CHAPTER 10

Summary and general discussion

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Preface

After describing the characteristics of bipolar disorder (BD), its burden of disease on afflicted persons and society, and the diagnostic and therapeutic struggles faced, this thesis started with a description of the main existent pathophysiological theories on BD. We explained that, based on the available knowledge, the immune system may play a key role in the pathophysiology of BD. Thus, this thesis aimed to clarify the role of the immune system in the pathophysiology of BD via several different approaches. In this discussion we will put our findings into perspective. We start by summarizing our main findings. Then, we consider some methodological strengths and limitations. Subsequently, we place the findings within the perspective of other recent neuroscientific developments and indicate the implications for clinical practice. And finally, we suggest starting-points for future research in this field and end with some concluding remarks.

Summary of main findings

This thesis includes two parts: the peripheral immune system and the neuroimmune system.

Part 1: Peripheral immune system

The first part of the thesis centered around the function of the peripheral immune system in BD, focusing on monocyte pro-inflammatory gene-expression and C-reactive protein (CRP), using bio assay techniques.

Association between monocyte gene-expression and clinical features

Initially, we presented our study on the associations between an extensive set of clinical features and quantitative PCR (qPCR) measured monocyte gene expression in BD (chapter 2). Our a-priori hypothesis that lifetime psychotic features would be associated with the pro-inflammatory monocyte gene expression could not be confirmed. However, based on our newly developed feature-expression heat map method (see also chapter 3) we visualized the following interesting findings in patients with: a possible relation between pro-inflammatory gene expression and manic symptomatology, a differential immune activation related to an earlier age at onset, an increased immune system dysregulation during the course of the disorder, and support for the concept of an immune suppressive action of some of the mood regulating medications.

In chapter 3 we described the newly developed feature-expression heat map method: a combined presentation of effect size and statistical significance in a graphical...
method, added to the ordering of the variables based on the *effect-ordered data display* principle. To visualize the associations of two sets of variables, adapted heat maps are drawn, displaying in the columns the preceding variable set, and in the rows the subsequent variable set. Each are ordered to facilitate the visual identification of meaningful clusters of association later on. An underlying cluster analysis tree may be added to one or both of the axes. In the feature-expression heat maps the associations between preceding and subsequent variables are represented by circles, visualizing the measure of effect size and the statistical significance of the analyses. This combination aids in the visual recognition of association patterns in complex systems, e.g. pathophysiological models.

**Monocyte gene-expression: state or trait?**
To investigate whether the qPCR measured monocyte pro-inflammatory gene-expression is more related to mood state or a marker for disease (trait) we performed the next study, in which we presented the results of the bipolar cohort of the MOODINFLAME study¹ (chapter 4). We demonstrated an elevated pro-inflammatory monocyte gene-expression in patients with experiencing a mood episode, as compared to both healthy controls (HC) and euthymic patients with. Furthermore, we found patients with experiencing a mood episode to have an increased inflammatory gene expression compared to when they were euthymic. This indicates that inflammatory gene expression in BD is related to the mood state, rather than being a trait marker.

**Does CRP predict outcome in clinical practice?**
Subsequently, we examined whether higher CRP levels predicted a worse BD outcome in a clinical setting, defined as a shorter time to relapse or a longer time to recover, depending on the mood state at baseline (chapter 5). We found no statistically significant association between CRP and a more unfavorable BD prognosis, suggesting that the application of CRP as a practical biomarker to predict outcome in a naturalistic outpatient care setting is not as straightforward as it may seem. In a first cross-sectional analysis, we could not distinguish a sub-group of patients with with an elevated baseline CRP level based on affective state. In the longitudinal analysis, no statistically significant association was found between higher CRP values and relapsing in either euthymic or non-euthymic patients, as well as when comparing them.
Part 2: Neuroimmune system
In the second part of this thesis we investigated the function of the neuroimmune system, focusing on microglia activation, using positron emission tomography (PET), magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), and diffusion tensor imaging (DTI) as neuroimaging techniques.

Previous PET/SPECT studies
We first presented a literature review of the previous PET / single-photon emission computed tomography (SPECT) research efforts on BD (chapter 6). The earliest PET/SPECT studies, mainly focusing on metabolism and blood flow, looked at various aspects of the metabolism based disease model in which prefrontal cortex (PFC) hypoactivity is accompanied by limbic hyperactivity. However, in its comprehensive form this model is probably not precise enough to account for most of the specific mood and cognitive disease features. Molecular imaging demonstrated the importance of serotonin transporter alterations in parts of the limbic system in BD and underscored the role of dopamine and cholinergic neurotransmission. We observed that most molecular imaging studies in BD have unique designs, extending our knowledge of the pathophysiological mechanisms, but also complicating comparisons between studies.

Microglial activation in the hippocampus
We subsequently performed a neuroinflammation PET study in BD (chapter 7) and demonstrated a statistically significant increased binding potential of $[^{11}C]-(R)$-PK11195 in the right hippocampus and a similar but trend level increased binding potential in the left hippocampus of bipolar I disorder (BD-I) patients as compared to healthy controls, indicative of microglial activation.

Associations between volume, metabolites and microglial activation
Next, we investigated the relations between volume, metabolites and microglial activation of the hippocampus in a contemporaneously executed PET/MRI study (chapter 8). Using MRS, we demonstrated a decreased concentration of N-acetylaspartate (NAA) + N-acetyl-aspartyl-glutamate (NAAG) in the left hippocampus of BD-I patients as compared to HC. Using volumetric MRI, we were not able to prove decreased hippocampal volumes between these groups after correcting for individual whole-brain volume variations.

In the subsequent explorative analyses that were executed in accordance with an a-priori analysis model, we identified a positive association between microglial activation and the NAA+NAAG concentration in the left hippocampus, indicating a positive relation between microglial activation and neuronal integrity in vivo. In these analyses, we furthermore found positive associations between alcohol use and NAA+NAAG concentration, and between microglial activation and the depression...
score, and a negative relation between the creatine (Cr) + phosphocreatine (PCr) concentration and experienced occupational disability. Duration of illness was also bilaterally associated with hippocampal volume.

**White matter microstructure disturbances and lithium usage**

Finally, using DTI we investigated white matter microstructure in BD-I and HC, and differences related to lithium usage among patients (chapter 9). In this study we could not demonstrate a difference in fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD) and axial diffusivity (AD) between BD-I patients and HC. Our null finding might suggest that white matter microstructure disturbances are less outspoken in patients with not experiencing mood symptoms. Lithium-using patients, when compared to non-lithium-using BD-I patients, amongst others demonstrated a higher FA and lower RD in the corpus callosum and left anterior corona radiata. Widespread clusters demonstrated negative FA associations and positive RD and MD associations with minor depressive symptoms. Differences in FA between patients using and not using lithium suggest a counteracting effect of lithium on white matter microstructural disturbances.

**Methodological considerations: limitations and strengths**

Each chapter of the thesis includes a discussion of the strengths and limitations of the specific issue addressed. Here we will highlight the most prominent limitations and strengths of the thesis as a whole.

**Multiple testing and two-step analysis approach**

Since pathophysiologically oriented research into psychiatric illness is intrinsically complex, often multiple variables are incorporated into the analysis models in an attempt to clarify the mechanisms involved. This can lead to an increased probability of false positive findings (type I error) in statistical analysis. This problem is especially important in confirmatory studies, where in the analyses a priori postulated hypotheses are either preserved or rejected. Because the conclusions in a confirmatory study are assumed to be robust, the consequences of a false positive finding are generally more far-reaching than those of a false negative finding (type II error). In contrast, exploratory studies have a distinct, hypotheses-generating goal. Therefore, the consequences of type I and II errors weigh differently in exploratory studies than in confirmatory studies; in exploratory studies it is the false negative findings that pose the greatest problem. In other words, it is more of a problem to miss clues for a novel hypothesis. Even if this means one needs to accept that there is a chance
of developing hypotheses based on findings that are not actually true. This problem is
dealt with in the scientific process whereby exploratory generated hypotheses must
be evaluated by confirmatory studies, and with the reservation that the results of ex-
ploratory studies should be reported as providing only preliminary information. In the
context of an exploratory study, one may argue that results should not be presented
statistically as positive or negative findings but rather should be presented only as
estimates with a confidence interval.

Although the following viewpoint is not unanimously held, multiple sources state that
multiplicity adjustment is not required in exploratory analyses (and in fact would in-
crease the risk of type–II errors), provided that these results are reported as providing
preliminary, indicatory information on relationships\(^2\text{--}^4\).

We reckon hypotheses-generating exploratory studies to have their own merits next
to hypothesis-driven confirmatory studies. We therefore constructed several of our
studies (chapters 2, 7, 8) in two parts: a hypothesis-driven group analysis part with
confirmatory objectives and an exploratory part with results that do not claim to
provide rigorous evidence, but have an exclusively hypothesis-generating goal.

When dealing with the problem of multiplicity of data in our studies, we followed
several somewhat different strategies. In most studies we applied correction for the
false discovery rate (FDR), as described by Benjamini and Hochberg\(^5\text{,}^6\) (chapters 2,
7, 8). The FDR method has several advantages over the Bonferonni method, which
is regarded as being more conservative\(^2\text{,}^7\). In chapter 4 we followed a different ap-
proach because the expression of the individual pro-inflammatory genes is not really
independent; we have here instead measurements of a coherent pro-inflammatory
cell function. To obtain a single measure of pro-inflammatory gene activation in
monocytes the expression of multiple genes was reduced to a gene score, leav-
ing further adjustments unnecessary. In chapter 5 we used simple measurements
and applied no adjustment for multiple testing. The complex volumetric and DTI
neuroimaging techniques in chapters 8 and 9 used specific methods to deal with
multiplicity, based on cluster finding\(^8\text{,}^9\).

**Study design issues**

As with most pathophysiological research in humans, the studies in this thesis are
limited by several design issues.

**Cross-sectional case–control designs**

Cross-sectional case–control designs, as applied in most of the studies in this thesis,
have the advantage that they are relatively simple to organize. They are, however,
suboptimal for analyses of cause and effect and therefore in the scientific process
should be followed by a prospective cohort study\(^10\). The distinctive feature of a pro-
spective cohort study is that at the time of collecting baseline exposure information,
none of the subjects have developed any of the outcomes of interest and subjects are followed longitudinally. After a period of time one can investigate if and when they become diseased and whether their exposure status changes outcomes. In this way the prospective cohort study data can be used to answer many questions about the associations between “risk factors” and disease outcomes.

Sample size
Another complicating factor is that the studies in this thesis are intrinsically limited in patient sample size because of careful ethical and economic considerations. Unarguably, the research would have profited from a larger sample size, thereby increasing its statistical power, while enabling comparisons between subgroups. This limitation is also related to the problem of the risk of increased type I-errors in multiple analyses, as described above.

Naturalistic design
Naturalistic designs in which patients are treated regularly and no interventions are carried out by the investigators, have the advantage that in general they are easier to perform and are less burdensome for patients with this serious psychiatric disorder. However, they do not take into account the possible confounding effect of concomitant medication use. In the studies of this thesis, all patients were treated naturalistically and none of them was ‘medication naive’; this may have led to medication effects in the observations, revealing false findings or concealing true ones. For instance, and very relevant, it is known that most mood stabilizing medications, including lithium, anticonvulsants and antipsychotics, as well as several antidepressants (SSRI’s, clomipramine, imipramine, MAO inhibitors) have an effect on the immune system\textsuperscript{11–16}. In general, their effects are thought to be immunosuppressive in nature. Thus, it can be argued that in the present study most medications would actually have reduced the effect of the observations or may even have concealed them.

In this light it must be noted, contrary to the communis opinio, studies with medication-naïve patients and patients in whom medication was stopped (washout studies) are also not without their flaws. The obvious advantage of studies with medication-naïve patients is the exclusion of these medication effects. The question arises, however, in how far the selection of these patients who are able to function without medication, interferes with the investigated mechanism (i.e. affecting the internal validity) and limits the generalizability (i.e. affecting the external validity). In washout studies one could argue that the withdrawal effects interfere with the investigated mechanism.
Pieces of the immunological puzzle

The studies in this thesis focus, among other things, on monocyte gene-expression, CRP and TSPO receptor presentation, which are select parts of the complex immune system. Therefore, generalized statements should be considered in that regard, e.g. the gene-expression studies investigated only inflammatory gene expression of monocytes, which make up around 2-8% of the total white blood cell population. The original selection design of these gene-expression studies was based on the study of Padmos et al., who found these specific signature genes, possibly ruling out other important genes.

In the PET studies, increased $[^{11}C]$-(R)–PK11195 binding to the TSPO receptor in the brain is traditionally related to microglia activation. It is important to note that the TSPO receptor can also be expressed in astrocytes, potentially influencing the $[^{11}C]$-(R)–PK11195 binding potential signal. However, because both cells are known to contribute to neuroinflammation, it can be argued that regardless of whether activated microglia cells or astrocytes are responsible for the increased TSPO expression, the increased $[^{11}C]$–(R)–PK11195 binding most likely represents a neuroinflammatory process.

Innovative techniques

Despite these limitations, we do consider the studies in this thesis to have several key and innovative strengths and they provide important findings to guide further hypothesis-forming and serve as a starting-point for future studies.

Our study of the associations between clinical features and monocyte gene expression was the first study to investigate the associations between psychotic, manic, and depressive symptoms and gene expression in such an extensive fashion and can be regarded as a next step in the converging approach between immunology and psychopathology. Especially, the feature-expression heat map method which we developed proved to be a useful graphical instrument to visually explore associations in complex biological systems where one-way direction is assumed; this method has also been adopted by other research groups.

Furthermore, using novel, innovative techniques we were able for the first time to investigate microglia activation in vivo, thereby providing direct evidence for the neuroinflammation theory, which has been reviewed extensively in the literature, but thus far solely based on studies using indirect measurements. Moreover, we were also able to investigate associations between microglia activation, metabolites and volume of the hippocampus. Finally, we were one of the first research groups to focus on the effect of lithium on white matter microstructure in BD.
Main findings in perspective

The aim of this thesis was to clarify the role of the immune system in the pathophysiology of BD via several different approaches. In this section we will place those findings within the perspective of other recent neuroscientific developments and suggest starting-points for future research.

Biomarkers or dysfunctional processes: bankruptcy of the biomarker approach?

The current psychiatric diagnostic systems are descriptive taxonomies based on classifications of phenomenology (Diagnostic and Statistical Manual, DSM; International Statistical Classification of Diseases and Related Health Problems, ICD). Psychiatric disorders thus traditionally lack the biological foundation that is an essential part of medical disease. Since the 1960s, in attempts to extend the diagnostic and treatment options for psychiatric patients, biological psychiatric research has sought incessantly to support the diagnostic system with robust psychopathophysiological models. Examples of such models for BD are described in chapter 1. Unfortunately, all these research efforts have yet to yield the promised clinical significance: clinical biomarker tests are still not available and novel pharmaceutical treatments appear only sparsely.

Back in 2009 the MOODINFLAME project started off with the development of biomarkers for mood disorders as one of its important goals. Before the start of this project, Padmos et al. defined a promising monocyte pro-inflammatory gene-expression signature that was able to discriminate patients with BD from HC. The study population in the Padmos study consisted of a mixed patient sample with euthymic, depressed and manic patients. In this study the signature was also found to be present in the offspring of patients with bipolar disorder, thereby fulfilling some of the criteria of an endophenotype. Endophenotypes are, by definition, more related to the underlying genotype than to the ultimate phenotype. Endophenotypes should be consistently associated with the illness and represent persistent “trait” rather than episodic or “state” features. By definition, they also should be found at a higher rate in high-risk individuals, such as non-affected first-degree family members, than in the general population.

Further elaborating on this, endophenotypes in psychiatry could function as diagnostic biomarkers. Biomarkers are conventionally defined as characteristics that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Thus in general, biomarkers may be used as an aid to diagnose a disease, or to predict or follow treatment response. In medicine as a whole, biomarkers may include hormones, proteins and genetic markers at the levels of DNA and RNA, but also...
structural and functional alterations that can be visualized with imaging techniques. In the process of further validating the monocyte pro-inflammatory gene-expression signature as a biomarker, the first problems arose with regard to the specificity of the test. Drexhage et al. demonstrated that the gene-expression was also increased in schizophrenia (SZ), albeit a bit differently. In a follow-up analysis we found indications that some of the gene-expression was associated with manic symptomatology (chapter 2). When analyzing the MOODINFLAME gene-expression results, already having abandoned the biomarker hypothesis, we found monocyte pro-inflammatory gene-expression not significantly different in euthymic MOODINFLAME BD patients, compared to HC (chapter 4). Instead, increase of the pro-inflammatory gene-expression was more associated with being in a mood episode (i.e. ‘state’). Furthermore, the use of CRP, which is considered to be another promising candidate as a biomarker for psychiatric disorders, for predicting prognosis in clinical practice also proved to be not as straightforward as it may have seemed (chapter 5).

The process of initially promising biomarker candidates for psychiatric disorders that subsequently fail in validation steps is more a rule than an exception. Although at first glance this may seem problematic and unfortunate, it may not be an entirely bad thing. Replication is not only important when findings are reproduced, it is equally vital when results are nuanced or not reproduced. In other words, while innovation points out possible new paths, replication points out likely paths, and progress relies on both. Undeservingly, performing replication studies is underappreciated. In the scientific enterprise individual scientists are often prompted to prioritize novelty findings from explorative studies, even when not yet confirmed, over findings from replication studies. Grant applications focusing on pioneering work instead of replication studies are known to have a higher chance of getting accepted. And a related problem is getting the findings from replication studies published. This is one of the reasons behind publication bias: journal reviewers and editors may dismiss a new test of a published idea as uninspired.

Besides these problems, it has been argued that the development of neurobiological markers for mood disorders has been impeded by several factors: complexity of the brain (in both normal physiology and pathophysiology), difficulties with access to brain tissue, and relatively poor permeability of the brain to investigational neuroimaging ligands because of the blood-brain-barrier. Difficulties with regard to the blood-brain-barrier and with access to cerebral tissue are inherent to the biological nature of the structure and are deemed irresolvable. The notion that the brain is an extremely complex structure can, however, provide a way to understand why biomarker development for our current psychiatric disorder classifications is problematic.

The dysfunctional processes in psychiatric disorders are not limited to one physiological level, e.g. the cellular level or neuronal circuit level. To the contrary, these dysfunc-
tional processes are involved in and influence all the physiological levels of the central nervous system, i.e. the genetic, molecular, cellular, neuronal circuits and phenomenological levels. This multilevel physiology of the brain is called spatial complexity\textsuperscript{47}. An example of this multilevel pathophysiology for BD can be found in figure 1, emphasizing that for a complete understanding of the pathophysiology of BD, its neurobiology must be addressed at different physiological levels\textsuperscript{48}. A problem with many of the simpler versions of the pathophysiological models described in chapter 1 is that they remain locked within one physiological level. In addition, the manifestations of the illness on each physiological level that are investigated as potential biomarkers are often dichotomized into either affected or not-affected, corresponding to a medical model for disease. Yet in the CNS functional

**FIGURE 1**

Multiple pathophysiological levels in bipolar disorder

For a complete understanding of the pathophysiology of bipolar disorder, its neurobiology must be addressed at different physiological levels (i.e., molecular, cellular, systems, and behavioral). Bcl-2 = B-cell leukemia/lymphoma; BDNF = brain-derived neurotrophic factor; CREB= cAMP response element binding protein; ERK = extracellular receptor-coupled kinase; GSK-3 = glycogen synthase kinase-3; MAP kinase = mitogen-activated protein kinase; MARCKS = myristoylated alanine-rich C kinase substrate; PKC = protein kinase C; proteome = the population of cellular protein species and their expression level; transcriptome = the population of cellular messenger RNA species and their expression level. (Source: Manji and Lenox\textsuperscript{48}, reprinted with permission)
pathophysiology, the illness manifestations are probably much more gradual due to the multiple complex interactions\textsuperscript{47}, and such dichotomizations should be considered inadequate or false. As an example with regard to neuroinflammation, i.e. the activated, inflamed state of the immune system as a cause of psychiatric illness in general and BD specifically, this concept can be considered an oversimplification of a complex system in which in fact various (stimulating and inhibiting) aspects are dysregulated.

Based on the spatial physiological complexity of the brain alone, one could argue that endeavors to find pure diagnostic biomarkers for the current psychiatric disorders will probably prove to be unfruitful. However, this does not necessarily mean that observing alterations in bioassays, thereby demonstrating the activity of a dysfunctional state, would not be helpful in predicting or following treatment response. Thus, bioassays would not replace clinical diagnosis, but support and supplement it in the process of treatment decision-making\textsuperscript{36,48}.

**Multidimensional psychopathology: Crossing the Kraepelinian dichotomy**

The false dichotomy problem in our present pathophysiological models is not limited to biological observations. Tracing back to the medical disease model, psychiatric disorders as diagnostic entities are also approached dichotomously in our current diagnostic systems. In other words: persons are either ill, and are called patients, or they are not.

This oversimplification has some overlap with the Descartian dualism and the state of neuropathology in the late nineteenth century, when disorders were divided into those involving functional or organic pathology\textsuperscript{60}. However, a vast amount of neuroscientific evidence has demonstrated the functional – organic dualism to be false\textsuperscript{51,52}. One could even go as far as argue that all psychiatry is biological psychiatry. Or even more extreme: the most effective therapies should be those grounded in biology.

In reality, psychiatric patients present with diverse psychological symptoms (phenomenology) that vary in form and severity, between patients and in the course of time. Historically based on observations, clinicians have recognized prototypic patterns in these symptom presentations, which have evolved into diagnostic classifications that are ordered into categories, i.e. the categorical diagnostic approach. Although improved over time, inter-operator validity is still a matter of debate\textsuperscript{53}. In addition, these diagnostic classifications still largely ignore the unique role of individual symptoms and, consequently, potentially important information is lost\textsuperscript{54}. Besides oversimplified stratification of the symptoms, information about the course of the disorder is also only sparsely utilized in recent versions of the DSM (DSM-III, DSM-IV en DSM-5) and ICD (ICD-9 and ICD-10), respectively.
Starting more than a decade ago, researchers proposed a more dimensional diagnostic approach\textsuperscript{36,55,56}, linking psychiatric symptoms more continuously to the underlying pathophysiology (see figure 2). In an attempt to expand on the dimensional diagnostic approach, the United States National Institute of Mental Health (US NIMH) has initiated the Research Domain Criteria (RDoC) project, which aims to “develop, for research purposes, new ways of classifying mental disorders based on dimensions of observable behavior and neurobiological measures”.

Emil Kraepelin (1856–1926) was the first to make the distinction between the two disorders BD and SZ in what has since been known as the Kraepelinian dichotomy. This dichotomy was long clinically appreciated because it enabled psychiatrists to come to a clear diagnosis in complex clinical settings\textsuperscript{55} and guided them in decision making for pharmacological treatment: treatment with mood stabilizers in BD and antipsychotics in SZ. Yet, two problems arose. First, as many psychiatrists experienced, no point of rarity exists between these diagnoses\textsuperscript{57}, which among other ‘solutions’ resulted in the intermediate classification of schizo-affective disorder. Secondly, the use of antipsychotics in particular has crossed the Kraepelinian dichotomy; these are nowadays also frequently used in BD for manic episodes, and some for depressive episodes\textsuperscript{58,59}.

Besides the clinical cracks in the Kraepelinian model, there is at present multiple evidence for shared pathophysiological mechanisms between BD and SZ. Most, if not all of the pathophysiological BD models described in chapter 1 have variants in SZ, and MDD\textsuperscript{51}. Furthermore, genome-wide association studies (GWAS) have demonstrated the existence of genetic single nucleotide polymorphisms that influence the risk of both SZ and BD\textsuperscript{56,60}. In addition, as described above, Drexhage et al. demonstrated that monocyte pro-inflammatory gene-expression was increased in both BD and SZ\textsuperscript{41}.

On the other hand, it would also be too simplistic to argue that both BD and SZ are the same disorder. There was some distinction between BD and SZ in the gene-expression investigated by Drexhage et al.\textsuperscript{41}. In addition, pre-schizophrenic children are characterized by cognitive and neuromotor impairments, which are not shared (at least not to the same extent) by children who later develop BD\textsuperscript{61}. Finally, there are quite consistent neuroanatomical differences between BD and SZ, e.g. hippocampus volumes are typically decreased in patients with SZ, whereas in most studies hippocampus volumes in patients with BD are not distinguishable from those in HC\textsuperscript{62,63}.

Researchers in genetics, neurobiology and population epidemiology are increasingly inclined to adopt a continuous dimensional diagnostic approach to the variation in symptomatology in order to increase the validity of the diagnostic system. However, clinicians tend to maintain the categorical approach embodied in current classifications such as DSM-5 and ICD-10\textsuperscript{64}. This should not be frowned upon. Current categorical classification systems have indisputably facilitated the development of
Recent studies suggest that some of this data suggesting that there are relatively specific as well as shared risk factors. These findings are fully consistent with earlier genetic studies providing clear evidence for the existence of non-shared genetic variation within and between individuals. Although there is emerging evidence that CNVs have some specificity, the relationship between clinical expression and genetic susceptibility might be due to structural genomic variation (CNVs).

Although we can reject a simple model of separate, unrelated causes of mental retardation, autism and other neurodevelopmental disorders and challenges to the view that these are completely unrelated diagnostic entities. Some cases are highly specific (e.g., Rett’s syndrome), while others are not (e.g., autism). However, we present the data as one large family study that demonstrated a substantial overlap between schizophrenia and autism, and, hence, underlying biological mechanisms. For example, the relationship between clinical expression and genetic susceptibility, disease category that is undifferentiated with respect to the disease categories, the data do not support a model of a single specific cause and are not the same clinical entity. However, there is a gradient of decreasing neurodevelopmental impairment between schizophrenia (to date, variants influencing bipolar disorder seem to be smaller, less likely to be deletions, and have smaller effect size than those associated with schizophrenia). The Kraepelinian dichotomy – going, going . . . but still not gone, and reciprocal increasing gradient of proportion of episodic affective disturbance. (Source: Craddock and Owen, reprinted with permission)

This is a simplified model of a highly complex set of relationships between genotype and clinical phenotype. Starting at the level of genetic variation, DNA structural variation is particularly useful for genetic studies, and there is evidence that DNA structural variants, particularly involving DNA loss, are more likely to affect receptor subunit genes. Single gene variants, particularly involving DNA loss, are more likely to affect receptor subunit genes. In general, even single base-pair changes in a gene may influence multiple biological systems because genes typically have multiple functions and produce proteins that interact with multiple other proteins. For simplicity, only an example of a variant has been shown that influences three biological systems (blue asterisk and arrows) and another that influences only one system (black asterisk and arrow). Variation in the relevant biological systems is influenced by genotype at many genetic loci and by environmental exposures/experiences, both historically during development and currently, to influence the dynamic state of the systems. The relevant biological systems influence the neural modules that comprise the key relevant functional elements of the brain (shown as solid turquoise circles). Typically, multiple biological systems influence each neural module. The (abnormal) functioning of the neural modules together influences the domains of experienced psychopathology and ultimately the clinical syndromes. Some important clinical syndromes have been ordered along a single major axis with a gradient of decreasing proportional neurodevelopmental contribution to causation and reciprocal increasing gradient of proportion of episodic affective disturbance. (Source: Craddock and Owen, reprinted with permission)
many psychiatric treatment options, thereby demonstrating almost indispensable clinical utility\textsuperscript{64}. Therefore, altering the diagnostic system towards a dimensional approach in clinical practice would mean a major paradigm shift, requiring not only the investigation of connections between symptoms (phenomenology) and the underlying neuronal circuits, and cellular, molecular and genetic alterations, but also evidence for the prediction of treatment efficacy for the existing psychiatric treatments, relating them to this approach.

**Bipolar disorder as a glial neurodevelopmental disorder with late clinical presentation**

Traditionally, biological psychiatric research and its pathophysiologic models focused mainly on the neuronal part of the CNS, e.g. brain region activation and neuronal neurotransmission, thereby ignoring the other predominant portion, namely the neuroglia, which consist of glial cells\textsuperscript{65}. As indicated by their name, which translates from Greek as “glue”, glial cells were long thought to be of use mainly as structural supporting cells for the neurons: holding them in place, supplying them with nutrients and oxygen and destroying pathogens. However, at the turn of the century research began to demonstrate that glial cells have important functions in neurodevelopment and synaptic function\textsuperscript{66,67}.

The glial cell population consists mainly of oligodendroglia, astrocytes and microglia. Oligodendroglia create myelin sheaths around neuronal axons to give support and to increase the axonal transmission speed. In addition they provide trophic support by producing glial cell line-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF) and insulin-like growth factor-1 (IGF-1)\textsuperscript{68}. In humans, astrocytes are now known to perform a multitude of functions such as, but not limited to: providing metabolic support as a lactate and glycogen energy buffer, vasomodulation by regulating blood flow\textsuperscript{69}, promoting myelinating activity of oligodendroglia\textsuperscript{70,71}, regulating nervous system repair\textsuperscript{72,73}, and several kinds of signal transmission modulation, including modulation of synaptic transmission\textsuperscript{74} and regulation of ion concentration in the extracellular space\textsuperscript{75}. Microglia are the resident macrophages of the brain and spinal cord, and thus act as the first and main form of active immune defense in the CNS, constantly scavenging the CNS for plaques, damaged neurons and infectious agents. Besides functions relating to the immunoresponse, microglia play an important role in maintaining homeostasis. As with peripheral macrophages, microglial activation could be in an inflammatory sense (M1 macrophages), an anti-inflammatory sense (M2 macrophages), and a regenerating/tissue support sense (M2b macrophages). Animal models demonstrated that microglia are also involved in tissue regeneration and play an active role in neuronal support, i.e. the development of mature synapses during embryogenesis\textsuperscript{76}, pruning synapses postnatally\textsuperscript{77}, regulating neurogenesis\textsuperscript{78} and inducing apoptosis\textsuperscript{17}. 
It may well be the case that some microglial cells induce apoptosis, while others actively facilitate neurogenesis.

Over the last few years these individual parts of the neuroglia have received increasing scientific attention in research on functional CNS disorders, including neurodegenerative disease\textsuperscript{79}, neurodevelopmental disorders\textsuperscript{80} and psychiatric disorders in the strict sense\textsuperscript{81}. In investigating BD in this thesis, we demonstrated activated microglia to be present in the (right) hippocampus of BD (chapter 6). We furthermore revealed a positive relationship between microglial activation and neuronal integrity, consistent with the concept of microglial function differentiation in vivo (chapter 7), thereby underpinning the concept of a key role for neuroglia in BD pathology.

The concept of neuroplasticity, which is the ability of the brain to adapt during adult life, may be of use in understanding glial dysregulation in psychiatric disorders. Based on the principles of Darwinian selection\textsuperscript{82}, the human brain displays an exceptional amount of plasticity, facilitating the adaptability characteristic of the human species\textsuperscript{83}. Recently, evidence arose linking astrocyte anatomical and functional exaptations, and genetic variations, to the complexity of the brain, which seems to be a distinctive feature in humans compared to other species\textsuperscript{84}. In addition, a decreased density of glial cells across all layers was found in a post mortem morphometric study of the supragenual anterior cingulate cortex in mood disorder patients\textsuperscript{85}. Elaborating on this line of thought, it could be hypothesized that glial dysregulation in BD, among other things, leads to a diminished neuroplastic potential\textsuperscript{86,87}. In this respect, it is noteworthy to emphasize that lithium, valproate and antidepressants indirectly regulate a number of pathways involved in cell survival and thereby bring about some long-term beneficial effects on this decreased neuroplastic potential\textsuperscript{88}.

As stated above, one of the essential functions of glial cells lies within the process of neurodevelopment\textsuperscript{66}. Neurodevelopment, the process of creating a mature nervous system able to cope with its complex tasks, can be considered to be the temporal complexity of the brain, another form of CNS complexity in addition to the already mentioned spatial complexity\textsuperscript{47}. In SZ research it has become clear that those patients demonstrate neurodevelopmental problems. For some time this was considered to be a distinctive feature of this disorder, as compared to BD\textsuperscript{86}. However, more recently quite compelling scientific evidence became available which also indicated neurodevelopmental aberrations in BD\textsuperscript{89}. The average total brain volume of patients with bipolar disorder was quite consistently found to be relatively small compared to HC, both in adults\textsuperscript{90} and adolescents\textsuperscript{91}. Furthermore, there are reports of cell migration abnormalities in the neocortex of patients with BD\textsuperscript{92,93}. Supporting this notion, in chapter 2 we demonstrated a possible association between an earlier age at onset and increased pro-inflammatory monocyte gene-expression. This is in agreement with several other studies demonstrating increased morbidity in BD patients with an earlier age at onset\textsuperscript{84–96}. In the Dutch bipolar offspring study, Mesman et al. demon-
strated that during adolescence, bipolar offspring showed increased inflammatory gene expression in monocytes, high serum pentraxin-related protein 3 (PTX3, an acute phase response protein) levels, but normal chemokine (C-C motif) ligand 2 (CCL2, a chemotactic protein) levels. In adolescence BDNF levels were decreased, while S100B levels, a marker for astrocytes, were normal. Interestingly at adulthood, circulating monocytes had lost their activation state, but CCL2 levels remained increased and both BDNF and S100B were now increased. The study suggests an aberrant neuro-immune state in bipolar offspring, following a dynamic course from adolescence into adulthood.\textsuperscript{97}

Based on these findings it can be hypothesized that neurodevelopmental disorganization, originating in glial dysregulation, may be a fundamental feature of BD which precedes the revelation of the clinical disorder. In BD, the brain probably temporarily overcomes this disorganization via compensation strategies, which are adequate for seemingly unaffected functioning during childhood, although in fact in hindsight many patients appear to have had prodromal symptoms.\textsuperscript{98} Later on, these compensation strategies prove to be inferior to cope with excessive stress in adulthood, leading to psychic decompensation states such as manic, depressive and psychotic episodes (figure 3). Indeed, it has long been known that biological and psychosocial stress influence the nervous system and innate immunity through persistent activation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal-medullary (SAM) axis, while on the other hand, chronic secretion of glucocorticoids and noradrenaline from the HPA and SAM axes can have profound effects on many immune functions.\textsuperscript{99} In these stress-induced decompensation

**FIGURE 3**

Psychiatric and biological course of bipolar disorder

Schematic representation of the activation of the inflammatory response system (IRS) and the development of psychiatric symptoms during the course of bipolar disorder.
Implications for clinical practice

Addition to current diagnostic and treatment options?
As is intrinsic to pathophysiological research, this thesis did not give rise to direct diagnostic and treatment options, although the development of a diagnostic biomarker for BD was one of the initial goals, as discussed above. However, it is our hope that its main merit lies in its contribution of additional pieces of the puzzle that will eventually provide a clearer vision on the causes and mechanisms of psychiatric disorders. Based on the discussion above, some recommendations can be provided for clinical practice.

Gradual transformation to a more dimensional diagnostic system
First, while many researchers are cautiously moving towards a more continuous dimensional diagnostic approach in order to increase the validity of the diagnostic system, clinicians prefer to hold on to the classical categorical approach, because of its accepted clinical utility. Yet, reconciliation between validity and utility properties of the psychiatric diagnostic system is a vital goal to strive for, from the perspectives of both research and clinical practice. Following research, professionals in the clinical field should keep a close eye on dimensional diagnostic developments and be receptive for gradual, evolutionary dimensional alterations of the diagnostic system. This requires some re-evaluation of the status of our current diagnostic system, which is held in high esteem. The DSM and ICD are not nature-given, but man-made and have their utilitarian merits and their validation flaws. We should therefore be transparent about these uncertainties, both in our own stance towards the diagnostic system and in the communication with our patient. This needs to be done carefully without completely removing the basis of diagnosis, which is essential for both research and for clinical psychiatric practice.

One move towards such a goal is to reappraise the vulnerability concept in our diagnostic systems. In the current psychiatric population, amongst others, patients with psychotic and manic vulnerability can be recognized. In this respect, patients with a manic vulnerability can be defined as patients that have had a previous manic or hypomanic episode, have a higher risk for recurrence (and ideally even first occurrence) of manic and depressive episodes, and can have cognitive problems, resulting in functional disabilities in daily life. Minimizing the effects of this vulnerability by
maximizing resilience is the target of the treatment approaches in these patients, requiring amongst others, psycho-educational programs, medication and psycho-therapy\textsuperscript{103}. The vulnerability concept better grasps the developmental properties of the disorders and, because of its normalizing character, probably would facilitate the patient’s acceptance of having a psychiatric disorder.

**Promising immune system related treatment options for bipolar disorder**

Based on the growing evidence for a role of the immune system and the neuroglia in BD pathophysiology, existing immune system targeting drugs have been investigated for treatment efficacy in BD and other disorders\textsuperscript{104}. N-acetyl-cysteine (NAC), non-steroid anti-inflammatory drugs (NSAID) and omega 3 fatty acids (O3FA) have gained the most scientific attention. Although at this time there is inadequate evidence to support its widespread use, NAC shows the most promising results, being successful in lowering depressive symptomatology in four of five trials\textsuperscript{104,105}. It is usually well tolerated and possesses anti-inflammatory effects, possibly via inhibiting NF-κB and modulating cytokine synthesis, and enhances the availability of glutathione, thereby affecting the NMDA and AMPA receptors\textsuperscript{105}. Both NSAIDs and O3FAs produced conflicting results\textsuperscript{104}. Some studies in psychosis did report a beneficial effect of celecoxib, a cyclooxygenase-2 inhibiting NSAID, especially in the early stage of illness onset\textsuperscript{104,106,107}. Of the studies on O3FAs one neuroimaging study observed a short-term effect in membrane fluidity and neuronal activity in BD, but this effect was not reflected by a change in depression score\textsuperscript{108}. O3FAs were, however, able to reduce the risk of progression to psychosis in another study with individuals at high risk for psychosis, emphasizing the importance of timely treatment\textsuperscript{109}. It has to be noted that these findings are also interesting in light of the above discussion of the Kraepelinian dichotomy.

**Future research perspectives**

When establishing the spatial and temporal complexity of the brain as important contributors hampering pathophysiological research in biological psychiatry, several important points for further research in BD need to be addressed.

**Study design**

First, to elucidate the associations between various biological and phenomenological observations, aberrations on the individual physiologic levels of functioning need to be compared to each other, thereby stressing the importance of multimodal studies using multiple bioassay (genetics, cellular), neuroimaging (neuronal circuits) and interview (phenomenological) techniques.
Second, given the limitation of the dimensional diagnostic approach, one should invest in precise descriptions of the psychiatric phenomenology with predefined dimensions (e.g. manic, depressive, anxiety, and psychotic), using individual symptom profiles at the moment when the observational data are acquired. Besides profiling, stratification of the patients by means of staging techniques can help in coping with the (temporal) complexity of the brain, without falling into dichotomization\textsuperscript{110,111}. Research has already demonstrated some prognostic biomarkers to be useful as an adjunctive tool in staging BD\textsuperscript{112}.

Third, longitudinal studies acquiring at least two time points (and preferentially more) are essential for elucidating cause and effect associations, both within and between the various pathophysiological levels. In addition, they are also essential for improving our understanding of the developmental aspects of BD. When attempting to elucidate the developmental aspects of BD, offspring studies are of special importance; these studies not only provide an elegant and valid method to study the familial transmission of BD, but they also make possible the identification of the early trajectory of BD and the earliest aberrations\textsuperscript{97}. Twin studies provide another perspective, revealing the absolute and relative importance of environmental and genetic influences on the development of BD\textsuperscript{113}. Last but not least, randomized trials are longitudinal studies that can provide the highest level of causal relationships, as in many other areas of research.

Finally, due to difficulties with accessing the brain tissue, complex cross-sectional and longitudinal studies in humans ultimately will not be able to answer all the research questions. Therefore, animal and laboratory models provide essential contributions, although they obviously must meet the highest ethical standards. Many of the more recent developments in biological psychiatric research would not have been possible without the scrutinizing research performed on these models to reveal the fundamental biological and biochemical processes involved.

**Pharmacological considerations**

**Focus on relapse prevention in psychoimmunological treatment**

As discussed above, based on current research it can be reasoned that glial cell dysregulation, together with inadequately coped neurodevelopmental disorganization, plays an important part in BD pathophysiology, where stress induced decompensation states give rise to neuronal circuit aberrations and metabolic alterations that are associated with mood episodes. Contrary to many of the current pharmacological treatments that have their main effect on these decompensation states, it can be argued that the goal of immune system targeting drug candidates should be to improve the stability of the dysregulated glial cells. In doing so these potential psychoimmunological treatment strategies should probably primarily target the prevention of relapses or exacerbation of the disorder.
Interestingly, this is something lithium already does. Lithium is renowned for its efficacy in treating BD, where response to lithium can almost be considered pathognomonic, and for its multiple points of action. Lithium acts on several second messenger systems that underpin its regulatory effects on neurotransmission and its neuroprotective properties. It modulates neurotransmission via several mechanisms. Over time, these processes modify gene transcription within the cells and yield long-lasting mood stabilization. Additionally, lithium reduces the oxidative burden caused by mood episodes and protects against apoptosis by promoting neuroprotective pathways and facilitating the actions of neuroprotective proteins. Furthermore, it inhibits glycogen synthase kinase 3 beta (GSK-3β), which besides regulating glycogen synthesis, is also involved in gene transcription, synaptic plasticity, cell structure and resilience. Finally, lithium also inhibits pro-apoptotic proteins and processes, e.g. autophagy. It has been suggested that the unifying effect of all these functions lies within neurotrophic actions on neuronal and glial cells.

To increase our understanding of the multilevel BD pathophysiology, it is important to investigate longitudinally the versatile therapeutic actions of lithium not only on the cellular level, but also on the neuronal circuits level, and link these to the phenomenological alterations, both before and during or during and after lithium treatment.

**Concluding remarks**

As a consequence of the research techniques that have become available over the last few decades and the insights that are thereby provided, these are very exciting times to perform psychopathophysiological research, pushing our knowledge forward, always with the ultimate goal to further improve the lives of our patients. With this thesis we hope to contribute additional pieces of the puzzle, eventually providing a clearer vision of the causes and mechanisms of psychiatric disorders than we have today. The neuroscientific research in this thesis adds to the important perception that glial cells play an important, and perhaps central, role in BD pathophysiology. Furthermore, increasingly evidence arises emphasizing the importance of looking at BD from a neurodevelopmental and transdimensional perspective to better understand its origins and its course. Understanding the spatial and temporal complexity of the brain, and the implications that these complexities pose for our thinking about BD pathophysiology, are essential to achieve further advancement in this field of research, unravelling the dysregulated brain.
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