions to allopregnonlone are postulated to occur in 20% of individuals, with 2-3% experiencing more severe emotional reactions (85-88). These prevalence rates are in line with those seen for premenstrual syndrome and premenstrual dysphoric disorder, respectively(83,85). This is supported by evidence indicating that women with PMDD display altered sensitivity to other GABAa receptor modulators such as barbutuates and alcohol in their late luteal phase (86,88-90). Dehydroepiandrosterone sulfate, is a neuroactive metabolite of DHEA and another GABA-a receptor modulator (91). DHEAS also modulates GABA-a receptors and influences serotonin neuron firing and availability (91).

1.2.2. Bipolar Disorder and Menstrual Cycle Hormonal Fluctuations

As discussed above, estradiol (E2) and progesterone (P4) mediate a cascade of neuroendocrine functions, which have implications on both the structure, and function of the central nervous system (CNS). Studies have shown that E2 and P4 regulate the availability and function of monoamines, neurogenesis, inflammatory processes and play a role in cognitive and affective regulation(71,92). Hormones may act to influence BD through (i) the influence of hormones on modulation of neurotransmitter systems, which may be of particular importance as therapeutic agents used to achieve remission from affective illness phases may also act to stabilize these neurotransmitter regions(93); (ii) through sex hormone binding to brain regions associated with affective and cognitive processes that are also implicated in the pathophysiology of BD (12,14,76,80). In this capacity, women with affective disorders such as BD may be more vulnerable to the actions of hormones on the CNS than women without a history of psychiatric disorders. Despite this influence and the higher rates of premenstrual exacerbation of mood in women with BD(61), little is known about the impact of sex hormone fluctuations associated with the menstrual cycle on BD.

In a study by Reynolds-May et al., women with BD (n=103) were followed for three consecutive menstrual cycles, during which serum hormone levels, ovulation and biochemical markers were tracked through measurement of sex hormones (testosterone, estradiol, dehydroepiandrosterone sulphate (DHEAS), prolactin, follicle stimulating hormone, lutenizing hormone, 17-hydroxyprogesterone) (94). In this sample, levels of DHEAS and 17-hydroxyprogesterone were lower in BD than in the control group (94). Levels of estradiol did not differ between groups (94). Further, 80% of women taking an atypical antipsychotic endorsed a greater history of current or past menstrual abnormalities compared to 55% of women not taking atypical antipsychotic medication (94). Additionally, women with BD reported greater stress or exercise induced amenorrhea than controls (BD: n= 22%, CTRL: n = 8%) (94). Another study on DHEAS and pregnanolone in BD found that DHEAS levels correlated with performance on the Brief Assessment of Cognition in Affective Disorders (BACA) when controlling for age and years of education (95). Further, DHEAS was also correlated with symptoms of mania in this population(95).

1.3 Premenstrual Dysphoric Disorder

Premenstrual Dysphoric Disorder (PMDD) is a mood disorder, characterized by symptoms in affective, cognitive, behavioural and somatic domains, occurring in the late luteal phase of the menstrual cycle and ameliorate in the follicular phase. In contrast to PMS in order to meet criteria for a diagnosis of PMDD, an individual must present with one or more of the following symptoms: (i) marked affective liability – often recounted by subjective report as mood swings, sensitivity to rejection or feeling sad or tearful; (ii) marked irritability or anger; (iii) marked depressed mood, feelings of hopelessness or self-deprecating thoughts; (iv) marked anxiety and tension. In addition to this, five or more symptoms from the following list must be experienced: (i) anhedonia; (ii) subjective difficulty in concentration; (iii) lethargy, marked

fatigability; (iv) marked change in appetite, including specific cravings or overeating; (v) changes in sleep – defined as hypersomnia or insomnia; (vi) a subjective sense of being overwhelmed or having a loss of control; (vii) somatic complaints – breast tenderness, bloating, swelling, joint or muscle pain. The symptoms described must be associated with clinically significant distress and interference in both social and occupational domains. It must not be an exacerbation of another psychiatric disorder or medical condition, and must be confirmed by at least two months of prospective charting (1).

1.3.1. Prevalence, Course and Comorbidities

While it is suggested that 20-50% of women experience moderate to severe premenstrual symptoms, approximately 3-9% of women of reproductive age experience PMDD (59,96). Estimates from twin studies suggest that it carries a heritability of 44-56% (97,98). A large community-based study suggested that women with PMDD were more likely to develop psychiatric comorbidities including anxiety disorders (47.4%), mood disorders (22.9%) and somatoform disorders (28.4%). From this sample, 26.5% of women with PMDD had no psychiatric comorbidity (65). PMDD causes significant impairment in functioning and diminished quality of life; thus, it is estimated to be responsible for 14.5 million disability-adjusted life years (DALYs) (99,100). Further, PMDD is associated with a greater history of stressful life events (101,102), sexual abuse (103,104) and high levels of daily life stress (87,105).

1.3.2. Pathophysiology of PMDD

In women with PMDD, symptoms arise from sensitivity to normal hormonal fluctuations associated with the menstrual cycle (87). Several theories have been suggested to help explain the aetiology of PMDD (84,87,90,106,107). Among them, women with PMDD report a reduced GABA-a receptor sensitivity to allopregnanolone (86,90). Women without the disorder report

decreased anxiety and depressive symptoms upon allopregnanolone administration (86,90) . However, women with PMDD display paradoxical effects of high doses of allopregnanolone administration, as they report greater anxiety and depressive symptoms – thereby suggesting a relationship between alloprenanolone sensitivity and premenstrual mood symptoms in PMDD (86,90). This is also explained by the lag time of 4-5 days between onset of symptoms and peak of luteal steroids (85,90). Lag time between allopregnanolone peak and symptom presentation may suggest the importance of and occurrence of protein synthesis in symptom development (86).

Serotonin has also been postulated to play a role in the pathophysiology of PMDD due to the complex relationship between sex hormones and neurotransmitters (87,90,99). Support for this hypothesis comes from the use of selective serotonin reuptake inhibitors (SSRIs) as treatment to alleviate menstrual symptoms in women with PMDD (108), and literature highlighting lower whole blood serotonin levels in women with PMDD compared to controls in the late luteal phase (109). Further, the genetic and psychosocial factors discussed above also play into the development and pathophysiology of PMDD (87,107,110).

1.3.3 Neuroimaging in PMDD

Functional and structural MRI literature on women with PMDD is sparse. To our knowledge, there is currently no literature exploring either cortical thickness or resting state functional connectivity (Rs-FC) in women with PMDD compared to controls. This knowledge is critical to informing neurobiological models of PMDD and should be investigated. Structurally, women with PMDD display greater volume in the posterior cerebellum (111) and increased gray matter density of the left hippocampus (112) compared to controls. Interestingly, increased activation in the right cerebellar vermis has also been found in women with PMDD compared to controls using positron emission topography (PET) with fluorodeoxyglucose (113). This further supports the role of the cerebellum in the pathophysiology of PMDD. Recent literature has

highlighted that the cerebellum may play a complex role in emotional regulation and mood disorders through the influence of cerebro-cerebellar neural pathways and feedback loops (114). Decreased gray matter density has been reported in the parahippocampal gyrus (112).

Task based fMRI studies support the notion of enhanced processing of stimuli with a negative emotional valance. Compared to controls, women with PMDD display greater medial and dorsolateral PFC activity during anticipation of negative images (115), greater amygdala activation induced by exposure to negative words, and lower nucleus accumbens activity following positive word exposure (116) in the luteal vs. follicular phases. Further during a Go/NoGo task women with PMDD reported enhanced activity in the left insula and less activity in parietal regions compared to controls in luteal vs. follicular phases (117).

1.4 Magnetic Resonance Imaging

Magnetic resonance imaging is a useful tool for investigating differences in brain structure and function and is often used to inform neurobiological models of disease and pathophysiology. Magnetic resonance imaging capitalizes on the quantum mechanism properties of spin angular momentum of protons in hydrogen atoms (118,119). In a standard MRI, a magnetic field causes hydrogen atom protons to align with the scanners magnetic field (118,119). Radio waves emitted at the larmor frequency (the product of the strength of the magnetic field and gyromagnetic ratio) are absorbed by the hydrogen protons and causes alterations in their spin angular momentum (118,119). When the radio frequency pulse has concluded, the protons release the absorbed energy, which is detected by the scanner machinery and used to create images (118,119). Anatomical localization of structures is accomplished through a magnetic gradient created through the applied combinations of three orthogonal magnetic gradient fields (118,119).

Functional magnetic resonance imaging (fMRI) provides an indirect measure of neuronal activity through visualization of the blood-oxygen-level-dependent (BOLD) signal; which

capitalizes on the magnetic susceptibility of blood and the hemodynamic response (118,119). The magnetic susceptibility of blood originates from the magnetic properties of haemoglobin; which is diamagnetic when oxygenated and paramagnetic when deoxygenated – thereby leading to suppression of the MR signal (118,119). The resulting contrast between oxygenated haemoglobin and deoxygenated haemoglobin creates inhomogeneity in the magnetic field results in an MR signal that is slightly altered (118,119). Further, the hemodynamic response is a culmination of a series of events that allow for visualization of the BOLD signal (118,119). First, there is an increase in regional blood flow that follows a brief period of neuronal activity (118,119). This increase is greater than the amount required to replenish the depleted oxygen and more oxygenated, thereby changing the ratio of deoxygenated to oxygenated haemoglobin (118,119). As this occurs, magnetic field distortions are reduced and the local MR signal increases slightly allowing for visualization of the BOLD signal (118,119).

1.5 Main Aims

The literature presented above describes the clinical features, pathophysiology and neurobiology of BD and PMDD, as well as, the influence of sex hormone fluctuations associated with the menstrual cycle on the brain. Several consistent themes can be extracted from the literature, which are shared across both disorders and may be susceptible to the influence of hormonal fluctuations. Clinically, affective dysregulation and emotional lability are hallmarks of both BD and PMDD(1,12,87,120). Feelings of irritability, depressed mood, anhedonia and cognitive symptoms (decreased concentration, racing thoughts) are commonly reported during the late luteal phase in PMDD and episodic illness phases in BD (1). From a regulatory perspective, both manic/hypomanic and depressive episodes in BD and the late luteal phase in PMDD are characterized by distinct changes in energy and regulatory processes such as sleep and appetite (1). From a neurobiological perspective, both disorders follow a similar pattern of neurobiology;

with aberrant structure or functional connectivity reported in the prefrontal cortex (14,115), limbic regions (amygdala and hippocampus) (112,121) and cerebellar vermis (14,122,123). The prefrontal cortex and limbic system are also regions influenced by menstrual cycle hormone fluctuations (92,124-126), and thus may exploit the trait-based pathology of BD, in individuals both with and without PMDD. Moreover, at the cellular level hormonal fluctuations also act to influence neurotransmitter systems, which are implicated in both disorders, through the influence of E2 on 5HT systems and allopregnanolone and DHEAS on GABAa receptors (87,107,127-129). The shared clinical and neurobiological features of these disorders may suggest a potentially shared aetiology, and may help to explain the presence of PMDD in BD relative to controls.

In order to examine the overarching theme of comorbid BD and PMDD, we first assessed the impact of hormonal fluctuations on Rs-FC across the menstrual cycle (Chapter 2). We then systematically reviewed literature on Rs-FC in BD to assess the trait-based pathology of BD and if sex or menstrual phase were accounted for in current literature (Chapter 3). Following this, we explored the trait-based pathology of BD in women controlling for menstrual cycle hormone fluctuations by studying Rs-FC during the mid-follicular phase (Chapter 4, 5). In Chapter 6, we examine the clinical, structural and functional correlates of comorbid BD and PMDD by studying four groups of women in the mid-follicular and late luteal menstrual phases.

The overarching goal of this thesis was to examine the influence of hormonal fluctuations associated with the menstrual cycle on structural and functional connectivity on healthy women with and without PMDD and with and without BD. To date, there is no literature examining (i) the influence of menstrual cycle hormone fluctuations on Rs-FC in healthy women; (ii) the trait-based pathology of BD during euthymia controlling for sex and menstrual phase; (iii) the neural correlates of BD and comorbid PMDD. Thus, this thesis strived to address significant gaps in the literature and contributes to both clinical and imaging fields of research.

1.6. Objectives

The specific objectives of this thesis were as follows:

1. To examine the influence of E2, P4, allopregnanlone and DHEAS on Rs-FC through the menstrual cycle in healthy women
2. To examine the trait-based pathology of BD in women while controlling for menstrual phase
3. To investigate the neural correlates of comorbid BD and PMDD across the menstrual cycle

1.7. Hypotheses

Based on literature reviewed, hypotheses for each objective are outlined below:

1. We postulate patterns of functional connectivity will occur between regions of the brain dense in sex hormone receptors in both menstrual phases.
2. Aberrant patterns of functional connectivity in BD will present between brain regions associated with emotional regulation and cognition.
3. Participants with comorbid BD and PMDD will display differences in brain structure and patterns of Rs-FC than those without PMDD. Differences may be concentrated to regions associated with the pathophysiology of BD, PMDD or known binding sites of E2 and P4.

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Chapter 2: The Influence of Endogenous Estradiol, Progesterone, Allopregnanolone and Dehydroepiandrosterone Sulfate on Brain Resting State Functional Connectivity Across the Menstrual Cycle

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2.1 Abstract

Objective: To study (1) brain resting state functional connectivity (Rs-FC) in a well-characterized sample of healthy women in the mid-follicular and late-luteal phases of the menstrual cycle and (2) to examine the correlation between endogenous estradiol, progesterone, allopregnanolone and dehydroepiandrosterone sulfate and patterns of Rs-FC across the menstrual cycle.

Design: We studied the Rs-FC of the default mode network, salience network, meso-paralimbic network, fronto-parietal network, visual network and sensorimotor network in the mid-follicular and late luteal phases. Serum levels of estradiol, progesterone, allopregnanolone and dehydroepiandrosterone sulfate were correlated to patterns of functional connectivity.

Setting: University medical center

Patients: 25 healthy women with regular menstrual cycles

Intervention(s): None

Main Outcome Measure(s): Functional connectivity of key brain networks at rest and correlations of hormones to Rs-FC in the mid-follcuar and late luteal menstrual phases.

Results: There were no differences in Rs-FC between the mid-follicular and late luteal menstrual phases using either ICA or SBA. However, specific correlations between each hormone and patterns of functional connectivity were found in both menstrual cycle phases.

Conclusions: It seems that the association between female sex hormones and brain Rs-FC is menstrual cycle phase-dependent. Future studies should examine the cognitive and behavioral correlates of this association in regularly cycling women.

2.2. Introduction

Estradiol (E2) and progesterone (P4) mediate a cascade of neuroendocrine functions, with structural and functional implications to the central nervous system (CNS). Previous studies have shown that E2 and P4 regulate the availability and function of monoamines, neurogenesis, inflammatory processes and also play a role in regulating cognitive and affective processes (1-4). Sex hormones have also been suggested to play a role in functional cerebral asymmetries by influencing inter-hemispheric inhibition of the dominant on the non-dominant brain hemisphere (5).

E2 and P4 are present in various regions of the CNS: post-mortem studies investigating estrogen receptor-alpha and estrogen receptor-beta mRNA expression have found greatest localization in the hippocampal formation, claustrum, cerebral cortex, amygdala, hypothalamus subthalamic nucleus and the thalamus (4,6-8). Although there is no literature that investigates mRNA expression in the human brain, a post mortem study reported high concentrations of P4 in the amygdala, hypothalamus and cerebellum (9). In regularly cycling women, allopregnanolone (ALLO), a neuroactive metabolite of progesterone, also varies across the menstrual cycle with the corpus luteum serving as the primary site of contribution, and a modest amount being synthesized in the brain (10,11). ALLO is hypothesized to regulate mood and cognition through the modulation of GABA-a receptors and by exerting regulatory effects on serotonin (5-HT) and noradrenaline (12,13). Another important moderator of neurotransmitter systems and thereby emotion and cognition is Dehydroepiandrosterone sulfate (DHEAS), a neuroactive metabolite of dehydroepiandrosterone (DHEA) (14). DHEAS also modulates GABA-a receptors and influences 5-HT neuron firing and availability (14). Administration of both DHEAS and ALLO have been associated with decreased Rs-FC between brain areas involved in mediating anxious and depressive behavior (15). Studies using task-based fMRI and structural MRI have found changes

in regions critical to emotional regulation and cognition throughout the menstrual cycle, emphasizing the influence of sex hormones on the CNS (for review see (3,11,16). The influence of sex hormones on resting state functional connectivity (Rs-FC) has been less investigated and may be useful in providing a picture of intrinsic brain activation patterns in response to endogenous hormones free of task related bias.

Rs-FC is commonly studied through the use of seed-based analysis (SBA) and independent component analysis (ICA). SBA is a hypothesis driven approach, through which the blood-oxygen-level dependent (BOLD) signal of a predefined seed region is correlated with the BOLD signal of other regions in the brain. On the other hand, ICA is an exploratory, data-driven approach, which maximizes statistical independence by highlighting patterns of BOLD signal that are independent from one another (17). Both SBA and ICA approaches are commonly used to visualize the functional connectivity of resting state networks (RSNs). The synchronous use of both methods provides visualization of the functional connectivity of RSNs, and the connectivity of *a priori* seed regions integral to their function and maintenance.

Rs-FC literature examining the effects of hormonal fluctuations across the menstrual cycle is sparse and largely inconsistent. Two studies using repeated-measures across the follicular, ovulatory and mid-luteal phases found no differences in the functional connectivity of RSNs between menstrual phases (18,19). Petersen and colleagues scanned two different groups of women, one in the early follicular and the other group in the late luteal phase, and found increased functional connectivity of the angular gyrus of the default mode network (DMN) (20). Further, a recent study examining volume and functional connectivity of the hippocampus across the menstrual cycle scanned the same group of women at four points of their menstrual cycle (early follicular, late follicular, ovulation and late luteal). They reported increased functional connectivity of both the right and left hippocampus in the late follicular phase

compared to the early follicular and late luteal phases (21). A longitudinal study scanned the same woman (n=1) 32 times over the course of four menstrual cycles and reported a correlation of P4 levels with intrinsic connectivity changes in the right dlPFC and bilateral sensorimotor cortex (22). Finally, a SBA study found sex- and estrogen level-dependent influences in Rs-FC in the left and right laterobasal and centromedial amygdala (23). Here it is important to note that none of these studies assessed or controlled for the presence of premenstrual symptoms or premenstrual dysphoric disorder (PMDD). This may be problematic because PMDD has been associated with alterations in brain functional connectivity and in affective and cognitive processing during the late luteal phase (24-28).

The influence of sex hormones on functional networks involved in cognitive, emotional and self-referential processing is vital to our understanding of brain activity during periods of endogenous hormonal fluctuation. Numerous RSNs have been identified. Among those, six commonly studied networks include the default mode network (DMN), salience network (SN), fronto-parietal network (FPN), meso-paralimbic network (MPN), visual network (VN) and sensorimotor network (SMN) (*Table 1)* (29-32).

The objectives of the present study were to examine (1) the Rs-FC of the six RSNs mentioned above, and (2) the influence of E2, P4, ALLO and DHEAS on Rs-FC during the mid-follicular and late luteal menstrual phases in a well-defined sample of healthy, naturally cycling women with no PMDD. We hypothesized that sex hormones will correlate with Rs-FC in brain regions that are dense in hormone receptors, such as the limbic system and prefrontal cortical regions specific to each menstrual phase. Based on previous studies with repeated-measures design (18,19), we also hypothesized that there would be no differences in Rs-FC of the 6 networks studied through either ICA or SBA between menstrual phases.

2.3 Methodology

2.3.1. Participants

This study was approved by the Hamilton Integrated Research Ethics Board (HiREB) and adhered to the tenets of the Declaration of Helsinki. All participants provided informed written consent. Participants were recruited through community-based advertisements in the Hamilton and Halton Regions, Ontario, Canada.

Twenty-five right-handed women between 16-45 years of age, with regular menstrual cycles (25-32 days) were enrolled. Exclusion criteria included: (1) current or recent (last 3 months) use of systemic hormonal treatment; (2) Pregnancy; (3) contraindications for MRI; (4) history of head trauma resulting in a loss of consciousness; (5) neurological disorders affecting cognition; (6) current or recent (6 months) alcohol or drug abuse or dependence; (7) a lifetime history of any psychiatric disorder according to the SCID-I. Regularly cycling women using levonorgestrel intrauterine device were allowed in the study due to its primarily localized hormonal effect. All women performed at least 2 months of prospective symptom charting using the Daily Record of Severity of Problems (DRSP) (33). Women with greater than a 30% change in the four core PMDD symptoms in their late luteal phase from their mid-follicular phase were excluded. To further ensure women did not present with significant mood or anxiety symptoms, the clinician-rated Montgomery-Asberg Depression Rating Scale (MADRS) (34) and the State Trait Anxiety Inventory (STAI) (35) were used to identify symptoms of depression and anxiety in each menstrual phase.

2.3.2. Study Design

Study participation was comprised of three visits to St. Joseph's Healthcare Hamilton. The first visit consisted of administration of the SCID-I (36) by a psychiatrist or trained PhD

student, followed by a psychiatric and gynaecological history. The second and third visits took place during the mid-follicular phase (days 5-10) and/or late luteal phase (last 5 days before bleeding) of the menstrual cycle. Approximately half of the study participants began with their mid-follicular scan and half with their late luteal. Menstrual cycle phase was confirmed and diagnosis of PMDD was ruled out by 2 months of prospective charting using the DRSP and hormonal assays. Visits two and three included an MRI scan, collection of a blood sample for hormonal assay and completion of validated clinical questionnaires, as described below.

2.3.3. Hormone Assays

Immediately following both MRI scans, 10 ml of whole blood was collected in serum tubes. The blood was clotted at room temperature for 45 minutes, and centrifuged at 20^{o}C for 15 minutes at 3000 rpm. Four serum aliquots were obtained and frozen at -80^{o}C until assayed. Serum was assayed for P4, E2 and DHEAS using commercially prepared solid phase enzyme-linked immunosorbent assay (ELISA) kits, purchased from ALPCO Diagnostics, Salem, NH. In addition, samples were assayed for ALLO, also using ELISA technique purchased from Kiamiya Biomedical Company, Seattle, WA. All serum samples were assayed in duplicate following the manufacturer's protocol, with a fresh aliquot for each analyte. The inter-assay variations for P4, E2, DHEA-S and ALLO were 11.3%, 8.7%, 9.2% and 6.0% respectively. The intra-assay variations were 10.4%, 7.7%, 9.3% and 11.7% and the sensitivities were 0.1 ng/ml, 10 pg/ml, 0.005 ug/ml and 0.52 ng/ml, respectively. A staff gynecologist (D.C.) confirmed that hormone levels were within physiological range for each menstrual phase.

2.3.4. MRI Protocol

2.3.4.1. Image Acquisition and Preprocessing

Images were acquired using a GE whole body short-bore 3T scanner with 8 parallel receiver channels (General Electric, Milwaukee, WI, USA). Anatomical images were acquired with high-resolution T1 weighted images (gradient-echo inversion-recovery sequence, TR=1.6s, TE=5ms, matrix 256x256x128, FOV 220x220mm, slice thickness 1mm). Functional resting state imaging was completed using a T2* interleaved echo-planar imaging (EPI) sequence with TR=2000ms, TE=40ms, flip angle=60°, 4mm thick, 29 axial slices, matrix 64x64 resolution over 256 mm FOV). Of the 129 volumes acquired, the first 2 were discarded to account for T2 stabilization effects. Once positioned in the scanner participants were instructed to "Lay still, relax and try not to think about anything in particular" as they looked at a fixation point. Anatomical and resting state scans took place over 10 minutes and were followed by functional tasks that will be published at a later date.

The resting state and anatomical MRI data were preprocessed using the Statistical Parametric Mapping Software SPM12 (http://www.fil.ion.ucl.ac.uk/spm). Imaging data was obtained in DICOM file format and converted to NIFTI. Anatomic data was segmented to white matter (WM), cerebrospinal fluid (CSF) and grey matter (GM) using affine regularization (light bias regularization=0.001, Bias FWHM = 60mm cut off), according to the ICBM space template for European Brains (37). Subsequent to this, images were normalized to standard space using a 4th Degree B-Spline Interpolation and resampled to 1mm x 1mm x 1mm voxels (38). Deformation fields created during the segmentation process were used during normalization for increased precision in the

alignment of images (39). Resting state images were then motion corrected; estimated using 2nd Degree B-Spline Interpolation and replaced using 4th Degree B-Spline Interpolation (40). Images with motion greater than 3mm in the translational plane and 3 degrees in the rotational plane were discarded. Co-registration of functional images with anatomical images was estimated using the Normalized Mutual Information Function (39). Images were spatially smoothed to increase the signal to noise ratio with a 8 mm FWHM Gaussian filter (41).

2.3.4.2. Seed-Based Analysis (SBA)

SBA was completed using the CONN toolbox v.15.d (https://www.nitrc.org/projects/conn). Subject specific maps of CSF and WM were used as nuisance regressors. The aCompCor strategy was employed within CONN to control for the effects of physiological motion and residual head movement (42). The function images were then temporally band-pass filtered (0.008-0.09 Hz). The posterior cingulate cortex (PCC), bilateral dorsolateral prefrontal cortices (dlPFC) bilateral anterior insula, bilateral amygdalae, visual cortex (V1) and bilateral somatosensory cortices (BA 2) were used as seed-points for the DMN, FPN, SN, MPN, VN and SMN respectively, in a ROI-ROI analysis using the Brodmann Area and Harvard-Oxford Atlas available with CONN (43). Statistical analyses were completed using CONN using a two-sample t-test comparing activation between groups. Activation in regions of interest were corrected for multiple comparisons using False Discovery Rate (FDR; $p<0.05$). Serum levels of E2, P4, ALLO and DHEAS from the mid-follicular and late luteal menstrual phases were added as second-level covariates in CONN and their correlation to patterns of functional coupling were assessed through second-level analysis.

2.3.4.3. Independent Component Analysis (ICA)

Group ICA was performed using GIFT software, version 3.0a (http://mialab.mrn.org/software/gift/index.html). Detailed descriptions of methods employed in this program have been published elsewhere (17). The minimum description length (MDL) criteria were used to determine 34 independent components (ICs). Subject-specific data was reduced from 129 time-points to 51 time-points and further to 34 ICs using subject-specific principle component analysis. ICs were estimated using the Infomax algorithm and repeated in ICASSO 10 times (44). Group level spatial and time course maps were then back-constructed using the GIGA method and calibrated into z-scores, normalized across all subjects. Networks of interest were identified by visual inspection and supported by previous literature (29-32, 45).

Spectral characteristics (dynamic range and low frequency to high frequency power ratio) of the 3 identified networks were examined using the same procedure as Allen et al (45). Dynamic range was estimated as the difference between the peak spectral power and minimum spectral power at frequencies to the right of the peak. The low frequency to high frequency power ratio was below 0.10 Hz to the integral of power between 0.15 Hz and 0.25 Hz. This criterion was used to confirm that all networks analyzed were dominated by low frequency BOLD fluctuations in the 0.01 - 0.1 Hz range (45)

Statistical analysis was completed with SPM12 using the subject-specific z-maps of the 6 RSN studied (*Figure 1*). The DMN and FPN were split and each found between two components, the anterior and posterior DMN and right and left FPN. Two-sample t-tests were performed on the 8 components of interest to compare network connectivity

between groups. A p-value of <0.05 (Family-wise error, FWE) was considered significant.

2.3.4.4. Statistical Analysis

Statistical analyses of hormonal levels and clinical questionnaires were completed using R version 3.1.2. (https://www.r-project.org). Each clinical questionnaire was scored according to its respective criteria and an overall score was obtained for each participant. A Shapiro-Wilks test was used to determine whether the clinical variables were normally distributed. Hormonal levels were analyzed using a paired t-test, Wilcoxon rank test, or two-sample t-test where applicable. A p-value <0.05 was considered significant.

2.4. Results

2.4.1. Demographic Characteristics

Our sample was on average 27 years old, had approximately 17 years of education and a healthy BMI *(Table 2)*. Nine women (36%) had never used hormonal contraception. DRSP, MADRS and STAI scores did not change across the menstrual cycle. E2, P4, ALLO and DHEAS levels were all within expected normal physiological range *(Table 2)*.

2.4.2. Functional Connectivity

Rs-FC was analyzed using a multimodal approach through the use of both SBA and ICA to obtain a more comprehensive picture of Rs-FC throughout the menstrual cycle. Consistent with our hypothesis, we did not find any differences in Rs-FC between late luteal and mid-follicular phases of the menstrual cycle using a SBA when using the PCC, primary visual cortex, primary somatosensory cortex, bilateral dlPFC, bilateral anterior insula, and bilateral amygdalae as seed points for their respective networks (all $p>0.05$, FDR-corrected). Similarly, we did not find differences in Rs-FC between menstrual phases when using ICA; no differences were found in the DMN, FPN, SN, MPN, VN and SMN between menstrual phases (all $p>0.05$, FWE-corrected).

2.4.3. Hormonal Correlations to Functional Connectivity

To investigate the potential influence of hormonal fluctuations across the menstrual cycle on Rs-FC we correlated levels of E2, P4, ALLO and DHEAS to seed-based Rs-FC. *Figure 2* depicts the influence of each hormone on brain functional coupling. In the mid-follicular phase, we found that the hormones were correlated with 9

patterns of functional connectivity (7 of which were negative correlations, and 8 of which were bidirectional). A substantially greater amount of correlations were seen during the late luteal phase, where 47 correlations between hormones and functional connectivity were identified (32 of which were bidirectional, and 28 were positive correlations). The left primary auditory cortex (BA 42) displayed the greatest number of correlations (8 correlations) with DHEAS in the late luteal phase. Hormones were correlated with Rs-FC between components of all RSNs studied *(Supplementary Figure 2*; all FDR-corrected).

2.5. Discussion

This study was the first to use a multimodal approach to investigate differences in Rs-FC across the menstrual cycle in a well-characterized sample of healthy women using repeated measures. This study expands the current body of literature on endogenous hormonal fluctuations by correlating E2, P4, ALLO and DHEAS levels with patterns of Rs-FC within the mid-follicular and late luteal phases. Consistent with our hypotheses, multiple patterns of brain functional coupling were correlated with each of the hormones within each menstrual phase, and to a much greater degree in the late-luteal phase, as discussed below. Our study provides evidence supporting an association between endogenous hormones and resting state activation in cortical and subcortical regions implicated in cognitive and emotional processes within the mid-follicular and late-luteal menstrual phases. The exact physiological relevance of this association between hormonal levels and brain function remains to be determined. Since women in our sample did not report symptoms of PMS, this pattern of brain connectivity was not associated with clinically significant changes in mood. This is consistent with previous functional MRI studies revealing menstrual phase-specific patterns of functional connectivity in subcortical and cortical regions that were modulated, in part, by endogenous E2 and P4 levels (3,16).

Research on the impact of the menstrual cycle on Rs-FC in healthy women is sparse. Our ICA findings showing no differences in functional connectivity between menstrual phases are consistent with two previous studies that also used repeated-measures design. Specifically, De Bondt and colleagues scanned 18 women during their follicular, ovulatory and luteal phases and found no differences in the functional connectivity of the DMN or central executive network (CEN) using ICA, between each of the three phased studied (19). A similar three-phase study design was used by Hjelmervik and colleagues, in which 16 healthy women were scanned during their menstrual (days 2-4), follicular (days 8-12) and luteal (20-22) phases (18). No significant differences in functional connectivity of the left and right dorsal networks, ventral network and anterior network were found between menstrual cycle phases using ICA. To our knowledge, the only study that found menstrual phase-specific differences in Rs-FC using ICA actually compared two different groups of women (20). In this study, women in the follicular phase displayed heightened ACC activity, a critical aspect of the executive control network, relative to a different group of women scanned in the luteal phase (20). Thus, this latter study may be methodologically flawed in its attempt to provide a true picture of changes in neural activation through the menstrual cycle given its lack of repeated measures.

2.5.1. Estradiol (E2)

During the mid-follicular phase, E2 levels were negatively correlated with Rs-FC between the bilateral entorhinal cortices (pEC-L, pEC-R), brain regions that are part of the MPN. In the late luteal phase, E2 levels were positive correlated with functional coupling between the left amygdala, bilateral somatosensory cortices and right motor cortex, brain areas associated with the MPN and SMN. This finding may suggest an influence of E2 on communication between the amygdala, an area dense in E2 receptors, and the somatosensory cortex, an area that has been implicated in human perception of emotional empathy (47,48). Previous studies have suggested

that mirroring - creating somatosensory representations of another individual's actions - is central to the development of emotional empathy (47,48). Interestingly, it has been found that females display greater activation in brain regions containing mirror neurons during empathetic face-to-face interaction compared to men (49). It is conceivable that this phenomenon may be, at least in part, modulated by differences in sex differences in hormonal milieu.

2.5.2. Progesterone (P4)

P4 displayed different patterns of correlations in each phase studied: negative correlations with MPN regions were found during the mid-follicular phase and positive correlations with FPN regions were observed in the late luteal phase. P4 also displayed menstrual cycle phase-specific correlations with DMN regions; this was exemplified by the negative correlation between P4 and left inferior temporal gyrus (ITG)-left fusiform gyrus (FG) coupling in the mid-follicular phase, and positive correlation between left-ITG-left dorsal frontal cortex (dFC) coupling in the late luteal phase. Previous research showed that P4 administration decreased activity of the FG, an area critical to face expression processing, which may consequently impair emotional memory (50). Moreover, considering that the inferior temporal cortex (ITC) plays an important role in the storage of visual long-term memory (51), this pattern of coupling may suggest a role for P4 in long-term emotional and visual memory processing. Studies in non-human primates show that top-down modulation of temporal regions controlling memory formation is also mediated by dorsal frontal regions (51). In this context, the positive correlation between P4 levels and left-ITG-left-dFC coupling could also explain how P4 may influence memory formation. This hypothesis is consistent with previous findings showing increased engagement of the left inferior frontal gyrus (IFG) during verbal and implicit memory tasks during the mid-luteal phase, a time of high progesterone (3,52,53).

2.5.3. GABA-mediating Hormones: ALLO and DHEAS

ALLO showed distinct correlations with different DMN regions in each menstrual cycle phase. In the mid-follicular phase, serum ALLO levels were negatively correlated with posterior cingulate cortex (PCC) and somatosensory association cortex (S2) coupling, brain regions involved in self-referential processing and empathy. In the late luteal phase, ALLO levels were correlated with widespread patterns of coupling between the medial prefrontal cortex (mPFC) and many cortical areas including the primary (V1) and associative visual (V2, V3) cortex, central regions of the visual network (VN). The associative visual cortex and its relationship with GABA is well-established, and has been highlighted by research in healthy women, as well as individuals with PMDD, and depressive and anxiety disorders (54-57). Notably, Epperson and colleagues studied GABA concentration in the occipital cortex using MR spectroscopy and found that in healthy women, cortical GABA levels drop from the follicular to mid and late luteal phases, which is consistent with our finding of a negative correlation between ALLO and VN-related coupling in the late luteal phase (56). Moreover, ALLO was positively correlated with mPFC-bilateral pEC coupling, a limbic region with neural connections projecting to the hippocampus. The correlations of ALLO with mPFC, and thereby DMN activity, may be related to the positive influence of ALLO and GABAa binding in the cortex of limbic regions and negative influence between cognitive and visual regions inherent to higher order cognitive processes. DHEAS, another GABAa mediating hormone, displayed a complex web of correlations with various coupling patterns in the late luteal phase largely stemming from the primary auditory cortex (A1), located in a key hub of the MPN. This may highlight the role of DHEAS in mediating bottom up processing of VN, DMN, SN, SMN and cognitive areas by acting on the MPN (58).

The limitations of our study deserve attention. First, the DRSP is a self-administered tool used to chart symptoms of PMS across the menstrual cycle. It is possible that women may provide

an inaccurate account of their premenstrual symptoms or that use of the DRSP may be skewed by stressful life events, which the DRSP has no place to document. However, the use of a clinician-rated scale (MADRS) increased our confidence in the self-reported results. Second, Rs-fMRI only provides an indirect measure of spontaneous neuronal activity in the ultraslow frequency range (0.01 - 0.1 Hz Hz) (45). However, studies combining fMRI and electroencephalogram (a direct measure of electrical neuronal activity in broader and higher frequency ranges) were able to identify common hemodynamic and electrical oscillations in the brain at rest (see review, 59). Furthermore, the Rs-fMRI technique is based on an oversimplified assumption that BOLD activation measured is static through the entire scanning paradigm, inability to control participant's memory in the scanner and participant's ability to remain awake during the entire scanning paradigm (60, 61). Although participants were advised to not think about anything in particular and remain awake with their eyes focused on a fixation point throughout the duration of the brain scan, there was no objective measure (e.g. simultaneous electroencephalogram, eye tracking) to confirm that they followed these instructions. In addition, our SBA and hormonal findings are based on correlations and cannot imply causation; in other words, we cannot determine that the hormone level directly changed or modulated the patterns of functional coupling seen, and consequently our results should be interpreted accordingly. All Rs-FC analyses were completed using strict methodology; multiple comparison correction specific to analysis technique used was employed to provide a conservative estimate of correlations between hormones and functional connectivity, and functional connectivity between menstrual phases. Due to the challenges noted in the use of FWE-correction for cluster-wise inference, FDR-correction was used to correct for multiple comparisons in all SBA and ROI-based hormonal correlations to functional coupling (62, 63). FWE-correction was used to correct of for multiple comparisons in the ICA voxel-based inference.

In conclusion, we found robust, menstrual phase-specific correlations of hormonal levels with patterns of Rs-FC. However, in a sample of healthy women experiencing minimal premenstrual symptoms, these patterns of brain connectivity were not associated with any clinically significant changes in mood. In addition, using both ICA and SBA we found no differences in Rs-FC between the mid-follicular and late-luteal phases in women with no history of PMDD. Future studies should explore if this interaction between hormonal levels and resting-state brain activation may influence cognitive, behavioral and/or emotional processes in regularly cycling women.

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Table 1: Resting state networks studied and corresponding brain and seed regions

Table 1: Resting state networks studied and corresponding brain and seed regions			
Network	Goal	Brain Regions	Seed Region (SBA)
Default Mode Network (DMN)	Activated during internally directed thoughts/self referential processing. Strongly deactivated during goal/task directed activity	Medial prefrontal cortex, posterior cingulate cortex, precuneus , lateral parietal cortex/ angular gyrus, inferior temporal gyrus	Posterior Cingulate Cortex (PCC)
(Meso/Paralimbic Network) (MPN)	Processing of emotional and introspective information	Amygdala, hippocampus, parahippocampal gyrus, temporal poles	Left and Right Amygdala
Left/Right Fronto/Parietal Networks (FPN)	Right is responsible for cognitive control and attention Left is responsible for language processing and working memory	Lateral prefrontal regions, inferior parietal cortex	Left and Right Dorsolateral Prefrontal Cortex (dlPFC) (BA 46)
Salience Network (SN)	Detection of salient stimuli	Anterior insula, anterior cingulate cortex	Left and Right Insular Cortex (BA 13)
Visual Network	Attention to visual stimuli	Primary and association visual cortices	BA 17, 18, 19 (V1, V2, V3)
Sensorimotor Network	Execution of motor actions and somatosensory perception	Supplementary motor area, sensorimotor cortex and secondary somatosensory cortex	BA 1-7

Table 2: Demographic characteristics of study sample (n=25): All hormone levels were found to be within normal range for each menstrual phase assessed.

Table 2: Demographic characteristics of study sample (n=25)			
Age, mean (SD)	27.4 (7.7)		
BMI, mean (SD)	23.2 (3.3)		
Years of Education, mean (SD)	16.9 (2.6)		
Age at Menarche, mean (SD)	11.9 (1.4)		
Clinical Variables, mean (SD)	**Mid-Follicular**	**Late-Luteal**	***p* value**
MADRS	2.6 (2.9)	2.2 (2.9)	0.81
STAI-State	29.2 (6.8)	31.0 (7.3)	0.23
STAI-Trait	30.1 (7.3)	30.7 (9.4)	0.44
Hormone Levels, mean (SD)			
Estradiol (pg/ml)	70.58 (45.6)	85.7 (51.5)	p<0.0001
Progesterone (ng/ml)	1.30 (0.92)	4.9 (3.0)	0.219
Allopregnanolone (ng/ml)	4.12 (1.61)	4.89 (2.01)	0.173
DHEAS (ug/dl)	163.5 (77.2)	155.9 (73.9)	0.241

Figure 1: Networks of interest identified through ICA: One-sample t-test maps of networks of interest, ($p<0.05$, FWE-corrected). A. Anterior DMN; B. Posterior DMN; C. Right FPN; D. Left FPN; E. MPN; F. VN; G. SN; H. SMN.

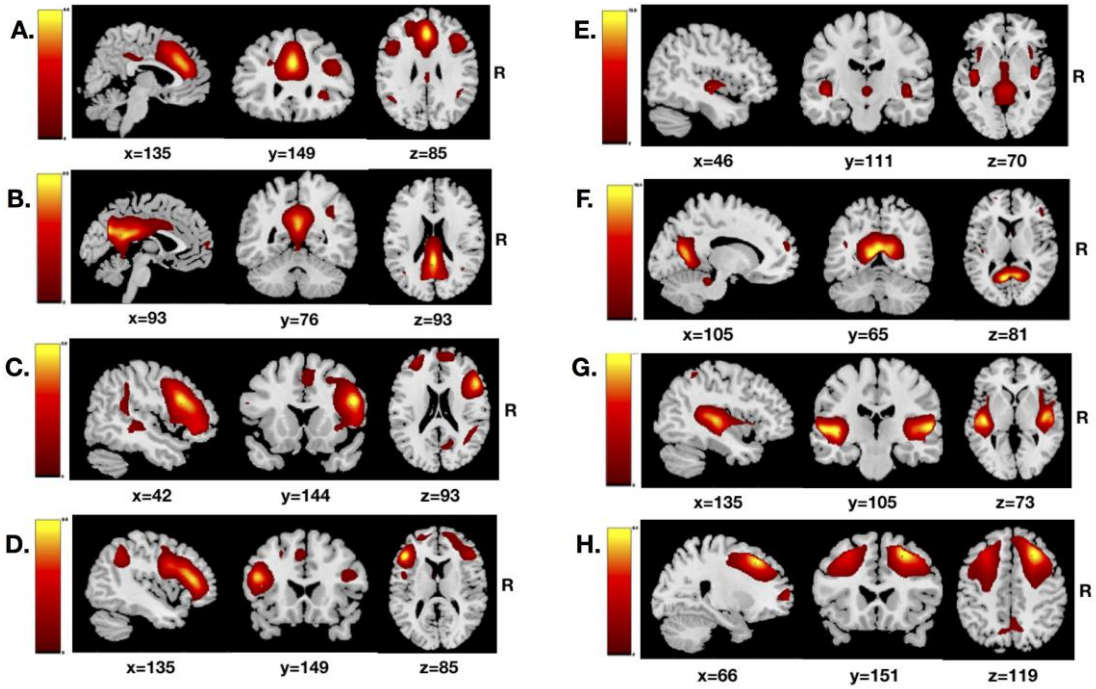
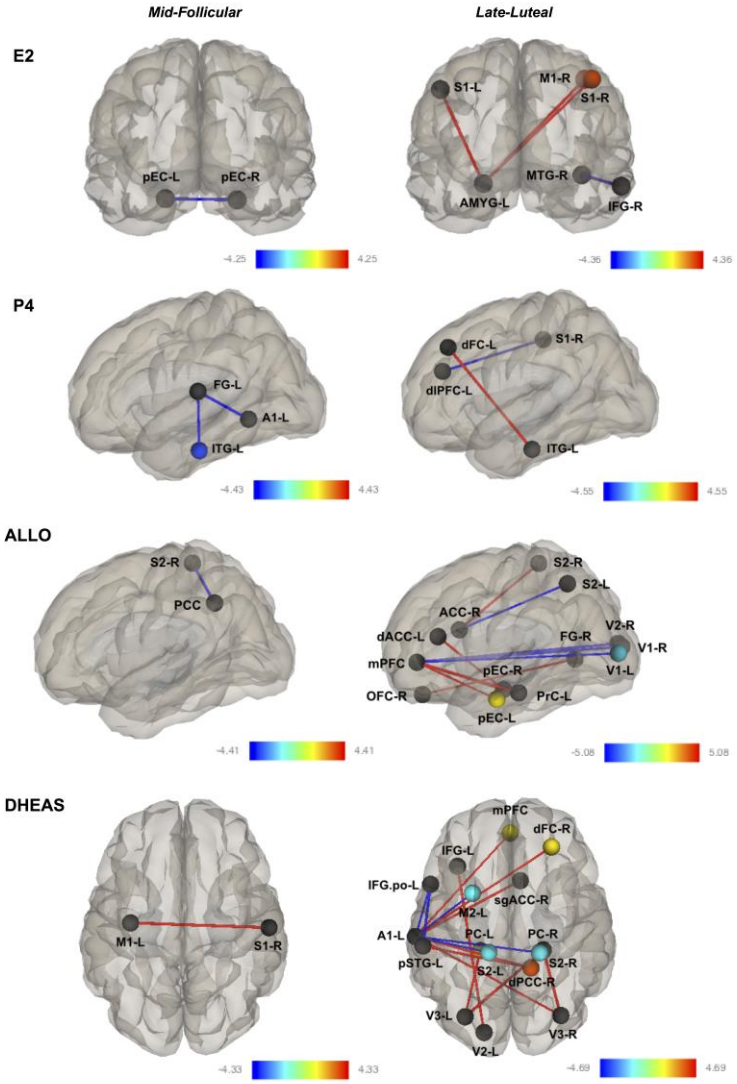


Figure 2: Correlations between hormones and functional coupling in the mid-follicular and late luteal phase: Negative correlations between hormones and patterns of coupling are represented in blue, positive correlations represented in red. Intensity of correlations (T values) are represented in the colour legend, specific to menstrual phase and hormone. A detailed list of correlation specific ROIs, Brodmann Areas, R and T values can be found in Supplementary Material 2.



Supplementary Figure 1: Statistical profile of menstrual phase specific hormone correlations with patterns of functional coupling

Supplementary Figure 1: Correlation between menstrual phase specific hormone levels and key regions of networks of interest								
Network	**P4**		**E2**		**ALLO**		**DHEAS**	
	F	L	F	L	F	L	F	L
DMN	✓	✓			✓	✓		✓
SN						✓		✓
FPN		✓						✓
MPN	✓		✓	✓		✓		✓
SMN		✓		✓	✓	✓	✓	✓
VN						✓		✓

Supplementary Figure 2: Correlation between menstrual phase specific hormone levels and key regions of networks of interest: Hormone levels were correlated with key regions of networks studied in the mid-follicular phase (F) and late luteal phase (L).

Phase and Hormone	Seed	Brodmann	Target	Brodmann	T-Value	R-Value	P-Value
Follicular: P4	FusiformG	37.L	A1	42.L	-4.43	-0.678534664761348	0.019
Follicular: P4	A1	42.L	FusiformG	37.L	-4.43	-0.678534664761348	0.019
Follicular: P4	A1	42.L	ITG	20.L	-3.83	-0.624032638327588	0.043
Follicular: E2	pEC	28.L	pEC	28.R	-4.25	-0.66323316744474	0.035
Follicular: E2	pEC	28.R	pEC	28.L	-4.25	-0.66323316744474	0.035
Follicular: ALLO	PCC	NA	S2	5.L	-4.41	-0.676876539267981	0.02
Follicular: ALLO	PCC	NA	S2	5.L	-4.41	-0.676876539267981	0.02
Follicular: DHEAS	S1	1.R	M1	4.L	4.33	0.670139747621005	0.024
Follicular: DHEAS	M1	4.L	S1	1.R	4.33	0.670139747621005	0.024
Luteal: P4	S1	2.R	dlPFC	9.L	-4.18	-0.657047553328204	0.036
Luteal: P4	dlPFC	9.L	S1	2.R	-4.18	-0.657047553328204	0.036
Luteal: P4	ITG	20.L	DFC	8.L	4.55	0.688269074129324	0.014
Luteal: P4	DFC	8.L	ITG	20.L	4.55	0.688269074129324	0.014
Luteal:E2	S1	2.L	AMYG	NA	4.32	0.669285790534408	0.025
Luteal:E2	MTG	21.R	IFG	47.R	-4.36	-0.672685731857913	0.023
Luteal:E2	IFG	47.R	MTG	21.R	-4.36	-0.672685731857913	0.023
Luteal:E2	AMYG	NA	S1	2.L	4.32	0.669285790534408	0.025
Luteal:E2	AMYG	NA	M1	4.R	4.02	0.642395505093554	0.027
Luteal:E2	AMYG	NA	S1	3.R	3.67	0.607721683495268	0.042
Luteal: ALLO	mPFC	NA	V2	18.R	-5.08	-0.727152204030118	0.002
Luteal: ALLO	mPFC	NA	pEC	28.R	4.92	0.716085140480157	0.002
Luteal: ALLO	mPFC	NA	V1	17.R	-4.44	-0.679359858396917	0.004

Luteal: ALLO	mPFC	NA	PC	35.L	4.43	0.678534664761348	0.004
Luteal: ALLO	mPFC	NA	V1	17.L	-3.71	-0.611873916703507	0.02
Luteal: ALLO	mPFC	NA	pEC	28.L	3.52	0.59169776364379	0.03
Luteal: ALLO	ACC	33.R	S2	7.L	-4.06	-0.646126661299569	0.04
Luteal: ALLO	ACC	33.R	S2	5.L	4.47	0.68182005368301	0.01
Luteal: ALLO	FusiformG	37.R	OFC	11.R	4.16	0.655255440231881	0.03
Luteal: ALLO	PC	35.L	mPFC	NA	4.43	0.678534664761348	0.004
Luteal: ALLO	S2	7.L	ACC	33.R	-4.06	-0.646126661299569	0.04
Luteal: ALLO	S2	5.L	ACC	33.R	4.47	0.68182005368301	0.01
Luteal: ALLO	dACC	32.L	pEC	28.R	4.21	0.659714983968856	0.03
Luteal: ALLO	pEC	28.R	dACC	32.L	4.21	0.659714983968856	0.03
Luteal: ALLO	pEC	28.R	mPFC	NA	4.92	0.716085140480157	0.005
Luteal: ALLO	V2	18.R	mPFC	NA	-5.08	-0.727152204030118	0.003
Luteal: ALLO	V1	17.R	mPFC	NA	-4.44	-0.679359858396917	0.019
Luteal: ALLO	OFC	11.R	FusiformG	37.R	4.16	0.655255440231881	0.038
Luteal: DHEAS	pSTG	NA	IFC.po	44.L	-4.37	-0.673529123007219	0.02
Luteal: DHEAS	pSTG	NA	dPCC	31.R	3.85	0.626016260162602	0.04
Luteal: DHEAS	IFG	NA	V2	18.L	4.16	0.655255440231881	0.038
Luteal: DHEAS	IFC.po	44.L	pSTG	NA	-4.37	-0.673529123007219	0.02
Luteal: DHEAS	A1	42.L	sgACC	25.R	4.32	0.669285790534408	0.012
Luteal: DHEAS	A1	42.L	dPCC	31.R	3.72	0.612904147557447	0.03
Luteal: DHEAS	A1	42.L	dFC	8.R	3.67	0.607721683495268	0.03
Luteal: DHEAS	A1	42.L	V3	19.R	3.54	0.593876143761428	0.035
Luteal: DHEAS	A1	42.L	S2	5.R	-3.42	-0.580609179869253	0.035
Luteal: DHEAS	A1	42.L	IFC.po	44.L	-3.37	-0.574940517383295	0.035
Luteal: DHEAS	A1	42.L	S2	5.L	-3.33	-0.570345132910266	0.035
Luteal: DHEAS	A1	42.L	M2	6.L	-3.26	-0.562172585947854	0.035
Luteal: DHEAS	A1	42.L	mPFC	NA	3.26	0.562172585947854	0.035

Luteal: DHEAS	PC	27.R	V3	19.L	4.69	0.699174219170907	0.006
Luteal: DHEAS	PC	27.R	V3	19.R	4.56	0.68906393355529	0.006
Luteal: DHEAS	PC	27.R	ITG	20.L	3.51	0.590603687514553	0.04
Luteal: DHEAS	sgACC	25.R	A1	42.L	4.32	0.669285790534408	0.02
Luteal: DHEAS	V3	19.R	PC	27.R	4.56	0.68906393355529	0.013
Luteal: DHEAS	V2	18.L	IFG	NA	4.16	0.655255440231881	0.03

Chapter 3: Resting State Functional Connectivity in Bipolar Disorder during Clinical Remission: A Systematic Review

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3.1 Abstract

Introduction: Bipolar Disorder (BD) is chronic and debilitating. Studies investigating resting state functional connectivity (Rs-FC) in BD may help to inform neurobiological models of illness.

Methods: We conducted a systematic review with the following goals (1) summarize Rs-FC literature in BD during clinical remission (euthymia) compared to healthy controls (CTRL); (2) critically appraise literature and research gaps; and (3) propose directions for future research. Pubmed/Embase, PsychInfo, CINAHL and grey literature were searched up to April 2017.

Results: Twenty-three studies were included. Consistent patterns of Rs-FC were identified using seed based analysis (SBA) and independent component analysis (ICA). The most consistent finding was the absence of differences in Rs-FC of the default mode network (DMN), frontoparietal network (FPN) and salience network (SN) using ICA between BD and CTRL. Two studies with a bipolar sample entirely positive for psychosis history were exceptions, and reported DMN hypoconnectivity. Studies using SBA largely reported aberrant Rs-FC with the amygdala, ventrolateral PFC, cingulate cortex and medial PFC in BD compared to CTRL. Few studies used regional homogeneity (ReHO) and amplitude of low-frequency fluctuations (ALFF).

Conclusions: Stability of the DMN, SN and FPN may reflect a state of bipolar remission. Further, DMN hypoconnectivity may reflect a positive history of psychosis in patients with BD compared to controls; highlighting a potentially different neural phenotype of psychosis in BD. Rs-FC changes between the amygdala, PFC and cingulate cortex may reflect (i) a neural correlate of sub-threshold symptoms experienced during BD euthymia; (ii) the trait-based pathophysiology of BD.

3.2. Introduction

Bipolar Disorder (BD) is a major mental illness characterized by discrete periods of depression and mania (Bipolar Disorder type I; BD-I) or hypomania (Bipolar Disorder type II; BD-II), including changes in sleep, appetite and psychomotor activity (Green et al. 2007; Strakowski et al. 2012; Wessa et al. 2014). Due to its severity, chronicity and early age of onset, BD is considered the 5th leading cause of disability among mental health and substance use disorders (Ferrari et al. 2016). BD also carries a significantly elevated risk of suicide and psychiatric comorbidity which further contribute to its illness burden (Merikangas et al. 2011). Cognitive impairment and emotional lability are common clinical features of BD and these features are present not only during acute mood episodes, but also during periods of clinical remission (euthymia) (Olley et al. 2005). Advancement in neuroimaging techniques in recent decades have led to an increase in the use of functional magnetic resonance imaging (fMRI) in the study of brain activation and connectivity patterns in BD (Townsend and Altshuler 2012; Vargas et al. 2013). To a large degree, resting state functional connectivity (Rs-FC) and task-based fMRI studies of BD patients during acute mood episodes have consistently found abnormal activity in brain regions implicated in cognitive and emotional processing. However, research during the euthymic phase has been less consistent (Townsend and Altshuler 2012; Vargas et al. 2013). Based on neuroimaging and post-mortem research, a number of neurobiological models of BD have been proposed, the majority of which suggest that BD is associated with dysfunction in dorsal and ventral neural streams (Phillips and Vieta 2007; Strakowski et al. 2012; Phillips and Swartz 2014). The dorsal network plays an integral role in mediating cognitive processing and executive functioning; it is typically comprised of the dorsolateral prefrontal cortex (dlPFC), ventrolateral prefrontal cortex (vlPFC), dorsal anterior cingulate cortex (dACC) and hippocampus. The insula, amygdala, ventral striatum, ventral anterior cingulate cortex (vACC)

and ventromedial prefrontal cortex are involved in implicit aspects of emotional regulation and encompass the ventral neural stream (Phillips and Vieta 2007; Strakowski et al. 2012; Phillips and Swartz 2014).

Rs-FC measures alterations in the blood-oxygen-level-dependent (BOLD) signal across the brain in the absence of specific engagement in cognitive or emotional tasks (Fox and Raichle 2007). Participants commonly gaze at a fixation point or lay with their eyes closed for the duration of the scan. Therefore, Rs-FC provides an indirect measure of neuronal activation patterns that occur without the influence of task or emotional or cognitive processing.

Rs-FC is commonly examined through the use of independent component analysis (ICA), seed-based analysis (SBA) and by investigating localized properties of spontaneous activity, such as amplitude of low frequency fluctuation (ALFF), fractional ALFF (fALFF), and regional homogeneity (ReHo). ICA is an exploratory, data-driven approach that maximizes statistical independence by constructing spatial maps of BOLD signal time-courses that are independent from one another (Calhoun et al. 2009). SBA is a hypothesis driven approach, which correlates the BOLD activation in a predefined "seed" region with activation during the same time course in other brain regions (Whitfield-Gabrieli and Nieto-Castanon 2012). ReHo measures the functional connectivity of a given voxel in the brain and its neighborhood voxels (Zang et al. 2004), and ALFF/fALFF are used to detect regional changes in spontaneous brain activity by measuring the amplitude of low frequency fluctuations in BOLD signal (Zou et al. 2008; Zhou et al. 2010). While these techniques assume that functional connectivity remains static through the resting state scan, dynamic functional connectivity (dFNC) is based on the principle that dynamic changes in Rs-FC occur through the course of a RS fMRI scan (Calhoun 2014). The diversity in these different methodologies used to analyze brain activation at rest often makes it difficult to establish consensus among related studies in the literature. A systematic review of the breadth of

findings would help in determining consistent patterns of brain connectivity reported in BD at rest, identify the inconsistencies and the main gaps in the literature, and ultimately guide future research. Thus, the aim of this current systematic review was to (1) systematically review the current literature regarding Rs-FC in BD during clinical remission (euthymia); (2) provide a critical appraisal of the literature in this field including the research gaps; and (3) propose directions for future research.

3.3 Methodology

This systematic review was formulated in accordance with the Preferred Reporting Items for Systematic review and Meta-Analysis (PRISMA) guidelines (Moher et al. 2009).

3.3.1 Eligibility Criteria

This systematic review included original studies with a principal objective of investigating Rs-FC in individuals with BD during euthymia, as well as Rs-FC studies reporting sub-analyses containing a well-identified euthymic BD population. Studies were included if: (1) the sample had a clearly defined diagnosis of BD, according to validated diagnostic tools; (2) at least a subset of the Rs-FC results were solely with reference to a euthymic population; (3) there was a healthy control comparison group, with no lifetime psychiatric history; (4) at least one of the following techniques were used: SBA, ICA, ReHo, ALFF, fALFF, or dFNC; (5) the cluster size was over 50 voxels for significant results (Wang and Li 2013); (6) appropriate control for multiple comparisons and/or statistical thresholds was used; and (7) the age of the sample was 18 years or over. Paediatric studies were excluded from this review. The study sample should also have been free of neurological disorder or learning disabilities.

3.3.2 Information Sources

Relevant studies published in English language were identified from Pubmed/MEDLINE, EMBASE, PsycINFO and grey literature, with no time restriction. This search was conducted again prior to submission for publication to ensure that literature contained in the final publication encompassed all current and relevant literature. To maximize literature retrieved the search terms "Bipolar Disorder", "Bipolar Affective Disorder", "Bipolar I Disorder", "Bipolar II Disorder", "Cyclothymia/Cyclothymic Disorder", "Rapid-Cycling Bipolar Disorder", "Bipolar Depression", and "Bipolar Mania" were used with the Boolean Operator OR. Further, the terms "Resting State Functional Connectivity", "Functional Connectivity", "Resting State Network", and "Functional Magnetic Resonance Imaging", were joined with the Boolean Operator OR and connected to the search terms above using the Boolean Operator AND. A detailed search strategy is available in Appendix 1.

Since many publications on Rs-FC in BD contain sub-analysis with a euthymic population, we did not use "Euthymic", "Euthymia", "Remission" or "Inter-critical" as search terms, to avoid unnecessarily excluding literature with sub-sample analysis. Co-authors and a librarian reviewed search strategies. Only original articles were included in this systematic review. Reviews, case reports and conference abstracts were excluded. Repeated articles and duplicate searches were removed.

3.3.3 Data Screening and Collection

Two reviewers (S.S. and M.S.) independently reviewed and selected papers, based on titles and abstracts. Data was then extracted into a predetermined data extraction form, (see Appendix 2). Reviewers recorded the following information (1) Study characteristics: first author, year of publication, journal; (2) Demographic Information: sample size, measures used to confirm diagnosis and clinical definition of remission/euthymia; (3) Neuroimaging information: Scanner

model and type, technique used, pre-processing and analysis programs used (4) Description of results, with Montreal Neurological Institute (MNI) or Talairach coordinates, cluster size and probability values (t/p/z values) where applicable. Further, studies investigating the neural correlates of psychosis often use a sample of BD with and without psychosis and schizophrenia. Due to the scope of this review, comparisons were only made between BD and healthy control groups. In the case of discordance between the two reviewers, a third reviewer (L.M.) was consulted.

3.4. Results

3.4.1 Study Selection

Our initial search identified 2125 possible research studies. Following screening of titles and removal of duplicate entries 86 studies remained. The abstracts of these remaining studies were screened and yielded 59 full-text papers, which were then screened further for inclusion in the study. Data was extracted from 23 papers. The primary reasons for exclusion of identified literature were the absence of a euthymic sample or sub-sample and use of task-based or use of an MRI analysis method other than Rs-FC.

3.4.2 Sample Population

Out of the 23 studies included, only 8 had a primary objective of studying Rs-FC in bipolar euthymia compared to controls (Table 1). The remaining 15 used an additional comparative group, which included: 9 with schizophrenia or schizoaffective disorder, 1 with borderline personality disorder, 2 with mixed mood states, 2 with BD mania and 1 with BD depression. Each of these 15 studies contained sub-analysis from which results were extracted for the purpose of this review.

Cumulatively, 897 patients with BD and 1030 healthy controls were analyzed from these 23 studies. Two of the 23 studies assessed a sample of women only, of which only one controlled for menstrual phase by scanning individuals in the mid-follicular phase – a period when hormonal fluctuations are least likely to influence mood. Individuals with BD were on average 34 years of age with a standard deviation of 5.26 years. All studies confirmed a diagnosis of BD using validated diagnostic tools such as the Structured Clinical Diagnostic Interview for DSM-IV (First 2002) or the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al. 1997). Only 3 studies imposed a maximum cut off score on the Young Mania Rating Scale (YMRS) (Young et al. 1978) and the Hamilton Depression Inventory (HAMD) (Hamilton 1960) or Montgomery Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg 1979) in addition to use of the SCID or MINI. Additionally, 3 studies used the Beck Depression Inventory (BDI-II) (Hautzinger et al. 2006) and the Beck Rafaelsen Mania Scale (BRMAS) (Bech 1981) with clinically meaningful cut offs of 18 and 7, respectively. Most studies reported inclusion and exclusion criteria and confirmed euthymia using well-validated measures. Criteria for inclusion in either the BD or HC groups were similar across research studies. BD subjects were stable for a minimum of 2 weeks to 2 months prior to scanning, adding some element of heterogeneity to the study sample. However, as mentioned previously, euthymia was indeed confirmed in all cases using well-validated measures, such as the SCID or MINI (First 2002; Sheehan et al 1997). Two studies (Rashid et al. 2014; Rey et al. 2016) neglected to report detailed inclusion or exclusion criteria for BD subjects.

Current available literature largely comprised studies of BD-I subjects with three studies using a mixed sample and one studying a sample with BD-II. Psychosis history has been found to alter Rs-FC in patients with BD and as a result should be reported in the demographics section of manuscripts. Thirteen studies did not report their participants' history of psychosis, whereas 6

studies used a sample with BD-I of which all patients endorsed lifetime history of psychotic symptoms, 4 studies contained samples with a partial history of psychosis and studies by Anticevic at el. made comparisons between a sample of patients with and without a history of psychosis (Anticevic et al. 2013; Anticevic et al. 2014b; Anticevic et al. 2014a).

Little is known about the influence of psychotropic medications on Rs-FC. Therefore a sub-analysis reporting the effect of psychotropic medication on Rs-FC results in BD euthymia would serve as an important tool for highlighting medication effects. Out of the 23 papers included in this review, 12 contained sub-analyses reporting the effects of psychotropic medications on Rs-FC. This was most commonly done by converting psychotropic medications to chlorpromazine equivalent doses and/or through the method utilized by Hassel et al. (Hassel et al. 2008). Although 11 studies found no influence of medication on Rs-FC, 1 found minimal effects of antipsychotics on ALFF/fALFF in the slow-4 bandwidth resulting in reduced ALFF in the lingual/precuneus region (as outlined in Table 2) (Meda et al. 2015). Three studies contained neither sub-analysis nor reported any participant medication history (Mamah et al. 2013; Rashid et al. 2014; Du et al. 2015).

3.4.3 Independent Component Analysis

Eight out of the 23 studies used ICA to investigate differences in Rs-FC between BD and HC (see Table 2, Figure 1). Among them, 17 networks of interest were identified. The default mode network (DMN), fronto-parietal networks (FPN) and salience networks (SN) were the most commonly studied and yielded largely consistent results. No differences in Rs-FC of the DMN between BD and HC were found in 6 out of 8 studies (Mamah et al. 2013; Rashid et al. 2014; Yip et al. 2014; Das et al. 2014; Lois et al. 2014; Syan et al. 2017), while two studies reported hypoconnectivity in BD participants relative to controls: Brady et al. reported hypoconnectivity in the anterior aspect of the DMN, largely concentrated in dorsal frontal regions (Brady et al. 2017),

while Khadka et al. reported it in the posterior DMN, with the left and right cingulate gyrus and left and right precuneus showing the greatest hypoconnectivity relative to HC (Khadka et al. 2013). It is important to note that the two aforementioned studies were the only ones to include solely BD-I subjects with a positive history of psychosis.

As subthreshold symptoms and minor cognitive impairment may be hallmarks of bipolar euthymia (Olley et al. 2005; Sole et al. 2017), the FPN is often investigated at rest. Although previous studies using SBA have found differences in the functional connectivity of the dlPFC at rest, studies using ICA to investigate Rs-FC of the entire FPN at rest found no differences in Rs-FC between BD and controls (Mamah et al. 2013; Rashid et al. 2014; Yip et al. 2014; Das et al. 2014; Lois et al. 2014; Syan et al. 2017). This result provides support to the notion that resting state network stability, seen through the use of ICA, may be a hallmark of bipolar euthymia. In other words, the brain correlates of an euthymic, stable mood state may be reflected through brain-wide resting state network stability. A similar pattern was seen with respect to the salience network, which was investigated in 3 studies and consistently showed no differences between BD and control subjects (Mamah et al. 2013; Yip et al. 2014; Das et al. 2014; Lois et al. 2014). The meso-paralimbic network (MPN) and the temporo-insular network (TIN) were investigated in 4 studies (Khadka et al. 2013; Yip et al. 2014; Lois et al. 2014; Syan et al. 2017). While 2 studies did not find differences in Rs-FC between BD and HC (Lois et al. 2014; Syan et al. 2017), Khadka et al. reported increased activation within the left uncus in BD compared to controls (Khadka et al. 2013). Yip et al. investigated the TIN in a medication naïve sample of BD-II patients and found increased engagement of the right caudate, left precentral gyrus, left postcentral gyrus, left inferior frontal gyrus, left supplementary motor area, bilateral putamen and bilateral insula in BD compared to controls (Yip et al. 2014). However, these results should be interpreted with caution as although the sample had been “euthymic” according to study criteria,

the lack of treatment with mood stabilizers or antipsychotics suggests that this specific population may have a less severe course of bipolar illness and, therefore, may not be comparable with majority of other study populations.

Less frequently studied networks included the CER, PLN, Precuneus Network, vmPFC Network, MN, VPN, and ECN; all of which have been associated with no significant differences in Rs-FC between BD and controls (Mamah et al. 2013; Khadka et al. 2013; Yip et al. 2014; Das et al. 2014). Moreover, single studies have found that DAN, CO, BGN and FON were associated with hypoconnectivity between brain regions outlined in Table 2 (Mamah et al. 2013; Khadka et al. 2013; Brady et al. 2017). Since only one study reported on each of these specific networks, we are unable to speculate if abnormal connectivity in the aforementioned networks is a true reflection of the trait based pathology of BD. More studies investigating these specific networks in euthymic BD subjects are needed. The same is true for the single SMN reporting hyperconnectivity between the superior frontal gyrus (SFG) and medial frontal gyrus (MFG) in BD relative to controls (Khadka et al. 2013).

As shown above, though between-group differences in RSN functional connectivity are typically negative, abnormal patterns of functional connectivity *between* RSNs (intra-network connectivity) have been reported to a greater degree between BD and HC. For instance, Das et al. reported that coupling between the DMN-Precuneus and SS-vmPFC networks was increased in BD compared to controls, although differences did not withstand multiple comparison correction (Das et al. 2014). Lois et al. found increased functional connectivity between the meso/paralimbic and the right frontoparietal network in bipolar disorder ($p<0.001$-FDR-corrected) (Lois et al. 2014). Mamah et al. reported less connectivity between the CO-CER and CO-SN in BD relative to controls ($p<0.01$) (Mamah et al. 2013). Thus, the 3 studies that investigated intra-network Rs-FC between BD and HC reported differences in functional connectivity.

Dynamic causal modeling (DCM) was employed by one study: Rashid et al. investigated both the static and dynamic functional connectivity of RSN (Rashid et al. 2014). Although no differences were found in the functional connectivity of the DMN, VN, SMN, CER or subcortical regions between groups, results from DCM analysis revealed less connectivity between two parietal components: the paracentral and superior parietal lobule (dynamic state 4), in BD compared to controls. Moreover, greater connectivity was found in BD relative to HC between the temporal component (bilateral fusiform gyrus) and a parietal component (left supramarginal gyrus) (dynamic state 4). No differences in functional connectivity between HC and BD were found in any other dynamic states (Rashid et al. 2014).

Du et al. used the novel method of guided independent component analysis (GIG-ICA) to identify patterns of functional connectivity at rest that are unique to a particular psychiatric illness and are able to discriminate between BD and other illnesses. GIG-ICA involves the estimation of resting state components using an algorithm that increases both the correspondence of intrinsic components between subject groups and the independence of intrinsic networks specific to each subject (Du et al. 2015). This particular study found that the insular cortex was a discriminatory region between euthymic BD and healthy controls using the GIG-ICA method.

3.4.4. Seed Based Analysis (SBA)

SBA correlates the BOLD signal of an a priori chosen "seed" region with other temporally significant changes in BOLD signal throughout the brain (Whitfield-Gabrieli and Nieto-Castanon 2012). Thirteen studies used SBA to investigate differences in Rs-FC in individuals with BD compared to controls; three of which included sub-analysis to explore differences in functional connectivity based on a history of psychosis (Anticevic et al. 2013; Anticevic et al. 2014b; Anticevic et al. 2014a). Overall, the results from SBA are largely heterogeneous and lack the consistency and generalizability of results obtained from ICA due to

the wide variety of seed regions chosen for analysis. All regions of Interest (ROIs) selected in individual studies are described thoroughly in Table 2.0, and the seed regions most commonly studied are described in greater detail below.

3.4.4.1.Bilateral and Left and Right Amygdala

It has been well established that the amygdala plays a role in emotional regulation and the trait-based pathology of BD. The left and right amygdala have been used as a priori seed regions in five of the thirteen SBA studies (Torrisi et al. 2013; Anticevic et al. 2013; Rey et al. 2016; Brady et al. 2016; Syan et al. 2017). No differences in the Rs-FC of the amygdala were found at rest between BD and HC (Rey et al. 2016; Syan et al. 2017). However, two studies found decreased connectivity between the amygdala and other regions of the brain in BD relative to controls (Anticevic et al. 2013; Brady et al. 2016). More specifically, decreased connectivity was found between the bilateral amygdala and (i) right BA5; (ii) left SMA; (iii) right SMA; (iv) left BA5; and between the right amygdala and (i) right BA5; (ii) right SMA; (Brady et al. 2016) and between the amygdala and dlPFC (Anticevic et al. 2013). On the other hand, increased connectivity between the amydgala and mPFC was reported in the same population (Anticevic et al. 2013) and between the right amygdala and right vlPFC (Anticevic et al. 2013). Interestingly, amygdala hyperconnectivity has been frequently reported in bipolar mania and to a lesser degree in bipolar depression (Townsend and Altshuler 2012), and thus the absence of hyperconnectivity may reflect a state of functional connectivity adopted during euthymia (Townsend and Altshuler 2012).

3.4.4.2 Medial Prefrontal Cortex

The medial PFC (mPFC) is a central node of the DMN and has been postulated to play a role in emotional regulation via the generation of anticipatory responses preceding emotional

events (Ochsner et al. 2009). Aberrant connectivity of the DMN has been reported in bipolar and unipolar depression; coupled with its role in emotional regulation and the DMN, this makes the mPFC a logical a priori ROI to study in the pathophysiology of BD (Vargas et al. 2013; Liu et al. 2015; Mulders et al. 2015).

Two studies used the mPFC as a ROI through SBA (Favre et al. 2014; Rey et al. 2016) and one study found differences in functional connectivity of the mPFC while one did not. Favre et al found mPFC activity was significantly correlated to the right amygdala in BD but not in HC (Favre et al. 2014). Moreover, in BD, mean duration of illness and mPFC-right amygdala functional connectivity was significantly correlated. Additionally, in BD increased functional connectivity between the mPFC and right dlPFC was reported relative to controls (Favre et al. 2014). Anti-correlation between the mPFC and right dlPFC was seen in HC but was not significant in BD. Rey et al. also investigated the Rs-FC of the mPFC however did not find differences in functional connectivity between HC and BD (Rey et al. 2016).

3.4.4.3 Dorsolateral Prefrontal Cortex

The dlPFC is a brain region central to high-order cognitive abilities and higher-order thinking. Due to its large role in central executive and fronto-parietal RSNs, the dlPFC has been explored as a ROI through SBA. While the aforementioned studies have described aberrant connectivity between the mPFC and dlPFC and between amygdala and dlPFC, the dlPFC was not used as a primary ROI in any of these studies. Our group used the dlPFC as a primary ROI through a SBA and we found increased Rs-FC between the dlPFC and the brainstem ($p=0.03$, FDR-corrected) (Syan et al. 2017). We postulated that this pattern of functional connectivity might reflect heightened activity of top-down and/or bottom-up processes between cortical regions and primitive neural regions involved in autonomic nervous and neurotransmitter system modulation. Further, it has been widely shown that cognitive difficulties, particularly in executive

functioning and verbal memory, often persist in between acute episodes in BD (Solé et al. 2011; Sole et al. 2017). Thus, it is conceivable that abnormal patterns of functional connectivity between the dlPFC and other brain regions may reflect some neural component of these persistent cognitive difficulties.

3.4.4.4 Ventrolateral Prefrontal Cortex/ Inferior Frontal Gyrus

The vlPFC/IFG was one of the most commonly studied ROI within the reviewed SBA literature and was investigated in four studies, with some mixed results being reported. Torissi et al. found increased connectivity between the right amygdala and right vlPFC. This pattern of connectivity was not correlated with any clinical variable tested – illness duration, number of depressive or manic episodes or HDRS/YMRS scores (Torrisi et al. 2013). Rey et al. found decreased connectivity between the sgACC and vlPFC, as discussed below (Rey et al. 2016). Brady et al. found no differences in Rs-FC of the right or left vlPFC (Brady et al. 2016). Moreover, Oertel-Knochel et al. found decreased functional connectivity of the left IFG and the left hippocampus ($p<0.001$, cluster-level correction) (Oertel-Knöchel et al. 2015).

3.4.4.5 Orbitofrontal Cortex

The OFC plays a well-established role in emotional regulation and impulsivity and has been widely implicated in the neurobiology of BD (Wessa and Linke 2009; Nusslock et al. 2012). The only study that tested the OFC and a primary ROI in Rs-FC found no differences in connectivity between euthymic BD subjects and controls (Brady et al. 2016).

3.4.4.6 Cingulate Cortex

Subdivisions of the cingulate cortex were investigated in five studies, each making a unique contribution to the knowledge on the trait-based pathophysiology of BD (Magioncalda et al. 2014; Anticevic et al. 2014a; Rey et al. 2016; Brady et al. 2016; Syan et al. 2017). Anticevic et

al explored the Rs-FC of the vACC between BD with and without a history of psychosis and HC. They found decreased connectivity between mPFC and vACC in BD patients with lifetime psychosis relative to HC and increased connectivity in BD patients without psychosis between mPFC and vACC relative to HCs (Anticevic et al. 2014a). Our group studied Rs-FC through the DMN using both ICA and SBA, and found increased coupling between the PCC and angular gyrus relative to HC, however as mentioned above, no differences in the functional connectivity of the DMN were observed using ICA (Syan et al. 2017). In a recent study by Rey et al., increased coupling between the left subgenual ACC (sgACC) and PCC, and increased coupling between the left and right sgACC were found within BD compared to controls; however in the same population, decoupling was found between the right sgACC and right vlPFC (d'=0.58, p=0.04). Also worth mentioning is that the pattern of coupling between the right amygdala and right vlPFC reported by Torissi et al. was mediated by the ACC (Torrisi et al. 2013). Further, two studies reported no difference in ACC connectivity between BD and controls (Magioncalda et al. 2014; Brady et al. 2016).

3.5 Discussion

Investigating Rs-FC during inter-episodic periods of BD may contribute to our understanding of the neurobiology of BD; common patterns of Rs-FC may highlight regions implicated in its pathophysiology, and/or markers of bipolar euthymia. Upon a systematic review of the literature, we found that studies using ICA to examine the functional connectivity of the DMN, FPN and SS, largely show that patterns of functional connectivity are not distinguishable between BD subjects and healthy controls. Notably, the two studies (out of the 8 that were investigated) reporting hypoconnectivity between nodes of the DMN in BD relative to healthy controls included only individuals with a positive history of psychosis. Therefore, the reported hypoconnectivity of the DMN may reflect a positive history of psychosis. Aberrant patterns of

DMN connectivity are well documented in bipolar mania and in schizophrenia during task-based and resting state fMRI (Garrity et al, 2007; Hu et al. 2016; Pomarol-Clotet et al. 2008; Sambataro et al., 2010; Salgado-Pineda et al, 2011), and in acute mood states of BD (for review (Vargas et al. 2013)). Modulation of DMN connectivity with antipsychotic medication has also been reported (Sambataro et al., 2010). The DMN is central to spontaneous cognition, self-referential processing and emotional regulation (Fox and Raichle 2007; Andrews-Hanna et al. 2010). The absence of aberrant connectivity in DMN functional connectivity found in most studies in euthymic BD subjects may reflect a normalization of DMN activity in between acute mood episodes. Psychotic symptoms may alter the integrity of the DMN and lead to persistent hypoactivation even during periods of euthymia. Despite the common presence of subjective cognitive impairment in individuals with BD, our review suggests that the FPN and SN functional connectivity are largely similar between BD subjects and controls. Therefore, it is possible that stability of the DMN, FPN and SN using ICA may reflect stabilization of RSN and a state of remission in BD.

Contrary to the results from ICA, studies employing SBA found hyper/hypo-activation of important regions of the DMN and FPN such as the mPFC, PCC and dlPFC. These regions are central to emotional regulation, self-referential processing and executive functioning (Fox and Raichle 2007; Andrews-Hanna et al. 2010): deficits in all of which are reported during euthymic periods in BD (Olley et al., 2005). Since SBA largely investigates functional connectivity between distinct regions of the brain (seed points) and other voxels in the brain (seed-to-voxel) or other brain regions (ROI-ROI), this technique may be more useful at capturing smaller scale changes in Rs-FC, such as those between brain regions. Smaller scale/seed-based changes in Rs-FC may also be more susceptible to the influence of characteristics of the patient population. In this capacity, heterogeneity in the patient populations used may contribute to the differences in functional connectivity reported in SBA. While each patient group included from the literature in

this review was comprised of euthymic samples, differences in psychotropic medication use, illness duration, history of psychiatric co-morbidities and severity of sub-threshold symptoms may have influenced the results. These factors should be carefully considered when interpreting functional connectivity results, from individual studies and in the framework of this review. With respect to SBA, postulate that aberrant patterns of DMN functional connectivity may reflect (i) a neural correlate of subthreshold symptoms or to a similar degree, differences in psychiatric comorbidities or medication use; and/or (ii) sustained patterns of Rs-FC present during bipolar euthymia that may contribute to its trait-based pathology.

The MPN and its primary seed point, the amygdala, play an important role in emotional regulation (Townsend and Altshuler 2012), and this network has largely been implicated in the pathophysiology of BD (Strakowski et al. 2012; Townsend and Altshuler 2012; Phillips and Swartz 2014b). However, results from ICA studies in BD euthymia are conflicting: with 2 out of 4 studies reporting no difference in Rs-FC of the MPN, and two studies reporting hyperconnectivity of nodes of the MPN/TIN, one study being conducted in an antipsychotic naïve population (Yip et al. 2014), and the other in individuals with a positive history of psychosis (Khadka et al. 2013). The functional connectivity of the amygdala, a central node of the limbic network was explored in 5 studies using SBA, also yielding mixed results with two studies reporting no differences between BD and controls and three reporting hyperconnectivity between the amygdala and the vlPFC, medial PFC and somatosensory association cortex and decoupling with the dlPFC. As mentioned above, heterogeneity in variables associated with the patient population (illness history, number of episodes, psychotropic medication use) may help to explain these differences in functional connectivity.

Numerous studies have investigated the role of the cingulate cortex in emotional regulation and its role in both dorsal and ventral streams of cognition (Ochsner and Gross 2005;

Ochsner et al. 2009; Wessa et al. 2014b; Phillips and Swartz 2014). The cingulate cortex is split into many anatomical and functional regions; each of which making a unique contribution to emotional regulation, cognition, and the trait-based pathology of various psychiatric illnesses (Ochsner and Gross 2005; Ochsner et al. 2009; Wessa et al. 2014b; Phillips and Swartz 2014b). Studies included in the review highlighted patterns of altered functional connectivity in 5 key sub-regions of the cingulate cortex: the PCC, sgACC, vACC, pACC and in one instance the entire ACC. Functional coupling of specific nodes of the cingulate cortex in BD relative to controls may reflect trait-based pathology of BD that highlights (i) compensatory neural activity responsible for maintaining a state of remission; and/or (ii) an increased vulnerability to develop acute mood states associated with BD.

3.5.1. Limitations

Study Limitations and Future Considerations

An important limitation of these findings is related to certain aspects inherent of the Rs-FC technique. First, Rs-FC provides an *indirect* measure of neuronal activity in an ultralow frequency typically ranging from 0.01-0.10 Hz (Allen et al. 2011). Therefore findings from Rs-FC studies must be interpreted within this framework. Moreover, commonly used Rs-FC techniques (SBA, ICA, ALFF, ReHo), rely on the oversimplification that BOLD activation is static through the entire scanning paradigm (Cole et al. 2010; Daliri and Behroozi, 2014). An exception to this is a using a dynamic causal modeling or sliding window approach (Calhoun 2014). Further, participants are told at the beginning of many resting state scanning paradigms to keep their eyes open, fixed on a fixation cross/point and try not to think of anything in particular. However, many studies do not use objective measures such as simultaneous electroencephalograms or eye tracking to confirm this (Cole et al. 2010; Daliri and Behroozi, 2014). Also although participants are often advised to keep a "clear mind" through the session we do not know if participants are

able to do this throughout the scan. Additionally, it is important to consider that seed based analyses are limited by the location and size of the ROI used across subjects. Although most studies included pre-processing steps such as normalization and segmentation to mitigate these effects, they should still be carefully considered as a potential limitation of SBA studies (Du et al. 2012).

In order to achieve a sustained period of clinical stability, most individuals with BD need to be managed pharmacologically on a mood-stabilizing regimen and, therefore, studies involving euthymic subjects typically recruit individuals on medications (Phillips and Swartz 2014). Thus, the effect of psychotropic medication is a common limitation of neuroimaging research on bipolar euthymia. Nevertheless, many studies investigating Rs-FC in BD including our previous work, have ruled out the influence of medication (Torrisi et al. 2013; Lv et al., 2016; Anticevic et al. 2013; Reinke et al. 2013; Lois et al. 2014; Anticevic et al. 2014; Oertel-Knöchel et al. 2015; Liu et al. 2015; Brady et al. 2016; Brady et al. 2017; Syan et al. 2017). We encourage authors to report complete medication history and conduct sub-analysis to analyze the effect of medication on Rs-FC, so that we are able to better understand the effects of psychotropic medication on Rs-FC. We also encourage future studies specifically designed to investigate the potential effects of psychotropic medications on Rs-FC.

Patient characteristics, such as body mass index, subthreshold mood/anxiety symptoms and lifetime history of psychosis may be also associated with certain discrepancies between available studies (Bond et al. 2011; Anticevic et al. 2014; Bond et al. 2016). Many studies did not report psychosis history nor did they conduct sub-analysis to assess the potential influence of psychosis on Rs-FC in their sample. Notably, BMI is known to affect brain structure in individuals with BD (Bond et al. 2011); but the effect of BMI on Rs-FC in BD is, however, largely unknown - future studies are warranted. Another important knowledge gap is the degree to

which the presence of subclinical/subthreshold symptoms may affect Rs-FC (Olley et al. 2005). We encourage future studies to provide a detailed report on the scores of clinical measures to allow findings to be interpreted in the framework of clinical characteristics experienced by the specific study populations.

This systematic review also highlighted the lack of control for sex, menstrual cycle phase or menstrual cycle disorders in studies that investigated women within reproductive age. This may be important for numerous reasons: the clinical course of BD has shown to progress differently in men and women (Miller, 2014), with women reporting greater symptoms of depression and more lability in mood resulting from hormonal fluctuations (Frey and Dias, 2013); and an increasing body of literature that has found women with BD report higher rates of premenstrual syndrome and premenstrual dysphoric disorder than controls (Fornaro and Perugi 2010; Choi et al. 2011; Dias et al. 2011; Teatero et al. 2013).

Systematic Review Limitations

This systematic review provided a concise review of the Rs-FC literature in bipolar euthymia, however is not without limitations. A major limitation was the considerable heterogeneity in analytical approach used. In studies using a SBA in particular, the regions of interest used were quite diverse making it challenging to compare results between studies. Further, the size and location of ROIs also varied between studies and may have contributed to the diversity in results. As a result, we were unable to conduct a meta-analysis. In addition, we excluded studies on paediatric BD. As a result, this review encapsulates literature on a distinct phase of BD in adults and although it contributes to providing an overview of bipolar remission it cannot be generalized to younger populations.

3.6 Conclusion

In conclusion, stability of DMN, FPN and the SN was a consistent finding in ICA studies, with the exception of studies with an entire sample endorsing a positive history of psychosis, which may reflect patterns of connectivity similar to those seen during bipolar mania or schizophrenia. We postulate that the stability of resting state networks may be neural correlated of a state of clinical remission in BD, whereas history of psychosis may be reflected by instability of the DMN, which seems to persist in remission. Results from SBA studies were significantly more diverse and discrepant due, at least in part, to the heterogeneity in patient populations and localization of ROIs. Changes in resting state functional connectivity between neural regions central to the pathophysiology of BD such as the amygdala, PFC and cingulate cortex may reflect (i) a neural correlate of sub-threshold symptoms experienced during BD euthymia; (ii) a compensatory mechanism of neural activity that is underlying the stability of RSN using ICA; and/or (iii) a reflection of the trait-based pathophysiology of BD.

3.7 References

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Figure 1: Summary of ICA Study Findings Between BD and CTRL

	DMN	FPN	SN	MPN	TIN	CO	CER	DAN	BGN	SMN	PLN	FON	Prec.	vmPFC	VPN	MN	ECN
Brady	Hypo	-	-	-	-	-	-	Hypo	-	-	-	-	-	-	-	-	-
Das	N.S.	N.S.	N.S.	-	-	-	-	-	-	-	-	-	N.S.	N.S.	-	-	-
Lois	N.S.	N.S.	N.S.	N.S.	-	-	-	-	-	-	-	-	-	-	-	-	-
Mamah	N.S.	N.S.	N.S.	-	-	Hypo	N.S.	-	-	-	-	-	-	-	-	-	-
Khadka	Hypo	-	-	Hyper	-	-	N.S.	-	Hypo	Hyper	N.S.	Hypo	-	-	-	-	-
Rashid	N.S.	N.S.						-									
Syan	N.S.	N.S.	-	N.S.	-	-	-	--	-	-	-	-	-	-	-	-	-
Yip	N.S.	N.S.	N.S.	-	Hyper	-	-	-	-	-	-	-	-	-	N.S.	N.S.	N.S.
Du	Used GIG-ICA																

Hypo – Hypoconnectivity; N.S – Not Significant; Hyper – Hyperconnectivity; DMN – Default Mode Network; FPN – Fronto-Parietal Network; SN – Salience Network; MPN – Mesoparalimbic Network; TIN - Temporo-Insular Network; CO - Cingulo-opercular; CER - Cerebellum/Midbrain Network; DAN – Dorsal Attention Network; BGN - Fronto-thalamic/Basal Ganglia Network; SMN – Sensorimotor Network; PLN – Fronto-Temporal-Paralimbic Network; FON - Fronto-occipital Network; Prec – Precuneus Network; vmPFC – vmPFC Network; VPN – Visual Processing Network; MN – Motor Network; ECN – Executive Network

Table 1: Summary of Neuroimaging Studies

First Author and Year	Bipolar Sample							
	Sample (n)	**Subtype**	**Hx of Psychosis**	**Sex (M/F)**	**Age (S.D.)**	**Years of Education (SD)**	**Control Subjects (n)**	**Other Comparative Groups**
DCM/ICA								
Rashid et al. (2014)	38	NA	NA	18/20	38.96 (10.9)	NA	61	SCZ or SAD (n=60)
Independent Component Analysis (ICA)								
Brady et al. (2016)	24	BD-1	Yes (n=24)	16/8	30.9 (11.9)	NA	23	BD[Mania] (n=28)
Du et al. (2015)	20	NA	Yes (n=20)	8/12	31.2 (9.5)	NA	20	SCZ (n=20), SADM (n=20), SADD (n=13)
Das et al. (2014)	16	NA	NA	0/16	35.6 (10.71)	15.87 (1.51)	13	Borderline Personality Disorder (n=14)
Lois et al. (2014)	30	BD-1	Yes (n=13)	13/17	40.8 (9.43)	14.97 (2.58)	35	No
Mamah et al. (2013)	35	NA	NA	16/19	24.9 (3.75)	14.1 (2.4)	33	SCZ (n=25)
Khadka et al. (2013)	64	BD-1	Yes (n=64)	35/29	35.1(11.2)	NA	118	SCZ (n=70)
Yip et al. (2014)	15	BD-2	NA	7/8	23.1 (3.7)	NA	20	No

Seed-Based Analysis (SBA)								
Anticevic et al (2012)	68	BD-1	Psychosis Hx (34), Without Psychosis (34)	Psychosis Hx (n=13/21) No Hx Psychosis (n=8/26)	Psychosis Hx: 34.0 (10.8) No Hx Psychosis: 29.9 (11.9)	Psychosis Hx: 13.9 (1.6) No Hx Psychosis: 14.4 (2.0)	51	No
Anticevic et al (2013)	73	BD-1	Psychosis Hx (33), Without Psychosis (40)	Psychosis Hx (n=12/21) No Hx Psychosis (n=8/32)	Psychosis Hx: 34.2 (10.9) No Hx Psychosis: 30.2 (11.5)	Psychosis Hx: 13.9 (1.6) No Hx Psychosis: 14.4 (2.1)	56	SCZ (n=73)
Anticevic et al (2014)	73	BD-1	Psychosis Hx (33), Without Psychosis (40)	Psychosis Hx (n=12/21) No Hx Psychosis (n=8/32)	Psychosis Hx: 34.2 (10.9) No Hx Psychosis: 30.2 (11.5)	Psychosis Hx: 13.9 (1.6) No Hx Psychosis: 14.4 (2.1)	56	SCZ (n=73)
Brady et al. (2016)	24	BD-1	Yes (n =24)	16/8	30.9 (11.9)	NA	23	BD [Mania] (n=23)
Favre et al. (2014)	20	Mixed (BD-I n=13; BD-II n=5; BD-NOS n=2)	NA	9/11	42 (10.7)	NA	20	No
Konchel et al. (2014)	21	BD-1	NA	12/9	35.7 (10.7)	14.7 (2.4)	21	SCZ (n=21)
Lv et al., (2016)	19	NA	NA	10/9	27.8 (6.7)	13.3 (2.7)	28	BD [Depressed] (n=23)
Magioncalda et al., (2015)	11	NA	NA	NA	NA	NA	40	BD [Depressed]

								(n=11), [Mania] (n=11), [Mixed] (n=7)
Oertel-Knöchel et al. (2015)	21	BD-1	NA	12/9	35.7 (10.7)	14.7 (2.4)	20	No
Rey et al. (2016)	15	BD-1 (n=7), BD-2 (n=3), BD-NOS (n=3), BD Rapid Cycling (n=1)	NA	6/9	41.4 (9.6)	NA	15	BD [Non Euthymic] (n=12)
Reinke et al. (2013)	21	BD-1	NA	12/9	35.7 (10.7)	NA	20	No
Syan et al. (2017)	32	BD-1 (n=18), BD-2 (n=14)	NA	0/32	29 (8.07)	15.6 (2.6)	36	No
Torissi et al. (2013)	20	BD-1	NA	10/10	42.1 (11.4)	14.1 (1.9)	20	No
Absolute & Fractional Amplitude of Low Frequency Fluctuations (AALF)								
Meda et al. (2014)	180	BD-1	Yes (n=180)	58/122	36.94 (13.0)	NA	242	SCZ (n=220), SAD (n=147), BDR (n=134), SCZR (n=150), SADR (n=126)

Lui et al. (2014)	57	BD-1	Yes (n=57)	18/39	34 (13)	14 (3)	59	SCZ (n=37), SCZR (n=38), BDR (n=28)

BD- Bipolar Disorder; SCZ – Schizophrenia; SAD- Schizaoaffective Disorder; SADM- Schizoaffective Disorder, Manic subtype; SADD- Schizoaffective Disorder, Depressed subtype; BDR- Healthy Relative of Bipolar proband; SCZR- Healthy relative of schizophrenic proband; SADR- Healthy relative of schizoaffective probra

Table 2: Summary of Neuroimaging Results

Study	Methodology				Results
First Author and Year	**Diagnosis of BD**	**Inclusion Criteria**	**Medication Information**	**Region of Interest/Network Studied**	**Major Results**
DCM/ICA					
Rashid et al. (2014)	Chart Review and Structured Clinical Interview for DSM-IV	Not available No patients were acutely ill at the time of scanning	Not available	VSN, CC, AUD, SMN, DMN, CER, Subcortical Network/Regions	(i) No differences in static functional connectivity between BD and HC in the VN, SMN, DMN, CER or Subcortical regions. (ii) BD<HC: Lower connectivity was found in BD between two parietal components, paracentral and superior parietal lobule (dynamic state 4). (iii) BD>HC: Greater connectivity was found between the temporal component (bilateral fusiform gyrus) and a parietal component (left supramarginal gyrus) (dynamic state 4) in BD. No differences in functional connectivity between HC and BD were found in any other dynamic states, and

					symptomology and functional connectivity were not correlated in this sample.
Independent Component Analysis (ICA)					
Brady et al. (2017)	Structured Clinical Interview based on DSM-IV	BD: (i) 18-65 years of age; (ii) no history of neurological illness; (iii) not currently pregnant or lactating; (iv) no electroconvulsive therapy within 3 months of study enrolment; (v) no history of head trauma resulting in a loss of consciousness for greater than a few minutes; (vi) no MRI contraindication HC: No current or lifetime history of any Axis 1 psychiatric disorder	Anticonvulsants: (9) Antipsychotics: (15) Benzodiazepenes: (4) Lithium: (16) Antidepressants: (1) Sub-analysis revealed no significant effect of medication on Rs-FC analysis (p-FDR<0.05)	Exploratory whole brain analysis Results found in DAN and DMN	(i) BD<HC: Hypo-connectivity in the dorsal attention network (DAN) in BD relative to HC. (ii) BD<HC: Dorsal frontal hypoconnectivity in the default mode network (DMN) in BD relative to HC. (iii) Additional uncorrected ROI-ROI results between BD and HC are described in the manuscript.
Du et al. (2015)	DSM-IV-TR	BD: (i) stable and consistent medication dose for 4 weeks or longer.	Medication history not available	FPN (R/L), DMN (1,2,3), SN, PN, ARN, VRN, VSN, CER, SMN	(i) The insular cortex was identified as a discriminatory region between HC and BD using the GIG-ICA technique.
Das et al. (2014)	Structured Clinical Interview based on DSM-IV	BD: (i) euthymic state; (ii) no current hospitalization; (iii) no substance abuse; (iv) no history of traumatic head injury; (v) no neurological illness; (vi) no learning or developmental disorders or poor English proficiency HC: No history of psychiatric illness	Unmedicated (3), Antidepressants (7), Antipsychotics (6), Mood stabilizers (3), Lithium (5) No medication sub-analysis reported	FPN (R/L) Precuneus, DMN Social Salience vmPFC	(i) No differences in network connectivity (FPN (R/L), Precuneus, DMN, Social Salience, vmPFC) between BD and HC. (ii) BD>HC: Internetwork differences: coupling between the DMN-Precuneus and SS-vmPFC

					networks was increased in BD compared to controls. Differences did not withstand multiple comparison correction. (iii) Increased coupling in BD between the SS-vmPFC networks was positively correlated with lack of emotional clarity ($r = 0.605$, $p = 0.029$). (iv) Increased coupling in BD between the DM-Precuneus was negatively correlated with lack of emotional awareness scores ($r = -0.574$, $p = 0.040$). Both iii and iv refer to correlations with sub-scores from the Difficulties in Emotion Regulation Scale (DERS).
Lois et al. (2014)	Structured Clinical Interview for DSM-IV Axis I Disorders	All: (i) over 18 years of age; (ii) no history of neurological disorder or head trauma with a loss of consciousness; (iii) do not meet common MRI exclusion criteria BD: (i) did not meet criteria for another Axis-I mental disorder within the last 6 months; (ii) if they had lifetime diagnosis of rapid cycling, schizoaffective disorder or schizophrenia	Medication loads for Bipolar I patients was 3.1 with a standard deviation of 2.35 No correlation between functional connectivity and medication load	aDMN, pDMN, FPN (L/R), SN MPN	(i) No differences in functional connectivity between BD and HC in any of the networks of interest. (ii) BD>HC: Increased functional connectivity between the meso/paralimbic and the right frontoparietal network in BD.

		HC: (i) no current or lifetime history of any Axis -1 psychiatric illness or any psychotropic medication.			(iii) The abnormal connectivity pattern in bipolar patients did not correlate with variables related to the clinical course of the disease.
Mamah et al. (2013)	Structured Clinical Interview for DSM-IV Axis I Disorders	All: (i) did not meet DSM-IV criteria for substance dependence or severe/moderate abuse during 6 months preceding study enrolment; (ii) had no clinically unstable or severe general medical disorder; (iii) no history of head injury with documented neurological sequelae or less of consciousness; (iv) met DSM-IV criteria for mental retardation BD: (i) clinically stable for at least two weeks. HC: (i) no current or lifetime history of psychotic or mood disorder, or first degree family members with a psychotic disorder	Medication history not available	DMN, FPN CO, CER SN	(i) BD<HC: BD had lower within network connectivity of the CO than HC. (ii) BD<HC: Connectivity between the CO-CER and CO-SN was lower in BD than HC.
Khadka et al. (2013)	Structured Clinical Interview for DSM IV	BD: (i) met criteria for bipolar I disorder with psychosis; (ii) clinically stable with consistent medication for at least 4 weeks prior to study enrolment	Mood Stabilizer (44) Typical Antipsychotics (2) Atypical Antipsychotics (36) Benzodiazepines (11) Anticholinergics (4) SSRIs (16) Tricyclics/MAOs (13), Psychostimulants (4)	FON, CER BGN, MPN pDMN, PLN, SMN	(i) BD<HC: decreased functional connectivity in FON (left cuneus, left lingual gyrus) BGN (right thalamus) pDMN (left and right cingulate gyrus, left and right precuneus) in BD compared to HC. (ii) HD>HC: BD greater connectivity than HC in:

			No medication sub-analysis could be completed		MPN (left uncus) SMN (right SFG, right MFG) (iii) No differences in the connectivity of the CER or PLN between HC and BD.
Yip et al. (2014)	Mini-International Neuropsychiatric Interview (MINI)	All: (i) no history of head injury, neurological condition or MRI contraindication HC: (i) no history of psychotropic medication use; (ii) no history of hypomanic experiences as defined by the Mood Disorders Questionnaire (MDQ); (ii) no current or past psychiatric disorder. BD: (i) no current major depressive, manic or hypomanic episodes at the time of scanning; (ii) no use of any current psychotropic medication or exposure to antipsychotic agents or mood stabilizers; (iii) no other psychiatric disorder (excluding BD and anxiety disorders).	Entire sample was naïve to antipsychotic or mood stabilizing medication and unmedicated at the time of scanning	DMN, VPN (1,2), TIN, MN, ECN, FPN (R/L)	(i) BD>HC: BD had increased engagement of the regions of the TIN compared to HC: right caudate, left precentral gyrus, left postcentral gyrus, left inferior frontal gyrus, left supplementary motor area, bilateral putamen and bilateral insula. (ii) No significant differences were found between BD and HC in any of the other networks of interest studied.
Seed-Based Analysis (SBA)					
Anticevic	Structured	All: (i) No history of a major medical or	Mood Stabilizers	Seed based	(i) BD<HC: decreased

et al. (2013) Global Prefrontal and Fronto-amygdalar Dysconnectivity in Bipolar 1 Disorder with Psychosis Hx	Clinical Interview for DSM-IV	neurological condition (e.g., epilepsy, migraines, head injury with loss of consciousness) (ii) IQ> 80 on Weshler Abbreviated Intelligence Scale BD: (i) Bipolar I diagnosed using SCID for DSM-IV by MA or PhD level research clinicians (ii) Co-morbid Axis I disorders and substance use disorders (in remission for at least 6 months) were allowed HC: (i) No Axis I diagnoses in lifetime as per SCID-NP (ii) No history of mood or psychotic symptoms in first degree relatives	(53%) Anti-depressants (43%) Atypical Anti-psychotics (34%) Anxiolytics (35%) Lithium (16%) Unmedicated (16%) Medication type did not alter results when used as a covariate	Amygdala was correlated with all PFC voxels using a Global Brain Connectivity method, restricted to PFC (rGBC)	connectivity between mPFC and rGBC in BD compared to HC. (ii) BD>HC increased connectivity between amygdala and mPFC in BD compared to HC. (iii) BD<HC decreased connectivity between amygdala and dlPFC in BD compared to HC.
Anticevic et al. (2012)	Structured Clinical Interview for DSM-IV	All: (i) No history of a major medical or neurological condition (e.g., epilepsy, migraines, head injury with loss of consciousness) (ii) IQ> 80 on Weshler Abbreviated Intelligence Scale BD: (i) Bipolar I diagnosed using SCID for DSM-IV by MA or PhD level research clinicians (ii) Co-morbid Axis I disorders and substance use disorders (in remission for at least 6 months) were allowed HC: (i) No Axis I diagnoses in lifetime as per SCID-NP (ii) No history of mood or psychotic symptoms in first degree relatives	BD with Psychosis: Mood Stabilizers (15%) Antidepressants (33% Atypical Antipsychotics (45%) Lithium (24%) Typical Antipsychotic (2%) Unmedicated (15%) BD without Psychosis: Mood Stabilizers (18%) Antidepressants (50%) Atypical Antipsychotics (25%) Lithium (12%) Typical	vACC	(i) BD<HC: Decreased connectivity between mPFC and vACC in BD patients with psychosis history relative to HC. (ii) BD>HC: Increased connectivity in BD patients without psychosis between mPFC and vACC relative HC.

			Antipsychotic (0%) Unmedicated (18%) Medication sub-analysis results: there was no effect of medication on Rs-FC results		
Anticevic et al. (2014)	Structured Clinical Interview for DSM-IV	All: (i) No history of a major medical or neurological condition (e.g., epilepsy, migraines, head injury with loss of consciousness) (ii) IQ> 80 on Weshler Abbreviated Intelligence Scale BD: (i) Bipolar I diagnosed using SCID for DSM-IV by MA or PhD level research clinicians (ii) Co-morbid Axis I disorders and substance use disorders (in remission for at least 6 months) were allowed HC: (i) No Axis I diagnoses in lifetime as per SCID-NP (ii) No history of mood or psychotic symptoms in first degree relatives	BD with Psychosis: Mood Stabilizers (15%) Antidepressants (33% Atypical Antipsychotics (45%) Lithium (24%) Typical Antipsychotic (2%) Unmedicated (15%) BD without Psychosis: Mood Stabilizers (18%) Antidepressants (50%) Atypical Antipsychotics (25%) Lithium (12%) Typical Antipsychotic (0%) Unmedicated (18%) No sub-analysis in BD reported	Thalamic nuclei: Mediodorsal (MD) Nucleus and the Lateral Geniculate Nucleus	***Bipolar with Psychosis vs HC:*** (i) BD with Psychosis>HC: Coupling between the MD thalamic nucleus and the right superior temporal gyrus [BA22], left superior temporal gyrus [BA41], left insular cortex [BA13], and left precentral gyrus [BA4] in BD with psychosis relative to HC. (ii) BD with Psychosis<HC Decoupling between the MD thalamic nucleus and the precuneus. (iii) BD with Psychosis>HC: Coupling between the right superior temporal gyrus [BA22], right superior temporal gyrus [BA41], right precentral gyrus [BA6]. (iv) BD with Psychosis<HC: Decoupling between the

					LGN and the right thalamus, and anterior cingulate [BA32]. ***Bipolar without Psychosis vs HC:*** (i) BD without Psychosis>HC: Coupling between the MD thalamic nucleus and the insular cortex [BA13], anterior insular cortex [BA13], and right precentral gyrus [BA4]. (ii) BD without Psychosis<HC: Decoupling between the MD thalamic nucleus and the precuneus [BA7]. (iii) BD without Psychosis<HC: Decoupling between the LGN and the right thalamus, right anterior cingulate cortex. (iv) BD without Psychosis>HC: Coupling between the LGN and the right superior temporal gyrus [BA41], right precentral gyrus [BA6].
Brady et al. (2016)	Structured Clinical Interview	BD: (i) 18-65 years of age; (ii) no history of neurological illness; (iii) not currently pregnant or lactating; (iv) no	Anticonvulsants: (9) Antipsychotics: (15) Benzodiazepenes: (4)	Bilateral amygdala (and included	(i) BD<HC: Decreased connectivity between the bilateral amygdala and (a)

	based on DSM-IV	electroconvulsive therapy within 3 months of study enrolment; (v) no history of head trauma resulting in a loss of consciousness for greater than a few minutes; (vi) no MRI contraindication HC: no current or lifetime history of any Axis 1 psychiatric disorder	Lithium: (16) Antidepressants: (1) Sub-analysis revealed no significant effect of medication on Rs-FC analysis (p-FDR<0.05)	individual L/R seeds), bilateral OFC, bilateral ventral striatum, vlPFC (L/R) and ACC.	right BA5; (b) left SMA; (c) right SMA; (d) left BA5. Cluster size p-value <0.001. (i) BD<HC: Decreased connectivity between the right amygdala and (a) right BA5; (b) right SMA. Cluster size p-value <0.001.
Favre et al. (2014)	Structured Clinical Interview for DSM IV	All: (i) no history of alcohol or drug abuse; (ii) no current or past neurological or medical diseases that affect cognition; (iii) no history of head trauma with a loss of consciousness; (iv) no MRI contraindications BD: (i) euthymic for at least one month prior to scanning and MADRS<15 and YMRS<7; (ii) no other Axis I psychiatric disorder or electroconvulsive therapy during the previous year HC: (i) no current or lifetime history of psychiatric disorder; (ii) no family history of psychiatric disorders; (iii) no medical treatment affecting cerebral activity.	Lithium (80%) Anticonvulsants (60%) Antidepressants (35%) Atypical Antipsychotics (5%) No sub-analysis reported	mPFC and mPFC-amygdala connectivity	(i) BD>HC: Increased functional connectivity between the mPFC and right dlPFC (p<0.05, cluster-level FWE correction) in BD compared to HC. (ii) Anti-correlation between the mPFC and right dlPFC in HC (mean r= -0.25, p<0.001), but not significant in BD. (iii) mPFC activity was significantly correlated to the right amygdala in BD (r=0.08, p=0.002) but not HC (r=0.01, p-0.48). (iv) In BD, mean duration of disorder and mPFC-right amygdala functional connectivity was significantly correlated (r=0.46; p=0.04).
Konchel et al. (2014)	Structured Clinical Interview	BD: (i) no comorbid Axis-I or II disorders; (ii) BDI-II<18 and BRMAS<7; (iii) stable mood state (iv)	Mood Stabilizers: 21 Antidepressants: 2	Hippocampus	(i) BD<HC: Decreased functional connectivity between the hippocampus

	for DSM IV (SCID-I and SCID-II; German version)	no changes in medication within the month preceding study enrolment HC: (i) no current drug-abuse (ii) history of neurological disease (iii) no history of Axis-1 or II disorders; (iv) ability to provide consent and a family history of affective or psychotic disorders	Mean duration of medication: 6.26 years No sub-analysis reported for effect of medication on Rs-FC		and the left frontal lobe in BD compared to HC.
Lv. et al. (2015)	Structured Clinical Interview for DSM IV	All: (i) age between 18-45 years; (ii) completed 9 or more years of education; (iii) right handed; (iv) no history of neurological disease or other physical illness; (v) history of electroconvulsive therapy; (vi) no history of drug or alcohol abuse; (vii) no psychiatric comorbidities: schizoaffective disorder, personality disorders, and mental retardation; (viii) no contraindications for MRI BD: (i) does not meet criteria for current manic, hypomanic, or depressive mood according to the SCID, HAMD Score of ≤8 and YMRS ≤6 within 6 months preceding study enrolment. HC: (i) no current or lifetime history for any psychiatric disorder	Increased connectivity strength between the right middle cingulate gyrus and the right supramarginal gyrus in BD was positively correlated with lithium doses. No other significant interaction was found between dosage of other psychotropics and functional connectivity strength	Whole brain analysis SBA of Broca's Area (IFG: pars opercularis and pars traiangularis) and Wernicke's area (LSTG, LMTP and LANG).	(i) No significant differences in functional connectivity between BD and HC in any of the seed points studied: IFG (pars opercularis, pars traiangularis) LSTG, LMTP and LANG. (ii) BD>HC: increased connectivity between the left insula and LANG ($p<0.001$, uncorrected).
Magioncalda et al., (2015)	Mini-International Neuropsychiatric Interview (MINI)	All: (i) age 18-60; (ii) no history of schizophrenia, mental retardation, dementia, other cognitive disorders; (iii) no history of severe or decompensated somatic diseases or neurological diseases; (iv) no history of head injury with loss of consciousness >5 minutes; (v) no current alcohol or substance	Mood Stabilizers: (35) Antidepressants: (11) Antipsychotics (24) Benzodiazepines (12) Unmedicated: 1 No medication sub-	pACC	(i) subgroup analysis yielded no significant results between euthymic BD and HC.

	Structured Clinical Interview for DSM IV-Axis II Personality Disorders Structured Interview for Mood Disorder-Revised	abuse; (vi) no history of alcohol or substance dependence; (vii) no history of abuse of synthetic drug/new drug abuse; (viii) not pregnant or lactating; (ix) right handed; (x) no MRI contraindications; (xi) no previous treatment with electroconvulsive therapy, chemotherapy or brain radiotherapy BD: (i) HAMD score of <8, YMRS score of <8 HC: (i) no current of lifetime psychiatric history	analysis for BD reported		
Oertel-Knöchel et al. (2015)	Structured Clinical Interview for DSM-IV (SCID-I and SCID-II; German version	BD: (i) no comorbid Axis-I or II disorders; (ii) BDI-II<18 and BRMAS<7; (iii) stable mood state (iv) no changes in medication within the month preceding study enrolment HC: (i) no current drug-abuse (ii) history of neurological disease (iii) no history of Axis-1 or II disorders; (iv) ability to provide consent and a family history of affective or psychotic disorders	Mood Stabilizers: 21 Antidepressants: 3 Mean duration of medication: 6.26 years No effect of medication on Rs-FC	Left Middle/Superior Frontal Gyrus, Left IFG	(i) BD<HC: Decreased functional connectivity of the left middle/superior frontal gyrus and the bilateral medial frontal gyrus and the left and right superior and middle temporal gyrus ($p<0.001$, cluster-level correction) in BD relative to HC. (ii) BD>HC: Increased functional connectivity of the left middle/superior frontal gyrus and the bilateral dorsal cingulate cortex ($p<0.001$, cluster-level correction) in BD compared to HC. (iii) BD<HC: Decreased functional connectivity of the left IFG and the left

					hippocampus ((p<0.001, cluster-level correction).
Rey et al. (2016)	DSM-IV TR criteria & Mini-International Neuropsychiatric Interview (MINI)	HC: (i) no history of neurological illness; (ii) no history of Axis 1 psychiatric disorders as assessed by the MINI; (iii) not taking any drug.	Mood Stabilizers: (9) Antipsychotics: (7) Antidepressants: (6) Benzodiazepines: (4) Psychostimulats: (1) No medication sub-analysis reported	Amygdala (L,R), sgACC (L,R), PCC, mPFC, vlPFC (L,R)	(i) BD<HC: Decreased functional connectivity between the left sgACC and PCC. (ii) BD<HC: Decreased functional connectivity between the right sgACC and right vlPFC. (iii) BD>HC: Increased functional connectivity between the left and right sgACC in BD relative to HC.
Reinke et al. (2013)	Structured Clinical Interview for DSM-IV Axis I Disorders	BD: (i) no comorbid Axis-I or II disorders; (ii) BDI-II<18 and BRMAS<7; (iii) stable mood state (iv) no changes in medication within the month preceding study enrolment HC: (i) no current drug-abuse (ii) history of neurological disease (iii) no history of Axis-1 or II disorders; (iv) ability to provide consent and a family history of affective or psychotic disorders	Specific medication history not reported in paper. There were no significant relationships between the indices of medication and the results in resting state neuronal activation (p > 0.05)	Auditory Cortex: Heschl's Gyrus, Planum Temporale (PT)	(i) BD<HC: Decreased functional connectivity between bilateral Heschl's gyrus the left middle temporal gyrus (BA 22) in BD relative to HC. (ii) BD<HC: Decreased functional connectivity between the bilateral PT and the right superior and middle temporal gyrus in BD relative to HC. (iii) BD>HC: Increased functional connectivity between the PT and right inferior frontal/precentral gyrus and the insula in BD

					relative to HC.
Syan et al. (2017)	Structured Clinical Interview for DSM-IV Axis I Disorders	All: (i) no use of systemic hormonal treatment within 3 months of study enrolment; (ii) not currently pregnant; (iii) no contraindication for MRI; (iv) no history of head trauma resulting in a loss of consciousness; (v) no neurological disorders affecting cognition; (vi) no current or recent (6 months) alcohol drug abuse or dependence; (vii) no unstable medical conditions. BD: (i) no current depressive, manic or hypomanic episode; (ii) no changes in psychotropic medications or mood state with in 2 months prior to enrolment. HC: (i) no current or lifetime history of psychiatric disorder	Lithium (3), Anticonvulsants (15), Anxiolytics (6), Antipsychotics (16), Antidepressants (12), Sleep aids (2), Unmedicated (7). Sub-analysis found no effect of medication load on Rs-FC analysis.	ICA – DMN, FPN, MPN SBA – PCC, right and left dlPFC (BA 46), right and left amygdala	(i) BD>HC: Increased functional connectivity between the PCC and AG in BD vs HC. (ii) BD>HC: Increased functional connectivity between the right dlPFC and brainstem in BD vs. HC. (iii) No differences in functional connectivity between groups within networks using ICA (iv) In the BD group only, PCC-AG coupling was positively correlated with state anxiety (r_S=0.39; P=.028)
Torissi et al. (2013)	Structured Clinical Interview for DSM-IV	All: (i) right-handedness; (ii) no neurological illness; (iii) no metal implants; (iv) no history of skull fracture or head trauma with loss of consciousness > 5 minutes. BD: (i) no other current Axis I psychiatric disorder HC: (i) current or lifetime history of psychiatric disorders (including substance abuse); (ii) not taking medications for any medical reasons	Antipsychotics (15%), Antidepressants (75%), and Anticonvulsants (Valproic acid: 25%, Lamotrigine: 20%). Medication sub-analysis reported no significant effect of medication on observed results	Amygdala (L/R), vlPFC (L/R)	(i) BD>HC: Increased connectivity between the right amygdala and right vlPFC in BD vs HC. This pattern of connectivity was not correlated with any clinical variables – illness duration, number of depressive or manic episodes or HDRS/YMRS scores. (ii) There were no

					differences in whole brain connectivity between BD and HC in the primary somatosensory cortex (BA 1), auditory cortex (BA 41,42) or the primary visual cortex (BA17). (iii) ACC mediated the effect in (i) (Sobel Test, Z=7.88)
Absolute & Fractional Amplitude of Low Frequency Fluctuations (AALF)					
Meda et al. (2014)	Chart Review and Structured Clinical Interview for DSM-IV-TR (SCID I/P)	BD: all participants were stable for at least 1 month prior to scanning and on stable medications	Unknown Medication History (4) Medication Naive (8) Not Medicated (14) Antipsychotics (124) Mood stabilizer (122) Antidepressant (82) Anxiolytic (55) Anticholinergic (15) Stimulants (18) Miscellaneous (5) Medication sub-analysis results: No significant effects of CPZ equivalents were noted in ALFF/fALFF. There was no effect of medication doses for slow-5 and slow-4, however minimal effects were present	NA	(i) BD<HC: BD displayed decreased power in the medial frontal gyrus and ACC (Slow 5). (ii) BD>HC: BD displayed increased power in the inferior/middle temporal gyrus, uncus and parahippocampus relative to HC (Slow 4). (iii) BD<HC: Reduced power was seen in the pre and post central gyri in BD vs. HC.

			with antipsychotics on slow-4 (reduced ALFF in the lingual/precuneus region)		
Lui et al. (2014)	Structured Clinical Interview for DSM-IV	All: (i) no history of significant neurological or systemic illness; (ii) negative urine drug screen for common drugs of abuse on the day of testing; (iii) no diagnosis of substance abuse in the prior 30 days, or substance dependence in the prior 6 months; (iv) not currently pregnant; (v) no head translation or rotation movement during scanning >1.5mm. HC: (i) free of Axis I psychiatric disorders and not taking psychoactive medications. BD: (i) clinically stable for one month prior to study participation; (ii) stable medication for treatment for one month prior to testing	Mood Stabilizers (38) Mean chlorpromazine equivalent daily dose (236, SD 249) Correlational analysis of MRI data and medication was not significant	Regions displaying differences in ALFF were used as seed points in a whole brain functional connectivity analysis	(i) BD<HC: Decrease ALFF in the left-OFC and left-ACC in BD relative to HC. (ii) BD>HC: increased functional connectivity between the right-thalamus and the left insula, left-pre and postcentral gyri and right SFG; between the right thalamus and the bilateral cuneus; between the left ACC and left precuneus ($p<0.05$, AlphaSim to correct for multiple comparisons) in BD compared to HC.

Fronto-occipital Network (FON), Cerebellum/Midbrain Network (CER), Posterior Default Mode Network (pDMN), Anterior Default Mode Network (aDMN), Paralimbic Network (PL), Fronto-thalamic/Basal Ganglia Network (BGN), Salience Network (SN), Sensorimotor Network (SMN), Right Fronto Parietal Network (rFPN), Left Fronto-Parietal Network (lFPN), Parietal Network (PN), Auditory Related Network (ARN), Vision Related Network (VRN), Visospatial Network (VSN). Auditory Network (AUD), Cognitive Control Network (CC), Temporo-Insular Network (TIN), Motor Network (MN), Executive Control Network (ECN). Superior Frontal Gyrus (SFG), Middle Frontal Gyrus (MFG), Orbitofrontal Cortex (OFC), Anterior Cingulate Cortex (ACC), Pre-Frontal Cortex (PFC), Ventrolateral Prefrontal Cortex (vlPFC). German Version Bech Rafaelsen Mania (BRM), Beck Depression Inventory-II (BDI-II), Posterior Cingulate Cortex (PCC), Anterior Cingulate Cortex (ACC), Ventral Anterior Cingulate Cortex (vACC), Medial Prefrontal Cortex (mPFC), Left Superior Temporal Gyrus (LSTG), Left Middle Temporal Gyrus (LMTG), Left Angular Gyrus (LANG).

Chapter 4: Resting State Functional Connectivity in Females with Bipolar Disorder During Clinical Remission

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4.1. Abstract

Objectives: Periods of euthymia in bipolar disorder (BD) serve as a valuable time to study trait-based pathophysiology, and the use of resting state functional connectivity (Rs-FC) can aid in the understanding of BD pathophysiology free of task or mood state biases. The present study investigated two unexplored areas of Rs-FC research in bipolar remission: (1) Rs-FC in females, controlling for potential influence of premenstrual symptoms (2) the use of both independent component based analysis (ICA) and seed based analysis (SBA) to investigate Rs-FC.

Methods: We investigated Rs-FC of the default mode network, meso-paralimbic network and fronto-parietal network in a sample of 32 euthymic women with BD and 36 age-matched controls during their mid-follicular phase of the menstrual cycle. Rs-FC was assessed with ICA and SBA using the PCC, amygdala and dlPFC as seed points for their respective resting state networks.

Results: In BD, compared to controls, SBA analyses revealed increased coupling between: PCC-angular gyrus (p=0.002, FDR-corrected) and right dlPFC-brainstem (p=0.03, FDR-corrected). In BD only, PCC- angular gyrus coupling was correlated with anxiety symptoms. Group differences in Rs-FC using ICA did not survive multiple comparisons.

Conclusions: Negative findings from whole-brain ICA Rs-FC may reflect a state of clinical remission in BD. Heightened activation between PCC-angular gyrus and dlPFC-brainstem may reflect (1) an abnormal trait integration of affective information during clinical remission and/or (2) an adaptive compensatory mechanism required for clinical stabilization.

4.2. Introduction

There is considerable evidence suggesting that the state of clinical remission (euthymia) in bipolar disorder (BD) is often accompanied by subthreshold symptoms such as emotional lability and cognitive impairment (1-3). These clinical findings are in line with neurobiological models of BD including dysregulation in emotional and cognitive networks such as hypoactive dorsal and hyperactive ventral neural streams (4-11). The dorsolateral PFC (dlPFC), ventrolateral PFC (vlPFC), dorsal anterior cingulate cortex (dACC) and hippocampus encompass the dorsal neural stream (4-7, 11, 12), integral to mediating cognitive control and voluntary subprocesses of emotional regulation. The ventral system is comprised of traditional "limbic" regions, such as the insula, amygdala, ventral striatum, ventral anterior cingulate cortex (vACC) and ventromedial prefrontal cortex (vmPFC) and is involved in implicit aspects of emotional regulation (4-7, 11, 12). Abnormal patterns of functional connectivity involving regions in both neural systems have been reported during task-based and resting state fMRI studies in BD across all mood states (13,14). Patterns of resting state functional connectivity (Rs-FC) have shown greatest dysregulation during periods of mania and depression; literature during euthymia is more sparse and conflicting (13,14). The study of emotional and cognitive regions during euthymia may provide a unique picture into the compensatory mechanisms required to maintain a remitted clinical state; it may also be useful in the understanding of the impact of subclinical symptoms that are frequently observed in euthymic individuals with BD.

Rs-FC is commonly studied through the use of independent component analysis (ICA) or seed based analysis (SBA). ICA is an exploratory, data-driven approach used to visualize resting state network (RSN) connectivity (12). SBA is a hypothesis driven approach, which correlates the activation of a predefined "seed" region with activation in other brain regions. The synchronous use of both methods provides visualization of functional connectivity of RSNs as well as

connectivity of seed regions integral to the functioning and maintenance of the RSNs. Although sparse, previous studies examining Rs-FC in euthymic BD using SBA have found increased coupling between the amygdala-mPFC (13, 14), amygdala-vlPFC in BD type-I (15) and amygdala-right dlPFC (16). Use of the same approach, also found decoupling between the dlPFC-mPFC (14) and right dlPFC-amygdala (13). Although these results support the neurobiological hypothesis of persistent dysfunctional fronto-limbic connectivity in euthymic BD, most studies employing ICA to investigate Rs-FC of the default mode network (DMN), fronto-parietal network (FPN) and meso-paralimbic network (MPN) have consistently found no differences between euthymic BD and controls (17-20). An exception is a small study (n=15) of medication-naive subjects found increased activity of the bilateral insula and putamen comprising the temporo-insular network in individuals with BD type-II (20). In summary, together with several ICA studies showing abnormal Rs-FC in BD during manic and depressive states (10, 21-23), current ICA literature suggests that RSN stability may be a hallmark of bipolar euthymia.

The impact of sex on Rs-FC in BD is currently unknown. A recent study in healthy volunteers found different patterns of Rs-FC between men and women when using the laterobasal and centromedial nuclei of the amygdala as seed points (24). This is interesting as the amygdala is a well-established hub of functional dysregulation in BD and contains a wealth of estradiol receptors (4-6,8,10, 11, 16, 25). Also, increased Rs-FC has been reported in women relative to men in regions of the anterior network and right dorsal network (26), whereas men had stronger posterior cingulate cortex (PCC) coupling with the dlPFC than women (27). No sex differences have been found using an ICA of the salience network, central executive network and DMN (28). Taken together, these studies suggest that sex may influence certain brain networks related to Rs-FC. Although the influence of sex in Rs-FC in BD has not been studied, a recent review summarizing volumetric and task-based fMRI differences in limbic and frontal regions between

bipolar men and women suggested that sex may play an important role in brain structure and function in BD (29).

Clinically, BD women report more depressive and mixed episodes than men and are more prone to develop BD type-II and rapid cycling (30-33). Bipolar females also experience higher rates of comorbid post-traumatic stress disorder, eating disorders and personality disorders (33). Female-reproductive life events are also critical periods of mood worsening and relapse in BD. In particular, independent studies have shown higher rates of premenstrual worsening in women with BD (34-39). However, to our knowledge, no Rs-FC study has taken menstrual cycle phase into consideration in BD research. Thus, the objective of the present study was to investigate Rs-FC in a well-defined sample of women with BD during clinical remission. All women were investigated during the mid-follicular phase of the menstrual cycle to avoid potential premenstrual worsening of mood. We investigated the Rs-FC of the DMN, FPN and MPN using ICA and corresponding SBA of the major seed points of these networks, namely the PCC, bilateral dlPFC and bilateral amygdala. We hypothesized that females with BD will display differences in Rs-FC of selected seed points of RSN (PCC, dlPFC and amygdala) using SBA compared to matched controls. We do not anticipate finding any differences in Rs-FC of RSN using ICA.

4.3. Materials and Methods

4.3.1. Participants

This study was approved by the Hamilton Integrated Research Ethics Board (HiREB) and adhered to the tenets of the Declaration of Helsinki. All participants provided informed written consent before study entry. Participants were recruited through community-based advertisements in the Hamilton and Halton Regions, Ontario, Canada.

Sixty-eight right-handed women (HC: n=36, BD: n=32) between 16-45 years of age, with regular menstrual cycles were enrolled. Exclusion criteria for all participants included: (1) current or recent (last 3 months) use of any systemic hormonal treatment; (2) pregnancy; (3) contraindications for MRI; (4) history of head trauma resulting in a loss of consciousness; (5) neurological disorders affecting cognition; (6) current or recent (6 months) alcohol or drug abuse or dependence. Study participants were told that recreational drug use was also not allowed during the course of study participation. We did not perform drug tests on the day of the MRI scans. Exclusion criteria for BD subjects included: (1) current depressive, manic or hypomanic episode according to the SCID-I (40); (2) changes in psychotropic medications or mood state within 2 months prior to or during the study; (3) unstable medical conditions. Due to the very high rates of comorbid psychiatric conditions in BD we allowed lifetime but not current psychiatric comorbidities to provide a true reflection of individuals with BD. HC females were deemed not eligible for the study if they presented with a lifetime history of any psychiatric disorder according to the SCID-I.

4.3.2. Study Design

The first visit consisted of administration of the SCID-I followed by a demographic and brief gynaecological clinical history. The second visit took place during the mid-follicular phase (days 5-10) of their menstrual cycle. Menstrual cycle phase was confirmed by 2 months of prospective charting using the Daily Record of Severity of Problems (DRSP) (41) and hormonal assays. This visit included one MRI scan, collection of a blood sample for hormonal analyses and completion of validated clinical questionnaires, as described below.

4.3.3. Clinical Questionnaires

The Montgomery-Asberg Depression Rating Scale (MADRS) (42) and Hamilton Depression Rating Scale (HAM-D) (43) were administered to assess severity of depressive symptoms. The Young Mania Rating Scale (YMRS) was administered to assess severity of mania/hypomania (44). Disruptions in biological rhythms were assessed using the self-reported Biological Rhythms Interview of Assessment in Neuropsychiatry (BRIAN) (45). The Pittsburgh Sleep Quality Index (PSQI) was used to assess sleep quality (46) and the State and Trait Anxiety Inventory (STAI) was used to identify state and trait anxiety symptoms (47).

4.3.4. Hormone Assays

Immediately following the MRI scans, 10 ml of whole blood was collected in serum tubes to measure 17-β-estradiol and progesterone levels. Blood was clotted at room temperature for 45 minutes, and centrifuged at 4^{o}C for 15 minutes at 3000 rpm. Serum aliquots were obtained and frozen at -80^{o}C until assayed. Serum was assayed using commercially prepared solid phase enzyme-linked immunosorbent assay (ELISA) kits, purchased from ALPCO Diagnostics (Salem, NH). All serum samples were assayed in duplicate following the manufacturer's protocol, with a fresh aliquot for each analyte. The inter-assay variations for progesterone and estradiol were 11.3% and 8.7% respectively. The intra-assay variations were 10.4%, and 7.7%, and the sensitivities were 0.1 ng/ml and 10 pg/ml, respectively.

4.3.5. Imaging Acquisition and Preprocessing

Images were acquired using a GE whole body short-bore 3T scanner with 8 parallel receiver channels (General Electric, Milwaukee, WI, USA). Anatomical images were acquired with high-resolution T1-weighted images (gradient-echo inversion-recovery sequence, TR=1.6s, TE=5ms,

matrix 256x256x128, FOV 220 x 220mm, slice thickness 1mm). Functional resting state imaging was completed using a T2* interleaved echo-planar imaging (EPI) sequence with TR=2000ms, TE=40ms, flip angle=60°, 4mm thick, 29 axial slices, matrix 64x64 resolution over 256 mm FOV). Of the 129 volumes acquired, the first 2 were discarded to account for T2 stabilization effects. Once positioned in the scanner participants were instructed to "Lay still, relax and try not to think about anything in particular" as they looked at a fixation point. The entire scan took place for 10 minutes. We communicated with participants at the beginning and the end of the scans and confirmed that they remained awake with their eyes fixed on the fixation cross for the entire duration of the scan.

The resting state and anatomical MRI data were preprocessed using the Statistical Parametric Mapping Software SPM12 (http://www.fil.ion.ucl.ac.uk/spm). Imaging data was obtained in DICOM file format and converted to NIFTI. Anatomic data was segmented to white matter (WM), cerebrospinal fluid (CSF) and grey matter (GM) using affine regularization (light bias regularization=0.001, Bias FWHM = 60mm cut off), according to the ICBM space template for European Brains (48). Subsequent to this, images were normalized to standard space using a 4th Degree B-Spline Interpolation and resampled to 1mm x 1mm x 1mm voxels (49). Deformation fields created during the segmentation process were used during normalization for increased precision in the alignment of images. Resting state images were then motion corrected; estimated using 2nd Degree B-Spline Interpolation and replaced using 4th Degree B-Spline Interpolation (50). Images with motion greater than 3mm in the translational plane and 3 degrees in the rotational plane were discarded. Co-registration of functional images with anatomical images was estimated using the Normalized Mutual Information Function (51). Images were spatially smoothed to increase the signal to noise ratio with a 8 mm FWHM Gaussian filter (52).

4.3.6. Seed Based Analysis (SBA)

SBA was completed using the CONN toolbox v.15.d (https://www.nitrc.org/projects/conn). Diagnosis was used as a factor and age as a covariate. Subject specific maps of CSF and WM were used as nuisance regressors. The aCompCor strategy was employed within CONN to control for the effects of physiological motion and residual head movement (53). The function images were temporally band-pass filtered (0.008-0.09 Hz). The PCC, bilateral dlPFC and bilateral amygdala were used as seed-points for the DMN, FPN, and MPN respectively, in a ROI-ROI analysis using the Brodmann Area and Harvard-Oxford Atlas available with CONN (54). Second level statistical analyses were completed using two-sample *t-tests* comparing connectivity as measured by correlation in BOLD time course between groups. FDR-corrected activations with $p<0.05$ were considered significant. Since BD and HC subjects differed in BMI, we tested any potential effects of BMI on Rs-FC by (1) adding BMI as a second-level covariate and (2) directly analyzing the effect of BMI for particular regions of interest within groups.

4.3.7. Effect of Medication on Rs-FC

Additional analysis was completed to assess the potential effect of medication load on Rs-FC in bipolar subjects according to previous imaging studies investigating dose equivalences (55-57). Antipsychotics, mood stabilizers, antidepressants and anxiolytics were coded to 0, 1 or 2 to represent absent, low or high dose medication groupings previously employed by Hassel and colleagues (55). Mood stabilizers and antidepressants were categorized into groups from 1-4 depending on medication dose (57). Based on this, individuals in category 1 or 2 were grouped in the low dose group (scoring a 1), and individuals in category 3 or 4 were grouped in the high dose group (scoring a 2). Further, antipsychotics were converted to chlorpromazine equivalent doses. Doses below or above the mean effective daily dose of chlorpromazine were coded as 1 or 2,

respectively. Individuals not taking antipsychotics were coded 0. Anxiolytics were also grouped into similar categories based on the recommended daily dosage found in the *Physician's Desk Reference*. In this case, the dose was coded as 0 if absent or present in very low dose with reference to the midpoint, 1 if around the midpoint and 2 if higher than the midpoint. Composite scores of medication load were then calculated and used as second-level covariates in CONN in an analysis using our a-priori seed points.

4.3.8. Independent Component Analysis (ICA)

Group ICA was performed using GIFT software, version 3.0a (http://mialab.mrn.org/software/gift/index.html). Detailed descriptions of methods employed in this program have been previously published (58). The minimum description length (MDL) criteria was used to determine 38 independent components (ICs). Subject-specific data was reduced from 127 time-points to 57 time-points and further to 38 ICs using subject-specific principle component analysis. ICs were estimated using the Infomax algorithm and repeated in ICASSO 20 times (59). Group level spatial and time course maps were then back-constructed using the GIGA method and calibrated into z-scores, normalized across all subjects. Networks of interest were identified by visual inspection and supported by previous literature (60-62).

Spectral characteristics (dynamic range and low frequency to high frequency power ratio) of the 3 identified networks were examined using the same procedure as Allen et al (62). Dynamic range was estimated as the difference between the peak spectral power and minimum spectral power at frequencies to the right of the peak. The low frequency to high frequency power ratio was below 0.10 Hz to the integral of power between 0.15 Hz and 0.25 Hz. This criterion was used to confirm that all networks analyzed were dominated by low frequency BOLD fluctuations in the 0.01 - 0.1 Hz range.

Statistical analysis was completed with SPM12 using the subject-specific z-maps of the 3 RSN studied (Table 1). Two-sample *t-tests* were performed on the 3 components of interest to compare network connectivity between groups. A p-value of <0.05 (Family-wise error) was considered statistically significant. BMI was added as a covariate using the MANCOVAN toolbox available in GIFT (http://mialab.mrn.org/software/mancovan/).

Statistical Analysis (Clinical Questionnaires)

Statistical analysis of clinical variables was completed using R version 3.1.2. (https://www.r-project.org). Clinical domains were analyzed using two-sample *t-tests* or a Mann-Whitney U test with a confidence interval of 95% where applicable. Variables were correlated using either Pearson or Spearman correlations where applicable. A p-value <0.05 was considered significant.

4.4. Results

4.4.1. Clinical Characteristics

BD females had higher BMI than controls (p<0.001), thus BMI was added as covariate in all analyses. Age and years of education were not different between controls and BD. As expected, even though all BD subjects were clinically in sustained remission, their MADRS, HAMD, YMRS, PSQI, BRIAN and STAI scores were higher than controls consistent with many studies showing that presence of sub-threshold/residual symptoms are common in the course of BD even during euthymia *(Table 2)*.

4.4.2. Seed-Based Functional Connectivity

In the DMN seed, we found increased coupling between the PCC and the right angular gyrus (AG) in BD relative to HC (p=0.002, FDR-corrected) (Fig.Ia/c). In the FPN seed points, we found increased coupling between the right dlPFC and the brainstem in BD relative to HC (p=0.03,

FDR-corrected) (Fig.Ib/d). There were no differences in Rs-FC between the MPN seeds right and left amygdala and any other brain regions. Importantly, we found no effects of medication load on Rs-FC of the bilateral dlPFC, amygdala or PCC.

4.4.3. ICA-Based Functional Connectivity

The 3 RSN of interest were identified among 3 components (Figure 2). Specifically, the DMN was comprised of the dorsal anterior cingulate cortex, medial prefrontal cortex, precuneus, posterior cingulate cortex, inferior temporal gyri and angular gyri. The MPN was dominated by bilateral amygdala, temporal, parahippocampal and hippocampal activation. The FPN included the dlPFC and vlPFC. Using ICA we found no differences in DMN, FPN, or MPN Rs-FC between BD females and controls (all $p>0.05$).

4.4.4. Clinical Correlations

Spearman correlations were used to examine correlations between clinical symptoms and the RSN that were significant in the SBA *(Table 3)*. In the BD group only, PCC-AG coupling was positively correlated with state anxiety ($r_S=0.39$; $p=0.028$). However, this result should be considered exploratory, as it would not withstand correction for multiple comparisons.

4.5. Discussion

We examined Rs-FC in three large-scale brain networks and also examined whole brain connectivity to selected nodes from these networks. More specifically, we investigated the Rs-FC of the DMN, FPN, and MPN using both ICA and SBA approaches to obtain a comprehensive picture of brain Rs-FC in a well-characterized sample of euthymic bipolar women. Using SBA, we found increased PCC-AG and dlPFC-Brainstem coupling in the BD group relative to controls. Further, PCC-AG coupling was positively correlated with the severity of state anxiety in BD. We found no differences in Rs-FC using amygdala as the seed point, as well as no

differences in Rs-FC using ICA, suggesting that the amygdala and ICA RSN stability may be hallmarks of clinical stability in BD.

Out of the three *a priori* defined seed points, the PCC and dlPFC showed increased Rs-FC in the BD group. The PCC and AG are two central areas of the DMN involved in introspective processing, emotional processing, memory and spontaneous cognition (e.g. mind wandering, day dreaming, self reflection) (63-65). Impairment in social and cognitive domains have been widely reported in BD during euthymia (1-5,8). Therefore, greater coupling between PCC-AG may serve to guide certain cognitive functions such as working memory, semantic processing and internally directed and social cognition, thereby mitigating the severity of these impairments and contributing to a state of remission. On the other hand, we found that PCC-AG coupling was correlated with state anxiety, suggesting that this heightened connectivity may be associated with subsyndromal levels of anxiety during euthymia. This hypothesis is supported by a recent study that found a negative correlation between posterior regions of the DMN, including the PCC and AG, and anxiety in healthy volunteers (65).

Increased coupling between the right-dlPFC and brainstem can be interpreted taking a variety of factors into account. First, BD is associated with abnormalities in affective regulation, cognition, energy and regulatory processes such as sleep (5,66). These processes are, at least in part, influenced by primitive neural structures such as the brainstem and hypothalamus (66-67). The brainstem plays a central role in mediating emotional regulation via the autonomic nervous system, sensorimotor experience and neuroendocrine systems (66-67). It is also involved in complex top-down and bottom-up modulation of cortical and limbic/paralimbic circuitry through interaction with the amygdala and prefrontal regions; thereby contributing to emotional experience and regulation of emotional states (9,67). While much of this circuitry is known to be dysregulated in BD, previous research has largely neglected the involvement of the brainstem in

in Rs-FC in BD. Therefore, we postulate that increased Rs-FC between the right dlPFC and brainstem may reflect a heightened activity of top-down and/or bottom-up processes between prefrontal and primitive neural regions which may be associated with a state of clinical remission (5,9,66-67).

Contrary to our hypothesis, we did not find changes in the Rs-FC of the right or left amygdala in BD, which may reflect stability of the MPN during bipolar euthymia. This finding is consistent with the majority of studies investigating amygdala activation in euthymia during task-based conditions reporting no changes in amygdala activity (see review 11). It should be noted, however, that previous Rs-FC have found alterations in amygdala connectivity in BD. Previous studies have reported that BD type-I subjects during euthymia displayed increased amygdala-vlPFC coupling (15), and decreased amydgala-mPFC coupling in BD individuals with psychosis compared to those without psychosis and healthy controls (13). Further, another study using a sample of primarily BD type-I (BD1=13, BD2=5, NOS=2) described positive coupling between right amygdala and mPFC activity (14). Here it is important to highlight that all of these previous Rs-FC studies used a sample of both men and women, and none accounted for potential influence of menstrual cycle. This may be problematic when investigating amygdala activation specifically because both sex and estrogen levels can influence patterns of Rs-FC of the laterobasal and ventromedial amygdala (27, 28). Since none of the Rs-FC studies in BD thus far controlled for sex or menstrual cycle phase, previous reported Rs-FC amygdala activation may have been, at least in part, confounded by these variables.

We interpret the absence of whole-brain level group differences in the Rs-FC of the RSNs studied to reflect a state of clinical remission. This is consistent with almost all previous Rs-FC studies in euthymic BD using the ICA approach (17-20). The only exception is a recent study reporting increased activity in the temporo-insular network in a small sample (N=15) of

unmedicated BD type-II, although they did not find brain changes in connectivity in the other 7 RSNs examined, including DMN (20). Notably, studies investigating Rs-FC during acute mood episodes in BD have consistently found changes in brain connectivity (21-23). Thus, the presence of abnormal RSN connectivity during manic and depressive episodes and absence of changes in RSN connectivity during euthymia, as seen in our study and others, suggest that RSN stability may be a marker of clinical remission in BD.

4.5.1. Limitations

The limitations of our study must be discussed. First, the effects of psychotropic medications on Rs-FC are a common limitation in neuroimaging research in BD (55, 68). However, the vast majority of individuals with BD require medication to maintain sustained clinical stability because of the high risk of relapse associated with this condition (68). Although our sub-analysis has ruled out effects of psychotropics in our study sample, we still cannot completely rule out potential milder effects of psychotropics on Rs-FC. While few studies in healthy volunteers and unipolar depression have suggested that anxiolytics and antidepressants may affect functional connectivity (69-70), many studies investigating Rs-FC in BD, including ours, have ruled out the influence of mediations following correlational analysis with BOLD activation (71-72). The goal of our study was to investigate Rs-FC in a well-characterized sample of bipolar women during sustained clinical remission and we believe that this goal could only be realistically achieved by allowing a primarily medicated sample, and ensuring all bipolar participants were euthymic using well-validated diagnostic tools such as the SCID-I (40). Another limitation was that our sample included females with both BD type-I and BD type-II. Due to the nature of our study design, it would have been very difficult to exclude an entire subtype of BD while controlling for sex and menstrual phase. Nevertheless, we agree that there might be differences in Rs-FC between type-I and type-II BD females and future studies should investigate

this. Finally, our study was cross-sectional and, as a result, this study can only provide a one shot picture of disease state but not disease progression. Longitudinal studies in this area are necessary. Notable strengths include that this is the first study that (1) used both ICA and SBA to ascertain a holistic picture of RSN Rs-FC in the euthymic phase of BD; (2) studied a well-characterized sample of females with BD in sustained clinical remission; and (3) controlled for menstrual phase in BD.

4.6. Conclusion

In conclusion, our results suggest that the absence of differences in RSN connectivity through ICA may reflect a state of clinical remission, whereas increased connectivity between the PCC-AG (DMN activity), and the dlPFC-brainstem (FPN activity) may reflect trait-based pathology of BD. Since females with BD are more vulnerable to be affected by hormonal fluctuations (e.g. premenstrually, postpartum), studies comparing menstrual phases and other times of hormonal fluctuation would be useful in understanding the effects of endogenous hormones on Rs-FC in BD. Future research should also investigate if alterations in Rs-FC may be associated with clinical features known to be more prevalent in women such as mixed states, rapid cycling and BD-II diagnosis.

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Table 1: Resting state networks studied and corresponding brain and seed regions Brain regions composing networks were derived from well-established resting state literature (51-54).

Table 1. Resting state networks studied and corresponding brain and seed regions			
Network	Goal	Brain Regions	Seed Region (SBA)
Default Mode Network (DMN)	Activated during internally directed thoughts/self referential processing. Strongly deactivated during goal/task directed activity	Medial prefrontal cortex, posterior cingulate cortex, precuneus, lateral parietal cortex/ angular gyrus, inferior temporal gyrus	Posterior Cingulate Cortex (PCC)
(Meso/Paralimbic Network) (MPN)	Processing of emotional and introspective information	Amygdala, hippocampus, parahippocampal gyrus, temporal poles	Left and Right Amygdala
Left/Right Fronto/Parietal Networks (FPN)	Right is responsible for cognitive control and attention Left is responsible for language processing and working memory	Lateral prefrontal regions, inferior parietal cortex	Left and Right Dorsolateral Prefrontal Cortex (dlPFC) (BA 46)

Table 2: Demographic characteristics of study sample (N=68)

Table 2. Demographic characteristics of study sample (N=68)			
	BD (n=32)	HC (n=36)	P-value
Age	29.0 (8.07)	32.8 (8.32)	0.05
BMI	27.8 (5.71)	23.2 (3.74)	<0.001
Years of Education	15.6 (2.61)	16.5 (2.51)	0.13
MADRS, mean (SD)	8.34 (5.80)	4.00 (4.72)	0.001
HAMD, mean (SD)	5.66 (3.86)	2.28 (2.90)	0.0001
YMRS, mean (SD)	1.50 (1.39)	0.67 (1.12)	0.005
BRIAN, mean (SD)	42.59 (10.50)	30.22 (8.79)	<0.0001
PSQI, mean (SD)	7.03 (3.56)	4.50 (2.83)	0.003
STAI-State, mean (SD)	37.77 (11.81)	31.17 (8.70)	0.01
STAI-Trait, mean (SD)	44.94 (12.20)	31.34 (8.51)	<0.0001
BD subtype	BD-I = 18 BD-II = 14		
Age of illness onset, mean (SD)	18.63 (6.84)		
Mean number of psychiatric co-morbidities	1.4		
Psychotropic Medications			
Lithium	3		
Anticonvulsants	15		
Antipsychotics	16		
Anxiolytics	6		
Antidepressants	12		
Sleep Aids	2		
Unmedicated	7		
Mean # of psychotropic medications	1.8		

Table 3: Spearman correlation coefficient between significant SBA and clinical symptoms in bipolar disorder females and controls
* p<0.05, uncorrected
** p<0.05, Bonferroni-corrected

Table 3: Spearman correlation coefficients between significant SBA and clinical symptoms in bipolar disorder subjects and controls				
Component Combination	BD		HC	
	Correlation Coefficient	p-value	Correlation Coefficient	p-value
PCC/AG - HAMD	0.06	1	-0.26	0.66
PCC/AG – YMRS	0.1	1	-0.38*	0.024*
PCC/AG – BRIAN	0.27	0.72	-0.03	1
PCC/AG – STAI State	0.39*	0.028*	0.15	1
PCC/AG – STAI Trait	0.24	0.96	0.15	1
dlPFC-r/Brainstem - HAMD	0.01	1	-0.25	0.75
dlPFC-r/Brainstem – YMRS	0.16	1	-0.48*	0.019*
dlPFC-r/Brainstem – BRIAN	-0.31	0.45	0.04	1
dlPFC-r/Brainstem – STAI State	-0.27	0.74	0.06	1
dlPFC-r/Brainstem – STAI Trait	-0.27	0.68	-0.02	1

Figure 1: Significant differences in resting state functional connectivity between bipolar disorder and healthy females using Seed-Based Functional Connectivity analysis. Black circle shows seed point and red circle shows target ROI region. A and C: Increased coupling of the posterior cingulate cortex (PCC) and right angular gyrus (AG) in BD subjects compared to controls (p=0.002, FDR-corrected). B and D: Increased right dorsolateral prefrontal cortex (dlPFC) and brainstem coupling in BD (p=0.03, FDR-corrected). Brainstem seed MNI coordinate: 17, -34, -28 mm.

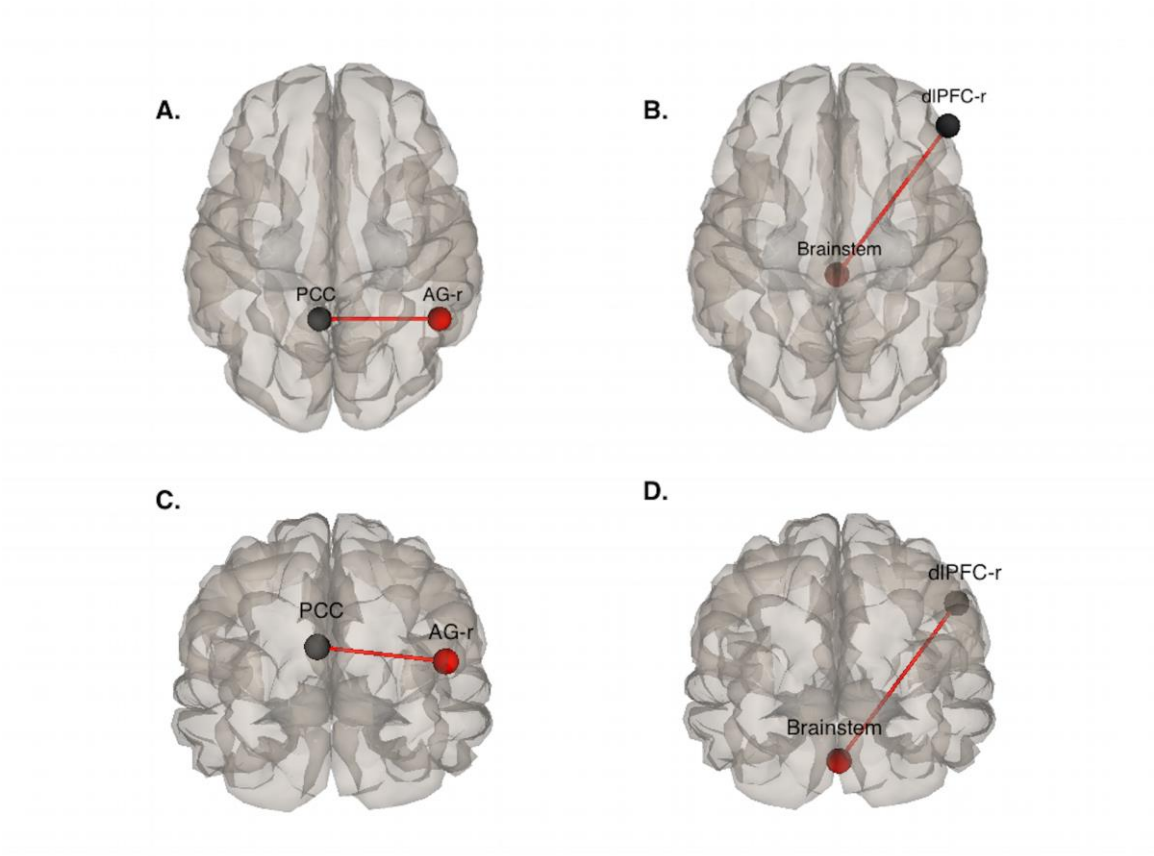
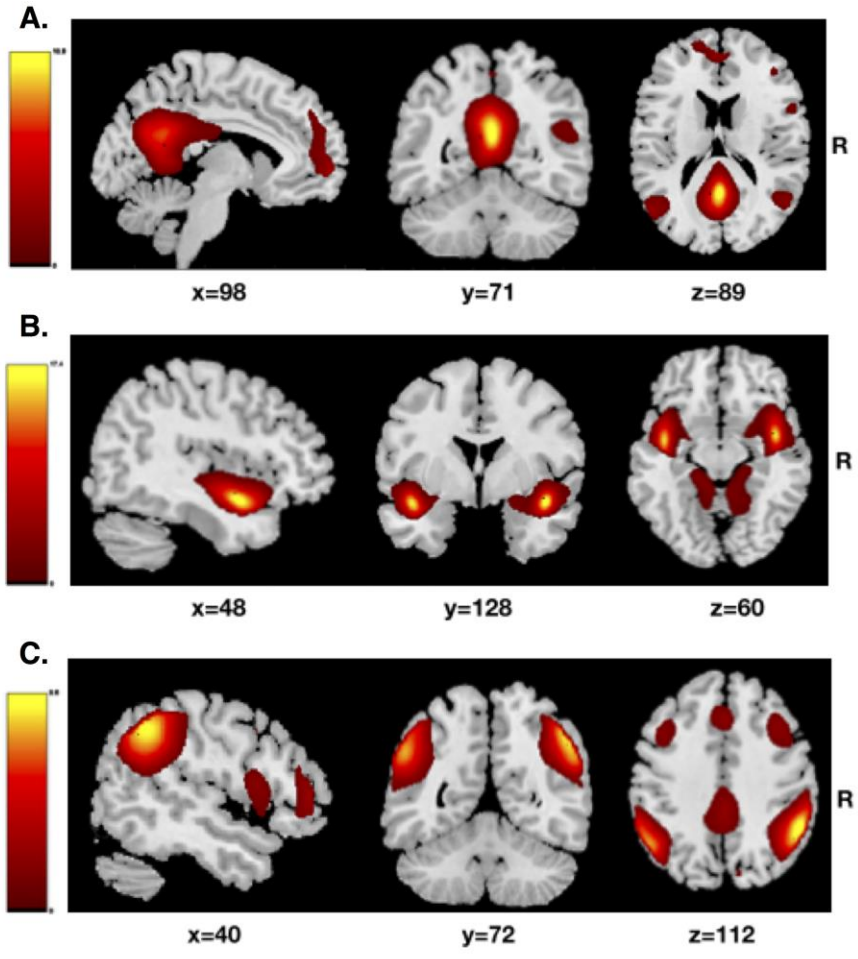


Figure 2: Networks of interest identified through Independent Component Analysis: One-sample t-test maps of networks of interest, ($p<0.05$, FWE-corrected). A. Default Mode Network (DMN); B. Meso-Paralimbic Network (MPN); C. Fronto-Parietal Network (FPN). X, Y and Z represent MNI coordinates of the representative image of each network. Scales represent intensity of BOLD signal (t-values).



Chapter 5: Changes in Somatosensory Cortex in Euthymic Bipolar Patients

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5.1. Abstract

Objective: Current evidence from neuroimaging data suggests possible dysfunction of the fronto-striatal-limbic circuits in patients with Bipolar Disorder (BD). Somatosensory cortical function has been implicated in emotional recognition, risk taking, and affective responses through sensory modalities. The present study investigates anatomy and function of the somatosensory cortex in euthymic bipolar women.

Method: 68 right-handed euthymic women (BD=32 and HC=36) between 16-45 years of age underwent high-resolution anatomical and functional MRI during the mid-follicular menstrual phase. The somatosensory cortex was used as a seed region for resting-state functional connectivity (Rs-FC) analysis. Voxel-based morphometry (VBM) was used to evaluate somatosensory cortical grey matter (GM) volume between groups.

Results: We found increased Rs-FC between somatosensory cortex and insular cortex, inferior prefrontal gyrus, and frontal orbital cortex in euthymic BD subjects compared to healthy controls. VBM analysis showed decreased GM in the left-somatosensory cortex in the BD group. Whole-brain VBM analysis controlled by age did not reveal any additional significant difference between groups.

Conclusions: This study is the first to date to evaluate anatomy and function of somatosensory cortex in a well-characterized sample of euthymic BD females. Anatomical and functional changes in somatosensory cortex in this population might contribute to the pathophysiology of BD.

Key Words: Resting-state, Female, fMRI, VBM, Somatosensory Cortex, Euthymia, Bipolar Disorder

5.2. Introduction

Bipolar disorder (BD) is a chronic mental illness characterized by the presence of episodic mood symptoms (depression and hypomania/mania) with a prevalence of 1-4% in the general population (1,2). Despite there being a similar transcultural presentation and its high heritability rates (3-6), the exact pathophysiology of BD is still undetermined. Probably the most accepted and replicated neurobiological model from neuroimaging studies suggest possible dysfunction of the fronto-striatal-limbic circuits in the brains of individuals who suffer from BD (7-11).

Resting-state functional connectivity (Rs-FC) is an imaging technique that measures spontaneous low-frequency oscillations of blood-oxygen-level dependent (BOLD) signal to determine points of high temporal correlation between two brain regions in the absence of a specific task (12). Resting-state seed-based analysis provides a functional connectivity map of brain voxels that are correlated through resting-state time-series using a predefined "seed" region (13). Investigating spontaneous brain activity in remitted BD subjects (euthymia) might provide an important understanding into the pathophysiology of the disease. Previous studies of Rs-FC in euthymic BD subjects have mainly studied connections between limbic and cortical regions (14) and used the amygdala as a seed point. Studies have shown abnormal connectivity between amygdala and prefrontal regions (15,16), amygdala and posterior cingulate cortex (17), and amygdala and supplemental motor area and Brodmann area 5 (18).

Our group recently studied a well-characterized sample of females with BD during euthymia to investigate Rs-FC of critical brain networks including the default mode, fronto-parietal and meso-paralimbic networks (19). Here we set out to further investigate potential anatomical and functional changes of the somatosensory cortex in euthymic BD individuals using

voxel-based morphometry (VBM) and seed-based Rs-FC. The somatosensory cortex or postcentral gyrus corresponds to Brodmann areas (BA) 3, 1, and 2 (20), and not only receives and integrates sensory information from the body (21) but is also involved with the capacity to sense internal bodily responses (22), recognition of emotional facial expression (23) and risk-taking behavior in females (24). Notably, a recent large neuroimaging meta-analysis of over 6000 subjects revealed that the somatosensory cortex is one of the sensory regions involved with affective-related activity, suggesting that brain regions originally thought to be involved solely with sensory processes may be also involved with affective regulation (25). Abnormal anatomical volume or function of the somatosensory cortex has been reported in depression (26-28), anxiety disorders (29), schizophrenia (30,31), and obesity (32). Our group has recently reported increased cortical thinning in the somatosensory cortex of high-risk adolescents with a parent with BD (33). However, to the best of our knowledge no imaging study has specifically focused on somatosensory structure and function in individuals with BD.

Aims of the Study

The objective of the present study was to evaluate the gray matter volume and Rs-FC of the somatosensory cortex in a well-characterized sample of euthymic women with BD. We hypothesized that women with BD will display 1) decreased in grey matter volume in the somatosensory cortex, and 2) abnormal Rs-FC between somatosensory cortex and limbic regions associated with affective regulation compared to age-matched controls.

5.3. Material and Methods

5.3.1 Participants

The present study was approved by the Hamilton Integrated Research Ethics Board (HiREB) and was conducted in accordance with the Helsinki Declaration. All participants provided written consent to participate in the study. Participants were recruited through community-based advertisements in the Hamilton and Halton regions, Ontario, Canada. Subjects were 68 right-handed women (32 subjects diagnosed with BD and 36 healthy controls) between 16-45 years of age, with regular menstrual cycles (25-32 days). Exclusion criteria for BD subjects included: (1) current mood episode (depression, hypomania/mania); (2) current psychiatric comorbidities; (3) changes in psychotropic medications or mood state within 2 months prior to study entry; and (4) presence of unstable medical conditions. Exclusion criterion for healthy controls (HC) was the presence of any lifetime psychiatric disorders and unstable medical conditions. Exclusion criteria for all women included: (1) current or recent (last 3 months) use of any systemic hormonal treatment; (2) pregnancy; (3) contraindications for MRI; (4) history of head trauma resulting in a loss of consciousness; (5) neurological disorders affecting cognition; and (6) current or recent (in the last 6 months) alcohol or drug abuse or dependence.

Psychiatric history was evaluated using the structured interview SCID-I for DSM-IV-TR (34) followed by a gynecological clinical history. Magnetic resonance imaging (MRI) data was acquired during the mid-follicular phase (days 5-10) of the menstrual cycle to avoid potential premenstrual worsening of mood (35,36). Menstrual cycle phase was confirmed by two consecutive months of prospective charting using the Daily Record of Severity of Problems (37) and sex hormonal assays (19). Clinical psychiatric questionnaires included the Montgomery-Asberg Depression Rating Scale (MADRS) (38) to asses severity of depressive symptoms, the

Young Mania Rating Scale (YMRS) to assess severity of hypomanic/manic symptoms (39), and the State Trait Anxiety Inventory (STAI) to assess anxiety symptoms (40).

5.3.2 Data acquisition

MRI data was acquired using a GE whole body short-bore 3T scanner with 8 parallel receiver channels (General Electric, Milwaukee, WI, USA). Anatomical images were acquired with high-resolution T1-weighted images (gradient-echo inversion-recovery sequence, TR=1.6s, TE=5ms, matrix 256x256x128, FOV 220 x 220mm, slice thickness 1mm). Eyes-open functional resting-state imaging data was acquired using a T2* interleaved echo-planar imaging (EPI) sequence with TR=2000ms, TE=40ms, flip angle=60°, 4mm thick, 29 axial slices, matrix 64x64 resolution over 256 mm FOV. Once positioned in the scanner subjects were instructed to "lay still, relax and try not to think about anything in particular" as they looked at a fixation point. The full imaging scan lasted 10 minutes.

5.3.3 Data analysis

5.3.3.1 Resting-state seed-based analysis

The anatomical and functional resting-state MRI data were preprocessed using the Statistical Parametric Software SPM12 (http://www.fil.ion.ucl.ac.uk/spm). Imaging data was obtained in DICOM file format and converted to NIFTY-1. High-resolution T1-weighted anatomic data was segmented into grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF) using affine regularization (light bias regularization=0.001, Bias FWHM = 60mm cut off), according to the ICBM space template for European Brains (41). Subsequently, images were normalized into standard space using a 4th Degree B-Spline Interpolation and resampled to 1mm x 1mm x 1mm voxels (42). Deformation fields created during the segmentation process were used

during normalization for increased precision in the alignment of images (43). Resting-state time-series were then realigned to correct for minor head motions during scan session (motion greater than 3mm in the translational plane and 3 degrees in the rotational plane were discarded). Realignment parameters included 2nd Degree B-Spline Interpolation for estimation and 4th Degree B-Spline Interpolation for reslicing (44). Co-registration of functional images with anatomical images was estimated using the Normalized Mutual Information Function (43). Images were spatially smoothed to increase the signal to noise ratio with a 8 mm full-width-half-maximum (FWHM) Gaussian filter (45).

After preprocessing, resting-state fMRI (Rs-fMRI) seed-based analysis was carried out using the CONN toolbox v.15.d (https://www.nitrc.org/projects/conn) with diagnosis as a factor and age as a covariate. Subject specific maps of WM and CSF were used as nuisance regressors. The aCompCor strategy was employed within CONN to control for the effects of physiological motion and residual head movement (46,47). The first two volumes were discarded to account for T2 stabilization effects. The function images were then temporally band-pass filtered (0.008-0.09 Hz). The somatosensory cortex was used as seed-point in a ROI-ROI analysis using the Brodmann Area and Harvard-Oxford Atlas available with CONN (48). First and second level statistical analyses were completed using CONN using two-sample *t-tests* comparing functional activation between groups. Brain Rs-FC was corrected for multiple comparisons using the False Discovery Rate (FDR; $p<0.05$).

Since BD and HC subjects differed in BMI, MADRS, YMRS and STAI scores (Table 1), we tested any potential effects of BMI and these clinical variables on Rs-FC by adding BMI and MADRS, YMRS and STAI scores as second-level covariates.

In order to evaluate the potential effect of medication on Rs-FC in BD subjects, medication load was analyzed according to the method described by Hassel et al (49). Briefly, psychotropic medication load was calculated by categorizing each medication according to its class (lithium, anticonvulsants, antidepressants, antipsychotics, and anxiolytics) and dose (49-52). Then the composite score of total medication load for each BD subject was added as a covariate in the Rs-fMRI second level analysis.

Additionally, one study reported an association between Rs-FC in somatosensory cortex and current or past history of psychosis in BD subjects (15). To examine the potential effect of history of psychosis on Rs-FC in our sample, we also added the presence of psychotic episode in the past as a second-level covariate.

5.3.3.2 Voxel-based morphometry analysis

Grey matter voxel-based morphometry analysis was conducted using the Voxel-Based Morphometry (VBM8) toolbox (http://www. neuro.uni-jena.de/vbm/) in SPM 12. During preprocessing high-resolution T1-weighted anatomical images were bias-corrected, registered into a standard space (MNI template) using linear (12-parameters affine) and non-linear transforms (41), and cerebral tissue was segmented into GM, WM, and CSF. Quality control of preprocessed data was done by visually inspecting for artifacts and assessing the homogeneity of variance and covariance matrices using VBM8 toolboxes. Images were then spatially smoothed using an 8 mm FWHM Gaussian filter. The volume of the somatosensory cortex was compared between groups using a general linear model (GLM) using group as a factor and age as a covariate. Grey matter volumes were corrected for multiple comparisons using the False Discovery Rate (FDR; $p<0.05$).

5.3.3.3 Statistical analysis

Statistical analysis of demographic and clinical variables was carried out using R (version 3.1.2, https://www.r-project.org). Shapiro-Wilks test and Bartlett's test were used to evaluate normal distribution of continuous variables and homogeneity of variances between groups, respectively. Clinical domains were analyzed using two-sample *t-tests* or a Mann-Whitney U test when applicable. A p-value <0.05 was considered statistically significant.

5.4. Results

5.4.1 Demographics and clinical data

Demographics and clinical data are presented in table 1. There was no difference in age (P>0.05) or years of education (P=0.13) between BD subjects and healthy controls. Although BD subjects were clinically stable and euthymic for a minimum of 2 months, they showed higher depressive and hypomanic/manic symptoms. In addition, BD subjects had higher average BMI than controls (P<0.001). Eighteen BD subjects had a diagnosis of BD type I and 14 were BD type II. The average age of illness onset was 18.63 (± 6.8). Further, the average number of psychotropic medications used was 1.8 medications. The most common psychotropic was atypical antipsychotics (used by 16 subjects), followed by anticonvulsants (15), antidepressants (12), anxiolytics agents (6), lithium (3) and sleeping aids (2).

5.4.2 Seed-based functional connectivity

Using somatosensory cortex as a seed point, euthymic BD subjects showed an increased Rs-FC between right somatosensory cortex and insular cortex (BA 13, beta coefficient 0.25, t=4.41, p=0.008 FDR-corrected), inferior prefrontal gyrus (BA 47, beta coefficient 0.21, t=4.10, p=0.012 FDR-corrected), and orbitofrontal cortex (BA 10, 11, and 47, beta coefficient 0.20,

t=3.74, p=0.028 FDR-corrected) (Figure 1). There was no effect of BMI, MADRS, YMRS or STAI scores on Rs-FC.

5.4.3 Voxel-based morphometry

Voxel-based morphometry (VBM) analysis showed decreased grey matter volume in the left somatosensory cortex in the BD group compared to controls (MNI coordinates: -62, -13, 28; cluster size=298 voxels; peak-level p unc<0.001; cluster-level p unc<0.05) (Figure 2).

5.5.Discussion

The main findings of the present study were that euthymic BD females showed increased Rs-FC between the right somatosensory cortex and fronto-limbic regions involved with affective regulation (insular cortex, inferior frontal gyrus and orbitofrontal cortex). Furthermore, voxel-based morphometry revealed decreased grey matter volume in the left somatosensory cortex in the BD group compared to controls.

Most seed-based Rs-FC studies in euthymic BD have focused on functional connectivity using the amygdala as a seed point. Overall, these studies support the view that euthymic BD subjects present abnormal Rs-FC between amygdala and prefrontal cortical regions (15-18). Functional changes in somatosensory cortex in BD have been demonstrated using task-based fMRI techniques. Malhi et al. (2007) used similar inclusion criteria (i.e., only euthymic female BD subjects) to investigate brain changes using a modified word-based memory task to implicitly affective changes (53). They found less activation in the right somatosensory cortex, left inferior parietal lobule, right thalamus and left putamen in euthymic BD subjects compared to controls when induced to negative affect, which supports that changes in somatosensory activation occur during affective/emotional processing. In another study, task-based fMRI for attention with

emotional distracters was used to evaluate depressed BD participants, individuals with major depressive disorder (MDD) and healthy controls (HC). The depressed BD group showed decreased BOLD activation in occipital lobes, lingual gyrus, and middle temporal gyri compared to the MDD group and HC. However, during the attentional task depressed BD subjects showed less activation in the somatosensory cortex compared to MDD. Interestingly, increased activation in somatosensory cortex was the only finding that differentiated MDD and HC (54). Thus, both task-based fMRI studies in BD subjects suggest that changes in activation of the somatosensory cortex in BD during verbal memory and attentional tasks.

Functional changes in somatosensory cortex were also found during mood episodes in BD. One study investigated the correlation between whole-brain Rs-FC, Default Mode Network (DMN) and the Bipolarity Index (BI) (55) in 15 subjects with MDD and 15 with BD type I. They found a negative correlation with BI and DMN in the left postcentral gyrus (56). A study which included 26 depressed BD participants using amplitude of low-frequency fluctuations (ALFF)(57) of Rs-fMRI showed patients with BD depression had decreased ALFF in the left postcentral gyrus, the left parahippocampal gyrus and the cerebellum (58). Altinay et al. (2016) evaluated Rs-FC using striatal regions as seed points in unmedicated BD patients (during episodes of depression and hypomania/mania) and healthy controls. They found that only in the BD depression group there was an increased Rs-FC between the putamen and somatosensory areas (59). Overall, Rs-FC changes in somatosensory cortex in BD subjects during a depressive episode have been correlated to abnormal connectivity with the striatum and other limbic regions specifically during depressive episodes.

As far as grey matter volume in adult euthymic BD subjects, studies have mainly reported decreased GM volume in fronto-limbic regions in BD patients, with the exemption of a single study that did not find any difference in GM volume between euthymic BD participants and

healthy controls (60). Almeida et al. (2009) found reduced GM volume in the rectal gyrus, parahippocampal gyrus and left putamen in euthymic BD patients (61). Interestingly, GM in the rectal gyrus had a significant group by gender by trait anxiety interaction. Lyoo et al. (2006) enrolled 25 BD participants and evaluated GM cortical thickness using voxel-wise and region-of-interest (ROI) analyses. Using a voxel-wise approach they found cortical thinning in somatosensory cortex, middle frontal cortex, pregenual and dorsal anterior cingulate cortex, posterior cingulate cortex, and middle occipital cortex compared to healthy controls. ROI analysis confirmed findings from voxel-wise analysis and revealed a negative correlation between cortical thickness and illness duration on the right somatosensory cortex and in the left middle frontal cortex (62). One study reported that BD subjects showed decreased GM volume in the ventromedial PFC compared to controls. However only BD type-I group presented widespread GM reductions in the frontal, temporal, parietal and parahippocampal cortices compared to controls. Interestingly, this study also reported decreased GM volume in the right postcentral gyrus (somatosensory cortex) in BD compared to controls (63). Adler et al. (65) found increased GM volume in a BD participant group in several regions including the anterior cingulate, ventral PFC, fusiform gyrus and primary and supplementary motor cortex. They also found decreased GM volume in the superior parietal lobule in the euthymic BD group. One study in a pediatric BD population in different mood states (BD=32) using ROI analysis showed decreased GM volumes bilaterally in the parietal lobe and on the left temporal lobe. Within the parietal lobe, the somatosensory cortex was significantly smaller in children with BD compared to healthy controls (67). Pharmacotherapy with lithium was also suggested to have an effect in somatosensory cortex in one study: GM volume of the somatosensory cortex, the subgenual anterior cingulate gyrus, hippocampus, amygdala complex and the insula was greater in BD patients on lithium treatment compared to other mood stabilizers (68). In summary, consistent with our finding, a number of

previous studies have consistently found decrease grey matter volume or thinning in the somatosensory cortex of individuals with bipolar disorder. However, most of these studies seem to have ignored the potential relevance or this particular brain region in the neurobiology of BD despite positive findings.

The somatosensory cortex has been directly implicated in the recognition of emotions from facial expressions. A study including 108 subjects with focal brain lesions assessed the recognition and naming of six basic emotions from facial expressions. The right somatosensory cortex was found to be a critical component along with the amygdala and right visual cortices in retrieving socially relevant information from faces (23). Zhou et al. (2014) evaluated the risk propensity in males and females using Rs-FC and found that general risk propensity was different between the sexes, with the right secondary somatosensory cortex being involved in risk processing in women only (24). The somatosensory cortex was also found to be involved in decision making processes when using emotional and value-based tasks compared to a mathematical approach (69). A recent neuroimaging meta-analysis examined the connection between affective responses through sensory modalities. They observed that in the somatosensory cortex the affect-related activity was greater for auditory, olfactory, gustatory, and somatosensory inputs (25). Taken together, these results strongly suggest that the somatosensory cortex plays an important role in emotional processing and regulation, and perhaps in impulse control.

Our findings of increased spontaneous Rs-FC between the somatosensory cortex and fronto-limbic regions (insular cortex, inferior frontal gyrus, and orbitofrontal cortex) might also be associated with changes in associative white matter tracks in BD. The somatosensory cortex is anatomically connected with fronto-cortical regions and the cingulate cortex mainly through the cingulum tract, the superior longitudinal fasciculus, and anterior arcuate fasciculus (70). Interestingly, these association tracks have been consistently shown to be disrupted in BD (71).

We speculate that the changes we found in the somatosensory cortex in the present study may in part contribute to the symptomatology or represent part of the psychopathology of BD.

The limitations of the present study include the cross-sectional design that prevents any interpretation related to cause and effect. We were not able to evaluate the medication effects due to the limited sample size, therefore we cannot control for the effect of psychotropic medication on Rs-FC results. A further limitation is that the population is composed of only females. Whether or not our results are also applicable to males it remains to be determined.

5.6 Conclusions

This study is the first to specifically examine the structure and function of the somatosensory cortex in a well-characterized sample of euthymic BD females compared to age-matched controls. We found increased Rs-FC between the somatosensory and insular cortex, inferior prefrontal gyrus, and frontal orbital cortex in euthymic BD subjects compared to controls. We also found decreased grey matter volume in the left somatosensory cortex in the BD group. Future research should consider using emotional regulation tasks along with fMRI to evaluate the impact of changes in somatosensory connectivity, and the inclusion of male subjects to investigate sex differences. The somatosensory cortex may be an interesting target for future research using neurostimulation, such as repetitive transcranial magnetic stimulation, in the treatment of BD.

5.7 References

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Table 1 Demographics: Each variable is presented as mean (standard deviation)
*p<0.05; **p<0.001

Table 1.0: Demographics			
Variable	**BD (n=32)**	**HC (n=36)**	**P-value**
Age	29.0 (8.07)	32.8 (8.32)	0.05
BMI	27.8 (5.71)	23.2 (3.74)	<0.001**
Years of Education	15.6 (2.61)	16.5 (2.51)	0.13
MADRS	8.34 (5.80)	4.00 (4.72)	0.001**
HAMD	5.66 (3.86)	2.28 (2.90)	0.0001**
YMRS	1.50 (1.39)	0.67 (1.12)	0.005**
STAI-State	37.77 (11.81)	31.17 (8.70)	0.01*
STAI-Trait	44.94 (12.20)	31.34 (8.51)	<0.0001**

Figure 1. Schematic representation of the resting-state fMRI analysis using the somatosensory cortex as seed point (S1-R). Euthymic bipolar patients showed an increased functional connectivity between right somatosensory cortex (S1-R) and right insular cortex (IC-R), inferior prefrontal gyrus (IFG-R), and frontal orbital cortex (Forb-R) (all analysis $p<0.05$, FDR-corrected). Lower row shows the effect size of the connectivity between the sensorimotor cortex and (A) the insular cortex, (B) the inferior prefrontal gyrus, and (C) the frontal orbital cortex in Bipolar subjects and healthy controls.

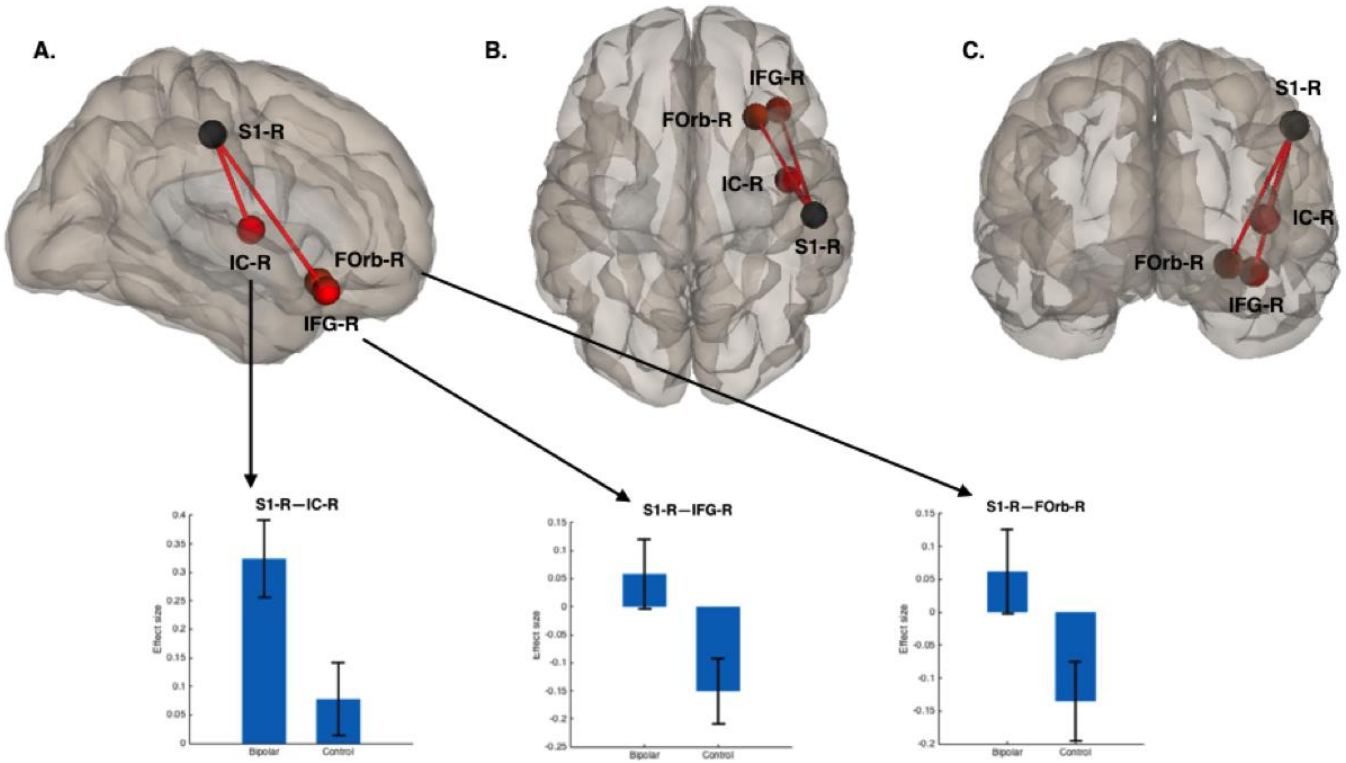
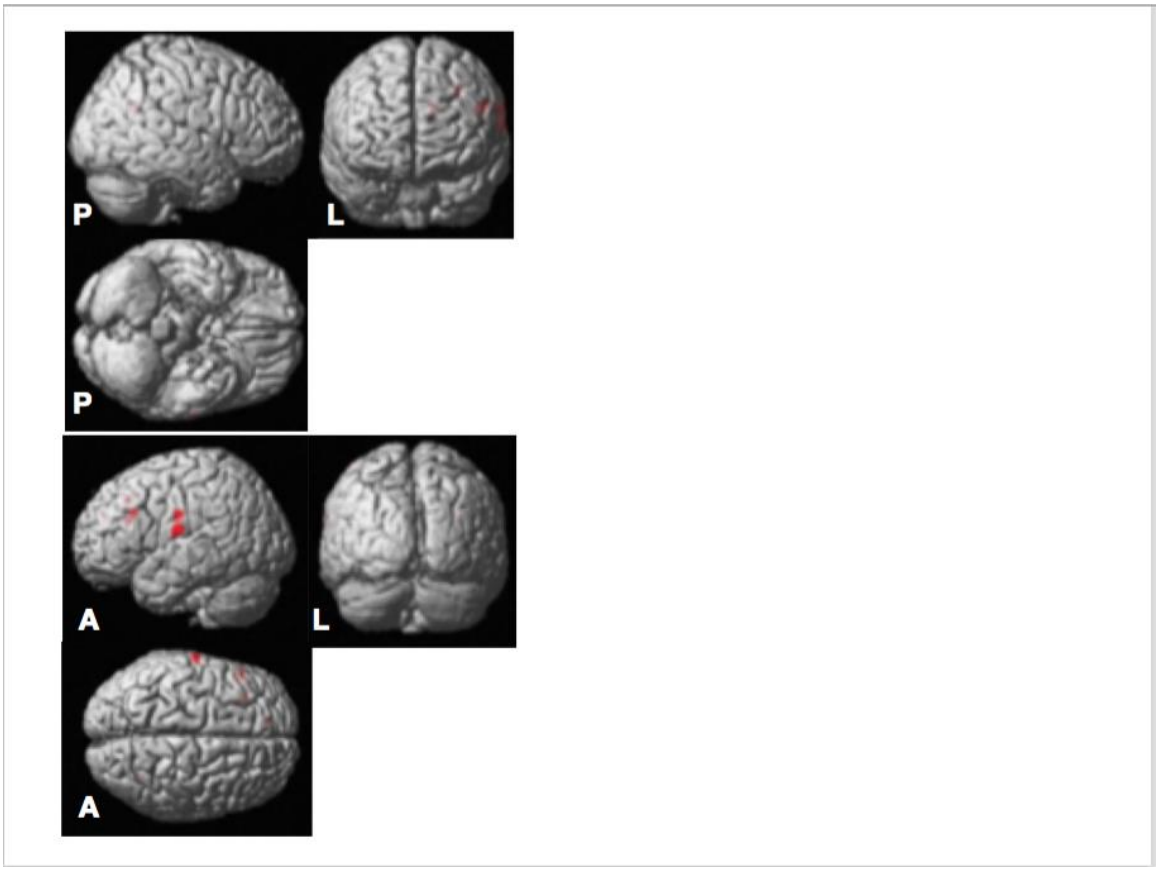


Figure 2. Voxel-based morphometry analysis showed decreased grey matter in the left postcentral gyrus in the bipolar group compared to healthy controls (peak-level p unc<0.001; cluster-level p unc<0.05).



Chapter 6: Clinical, Structural and Functional Correlates of Comorbid BD and PMDD

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6.1. Abstract

Introduction: Hormonal fluctuations associated with the female reproductive life events may precipitate or worsen affective episodes in women with bipolar disorder (BD). Previous studies have shown that women with BD report higher rates of premenstrual dysphoric disorder (PMDD) than controls; and BD women who report premenstrual worsening display a worse course of their bipolar illness. Interestingly, there is considerable overlap between brain regions implicated in the pathophysiology of BD and those influenced by female sex hormones – suggesting that hormonal fluctuations may have the potential to influence or alter the brain networks thought to be dysfunctional in BD. Despite this, the neural correlates of co-morbid BD and PMDD have not been investigated. In this study, we investigated clinical and neurobiological correlates of BD and PMDD using structural and functional MRI.

Methodology: Eighty-five (CTRL, n=25; PMDD, n=20; BD, n=21; BDPMDD, n=19), regularly cycling women, not on hormonal contraception, were scanned twice: During their mid-follicular and late luteal menstrual phases. We investigated resting-state functional connectivity (Rs-FC), cortical thickness and subcortical volumes of brain regions associated with the pathophysiology of BD. All women with a diagnosis of BD were euthymic for at least two months prior to study entry.

Results: Women in the BDPMDD group displayed greater disruption in biological rhythms and more subthreshold depressive and anxious symptoms through the menstrual cycle compared to other groups. Rs-FC was increased between the L-hippocampus and R-frontal cortex and decreased between the R-hippocampus and R-premotor cortex in BDPMDD vs BD (FDR-corrected, $p<0.05$). Cortical thickness analysis revealed decreased cortical thickness of the L-pericalcarine, L-superior parietal, R-middle temporal, R-rostral middle frontal and L-superior

frontal, as well as, increased cortical thickness of the L-superior temporal gyri in BDPMDD compared to BD. We also found increased left-caudate volume in BDPMDD vs BD ($p_{corr}<0.05$).

Conclusions: Women with BD and co-morbid PMDD display a distinct clinical and neurobiological phenotype of BD, which suggests differential sensitivity to hormonal influence that may potentially warrant course specifiers to capture the phenotypic differences and the additional burden of disease experienced in this population.

6.2. Introduction

Bipolar Disorder (BD) is estimated to affect approximately 1-4% of the population (1). It carries a significant burden of illness due to its chronicity, early age of onset and severity (1,2). Litertaure highlights that both men and women are equally affected by BD-type-I and over represented in the BD type-II and rapid cycling BD subtypes (3-6). Clinically, research shows the course of BD presents different between men and women with women reporting a greater frequency of depressive and mixed episodes than men (3,5-7) and experiencing greater psychiatric comorbidities (4,5,8). Female reproductive events have the potential to act as critical windows of mood worsening in women with BD; this may precipitate the onset of a mood episode and create a greater potential for relapse (5,9,10). This is exemplified during pregnancy and the postpartum period; a large study investigating 2252 pregnancies found that 23% of women with BD reported an affective episode during pregnancy and 52% during the post partum period (11). Literature estimates that the postpartum period carries a risk of psychosis 100 times greater than that of the general population in women with BD (12). Evidence from research examining the menopausal transition in women with BD also suggests that affective episodes may be entrained to the menopausal transition (13-15).

Mood instability during the premenstrual phase in women with BD has been reported across numerous studies (16). Women with BD display high rates of premenstrual syndrome (PMS); with studies estimating that approximately 51%-68% of women with BD report mood symptoms during the premenstrual period (6,14,17,18). Choi et al. investigated premenstrual exacerbation among women with BD to find that 51.6% of women with BD type-II displayed moderate to severe premenstrual syndrome as compared to 23.3% of women with BD type-I and 19.7% of healthy controls (18). Further, Fornaro and Perugi investigated the impact of premenstrual dysphoric disorder (PMDD) in a sample of 92 women with BD (19). In their

sample, 27.2% of women met criteria for PMDD according to a clinical semi structured interview. This subset of women with BD and co-morbid PMDD displayed a higher number of axis I co-morbidities than those without PMDD (19). Common co-morbidities associated with PMDD in this population included history of post-partum depression, obsessive-compulsive disorder and body dysmorphic disorders (19). Dias and colleagues conducted a large prospective study, which found that women with a diagnosis of BD and history of premenstrual exacerbation of mood have a worse course of their bipolar illness. This was characterized by shorter time to relapse, and greater symptom severity - to a greater extent for depressive symptoms (20). Further studies with a primary objective of examining prevalence of PMDD in community based samples have also highlighted its association with BD. Wittchen and colleagues reported that women with PMDD found that they are 8 times more likely to have a diagnosis of BD (21). It is important to note that smaller studies have failed to find an association with BD and PMS (22-25). In a recent study of a large sample of women with BD (N=1,099) we found that women who met DSM-5 provisional diagnosis of PMDD had an earlier onset of bipolar illness, higher rates of rapid cycling, increased number of mood episodes, and higher rates psychiatric co-morbidities (113). Notably, in this study there was a closer gap between BD onset and age of menarche in women with co-morbid PMDD, which points toward a potential link between puberty/hormones and BD onset in this population.

Structural and functional MRI techniques are useful tools to elucidate the neurobiological underpinnings of BD and potential vulnerabilities created by hormonal fluctuations. Neurobiological models of BD, hypothesize that the pathophysiology of BD is related to dysregulation in neural pathways involved in emotional control and processing (26-29). This is likely associated with a loss of top-down prefrontal modulation of limbic circuitry and aberrant functioning two interrelated networks responsible for mediating emotional regulation: (1) lateral

prefrontal cortical system (ventrolateral PFC, mid and dorsal anterior cingulate cortex (ACC), ventromedial striatum, globus paladus and thalamus); and (2) medial prefrontal cortical system (ventromedial PFC, subgenual ACC, nucleus accumbens, globus palladus and thalamus) (27-29). In complex emotional states, both networks function in synchrony to modulate the activity of the amygdala (28,29). It has been hypothesized that an imbalance between these two neural streams may lead to the onset of affective episodes and clinical symptoms experienced in BD.

Interestingly, there is considerable overlap between regions implicated in the neurobiology of BD and those affected by sex hormone fluctuations. Female sex hormones such as estradiol (E2) and progesterone (P4) bind to various regions of the cerebral cortex and subcortical gray matter regions (amygdala, thalamus, hypothalamus, hippocampal formation) (30-34). Our group recently published a study investigating the hormonal correlates of Rs-FC across the menstrual cycle in healthy women (35). We found that E2, P4, allopregnanlone and DHEAS are correlated with patterns of functional coupling throughout the cerebral cortex (35). Therefore it is plausible that in women with BD and comorbid PMDD, hormonal fluctuations associated with the menstrual cycle may activate or exploit mechanisms or neural circuitry which is implicated in pathophysiology of BD; thereby causing worse outcomes in this subset of women.

Despite strong support for the influence of hormonal fluctuations associated with reproductive milestones on BD, neuroimaging literature in these populations is sparse. To date, no brain imaging has been conducted in women with BD and PMDD. The primary goal of this study is to examine the clinical, structural and functional correlates in a group of well-characterized women with co-morbid BD and PMDD using Rs-FC, cortical thickness analysis and automated subcortical segmentation. The secondary goals of this study were to investigate (1) the influence of PMDD on brain structure and function in healthy women; (2) differences in hormonal levels throughout the menstrual cycle between women with and without PMDD and with and without

BD. We hypothesize that women with BD and comorbid PMDD will display a worse clinical profile and structural and functional differences across the menstrual cycle compared to BD women without PMDD.

6.3. Methodology

6.3.1. Participants

This study was approved by the Hamilton Integrated Research Ethics Board (HiREB) and adhered to the tenets of the Declaration of Helsinki. All participants provided informed written consent. Participants were recruited through community-based advertisements in the Hamilton and Halton Regions, Ontario, Canada.

Eighty-five women between 16-50 years of age, with regular menstrual cycles (25-32 days) were enrolled. Participants were split into four groups based on their history of BD and PMDD: (1) healthy controls with no history of PMDD (CTRL); (2) women with PMDD but no other psychiatric diagnosis (PMDD); (3) BD with no history of PMDD (BD); (4) BD with co-morbid PMDD (BDPMDD).

Exclusion criteria included: (1) current or recent (previous 3 months) use of systemic hormonal treatment; (2) pregnancy; (3) contraindications for MRI; (4) history of head trauma resulting in a loss of consciousness; (5) neurological disorders affecting cognition; (6) current or recent (previous 6 months) history of alcohol or drug abuse or dependence; (7) unstable general medical conditions. Regularly cycling women using levonorgestrel intrauterine device were allowed in the study due to its primarily localized hormonal effect. All women performed at least 2 months of prospective symptom charting using the Daily Record of Severity of Problems (DRSP) in order to confirm or rule out PMDD, as per DSM-5 diagnosis (39). Study participants were informed that recreational drug use was not allowed during the course of study participation

and all participants agreed to comply with this request. We did not perform drug testing on the day of the MRI scans.

Inclusion criteria for participants with BD (both BD and BDPMDD groups) included: (1) a diagnosis of BD according to the SCID-I (SCID-IV-TR) (40); (2) no current depressive, manic or hypomanic episodes according to the SCID-I (DSM IV-TR); (3) no changes in psychotropic medications or mood state within 2 months prior to or during the study. Due to the exceptionally high rates of comorbid psychiatric conditions in BD, a lifetime but not current history of psychiatric comorbidities was allowed to provide a true reflection of individuals with BD. Further, due to the high rates of co-morbid psychiatric conditions in PMDD, a lifetime history of a single major depressive episode, past history of generalized anxiety disorder or posttraumatic disorder was allowed so long as the individual was fully remitted for a minimum of 6 months prior to study entry.

Exclusion criteria for participants in the CTRL group included: (1) a lifetime history of any psychiatric disorder according to the SCID-I; (2) greater than a 30% change in the four core symptoms of PMDD in their late luteal phase from their mid-follicular phase according to the DRSP. Women with BD without PMDD (BD group) also did not display greater than a 30% change in the four core symptoms of PMDD in their late luteal phase from their mid-follicular phase according to the DRSP (39). A diagnosis of PMDD (both in PMDD and BDPMDD groups) was established by two independent licensed psychiatrists blinded by subjects' group status (B.F., L.M.) using a minimum of two months of prospective daily symptom charting as confirmed by the DRSP (9,39).

6.3.2. Study Design

Study participation was comprised of three visits to St. Joseph's Healthcare Hamilton. The first visit consisted of administration of the SCID-I by a psychiatrist or trained PhD

candidate, followed by a psychiatric and gynecological history. The second and third visits took place during the mid-follicular phase (days 5-10 of the menstrual cycle) or the late luteal phase of the menstrual cycle (7 days preceding menses). Approximately half of the study participants began with their mid-follicular visit and half with their late luteal visit. Menstrual cycle phase was confirmed using hormonal analysis. Visits two and three included an MRI scan, collection of a blood sample for hormone analysis and completion of validated clinical questionnaires as described below.

6.3.3. Clinical Questionnaires

The Mongomery-Asberg Depression Rating Scale (MADRS) (41) and Hamilton Depression Rating Scale (HAM-D) (42) were administered to assess severity of depressive symptoms. The Young Mania Rating Scale (YMRS) (43) was administered to assess the severity of manic/hypomanic symptoms through the menstrual cycle. Disruptions in biological rhythms were investigated using the self-reported Biological Rhythms Interview of Assessment in Neuropsychiatry (BRIAN) (44). The Pittsburgh Sleep Quality Index (PSQI) was used to asses sleep quality (45) and the State Trait Anxiety Inventory (STAI) was used to identify state and trait-based anxiety symptoms (46).

6.3.4. Hormonal Analysis

Immediately following both MRI scans, 10 ml of whole blood was collected in serum tubes. The blood was clotted at room temperature for 45 minutes, and centrifuged at 20^{o}C for 15 minutes at 3000 rpm. Four serum aliquots were obtained and frozen at -80^{o}C until assayed. Serum was assayed for P4, E2 and DHEAS using commercially prepared solid phase enzyme-linked immunosorbent assay (ELISA) kits, purchased from ALPCO Diagnostics, Salem, NH. In addition, samples were assayed for ALLO, also using ELISA technique purchased from Kiamiya

Biomedical Company, Seattle, WA. All serum samples were assayed in duplicate following the manufacturer's protocol, with a fresh aliquot for each analyte. The inter-assay variations for P4, E2, DHEA-S and ALLO were 11.3%, 8.7%, 9.2% and 6.0% respectively. The intra-assay variations were 10.4%, 7.7%, 9.3% and 11.7% and the sensitivities were 0.1 ng/ml, 10 pg/ml, 0.005 ug/ml and 0.52 ng/ml, respectively. A licensed gynecologist (D.C.) confirmed that hormone levels were within physiological range for each menstrual phase.

6.3.5. MRI Protocol

6.3.5.1 Image Acquisition

Images were acquired using a GE whole body short-bore 3T scanner with 8 parallel receiver channels (General Electric, Milwaukee, WI, USA). Anatomical images were acquired with high-resolution T1 weighted images (gradient-echo inversion-recovery sequence, TR=1.6s, TE=5ms, matrix 256x256x128, FOV 220x220mm, slice thickness 1mm). Functional resting state imaging was completed using a T2* interleaved echo-planar imaging (EPI) sequence with TR=2000ms, TE=40ms, flip angle=60°, 4mm thick, 29 axial slices, matrix 64x64 resolution over 256 mm FOV). Once positioned in the scanner participants were instructed to "Lay still, relax and try not to think about anything in particular" as they looked at a fixation point. Anatomical and resting state scans took place over 10 minutes and were followed by functional tasks that will be published at a later date.

6.3.5.2 Preprocessing for Resting State Functional Connectivity

The resting state and anatomical MRI data were preprocessed using the Statistical Parametric Mapping Software SPM12 (http://www.fil.ion.ucl.ac.uk/spm) and CONN Functional Connectivity Toolbox Verson 17e (https://www.nitrc.org/projects/conn) (47). Imaging data was obtained in DICOM file format and converted to NIFTI using SPM and then uploaded to CONN

for further preprocessing. The default preprocessing pipleline for volume-based analyses was optimized to preprocess both structural and functional scans. Briefly, structural scans were translated and centered to (0,0,0) coordinates and subsequently underwent direct segmentation (gray matter, white matter and cerebrospinal fluid) and MNI normalization. Functional scans were realigned and unwarped (motion estimation and correction), and translated and centered to (0,0,0) coordinates. Outliers were identified as volumes with greater than 2mm of motion in the translational plane, and were detected using the ART toolbox and added as condition files for denoising. Images with motion greater than 3mm in the translational plane and 3 degrees in the rotational plane were discarded. Finally, functional data was segmented (GM, WM, CSF), normalized to MNI space and spatially smoothed to increase the signal to noise ratio with an 8mm FWHM Gaussian filter.

6.3.5.3. Seed Based Analysis (SBA)

SBA was completed using the CONN toolbox version 17e (47). Subject specific maps of CSF and WM were used as nuisance regressors during the denoising step of analysis. The aCompCor strategy was employed within CONN to control for the effects of physiological motion and residual head movement. The functional images were then temporally band-pass filtered (0.008-0.09 Hz). The following regions were used as seed points in a seed to voxel analysis: (i) right and left vlPFC; (ii) right and left vmPFC; (iii) right and left amygdala; (iv) right and left hippocampus; (v) right and left postcentral gyrus; (vi) precuneus; (vii) subcallosal cortex. They were taken from the FSL Harvard-Oxford Atlas available with CONN. Statistical analysis was performed using an analysis of variance (ANOVA) in the mid-follicular and late luteal menstrual phases. Seed points with significant clusters were then explored using post hoc testing. A voxel height threshold of $p<0.001$ and cluster threshold of 0.05-FDR corrected was used to identify significant clusters that responded to regions of interest. Since BMI differed between groups, we

tested any potential effects of BMI on Rs- FC by (i) adding BMI as a second- level covariate and (ii) directly analyzing the effect of BMI for particular regions of interest within groups. The same subanalysis was completed for psychosis history and medication load, as these variables may have an effect on Rs-FC.

6.3.5.4. Potential Effects of Medication on Rs-FC

Additional analyses were completed to assess the potential effects of psychotropic medications on Rs-FC in bipolar subjects according to previous imaging studies investigating dose equivalences (48-50). Antipsychotics, lithium, anticonvulsants, antidepressants and anxiolytics were coded to 0, 1 or 2 to represent absent, low or high dose medication groupings previously employed by Hassel and colleagues (48). Lithium, anticonvulsants and antidepressants were categorized into groups from 1-4 depending on medication dose (50). Based on this, individuals in category 1 or 2 were grouped in the low dose group (scoring a 1), and individuals in category 3 or 4 were grouped in the high dose group (scoring a 2). Antipsychotics were converted to chlorpromazine equivalent doses. Doses below or above the mean effective daily dose of chlorpromazine were coded as 1 or 2, respectively. Individuals not taking antipsychotics were coded 0. Anxiolytics were also grouped into similar categories based on the recommended daily dosage found in the *Physician's Desk Reference* (51). In this case, the dose was coded as 0 if absent or present in very low dose with reference to the midpoint, 1 if around the midpoint and 2 if higher than the midpoint. Composite scores of medication load were then calculated and used as second-level covariates in CONN in an analysis using our a-priori seed points.

6.3.5.5 Cortical Thickness and Subcortical Volume Analysis

Cortical thickness and subcortical volume analysis was conducted using the FreeSurfer Software Version 5.3.0. (http://surfer.nmr.mgh.harvard.edu/). The FreeSurfer Software uses a

semi-automated process, which is described in full detail in (52,53). Briefly, non-brain tissue is removed through a process known as skull stripping, images are transformed into Talairach space, signal intensity is corrected and normalized, tissue is classified into WM, GM and CSF, and the white matter and gray matter boundaries are tessellated. Each subject's images were manually inspected to ensure that the GM/WM and GM/CSF boundaries were correctly identified. Images with inaccuracies in boundary identification were manually corrected and reprocessed. Subsequently, data was normalized and smoothed using a 10 mm full-width-half-maximum Gaussian kernel. Cortical thickness was measured as the closest distance between the GM/WM boundary and the GM/CSF boundary at each vertex on the tessellated surface.

6.3.5.6 Cortical Thickness Statistical Analysis

Statistical analysis was completed in FreeSurfer qdec. Cortical thickness was compared between groups using a general linear model using age and BMI as covariates, using a different slope different onset (DODS) approach. All clusters were required to have a minimum size of $50mm^2$. Results were Bonferroni corrected for multiple comparisons within each comparison and across both hemispheres to reduce the risk of type-1 errors.

6.3.5.7. Subcortical Volume Analysis

The volume of subcortical gray matter regions was extracted from FreeSurfer (automated segmentation) and corrected for intracranial volume. Volumes across regions of interest were compared between groups using an ANCOVA with BMI as a covariate and explored using post hoc analyses and multiple comparison corrections. Regions of interest were (i) left and right caudate; (ii) left and right putamen; (iii) left and right hippocampus; (iv) left and right amygdala; (v) left and right thalamus; (vi) left and right accumbens area; (vii) left and right ventral diencephalon (DC)

6.4. Results

6.4.1. Clinical Results

Participants in the BDPMDD group had a significantly higher BMI than CTRL (p_{CORR}=0.001) or PMDD (p_{CORR}=0.009). As a result, BMI was added as a covariate in all imaging analyses. Years of education (p=0.09) and age (p=0.05) were not different between groups. Neither medication load (p=0.86), age of onset (p=0.81), or number of comorbid conditions (p=0.94) were significantly different between the BD and the BDPMDD groups as seen in Table 1.

Consistent with our hypothesis, clinical data showed a stepwise progression of severity from the CTRL group to the BDPMDD group (Table 2). Between-group comparisons (Bonferroni corrected for multiple comparisons) are shown in Table 3. Notably, although participants in both the BD and BDPMDD groups are in the euthymic phase of their bipolar illness they still demonstrated trending differences in their follicular MADRS scores (p_{CORR}=0.075) and significant differences on the follicular BRIAN scores; potentially highlighting the impact of comorbid PMDD on BD. These group differences were also seen in the luteal phase among BRIAN scores (p_{CORR}=0.018) and STAI state scores (p_{CORR}=0.017).

We did not find differences in P4, E2 or DHEAS across groups in either the mid-follicular or late luteal menstrual phases. However, allopregnanolone was higher in PMDD subjects compared to CTRL in both menstrual phases (mid-follicular, p_{CORR}=0.026, late luteal phase, p_{CORR}=0.024) and compared to BD in the mid follicular phase (p_{CORR}=0.033).

6.4.2 Resting State fMRI Results

We investigated whole brain differences in Rs-FC using a seed to voxel approach. In the mid-follicular phase, we found differences between groups using the subcallosal cortex as our

seed point and a cluster in the angular gyrus (k= 316; X=-42, Y=-68, Z=+14; p=0.0013, FDR-corrected). We examined group differences in Rs-FC in the late luteal phase and found significant clusters using the precuneus and right and left hippocampus as seed points (Table 4). In the late luteal phase, we found differences in Rs-FC associated precuneus and a cluster in the right frontal cortex (BA8) (k=220; X=+10, Y=+44, Z=+46; p=0.011, FDR-corrected). Further when using the right hippocampus as a seed we found a cluster in the right premotor cortex (BA6) (k=166; X=+64, Y=+02 Z=+06; p=0.044, FDR-corrected). We found the greatest number of clusters when using the left hippocampus as a seed point, which yielded three significant clusters: (i) right somatosensory association cortex (BA5) (k=140; X=+10, Y=-30, Z=+50; p=0.037, FDR-corrected); (ii) right frontal cortex (BA8) (k=148; X=+00, Y=-34; Z=+62; p=0.037, FDR-corrected); (iii) right somatosensory cortex (BA1) (k=188; X=+48, Y=-34, Z=+62; p=0.033, FDR-corrected).

Post Hoc analyses revealed between group differences in functional connectivity between seed points and clusters, shown in Table 5. Most notable are the differences between BD and BDPMDD. We found increased connectivity between the right hippocampus and the left premotor cortex (k=201; X=+64, Y=+02 Z=+06; p=0.029, FDR-corrected) in BD compared to BDPMDD, and decreased connectivity in BD compared to BDPMDD between the left hippocampus and the right frontal cortex (k=165; X=+02, Y=+34 Z=+44; p=0.048, FDR-corrected). All post hoc analyses results are reported in Table 5. Moreover, we no found differences in whole brain connectivity using the following seed points: (1) right and left vlPFC; (2) right and left vmPFC; (3) right and left amygdala; and (4) right and left postcentral gyrus. We did not find any significant effect of medication load, psychosis, or BMI on any of the Rs-FC results.

6.4.3. Cortical Thickness

6.4.3.1. Group differences between BD and BDPMDD

We identified six clusters representing differences in cortical thickness between BD and BDPMDD groups, reported in Table 6. Compared to BD, BDPMDD displayed cortical thinnning in the following regions: (1) left pericalcarine gyrus (p_{CORR}=0.007); (2) left superior parietal gyrus (p_{CORR}=0.0024); (3) right middle temporal gyrus (p_{CORR}=0.0002); (4) right rostral middle frontal gyrus (p_{CORR}=0.0006) and (5) left superior frontal gyrus (p_{CORR}=0.040). Further, we found increased thickness in BDPMDD compared to BD in the left superior temporal gyrus (p_{CORR}=0.045).

6.4.3.2. Group differences between CTRL and PMDD

The PMDD group displayed cortical thinning compared to CTRL in the following regions: (1) left superior temporal gyrus (p_{CORR}=0.048); (2) left middle temporal gyrus p_{CORR}=0.020); (3) right inferior temporal gyrus (p_{CORR}=0.021); (4) right pars opercularis (p_{CORR}=0.010); (5) left post central gyrus (p_{CORR}=0.018); (6) right superior frontal gyrus (p_{CORR}=0.004); (7) right middle temporal (p_{CORR}=0.039).

6.4.3.3. Subcortical Volume Results

We investigated the volume of several subcortical regions of interest between groups in each menstrual phase, including right and left caudate; left and right putamen; left and right hippocampus; left and right amygdala; left and right thalamus; left and right accumbens area; and left and right ventral DC. We only found significant between group differences in the left caudate during the late luteal menstrual phase. Post hoc analyses revealed that these results were driven by increased volume in the left caudate in BDPMDD compared to BD (p_{CORR}=0.007). We did not

find any other differences in the volume of other regions of interest in either menstrual phase between groups.

6.5. Discussion

We assessed clinical, hormonal, structural and functional correlates of BD and co-morbid PMDD in a well-defined sample of euthymic women. Results from our clinical scales highlight a stepwise progression of severity across groups from the CTRL to BDPMDD groups across all clinical measures in both menstrual phases. More specifically, women with BD and co-morbid PMDD displayed greater subthreshold depressive and anxious symptoms, as well as more subjective disruption in biological rhythms and worse sleep quality than women with BD, PMDD and CTRL. Results from our seed to voxel analyses highlighted distinct patterns of Rs-FC between participants with a diagnosis of BD and co-morbid PMDD and those with BD without a diagnosis of PMDD in the left and right hippocampus with clusters in the premotor and frontal cortices. Structural analysis revealed differences in cortical thickness between BD and BDPMDD groups in left pericalcarine gyrus, left superior parietal gyrus, right middle temporal gyrus, right rostral middle frontal gyrus, left superior frontal gyrus and left superior temporal gyrus. Finally, through automated segmentation of subcortical regions we found increased volume of the left caudate in the late luteal phase in BDPMDD compared to BD. Taken together, these results highlight the impact of comorbid PMDD on the clinical and neurobiological profile of BD.

6.5.1. Clinical Scales

We found that the BDPMDD group reported significantly higher scores than the CTRL group in every clinical measure, in both menstrual phases. This is of particular importance as all of the women in our study with a diagnosis of bipolar disorder were clinically euthymic for a minimum of two months before study entry. The presence of subthreshold depressive and anxious

symptoms, as well as disturbances in sleep and biological rhythms during the mid-follicular phase may render these women more vulnerable to relapse since it is well established that the severity of subthreshold symptoms is associated with higher risk of relapse. This supports vast literature describing the presence of subthreshold affective and cognitive symptoms, which commonly persist in between mood episodes (54-56). For instance, a meta-analysis by Jackson et al. found that disruptions in sleep were the most common prodrome of manic and sixth of depressive episodes (59). This may highlight susceptibility of women with bipolar disorder and comorbid PMDD to develop more frequent mood episodes and contribute to their burden of illness. These findings support a study by Dias et al., which found that women with BD and premenstrual mood worsening represent a phenotype of bipolar disorder that is more symptomatic and relapse prone (20). This is also consistent with our recent study showing increased illness burden in women with BD and co-morbid PMDD. These women had a larger number psychiatric comorbidities, higher rates of rapid-cycling, earlier age of onset, increased number of hypomania and mania episodes and greater disruption of mood while taking oral contraceptive pills and through the perinatal period (113).

6.5.2. Hormone Analysis

We did not find differences in serum levels of E2, P4, Allopregnanolone or DHEAS between control groups (CTRL, PMDD) and patient groups (BD, BDPMDD). The influence of neurosteroids on mood and psychiatric illness may be of particular importance in bipolar disorders, as some mood stabilizing medications used to treat bipolar disorder are also GABAa receptor modulators (60,61). Previous literature on serum levels of DHEAS between patients with bipolar disorder and healthy controls are mixed (60), with studies reporting no differences, decreased levels in patients compared to controls (62), and increased levels in the posterior cingulate and parietal cortex in a post mortem study (63). Some studies have also found that

changes in DHEAS may precipitate the onset of, and be correlated to symptoms of mania (64,65). Literature also reports higher levels of progesterone and allopregnanolone in the late luteal phase in patients with BD, compared to patients with a diagnosis of major depressive disorder or healthy controls in the same menstrual phase (66); However they found these hormonal levels did not correlate with manic or depressive symptomology (67).

6.5.3. Resting State Functional Connectivity

We identified differences in Rs-FC between BD and BDPMDD groups using a seed-to-voxel analysis using the right and left hippocampus as seed points in the late luteal phase. Aberrant prefrontal-limbic circuitry is well documented in bipolar disorder; many of the brain regions in this circuitry are also influenced by hormonal fluctuations – the hippocampus as a primary example. The hippocampus is central to the trait-based pathology of bipolar disorder and regulation of mood and cognitive (memory, encoding and retrieval) and emotional processes (26,29,68,69). It also is a prominent site of E2 and 5HT receptors (31,70), has high expression of BDNF (60) and plays an inhibitory role in stress response (71) and HPA axis regulation (72,73). Literature also suggests that progesterone-derived neurosteroids may play an important role in mediating neuronal plasticity (neural survival, neurogenesis) in the hippocampus (60). Further, structural changes have been observed in the hippocampus across the menstrual cycle as studies in healthy women report both decreased gray matter in the anterior hippocampus and increased volume in the bilateral hippocampi during the follicular phase (74,75). FMRI studies in healthy women highlight increased functional coupling at rest between the bilateral hippocampi and bilateral superior parietal lobe in the late vs. early follicular phase, and decreased activity of the hippocampus in the luteal phase with response to emotional faces (76).

In this capacity, the increased functional coupling between the left hippocampus and right frontal cortex in BDPMDD compared to controls may suggest (1) subthreshold symptoms of

PMDD in women with BD; (2) compensatory changes in functional connectivity resulting from the late luteal menstrual phase in women during the "hormonal sensitivity" period (PMDD); and/or (3) a functional trait marker of the added weight of PMDD on the pathophysiology of BD.

Interestingly, we found decreased coupling between the right hippocampus and the left premotor cortex in the late luteal phase in BDPMDD compared to BD. Previous studies using PET imaging have reported increased activity of the premotor cortex during "sadness" inducing paradigms in remitted but not depressed patients with bipolar disorder (77), but decreased activity in patients with major depressive disorder (78,79). Further, a recent study reported that decreased connectivity of the somatomotor network, which encompasses the premotor cortex (as seen in the BDPMDD group), is present in bipolar depression and may represent aberrantly slow flow of inner time – a common feature of depression (80). Therefore, we hypothesize that this pattern of functional coupling in BD vs. BDPMDD may highlight the impact of comorbid PMDD in the late luteal phase in women with bipolar disorder. In women with BD and comorbid PMDD, we may see changes in Rs-FC which mirror bipolar depression. These findings also emphasize the need for greater research to explore the impact of the endogenous hormonal dynamics on women with BD.

6.5.4. Structural MRI (Cortical Thickness and Automated Subcortical Segmentation

We found decreased thickness in several frontal and temporal gyri central to the default mode and cognitive networks, in BDPMDD compared to BD; one exception was the left superior temporal gyrus, which was thicker in individuals with BDPMDD. Studies investigating cortical thickness in BD report decreases in thickness in the bilateral superior frontal gyri, superior parietal gyrus, middle temporal gyrus and pericalcarine gyri compared to controls (112). As these regions are thinner in individuals in the BDPMDD group than BD group, this suggests that having a diagnosis of BD and comorbid PMDD may have an even worse impact on brain structure. This

also may help explain the affective lability reported in our clinical data and in literature in women with BD and comorbid PMDD. This was further reinforced as women in the BDPMDD group had increased gray matter volume of the caudate nucleus compared to the BD group. The caudate nucleus facilitates cross talk between prefrontal cortical networks and subserves behavioral adaptations required for achievement of complex goals (28,88). Studies investigating caudate volume between bipolar disorder and controls have reported mixed findings with no differences (89,90) and decreased volume compared to controls (91). Our seed to voxel analysis results show increased prefrontal-limbic activity, which we postulate may be mediated by both the dorsal and ventral prefrontal cortical networks involving the striatum (28). When taken in conjunction with our Rs-FC findings, increased caudate volume in BDPMDD could reflect heightened activity of prefrontal-cortical circuitry central to the trait-based pathology of BD, which seems to be exacerbated in the late luteal phase.

6.5.5. Secondary Aims – PMDD Group

Our secondary aims were to explore the clinical, structure and functional correlates of PMDD in healthy women. Participants in the PMDD group displayed significantly greater scores on all clinical scales in their late luteal phase, with the exception of STAI-Trait Scale (46). Thereby reflecting the substantial impact of PMDD on mood, sleep quality and biological rhythms. Moreover, we found that women with PMDD displayed significantly greater levels of allopregnanolone than controls in both menstrual phases. Allopregnanlone is known to induce negative mood in women with PMDD and may aid in explaining the depressive and anxious symptoms experienced in this population (72,92,93). Interestingly, women with PMDD displayed similar scores on clinical scales in both menstrual phase to women with a diagnosis of BD and no PMDD; specifically, both groups of women endorsed comparable depressive and anxious symptomology in the follicular phase of the menstrual cycle. This suggests remitted BD and

PMDD may display a similar level of impact on circadian rhythms, sleep quality, and development of anxious and depressive symptoms.

Further, we found differences in cortical thickness and Rs-FC between CTRL and PMDD groups. Women in the PMDD group displayed decreased cortical thickness between seed points and clusters associated with regions in the default mode, limbic and sensory motor networks and increased Rs-FC between regions central to default mode, salience, and cognitive networks. In the late luteal phase, we found increased Rs-FC between the precuneus (seed) and frontal cortex and angular gyrus in the PMDD vs CTRL group. The precuneus and angular gyrus are key structures of the DMN, which plays a role in social cognition, autobiographical memory and self-referential processing (94,95); changes in DMN activity have been consistently reported in many psychiatric conditions (96,97). Hyperconnectivity in nodes of the DMN may reflect a tendency towards rumination, increased processing of threatening stimuli and impairment in switching between task-positive and negative networks (97). Further, differences in Rs-FC of the DMN reflects its structural integrity (98), which we also see with our results as decreased thickness was seen in the inferior and middle temporal gyri. Our structural and functional MRI results suggest compromised integrity of the DMN in women with PMDD compared to controls.

Neuroimaging literature on women with PMDD is sparse (99), with no previous studies to date investigating cortical thickness in PMDD. Studies using fMRI have found increased dlPFC activation during a working memory task, which also correlated with disease dimensions (100) and in patients with PMS increased fALFF in the bilateral precuneus, left hippocampus and inferior temporal cortex, and decreased fALFF in the anterior cingulate cortex and cerebellum in the late luteal phase (101). Overall, neurobiological models of PMDD suggest in the late luteal phase there is an increase in negative and decrease in positive processing and reduced top-down prefrontal modulation of limbic circuitry (102). Our results are consistent with these models as

they highlight aberrant structure and functional connectivity in regions integral to affective processing and may mediate the onset of symptoms in the late luteal phase.

6.5.6. Limitations

The limitations of our study deserve attention. First, the DRSP is a self-administered tool used to chart symptoms of premenstrual syndrome across the menstrual cycle (39). It is possible that women may provide an inaccurate account of their premenstrual symptoms or that use of the DRSP may be confounded by stressful life events. In both groups with a diagnosis of BD (BD and BDPMDD groups), symptoms reported on the DRSP may also be confounded by exacerbation of their bipolar illness. However, the use of mood, sleep and biological rhythms questionnaires in both menstrual phases increased our confidence in self-reported results.

Second, Rs-fMRI provides an indirect measure of spontaneous neuronal activity in the ultralow frequency range (0.01-0.10 Hz) (104). The fMRI techniques used in this paper, seed to voxel and ROI-ROI are based on an oversimplification that BOLD activation measured is static through the duration of the scanning paradigm (105,106). Further, results may be confounded by the participant's ability to remain awake for the duration of the scan, and inability to control the participant's memory in the scanner. Although we advised participants not to think about anything in particular, and remain awake with their eyes fixed at the fixation point throughout the entire resting state brain scan, no objective measures such as simultaneous electroencephalogram or eye tracking, were in place to confirm that participants followed our instructions.

Third, the effects of psychotropic medications on Rs-FC are a common limitation of fMRI research in BD (27,107). Studies investigating Rs-FC in BD, including our current and previous work (81), have ruled out the influence of medication following correlation analysis with BOLD activation (82,86,108-111) . The primary goal of this study was to investigate brain structure and function of co-morbid BD and PMDD. We believe this goal could have only been

achieved with a primarily medicated sample, by ensuring that all participants with BD were euthymic throughout the study (40).

Another limitation of our sample was that it comprised both women with BD type-I and BD type-II. We encourage future work on differences in Rs-FC between women with BD type-I and BD type-II. Finally, it is important to note that our study was cross-sectional and as a result this study only reflects a current picture of disease state and not disease progression. Longitudinal studies in this area are required to provide a picture of disease burden and progression in women with BD and comorbid PMDD.

6.7. Conclusions

In conclusion, our study was the first to examine the neurobiological profile of BD and comorbid PMDD in a well-defined sample of euthymic women with BD using prospective symptom charting. We found differences in the thickness of the cerebral cortex in regions critical to emotional regulation and cognition, as well as volumetric enlargement of the left caudate in the late luteal phase of participants in the BDPMDD group compared to BD group. Further, results from Rs-FC analysis highlight differences in brain regions dense in E2 and 5HT receptors, which may be liable to hormonal influence and are also implicated in the pathophysiology of bipolar disorder.

In individuals with BD a co-morbid diagnosis of PMDD may act to exploit the trait-based pathology of the disease, even in euthymic illness phases. This may predispose women to the onset of affective episodes and help explain the suggested worse course of illness and clinical profile in women with both diagnoses. When taken in the context of other literature on this population, our results suggest that women with BD and co-morbid PMDD display a distinct clinical and neurobiological phenotype of BD involving sensitivity to hormonal influence, which

may potentially warrant course specifiers to capture the phenotypic differences and the additional burden of disease experienced in this population.

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Table 1: Patient Demographics

Table 1: Patient Demographics					
	HC (n=25)	**PMDD (n=20)**	**BD (n=21)**	**BDPMDD (n=19)**	**P value**
Age	27.44 (7.74)	31.80 (7.33)	33.57 (8.04)	31.74 (7.91)	p=0.054
BMI	23.24 (3.29)	24.02 (4.32)	26.76 (5.96)	29.46 (5.11)	p<0.001
Years of Education	16.94 (2.64)	17.23 (3.55)	16.40 (2.82)	15.05 (2.07)	p=0.091
Illness History					
Age of Onset			18.3 (7.74)	17.1 (5.85)	p=0.817
Average Number of Co-morbidities			1.27 (1.35)	1.55 (1.68)	p=0.943
Diagnosis			BD1: 12 BD2: 9	BD1: 7 BD2: 12	
History of Psychosis			3	7	
Psychiatric Medications					
Lithium			3	1	
Anticonvulsants			7	8	
Antipsychotics			10	8	
Anxiolytics			6	10	
Antidepressants		2	5	4	
Sleep Aids			2	3	
Mean # of Psychotropic Meds/Medication Load			2.05 (1.02)/ 2.48 (1.83)	2.50 (1.22) 2.32 (1.70)	p=0.868

Table 2: Clinical Scale Scores Between Groups

Table 2: Clinical Scale Scores Between Groups					
Clinical Scale (SD)	HC	PMDD	BD	BDPMDD	P value
MADRS-F	2.60 (2.99)	5.40 (5.40)	6.33 (5.47)	10.79 (5.50)	p<0.001
MADRS-L	2.16 (2.90)	14.68 (7.80)	9.24 (6.86)	14.79 (7.28)	p<0.001
HAMD-F	1.44 (1.69)	3.25 (3.37)	4.43 (3.71)	6.58 (4.23)	p<0.001
HAMD-L	1.20 (1.58)	9.37 (5.12)	5.95 (3.97)	9.16 (4.50)	p<0.001
YMRS-F	0.48 (1.00)	0.75 (1.12)	1.48 (1.29)	1.37 (1.67)	p=0.020
YMRS-L	0.60 (1.15)	1.89 (1.56)	1.38 (1.43)	2.53 (1.65)	p<0.001
BRIAN-F	27.28 (6.36)	35.45 (11.25)	37.48 (9.26)	47.79 (8.99)	p<0.001
BRIAN-L	28.48 (8.41)	40.61 (12.26)	38.35 (12.46)	48.50 (8.42)	p<0.001
PSQI-F	4.28 (2.42)	5.05 (2.87)	5.95 (3.03)	7.95 (3.61)	p=0.005
STAI-State-F	29.16 (6.84)	31.80 (9.47)	33.40 (9.49)	40.58 (14.25)	p=0.034
STAI-State-L	31.04 (7.27)	43.47 (13.07)	34.10 (9.19)	45.84 (13.97)	p=0.001
STAI-Trait-F	30.08 (7.28)	33.95 (9.19)	41.70 (10.27)	47.63 (15.17)	p<0.001
STAI-Trait-L	30.67 (9.43)	38.63 (11.73)	41.95 (11.59)	48.63 (14.07)	p<0.001

Table 3: Group Differences in Clincal Scales

Table 3: Group Differences in Clinical Scales						
	BD-BDPMDD	BD- CTRL	BD-PMDD	BDPMDD-CTRL	BDPMDD-PMDD	CTRL-PMDD
MADRS-F	p=0.050	p=0.105	p=0.974	p<0.001	p=0.017	p=0.230
MADRS-L	p=0.081	p=0.001	0.118	p<0.001	p=0.999	p<0.001
HAMD-F	p=0.364	p=0.021	p=0.745	p<0.001	p=0.040	p=0.194
HAMD-L	p=0.102	p<0.001	p=0.112	p<0.001	p=0.999	p<0.001
YMRS-F	p=0.964	p=0.014	p=0.206	p<0.173	p=0.621	p=0.822
YMRS-L	p=0.142	p=0.182	p=0.883	p<0.001	p=0.421	p<0.001
BRIAN-F	p=0.012	p<0.001	p=0.677	p<0.001	p<0.001	p=0.003
BRIAN-L	p=0.018	p=0.002	p=0.900	p<0.001	p=0.120	p<0.001
PSQI-F	p=0.975	p=0.116	p=0.874	p=0.044	p=0.652	p=0.462
STAI-State-F	p=0.357	p=0.492	p=0.948	p=0.036	p=0.272	p=0.893
STAI-State-L	p=0.017	p=0.75	p=0.066	p<0.001	p=0.955	p=0.003
STAI-Trait-F	p=0.824	p=0.001	p=0.085	p<0.001	p=0.009	p=0.510
STAI-Trait-L	p=0.416	p=0.002	p=0.849	p<0.001	p=0.095	p=0.052

Table 4: Hormone Levels in the Mid-Follicular and Late Luteal Menstrual Phases

Table 4: Hormone Levels in the Mid-Follicular and Late Luteal Menstrual Phases					
Hormone Levels	HC	PMDD	BD	BDPMDD	P value
Follicular Phase					
P4	1.66 (2.08)	1.23 (0.91)	1.66 (4.15)	1.03 (0.84)	p=0.127
E2	69.71 (45.2)	87.27 (38.2)	73.71 (57.3)	76.34 (51.6)	p=0.220
Allo	4.13 (1.60)	13.58 (14.8)	4.97 (4.01)	5.82 (4.25)	p=0.010
DHEAS	163.56 (77.2)	161.67 (93.1)	160.63 121.9	129.27 (79.2)	p=0.506
Luteal Phase					
P4	4.53 (2.82)	3.72 (2.10)	5.25 (11.0)	4.20 (3.62)	p=0.505
E2	86.54 (51.3)	106.13 (50.7)	87.18 (73.0)	78.77 (39.5)	p=0.187
Allo	4.88 (2.02)	13.45 (11.7)	5.54 (4.16)	5.58 (4.78)	p=0.012
DHEAS	155.92 (73.9)	149.86 (75.9)	166.14 (156.8)	124.44 (84.3)	p=0.605

Table 5: Rs-FC Group Differences in the Mid-Follicular Phase

Table 5: Rs-FC Group Differences Across Groups in the Mid-Follicular Phase				
Seed Region	**Cluster Region**	**Peak Coordinates X, Y, Z**	**Clustersize**	**Clustersize P-Value**
R-FP	N/S			
L-FP	N/S			
R-IFG	N/S			
L-IFG	N/S			
R-OFC	N/S			
L-OFC	N/S			
R-PreCG	N/S			
L-PreCG	N/S			
scACC	L-BA 39	-42 -68 +14	316	p=0.0013
R-Hippo	N/S			
L-Hippo	N/S			
R-Amyg	N/S			
L-Amyg	N/S			

Table 6: Rs-FC Group Differences in the Late Luteal Phase

Table 6: Rs-FC Group Differences Across Groups in the Late Luteal Phase				
Seed Region	Cluster Region	Peak Coordinates X, Y, Z	Clustersize	Clustersize P-Value
R-FP	N/S			
L-FP	N/S			
R-IFG				
L-IFG	N/S			
R-OFC	N/S			
L-OFC	N/S			
R-PreCG	N/S			
L-PreCG	N/S			
scACC	N/S			
R-Hippo	Right BA6	+64 +02 +06	166	p=0.044
L-Hippo	Right sensory association cortex (BA-5 Right BA-8 Right – primary sensory cortex (BA-1)	+10 -30 +50 +00 +34 +44 +48 -34 +62	140 148 188	p=0.037 p=0.037 p=0.033
R-Amyg	N/S			
L-Amyg	N/S			
Precuneus	Right BA-8 frontal cortex	+10 +44 +46	220	p=0.011

Table 8: Differences in Cortical Thickness Between Groups

Table 8: Differences in Cortical Thickness Between Groups				
	Size mm^2	Peak Coordinates X, Y, Z	Peak T Value	P_{Cor} Value
CTRL vs. PMDD				
CTRL>PMDD				
L Superior temporal	342.94	-48.6 -1.5 -24.6	3.0244	0.0484
L Middle temporal	234.61	-64.3 -29.3 -14.8	3.3301	0.0209
R Inferior temporal	225.66	46.0 -6.7 -40.9	3.4449	0.021
R Pars opercularis	156.04	49.1 13.4 5.6	3.7015	0.0105
L Postcentral	144.07	-51.4 -18.0 49.7	3.3799	0.0187
R Superior frontal	112.48	7.8 15.8 60.9	4.0091	0.0045
R Middle temporal	98.68	65.2 -32.5 -12.7	3.2138	0.039
CTRL vs. BD				
CTRL>BD				
R Pars orbitalis	171.35	31.2 45.1 -11.5	3.4117	0.0068
L Precuneus	91.69	-7.1 -53.3 52.3	3.1303	0.0165
L Supramarginal	80.57	-44.9 -34.1 19.5	3.0149	0.022
R Pars opercularis	66.00	46.3 14.2 6.8	2.7132	0.0416
L Inferior parietal	65.73	-40.1 -69.6 17.7	2.7125	0.049
R Inferior temporal	60.82	53.0 -30.2 -20.6	2.8701	0.028
R Superior parietal	58.36	13.0 -69.7 53.7	2.9885	0.0208
CTRL vs. BDPMDD				
CTRL>BDPMDD				
L Insula	262.25	-32.1 14.7 -7.0	4.0548	0.0022
R Middle temporal	220.89	45.6 -61.0 6.4	4.7045	0.0004
R Medial orbitofrontal	148.09	11.1 28.0 -17.8	3.5026	0.0048
L Pars triangularis	91.66	-46.0 32.6 8.4	3.3432	0.0209
L Rostral middle frontal	85.62	-40.2 27.7 23.7	3.5982	0.0099
R Cuneus	80.53	7.5 -83.8 24.4	2.6684	0.0444
R Superior frontal	69.04	7.6 17.0 59.0	3.5556	0.004
PMDD vs. BD				
PMDD<BD				
R Precuneus	227.04	24.3 -62.1 8.2	3.3787	0.0234
R Lateral Occipital	213.97	44.4 -78.3 -12.0	4.1051	0.0026
L Middle frontal	199.42	-62.1 -33.8 -16.2	3.0880	0.0390
L Superior temporal	179.81	-56.6 -1.4 -5.0	3.0037	0.0490
L Superior frontal	150.60	-11.4 59.7 19.9	3.8683	0.0050
L Pars triangularis	118.88	-44.5 26.5 5.4	3.9493	0.0040
R Inferior Parietal	112.13	47.8 -47.1 20.9	3.9837	0.0039

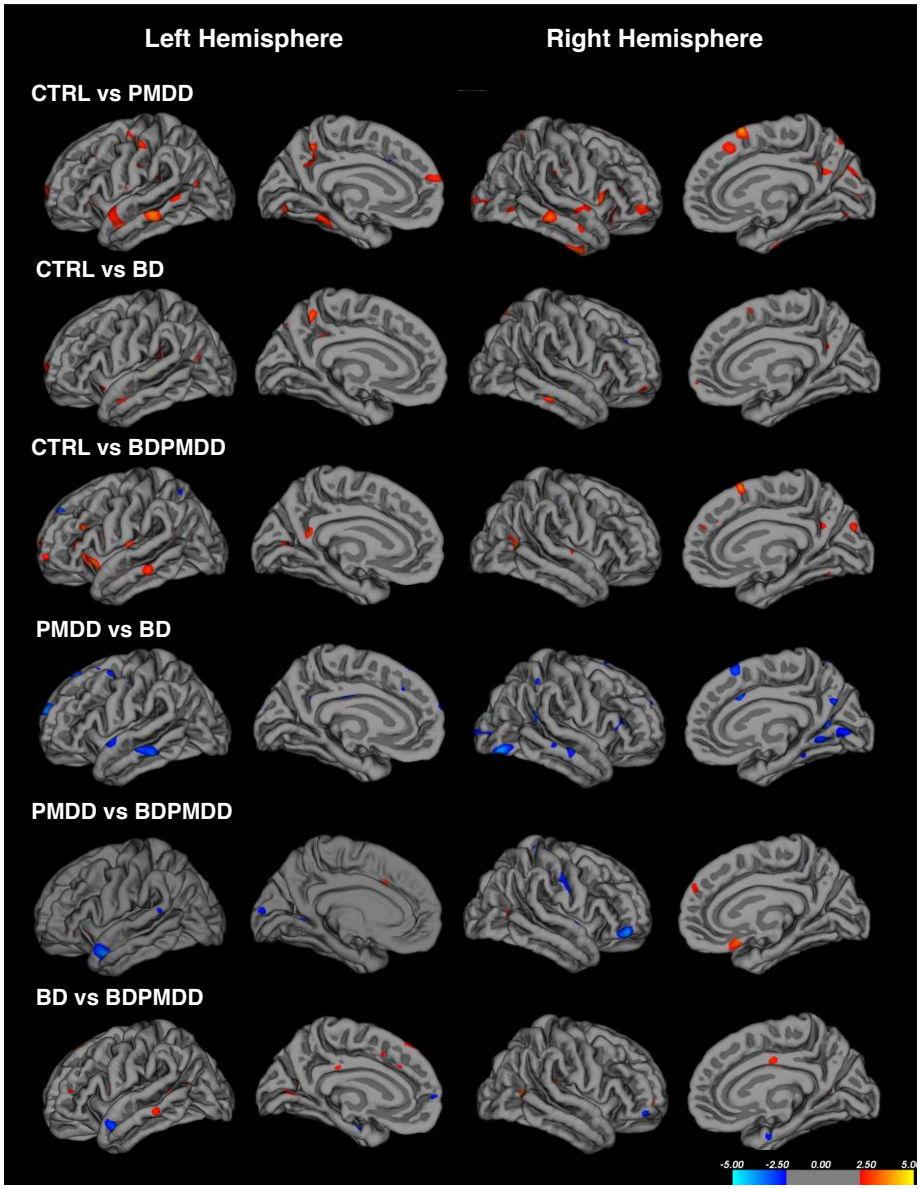
L Superior frontal	75.85	-10.0 33.4 51.2	3.3175	0.0210
R Caudal Anterior Cingulate	66.03	6.8 17.5 32.0	3.2138	0.0364
PMDD vs. BDPMDD				
PMDD>BDPMDD				
R Medial oribitofrontal	126.21	12.6 26.6 -16.2	3.5949	0.0060
R Medial orbitofrontal	58.92	7.0 15.9 -15.4	3.0872	0.0246
R Inferior parietal	56.52	44.0 -61.7 6.6	3.0320	0.0282
BDPMDD>PMDD				
L Superior Temporal	215.50	-48.3 4.9 -24.7	3.5025	0.0039
R Pars Orbitalis	137.85	43.8 44.6 -9.7	3.7023	0.0048
L Lingual	100.15	-23.5 -59.9 0.1	3.3728	0.0057
R Superior parietal	86.37	26.7 -69.2 28.6	2.9995	0.0306
BD vs. BDPMDD				
BD>BDPMDD				
L Pericalcarine	417.43	-13.1 -74.0 2.8	3.5462	0.0072
L Superior parietal	236.87	-20.7 -62.4 36.5	3.9530	0.0024
R Middle temporal	223.90	45.6 -61.0 6.4	4.8981	0.0002
R Rostral middle frontal	105.38	25.3 49.3 0.3	4.0143	0.0006
L Superior frontal	88.44	-10.0 34.2 50.9	2.8887	0.0402
BDPMDD>BD				
L Superior temporal	112.82	-48.7 4.8 -24.0	2.8448	0.045

Table 7: Differences in Resting State Functional Connectivity Across Groups

Seed Region	Group/Change	Area	Coordinates (X Y Z)	T min	Clustersize	Cluster wise P-value
Follicular Phase						
Subcallosal Cortex						
	PMDD>CTRL	L-Angular Gyrus: (BA 39)	-48 -64 +14	3.29	706	p<0.001
		L- Frontal Cortex (BA 8)	-34 +06 +36	3.29	240	p=0.017
	BD>PMDD	L-Inferior Frontal Gyrus: pars opercularis (BA 44)	-56 +06 +16	3.31	259	p=0.031
Luteal Phase						
R-Hippocampus						
	BDPMDD>PMDD	L-Frontal Cortex (BA 8) R-Dorsolateral Prefrontal Cortex (BA 9)	-42 +16 +44 +12 +42 +40 -20 +44 +42	3.33	539 276 197	p=0.0004 p=0.011 p=0.031
	PMDD>BDPMDD	R-Primary Motor Cortex (BA 4) L-Primary Motor Cortex (BA 4)	+66 +00 +14 -50 -08 +12	3.33	297 229	p=0.007 p=0.011
	BD>BDPMDD	R-Premotor Cortex (BA 6)	+64 +02 +06	3.32	201	p=0.029
L-Hippocampus						
	PMDD>CTRL	R-Sensory Association Cortex (BA 5) L-Sensory Association Cortex (BA 5)	+10 -30 +50 -28 -40 +52	3.29	1374 243	p<0.001 p=0.024
	BD>CTRL	-	+16 -40 +58	3.29	326	p=0.020

	BDPMDD>BD	R- Frontal Cortex (BA 8)	+02 +34 +44	3.32	165	p=0.048
	PMDD>BDPMDD	L-Somatosensory Cortex (BA 1)	-60 -18 +50	3.33	431	p=0.016
Precuneus						
	PMDD>CTRL	R-Frontal Cortex (BA 8) R-Angular Gyrus (BA 39) L-Frontal Cortex (BA 8)	+10 +44 +46 +42 -62 +20 -16 +46 +50	3.29	627 408 202	p<0.001 p=0.002 p=0.035

Figure 1: Cortical thickness across study groups in the mid-follicular menstrual phase: Cortical thicknening and thinning is represented with reference to the first group; red = increased cortical thickness, blue = decreased cortical thickness



Chapter 7: Future Directions

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

Periods of hormonal fluctuations have the potential to act as windows of vulnerability for the entrainment of affective episodes in patients with bipolar disorder (BD) (1-3). Out of the major reproductive milestones studied, there is considerable evidence confirming this hypothesis during the perinatal period (3-5). However, there is substantially less literature investigating the premenstrual period in women with BD and comorbid premenstrual dysphoric disorder (PMDD), and the menopausal transition. This is of particular importance as research suggests a diagnosis of PMDD may be an independent predictor of development of depressive episodes during the menopausal transition (6,7). The following body of work aims to further examine the neurobiological correlates of hormonal fluctuations associated with the menstrual cycle on resting state functional connectivity (Rs-FC) and emotional regulation in women with BD and comorbid PMDD. Finally, it aims to assess the menopausal transition in women with BD through a systematic literature review. A brief description and preliminary results of each study is below.

7.1. Emotional Regulation During an Emotional Stroop Paradigm in Women with BD and comorbid PMDD

7.1.1. Aims

Dysregulation in emotional regulation and processing is a common feature of BD (8-11). This is supported by neuroimaging literature, which reports aberrant structural and functional connectivity in brain regions and networks associated with emotional and cognitive regulation (8-12). Interestingly, there is considerable overlap between, regions implicated in the

pathophysiology of BD and those modulated by sex hormones. Further, literature on the premenstrual period in BD highlights both entrainment and exacerbation of affective episodes (13), suggesting aberrant functional connectivity of brain regions implicated in the pathophysiology of BD. The ability to monitor and regulate emotional conflict may also be altered in the late luteal phase in women with BD and comorbid PMDD. Therefore, we sought to investigate differences in conflict monitoring and resolution during an emotional Stroop task across four diagnostic categories of women (as established in Chapter 6): CTRL, PMDD, BD, BDPMDD.

7.1.2. Methods

Please refer to sections 2.1-2.5.1 for details methods on participant information, study design, clinical questionnaires and hormone analysis. Four groups of women completed the emotional Stroop fMRI task: CTRL (n=24), PMDD (n=19), BD (n=20), BDPMDD (n=18).

Image Acquisition

Images were acquired using a GE whole body short-bore 3T scanner with 8 parallel receiver channels (General Electric, Milwaukee, WI, USA). Anatomical images were acquired with high-resolution T1 weighted images (gradient-echo inversion-recovery sequence, TR=1.6s, TE=5ms, matrix 256x256x128, FOV 220x220mm, slice thickness 1mm). Functional blood oxygen level dependent (BOLD) images were acquired using a T2* interleaved echo-planar imaging (EPI) sequence with a TR=3000, TE=35ms, flip angle=90°, 3mm thick (no skip), 36 axial slices, matrix 64x64 resolution over 256 mm FOV).

Emotional Conflict Task

The emotional conflict task (14) used consists of 148 happy or fearful faces with the words HAPPY or FEAR written across them in red letters, thereby creating congruent (word matches the facial expression) and incongruent (word does not match the facial expression)

stimuli. Each stimulus was presented for 1000 milliseconds, with a jittered inter-stimulus interval of 3000-5000 milliseconds during which a fixation cross was shown. Equal stimulus pairings of congruent-congruent, congruent-incongruent, incongruent-congruent and incongruent-incongruent were shown.

Image Preprocessing

Functional and anatomical MRI data were preprocessed using the Statistical Parametric Mapping Software SPM12 (http://www.fil.ion.ucl.ac.uk/spm) and CONN Functional Connectivity Toolbox Verson 17e (https://www.nitrc.org/projects/conn) (15). Imaging data was obtained in DICOM file format and converted to NIFTI using SPM and then uploaded to CONN for further preprocessing. The default-preprocessing pipeline for volume-based analyses was optimized to preprocess both structural and functional scans. Briefly, structural scans were translated and centered to (0,0.0) coordinates and subsequently underwent direct segmentation (gray matter, white matter and cerebrospinal fluid) and MNI normalization. Functional scans were realigned and unwarped (motion estimation and correction), and translated and centered to (0,0,0) coordinates. Outliers were identified as volumes with greater than 2mm of motion in the translational plane, and were detected using the ART toolbox and added as condition files for denoising. Images with motion greater than 3mm in the translational plane and 3 degrees in the rotational plane were discarded. Finally, functional data was segmented (GM, WM, CSF), normalized to MNI space and spatially smoothed to increase the signal to noise ratio with a 8mm FWHM Gaussian filter.

Statistical Analysis

Statistical analysis was conducted using a similar procedure to a previous study from our group using this same fMRI task (16). Functional connectivity was compared between the mid-

follicular and late luteal menstrual phases, within groups using a series of paired t-tests. Motion regressor files were used as covariates in first level analysis.

7.1.3 Preliminary Results

Preliminary results show brain activation during emotional Stroop performance between menstrual phases in each of the four groups studied. Analysis of behavioural data, and both conflict monitoring and resolution conditions is currently in progress.

We did not find differences in functional connectivity during emotional Stroop performance in healthy controls or the BD (without comorbid) PMDD group between menstrual phases. Increased BOLD activation during emotional stroop performance was found in the luteal vs mid-follicular menstrual phase in both the PMDD and the BDPMDD groups (Table 1).

Table 1: Differences in Functional Connectivity During Emotional Stroop Task Performance Between the Mid-follicular and Late Luteal Menstrual Phases

Cluster Region	Cluster Size (k)	Peak Coordinates X, Y, Z	T-Value	P-Value (uncorrected)
Premenstrual Dysphoric Disorder (PMDD)				
L-Posterior Cingulate Cortex (BA23)	2929	-8, -58, 6	2.56	p=0.010
R-Primary Motor Cortex (BA 4)	107	60, -4, 12	2.42	p=0.013
L-Primary Auditory Cortex (BA 41)	126	-36, -26, 10	2.03	p=0.028
L-Visual Association Cortex (BA 19)	72	-32, -80, 28	1.95	p=0.033
Bipolar Disorder Comorbid Premenstrual Dysphoric Disorder (BDPMDD)				
L-Dorsolateral Prefrontal Cortex (BA 10)	56	-12, 60, 14	1.85	p=0.040

7.2. The Influence of Hormonal Fluctuations Associated with the Menstrual Cycle in Women with BD and Comorbid PMDD

7.2.1. Aims

Literature supports that women with BD and premenstrual exacerbation display a worse course of their bipolar illness, as characterized by more frequent depressive episodes, hospitalization and overall worse prognosis. It is hypothesized that women with PMDD display sensitivity to endogenous hormone fluctuations, thereby mediating the onset of clinical symptoms experienced in the late luteal phase. Women with BD and comorbid PMDD may display additional sensitivity as regions of the brain dense in sex hormone receptors are also implicated in the pathophysiology of BD. Despite this, the influence of hormonal fluctuations on Rs-FC in women with BD and PMDD has not been studied. In this study, we aim to correlate endogenous levels of E2, P4, ALLO and DHEAS to Rs-FC in women with BD and BDPMDD (as categorized in Chapter 6).

7.2.2. Methods

Please refer to sections 2.1-2.5.2 for detailed methods on participant information, study design, clinical questionnaires, hormonal analysis and MRI and fMRI image acquisition and pre-processing. Serum levels of E2, P4, ALLO and DHEAS from both the mid-follicular and late luteal menstrual phases will be added as second-level covariates in CONN to investigate the correlation between endogenous hormonal levels and patterns of functional coupling in these populations. Results will be considered significant below a threshold of $p<0.05$, FDR-corrected.

7.2.3. Results

In progress.

7.3. Bipolar Disorder and the Menopausal Transition: A Systematic Review

7.3.1 Introduction/Aims

Menopause marks the beginning of reproductive senescence in midlife women. During this time, ovulation ceases, leading to a progressive decline in circulating levels of endogenous sex hormones, such as 17-β-Estradiol (E2) (17). E2 is known to modulate monoaminergic systems in part through synthesis and availability of serotonin, noradrenaline and dopamine (18,19). Fluctuating levels during the menopausal transition result in (1) neuroendocrine changes in E2 receptor-dense brain regions (hippocampal formation, claustrum, cerebral cortex, amygdala, hypothalamus subthalamic nucleus and thalamus) (20-22); (2) aberrant functioning of neurotransmitter systems (serotonergic, adrenergic, dopaminergic), ultimately mediating the onset of menopausal symptoms (VMS, depressive and anxious symptomology, sleep and cognitive disturbances) (23-25). Neuroendocrine and neurotransmitter changes and menopausal symptoms have been associated with impairment in various cognitive domains (24,26). For example, objectively recorded moderate-severe VMS has been associated with delayed verbal memory (29) and subjectively reported moderate-severe VMS are predictive of lower retrospective memory functioning (30). Further, the menopause-associated decline in E2 has been associated with an increase in pro-inflammatory cytokines (TNF-α, Il-6, Il-1) and free radicals (NO) (31,32). Animal research suggests that E2 has anti-inflammatory effects on microglial activation (33), and this may be of increased importance in women with BD (34,35).

In the context of the menopausal transition, BD may lead to changes in the brain and central nervous system that may make it more difficult for the brain to adapt to E2 fluctuations, thus precipitating development of more severe menopausal symptoms or the onset of a mood episode during the perimenopausal period.

The extent of the current findings in the field suggests that a systematic review of the

evidence may be needed. The primary objective of this systematic review of peer-reviewed articles is to provide researchers and clinicians insight into the effect that menopause may have on female patients diagnosed with bipolar disorder.

7.3.2. Methods

Search Strategy

To identify relevant articles a comprehensive search strategy of relevant databases was implemented. Medical Subject Headings related to bipolar disorder and menopause were used in various combinations on Pubmed, MEDLINE and PsychINFO to identify relevant articles. References from articles in relationship to the topic of interest were reviewed to identify studies not identified by searching the databases. This step was used to evaluate the efficacy of the search strategy.

Study Selection Process

Two screening phases were implemented in determining included studies in the analysis: (i) an abstract and title-screening phase, where abstracts of articles were assessed; and (ii) a full text screening where the full article was assessed. Two reviewers SKS and RR assessed potential articles during both screening phases. Any disagreements were settled by an independent rater (LM). In order to determine the level of agreement a Kappa Statistic was calculated for both screening phases

Outcomes of Interest

To compare depressive to manic episodes during the menopausal transition, patient and caregiver reported worsening of symptoms were evaluated. Furthermore, the frequency of clinical visits in a depressed or manic state among patients with bipolar disease during perimenopause and post-menopause was compared.

Criteria for Inclusion/Exclusion in Study

Only observational studies were included the data analysis. These studies included prospective cohort studies with concurrent or historical controls, retrospective cohort studies, cross sectional studies and case-control studies. Case series and case reports were not included. Only studies that included participants with both menopause and bipolar disorder were included in the analysis. Articles that failed to verify diagnosis were excluded from the study. Bipolar disorder was defined as (i) bipolar I disorder, (ii) bipolar II disorder, (iii) cyclothymic disorder, (iv) another specified or (v) unspecific bipolar disorder. Only studies that were published in English were included in the review. Studies that analyzed menopause as a confounding factor were excluded from this review. Studies that measured the effects of treatment in menopausal women with bipolar women were also excluded. Only studies published in English were included in the review. Only articles published between 1800-2016 were searched.

Statistical analysis

In order to assess the level of agreement between reviewers, a Kappa statistic was calculated for both the abstract and title screening phase and the full text-screening phase (36). The following ranges were used for the interpretation for agreement, poor agreement (0.01-0.20), fair agreement (0.21-0.40), moderate agreement (0.41-0.60), very good agreement (0.61-0.80) and excellent agreement (0.81-0.99). Agreement greater than 0.60 would be considered significant for the collected findings (36). To determine effect size comparing depressed and manic bipolar symptoms, odds ratios were calculated. Odds ratios were calculated to compare comparing worsening of depressive symptoms to worsening of manic symptoms. In order to determine significance the Cochran-Mantel-Haenszel test was performed for pooled data with the continuity correction (37). Heterogeneity was evaluated by calculating the Cochrane chi-square test and the I^2 value. Since two outcomes of interest were included in this study multiple comparison error

must be accounted for. In order to account for multiple comparison error the bonferroni correction was used and the significance level was reduced to 0.025 (37).

7.3.3. Results

Through the search strategy 260 citations were identified (MEDLINE: 83, PsychINFO: 67, PubMed: 110). 104 of these citations were duplicated articles and were removed, leaving 156 articles. After the abstract and title screening, 113 articles were removed, leaving 43 articles. The Kappa agreement between reviewers was 81.3% identifying excellent agreement (36). Full text screening removed 30 articles, leaving 13 articles. The kappa agreement between reviewers for full text screening was 62.8%, identifying very good agreement (36). Reviewing reference lists identified no new studies suggesting the search strategy was robust.

Pooled analysis of patient and caregiver worsening of symptoms was performed for three studies including 116 participants. These findings indicate that patients and caregivers are 3.02 times more likely to report worsening of depressive symptoms rather manic symptoms over the menopausal transition (X^2=8.96, p<0.005, 95% CI:1.49-6.14). The Cochrane chi-square test (p=0.76) and the I^2 statistic (I^2=0.0%) both indicated non significant heterogeneity between studies evaluated (37).

For information extracted from studies please refer to Table 2, further details are in progress (38-49).

7.4. References

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Table 2. Menopause Bipolar Disorder Study Characteristics

First Author and Year	Patient Characteristics					Control Characteristics	
	Sample (n)	***Subtype***	***Sex (M/F)***	***Age (S.D.)***	***Years of Education (SD)***	***Control Subjects (n)***	***Other Comparative Groups***
Clancy et al, 1973	100	Not specified	Both	Not specified	Not Specified	No Control	225 patients with unipolar depression 200 patients with schizophrenia
Blehar et al, 1998	327	Bipolar I Disorder	Both (186 F and 141 male	41.2-42.1	Education beyond high school 56.5-70.2%	No Control	No other comparative group
Hu et al, 2016	66 newly diagnosed cases	Not specified	Female	Not Specified	Not Specified	Not applicable	Not applicable
Marsh et al, 2012	Peri/postmenopausal 249 Premenopausal 519	Bipolar I, Bipolar III and Bipolar NOS	Female	Postmenopausal 60.95(8.65) Perimenopausal 47.96(3.80) Premenopausal 33.09(3.21)	Not Specified	No Control	No other comparative group
Payne et al, 2007	777	Bipolar I and Bipolar II	Female	<30 years	Not Specified	163	1747 patients with unipolar depression
Gregory et al, 2000	8	Not specified	Female	Not specified	Not Specified	No Control	No other Comparative group
Marsh et al, 2105	Late or post menopause	Bipolar I, Bipolar	Female	Late or post menopause	Not specified	No Control	No other Comparative

	35 Early menopause 21	III and Bipolar NOS		50.6(4.7) Early menopause 45.0(3.4)			group
Sajatovic et al, 2006	30	Not specified	Female	50.4(3.0)	Not specified	No Control	36 patients with unipolar depression and 20 patients with schizophrenia
Sajatovic et al, 2003	6 patients and family members of patients	Not Specified	Not Specifed	51.2(11.8)	Not specifed	No Control	15 participants with or with family members with unipolar depression 8 participants with or with family members with schizophrenia 10 participants with or with family members with other specified disorder
Marsh et al, 2006	47	Bipolar I, Bipolar II, Bipolar NOS and Bipolar Rapid cycling	Female	45-55	Not Specified	No Control	No other Comparative group
Freeman et al, 2002	50	Bipolar I, Bipolar	Female	43.3(11.4)	Not Specified	No control	No other Comparative

		II, and Bipolar NOS					group
Perich et al, 2016	158	Bipolar I, and Bipolar II	Female	37.89(13.6)	Not Specified	No Control	No other Comparative group
Friedman et al, 2005	25	Not Specified	Female	45-55	14.2(2.7)	No Control	36 patients with unipolar depression 30 patients with schizophrenia

Chapter 8: General Discussion

8.1. Summary of Findings

Little is understood about the influence of hormonal fluctuations associated with the menstrual cycle on structural and functional brain connectivity. To an extent, female reproductive milestones, such the perinatal and perimenopausal periods may be thought of as neurological transition states; whereby the brain adapts to changes in the hormonal milieu, as well as downstream effects of hormonal changes (changes in neurotransmitter concentrations and resulting physiological and affective symptoms) [Brinton et al., 2015; Frey and Dias, 2013; Lokuge et al., 2010; Soares and Zitek, 2008]. While these periods may have the potential to precipitate psychiatric episodes, a subset of women with premenstrual dysphoric disorder (PMDD) may be vulnerable to more frequent and recurrent exacerbation of mood during their late luteal menstrual phase [Frey and Dias, 2013; Teatero et al., 2013]. In this contaxt, women with psychiatric illness may have greater difficulty adapting to hormonal changes associated with the reproductive lifespan, including smaller scale changes associated with the premenstrual period [Frey and Dias, 2013; Soares and Zitek, 2008]. Literature highlights that women with BD have higher rates of PMS and PMDD than controls [Choi et al., 2011; Dias et al., 2011; Fornaro and Perugi, 2010; Frey and Minuzzi, 2012; Teatero et al., 2013; Wittchen et al., 2002]. Notably, this subset of bipolar women with comorbid PMDD experiences a worse course of their bipolar illness [Dias et al., 2011; Slyepchenko et al., 2017].

The primary goal of this thesis was to investigate the influence of hormonal fluctuations associated with the menstrual cycle on brain structure and functional connectivity in women with BD and comorbid PMDD. This goal was accomplished through several studies, which

independently contribute to the literature and cumulatively illustrate the impact of menstrual phase, hormones and BD on the brain, and complexity of comorbid BD and PMDD.

In our first study (Chapter 2), we investigated differences in resting state functional connectivity (Rs-FC) between the mid-follicular and late luteal menstrual phases in women with no history of PMDD. We also examined the influence of estradiol (E2), progesterone (P4), allopregnanolone (ALLO), and dehydroepiandrosterone sulphate (DHEAS) on Rs-FC during the mid-follicular and late luteal menstrual phases in the same sample of women. Although we did not find overall differences in Rs-FC using either seed based or independent component analysis, we found robust hormone specific correlations between patterns of functional coupling and hormones in each menstrual phase. These patterns occurred in brain regions dense in hormone receptors and varied across menstrual phases [Brinton et al., 2008; Osterlund et al., 1998; Österlund and Hurd, 2001; Österlund et al., 2000]. The greatest number of correlations with functional coupling was seen in the late luteal phase with the GABAa receptor modulating hormones ALLO and DHEAS. This is interesting, as one hypothesis on the etiologic of PMDD highlights specifically the sensitivity of GABAa receptors [Backstrom et al., 2014; Pearlstein and Steiner, 2008; Vigod et al., 2010] . Therefore, we can begin to postulate that the increased influence of both ALLO and DHEAS to Rs-FC in the late luteal phase may provide insight to patterns of functional connectivity that may lead to the onset or worsening of clinical symptoms in women with PMDD.

Neuroimaging research on bipolar disorders often neglects to control for the influence of sex, menstrual cycle phase or menstrual cycle related disorders. Each of these factors may independently influence neuroimaging outcomes, as downstream effects of sex hormones can affect specific pathways implicated in the pathophysiology of BD. We sought to critically assess the literature to establish consensus on regions associated with the trait-based pathology of BD by

systematically reviewing literature on Rs-FC in bipolar euthymia. Through this study, we identified consistent patterns of Rs-FC using both ICA and SBA. There were no differences in either the default mode network (DMN), salience network (SN) or fronto-parietal network (FPN) between controls and BD using ICA. The exception being hypoactivation of the DMN in two studies, which consisted of a sample entirely positive for psychosis history [Brady et al., 2017; Khadka et al., 2013]. This may suggest that aberrant Rs-FC of the DMN in BD euthymia compared to controls may reflect a psychosis phenotype. Results from studies using SBA were largely heterogeneous and may reflect differences in sample population characteristics and variety of seed point used. This systematic review also highlighted a significant paucity in the literature on women with BD. Further, literature largely failed to control for sex, menstrual cycle phase or menstrual cycle disorders.

To address the paucity in the literature on women with BD, and assess the effect of menstrual cycle phase on Rs-FC in BD we conducted the first fMRI study to control for menstrual cycle phase in BD. Given the heterogeneity of results using ICA and SBA in literature on BD, we used both methods to investigate functional connectivity of the DMN, FPN and mesoparalimbic network (MPN) in the mid-follicular phase of women with BD with respect to controls with no previous psychiatric history. We did not find differences in the Rs-FC of either of these networks using ICA, however found increased coupling between nodes of the DMN and FPN using SBA. Increased coupling was seen between the posterior cingulate cortex and the angular gyrus, and between the right-dorsolateral prefrontal cortex and the brainstem. When taken in conjunction with the systematic review, these findings suggest that stability of key resting state networks using ICA may reflect or contribute to a state of euthymia in BD. Coupling of seed regions of the DMN and FPN may reflect the neural component of subthreshold and affective symptoms experienced during remitted illness phases, and/or the trait-based pathology of BD.

Deficits in emotional regulation and processing are critical components of both BD and PMDD [Strakowski et al., 2012; Vigod et al., 2010]; therefore prior to examining the impact of PMDD on BD, we examined functioning of regions key to emotional regulation during the mid-follicular phase. We investigated both the Rs-FC and volume of the somatosensory cortex, a region that has been increasingly implicated in emotional regulation in a well-defined sample of women with BD in remission compared to controls in the same menstrual phase [Nummenmaa et al., 2008]. We found increased functional connectivity between the somatosensory cortex and the following regions: insular cortex, inferior prefrontal gyrus and frontal orbital cortex in BD compared to controls. Further, voxel based morphometry analysis showed decreased gray matter in the somatosensory cortex in the same population compared to controls. This study further emphasized the impact of BD on regions influencing emotional regulation and cognitive processes and highlighted the pathophysiology of BD.

In our major study (primary aim), we investigated the clinical and neurobiological profile of comorbid PMDD on BD across a sample of four well-defined groups of women: CTRL, PMDD, BD, BDPMDD. Clinically, women with BD and comorbid PMDD displayed a worse course of illness and greater subthreshold symptoms irrespective of menstrual phase. Both structural and functional MRI analyses support this, as we found women with BD and co-morbid PMDD displayed different patterns of Rs-FC than women with BD without co-morbid PMDD and women with PMDD. Differences in cortical thickness were also found in regions central to emotional regulation and cognitive processing, suggesting that these regions may subserve symptom development in both BD and PMDD. In individuals with BD and comorbid PMDD we found increased connectivity between the left hippocampus and a large cluster centred around the right-frontal cortex (BA 8) and reduced connectivity between the right hippocampus and a cluster in the right-premotor cortex (BA 6). We also found decreased thickness in individuals with BD

and comorbid PMDD compared to without comorbid PMDD throughout the brain in the temporal, frontal, parietal and occipital cortices, the exception was the left-superior temporal gyrus, in which individuals with BD and comorbid PMDD had greater thickness and increased left caudate volume than those without comorbid PMDD. This foundational study on individuals with BD and comorbid PMDD highlights the impact of PMDD on the clinical and neurobiological profile of women with BD. Structural and functional imaging results also highlight the profound overlap between regions of the brain involved in the pathophysiology of BD and those influenced by sex hormones; either through direct binding of sex hormones or downstream effects of neurotransmitters [Bixo et al., 1995; Lokuge et al., 2010; Maletic, 2014; Osterlund et al., 2000; Osterlund et al., 1998; Phillips and Swartz, 2014; Strakowski et al., 2012; Weiser et al., 2008; Wessa and Linke, 2009].

8.2 Clinical and Neuroimaging Significance and Implications

The implications of the foundational work in this thesis span across various aspects of women's mental health, as well as clinical and neuroimaging disciplines. In general, this body of work highlights the influence of hormonal fluctuations across the menstrual cycle in women with and without PMDD and also makes a unique contribution to the existing literature the trait based pathology of bipolar disorders.

The significance and specific implications of this work have been discussed in depth in each specific chapter. To summarize, our study on the influence of hormonal fluctuations on Rs-FC across the menstrual cycle in healthy women with no history of PMDD was the first to correlate endogenous levels of E2, P4, ALLO and DHEAS to patterns of functional coupling [Syan et al., 2017]. This study provided unique insights into the activity of the brain and potential brain regions and functional relationships that may be susceptible to hormonal influence and modulation across the menstrual cycle. Since this study was conducted in a sample with no

lifetime psychiatric history, it may serve as a baseline for future studies investigating hormonal influence in psychiatric populations.

Our second study surveyed and critically appraised the literature on Rs-FC during bipolar euthymia. Given the heterogeneity in methods used to complete Rs-FC analysis a review was necessary and lacking in the existing literature on BD. This study provided a concise overview of networks and seed-points, which may be dysregulated in BD, compared to controls and highlighted the impact of psychosis on the functional connectivity of the DMN [Brady et al., 2017; Khadka et al., 2013]. This review addressed important issues beyond the trait-based pathology of BD such as, the effect of medication load on functional connectivity, need to report and medication load and psychosis history in patients with BD and paucity in the literature on women with BD and control for menstrual phase disorders.

Rs-FC was explored using both SBA and ICA in women with BD and age matched controls during their mid-follicular menstrual phase (Chapter 4). This was the first fMRI study to control for menstrual phase in bipolar disorders research, and as a result made a unique contribution to contributing to the trait-based pathology of BD in women. Chapter 5 extended on this by specifically examining the somatosensory cortex (postcentral gyrus) a region increasingly implicated in emotional regulation and processing, and risk taking behaviour [Nummenmaa et al., 2008; Uchida et al., 2015], using multimodal structural and functional imaging analyses during the mid-follicular phase. This study highlighted aberrant functional and structural architecture in the somatosensory cortex in women with BD during euthymia. May help elucidate patterns that underlie clinical deficits in emotional regulation and lability in mood seen throughout periods of euthymia and serve to guide future research using neurostimulation in the treatment of BD [Olley et al., 2005; Townsend and Altshuler, 2012; Vargas et al., 2013].

In chapter 6 we conducted the first study to examine the brain structure and function in women with BD and comorbid PMDD. Being a foundational study, it has broad implications to influence research on menstrual cycle phase disorders in BD, hormonal differences and trait-based pathology. This study illustrated the clinical and neurobiological impact of comorbid PMDD on BD; thereby encouraging future research into this population and suggests that course specifiers may be warranted to acknowledge this increased illness burden. We have no doubt that this study will serve as a baseline for and encourage, future research in this population of women.

8.3 Future Directions

The seventh chapter of this work was dedicated to providing a brief overview of studies currently in progress and work that will be included in future manuscripts. It builds upon the foundation of work created by this thesis and extends the knowledge of hormones to the menopausal transition and the influence of specific hormones on Rs-FC. Dysregulation in emotional regulation and processing is a common feature of both BD [Phillips and Swartz, 2014; Strakowski et al., 2012; Townsend and Altshuler, 2012; Wessa et al., 2014] and PMDD [Baller et al., 2013; di Scalea MD PhD and MD, 2017; Gingnell et al., 2013; Vigod et al., 2010] and thus may be further affected in women with comorbid BD and PMDD [Teatero et al., 2013]. As a result, we aim to investigate functional connectivity in women with BD and comorbid PMDD during conflict monitoring and resolution of an emotional Stroop paradigm [Etkin et al., 2006]. Preliminary results suggest there is greater connectivity in the dlPFC during the emotional Stroop paradigm than in the mid-follicular phase vs late luteal phase in BDPMDD; this may reflect increased recruitment of cognitive regions to address dissonance created by the paradigm in the late luteal phase. Second, the considerable overlap between regions involved in the pathophysiology of BD [Maletic, 2014; Strakowski et al., 2012], PMDD[Vigod et al., 2010] and sex hormone binding [Bixo et al., 1995; Osterlund et al., 2000; Osterlund et al., 1998; Weiser et

al., 2008] highlighted the need for research on the influence of hormones on Rs-FC in women with comorbid BD and PMDD. We also aim to investigate the influence of E2, P4, ALLO and DHEAS on Rs-FC, similar to the method completed in Chapter 2, in women with BD and comorbid PMDD. We hope this study will increase our knowledge about regions influenced by hormonal fluctuations in women with both conditions. Finally, periods of intense hormonal fluctuation may act to precipitate affective episodes in women with BD [Frey and Dias, 2013]. Specifically; changes in E2 may lead to an increase in pro-inflammatory cytokines, free radical creation and alterations in serotonergic systems [Au et al., 2016; Brinton et al., 2015; Lokuge et al., 2010; Mor et al., 1999; PhD and PhD, 2013; Soares, 2010; Vegeto et al., 2008]. The literature on the menopausal transition in women with BD suggests that a systematic review of the evidence is necessary. Our preliminary analysis has identified that women with BD endorse greater depressive rather than manic symptoms during the perimenopausal period.

8.4 Limitations

This study has several limitations, which should be acknowledged. Study specific limitations have been addressed in the discussion of every chapter. Broader limitations that transcend specific studies are further discussed below.

First, the DRSP is a self-administered tool used to chart symptoms of premenstrual syndrome across the menstrual cycle [Endicott et al., 2005]. This tool was used to capture a sense of premenstrual syndrome or PMDD and aid in menstrual cycle date tracking in all of our studies. It is possible that women may provide an inaccurate account of their premenstrual symptoms or that use of the DRSP may be confounded by stressful life events. In both groups with a diagnosis of BD (BD and BDPMDD groups), symptoms reported on the DRSP may also be confounded by exacerbation of their bipolar illness. We increased our confidence in the reports of the DRSP by administering additional premenstrual symptom questionnaires such as the Premenstrual

Syndrome Screening Tool and the SCID-PMDD [Steiner et al., 2003]. The use of mood, sleep and biological rhythms questionnaires in each study using the DRSP also increased our confidence in self-reported results [Giglio et al., 2009; Montgomery and Asberg, 1979].

Second, each study in this thesis, in some way, uses Rs-FC to capture differences between populations. Rs-fMRI provides an indirect measure of spontaneous neuronal activity in the ultralow frequency range (0.01-0.10 Hz) [Allen et al., 2011]. The fMRI techniques used in thisthesis, seed to voxel, ROI-ROI and ICA are based on an oversimplification that BOLD activation measured is static through the duration of the scanning paradigm [Cole et al., 2010; MR and M, 2014]. Further, results may be confounded by the participant's ability to remain awake for the duration of the scan, and inability to control the participant's memory in the scanner. Although, in our studies we advised participants not to think about anything in particular, and remain awake with their eyes fixed at the fixation point throughout the entire resting state brain scan, no objective measures such as simultaneous electroencephalogram or eye tracking, were in place to confirm that participants followed our instructions. The later was also a common limitation of Rs-fMRI studies we encountered through our systematic review.

Third, the effects of psychotropic medications on Rs-FC are a common limitation of fMRI research in BD [Phillips and Swartz, 2014; Phillips et al., 2008]. Several studies investigating Rs-FC in BD, including our work in this thesis, have ruled out the influence of medication following correlation analysis with BOLD activation [Anticevic et al., 2014; Anticevic et al., 2013; Brady et al., 2016; Brady et al., 2017; Lv et al., 2016; Oertel-Knöchel et al., 2015; Reinke et al., 2013; Torrisi et al., 2013]. The goal of many chapters of this thesis was to investigate brain structure and function of BD and ultimately BD and comorbid PMDD. We believe this goal could have only been achieved with a primarily medicated sample, by ensuring that all participants with BD were euthymic throughout the study. Another limitation of our

bipolar sample was that it comprised both women with BD type-I and BD type-II. Finally, it is important to note this thesis includes studies that are cross-sectional in design and as a result this study only reflects a current picture of disease state and not disease progression. We encourage researchers to expand upon the work laid out in this thesis and investigate disease burden and progression in women both in bipolar women with and without comorbid PMDD using longitudinal study designs.

8.5. Conclusions

In conclusion, the work contained in this thesis highlights the influence of menstrual cycle related sex hormone fluctuations on women with and without a susceptibility to develop PMDD (healthy women and those with BD). Importantly, our work supports the need to control for menstrual phase and PMDD diagnosis. Finally, this thesis contains the first neuroimaging study to investigate the neural correlates of BD and co-morbid PMDD. Differences in structural and functional connectivity, and the clinical profile of women with BD and those with comorbid BD and PMDD underlies the need for further investigation into the neural correlates of women in this sub-population and emphasizes the potential role of PMDD as a potential course specifier of BD.

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