Thesis

Associations of Brain Structural Characteristics with Clinical Parameters in Bipolar Disorder with Special Emphasis on Sex and Obesity

Submitted by

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Disclosure

Major parts of this dissertation have been published (Birner et al., 2015):

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Abbreviations

3-HK...3-Hydroxykynurenine

ACC...Anterior Cingulate Cortex

ALSN...The Anterior Limbic Striatal Networks

ANCOVA... Analysis of Covariance

BBB...Blood Brain Barrier

BD...Bipolar Disorder

BD I...Bipolar Disorder I

BD II...Bipolar Disorder II

BDNF...Brain Derived Neurotrophic Factor

BIPFAT...Bipolar Disorder and Fat Metabolism study

CCL...Chemokine Ligand

CXCL... Chemokine (C-X-C motif) Ligand

CSF...Cerebrospinal Fluid

DSM-IV… Diagnostic and Statistical Manual of Mental Disorders version IV by the American Psychiatric Association (APA)

DTI...Diffusion Tensor Imaging

FLAIR...Fluid Attenuated Inversion Recovery

fMRI...functional Magnetic Resonance Imaging

HC...Healthy Controls

HPA-axis...Hypothalamic–Pituitary–Adrenal axis

hsCRP...High Sensitive C-Reactive Protein

IDO-1...Indoleamine Dioxygenase-I

IFN...Interferon

IL-x…Interleukin-x (e.g. 1,6,10…)

IQR...Inter Quartile Range

KYN...Kynurenine

KYNA...Kynurenine Acid

Mdn...Median

MRI...Magnetic Resonance Imaging

OFC...Orbitofrontal Cortex

PFC...Prefrontal Cortex

SCID-I...Structured Clinical Interview of axis-I disorders according to DSM-IV

TIV…Total Intracranial Volume

TNF…Tumor Necrosis Factor

WMH...White Matter Hyperintensities

Zusammenfassung

Hintergrund/Ziele

Obwohl durchaus Evidenz vorhanden ist, dass Geschlecht und Adipositas die menschliche Gehirnstruktur beeinflussen können, wird dies in den meisten Bildgebungsstudien des Gehirns bei bipolarer affektiver Störung (BD) nicht in ausgewiesener Form berücksichtigt. Dies lieferte den Ansporn die folgenden Hypothesen in einer Kohorte von 124 Erwachsenen mit BD und 86 Kontrollpersonen (HC) zu testen: Erstens (I), in Individuen mit BD ist im Vergleich zu HC das Volumen der Hyperintensitäten der weißen Substanz (WMH) erhöht (Ia) und das Volumen der grauen Substanz von prädefinierten anterioren-limbisch-striatalen Netzwerken (ALSN), welche laut Experten-Konsensus als neuroanatomische Basis für BD angesehen werden können, reduziert (Ib), wobei auch Geschlechtsunterschiede vorliegen können. Zweitens (II), diese potentiellen hirnstrukturellen Abweichungen von der Norm der weißen (IIa) und grauen (IIb) Substanz werden durch Lebensstilfaktoren wie Übergewicht oder Adipositas mitbedingt. Und drittens (III), das Volumen der WMH (IIIa) und/oder das Volumen des ALSN (IIIb) sind mit klinischen Parametern und/oder der medikamentösen Therapie assoziiert.

Methoden

In der BIPFAT („Bipolar Disorder and Fat Metabolism")-Querschnittsstudie wurden bei den ProbandInnen unter anderem ein 3 Tesla-MRT des Schädels und ein diagnostisches Interview und eine ausführliche Anamneserhebung durchgeführt. Die WMH wurden vom Dissertanten semi-manuell mit der Hilfe eines eigens entwickelten Computerprogramms eingezeichnet und die Volumina der WMH berechnet. Das graue Volumen der ALSN wurde mit Hilfe des Programms Freesurfer, basierend auf kortikaler Rekonstruktion und volumetrischer Segmentierung, berechnet. Alle für die Statistik verwendeten Volumsvariablen wurden anhand des totalen intrakraniellen Volumens jedes Probanden/jeder Probandin normalisiert. Es wurden Hypothesen-abhängig Varianz- und/oder Korrelationsanalysen, kontrolliert für bekannte Störfaktoren, durchgeführt.

Ergebnisse

Betreffend WMH: (Ia) Individuen mit BD hatten signifikant mehr (F=3.968, p<0.05) WMH (Mdn=3710mm3; IQR=2961mm3) als HC (Mdn=2185mm3; IQR=1665mm3). BD Männer (Mdn=4095mm3; IQR=3295mm3) und BD Frauen (Mdn=3032mm3; IQR=2816mm3) unterschieden sich nicht signifikant im Volumen an WMH. (IIa) Es konnte keine Assoziation von WMH mit Lebensstilfaktoren festgestellt werden. (IIIa) Nur bei den BD Männern

korrelierte die Anzahl an manischen/hypomanen Phasen(r=0.75; p<0.001) als auch an depressiven Phasen(r=0.51; p<0.001) positiv mit dem WMH-Gesamtvolumen. Betreffend ALSN-Volumen: (Ib) Die Varianzanalyse (ANCOVA) ergab, dass Individuen mit BD verglichen mit Kontrollen ein signifikant reduziertes Volumen der ALSN aufwiesen (F=5.935, p=.016, Eta=.028) und Männer ein signifikant geringeres Volumen als Frauen zeigten (F=4.594, p=.033, Eta=.022). (IIb) Nach dem Hinzufügen der Body Mass Indices und des Zigarettenrauchens in die gleiche ANCOVA konnte dieser signifikante Gruppen- und Geschlechtsunterschied nicht mehr detektiert werden, wobei der Body Mass Index (BMI) die signifikante Störgröße darstellte (F=5.666, p=.018, Eta=.010). (IIIa) Individuen mit BD, die atypische Antipsychotika einnahmen, wiesen ein signifikant reduziertes Volumen der ALSN auf, verglichen mit Individuen mit BD, die keine derartige Medikation einnahmen (F=12.034, p=.001, Eta=.105).

Schlussfolgerungen

Bei Männern mit BD war das Gesamtvolumen an WMH stark mit der Anzahl manischer Phasen assoziiert, was für eine erhöhte Anfälligkeit der Männer im Bezug auf manische Symptome im Kontext von Veränderungen der weißen Substanz spricht. Unsere Resultate der reduzierten ALSN Volumina unterstreichen die Notwendigkeit, Parameter wie Adipositas und Geschlecht bei der Analyse bildgebender Verfahren des Gehirns im Kontext der BD und vermutlich auch anderer neuropsychiatrischer Erkrankungsbilder miteinzubeziehen. Da Individuen mit BD, die zum Testzeitpunkt mit atypischen Antipsychotika therapiert wurden, eine signifikante Reduktion des ALSN Volumens zeigten, entsteht aufgrund unserer Resultate ein zusätzlicher Anreiz, dieser Beobachtung durch longitudinale Studien weiter auf den Grund zu gehen.

Summary

Background/Aims

The majority of neuroimaging studies in bipolar affective disorder (BD) to date largely ignore the influence of sex and health issues, such as obesity, on the brain morphology of affected individuals. Hence, the current thesis tests the following hypotheses in 124 adults with BD and 86 healthy controls (HC):

(I) BD individuals exhibit an increased load of white matter hyperintensities (WMH) (Ia) as well as gray matter volume reduction in brain areas affected in BD (Ib), such as the anterior limbic striatal networks (ALSN), when compared to HC and between sexes;

(II), these morphological changes of white matter (IIa) and gray matter (IIb) will be related to life style related factors such as body weight (i.e. being overweight or obese);

(III) WMH-load (IIIa) and gray matter volume of the ALSN (IIIb) will correlate with clinical parameters (i.e. the number of disease episodes) and type of medication.

Methods

By applying a cross-sectional study design, 124 adult BD and 86 adult HC underwent 3T-MRI scans of the brain and clinical assessment. WMH were delineated using in-house developed software. Anatomical segmentation was performed by combined region growing and local thresholding following manual selection by a single instructed rater. Gray matter volume of the ALSN was calculated following cortical reconstruction and volumetric segmentation performed in Freesurfer version 5.3. All brain volumes were normalized to the individual total intracranial volume. ANCOVAs or correlation analyses were performed for hypothesis testing controlling for confounding factors like age.

Results

(Ia): Individuals with BD had a significantly higher value ($F=3.968$, $p<0.05$) of WMH (Mdn=3710mm3; IQR=2961mm3) than HC (Mdn=2185mm3; IQR=1665mm3); BD men (Mdn=4095mm3; IQR=3295mm3) and BD women (Mdn=3032mm3; IQR=2816mm3) did not significantly differ in their WMH-load;

(IIa) There was no association of WMH with lifestyle related factors was found;

(IIIa) In men only, the number of manic or hypomanic episodes ($r=0.75$; $p<0.001$) as well as depressive episodes ($r=0.51$; $p<0.001$) correlated positively with WMH-load;

(Ib) BD individuals had significantly reduced normalized gray volume of the ALSN compared to HC ($F=5.935$, $p=.016$, Eta=.028); men also had reduced normalized gray volume of the ALSN compared to women ($F=4.594$, $p=.033$, Eta=.022);

(IIb) After including Body Mass Index (BMI) and smoking into the same ANCOVA, the significant group differences disappeared, while BMI was found to be a significant confounding factor (F=5.666, p=.018, Eta=.010). (IIIa) BD individuals taking atypical antipsychotics had significantly reduced normalized gray volume of the ALSN compared to BD not taking atypical antipsychotics (F=12.034, p=.001, Eta=.105).

Conclusions: WMH-load was significantly associated with the number of manic episodes in male BD patients, suggesting that men are more vulnerable to develop manic symptoms in the presence of cerebral white matter changes. The finding of reduced ALSN volumes indicates the importance of including proxies of obesity and/or the metabolic syndrome (e.g. BMI, body fat content, or waist circumference) as well sex as moderating variables when analyzing morphological brain imaging data in BD. Moreover, BD individuals treated with atypical antipsychotic medication showed significantly reduced ALSN volumes compared to individuals with BD not being treated with atypical antipsychotics at the time of the study.

1. Introduction

1.1. Bipolar Disorder

Bipolar disorder (BD) is an affective disorder with a high burden of disease. BD refers to an episodic, recurrent pathological disturbance in mood, ranging from severe depression to massive elation (mania). The two main variants of BD are bipolar affective disorder I (BD I), which is defined by the occurrence of depressive and manic episodes during the course, as well as bipolar disorder II (BD II), which includes hypomanic and depressive episodes but never involves manic epsiodes with severe disturbances of daily living that are characteristic for BD I. Suicide (during a depressive episode) and potentially making life-devastating decisions (during a manic episode) belong to the greatest risks of those opposing (bipolar) mental states of the disease. In addition to its affective and sometimes psychotic symptomatology, BD can also lead to cognitive disturbances (Cardoso et al., 2015, Robinson, Ferrier, 2006) as well as somatic comorbidities (Goldstein et al., 2009, Diaz et al., 2009, McIntyre et al., 2010).

Besides its implications for the mental and physical health of the individual, it also affects psychosocial, occupational and financial functioning (Muller-Oerlinghausen, Berghofer & Bauer, 2002). The prevalence of BD is estimated at 2-5% in the general pulation with an average age of onset between 25 and 30 years (Dilling H, 2010).

The standard treatment usually involves longterm pharmacotherapy with mood stabilizing agents (lithium and/or antiepileptics and/or antipsychotics) combined with targeted psychotherapeutic interventions (e.g. psychoeducation) as well as the use of antidepressants as indicated (Kasper et al., 2013).

Diagnosis is limited to mental state examination and corroborative information (i.e. from relatives and partners). There are no known biomarkers associated with BD to guide diagnosis, intervention, and prognosis. Correctly diagnosing BD can take up to 10 years (Lish et al., 1994) as it often presents predominantly as unipolar major depressive disorder or other related conditions (Kasper et al., 2013). Particularly hypomanic episodes may pass unnoticed due to their low levels of severity.

While there are known pathophysiological findings associated with BD (i.e. changes in neurotransmitter balance, neuroinflammation, altered endocrine functioning, metabolic changes, etc.) the current thesis will focus on macro-structural brain changes associated with BD. The study was performed at the Department of Psychiatry and Psychotherapeutic Medicine in close cooperation with the Department of Neurology of the Medical University

of Graz (MUG). The thesis aims to elucidate the potential clinical implications of the respective findings taking into account gender and obesity and discuss these factors within the context of brain function and pathophysiology.

1.2. Brain Structure in Bipolar Disorder

Brain morphology can best be analysed in vivo with magnetic resonance imaging (MRI). Reasons for this include excellent spatial resolution, high sensitivity in detection of white matter changes, good delineation of gray and white matter, no radiation exposure, and the possibility to investigate brain structure and brain function simultaneously as in functional MRI (fMRI) (Reiser, Kuhn & Debus, 2006).

From a macro-structural perspective, gray and white matter volumes and cortical thickness are among the most commonly used measures in structural brain imaging studies. Normal ageing but also neurodegenerative conditions result in a progressive reduction of those measures (Sluimer et al., 2008). In BD, reported structural brain changes tend to be more heterogeneous across studies and are also somewhat subtle in nature (Ellison-Wright, Bullmore, 2010). This observation is supported by meta-analyses of published brain imaging studies showing rather low effect sizes for morphological differences found in BD individuals compared to healthy controls (HC) (Hibar et al., 2016).
The volumetric evaluation of white matter is generally problematic due to the confounding occurrence of white matter hyperintensities (WMH). This issue will be specifically addressed in chapter 1.2.4. For micro-structural analyses, diffusion tensor imaging (DTI) is the most commonly used approach to investigate the orientation and integrity of white matter trajectories. Table 1 summarises the findings of the most important studies published in the past ten years (Ellison-Wright, Bullmore, 2010, Hibar et al., 2016, Hibar et al., 2017, Arnone et al., 2009, Bora et al., 2010, Grunze, Meisenzahl & Grunze, 2013, Beyer et al., 2009, Kempton et al., 2008, Singh et al., 2008, Nortja et al., 2013, Pfeifer, Welge & Strakowski, 2008, Sexton, Mackay & Ebmeier, 2009, Ladouceur, Almeida & Birmaher, 2008, Hallahana et al., 2011, Foland-Ross et al., 2011, Rimol et al., 2010, Rajkowska, Halaris & Selemon, 2001, Lim et al., 2013, Pezzoli et al., 2018, Konopaske et al., 2014).

Table 1: Brain Structural Changes in Bipolar Disorder

Structural Changes in Bipolar Disorder (Review of current literature)	
Cerebral gray matter and ventricles	1. BD individuals (n=1710) showed decreased volume of the hippocampus, of the thalamus and by trend of the amygdala as well as enlarged lateral ventricles compared to HC (n=2594). Differences were only significant between BD I and HC, but not between BD I and BD II and not between BD II and HC. Larger thalamic volumes in BD individuals taking lithium (n=545). This is the best powered meta-analysis of subcortical structures to date (Hibar et al., 2016). 2. BD Individuals (n=1837) showed cortical thinning in the left pars opercularis, left rostral middle frontal gyrus, left fusiform gyrus, and average cortical thickness compared to HC (n=2538). Thinning was associated with illness duration. Thinning was more pronounced in patients taking antiepiletics. This is the best powered meta-analysis of cortical structures to date (Hibar et al., 2017). 3. BD Individuals (n=980) showed reduced gray volume in clusters localized bilateral insula, superior temporal gyrus and medial prefrontal cortex including the anterior cingulate cortex (ACC) compared to HC (n=1427) (Voxel based morphometry meta-analysis, 32 studies) (Wise et al., 2017). 4. Whole brain and prefrontal lobe volume reductions, increases in the volume of the globus pallidus and

lateral ventricles. Heterogeneity in amygdala volume across studies. Predominantly, age and illness duration determinated the magnitude of volume reductions (systematic review and meta-analysis, 69 studies) (Arnone et al., 2009).

5. Lateral ventricle enlargement, total gray matter volume increased among patients when the proportion of patients using lithium increased as well (meta-analysis, 96 studies) (Kempton et al., 2008).

6. Decreased volume of insular cortex and ACC in BD compared to HC (meta-analysis, 14 studies, n=366) (Ellison-Wright, Bullmore, 2010).

7. Gray matter reductions in BD occur in anterior limbic regions, strongest association of BD with ACC and fronto-insular cortex. Lithium treatment was correlated with ACC enlargement. Duration of illness was associated with increased gray matter in a cluster that includes basal ganglia, subgenual ACC, and amygdala (meta-analysis, 21 studies) (Bora et al., 2010).

8. Decreased amygdala volume in bipolar children and adolescents (Pfeifer, Welge & Strakowski, 2008).

9. Increased right lateral ventricular, left temporal lobe, and right putamen volume. Bipolar patients taking lithium display significantly increased hippocampal and amygdala volume compared with patients not treated with lithium and healthy comparison subjects. Cerebral volume reduction is

significantly associated with illness duration in BD (mega-Analysis, n=442) (Hallahana et al., 2011).

10. BD individuals (n=266) had significantly larger lateral and third ventricles, smaller whole brain, nucleus caudate and pallidum volumes as well as thinner cortex in small clusters in frontal, parietal and cingulate regions compared with HC (n=171). Lithium-free patients (n=91) showed significantly smaller total brain, thalamus, putamen, pallidum, hippocampus and nucleus accumbens volumes compared to patients treated with lithium (n=175) (Abramovic et al., 2016).

11. Euthymic BD patients off lithium (n=34) had significantly thinner gray matter in the left and right prefrontal cortex and the left anterior cingulate cortex. Thinning in these regions was more pronounced in patients with a history of psychosis (Foland-Ross et al., 2011).

12. BD I patients (n=139) showed cortical thinning, primarily in the frontal lobes and superior temporal and temporoparietal regions and subcortical volume reductions bilaterally in the hippocampus, the left thalamus, the right nucleus accumbens, the left cerebellar cortex, and the brainstem, along with substantial ventricular enlargements (Rimol et al., 2010).

13. Longitudinal structural neuro-imaging studies generally reveal losses of gray-matter volume in prefrontal, anterior cingulate, and subgenual cortex, with less consistent findings in the temporal lobe

	and subcortical regions (Systematic review including 22 longitudinal MRI studies in BD) (Lim et al., 2013). 14. Increased parahippocampal and prefrontal volume in children of individuals with BD (Singh et al., 2008, Ladouceur, Almeida & Birmaher, 2008).
Cerebral white matter	1. Increased amount of WMH (White matter hyperintensities) in two meta-analyses (Beyer et al., 2009, Kempton et al., 2008) 2. Decreased white matter volume in the posterior corpus callosum extending to white matter in the posterior cingulate cortex, no correlation with clinical parameters. (Meta analysis, 24 studies) (Pezzoli et al., 2018) 3. Heterogeneity in Diffusion Tensor Imaging findings; tendency to reduced regional fractal anisotropy (Nortja et al., 2013, Sexton, Mackay & Ebmeier, 2009)
Histological findings	1. Reduced neuronal and glia density in the prefrontal cortex and anterior gyrus cingula. Reduced number of dendrites in the hippocampus. (Rajkowska, Halaris & Selemon, 2001) 2. Reduced spine density, number of spines per dendrite and dendrite length in the dorsolateral prefrontal cortex (DLPFC) in BD (n=9), overlapping with a schizophrenia sample (n=14), compared to controls (n=19) (Konopaske et al., 2014)

Volume reduction and cortical thinning in BD appear to affect several brain regions (Arnone et al., 2009, Abramovic et al., 2016). Prefrontal areas, the cingulate cortex, insular cortex, thalamus, nucleus accumbens, fusiform gyrus and the hippocampus seem to be predominantely affected (Ellison-Wright, Bullmore, 2010, Hibar et al., 2016, Hibar et al., 2017, Hallahana et al., 2011, Wise et al., 2017). The lateral ventricles are consistently enlarged (Arnone et al., 2009, Kempton et al., 2008, Abramovic et al., 2016). Prefrontal cortex and cingulate cortex pathology is consistent with histological findings of reduced neural and glial density reported in in two post mortem studies (Rajkowska, Halaris & Selemon, 2001, Konopaske et al., 2014). The morphological changes become more pronounced with illness duration (Arnone et al., 2009, Hallahana et al., 2011), while lithium seems to exert neuroprotection to some degree, whereas antiepileptic drugs may accelerate cortical thinning (Hibar et al., 2016, Hibar et al., 2017, Kempton et al., 2008, Hallahana et al., 2011, Abramovic et al., 2016). White matter hyperintensities are more frequent in BD than in healthy subjects (Beyer et al., 2009, Kempton et al., 2008). Although microstructural findings are heterogeneous, there is some evidence for disturbed white matter microstructure (Nortja et al., 2013, Sexton, Mackay & Ebmeier, 2009). Particularly the recent ENIGMA study involving 1837 patients and 2538 controls (Hibar et al., 2016, Hibar et al., 2017) provided more clarity by identifying volume reductions in fronto-temporal, limbic and striatal structures in BD, although the respective effect sizes were small.

The next sections will review potential mechanisms driving these brain structural changes in BD, whether these changes are progressive in nature or already present prior to the onset of the clinical manifestation. Moreover, whether these changes relate to disease severity and cognitive decline will also be reviewed with reference to the brain function of the identified brain regions.

1.2.1. “Neuroprogression” – Neurodegenerative processes in BD

There is some evidence for neurodegeneration in BD. It was already Emil Kraepelin who described the progressive and accelerating course of “manic depressive insanity” (the historic term for BD) about 100 years ago (Berk et al., 2011). There is a comprehensive body of evidence supporting a pattern of reduced euthymic inter-episode intervals over time (Berk et al., 2011). More recurrent courses of BD are associated with higher rates of psychiatric comorbidities, difficulties in social adjustment and forensic issues (Berk et al., 2011) as well as with somatic comorbidities (Leboyer et al., 2012b). It is also often associated with

cognitive decline, occuring predominantly during affective episodes but also remaining in states of euthymia to a lesser degree (Cardoso et al., 2015, Robinson, Ferrier, 2006, Robinson et al., 2006). This decline seems to be more pronounced with illness chronicity and duration (Berk et al., 2011), suggesting that disease-related factors are driving regional brain atrophy (see Chapter 1.2. Table 1) over time along with the observed cognitive decline. In psychiatric research, the model of "neuroprogression" includes presumed underlying processes of neurodegeneration in chronic psychiatric disorders in conjunction with the observed accelerating course of disease (Fries et al., 2012).

Neuroinflammatory pathways as well as the presence of metabolites associated with stress response have been identified as potential neurotoxic factors impacting on the neurotrophic modulations of brain circuits (Berk et al., 2011, Fries et al., 2012, Kapczinski et al., 2017). Oxidative stress and inflammation as well as neurotoxic metabolites are known to damage neural tissue. Oxidative stress and inflammation can be detected in the blood stream with many of these molecules capable of passing the blood-brain barrier (BBB) (Kapczinski et al., 2017).

Alterations in different immune-inflammatory processes are evident in BD and inflammatory cytokines may pass the BBB and interact with patho-physiological domains relevant to BD as well as interlink with medical comorbidities, such as atherosclerosis, hypertension, diabetes and obesity (Leboyer et al., 2012b). Episodes of depression and mania are accompanied by the activation of immune inflammatory pathways with - compared to euthymic states – further increased levels of pro-inflammatory cytokines (Leboyer et al., 2012b, Anderson, Maes, 2015). Elevated inflammatory markers include interleukin-6, interleukin-10, and tumor necrosis factor (TNF) in early stages as well as high sensitive C-reactive protein (hs-CRP), interleukin-1, and the immune cell are attracting chemokines CCL 24, CCL 11 and CXCL 10 in later stages (Fries et al., 2012).

Oxidative stress is associated with a variety of pathophysiological processes, as it is capable of promoting inflammatory pathways, thus resulting in neurotoxicity and reduced neuroplasticity (Di Dalmazi et al., 2016). The process damages microstructures and creates novel epitopes, thereby activating secondary autoimmune responses to neurotransmitters and their receptors (Maes et al., 2013). Moreover, oxidative stress can cause DNA point mutations (Dutta et al., 2015) and is involved in atherosclerosis and other degenerative processes (Anderson, Maes, 2015, Maes et al., 2013, Bengesser et al., 2015). Oxidative stress cannot be measured directly as the the free radicales have a very short life span as the body quickly

detoxifies the harmful effects. However, there are quantifiable markers that can measure approximations of oxidative stress. Increased oxidative stress markers in BD include protein carbonyl content and 3-Nitrotyrosin in early stages of the disease as well as nitric oxide, thiobarbituric acid-reactive substances, glutathione reductase, glutathione S-transferase and total oxidative status (Fries et al., 2012).

As the topic revolves around the term stress, the hypothalamic–pituitary–adrenal (HPA)-axis cannot be neglected. HPA-axis hyperactivity occurs with BD, while abnormalities of glucocorticoid signaling are found in several key areas of the brain (Belvederi Murri et al., 2016). Furthermore, cortisol levels were found to be related to some structural and functional abnormalities in BD. HPA-axis dysfunctions might also increase the risk of relapse, disease progression, and cognitive decline (Belvederi Murri et al., 2016).

Another potential molecular mechanism for inflammatory-mediated neurodegeneration involves tryptophan metabolism (see Figure 1). Previous own work showed increased breakdown of tryptophan (the precursor of serotonin) towards neurotoxicity in 143 BD individuals versus 101 HC indexed by decreased levels of the neuroprotective kynurenine acid as well as increased the neurotoxic 3-hydroxikynurenine (3-HK) to kynurenine (KYN) and kynurenine acid ratios (KYNA) (Birner et al., 2017). These metabolic changes are related to poor cognitive functioning (Platzer et al., 2017). The pro-inflammatory state in BD is also linked to the kynurenine pathway (Johansson et al., 2013). The authors showed that pro-inflammatory cytokine treatment (with human recombinants of interleukin-1, interleukin-6, tumor-necrosis-factor and interferon-α (IFN-α)) promoted indoleamine dioxygenase-I (IDO-1) activation and further increase of the elevated 3-HK/KYNA ratio in cultures of skin fibroblasts. This observation suggests that pro-inflammatory states are linked to an even greater imbalance of kynurenine metabolites (Birner et al., 2017). Raisson et al. (Raison et al., 2010) showed a significant increase towards the KYN-pathway metabolism in combination with worsening depressive symptoms in 27 hepatitis C patients following IFN-α treatment (Birner et al., 2017). Our group also showed increased IDO-1 activity in euthymic BD patients compared to HC with greater increases noted in a subsample of overweight BD patients (Reininghaus et al., 2014). Further support is derived from preliminary findings in a small sample of BD subjects which suggests that reduced levels of KYNA and serotonin are associated with DTI-detected microstructural white matter integrity impairments (Poletti et al., 2018).

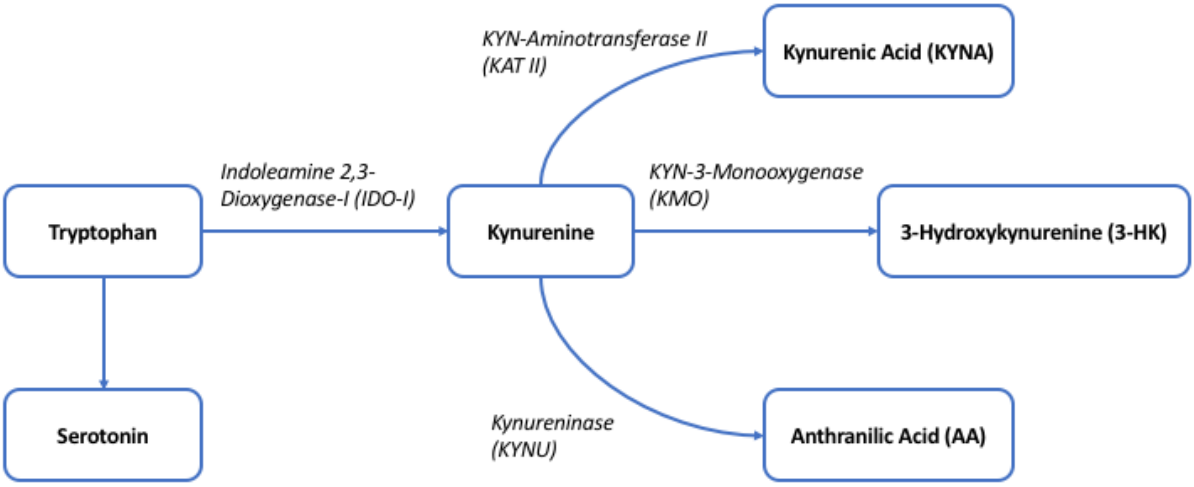

Figure 1: Part of the Tryptophan Metabolism. Showing a simplified pathway of the tryptophan metabolism towards the neuroprotective kynurenine acid and the neurotoxic 3-hydroxykynurenine. Enzymes are written in *italic.* Modified from our PLOS One publication (Birner et al., 2017).

The model of neuroprogression also involves neural plasticity and cellular resilience, presenting the ability of the brain cells to adapt to certain stressful environmental conditions. Neural plasticity and cellular resilience are suggested to be affected in BD by abnormalities in several intracellular signaling pathways including neurotransmitters, glutamatergic and glucocorticoid signaling, neurotrophic cascades, anti-apoptotic factors, cell survival pathways, and calcium signaling (Fries et al., 2012). The loss of cell plasticity might be a result of decreased neurotrophic support, as brain-derived neurotrophic factor (BDNF) levels are reduced in post-mortem brains of BD patients (Fries et al., 2012). Among other neurotrophic factors, BDNF is a prerequisite of neural functioning and cell survival as it is involved in neurogenesis and neural growth during developmental phases and continues in adulthood promoting dendritic growth and synaptic plasticity predominantly in the cortex and the hippocampus (Fries et al., 2012). Hence, brains of some individuals with BD might be on a disadvantage to structurally adapt to stressful or neurotoxic environmental conditions.

In summary, the neuroprogression model (see Figure 2) proposes that recurring affective episodes in combination with other stressors promote neurotoxic metabolism, neuroinflammation, and oxidation as well as neurotrophic modulation of brain circuits. These processes lead to neurodegeneration and structural brain abnormalities causing increased

biological vulnerability to stressors, thereby contributing to disease severity, cognitive decline, and worsening psychosocial functioning (Cardoso et al., 2015, Berk et al., 2011).

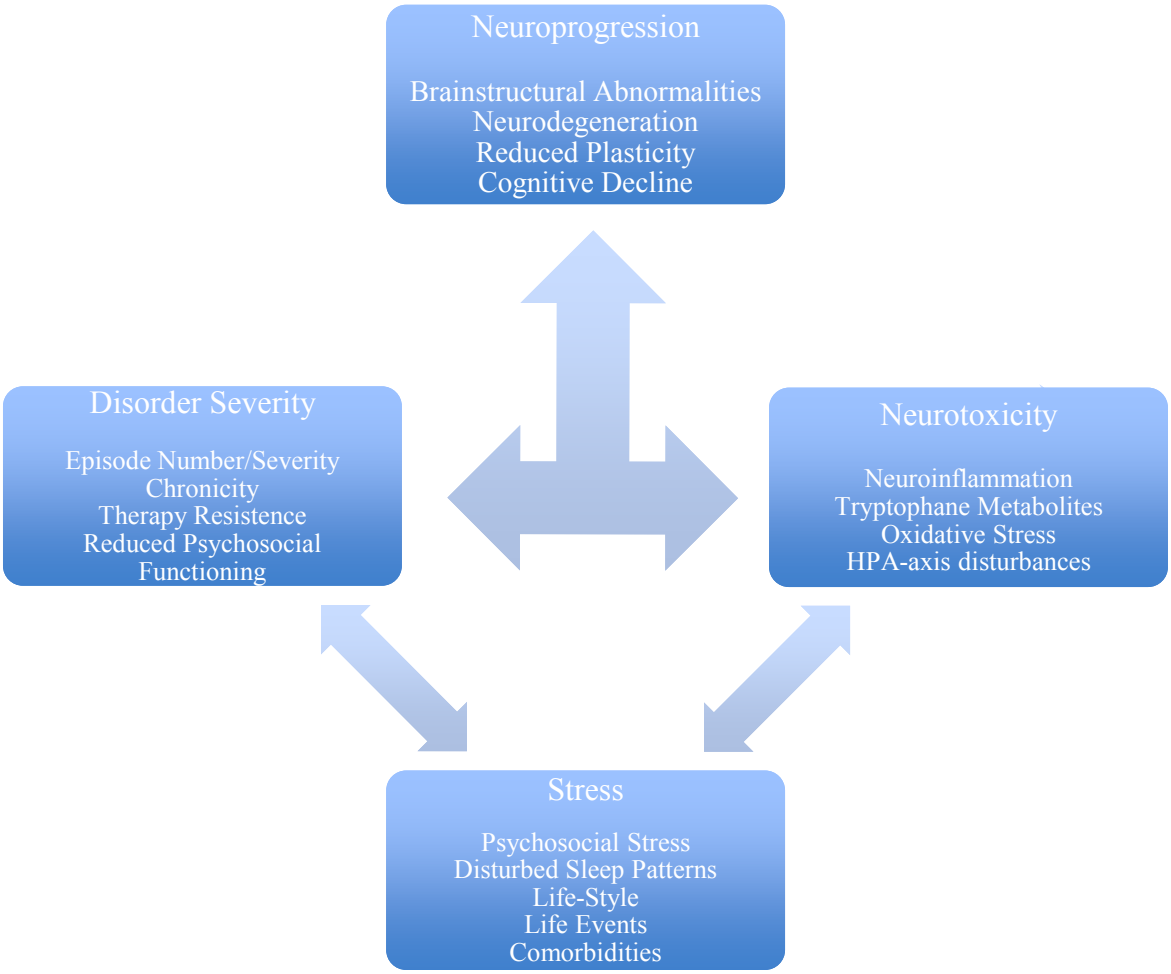

Figure 2: Model of Neuroprogression

1.2.2. The Anterior Limbic Striatal Networks – a Functional Neuroanatomical Basis for Bipolar Disorder

Many attempts have been made to localize the affective and cognitive dysfunction in BD. To get a better understanding of the involved brain structures, it seems mandatory to take a look at BD brain function. Strakowski and colleagues (Strakowski et al., 2012) proposed a model of networks of several prefrontal, limbic and striatal brain regions in a proposed consensus model of the functional neuroanatomy of BD. The networks include amygdala, thalamus, globus pallidus, ventromedial striatum, nucleus accumbens, anterior cingulate cortex (ACC), prefrontal cortex (PFC), and orbitofrontal cortex (OFC). These brain regions are known to be involved in emotional and cognitive processes. The networks are also interlinked via

connecting circuits of varying complexity (see Figure 3). As already shown in chapter 1.2 (Table 1), morphological abnormalities in most of these regions have been confirmed for BD. Strakowski and colleagues (Strakowski et al., 2012) proposed that disruption in early development (e.g. white matter connectivity, prefrontal pruning) of these networks lead to reduced connectivity and malfunctional modulation among prefrontal networks and limbic structures, thus increasing the vulnerability for mania while progressive changes in these circuits is promoting diseas progession in accordance with the model of neuroprogression.

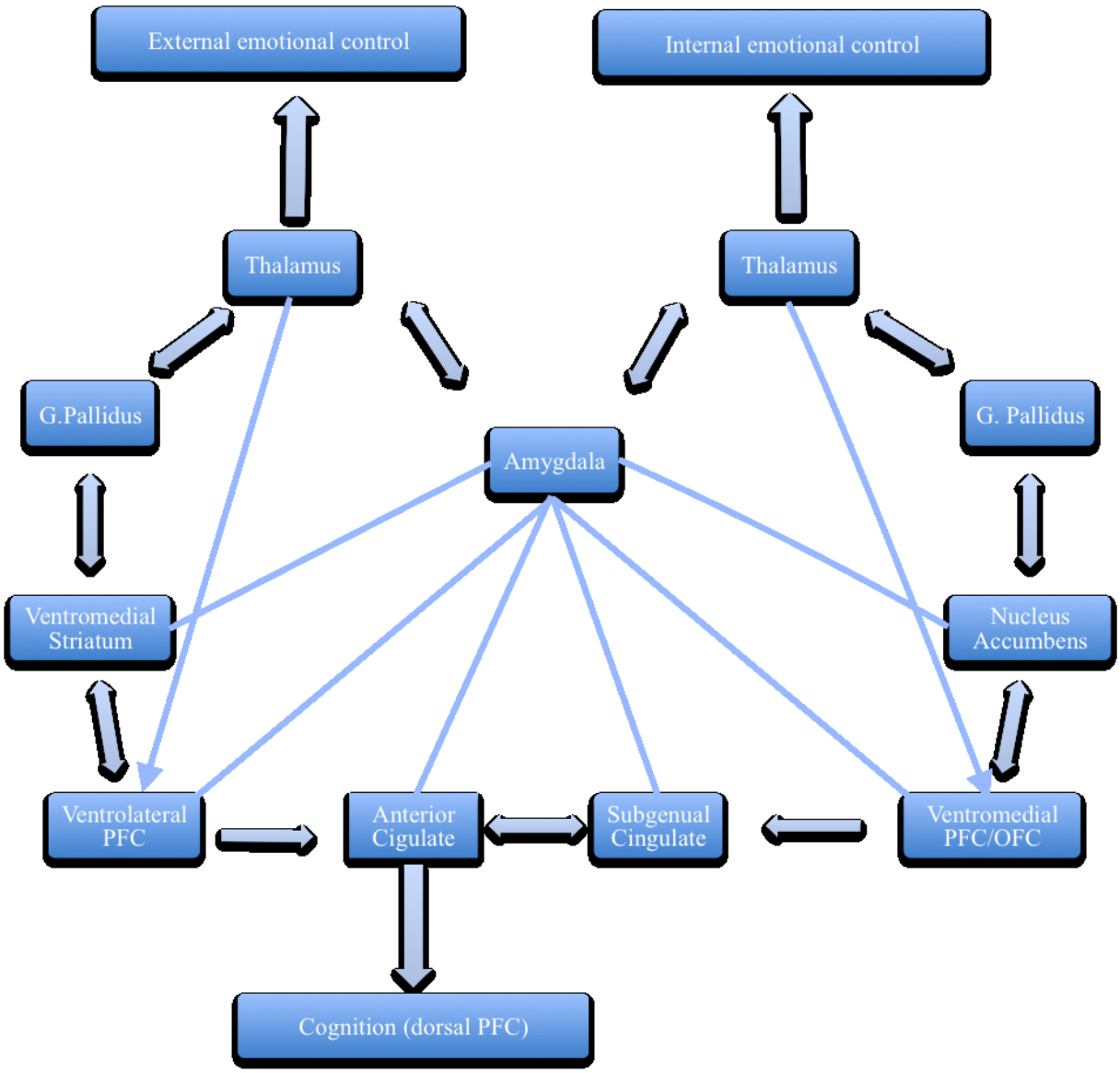

Figure 3: The anterior limbic striatal networks (ALSN): Based on the model of the functional neuroanatomy of bipolar disorder proposed by Strakowski and colleagues (Strakowski et al., 2012).

It is interesting to briefly review the way these experts came to their consensus as well as the reasoning to include the aforementioned compartments to the networks in more detail. This group, consisting of leading U.S. and U.K. experts, met in Miami, Florida in December 2010 with the goal to develop a consensus on the underlying functional neuroanatomy in BD to guide future research of neuroimaging. They were primarily focused on functional magnetic resonance (fMRI) and DTI studies on BD I subjects, as the available data regarding BD II had been very sparse to that date (which it still is). They started with the assumption that emotional systems most likely underlie BD, as the disease is diagnostically characterized by recurrent episodes of changed mood states (Strakowski et al., 2012). They based their discussions on two ventral prefrontal networks that appear to modulate emotional behavior (Blond, Fredericks & Blumberg, 2012, Yamasaki, LaBar & McCarthy, 2002, Phan et al., 2002), which are similarly organized by links of prefrontal regions to thalamic, striatal and pallidal areas by repetitious feedback loops that are converting information and modulating activation in limbic areas, especially the amygdala (Yamasaki, LaBar & McCarthy, 2002, Phan et al., 2002, Townsend, Altshuler, 2012). The first one of those networks emerges from the ventrolateral PFC and was thought to process external emotional stimuli, like affective face tasks (Townsend, Altshuler, 2012, Chen et al., 2011), while the second network had its starting point in the ventromedial PFC and OFC and was thought to process internal emotional cues that arise from inner feeling states like emotional responses to personalized events (Yamasaki, LaBar & McCarthy, 2002, Lane et al., 1997). In addition to these concepts of internal and external emotional control, many implicit and voluntary sub-processes have been identified to be centered either in the ventromedial or ventrolateral PFC (Phillips, Ladouceur & Drevets, 2008). The expert-group concluded to use these two networks as their primary focus in their task (Strakowski et al., 2012).

While these networks are repititious feedback and circular systems and therefore have no true point of origin, the evolutionary ancient limbic structure amygdala is seen as the core of those networks (see Figure 3). The amygdala is responsible for fight/flight reactions, emotion perception and regulation (Strakowski et al., 2012, Blond, Fredericks & Blumberg, 2012). While there is heterogeneity across fMRI studies of the amygdala in BD, amygdala activation is regularly found to be abnormal in response to diverse kinds of stimuli and is affected by mood state and emotional force of the task, which deems the dysregulation of the amygdala to be a neurophysiological feature of BD (Strakowski et al., 2012).

Amygdala function is modulated by different areas of the PFC. Decreased fMRI activations of predominantly ventrally localized PFC (which includes the OFC and ventrolateral PFC) areas

across different mood states and cognitive tasks have been reported in many studies, confirmed by meta analysis (Chen et al., 2011) and supported by positron emission tomography (PET) (Strakowski et al., 2012, Blumberg et al., 1999). Authors hypothesised that disturbed PFC functioning in combination with amygdala dysregulation sufficiently explains affective disease episodes like mania, depression or mixed episodes. Aberrant activation patterns were found in amygdala and PFC themselves and also within their connection (Strakowski et al., 2012). DTI-detected microstructural white matter abnormalities (Kafantaris et al., 2009, Sprooten et al., 2011, Versace et al., 2010, Caetano et al., 2008) were interpreted as a possible anatomical basis for this functional disturbance in connectivity between emotion-regulating structures which possibly predate the onset of BD and further progress with recurrent episodes over time (Blond, Fredericks & Blumberg, 2012). Basal ganglia also play an important role the proposed networks. Thalamus and striatum are seen as critical integrative structures allowing for the communication between the otherwise independent prefrontal networks (Strakowski et al., 2012). Especially in mania and euthymia, abnormal activation patterns in subcortical regions have been described (Blond, Fredericks & Blumberg, 2012). The last structure to complete the ALSN is the anterior cingulate cortex (ACC). The ventral part of the ACC, together with subgenual regions, is thought to process emotional stimuli, while the dorsal part of the ACC is thought to process cognitive stimuli (Strakowski et al., 2012). The ACC has shown to be activated differently through many studies in BD compared to HC (Altshuler et al., 2005, Liu et al., 2012) and has been described to be of reduced size in volumetric meta-analyses (Ellison-Wright, Bullmore, 2010, Bora et al., 2010).

Bilateral fMRI resting state abnormalities in have been reported ib BD individuals whereas left-lateral resting state abnormalities for characterizes individuals with unipolar major depression (Jiang et al., 2017). Phillips and Swartz (Phillips, Swartz, 2014) reviewed the literature and proposed similar networks of dysfunctional emotion processing and regulation as well as reward processing in BD. They proposed circuits involve the same structures as Strakowski and colleagues (Strakowski et al., 2012) but included hippocampal-amygdala circuits as also relevant.

Based on these excellent reviews, these functionally involved networks seem to be the best starting point to understand what structures are primarily affected in BD. As pointed out before, not only the gray matter but also the white matter connecting the relevant regions seems to be of high importance for these networks to maintain appropriate function. Thus,

disturbances in white matter structure are obviously capable to distort the processes of emotion regulation, leading us to the next chapter.

1.2.3 White Matter Hyperintensities

White matter hyperintensities (WMH) (or white matter lesions) appear as hyperintense bright spots in T2-weighted and fluid-attenuated inversion recovery (FLAIR) sequences on Magnetic Resonance Imaging (MRI) of the brain and are one of the most consistently reported brain abnormalities in individuals with BD (Beyer et al., 2009, Kempton et al., 2008). Conventionally, WMH have been classified by location into those that occur periventricular or in the deep white matter ranging in severity from "punctuate" to "confluent" (Fazekas et al., 1993). Individuals with BD are approximately 2.5 times more likely to have WMH compared to controls with deep WMH more common (Beyer et al., 2009, Kempton et al., 2008). However, WMH are not specific to BD as they are also become more prevalent with normal aging and are also more frequent in patients with cardio- and cerebrovascular disease (Birner et al., 2015). Moreover, they are also more frequent in various neuropsychiatric conditions, such as unipolar depression, schizophrenia, migraine and some forms of dementia (Palm-Meinders et al., 2012, Kruit et al., 2010, Rivkin et al., 2000, Grangeon et al., 2010, Wang et al., 2014, Barber et al., 1999). To our knowledge there is no evidence that the occurrence of WMH differs between the sexes. There are several possible non-mutually exclusive causes of WMH, including ischemia, demyelination, edema, and gliosis (Birner et al., 2015). Early confluent and confluent WMH more typically represent ischemic brain tissue damage (Fazekas et al., 1993) and progress over time (Schmidt et al., 2003, Birner et al., 2015). In otherwise healthy adults, the presence of WMH is generally associated with age and cardiovascular risk factors such as hypertension or smoking (O'Brien et al., 2006, Schmidt et al., 2011, Birner et al., 2015). Adults with BD are at increased risk of cerebro- and cardiovascular disease and hypertension compared to non-BD adults (Goldstein et al., 2009, Birner et al., 2015). Additionally, smoking behaviour and obesity/metabolic syndrome are occurring at higher rates in individuals with BD compared to HC (Diaz et al., 2009, McIntyre et al., 2010, Birner et al., 2015). However, there is no clear evidence for obesity specifically causing WMH. The cognitive decline, which is prevalent in both conditions, might be another bridge between WMH and BD (Robinson, Ferrier, 2006). Cognitive dysfunction, impairment of motor function and urinary control has been associated with WMH in otherwise healthy older subjects (Schmidt et al., 2011).

Beside their increased appearance in affective disorders in general, WMH are also predictors

for depressive symptoms in elderly healthy adults (O'Brien et al., 2006, Birner et al., 2015). Additionally, from an affective brainfunctional perspective, WMH are relevant as they were proposed to disturb connectivity between emotion regulating centers by being associated with increased limbic activations on facial expression affective-reactivity tasks in elderly depressed individuals compared to healthy elderly subjects (Aizenstein et al., 2011).
Several associations between clinical characteristics and WMH in BD have been described in the literature (Birner et al., 2015). Positive correlations with the number of hospitalizations and suicide attempts, and poor illness outcome as well as poorer treatment response, decreased performance on neuropsychological tests and impaired insight have been reported (Grangeon et al., 2010, Dupont et al., 1990, Moore et al., 2001, Serafini et al., 2010, Serafini et al., 2014, Pompili et al., 2007, Regenold et al., 2008, Birner et al., 2015).
Investigations of WMH in BD have relied on a categorical method of lesion description reporting the presence or absence of WMH and grading this presence on scales of differing reliabilities and lengths (Kapeller et al., 2003, Scheltens et al., 1998, Fazekas et al., 1987, Birner et al., 2015). Although volumetric analysis is common within the field of structural neuroimaging, this approach has rarely been applied for rating WMH in BD (Regenold et al., 2008, Regenold et al., 2005, Tighe et al., 2012) and consequently makes it interesting for further research (Birner et al., 2015).

1.2.4 Moderational factors in neuroimaging in BD

When analyzing brainstructural data, it is important to take potential moderational variables into account. Brain volumes progressively decline with ageing (Enzinger et al., 2005), thus making it perhaps the most important moderating variable when investigating disease effects on brain structure. Correcting for total intracranial volume (TIV) is another important measure to account for individuals head size as it is directly related to the brain's parenchymal volume (Wolf et al., 2003). Consequently, global and regional brain volumes have to be controlled for, or preferably normalized on, TIV, to investigate effects of other variables.
While age and TIV are usually controlled for in most neuroimaging studies, obesity and sex effects on brain volumes are receiving less attention in BD studies. The following sections will briefly explain why these two entities are interesting to be taken into consideration when investigating brain structural characteristics in psychiatric disorders like BD, schizophrenia or major depression.

1.2.4.1 Sex

On average, men have bigger headsizes and therefore higher TIVs and increased parenchymal volumes than women (Wolf et al., 2003, Sacher et al., 2013). Additionally, there are more subtle differences between male and female brains that deserve consideration on top of TIV. A meta-analysis of healthy individuals (Ruigrok et al., 2014) showed regional sex differences in volume and tissue density in amygdala, hippocampus and insula. The authors concluded that these results suggest candidate regions for the investigation of sex-related asymmetries in brain development and functional differences including those occurring in neurological and psychiatric conditions. Another meta-analyses (Sacher et al., 2013) highlighted increased gray matter volume in men, higher gray to white matter ratio in women, and differences in connectivity as well as activation patterns in amygdala, ACC, PCC, Insula, Thalamus, Globus pallidus, Putamen and the orbitofrontal cortex. The authors emphasized the importance of including sex as an important moderator variable when studying the human brain.

The ENIGMA meta-analysis (Hibar et al., 2016) reported a significant a sex by diagnosis interaction where BD women tended to have enlarged thalami compared to BD men which is conflicting with the results of Rimol et al. who did not find such an interaction (Rimol et al., 2010). Jogia et al. conducted a review of literature on sex – diagnosis interactions on brain structure and function in BD and pointed out that these interactions received limited attention in the past. They suggested that sex potentially modulates structure and function in regions that are likely to be involved in disease manifestation (Jogia, Dima & Frangou, 2012). Despite those observations, taking sex – diagnosis interactions into account does not seem to be a regularly approach in BD neuroimaging studies.

From a clinical perspective, men and women show similar prevalence rates of BD and also a similar age of onset while BD women show higher rates of BD II, hypomania, mixed episodes (episodes with manic and depressive symptoms at the same time) and rapid cycling (four or more affective episodes per year) compared to men (Diflorio, Jones, 2010). These clinical differences provide further rationale to consider potential sex differences when investigating biological underpinnings of BD.

1.2.4.2 Obesity and other Life Style related Factors

There is ample evidence implicating that obesity and/or the metabolic syndrome is associated with a more complex BD illness presentation and course (McIntyre et al., 2010, Leboyer et al., 2012a, Fagiolini et al., 2008, Fagiolini et al., 2005, Goldstein et al., 2013, Lackner et al., 2014) and poor cognition (Depp et al., 2014, Lackner et al., 2015, Yim et al., 2011). Coincidently, being overweight or obese is more common in BD than in HC affecting about 70 percent of BD patients (Diaz et al., 2009, McIntyre et al., 2010).
Moreover, elevated Body Mass Index (BMI) in otherwise healthy people is also associated with significantly decreased total brain and gray matter volumes even in adolescents and young adults. Also cognition is affected. Higher proportions of body tissue fat correlates with reduced regional brain volumes cognition-relevant and emotion-processing, such as the frontal lobes, hippocampus, putamen, caudate, precuneus, and thalamus as well as white matter volume reductions (Gunstad et al., 2008, Ward et al., 2005, Walther et al., 2010, Pannacciulli et al., 2006, Raji et al., 2010, Gustafson et al., 2004). Obese individuals also show more progressive brain volume loss over time compared to those with normal weight (Enzinger et al., 2005).

There are just a few studies investigating the potentially confounding effect on brain morphology and function in overweight or obese BD patients. For instance, Bond et al (Bond et al., 2011) reported reduced regional gray and white matter volumes (Bond et al., 2014) as well as disturbed white matter integrity (Kuswanto et al., 2014) in BDI individuals with elevated BMI following their first episode of mania. More recently, the same authors were unable to confirm an association of BMI with hippocampal volume in BD but found disturbed hippocampal neurochemistry when applying magnetic resonance spectroscopy (Bond et al., 2017).

At a molecular level, the consequences of obesity can be easily integrated into the neuroprogression model (see chapter 1.2.2). Obesity causes endocrine changes mediated by adipokine which is expressed in adipocytes. It has pro-inflammatory qualities and interacts with circadian rhythms. It also regulates sexual behavior. The serum levels are directly proportional to the amount of body fat (Aguilar-Valles et al., 2015, Park, Ahima, 2015). Adiponectin, on the other hand, which is also exclusively secreted in adipocytes, is reduced in obese individuals and characterise the metabolic syndrome (Ohashi et al., 2015). Adiponectin has anti-inflammatory properties. It increases insulin sensitivity and accelerates fat metabolism (Berg, Combs & Scherer, 2002, Qi et al., 2004, Kubota et al., 2007).

Additionally, there appears to be some evidence for adipokines being directly related to mood and cognition (Farr, Tsoukas & Mantzoros, 2015).

Despite all this evidence, overweight and obesity have not been considered to play a role in most of the neuroimaging studies in BD we are aware of (including all studies investigating gray substance changes highlighted in Table 1). Especially for the huge ENIGMA analyses (Hibar et al., 2016, Hibar et al., 2017) that contributed most of the data to our neuroimaging knowledge in BD, this might be an unknown limitation when interpreting the results.

Other life style-related factors, such as hypertension, diabetes, hyperlipidemia, and smoking also appear to be associated with morphological brain changes, although the empirical evidence remains limited (Friedman et al., 2014).

1.3. Aims/Hypotheses

The foregoing collection of observations concerning WMH and brain volumes in BD and the potentially moderational influence of sex and obesity on brain structure provided the impetus for exploring the following hypotheses in adults with BD in this analysis: First (I), BD individuals in our cohort exhibit a higher WMH-load (Ia) as well as volume reduction in the combined gray matter of the ALSN (Ib), slightly modified to the ones Strakowski and colleagues published (Strakowski et al., 2012), compared to healthy controls and this will differ between sexes. Second (II), these potential alterations of white matter (IIa) and gray matter (IIb) will be influenced by life style factors as concurrent overweight/obesity. And third (III), there will be associations of WMH-load (IIIa) and combined gray matter volume of the ALSN (IIIb) to clinical parameters and type of medication.

Concerning the analysis of WMH, we combined the volumes of periventricular and deep WMH, as a separation between this two types yielded issues of randomness, which will be addressed in the Methods part.

For brain volumetric analysis, we settled on the novel approach of using the combined gray matter volume of the regions involved in the ALSN (Strakowski et al., 2012) with the goal to create one unified and statistically convenient useable BD gray volume variable. With this variable, it is possible to test the aforementioned hypotheses based upon the following reasoning: First, with this approach we include the structural areas that actually seem to

matter the most in BD, and eliminate potential noise of redundantly analyzed gray substance, which seems more likely not to be involved in disturbed functions, disease presentation and/or progression. This decision is supported by the lion share of evidence presented by neuroimaging studies, as differences are usually found in areas contributing to these networks rather than global gray matter changes (see Chapter 1.2.). Second, we evade a statistical trap by not comparing multiple parameters in the context of a single center data collection combined with rather low effect sizes we expected based on the findings of recent meta-analyses (Hibar et al., 2016, Hibar et al., 2017, Wise et al., 2017). Thus, we avoid false negative results by not being forced to set a Bonferroni-corrected significance level.

Based on the model of Strakowski and colleges (Strakowski et al., 2012) we included the volumes of the following regions into our variable of interest: Amygdala, globus pallidus, putamen, thalamus, nucleus accumbens, caudate nucleus, anterior cingulate cortex (ACC), prefrontal cortex (PFC) and orbitofrontal cortex (OFC). Furthermore, we added the hippocampus (Phillips, Swartz, 2014), the parahippocampal gyrus (Aminoff, Kveraga & Bar, 2013) and the insular cortex (Ellison-Wright, Bullmore, 2010) to this variable, because these three are highly involved in cognitive processes and regulation of emotion and have been reported to be of abnormal volume in BD (Ellison-Wright, Bullmore, 2010, Hibar et al., 2016, Singh et al., 2008, Ladouceur, Almeida & Birmaher, 2008, Wise et al., 2017).

2. Methods/Materials

2.1. The BIPFAT Study

The study of brain structural characteristics in BD is an ongoing study that I conducted as part of the single centre "Bipolar Disorder and Fat Metabolism" (BIPFAT) study at the Medical University of Graz, Department of Psychiatry, that assesses demographic and clinical parameters as well as complete actual and lifetime psychiatric history using the Structured Clinical Interview according to DSM-IV (SCID I), history of medication, anthropometric measures (height, weight, circumference, measurement of areal fat thickness), fasting blood samples, neuropsychological testing, electroencephalogram EEG and magnetic resonance imaging (MRI) of the brain (Birner et al., 2015). All patients included were former in- or outpatients of the Medical University of Graz and had a diagnosis of BD I or BD II according to the DSM-IV criteria. Exclusion criteria were the presence of chronic obstructive pulmonary disease, rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, neurodegenerative and neuroinflammatory disorders (i.e. Alzheimer's, Huntington's and Parkinson's disorder, multiple sclerosis), haemodialysis and interferon-α-based immunotherapy. Further exclusion criteria for controls were the presence of lifetime psychiatric diagnoses (verified by SCID I) and first and second grade relationship to relatives with psychiatric disorders (Birner et al., 2015).

The study has been approved by the local ethics committee (Medical University of Graz, Austria) in compliance with the current revision of the Declaration of Helsinki, ICH guideline for Good Clinical Practice and current regulations (EK-number: 24-123 ex 11/12). For further information see our previous publication concerning the BIPFAT study (Birner et al., 2015, Birner et al., 2017, Bengesser et al., 2015, Platzer et al., 2017, Lackner et al., 2015, Queissner et al., 2018, Dalkner et al., 2017, Fellendorf et al., 2017, Bengesser et al., 2016, Reininghaus et al., 2016, Reininghaus et al., 2015, Bengesser et al., 2014).

2.2. MRI

MRI was performed on a 3T whole body scanner (TimTrio; Siemens Healthcare, Erlangen, Germany). The protocol includes an axial Fluid Attenuated Inversion Recovery (FLAIR) sequence (TR=10000ms, TE=69ms, inversion time=2500ms, number of slices=40, slice thickness=3mm, in-plane resolution=0.86x0.86 mm²) and a high resolution T1 weighted 3D sequence with magnetization prepared rapid gradient echo (MPRAGE) and whole brain

coverage (TR=1900ms, TE=2.19ms, inversion time=900ms, flip angle=9°, isotropic resolution of 1 mm) (Birner et al., 2015).

2.2.1 Estimation of WMH-load

Supra- and infratentorially located lesions of high signal intensity on FLAIR images were considered as WMH (Birner et al., 2015). The WMH types included were either of periventricular (caps, pencil-thin lining or halo) or of deep (punctuate, early confluent or confluent) localization and presentation. WMH were outlined on a computer using a custom written IDL program (Exelis Visual Information Solutions, USA; see Figure 4) (Birner et al., 2017). Following manual selection and outlining by a single instructed rater, lesion areas were segmented by a combination of region growing and local thresholding (Plummer, 1992). In anticipation of potential inter-rater variability, 15 cases from the study cohort were randomly chosen and rated again by the instructor, a neurologist who is highly experienced in identification, volumetry and rating of WMH (Birner et al., 2017). The neurologist was blinded to previous results and outlined WMH on FLAIR scans of the 15 cases using the same procedure as the main rater. WMH volumes of both raters were entered into SPSS. Subsequent intraclass correlation analysis yielded an intraclass correlation coefficient (ICC (2, 1)) of 0.938 (95%CI 0.570-0.984; p<.001). An ICC of 0.938 indicates high agreement and supports the reliability of WMH volumetry between the two raters. Lesion volume in mm3 was calculated using the program FSLMATHS (FSL, Oxford, www.fmrib.ox.ac.uk) by multiplying the lesion area with the slice thickness and normalized by total intracranial volume (TIV) (Birner et al., 2015). Segmentation of TIV and cortex volume from the T1-weighted high resolution MPRAGE scans was performed with the Freesurfer image analysis suite version 5.3., which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/) (Birner et al., 2015, Fischl, Dale, 2000, Fischl et al., 2004a).

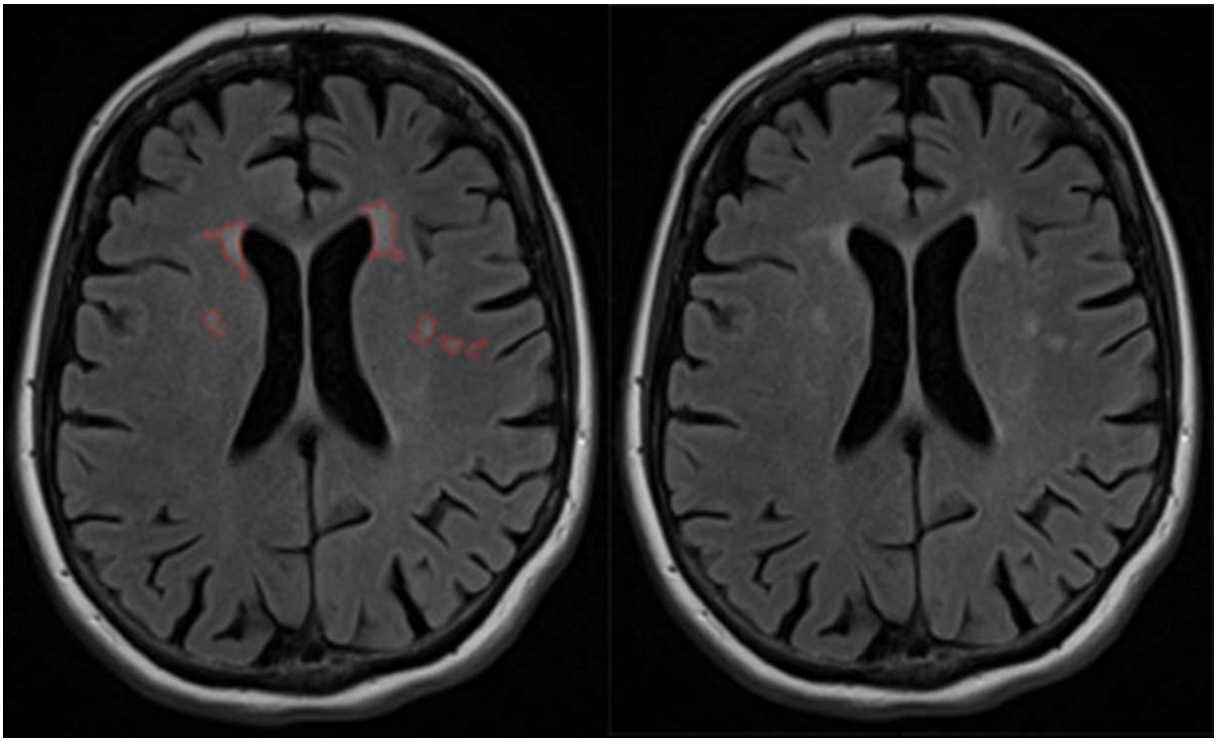

Figure 4: Representative axial FLAIR slice of one patient. The left image shows an example of lesion areas, outlined semi-automatically by the instructed rater using a custom written IDL program (Exelis Visual Information Solutions, USA). The right image shows the native FLAIR scan. Reused from our PLOS One publication (Birner et al., 2015).

We decided against a separation into deep and periventricular WMH as the drawing of rather random boundaries in the lesions themselves was often considered to be arbitrary, which was especially the case in large periventricular lesions inflating into the deep white matter as can be seen on Figure 5 (Birner et al., 2015).

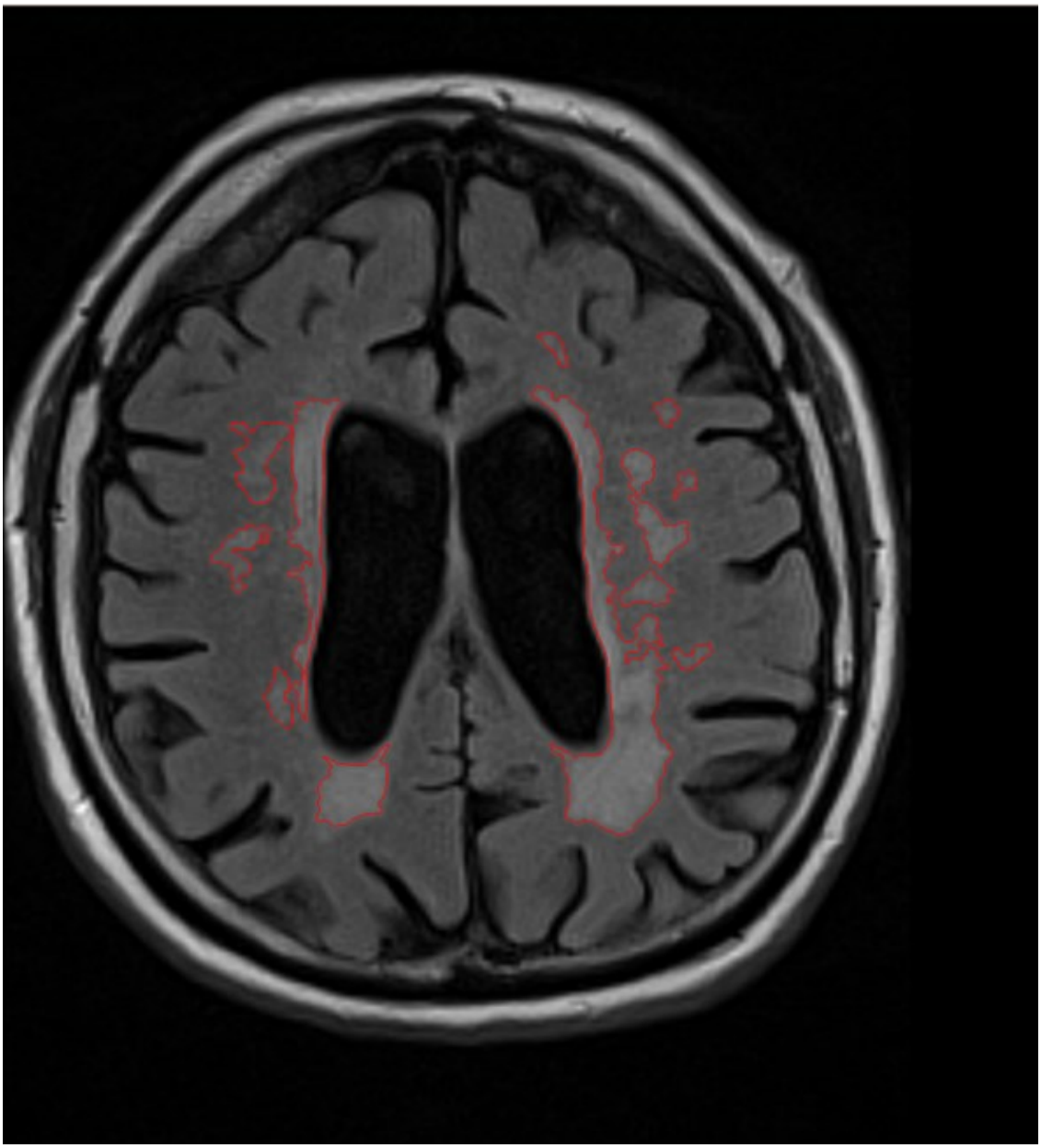

Figure 5: Axial FLAIR slice of one study individual. Periventricular WMH inflate into deep white matter and confluate with deep WMH. Reused from our PLOS One publication (Birner et al., 2015).

We were aware to exclude silent non-lacunar infarcts and lacunes from the analysis. Non-lacunar infarcts were defined as lesions with typical signal characteristics of infarcts following a typical vascular territory or being located in a border zone between two arterial supplied territories. Lacunes were defined as focal lesions involving the basal ganglia, the internal capsule, the thalamus, the brainstem or the white matter not exceeding a maximum diameter of 20 mm (Schmidt et al., 1999, Seiler et al., 2014). However, this was not necessary as none of our patients or HC showed any of those two ischemic lesion variants.

2.2.1 Estimation of Brain Volumes with Freesurfer

Cortical reconstruction and volumetric segmentation (see Figure 6) was performed with the Freesurfer image analysis suite, which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/). The technical details of these procedures are described in a variety of publications (Fischl, Dale, 2000, Fischl et al., 2004a, Fischl, 2012, Han et al., 2006, Dale, Fischl & Sereno, 1999, Reuter et al., 2012). The processing includes motion correction and averaging (Reuter, Rosas & Fischl, 2010) of volumetric T1 weighted images, removal of non-brain tissue (including stripping of the skull) using a watershed/surface deformation procedure (Segonne, Pacheco & Fischl, 2007), automated Talairach transformation, segmentation of the subcortical white matter and deep gray matter volumetric structures (including hippocampus, amygdala, caudate, putamen, ventricles) (Fischl et al., 2004b) intensity normalization (Sled, Zijdenbos & Evans, 1998), tessellation of the gray/white matter boundary, automated topology correction (Segonne, Pacheco & Fischl, 2007), and surface deformation following intensity gradients to place the gray/white and gray/cerebrospinal fluid borders at the location where the greatest shift in intensity is defining the transition to the other class of tissue (Fischl, Dale, 2000, Dale, Fischl & Sereno, 1999). Further steps include registration to a spherical atlas which is based on individual cortical folding patterns to match cortical geometry across subjects, parcellation of the cerebral cortex into units with respect to gyral and sulcal structure (Fischl et al., 2004b, Desikan et al., 2006) and creation of a variety of surface based data including maps of curvature and sulcal depth. This method uses both continuity and intensity input from the whole three dimensional MRI volume in segmentation and deformation procedures to create representations of cortical thickness, calculated as the nearest distance from the gray/white border to the gray/cerebral spinal fluid (CSF) border at each vertex on the tessellated surface (Fischl, Dale, 2000). The maps are created using spatial intensity gradients across tissue classes and are therefore not only dependent on signal intensity. The maps are not restricted to the voxel resolution of the original data and therefor are able to show submillimeter differences between groups. Procedures for these measurements have been validated against histological analysis (Rosas et al., 2002) and manual measurements (Kuperberg et al., 2003). Freesurfer morphometrics have shown good reliability across scanner brands and across magnetic field strengths (Han et al., 2006, Reuter et al., 2012).

For quality control, an imaging expert, who is trained in and familiar with Freesurfer, checked the appropriate registration, subcortical segmentations and cortical parcellations. Six

incorrectly registered data sets were removed. Because of favourable visual inspection results, no manual editing was done. After this quality check, the cortical and subcortical volumes among other variables (like cortical thickness and surface areas which are not part of this thesis) of each individual were computed with Freesurfer.

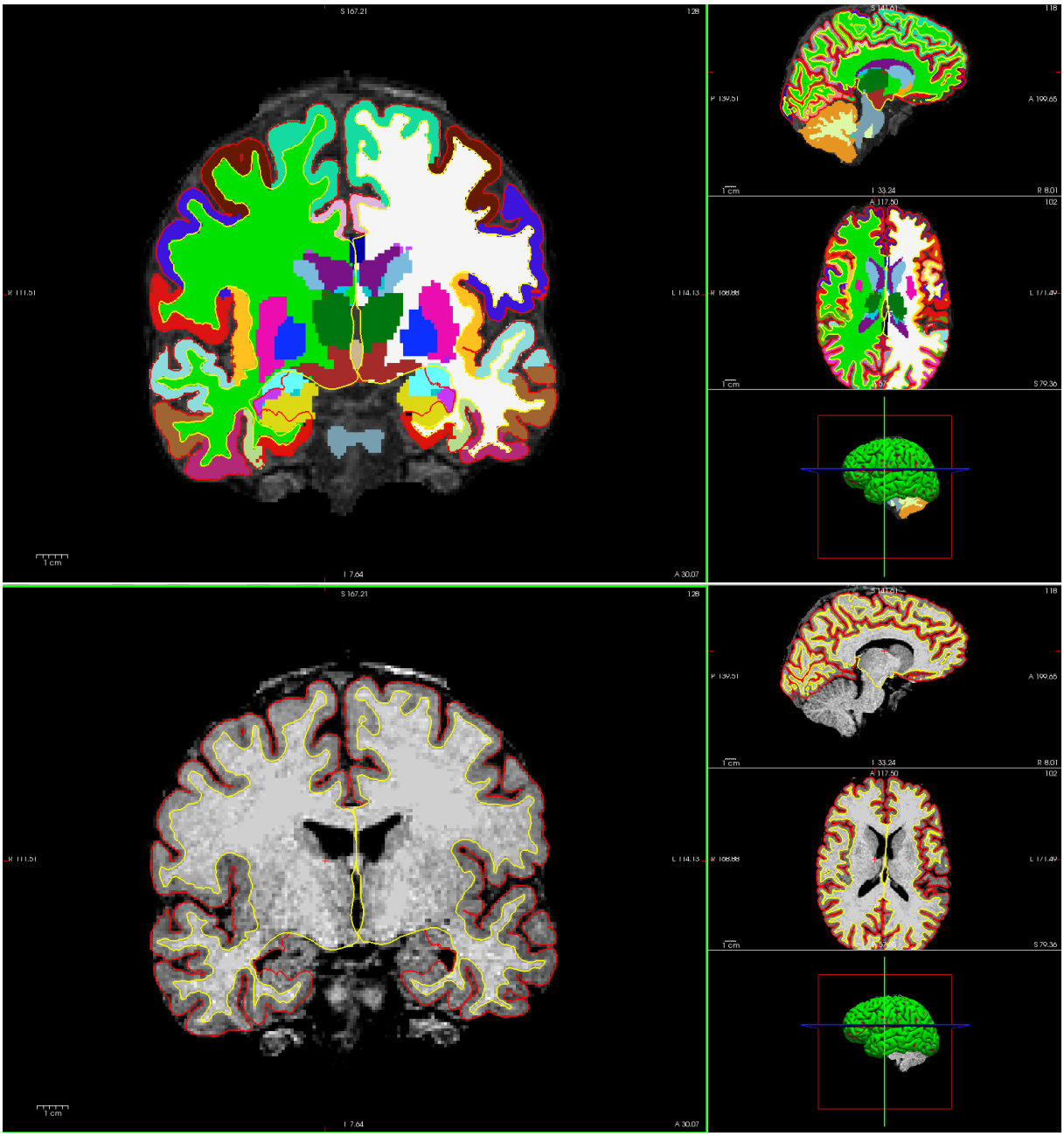

Figure 6: Freesurfer analyses opened in the programm Freeview. The probalistic registration to a spherical atlas is based on individual cortical folding patterns. Cortical thickness is calculated as the nearest distance from the gray/white border (yellow line) to the gray/cerebral spinal fluid (red line) border at each vertex on the tessellated surface

2.3 Statistics

For the calculation of differences in demographic characteristics between BD and HC, Student´s *t*-tests were performed for group comparisons of normally distributed variables. Categorical data sets were analyzed using chi-squared tests. Tests were two tailed and a value of $p < .05$ was considered statistically significant. For group differences, tests for normal distribution were obtained using Kolmogorov Smirnov test. In case of violation of normality, Mann-Whitney-U-tests were performed.

2.3.1. Statistics – White Matter Hyperintensities

For differences in WMH-load (Ia), BD patients and controls were analyzed using a univariate covariance analysis model (ANCOVA) controlling for the confounding factor age and additionally for BMI, smoking, and diabetes, as the groups differed in these parameters (Birner et al., 2015).

For partial correlation analyses within the whole BD group as well as in BD groups separated by sex (IIa and IIIa), variables of interest matching the required normal distribution of residuals confirmed visually by histograms have been used for calculation (normalized WMH with number of depressive episodes, number of manic episodes, illness duration, age of onset and BMI). For this purpose, normalized WMH volumes have been transformed by adding the number one and the use of a natural logarithm (Birner et al., 2015). After Bonferroni-correction for multiple comparison (including five variables) a value of $p<0.01$ was considered statistically significant. Partial correlation analyses of WMH were conducted controlling for age, hypertension, diabetes, smoking, migraine and medication (lithium, antiepilieptics and antipsychotics). Illness duration was additionally introduced as a control variable for number of affective episodes and age of onset (Birner et al., 2015).

2.3.2. Statistics – Volumes of the Anterior Limbic Striatal Networks

The volume of the ALSN was assessed by adding the volumes of the amygdala, the hippocampus, the globus pallidus, the putamen, the thalamus, the caudate nucleus, the ACC, the PFC, the OFC, the nucleus accumbens, the insular cortex and the parahippocampal gyrus. For all statistical calculations the volumes were normalized by dividing it through the individual's total intracranial volume and then multiplied by 10.000 (Buckner et al., 2004). To

test hypothesis Ib- “BD individuals in our cohort exhibit higher gray volume reduction compared to healthy controls in the ALSN and this potential difference might differ between sexes”, normalized volumes of the ALSN in BD patients and healthy controls were analyzed using a two-way ANCOVA set to BD vs. HC and male vs. female, controlling for the confounding factor age. In the next step to test hypotheses (IIb) “this potential brain volume alteration in BD will be mediated by life style factors as concurrent overweight/obesity”, we performed the same analysis (ANCOVA) again but introduced BMI and smoking as life style factors to the model to see how they modulate potential group effects. We did not include diabetes into the model, as it did not appear in HC. For hypotheses (IIIb) “there will be associations to clinical parameters and type of medication”, first, partial correlation analyses within the whole BD group as well as in BD groups separated by sex of variables of interest were conducted. All variables of interest included had to meet the required normal distribution of residuals confirmed visually by histograms. As a consequence, correlation was performed between normalized volume of the ALSN and number of depressive episodes, number of manic episodes, illness duration and BMI. Analyses were controlled for age, sex (when applicable), diabetes, smoking, alcohol dependency, medication (lithium, antiepileptic agents and antipsychotics) as well as BMI and illness duration. After Bonferroni-correction for multiple comparison (including four variables) a value of $p<0.0125$ was considered statistically significant. Second, to investigate potential volume differences in medication groups within BD, another ANCOVA set to sex (male/female), actual intake of lithium (yes/no), antiepileptics (yes/no) and atypical antipsychotics (yes/no) controlling for age, diabetes, smoking, BMI, alcohol dependency and illness duration was performed.

3. Results

3.1. Results – White Matter Hyperintensities (a)

3.1.1 Hypothesis Ia - Differences BD and HC in WMH-load, Clinical Parameters and Demographic Characteristics

Group differences in WMH between BD patients and controls are displayed in Table 2 (Birner et al., 2017). ANCOVA results indicated that individuals with BD had a significantly increased value of WMH-load compared to HC (*F(1/148)*=3.968, *p*=.048, *Eta*=.026; Median and IQR are shown in table 2).

Table 2: BD vs. HC; WMH-load, clinical parameters and demographic data

	BD (n=100)	**HC (n=54)**	***Statistics***	***p***
Male (%)	52%	43%	*χ 2=1.242*	*.265*
Age (years) (M, SD)	44 (14)	41 (16)	*U=-1.475*	*.140*
Body Mass Index (M, SD)	28 (5)	25 (4)	***t=3.695***	*.000*
Migraine (%)	22%	28%	*χ 2=.750*	*.386*
Hypertension (%)	29%	22%	*χ 2=.825*	*.364*
Smoking (%)	49%	24%	***χ 2=9.058***	*.003*
Diabetes (%)	7%	0%	***χ 2=3.960***	*.047*
Volume of WMH (mm³) (Mdn, IQR)[1]	3710 (2961)	2185 (1665)	***F=3.968***	*.048*

Note: Results are from *t*-tests (*t*), Chi square tests ($\chi 2$), Mann-Whitney-U-tests (*U*) and ANCOVA (*F*). (1) For ANCOVA, WMH volumes were normalized by individual total intracranial volume and controlled for age, diabetes, smoking and BMI. Statistically significant effects are marked **bold**. M=Mean, SD= Standard Deviation, Mdn= Median, IQR = interquartils range, p<.05 was considered as statistically significant. Reused from our PLOS One publication (Birner et al., 2015).

3.1.2 Sex Differences within BD

Descriptive baseline characteristics within the BD group, stratified by sex, are displayed in Table 3. In BD patients, there was neither a significant sex difference for WMH-load nor for demographic, clinical or vascular risk parameters (Birner et al., 2015).

Table 3: WMH-load, clinical parameters and demographic data in BD stratified by sex

	52 males	**48 females**	***Statistics***	***p***
BD I / BD II (%)	65% / 35%	65% / 35%	*χ 2=.007*	*.933*
Age (years) (M, SD)	44 (14)	44 (14)	*t=-0.24*	*.981*
Illness Duration (years) (M, SD)	19 (13)	20 (19)	*U=-.235*	*.814*
Age First Episode (years) (M, SD)	25 (12)	25 (9)	*U=-.366*	*.714*
Manic/hypomanic Episodes (M, SD)	11 (18)	8 (9)	*U=-.603*	*.547*
Depressive Episodes (M, SD)	12 (12)	16 (15)	*U=-.513*	*.608*
History of Suicide Attempts (%)	39%	28%	*χ 2=1.462*	*.227*
HAM-D (M, SD)	5 (4)	6 (5)	*U=-.573*	*.567*
YMRS (M, SD)	1 (2)	1 (2)	*U=-1.665*	*.095*
BDI (M, SD)	12 (9)	15 (12)	*U=-1.254*	*.210*
Body Mass Index (M, SD)	28 (4)	28 (6)	*t=-0.56*	*.955*
Antidepressant (%)	44%	31%	*χ 2=1.785*	*.182*
Lithium (%)	23%	10%	*χ 2=2.835*	*.092*
Antiepileptic (%)	25%	13%	*χ 2=2.534*	*.111*
Atypical Antipsychotic (%)	42%	40%	*χ 2=.077*	*.782*
Occasional Typical Antipsychotic (%)	12%	15%	*χ 2=.205*	*.651*
Permanent Typical Antipsychotic (%)	0%	0%	*n.a.*	*n.a*
Migraine (%)	23%	20%	*χ 2=.073*	*.787*
Hypertension (%)	31%	27%	*χ 2=.165*	*.685*
Smokers (%)	48%	50%	*χ 2=.037*	*.848*
Diabetes (%)	8%	6%	*χ^2=.080*	*.778*
Volume of WMH (mm³) (*Mdn*, IQR)[1]	4095 (3295)	3032 (2816)	*U=-.150*	*.133*

Note: Results from t-tests (t), Chi square tests (2) and Mann-Whitney-U-tests (U). (1) Volumes of WMH were normalised by individual total intracranial volume for the calculations. M=Mean, SD= Standard Deviation, Mdn= Median, IQR = interquartils range, n.a.= not applicable; p<.05 was considered as statistically significant. Reused from our PLOS One publication (Birner et al., 2015).

3.1.3 Hypothesis IIa and IIIa - Correlations of WMH-load with BMI and clinical parameters

The results of partial correlation analyses of WMH with clinical and demographic characteristics within the BD group are presented in table 4. WMH-load correlated positively with manic episodes and illness duration. In BD men, the number of manic/hypomanic episodes as well as depressive episodes correlated positively with WMH-load. In BD women, no significant correlations were found (Birner et al., 2015). There was no correlation with BMI.

Table 4: Partial correlation analyses of WMH-load with clinical parameters in BD

	WMH-load		
	BD (n=100)	BD male (n=52)	BD female (n=48)
Manic episodes[1]	**.420*** **	**.755*** **	-.101
Depressive episodes[1]	.226*	**.511*** **	.080
Age of onset[1]	-.142	.034	-.318*
Body Mass Index[2]	-.020	-.148	.083
Illness duration[2]	**.268** **	.348*	.074

Note: WMH have been normalized by the individual total intracranial volume and have been transformed by adding the number one and the use of natural logarithm; (1) controlled for age, hypertension, diabetes, smoking, migraine, lithium, antiepileptics, antipsychotics and illness duration; (2) controlled for age, hypertension, diabetes, smoking, migraine, lithium, antiepileptics and antipsychotics; Significant results after Bonferroni correction for multiple comparison (p<0.01) are shown in bold; p<0.05*, p<0.01**, p<0.001***. Reused from our PLOS One publication (Birner et al., 2015).

3.2. Results volume ALSN (b)

The investigation of brain volumes was performed around two years after the WMH-analysis. The cohort has therefore increased in size but all the 154 subjects of the previous WMH-analysis are included in the ALSN volume analysis as well.

3.2.1 Demographics

Descriptive baseline characteristics of BD and HC are displayed in table 5. Like the WMH-load analyzed cohort, BMI, diabetes and smoking differed significantly between individuals with BD and HCs.

Table 5: Demographic Characteristics and clinical parameters in BD and HC

	BD (n=124)	**HC (n=86)**	***Statistics***	***p***
Male (%)	49%	37%	*χ 2=3.600*	*.086*
Age (years) (M, SD)	43 (13)	42 (17)	*U=-.621*	*.534*
Body Mass Index (M, SD)	28 (6)	25 (4)	***t=4.503***	***.000***
Smoking (%)	50%	22%	***χ 2=16.691***	***.000***
Diabetes (%)	7%	0%	***χ 2=6.521***	***.011***
Alcohol dependency (%)	17%	-	n.a.	
Lithium (%)	33%	-	*n.a.*	
Antiepileptics (%)	29%	-	*n.a*	
Atypical Antipsychotics (%)	61%	-	*n.a*	

Note: Results from t-tests (t), Chi square tests (2) and Mann-Whitney-U-tests (U). Statistically significant effects are marked bold. (M=Mean, SD=standard deviation, n.a. = not applicable)

3.2.2 Hypothesis Ib – ALSN Volume BD vs. HC, male vs. female

ANCOVA results indicated that individuals with BD had significantly reduced normalized gray matter volume of the ALSN compared to HC (F=5.935, p=.016, Eta=.028). Furthermore, men had significantly reduced normalized gray volume of the ALSN compared to women (F=4.594, p=.033, Eta=.022). For visualization of data distribution see Figure 7.

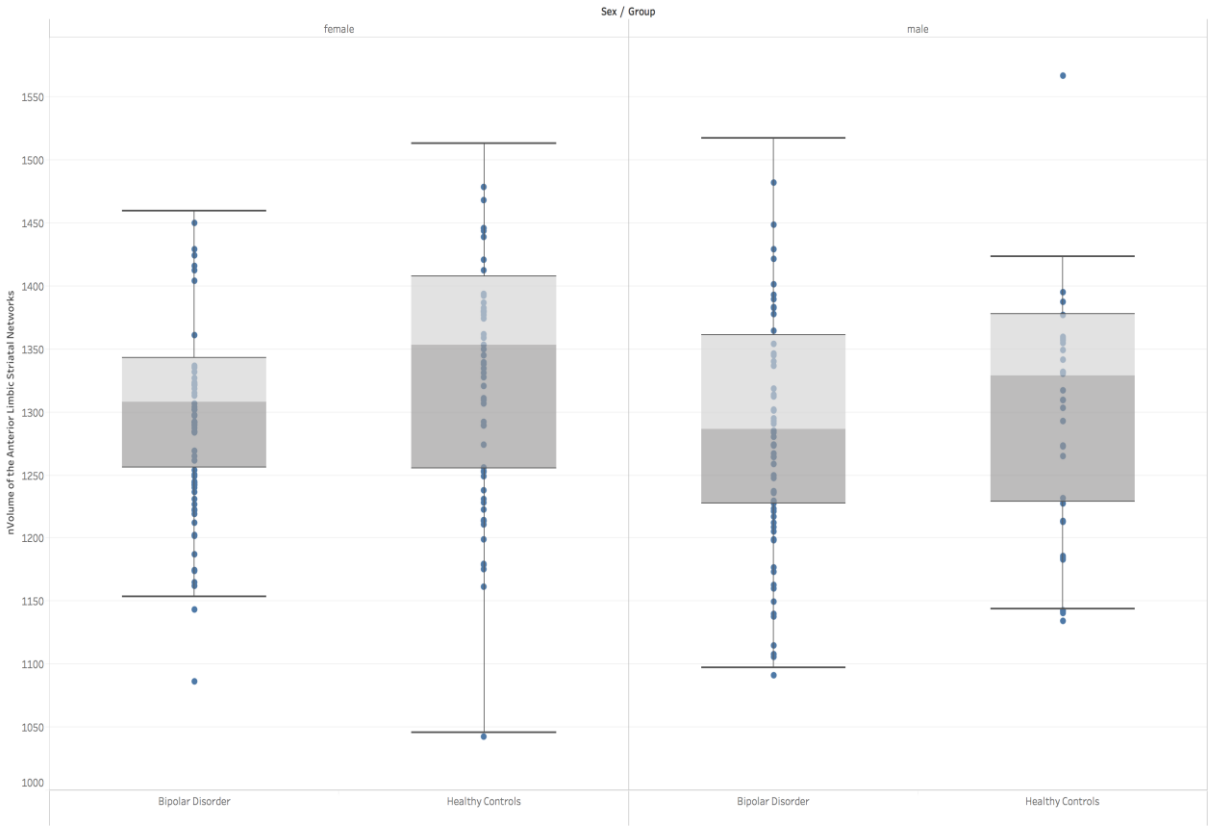

Figure 7: Boxplots of the gray volume of the anterior limbic striatal networks (ALSN) stratified by diagnosis and sex; normalized on the individuals total intracranial volume (y-axis) in 124 bipolar disorder subjects(61 male, 63 female), and 86 healthy controls (34 male, 52 female).

3.2.3 Hypothesis IIb – Inclusion of BMI and smoking into the model

After including BMI and smoking into the models, the significant differences disappeared, while BMI was found to be a significant confounding factor (F=5.666, p=.018, Eta=.010).

3.2.3 Hypothesis IIIb – Associations of ALSN volume with clinical parameters and medication

The results of partial correlation analyses are shown in table 6. After Bonferroni correction the calculation yielded no significant results.

Table 6: Partial correlation analysis ALSN volume

		Normalized gray Volume ALSN
BD total (n=124) [3]	Manic episodes[1]	.004
	Depressive episodes[1]	.108
	Illness duration[2]	-.145
	BMI [2]	-.203*
BD male (n=61)	Manic episodes[1]	.321*
	Depressive episodes[1]	.348*
	Illness duration[2]	-.133
	BMI [2]	-.123
BD female (n=63)	Manic episodes[1]	-.184
	Depressive episodes[1]	.201
	Illness duration[2]	-.213
	BMI [2]	-.288*

Note: The volume of the anterior limic striatal networks (ALSN) has been normalized by the individual total intracranial volume; (1) controlled for age, illness duration, BMI, medication, alcohol dependency/abuse and smoking; (2) same as (1), but not controlled for itself; (3) controlled for sex; *p<0.05; after Bonferroni correction (p<0.0125) there was no significant correlation.

ANCOVA results indicated that individuals with BD currently taking atypical antipsychotics had significantly reduced normalized grey volume of the ALSN compared to BD currently not taking atypical antipsychotics (F=12.034, p=.001, Eta=.105). This result was consistent for male and female individuals with BD. Permanent intake of antiepileptcs, lithium and antidepressants displayed no association to ALSN brain volumes. For visualization of the distributions see Figure 8.

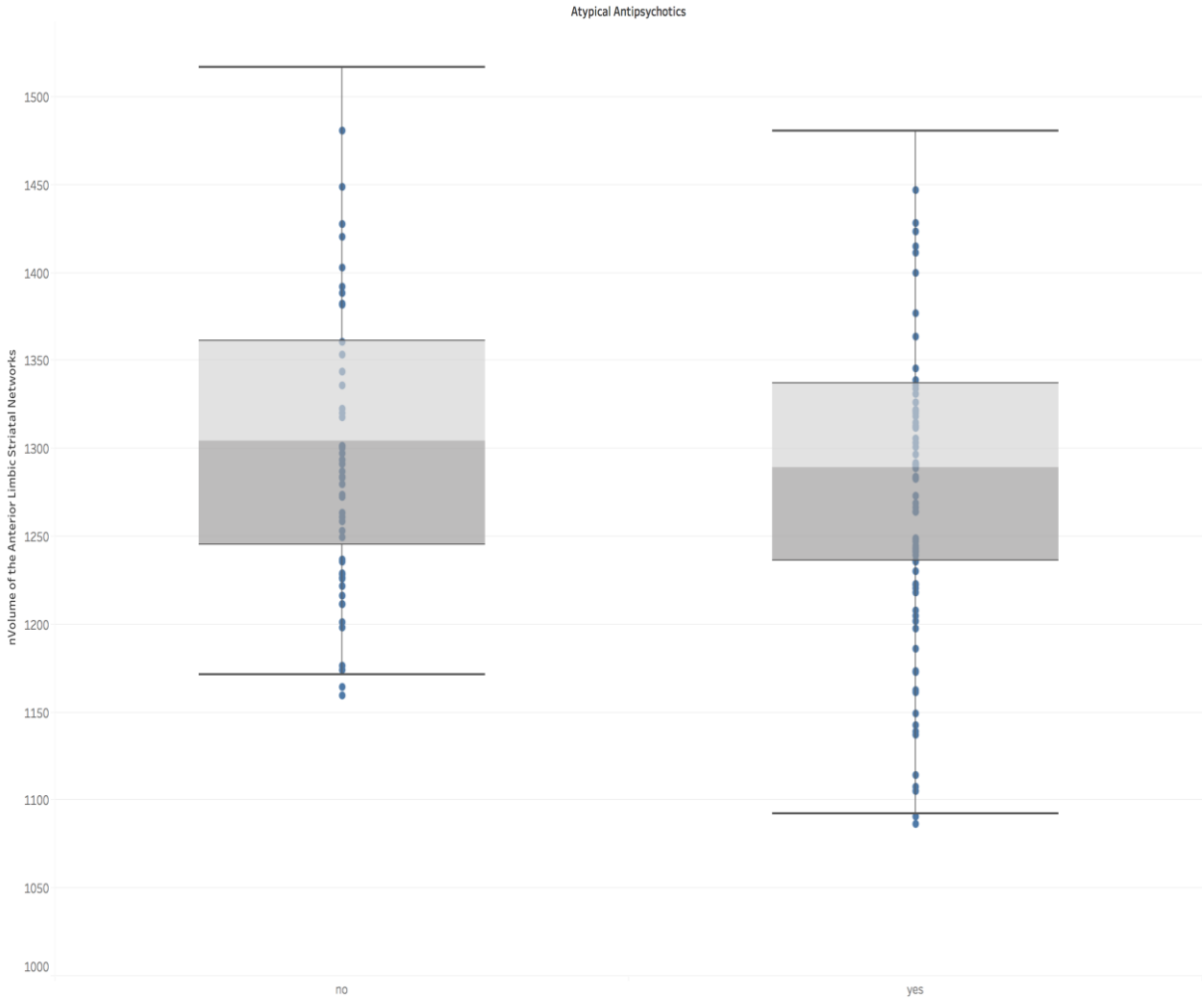

Figure 8: Boxplots of the gray matter volume of the anterior limbic striatal Networks (ALSN) stratified by use of atypical antipsychotics; normalized on the individuals total intracranial volume (y-axis) in 124 bipolar disorder subjects; 61% on atypical antipsychotics.

4. Discussion

We here aimed to investigate differences of WMH-load and gray matter volume of the ALSN in individuals with BD compared to HC, and to explore their relationship to clinical parameters while taking sex and overweight/obesity influences on human brain structures into account. Several interesting observations emerged from the analyses of our data.

Concerning WMH, first, we found an increased total volume of WMH in individuals with BD compared to HC. Secondly, we revealed a highly significant correlation between the number of affective episodes (manic and depressive) with the total volume of WMH in BD men, but not in BD women. And thirdly, illness duration was identified as a significant and independent factor associated with the volume of WMH. We could not identify significant associations of BMI or age of onset with total WMH-volume in BD (Birner et al., 2015). The result of increased volumes of WMH in BD compared to HC is in concordance with existing literature (Beyer et al., 2009, Kempton et al., 2008). Male and female individuals with BD in our cohort did neither differ significantly in the total volumes of WMH nor in the number of manic or depressive episodes. However, an association between the number of affective episodes and WMH-volume was only detected in male subjects. This sex effect might be a consequence of the neuroprotective influence of estradiol and/or progesterone (e.g. decrease of oxidative stress) which was repeatedly discussed in previous literature (Heron, Daya, 2000, Kiray et al., 2007, Moorthy et al., 2005, Requintina, Oxenkrug, 2005). Estrogen and progesterone might counterbalance certain neurodegenerative processes due to increased DNA repair, activation of antioxidative defense and interactions with BDNF (Requintina, Oxenkrug, 2005, Dietrich, Humphreys & Nardulli, 2013, Frey, Dias, 2014, Spence et al., 2013). Furthermore, decreased estrogen levels were associated with worse performance in cognitive tasks, especially in items testing short-term verbal memory in female individuals in previous studies (Shaywitz et al., 2003, Sherwin, 2003).

Despite the significant association between WMH and number of affective episodes in men, we do neither know the particularly protecting or harming factors for WMH in BD nor if WMH in men are causative for a poorer illness outcome nor if WMH stimulate clinical symptoms of manic and/or depressive episodes. In this context, it is interesting that severe WMH are proposed to be of ischemic origin, and may transform into true infarcts (Schmidt et al., 2011), which are capable to trigger depression and in rare cases also mania (Santos et al.,

2011). During the following years, about thirty percent of individuals with post-stroke mania develop a course of BD (Starkstein et al., 1991, Robinson et al., 1988, Robinson, 1997). Interestingly, a systematic review on stroke and mania involving 74 cases showed that men are approximately three times more likely to express post-stroke mania than women (Santos et al., 2011). Stroke in general is more prevalent in men (Truelsen et al., 2006, Appelros, Stegmayr & Terent, 2009), however this cannot explain the much higher occurrence of post-stroke mania in male individuals (Birner et al., 2015).

As a result of our foregoing observations we propose that the male brain may be more vulnerable to manic affective symptoms in the context of white matter changes, which implies that WMH might accelerate the course of BD in men. On the other hand, depression after stroke is not more common in men than in women (Burvill et al., 1995). We assume that the positive correlation with depressive episodes in our study is a consequence of the accelerated course of disease, as the number of manic episodes was directly and positively correlated with the number of depressive episodes (Birner et al., 2015).

BMI did not show a significant association with WMH in the present study. BMI can be regarded as a proxy of metabolic dysfunction but is not deterministic of metabolic dysfunction (Birner et al., 2015). The missing relationship between BMI and WMHs in our sample could be because no relationship exists (as there is no evidence in the literature for an association of BMI and WMH in healthy adults, this appears to be the most likely reason), or because the association between BMI and WMH in bipolar adults may only occur in individuals with elevated BMI and associated metabolic morbidity (e.g. dyslipidemia, hypertension etc.) (Birner et al., 2015).

Sex differences in clinical characteristics of BD have been repeatedly found. In one study (Kawa et al., 2005) more men than women reported mania as first illness episode at the onset of BD I. Rapid cycling might be slightly more common in women, while age at onset and number of affective episodes of each polarity did not differ between men and women in previous studies (Kawa et al., 2005, Tondo, Baldessarini, 1998, Hendrick et al., 2000). Men showed higher rates of comorbid substance abuse and gambling, while women reported higher rates of comorbid eating disorders, weight change, appetite change and insomnia during depression (Kawa et al., 2005, Birner et al., 2015). In a more recent systematic review Diflorio and Jones (Diflorio, Jones, 2010) concluded that women with BD show higher rates of BD II, hypomania, mixed episodes and rapid cycling compared to men, but do not differ in terms of clinical variables or treatment response. In a former analysis of our BD cohort, we reported that high frequencies of weight change, also called weight cycling, were

independently related to the number of affective episodes in women with BD only (Reininghaus et al., 2015). Furthermore, the shift towards the neurotoxic hydroxykynurenine arm of the kynurenine pathway was associated with poorer memory performance in men with BD only (Platzer et al., 2017), and male BD participants showed significantly higher peripheral markers of oxidative stress than female BD participants (Bengesser et al., 2015). Additionally, cognitive function in all domains were correlated positively with physical activity in BD women only (Fellendorf et al., 2017).

The analyses of our data concerning the gray matter volume of the ALSN in special consideration of sex, BMI, medication and clinical aspects yielded several interesting observations: First, we found a decreased normalized volume of the ALSN in individuals with BD compared to HC as well as in male subjects compared to female subjects when controlling for age only. Interestingly the group of female healthy controls showed the highest combined normalized gray matter volume in our study cohort. As shown on Figure 5 in the results section, the group of healthy females rises above the other shown three groups, who visually appear to be on a rather similar level, in terms of normalized ASLN volume.

Secondly, these group differences vanished when controlled for potential influences of BMI and smoking, while BMI was the moderating factor for this observation. When reviewing the literature of volumetric analysis in BD it does not appear that BMI was introduced as a controlling variable in the majority of statistical analyses. This is astonishing as there is knowledge in the literature that reveals obesity to be an independent factor for brain atrophy over time (Enzinger et al., 2005) and furthermore, obesity showed associations to reduced gray and white matter volumes as well as to microstructural characteristics in BD (Bond et al., 2011, Bond et al., 2014, Kuswanto et al., 2014, Bond et al., 2017) and in the general population (Gunstad et al., 2008, Ward et al., 2005, Walther et al., 2010, Pannacciulli et al., 2006, Raji et al., 2010, Gustafson et al., 2004). In our cohort, the gray matter volume difference appears only because of the elevated BMI in BD compared to HC. This goes in line with our principle hypothesis (The BIPFAT study) that obesity and BD are subserved by common underlying pathophysiological mechanisms. Possible reasons for the observation of the higher ALSN volumes in healthy females might include that, in our data set, the group of female healthy controls had a significantly lower BMI compared to the other groups. Contrasting to this explanation is the fact that in a further analysis, BMI did not correlate with gray volume in this female HC group at all. Additionally, the number of male HC (n=32) was

rather small compared to the other three groups. A better matching sample preferable in a bigger cohort would be helpful to perform a more conclusive analysis.

Thirdly, after correcting for multiple comparisons and controlling for confounding variables, there were no significant correlations of the volume of the ALSN with number of affective episodes, illness duration or BMI. However, we found a strong trend towards a negative correlation of ALSN volume to BMI in BD total and BD women, but not in BD men and a strong trend towards a positive correlation of ALSN volume to the lifetime number of manic and depressive episodes in BD men only, which appears to be a rather surprising association where we have no truly logically sound explanation now. One vague explanation might be a compensating hypertrophic mechanism to adapt to the impact of WMH, that also correlate with manic and depressive episodes in men only. However, as those trends did not reach a significant level, there is no need to over-interpret these results with more speculations.

Fourthly, individuals with BD receiving a continuous treatment of atypical antipsychotic medication at the time point of the study showed a highly significantly reduced gray matter volume of the ALSN compared to individuals with BD not receiving a treatment with atypical antipsychotics, even if controlled for influences of age, diabetes, smoking, BMI, alcohol dependency and illness duration. We did not include typical antipsychotics into the statistics, as not a single individual took a high potent antipsychotic like haloperidol. The low-potent antipsychotic Prothipendyl was commonly used for sleep disturbances on occasion but very rarely (in one patient) as a continuous treatment. The same applies to benzodiazepines. A recent review (McDonald, 2015) concluded that there is not enough evidence to propose that antipsychotic treatments have effects on brain volumes in BD. For example, in the mega-analysis of Hallahana (Hallahana et al., 2011) and colleagues of 442 BD individuals only 31 subjects were recorded to take atypical antipsychotics and 20 on typical antipsychotics, leading them to decide to not evaluate volume differences because of the small sample size and lack of information. However, this had been the status quo before newest evidence got published. The ENIGMA studies included numerous enough sample sizes to look at medication group differences and found significantly reduced cortical surface areas of large prefrontal areas in 504 individuals with BD on atypical antipsychotics compared to 994 individuals with BD off atypical antipsychotics (Hibar et al., 2017). As surface areas are one of the two factors multiplied to cortical volume (cortical thickness being the second factor) and the prefrontal cortex is the most voluminous structure of the ALSN, some alignment of our studies is suggested. Furthermore, looking at the supplementary data published, Hibar et

al. found reduced thalamic volumes taking group of BD individuals treated with atypical antipsychotic (Hibar et al., 2016). In a recent single-center analysis of cortical and subcortical measures, 114 BD individuals on antipsychotics showed reduced hippocampal volumes and enlarged third ventricle sizes compared to 142 BD Individuals off atypical antipsychotics (Abramovic et al., 2016). In rodents, studies using serial imaging with postmortem confirmation showed that haloperidol (typical antipsychotic) as well as olanzapine (atypical antipsychotic) led to significant decrease of brain volumes (Vernon et al., 2014, Vernon et al., 2012, Vernon et al., 2011). A study of macaque monkeys treated over 18 months with haloperidol or olanzapine detected an 8-11% fresh brain weight reduction, compared to control monkeys (Dorph-Petersen et al., 2005). This weight reduction was detected ubiquitous in the brain, but was higher attenuated in frontal and parietal lobes. In patients with first episode-schizophrenia subtle antipsychotic-dependent brain volume decrease was observed in a longitudinal design (Ho et al., 2011). This goes basically in line with a systematic review focusing on the effects of antipsychotic drugs on brain volumes, concluding that there is some evidence pointing out a potential volume reducing effect of antipsychotics (Moncrieff, Leo, 2010). Nevertheless, one argument against the idea of neurodegenerative qualities of atypical antipsychotics might be, that patients taking atypical antipsychotics were more severely affected by the disease and that disease related factors contribute to these different brainstructural characteristics instead of the medication. Our data provide no basement for this argument as it revealed no inverse correlation of brain volume with number of mood episodes, which might be an indicator of disease severity. Furthermore and in contrast, it revealed trends towards a positive correlation of brain volume to number of mood episodes in male BD individuals. The observed volume reduction was found in a BD group and might not be transferable to subjects suffering from a different psychiatric disease, like schizophrenia. It is also much harder to conduct an analysis like this in a large schizophrenia sample, as there are no alternative treatment options other than antipsychotics. In schizophrenia, it is also not clear if atypical antipsychotics are co-responsible beyond the disease itself for brain volume loss. I want to point out an older study of Owen and colleagues (Owens et al., 1985) using cerebral computed tomography in 112 chronic schizophrenia patients, who found a correlation between involuntary movements (a typical side effect of antipsychotics) and ventricle enlargement suggesting an influence of medication. BD on the other hand has three different groups of first-line mood stabilizing agents (lithium, antiepileptic agents and atypical antipsychotics) to choose from, which puts BD on an advantage to compare different groups of medications. As patients with BD are more vulnerable to the side effect of involuntary

movements on antipsychotic drug therapy compared to schizophrenia (Kasper et al., 2013), they might also be more vulnerable to these drug dependent brain changes. To point out some heterogeneity in the literature, the intake of antipsychotics also has been related to increases of subcortical volumes (Huhtaniska et al., 2017) and to left pallidum volume more specifically, published in a recent analysis (Hashimoto et al., 2017). A possible explanation of those findings might be a high occurrence of dopamine 2 receptors in striato-pallidal regions (Black, Gado & Perlmutter, 1997) leading to increased cell proliferation following dopamine blocking neuroleptic therapy (Beaulieu et al., 2007), and another one being increased blood flow in the striatum after antipsychotic administration (Lahti et al., 2005). Finally, some of the expected gray volume reducing effect of overweight/obesity might have been overlaid by the not fully understood implications that weight gain inducing antipsychotics have on brain structure.

4.1. Limitations

Concerning the WMH analysis, our results show no causal relationship between WMH and clinical symptoms as only correlations were studied. It is questionable if those hyperintensities are a result of comorbid conditions or are directly associated with the disorder itself and if WMH represent a biological risk factor for BD (Birner et al., 2015). Age is by far the most influential parameter contributing to WMH (Schmidt et al., 2011) which suggests that WMH in young subjects might not reflect a reliable indicator of the disease as the underlying pathology might not have had enough time to develop from a micro- to a visible macro-structural level (Birner et al., 2015). In line with this, there are conflicting results concerning the presence of WMH in children and adolescents with BD (Birner et al., 2015). Some studies show increased WMH already in young ages (Pillai et al., 2002, Lyoo et al., 2002, Botteron et al., 1992, Botteron et al., 1995) while others revealed no increased rate of WMH in young BD subjects without comorbid conditions (Gunde et al., 2011). Non-conventional more advanced MRI methods as DTI might provide more detailed insights, especially in younger subjects with BD (Birner et al., 2015). A further limitation is the fact that it is not possible to border large periventricular from confluent deep WMH precisely enough with volumetric analysis, which restricts is use in this point compared to the more commonly used rating scales (Kapeller et al., 2003).

Another issue that needs to be addressed is the acquisition of the number of disease episodes (lifetime manic and depressive episodes). The method used for this assessment was a

structural clinical interview (SCID-I) performed by a specialist of psychiatry or a clinical psychologist. It relies on the personal impression of the investigator related to the information provided by the medical history and the patients remembered episodes. Even though the interview is "structured", it arguably leaves some space for the investigators exploration style. This is not a bad thing, as its intention is to find the true answer for every item of the interview. On the other hand, there are factors that possibly can distort the actual number of the episodes recorded. The most common failure of this method is probably that mild episodes of hypomania and depression tend to be forgotten over time or may not even have been recognized at all. Persons with rather theatrical personality features or certain comorbid personality disorders might also narratively over-present bipolar related disease episodes. Investigators may differ in their judgment of what to count as criteria fulfilling episodes based on the way of their exploration technique and depth as well as on how they interpret the episode related information given by the patient, especially in terms of individual credibility.

The reasoning for the choice to analyze the combined gray volume of the anterior limbic striatal networks (ALSN) proposed by Strakowski and colleagues (Strakowski et al., 2012) was already explained in chapter 1.3. of the introduction part. Our slightly modified version of those ALSN contains about thirty percent of the total gray matter volume and based on the literature seems to be a more precise neuroanatomical base for BD than just using the total gray volume. Total gray matter volume does not seem like a very promising indicator for the suspected neuroprogressive and neurodegenerative processes in BD, as most studies showed volumetric differences in more specific regions, usually those found in our version of the ALSN (see chapter 1.2.).

However, as we do not understand the underlying mechanisms with certainty and this selection is primarily based on an expert consensus opinion model with a few evidence-driven custom additions from our side, this can only approximate the actual truth and therefore can be accounted as a limitation. The model itself might also exclude areas of the brain that are involved in key domains of BD. The original model itself concentrates on emotion regulation and processing, but did surprisingly not include the hippocampus for example, which was pointed out to be of importance in another review (Phillips, Swartz, 2014) and was the most affected subcortical structure from volume reduction in the best-powered meta-analysis so far (Hibar et al., 2016). The original model also did not include the insula and the parahippocampal gyri, which are both involved in emotion processing and cognition and have been described to show abnormal volumes in BD compared to HC in some studies (Ellison-

Wright, Bullmore, 2010, Singh et al., 2008, Ladouceur, Almeida & Birmaher, 2008). Furthermore, while emotional processing and regulation is certainly disturbed in BD, these might not be the only neural circuits relevant to the functional neuroanatomy of BD. Other domains that are disturbed in BD include cognition and vegetative functions as sleep, energy and sexual drive. Because of these reasons, we did not hesitate to add relevant regions to the studied volume variable. The evidence of cortical thinning of the fusiform gyrus (Hibar et al., 2017) was not available at the time this study got conducted and therefor wasn't included. While probably being more accurate than total volume, the use of a combined ALSN volume still sacrifices certain amounts of impact of small, but functional important regions as the amygdala and nucleus accumbens among others, compared to bigger structures as the prefrontal cortex.

Another critical aspect might be that we did not use the in Freesurfer integrated generalized linear model (GLM) that is able to calculate group differences on each and every cortical vertex and is able to control for multiple comparisons due complicated methods of permutation. That way, Freesurfer can produce cortical maps that show clusters of vertices that differ between groups or correlate with a specific variable above a set threshold of significance. Freesurfer cannot do the same with subcortical structures and therefor, these regions must be analyzed separately. The main reason for us to dismiss this method in this first analysis was that we wanted to keep things as simple as possible. We wanted to have one convenient variable to easily test specific hypotheses with classical statistics and were not looking for small super-significant clusters around the cortex and complex permutation based controlling for multiple comparisons. Nevertheless, this is an interesting approach to further analyze our data in a next step.

As already mentioned above, this analysis is also limited by the female subgroup of the HC, which had a lower BMI than the male HC subgroup. While there is no statistical significant difference, the female HC subgroup (n=54) also outnumbers the male HC subgroup (n=32) by trend.

Additionally, as our analysis is cross-sectional and we have no clear information on treatment time and baseline volumes before drug treatment in the studied subjects, which limits the weight of our medication related findings. Another limitation concerning the pharmacological treatment is that the group of atypical antipsychotics consists of seven different agents (amisulpride, aripiprazole, clozapine, olanzapine, quetiapine, risperidone and ziprasidone) and

we did not included dosages into the calculation, which should be considered as well. Furthermore, many BD individuals received combinations of psychopharmacotherapy across drug classes and according to the latest meta-analysis results each class was associated with different kind of brainstructural changes (Hibar et al., 2016, Hibar et al., 2017).

In comparison to high-powered meta-analyses, where usually different kinds of field strengths as well as different program versions of Freesurfer are used, which both are sources of heterogeneity between included samples, advantages of this study were the acquisition of data in a single center, same inclusion/exclusion criteria for all patients and the performance of the identical protocol on the same MRI scanner with 3 Tesla and the use of the same version of Freesurfer as well as the selection of all WMH with the same criteria by a single rater and the volumetric quantitative lesion segmentation allowing refined statistical analyses.

4.2 Conclusions

Concerning WMH, our results underline the increased occurrence of WMH in individuals with BD and their associations with variables of clinical course. A specific sex effect was revealed, as the number of affective episodes correlated positively with WMH-load only in male bipolar study participants. The moderational influence of sex suggests a potentially contributing role of endocrinological and/or pathogenic mechanisms that differently affect male and female individuals with BD. BMI did not show a relation to WMH in BD. We propose that men are more vulnerable to mania in the context of ischemic brain alterations as confluent sub-types of WMH or stroke, which should stimulate further research into this area (Birner et al., 2015).

Concerning the ALSN volumes, our results give additional reason to include proxies of obesity/metabolic syndromes (like BMI, body fat content, WHR) and sex as moderating factors for the analysis and interpretation of neuroimaging findings in BD and likewise even in other neuropsychiatric conditions that tend to present in a recurrent style of course such as schizophrenia, major depression, multiple sclerosis and epilepsy. Furthermore, it might even put a doubtful light on existing study results, as differences between BD and HC might have just been visible to the published extent because of the higher occurrence of overweight and obesity in the BD samples, which has not been controlled for.

In our cohort, individuals with BD that received continuous treatment of atypical antipsychotic medication at the time point of the study showed a highly significant reduced

gray volume of the ALSN compared to individuals with BD without atypical antipsychotics. This finding is only cross-sectional but receives some reinforcement from schizophrenia-centered studies, thus presenting additional reason for further research in this topic. A longitudinal design in a generously sized cohort, preferable drug-naive, over a long period of time with exact assessment of medication intake is needed to elucidate those potential pharmacologically effects on brain structure in BD. Additionally, microstructural assessment with DTI would be of great interest to include in this kind of study design.

4.3. Future Directions of Neuroimaging in BD

In my opinion, the most common problem for understanding the neuroimaging aspects of bipolar disorder comes from the heterogeneous presentations of the disease itself (i.e. BD I, BD II, BD n.o.s., rapid cycling, mixed episodes, psychotic features, cyclothymia, BD spectrum 0,5-VI…), the clinical overlapping in a dimensional perspective with other psychiatric disorder categories (schizophrenia, major depression, schizoaffective disorder, attention deficit hyperactivity disorder, some personality disorders, substance and behavioral dependencies and abuse) and the lack of longitudinal designs in highly powered cohorts in perfectly aligned multi-centered collaborating settings. Other confounders are the diverse neuroimaging methods themselves with the different ways to compute the data and to analyze them statistically. Especially fMRI data sets produced are of incredible size and are challenging to be filtered in an appropriate way.

Testing treatment response in longitudinal fMRI studies is believed to be a potentially fruitful approach (Strakowski et al., 2012). Advancement of cognitive probes in fMRI studies, in precisely defined subgroups and between mood states, combining imaging methods (fMRI, magnetic resonance spectroscopy, connectivity measures, DTI…) are other possible options to explore brain function and connectivity in BD in the near future.

As most studies seem to be of a BD vs. HC design, it is also not clear what kind of changes are expected being part of "generally mental ill" or are specific to BD, so more studies comparing narrower defined groups of BD to other major psychiatric disorders are needed for a possible better clarification. This should include huge cohorts for example setting groups like schizophrenia vs. schizoaffective disorder vs. BD I vs. BD II vs. major depression vs. HC. A very recent meta-analysis (Wise et al., 2017) revealed evidence for common and distinct patterns of gray volume alterations in BD and major depression which might have the potential for developing diagnostic biomarkers in the future.

The opposite road to the narrowing approach is to dismiss diagnostic categories altogether and concentrate on disturbed neural circuitry domains of emotion processing, emotion regulation and reward seeking among others (Phillips, Swartz, 2014).

Regardless of what path is taken, ideally this should be done in a longitudinal setting combining an overall assessment of brain structure, brain function, cognitive tests, complete clinical phenotyping, gene expression and different biomarkers on early onset and at risk populations and through most of the life-span, with regular follow-ups and additionally in acute affective episodes, distributed over perfectly accorded centers with a close to zero dropout rate. Obviously, this approach needs a lot of know-how and resources combined with high endurance in many people in numerous sites. But in the end this approach might finally yield information on dysregulated circuits combined with molecular biomarkers that improve our understanding of the biological underpinnings in BD and may have enough specifity and sensitivity to be helpful in clinical routine.

Of course, another approach is to use new imaging techniques focusing on microstructure, as the field of neuroimaging keeps constantly evolving. For example, Nazari and colleagues (Nazeri et al., 2017) performed a novel diffusion-weighted imaging analyzation approach of gray matter to investigate regional neuritic density differences in 29 BD subjects, 36 schizophrenia subjects and 35 HCs. The schizophrenia group differed significantly from HC in temporal areas while the BD group was intermediate between those two, but without statistical significance. Regardless of diagnosis, better performance in spatial working memory tasks was significantly associated with higher neuritic density, predominantly in the frontotemporal areas. The associations to disease severity in a dimensional perspective (schizophrenia>BD>HC) as well as to cognitive performance suggest that the utility of this method should further be researched.

Neuroimaging studies usually provide data on a group-level that are not clinically useful at all on an individual level, which might feel unacceptable to some criticists if you consider the huge amount of resources that were and still are invested in psychiatric focused neuroimaging research. But, another combination of approaches might be promising in creating data that might ultimately be helpful in an individual setting. This method is the combination of neuroimaging techniques with computerized pattern recognition approaches that create algorithms based on large data sets (“big data”) to recognize complex patterns to inform decision-making (Phillips, Swartz, 2014). A few studies that used a combination of these

techniques were able, based on patterns of neural activity, to give information that increased the likelihood to classify individuals into diagnostic categories including major depression versus BD (Mourao-Miranda et al., 2012a, Almeida et al., 2013, Grotegerd et al., 2013, Grotegerd et al., 2014). Even more, they were capable to differentiate young individuals with high genetic risk for BD from young individuals with low genetic risk for BD (Mourao-Miranda et al., 2012b), making this methodology one of the more interesting approaches for future research and replication studies as it actually yields the possibility of real clinical utility.

Besides fMRI, also structural MRI can be used in a pattern recognition approach. While fMRI might be more accurate for this kind of method, its use in clinical practice might be flawed by the longer measurement times and the difficulty for suffering patients to fulfill specific tasks (Kim, Na, 2018). The current state of the machine learning based-brain structural MRI approach is that the findings altogether are seriously restricted by confounders and sample sizes, thus again sophisticated classification of clinical and diagnostic subtypes and multi center-approaches are needed to test this promising methodology, while improvements in machine learning technology are necessary to integrate additional clinical data into the computing models (Kim, Na, 2018). It might not be a coincidence, as most of the research in BD presents correlations and no causations, that the only tool that might be useful on an individual level is based on correlations as well and we do not fully understand the underlying processes.

After all, the complexity of the human brain appears to be endless, especially when it comes to emotions. The neuroscientific fields investigating the neurobiological underpinnings of BD are putting together puzzle stones, one by one, sometimes removing already set stones and replace them with more promising looking ones. I really wonder if we ever get to see the whole picture and based on that, will be able to develop new detection, prevention or even treatment methods. As nobody knows how many and what puzzle stones are still missing and how many of the ones we believe to know about have been wrongly placed, I sometimes tend to be in line with the physician Emerson M. Pugh, who's quote from estimated 80 years ago I want to cite as the final sentence of my dissertation: "If the human brain were so simple that we could understand it, we would be so simple that we couldn't."

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