Bipolar or unipolar?
A brain teasing question
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Chapter 7

General discussion
The aim of this thesis was to identify differences in brain structure and brain function between major depressive disorder (MDD) and bipolar disorder (BD), which may provide useful candidate markers for differentiation between the two. Finding biomarkers for MDD versus BD differentiation is important: clinically both disorders can be hard to distinguish, due to comparable depressive symptomatology, and underreporting and delayed occurrence of (hypo)manic episodes. Early in the course of illness, BD subjects presenting with a major depressive episode are frequently misdiagnosed as MDD, which may result in inappropriate treatment, thereby worsening outcome and prognosis. We compared an MDD sample with relatively low risk of comprising late MDD-to-BD converters, and a BD sample comprising both BD-II and BD-I subjects. Importantly, whereas previous studies generally investigated subjects while using psychotropic medication (antidepressants and mood stabilizers, i.e. antipsychotics and anticonvulsants), our samples were free of such patients, thereby precluding confounding effects of psychotropic agents. Furthermore, we investigated two mood states, allowing us to distinguish between state and trait effects. In this chapter, we will discuss the main results of the studies described, as well as implications and recommendations for further research.

**Main results**

In *chapter two*, we reviewed existing literature on emotion regulation in depressed MDD, to complement the key review on this subject in BD. Although the lack of consistent positive findings and the great amount of null findings was striking, a general pattern of increased activity in emotion regulation areas during automatic regulation processes emerged, implying the need for recruitment of additional neuronal resources during the initial stages of emotion regulation. We speculated that during early, automatic stages, depressed MDD subjects may be capable of successful emotion regulation, but only by recruiting additional lateral prefrontal neuronal resources. However, during explicit voluntary control, when the emotional experience is already ongoing, this strategy of additional recruitment of lateral prefrontal structures seems to fail. Extensive comparison with emotion regulation in BD was – and still is - hampered by the lack of emotion regulation studies in *depressed* bipolar disorder.

In *chapter three*, we directly compared a voluntary emotion regulation process (distancing, i.e. becoming a detached observer in
order to achieve an emotional neutral state) between MDD and BD. In remitted BD, activity of the inferior frontal gyrus (IFG; extending to the middle frontal gyrus, MFG, BA 46/9) was increased compared to activity in remitted MDD and HC, paralleled by decreased emotion regulation success irrespective of the type of emotional picture presented (sad, fearful, happy). We interpreted this finding in BD subjects as an indication of ongoing compromised emotion regulation during remission, with ineffective utilization of the dorsolateral prefrontal cortex (DLPFC). In contrast, in remitted MDD subjects there were no behavioural or neural abnormalities, so therefore emotion regulation may normalize in MDD upon remission.

During the depressed state, the situation was more complex. Depressed MDD subjects performed equally badly at distancing from happy and from sad pictures, whereas depressed BD subjects performed badly at distancing from sad, but in contrast to our expectations, well at distancing from happy pictures. This finding was paralleled by differential rostral anterior cingulate cortex (rACC) activity: in depressed BD rACC activity decreased during distancing from happy, and increased during distancing from sad pictures; in depressed MDD, there were no rACC activity differences. These results may be explained by our finding that, whereas depressed BD subjects did experience happy pictures as happy and sad pictures as sad, depressed MDD subjects experienced both type of pictures as sad. Also, intensity of evoked emotions was less in depressed MDD than in depressed BD, or in other words, there was a blunting of emotional experience in depressed MDD. Thus, for depressed BD subjects, happy pictures evoked mood incongruent emotions, which may consequently have been easy to distance from. In addition, distancing from these mood incongruent emotions may have resolved an emotional conflict. Because the rACC is a region involved in processing of emotional conflict, the observed deactivation of the rACC is in line with this resolution of emotional conflict by distancing. Likewise, sad pictures evoked mood congruent emotions, complicating distancing; in turn, attempts to distance may have resulted in emotional conflict, thus activating the rACC. In depressed MDD subjects, on the other hand, both happy and sad pictures evoked mood congruent emotions, hence distancing from those pictures was equally difficult and there was no difference in rACC activity between happy and sad pictures.

In chapter four, we examined the neural correlates of visuo-spatial planning. Here, we found that increasing planning demands were
associated with increased parietal activity in depressed compared to remitted patients in general, and BD compared to MDD subjects. In addition, visuo-spatial planning *per se* was associated with increased fronto-striatal activity in depressed BD compared to depressed MDD. This indicates both quantitative (depressed BD requiring the most parietal activity to perform visuo-spatial planning, followed by depressed MDD, remitted BD and finally remitted MDD) and qualitative (state-dependent fronto-striatal hyperactivity during planning specifically in BD) differences between MDD and BD. In other words, both common (implying a mood disorder spectrum) and distinct (implying discrete MDD and BD entities) abnormalities exist.

In *chapter five*, we investigated structural cortical differences in MDD and BD. Our results indicate the presence of subtle subcortical volume changes associated with diagnosis, possibly especially in MDD subjects, with smaller thalamus and caudate and larger accumbens favouring an MDD diagnosis. Also, we found a general pattern of less gyrification and smaller surface area in MDD compared to BD in widespread regions, amongst others medial (pre)frontal, lateral (pre)frontal, temporal and parietal regions. Furthermore, evidence for state effects was strongest in MDD subjects, including frontal, parietal and lingual regions. Thickness was decreased in the temporal cortex in depressed MDD.

In *chapter six*, we applied multivariate pattern analysis (MVPA) to investigate whether we could classify individual subjects as being either MDD or BD. We used resting state functional and structural MRI data, since these are relatively easy to obtain and relatively stable compared to task-related functional MRI data, which increases future clinical applicability.2 Our major finding was that mood state is an important factor to consider for classification: both default mode network and structural data (volumes of emotion regulation regions) revealed that depressed MDD and BD subjects could be correctly classified above chance level, but that remitted MDD and BD subjects could not.

In summary, the studies described in this thesis provide support for distinctive behavioural, functional neural and structural neural abnormalities in medication-free MDD and BD on a group and on an individual level. Further on, we discuss the implications of these findings for the neural models for mood disorders, for the conceptual thinking about MDD versus BD and for the development of diagnostic tools.
Neural circuitry of mood disorders

Observed differences between MDD and BD were qualitatively and quantitatively heterogeneous, concerning different regions and different directions (hypo-/hyperactivity) depending on mood state and on the paradigm employed (emotion regulation, visuospatial planning), the modality (functional MRI, structural MRI) and the measure under investigation (functional activity, functional connectivity, thickness, area, gyrification). These issues complicate reconciliation and integration of findings into a unifying model or theory explaining these differences. Moreover, we aimed primarily to compare MDD and BD; in most cases, comparisons with healthy controls (HC) were done post hoc only for regions where differences were found. Although this approach reduces chance capitalization, additional regions of abnormal activity or structure common to MDD or BD may not be detected. Likewise, any comparisons of depressed and remitted states across or within the MDD and BD groups were performed post-hoc, precluding definite conclusions about state effects. With these limitations in mind, below we discuss our findings, in the context of contemporary neurobiological models of mood disorders.

Emotion regulation circuits in BD and MDD

Emotion dysregulation is considered one of the central features of mood disorders.4–6 Behavioural neurocognitive research suggests that depression interferes with voluntary (effortful) processes, but only minimally with automatic processes.5,7 The neural model of emotion regulation proposed by Phillips et al. allows for investigation of automatic and voluntary regulation processes.1 The literature reviewed in chapter two, using this model as a framework, supports the above hypothesis with regard to MDD subjects: findings indicated that automatic emotion regulation may be maintained in depressed MDD - but at the cost of compensatory lateral prefrontal recruitment, whereas this strategy appears to fail during voluntary emotion regulation.

In BD, the original review1 also suggested compensatory increased lateral prefrontal activity during automatic emotion regulation, but there were only few studies supporting this claim, none of which examined depressed BD subjects. In addition, findings from voluntary regulation studies in BD were inconclusive. Since then, little additional evidence has been provided. Most new studies focused on emotion processing or on euthymic/(hypo)manic subjects. The three
more recent studies that studied emotion regulation in depressed BD subjects reported heterogeneous results, especially during automatic regulation: either increased, increased, or equal prefrontal activity has been demonstrated.

Thus, given the paucity of (replicated) studies, the hypothesis of compensatory increased lateral prefrontal activity during automatic emotion regulation cannot be confirmed nor rejected. Regarding voluntary regulation, either equal or decreased prefrontal activity has been found, which may suggest a pattern in depressed BD comparable to that of depressed MDD subjects, i.e. an inability to generate compensatory lateral prefrontal activity when the emotional stimuli has already captured attention.

Our emotion regulation study (chapter three) demonstrated increased lateral prefrontal (DLPFC) activity during voluntary control in remitted BD subjects. We may speculate that, in contrast to depressed BD subjects, remitted BD subjects still have access to compensatory prefrontal activity also during voluntary regulation (which nevertheless fails, as implied by decreased emotion regulation success on a behavioural level). For remitted MDD subjects, such compensatory activity was not required to maintain normal performance. Together, this suggests that voluntary emotion regulation, compromised during depression in both MDD and BD, may return to normal levels during remission in MDD, but not in BD. Thus, BD subjects may be more severely affected than MDD subjects when considering emotion regulation disturbances.

It should be mentioned in this context is that we did not find any lateral prefrontal activity differences in depressed MDD and BD. This may reflect the inability to recruit compensatory mechanisms in both depressed MDD and BD during voluntary regulation, as described in the previous section and in chapter two. However, guided by the behavioural results and to prevent chance capitalization, we only tested whether neural activity associated with happy versus sad emotion regulation differed. This may have obscured possible overall DLPFC activity differences between MDD and BD during regulation of happy or sad emotions per se (i.e. regulate happy or sad versus attend happy or sad). Another possibility is the existence of equally abnormal lateral prefrontal activity in MDD and BD compared to HC, but comparisons with HC were only conducted post hoc (i.e. only for regions showing MDD versus BD differences).

Our findings furthermore suggest that emotion regulation differences in MDD and BD during depressive episodes (chapter three) were valence specific and may be secondary to a difference in
emotional appraisal (blunting and negative bias in MDD, normal appraisal in BD). Other studies investigating emotional appraisal support our appraisal findings, both on a neurocognitive/psychological level\textsuperscript{14–16} and a neuronal level.\textsuperscript{17} This differential mood reactivity may represent the vulnerability to switch to a (hypo)manic episode, or, related to this, a hyperthymic temperament.\textsuperscript{18–20} Tentatively, it may also be related to the notion that depression with atypical features, one of its characteristics being mood reactivity, is more common in BD, in contrast to depression with melancholic features, which may be more common in MDD.\textsuperscript{21}

Non-emotional cognitive circuits in BD and MDD

As discussed above, we did not find any DLPFC activity differences in depressed MDD and BD. Nevertheless, when investigating DLPFC functioning with a non-emotional cognitive task in chapter four, we did find indications for differential functioning in MDD and BD both during depression and remission. In secondary analyses, we found that increasing planning demands were associated with increased activity in depressed compared to remitted, and in BD compared to MDD subjects in a left DLPFC region previously implicated in depressed, but not remitted MDD.\textsuperscript{22} In addition, visuo-spatial planning \textit{per se} was associated with an interaction effect between mood state and diagnosis in another region of the left DLPFC and the right DLPFC (although in secondary analysis and in a different region). In BD, depression was associated with hyperactivity and remission with hypo-activity, whereas in MDD, depression was associated with hypo-activity and remission with hyperactivity.

In other words, mood state had opposite effects on prefrontal activity in MDD and BD during visuospatial planning. This finding is hard to interpret. Selection bias may have played a role, given the cross – sectional nature of our study: we may have included a depressed MDD or BD group with different “baseline” DLPFC activity compared to the remitted MDD or BD group. For example, in theory it is possible that the level of DLPFC activity of subjects included in the depressed BD group will increase and not decrease as soon as they achieve remission. Then, had the same group been tested in a longitudinal design, we would have found hyperactivity in remitted compared to depressed BD, just like in MDD, and no diagnosis by state interaction. Nevertheless, lateral prefrontal functioning appears disturbed also during non-emotional demands, in a disorder and possibly state specific way.
The parietal cortex also showed abnormally increased activity during visuo-spatial planning (chapter four), in a linear fashion across patient groups, with depressed BD subjects demonstrating the most pronounced increases. Like the DLPFC, the parietal cortex is not only involved in this specific cognitive task, but also in emotion regulation, e.g. in asserting attentional control in the presence of emotional distraction\textsuperscript{1,23} and in distancing from emotional stimuli.\textsuperscript{24} The need for increased activity in the absence of emotional stimuli may indicate a dysfunction which may also interfere with emotion regulation. It is, by the way, questionable to what extend cognitive tasks are really non-emotional in the context of mood disorders, since MDD and BD subjects often attribute emotional meaning to “neutral” tasks.\textsuperscript{25,26} Thus, alternatively, compensatory activity may have been required to overcome emotional interference.

Results regarding caudate activity in chapter four corroborate previous findings of striatal hyperactivity in depressed and (hypo) manic BD\textsuperscript{17,27–29} versus striatal hypoactivity in depressed MDD.\textsuperscript{17,27,30–34} Cortico-striatal circuits (or more general, cortico-basal ganglia circuits) subserve a variety of functions important in mood disorders (emotion and reward processing, motivation, motor and cognitive functions).\textsuperscript{27} Direct and indirect pathways exist. Briefly, the direct pathway results in increased cortico-thalamic excitation through striatal inhibition of the internal segment of the globus pallidus and the substantia nigra; the indirect pathway results in decreased cortico-thalamic excitation via striatal inhibition of the external segment of the globus pallidus and the subthalamic nucleus, which in turn leads to excitation of the internal segment of the globus pallidus and substantia nigra.\textsuperscript{27} Therefore, both striatal hyper- and hypoactivity may result in decreased thalamo-cortical excitation, in case of hyperactivity via the indirect pathway (in BD),\textsuperscript{35,36} and in case of hypoactivity via the direct pathway (in MDD)\textsuperscript{27}, thus differentially accounting for the affective, cognitive and motor symptoms during depressive episodes of both disorders. Tentatively, since striatal hyperactivity has also been found in (hypo)manic BD,\textsuperscript{17,27,29} one could argue that striatal hyperactivity may account for symptoms of (hypo)mania by increased thalamo-cortical excitation through the direct pathway.

**Individualized differentiation between BD and MDD by emotion regulation circuits**

Further support for differences between MDD and BD in the neural emotion regulation circuit is provided by the multivariate pattern
analysis (MVPA) study described in **chapter six**. Here, we found that the pattern of grey matter volumes of emotion regulation areas could be used to classify depressed MDD versus BD with an accuracy of 69% using a Gaussian process classifier (and a lower accuracy of 63% using a support vector machine [SVM]). Remitted MDD and BD could not be classified, however. This may imply either common volume abnormalities during remission, or a normalization of volume abnormalities upon remission in both MDD and BD. Since we were unable to classify either MDD or BD versus HC, neither during remission nor when depressed, it remains unclear to what extend grey matter volume patterns of emotion regulation areas in MDD and BD represent abnormalities.

**Alternative brain networks possibly relevant for distinction of BD vs MDD**

Critically, emotion regulation models are just one approach to explain neural mechanisms in mood disorders. Other models do exist, implicating comparable brain areas, that subserve slightly different functions dependent on the model’s perspective. For example, the model of Price and Drevets\(^{37}\) and the model of Pizzagalli\(^{38}\) emphasize the role of the medial prefrontal cortex, given its role in self-referential functioning as part of the default mode network (DMN). It has frequently been proposed that depression is related to an imbalance between the DMN and task-positive networks (e.g. fronto-parietal networks), reflected in increased rumination at the cost of cognitive functioning due to depletion of cognitive resources.\(^{38,39}\) In addition to grey matter volumes of emotion regulation areas, we used DMN functional connectivity to investigate the possibility of classifying MDD and BD subjects with multivariate pattern analysis (**chapter six**).

Findings were comparable to those using grey matter volumes of emotion regulation areas, showing that depressed, but not remitted, subjects could be discriminated. In support of this, a recent study comparing functional connectivity within the DMN demonstrated differences between depressed MDD and BD on a group level, i.e. increased functional connectivity in depressed MDD, but not BD.\(^{40}\) This study also found increased connectivity within the fronto-parietal network in depressed BD, but not MDD, which may corroborate our findings of differential fronto-parietal functioning between BD and MDD during emotion regulation and planning (**chapter three and four**). However, classification based on fronto-parietal connectivity was not possible in our dataset (**chapter six**). An explanation for the
latter may be a comparable fronto-parietal connectivity during rest despite activity differences during task performance in our sample. In turn, the lack of connectivity differences in our sample in contrast to connectivity differences found by Goya-Maldonato et al.40 may be due to differences in sample size between studies, or to the presence versus absence of medication effects.

**MDD and BD: what do observed differences mean?**

In summary, we found both behavioural ([chapter three](#)), functional ([chapter three, four, five](#)) and structural ([chapter five and six](#)) differences between medication-free MDD and BD. As discussed in the next section, these differences may provide clues for development of diagnostic tools. However, the finding of such differences also raises several questions.

**Scarring effects of mood episodes**

The question whether the above differences represent a vulnerability to develop (hypo)manic episodes or are the effect of scarring due to previous (hypo)manic episodes, is important for two reasons. First, to elucidate neuropathophysiological mechanisms, we need to distinguish between cause and consequence. Second, diagnostic uncertainty obviously exists in cases where (hypo)manic episodes have not yet occurred – although often hypomanic and sometimes even manic episodes go unrecognized or are not reported by the patient and thus will have left scarring effects by the time he or she seeks help for a depressive episode. If there is a major contribution of scarring, differences in persons without a previous (hypo-)manic episode will not be found, leaving no possibility to differentiate, despite the fact the patient is deemed to develop a bipolar course of illness.

Due to our cross-sectional design, we cannot answer this question; longitudinal studies in first-episode depression are needed to ascertain this. We may however speculate that structural inferior frontal gyrus (IFG) abnormalities are a vulnerability factor for development of (hypo)manic episodes. First, we found hypergyrification in BD specifically irrespective of mood state (although regional differences between depressed and remitted BD existed), which may indicate a trait abnormality in BD, but not in MDD ([chapter five](#)). In line with this, recent meta-analyses showed cortical thinning of
the IFG in established BD\textsuperscript{41} but not MDD,\textsuperscript{42} and another study using multivariate pattern analysis revealed a central role for IFG volume in the classification of MDD versus BD.\textsuperscript{46} Second, previous studies found volumetric abnormalities in the IFG in subjects at high risk for BD,\textsuperscript{43,44} indicating that abnormal IFG structure may be a vulnerability factor in BD. Third, in high-risk MDD studies, structural IFG abnormalities have not yet been found, which may indicate that abnormal IFG structure as a vulnerability factor is specific for BD. However, this hypothesis of IFG abnormalities being specific for BD is only tentative, since (a) absence of evidence is not equal to evidence of absence, (b) there are no studies directly comparing subjects at high risk for MDD versus BD, and (c) in established MDD and BD, indirect meta-analytic comparison revealed no volumetric IFG differences.\textsuperscript{45}

Another issue that is important to discuss here is the fact that our depressed BD and MDD subjects differed regarding the number of previous major depressive episodes. Hence, differences in depressed MDD versus BD subjects may also be explained by a vulnerability for or the effect of scarring due to high versus low recurrence rates of depressive episodes (see also further on).

**MDD and BD: spectrum disorders or not?**

Since the introduction of the unipolar-bipolar distinction in the 1960s, there has been an ongoing debate about the question whether MDD and BD categories indeed represent two separate disease entities or are part of a continuous unipolar-bipolar spectrum. To learn whether our results support one of the two concepts, it should be noted that we address the contemporary MDD-BD distinction according to the DSM-IV, i.e. based on polarity of mood symptoms. Thus, (hypo)manic symptoms being either present or absent during the course of disease would imply separate disease entities; a gradual increase in (hypo)manic symptoms from none in unipolar depression to full-blown mania in bipolar disorders would imply a unipolar-bipolar spectrum. Of note, as advocated by Goodwin et al, cyclicity (i.e. recurrence rate) may be more important in distinguishing a form of “pure” MDD from BD than mood polarity: more highly recurrent MDD patients may resemble BD patients with respect to several characteristics, like a positive family history for mania, an early age of onset, depression with atypical features, and prophylactic response to lithium.\textsuperscript{47}
Again, our results are inconclusive for several reasons, even if the different neurobiological profiles indeed reflect a difference in vulnerability for (hypo)manic derailment. First, we included subjects at the extreme ends of the spectrum, either with or without (hypo)manic episodes, also represented by high or low scores, respectively, at the Hypomania Checklist\textsuperscript{48} (assessing life time hypomanic symptoms) and Bipolarity Index\textsuperscript{49} (indicating the level of bipolarity). Thus, we could not correlate our imaging data with the degree of bipolarity. Although we did include BD-II subjects, groups were too small for a reliable comparison between MDD, BD-II and BD-I. Nevertheless, some findings of the visuo-spatial planning task may indicate that BD-II is not only intermediate between MDD and BD on a phenomenological level (i.e. severity of (hypo)manic symptoms), but also on a neurobiological level (\textit{chapter four}). In support, a small study investigating mood disorder subjects across the spectrum (i.e. MDD, BD-II, BD-I) found more pronounced structural abnormalities in BD-I than BD-II compared to MDD;\textsuperscript{50} another found correlations between Bipolarity Index scores and activity in several brain regions across MDD and BD subgroups.\textsuperscript{51} Second, the existence of a bipolar spectrum does not exclude the existence of a form of “pure” MDD, not only characterized by the complete absence of (hypo)manic subthreshold symptoms and/or (combinations of) other bipolar traits (high recurrence rate, positive family history etc.), but also by specific pathophysiology.\textsuperscript{52–55} Third, to what extent any similar neurobiological abnormalities associated with emotion regulation, planning, resting state and brain structure exist in medication-free MDD and BD was not investigated in this thesis: we only compared patient groups with healthy controls \textit{post hoc}, i.e. in those brain areas where differences between MDD and BD were found. Previous studies however do imply common abnormalities in (medicated) MDD and BD.\textsuperscript{8,45,56–62} Nevertheless, common abnormalities do not preclude the possibility that MDD and BD are distinct entities: the brain contains many final common pathways and some pathophysiological mechanisms may be shared, whereas others may be not.\textsuperscript{47}

\textbf{The nature of MDD versus BD depression}

Differential neurobiological profiles during depression may imply that the nature of depressive episodes in MDD versus BD is different. However, the vulnerability to develop (hypo)manic episodes may be considered a parallel but separate process, juxtaposed to the (vulnerability for) depression. If so, certain “disorder-specific”
neurobiological characteristics observed during the depressive episode may thus relate to this vulnerability for (hypo)manic episodes as an independent dimension, rather than to the depression itself. In other words, should the single dimension of “mood” (ranging from depressed to manic) be separated in two parallel dimensions: (a) “depression” (ranging from euthymic to depressed) and (b) “mania” (ranging from euthymic to manic), each characterized by their “own” pathophysiology, existing in parallel and interacting with each other?

This idea would be a return to pre-Kraepelin times, when mania and melancholia were predominantly considered separate diseases – although some 20th century psychiatrists (Kleist, Leonhard and Neele) maintained the distinction between mania, melancholia and bipolarity, considering the latter a combination of two unipolar conditions. The idea was renewed in 1999 by Joffe et al. and later supported by evidence reviewed by Cuellar et al., considering course, symptoms, neurobiology and psychosocial risk factors. Population studies and recent studies on family transmission of depression and mania also indicate that mania and depression may reflect separate underlying dimensions, since transmission of mania and depression were found to be largely independent. Also, different aspects of early childhood neurodevelopment were found to predict either manic or depressive symptoms in early adulthood, suggesting (partly) unique origins of manic and depressive symptoms.

Furthermore, depression has been proposed to be a nonspecific response to brain injury. In BD, such brain injury may be inflicted by mania itself (e.g. by the excitatory processes present during mania), or by the neurobiology underlying mania. From this perspective, BD depression may be an epiphenomenon. This would explain the frequent co-occurrence of depressive episodes in patients suffering from (hypo)manic episodes, both alternately and simultaneously (in mixed episodes). Indeed, the idea of depression being non-specific is not new either: Bleuler already considered affective symptoms in general as nonspecific, appearing in the context of a more or less severe psychotic spectrum disorder. A comparable view has been proposed by Hickie et al., considering mania a “bipolar” dimension of activation (ranging from high to low) rather than mood, to be separated from the independent “unipolar” dimensions of major depression (i.e. depressed mood) and psychotic syndromes. The concept of mania being a state of hyperarousal/increased activity is incorporated in the DSM-5 as the additional gate criterion of increased energy/activity, besides elated/euphoric mood or irritability.
Implications for diagnostic tools

Despite these psychopathological consideration regarding MDD and BD it is clinically still important to distinguish between both. Subjects who will never experience (hypo)manic episodes usually benefit from different treatment strategies than subjects who will. In chapter six we distinguished medication-free MDD and BD on an individual level and showed that mood state may be of vital importance when imaging data are to be used for the development of a diagnostic tool. Remitted MDD and BD subjects could not be classified correctly based on resting-state and structural MRI, while other MDD-BD differences, related to emotion regulation and cognitive functioning (chapter three and four), were also state-dependent. The possibility that neuroimaging differences observed during depression are in fact related to depression severity could not be investigated by us due to the inclusion of only moderately to severely depressed subjects in the depressed group. Ideally, a diagnostic tool is applicable for all stages and severity levels of a disease. Therefore, unless there is some way to control for possible confounders, a “disease”-specific characteristic should either be a trait, or independent from depression severity to be useful as a diagnostic tool. In the same vein, other important clinical characteristics to consider are recurrence rate, duration of illness, and previous medication use.

Another issue is the performance of the classifier in terms of sensitivity (true positives divided by number of subjects with the disease) and specificity (true negatives divided by the number of subjects without the disease). Sensitivity and specificity were only moderate when using resting state and structural MRI data (about 70%). For MDD and BD, ideally both high sensitivity and high specificity are required. Unjustly diagnosing a BD subject as MDD (low sensitivity/high specificity; i.e. many false negatives) would deny the patient effective treatment. However, incorrectly diagnosing an MDD subject with BD (high sensitivity/low specificity; many false positives) would expose the patient to treatment with medication (e.g. lithium) that is not only less likely to work, but also has more serious (and partly irreversible) side effects on the long term than antidepressants.

Furthermore, for clinical decisions, positive predictive value (true positives divided by the number of positive predictions) and negative predictive value (true negatives divided by the number of negative predictions) are more informative. They reflect the chance that the test result is correct, and not false-positive or false-negative.
However, whereas sensitivity and specificity are characteristics of the diagnostic test itself, positive and negative predictive value depend on the prevalence of the disease in the population under study. In other words, positive and negative predictive value calculated from our samples would only apply to a comparable population - MDD and BD subjects in a proportion rate of 1:2, which is not necessarily the situation in the clinical population where advanced diagnostic tests are required.

A more general area of concern is replicability of multivariate pattern analysis (MVPA) study results. While having the advantage of individual prediction over the group level results of mass univariate MRI analyses, basically the same problems inherent to the use of MRI data are encountered when using MVPA. The effects of using different scanners, different acquisition parameters, different scanning paradigms, small samples, heterogeneous samples based on categorical DSM diagnosis, and differences in feature selection methods, may all account for heterogeneous findings. Classifier performance should be confirmed in an independent testing sample, which means that the classifier resulting from training in one sample, performs more or less equally in another sample.

Redlich et al. were the only authors of a previous MDD versus BD study who so far actually tested this. In the training sample, MDD and BD subjects could be classified with 69% accuracy. However, performance declined to 64% in the test sample, despite similar clinical characteristics of the training and test samples. Furthermore, the classifier was also applied to our (medication-free) sample, resulting in a classification accuracy around chance level (unpublished results). This may again be due to differences between the samples (e.g. the medication-free status of our sample versus the medicated status of the Redlich-samples) especially because, as shown in chapter six, it was possible to correctly classify our MDD and BD subjects when training the classifier in our own sample. Still, this illustrates that replication of MVPA results is not straightforward, let alone clinical applicability.

Obviously, for reasons described above, because the features of the default mode network were sample-specific and because multivariate pattern analysis-results were uncorrected for multiple comparisons (i.e. MDDr versus BDr and MDDd versus BDd, for four different networks, thus eight comparisons) our findings are merely hypothesis-generating, showing that 1. imaging data may be useful for MDD versus BD classification, also if they are currently medication-free, and 2. mood state may be important.
Methodological considerations

We included specific subgroups of MDD and BD regarding several clinical characteristics (recurrence rate, illness duration, depression severity, comorbidity, family history of MDD subjects, medication-free status, response to antidepressants in MDD). This selection had the advantage of allowing the comparison between BD subjects and relatively “pure” MDD subjects with less risk of MDD-to-BD conversion, and without confounding effects of current medication use. Whilst this approach is valuable to find putative MDD-versus-BD markers, it also complicates straightforward translation towards clinical utility. In clinical practice, comorbidity or symptomatic overlap with other Axis I and/or personality disorders is the rule rather than the exception. On the other hand, since diagnosis was still based on DSM-IV categories (free of any assumptions regarding aetiology and allowing for several combinations of symptoms), it stands to reason that both the MDD and BD samples still comprised subtypes with regard to symptom constellations, aetiology and/or pathophysiology. The BD sample included both BD-I and BD-II subjects. Whether BD-II subjects may be considered intermediate between MDD and BD-I or represent a separate subgroup is still a matter of debate. Moreover, the depressed MDD and BD samples differed regarding number of previous episodes, so differences in neuroimaging profiles may also be (partly) be due to differences in recurrence rate, rather than differences in polarity. However, additional analyses regressing out the effect of previous episodes did not change the results (chapter three and four).

The inclusion of subjects that are not medication-naïve is also an important issue. Whereas some studies show that effects of medication on the brain are reversible,79,80 long-term effects cannot be ruled out. Medication-naïve subjects with established MDD and BD are, however, very rare and less representative for the general MDD and BD population. A well-known limitation of imaging studies also counts for our project: the small sample size. Low power may have obscured additional MDD-BD differences, reduced reliability of observed differences and inflated effects sizes of true differences. This general limitation in the field of neuroimaging likely contributes to replication failures and biased meta-analyses.81 Fortunately, there is a growing awareness of this problem. Currently sample sizes are increasing and since a few years several large-scale collaborative consortia, like the ENIGMA consortium, have started.
Another issue considers the cross-sectional nature of our studies which reduced our opportunity to disentangle whether observed differential profiles reflect vulnerability or scarring. Furthermore, state effects should be interpreted with caution: if the neuroimaging profiles of remitted patients are considered as “baseline”, it is possible that, by chance, the depressed groups would have had a different “baseline” had they been remitted; consequently, cross-sectional differences between remitted and depressed groups may not represent the change in profile that occurred within subjects.

Besides the fact that neuroimaging in general and (f)MRI in particular has limitations (e.g. it is still unclear how the BOLD-signal, used in fMRI, is related to underlying neural activity\(^{82}\)) it was recently discovered that assumptions used in MRI analysis software including SPM are not always met by real data, resulting in spurious findings.\(^{83}\) Freesurfer, used in the analysis of (sub)cortical structure, also has its drawbacks, since results have shown to vary between different software versions, workstations and operating systems.\(^{84}\)

**Recommendations for future research**

As we showed, neuroimaging may be useful for MDD-BD differentiation, although in a mood-state dependent way. However, the effects of depression severity, of scarring due to previous (hypo) manic episodes and of previous medication use remain unknown. To assess the effects of depression severity, investigation of subjects along a continuum of severity levels, ranging from mild to severe depression, is required. To confirm state effects, longitudinal within-subject studies comparing episodes of depression and remission are needed. Longitudinal studies are also warranted to investigate potential scarring effects, starting in first episode mood disorder patients and/or healthy, high risk relatives. Such longitudinal approach is also useful to further examine the effects of medication, as first-episode patients may be medication-naïve at the time of inclusion.

More generally, mechanisms underlying the vulnerability for and switch to mania, as well as the co-occurrence of and interactions between mania and depression need to be elucidated to further understand the pathophysiology of mood disorders. This is not only important for diagnostic purposes, but also for the development of treatment and prevention strategies.
Longitudinal studies may also identify disease-specific trajectories of (neuro)biological changes, which are more informative than cross-sectional differences, given the dynamic and developmental nature of psychiatric disorders. Furthermore, prospective studies in first degree relatives and offspring of MDD and BD subjects ("at risk" subjects) are vital to further discover vulnerability markers and confirm endophenotypical aspects of differences between MDD and BD.

To further examine the relationship between (hypo)mania and depression, investigating not only depressive, but also (hypo)manic symptoms on a continuum of severity is warranted. Importantly, matching on recurrence rate is essential for inferences regarding polarity when comparing MDD and BD, given the fact that BD is not only associated with (hypo)mania, but also with increased recurrence rate compared to MDD. Moreover, unipolar mania should be investigated. It has been suggested that the apparent rarity of unipolar mania is an artefact of clinical sampling and thus unipolar mania may be more prevalent than assumed. Examining mania in isolation might help clarifying its hypothetically unique biological pathway and allows for testing of the hypothesis of bipolar disorder as a multidimensional construct (i.e. comprising mania, depression, psychosis).

On the level of the brain, research would benefit from combining different neuroimaging techniques, thus integrating information from different levels of brain functioning. Moreover, as recognized for some time now, analysis techniques should go beyond investigating regional activity or anatomy. The brain can be conceptualized as organized in structural and functional networks interacting with each other (the human connectome) and psychiatric disorders are now considered disorders of brain connectivity rather than arising from dysfunctional brain regions in isolation.

However, the brain is not the only organ affected in mood disorders, given the high comorbidity with somatic disease (e.g. metabolic syndrome). It has been argued that mood disorders are in fact multisystem diseases. Investigating MDD and BD from this perspective would lead to a more comprehensive understanding of shared and/or differential pathophysiology.

Nevertheless, to really make progress we should probably abandon traditional DSM category based research. The current diagnostic system uses a categorical approach to classify psychiatric disorders, formulating discrete entities based on the presence of specific (combinations of) symptoms and phenomena. Notably, these
categories are descriptive constructs, not medical conditions based on underlying pathophysiology and aetiology. A paradigm shift is already going on, illustrated by initiatives like the Research Domain Criteria (RDoC), replacing categorical by dimensional approaches.\textsuperscript{92} Data-driven approaches (e.g. unsupervised machine learning) are also receiving increasing attention: for example, subgroups of bipolar patients have been identified based on neurocognitive data.\textsuperscript{93} Such approaches, free of any \textit{a priori} assumptions of categories, may eventually lead to identification of patient subgroups based on specific pathophysiological underpinnings, which in turn may aid in development of prevention and treatment strategies, as well as in predicting the course of disease. On the other hand, also without fully understanding the underlying pathophysiology of disorders, machine learning techniques may be applied directly to predict response to treatment or course of disease, as has already been demonstrated using supervised machine learning (see e.g.\textsuperscript{94–96}).

As a general recommendation, data sharing, standardization of assessments and collaboration between research groups should be promoted. In this way, it will be easier to obtain sample sizes large enough to yield reliable findings, and to test and improve analysis methods. This will be even more necessary given the recently reported limitations of widely applied statistical software.\textsuperscript{83} It may furthermore prevent publication bias and allow for external checks.\textsuperscript{81} As clearly pointed out by Button et al.,\textsuperscript{81} research that is unreliable is wasteful and ethically indefensible.
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